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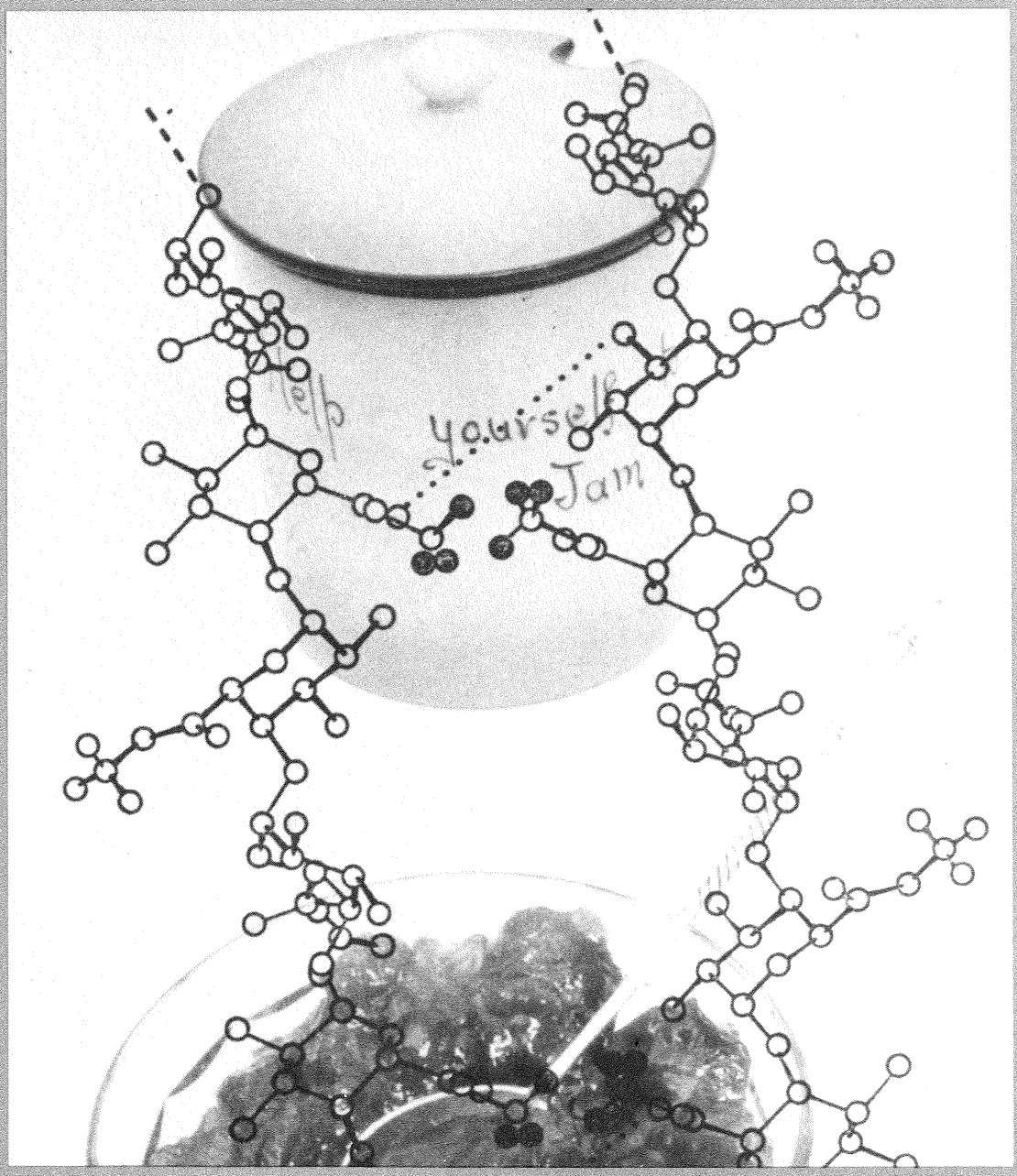

Food gels

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Introduction

Gels have a characteristic texture which has been exploited in foods for a long time. Boiled eggs, junket and yoghurt have obviously been eaten for thousands of years. Jellies specially prepared from gelatin first appeared in Europe in the fifteenth century. Jam was a Roman invention and a food served at feasts in medieval England was 'blancmange' – a savoury starch gel prepared from slivers of chicken boiled with rice and almond milk. The characteristic gel texture, a soft, elastic consistency, is obvious when gels are eaten or touched but is hard to define in physical terms. This review discusses recent developments in our understanding of the properties of gels and how this relates to their use in food and in the food industry.

Gels may contain as much as 99.9% water but have properties more typical of the solid state, particularly elasticity and rigidity. Their properties are the consequence of the formation of a three dimensional network of polymer molecules – proteins or polysaccharides – completely spanning the volume occupied by the gel (Fig. 1). The conditions required for the preparation of the gel, its stability, the shelf-life of the food and what it feels and tastes like to eat all ultimately depend on the nature of this network of polymer molecules and the molecular interactions which hold it together.

Gel forming polymers used in foods

The polymers most commonly used in the preparation of food gels are listed in Table 1. Their use depends partly on cost and partly on

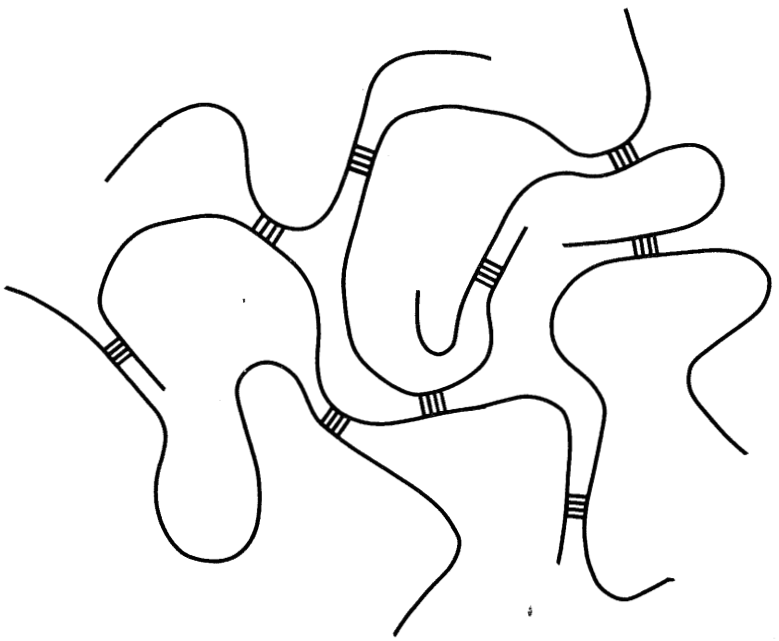

Fig. 1. Schematic diagram of a gel network. Hatched areas represent the junction zones

TABLE 1

The gel-forming polymers commonly used in foods

Polymer	Approximate cost relative to starch	Source	Chemical composition	Typical uses
Gelatin	5	Animal skins and bones	Protein: major amino acids are glycine and proline.	Dessert jellies, cake icings, confectionery, canned meat and fish.
Pectin	9	Citrus peel and apple pomace	Linear polymer of partly esterified D-galacturonic acid.	Jams and preserves, confectionery.
Carageenan	12	Seaweed *Chondrus crispus*	D-galactose and sulphated D-galactoses.	'Instant' desserts sugar-free dietetic jams.
Agar	21	Seaweed (many different species)	Agaropectin (a linear polymer of sulphated D-glucuronic acid) and agarose (a neutral polymer of agarobiose)	Pie fillings (withstands high temperatures), confectionery, canned meat and fish.
Alginates	10	Giant kelp, *Macrocystis pyrifera* or species of *Laminaria*	Copolymer of D-mannuronic acid and L-guluronic acid.	Dessert gels, puddings, pie fillings and artificial fruits for cakes and pies (withstands heat), confectionery.
Starch	1	Potato, wheat, rice, maize, cassava, sago, arrowroot	Polymers of D-glucose: amylose (linear) and amylopectin (branched)	Puddings, custard, pie fillings, confectionery.

the type of product – its pH, for example, the desired texture or whether the product has to withstand high temperature. The polymers used by the food industry in greatest quantity are starch, gelatin and alginate.

Gel networks

The key factor in gelation is the formation of cross-linkages between the polymer molecules which establish the gel network (Flory 1953). In the types of gel that occur in foods these cross-linkages are not permanent. The molecules are held loosely together by a large number of individually weak forces such as hydrogen bonds (Rees 1969). Many monomer units interact cooperatively and form a 'junction zone' – indicated by the hatched areas in Fig. 1. The structure and stability of these junction zones obviously depend on the chemical composition and molecular geometry of the gel-forming polymer.

Gelatin

Gelatin is a water-soluble protein formed by partial degradation of collagen from animal skins and bones. The basic collagen polymer unit (tropo-collagen) consists of three helical polypeptides wound around each other as shown in Fig. 2 (Rich and Crick 1961). Gelatin itself has a much lower degree of internal organization but when it gels the junction zones consist of regions of collagen-like triple helix (Veis 1964). The average number of amino-acid residues per junction zone is about 140, which represents 16 turns of triple helix (Oakenfull 1984).

Polysaccharides

Although polysaccharides have a less well-defined tertiary structure than proteins they often occur in nature in packed arrays of comformationally ordered chains – familiar examples are cellulose in wood or cotton or amylose and amylopectin in starch granules. In the gel-forming polysaccharides two types of structure are formed – helices or corrugated ribbons (Morris and Norton 1983).

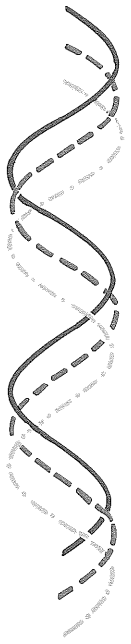

Fig. 2. The collagen triple helix forms the junction zones in gelatin gels.

Helices – A repeating monosaccharide unit as shown in Fig. 3 introduces a twist into the molecule. This type of linkage occurs in amylose and amylopectin, κ-carrageenan and agarose. The flexibility of polysaccharide chains is too restricted for them to form single-stranded helices equivalent to the protein α-helix but two molecules can form double helical structures as shown in Fig. 3. The junction zones in starch, carrageenan and agarose gels consist of lengths of polysaccharide chain twisted together in a double helix stabilized by hydrogen bonds (Rees 1969). The existence of these double helices has been inferred from spectroscopic measurements but nothing is yet known about their size or relative stability in the different gelling systems.

Corrugated ribbons – Alginates and pectins form these structures which result from the linkage geometry shown in Fig. 4. Chains packed together in this conformation have interstices or cavities which correspond in size to the ionic radius of Ca^{2+} (indicated by the filled circles in Fig. 4) and these structures are stabilized by calcium. In alginate gels, the junction zones consist of regions of corrugated ribbon structure made up from polyguluronate sequences (Rees 1969). Other monosaccharides in the polymer break up the ordered structure, promoting the formation of a gel network rather than complete aggregation (and consequently precipitation) of the polymer.

Pectin consists of partly esterified polygalacturonic acid which is structurally similar to guluronic acid. Low methoxyl (LM) pectins have most of their monomer units as unesterified, free D-galacturonic acid. This polymer interacts with calcium and gels under the same conditions as alginate. In high methoxyl (HM) pectins, however, most of the acid groups are esterified with methanol. Interaction with calcium is no longer structurally possible but hydrophobic interaction of ester methyl groups can now provide sufficient additional stabilization for the formation of junction zones if suitable additives, such as sucrose, are present (Oakenfull and Scott 1984). Segments from two polygalacturonic acid chains interact and the junction zones contain from 30 to 500 monomer units, depending on the degree of esterification.

The rheological properties of gels

Gels show complex viscoelastic behaviour (Mitchell 1976, 1980). In a perfectly elastic solid, strain responds immediately to stress, as

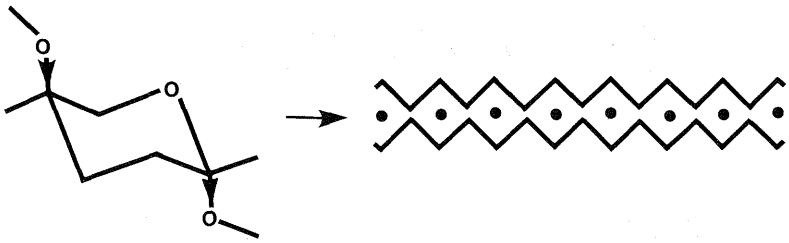

Fig. 3. The formation of helical structures by polysaccharides. The geometry of the linkage between the monosaccharide units induces a twist in the polymer chain. About six monosaccharide units complete one turn of the helix. The double helix structure is stabilized by hydrogen bonds.

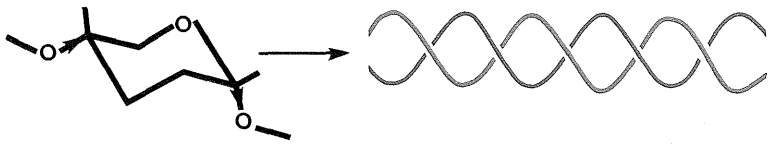

Fig. 4. The formation of corrugated ribbon structures by polysaccharides. The 1,4 diaxial linkage geometry between the monosaccharide units induces the polymer structure indicated schematically. Each straight line indicates one monosaccharide group. These structures are stabilized by calcium ions (indicated by the filled circles).

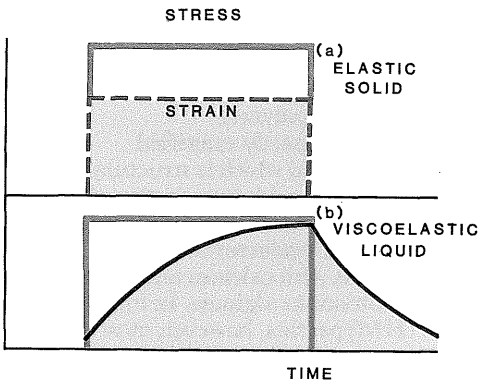

Fig. 5. The response to applied stress of (a) a perfectly elastic solid and (b) a viscoelastic material.

shown in Fig. 5(a). Gels, however, are viscoelastic liquids which means that they take a finite time to respond to an applied stress, as shown in Fig. 5(b). For food gels this time span is very long and for comparative purposes (for quality control, for example) gels can be set for the same period of time and measurements made following the same time course.

The various measurements that can be made are illustrated by Fig. 6 which shows the relationship between load (stress) and deformation (strain) for a slab of jelly compressed between two parallel plane surfaces. The initial slope gives the rigidity or shear modulus (G = stress/strain). The maximum load is the rupture strength (RS) of the gel and the deformation under a load of 0.5 RS is its cohesiveness. The shear modulus is the only one of these quantities which can at present be treated theoretically (Mitchell 1980) and related to polymer-polymer interactions and the formation of junction zones (Oakenfull 1984).

Several commercial instruments are available for the routine testing of gels and these, with their principles of operation, are listed in Table 2. They are all empirical tests and not necessarily related to the more fundamental measurements described in Fig. 6. They produce results more or less related to shear modulus (D and E), rupture strength (C) or a combination of both these parameters (A and B). Shear modulus and rupture strength are not linearly related, as is shown in Fig. 7. Consequently, different testing procedures may not rank a series of gels in the same order of gel strength (Mitchell 1976). If gels are to be characterized by a single parameter the test method chosen should give results which best reflect the gel property of interest, as discussed in the following section.

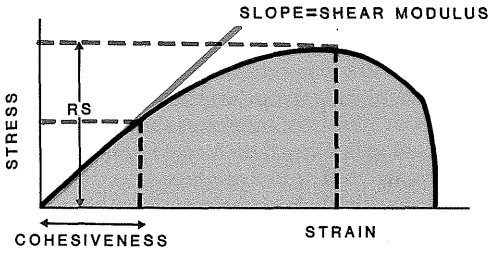

Fig 6. The relationship between stress and strain for a typical gel.

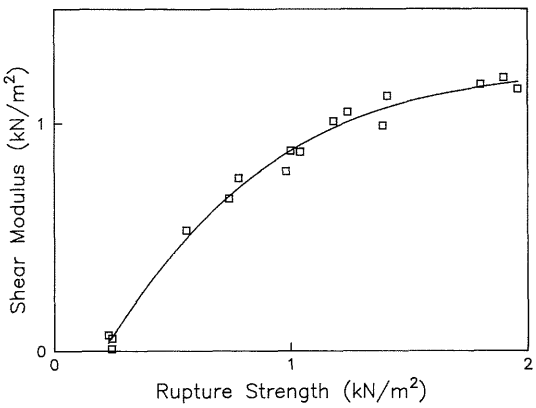

Fig. 7. The relationship between shear modulus and rupture strength for gels of high methoxyl pectin at a concentration of 5 g/kg in the presence of different concentration of ethanol.

The psychophysics of gels

Psychophysics is the quantitative study of the relationship between stimulus and perception. Within the context of food gels there are two areas of concern: (1) The relationship between the texture of the gel, as perceived by handling it or chewing it, and rheological measurements as described in the previous section and (2). The relationship between flavour and the analytical concentration of flavouring compounds combined with the rheological properties of the gel.

The texture of a gel is perceived by touch, sight and mouthfeel. It is described in terms such as hard, soft, elastic, brittle, tough, wobbly, stiff (Wood 1979). These various perceptions have been found to correlate with quite different rheological measurements as shown in Table 3. Perceived gel texture seems to be related to a combination of rheological

TABLE 2

Instruments for the routine testing of gels

Instrument	Principle of operation
Bloom Gelometer[A] Boucher Electronic Jelly Tester[B]	Measures the force required to push a plunger a fixed distance into the gel.
Marine Colloids Gel Tester[C]	Measures maximum force exerted by a plunger penetrating the gel at a constant velocity.
FIRA Jelly Tester[D]	The gel is set in a vessel containing a rectangular paddle. Measures the torque which must be applied to give a fixed (small) angular deflexion of the paddle.
Exchange Ridgelimeter[E]	Measures the distance that the gel slumps when removed from a standard mould.

[A]Precision Scientific Co., Chicago, IL., U.S.A.
[B]G. Stevens & Son Ltd., London, U.K.
[C]Marine Colloids Inc., Springfield, N.J., U.S.A.
[D]A. Gaydon & Co., Croydon, U.K.
[E]Sunkist Growers Inc., Ontario, CA., U.S.A.

TABLE 3

Comparison of sensory and rheological estimates of gel texture (Wood 1979)

Sensory testing method	Rheological measurement giving best correlation
Visible wobble, touch	Shear modulus
Brittleness in the mouth	Strain under maximum load
Gel strength in the mouth	Rupture strength

factors in a complex manner which is still far from understood (Sherman 1982). It is clear though that if a single rheological testing procedure is to be used to assess gel quality it should be chosen with care so as to correlate with the dominant sensory characteristics of the product.

Perception of flavour also depends on the rheological properties of the gel. Flavours presented in the form of a gel are less intense and more difficult to detect than when presented in equal concentration, in liquid solution (Mackey and Vallassi 1956). In a

comparative study of sweetness of different gels containing equal concentrations of sodium sucaryl, Marshall and Vaisey (1972) found that carrageenan gels were least sweet, starch gels were most sweet and gelatin and agar gels had intermediate sweetness. No single physical parameter, or group of parameters, adequately predicted the observed differences of sweetness.

Mixed gelling systems

Combinations of gelling hydrocolloids are often used in food products – either because a combination gives a desirable texture or when a polysaccharide is used to gel a meat or dairy product and protein is inevitably one of the components. In these systems there seems almost always to be a strong interaction between the polymers. Gelation is either inhibited or enhanced and the texture of the gel can be very different from those of the gels formed by the components singly.

Myoglobin and bovine serum albumin inhibit the formation of alginate gels but promote gelation of low methoxyl pectin (Hughes *et al.* 1980). Locust bean gum mixed with κ-carrageenan produces less brittle, more elastic gels than κ-carrageenan alone, the rupture strength and shear modulus increasing until the ratio of locust bean gum to κ-carrageenan reaches 1:1 (Christensen and Trudsoe 1980). Low methoxyl pectin, on the other hand, when added to κ-carrageenan produces no effect on the rupture stength or shear modulus of the gel but produces 'softer more palatable gels' according to sensory tests (Christensen and Trudsoe 1980). A synergistic interaction of fish and soya bean proteins has been observed (Furukawa *et al.* 1982) and similar effects occur in the formation of gels from egg white and soya bean protein (Jao *et al.* 1980).

The physical chemistry of these mixed systems is obviously very complex. At present we are confronted with a mass of empirical observations but have no theoretical framework for interpreting them.

Effects of added solutes

Food gels are likely to contain added solutes such as salt or sugar in sufficient concentration to affect gelation. In some cases particular solutes are require for the gel to form. Gels of alginate or low methoxyl pectin require the presence of Ca^{2+}; gels of high methoxyl pectin require sucrose at a concentration in excess of 55% by weight. More generally, solutes simply affect the texture of the gel, its melting temperature, or the minimum concentration of polymer required for gelation. This is again an area where there is a mass of empirical observations but little theoretical understanding.

Sucrose inhibits the gelatinization of starch (Glicksman 1969) but increases the shear modulus of gels formed by κ-carrageenan (Rey and Labuza 1981) or low methoxyl pectin (Glicksman 1969). Added electrolytes have little effect on gelatin gels (Veis 1964) but other gels may be sensitive to salts in general (i.e. to the ionic strength) or to specific ions. In the case of starch these effects are complex and depend on temperature and concentration as much as on the ionic species present (Glicksman 1969). Both sodium and potassium chloride increase the rupture strength of κ-carrageenan gels but potassium chloride has the greater effect by a factor of nearly 100 (Rey and Labuza 1981). There is currently much interest in reducing the sodium content of foods because of the association of high sodium intake with hypertension. Substituting potassium chloride for sodium chloride could have a powerful effect on the texture of a gel-based food and the product would probably need to be reformulated.

Nu-food

Gel-forming hydrocolloids can be used to give acceptable form and texture to low grade food materials such as fruit or vegetable pulp or comminuted meat or fish (Glicksman 1976). This would reduce waste and reduce the high cost of fruit and vegetable ingredients. Artificial cherries, prepared from alginate, were patented in 1946 and similar products now used commercially include apple slices, blackcurrants and onion rings. Low methoxyl pectin can be used instead of alginate and these gels have sufficiently high melting temperatures to be suitable for cakes and pies.

TABLE 4

Novel food gels (from Glicksman 1976)

Gelatinized beer
Artificial caviar
Artificial cherries and blueberries
Imitation low-calorie spaghetti
Fabricated meat balls and shrimps
Fabricated potato chips and other vegetables
Soft drink gels

Creative food technology has bestowed on humanity the products listed in Table 4 – not all of them have been commercially viable!

Research needs

There are three obvious major areas where more research is needed:

- More information is needed about the process of gelation at the molecular level. This is the key to understanding the complex behaviour of mixed gelling systems and how gels respond to different temperatures and different product formulations. It would also explain, and could probably be used to circumvent, the troublesome variations in product texture which can arise from small batch variations in the gelling polymers.
- The kinetics of gelation are of obvious technological importance but have not been studied systematically. The problem appears to be a lack of suitable experimental methods.
- More information is needed about the relationshp between gel texture, as perceived by the consumer, and rheological measurements. This is a conceptually difficult field of research which is still in its infancy.

References

Christensen, O., and Trudsoe, J. (1980). Effect of other hydrocolloids on the texture of kappa-carrageenan gels. *J. Texture Stud.* **11**, 137-47.

Flory, P. J. (1953). 'Principles of Polymer Chemistry'. (Cornell University Press: Ithaca, N.Y.)

Furukawa, T., Koyama, S., and Ohta, S. (1982). Interactions between fish protein and soy protein as related to their gel forming properties. *J. Jpn Soc. Food Sci. Technol.* **29**, 208-13.

Glicksman, M. (1969). 'Gum Technology in the Food Industry.' (Academic Press: New York.)

Glicksman, M. (1976). Hydrocolloid utilization in fabricated foods. *Cereal Foods World.* **21**, 17-20, 22-3, 26.

Hughes, L., Ledward, D. A., Mitchell, J. R., and Summerlin, C. (1980). Effect of some meat proteins on the rheological properties of pectate and alginate gels. *J. Texture Stud.* **11**, 247-56.

Jao, Y. C., Larkin, J. W., Chen, A. H., and Goldstein, W. E. (1980). Response surface analysis of egg white gelling properties in a meat loaf analog. *J. Food Process Eng.* **4**, 227-39.

Mackey, A., and Vallassi, K. (1956). The discernment of primary tastes in the presence of different food textures. *Food Technol.* **10**, 238-42.

Marshall, S. G., and Vaisey, M. (1972). Sweetness perception in relation to some textural characteristics of hydrocolloid gels. *J. Texture Stud.* **3**, 173-85.

Mitchell, J. R. (1976). Rheology of gels. *J. Texture Stud.* **7**, 313-39.

Mitchell, J. R. (1980). The rheology of gels. *J. Texture Stud.* **11**, 315-37.

Morris, E. R., and Norton, I. T. (1983). In 'Aggregation Processes in Solution'. (Eds E. Wyn Jones, and J. Gormally.) (Elsevier: Amsterdam.)

Oakenfull, D. (1984). A method for using measurements of shear modulus to estimate the size and thermodynamic stability of junction zones in non-covalently cross-linked gels. *J. Food Sci.* **49**, 1103-8.

Oakenfull, D., and Scott, A. (1984). Hydrophobic interaction in the gelation of high methoxyl pectins. *J. Food Sci.* **49**, 1093-102.

Rees, D. (1969). Structure, conformation and mechanism in the formation of polysaccharide gels and networks. *Adv. Carbohydr. Chem. Biochem.* **24**, 267-332.

Rey, D. H., and Labuza, T. P. (1981). Characterization of the effect of solutes on the water-binding and gel strength properties of carrageenan. *J. Food Sci.* **46**, 786-9.

Rich, A., and Crick, F. C. (1961). The molecular structure of collagen. *J. Mol. Biol.* **3**, 483-506.

Sherman, P. (1982). Gums and stabilisers for the food industry. Interactions of hydrocolloids. *Prog. Food Nutr. Sci.* **6**, 269-84.

Veis, A. (1964). 'The macromolecular chemistry of gelatin'. (Academic Press: New York.)

Wood, F. W. (1979). In 'Food Texture and Rheology'. (Ed. P. Sherman.) (Academic Press: London.)

The biotin content of Australian breakfast cereals and its role in the diet

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Introduction

Biotin (Fig. 1) is a water-soluble vitamin of the B-complex group. It is widely distributed in foods, but its concentration is low even in foods considered to be good sources (e.g. liver, kidney, yeast, egg yolk). With the exception of the bran component, cereals have a low biotin content. In foods biotin occurs either as a free vitamin or in a form bound to protein. Many forms of bound biotin (e.g. in wheat) are not biologically available to man or animals. Another source of biotin for man is synthesis of the vitamin by microorganisms in the gastrointestinal tract, but the extent of this synthesis and its importance to the overall biotin supply is not known.

Outright biotin deficiency symptoms in humans are encountered very infrequently and are caused by unbalanced diets or are experimentally induced (Bonjour 1977). The few reports of biotin deficiency in adults are all associated with a high intake of raw eggs. Avidin, a protein of raw egg white, is capable of binding biotin and thus rendering it biologically unavailable as a nutrient. Symptoms of biotin deficiency only appear after several weeks on a deficient diet and include a fine desquamation of the skin, dermatitis, mild depression and extreme lassitude. In infants less than six months of age, seborrhoeic dermatitis and Leiner's disease could be signs of biotin deficiency since both disorders are biotin responsive, whereas infantile eczema is not responsive to biotin treatment. Lowered circulating biotin concentrations and/or lowered urinary excretion of biotin have been reported in alcoholics, in pregnant and lactating women, in infants with inborn errors of metabolism, in the elderly and in athletes, suggesting these groups within the population may be at risk of biotin deficiency. Biotin is well tolerated by man and no side effects have been noted even at high doses.

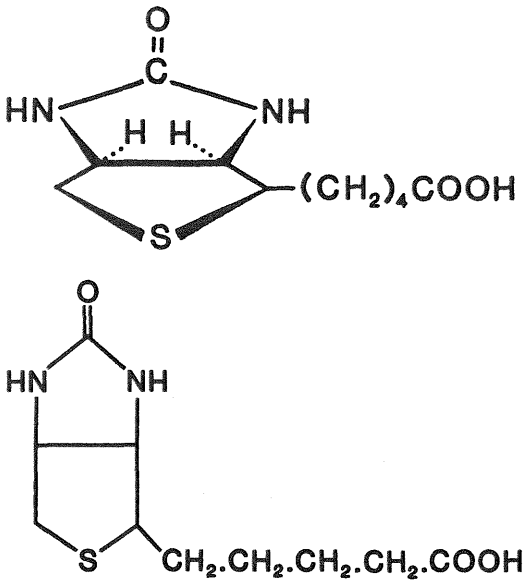

Fig. 1. Chemical structure of biotin

A marginal deficiency of biotin combined with stress has been shown to result in unexpected death of young chickens from a disorder known as "Fatty liver and kidney syndrome" (Pearson *et al.* 1976; Hood *et al.* 1976). Low hepatic concentrations of biotin have been demonstrated in victims of "Sudden infant death syndrome" (SIDS) (Johnson *et al.* 1980), suggesting a possible association between biotin and the aetiology of SIDS in human infants. The role of biotin in organisms is to function as the prosthetic group for various carboxylases and transcarboxylases. Under conditions of biotin deficiency in chickens (Hood *et al*, 1976) and yeast (Oura and

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Suomalainen 1978), the most sensitive point in metabolism is the carboxylation of pyruvate to oxaloacetate catalyzed by pyruvate carboxylase, a biotin-containing enzyme. Acetyl CoA carboxylase, also a biotin-containing enzyme important in the synthesis of fatty acids, is not as sensitive to a deficiency of biotin as is pyruvate carboxylase.

Since breakfast cereals are regularly included in the Australian diet, it is important that the biotin contents of these cereals are known.

This report documents the biotin content of 20 Australian breakfast cereals and of cereal components used in their manufacture.

Materials and methods

Twenty breakfast cereals from several manufacturers were purchased from a supermarket in the Sydney metropolitan area for analysis of biotin content. Cereal components isolated from wheat, rice and corn were supplied by CSIRO Wheat Research Unit, Ricegrowers' Co-operative Mills Limited, Kellogg (Aust) Pty Ltd and Corn Products-Fielders Pty Ltd. Subsamples of each cereal were ground in a Wiley Mill fitted with a 1 mm mesh screen and stored frozen in sealed containers.

Duplicate cereal samples (5 g) were hydrolysed in 50 ml of 2N H_2SO_4 for one hour at 121°C in an autoclave. All hydrolysates were neutralized with 20% NaOH, filtered, and the volume of filtered extract recorded before biotin assay. All reagents and calibrating procedures used in the radiochemical assay have been described previously (Hood 1975). The assay employs the principle of isotope dilution and is based on the competition between a known quantity of radioactive biotin and an unknown quantity of non-radioactive biotin for the binding site of avidin. After precipitation of the avidin-biotin complex the amount of excess ^{14}C-biotin remaining in the supernatant is measured. The concentration of biotin in the unknown sample is then calculated from the dilution of radioactive biotin by non-radioactive biotin. Linear regression analysis used in the calculation of the biotin content has been described more fully by Hood (1977). In contrast to the published biotin assay (Hood 1977), eight scaled dilutions of avidin were used rather than six dilutions in order to improve accuracy.

TABLE 1

Biotin content of breakfast cereals

Product	Major ingredient	Biotin (ng/g of product)	Water (%)
Rice Crispies[A]	Rice	32	3.3
Rice Bubbles[B]	Polished rice	8	2.0
Coco Pops[B]	Polished rice	24	3.1
Weet-Bix[C]	Whole wheat	69	3.8
Just Right[B]	Whole wheat	56	3.3
Weeties[A]	Whole wheat	70	2.9
Muesli Flakes[D]	Flaked whole wheat	50	4.8
Honey Smacks[B]	Pearled wheat	25	3.9
Puffed wheat[B]	Pearled wheat	43	3.0
All-Bran[B]	Wheat bran	167	2.3
Bran Flakes[B]	Wheat bran	125	4.5
Vital[A]	Wheat flour	109	3.9
Nutri-Grain[B]	Wheat flour	80	2.2
Frosties[B]	Corn	11	2.9
Corn Flakes[B]	Corn	15	2.4
Vitos E[D]	Rolled oats	146	2.4
Toasted Muesli[E]	Rolled oats	100	2.8
Rolled oats[E]	Rolled oats	136	7.2
Ossie Pops[A]	Sugar	36	2.6
Froot Loops[B]	Sugar	24	2.7

[A]Nabisco Pty. Ltd.
[B]Kellogg (Aust) Pty. Ltd.
[C]Sanitarium Health Food Co.
[D]White Wings Ltd.
[E]Home Brand, Woolworths Ltd.

Results and discussion

Breakfast cereals are produced from whole grain, milled fractions of whole grain or from fabricated mixtures by shredding, toasting, puffing, and flaking processes. Some vitamins, but not biotin, and minerals are added to most ready-to-serve breakfast cereals. Binders such as oils, sugar syrup or dextrins are often used in manufacture to incorporate vitamin and mineral mixtures into moist cereal. Alternatively, vitamins can be sprayed directly onto the cereal as a solution, suspension or emulsion. Published values of vitamin contents for a particular breakfast cereal can only represent an average value for that product and process, since variations in the natural content of grain, in processing conditions and in analytical methodology influence the actual vitamin content of processed food. The average biotin contents of 20 commercially available breakfast cereals are listed in Table 1.

Biotin concentrations varied from 7.6 ng/g of product in Rice Bubbles to 167 ng/g in All-Bran. Cereals based on rolled oats have an above average biotin content since they are whole-grain cereal products containing bran, germ and the aleurone layer. Although the brand names vary, all biotin contents are comparable to a recent survey of Canadian breakfast cereals (Hoppner and Lampi 1983) and early values published by Hardinge and Crooks (1961). Variation in biotin content is due to cereal type, presence of non-cereal components and to the fraction of cereal grain used in manufacture. Many breakfast cereals are low in biotin because cereal grain fractions which are low in biotin are used as starting ingredients. Biotin is unlikely to be destroyed during processing since it is stable in acid solution, in air, and to heat, although it is not stable in alkaline solution. All breakfast cereals analyzed had low water contents (Table 1).

Wheat bran and wheat germ contain high concentrations of biotin whereas flour is a poor source of biotin (Table 2). The biotin content of flour is variable depending on the extraction rate. When a series of flours of varying

TABLE 2

Biotin content of wheat fractions

	Biotin (ng/g)
Whole wheat	86.9
Bran	455.6
Germ	303.1
Flour S (high extraction rate)	32.1
Flour A (low extraction rate)	10.9

extraction rates is prepared in a mill, biotin content, and for that matter the contents of most nutrients, will increase as extraction rate increases. Biotin is higher in flour S than flour A (Table 2) due to a higher extraction rate in flour S, resulting in more fragments of bran and germ in the flour. Hinton *et al.* (1953) reported similar changes in the biotin content of flour as the extraction rate was increased.

Rice bran is also a good source of biotin (Table 3). As rice is polished (whitened) the biotin content decreases with each stage as a result of increasing amounts of bran being gently rubbed off the outside of the grain. White rice from the Inga variety retained more biotin due either to a varietal difference or to a grain structure from which it is more difficult to remove the bran layer.

TABLE 3

Biotin content of rice fractions from two varieties

	Biotin (ng/g)	
	Calrose	Inga
Paddy	57.7	57.3
Hulls	27.3	30.9
Bran	287.0	337.0
Brown rice	67.6	72.5
White (1st polish)	53.1	55.0
White (2nd polish)	20.3	39.0
White (3rd polish)	12.0	27.6

TABLE 4

Biotin content of corn fractions

	Biotin (ng/g)
Whole[A]	62.0
Fibre[A]	140.8
Germ[A]	95.6
Flour[A]	4.6
Corn Grit[B]	44.8
Hominy Corn[B]	181.0

[A]Supplied by Corn Products-Fielders Pty. Ltd.
[B]Supplied by Kellogg (Aust) Pty. Ltd.

During wet milling of corn, significant quantities of biotin are retained in the corn fibre fraction (Table 4) and consequently corn flour is a poor source of biotin. However, corn steep liquor, another byproduct of wet corn milling, is a rich source of biotin. Dry milling of corn for breakfast cereal manufacture produces two fractions, hominy corn being a better source of biotin than corn grit (Table 4).

Considerable variation exists in the biological availability of biotin from different cereal grains. Most biotin exists in a form which is bound to another food component; this biotin may not be released by gastric or intestinal digestion and hence passes through the gastrointestinal tract. Bioavailability of biotin using a chick growth assay (Frigg 1976) has shown that only a small portion of microbiologically assayed biotin in wheat is available to the chick. In contrast, all biotin found in corn and approximately 25% in oats is biologically available (Anderson *et al.* 1978).

Bioavailability studies for biotin have not been carried out in man. Biotin contents listed in this paper provide information on the total amount of biotin present in a cereal but give no indication on the amount of biotin which is available for absorption from the gastrointestinal tract.

Several of the B-complex vitamins, namely thiamin, riboflavin and niacin, are routinely added to most breakfast cereals to provide the recommended daily allowance in one serving of cereal. Breakfast cereals are not supplemented with biotin. During the year 1981-82, there were 118 865 tonnes of breakfast cereal available for consumption in Australia (Australian Bureau of Statistics 1983). On an apparent per capita basis, consumption can be calculated to be 21.5 g of breakfast cereal/person/day. Assuming an average biotin content of 66 ng/g of product (average of cereals in Table 1), then the average Australian consumes approximately 1.4 μg of biotin each day from breakfast cereals. There is likely to be considerable variation in the dietary intake of breakfast cereals as dictated by age and sex. Data collected in the Nutrition Canada Dietary Survey (Nutrition Canada 1977) indicates that males consume up to three times more breakfast cereal than females, but cereal consumption is low and similar for both sexes of young adults (20–39 years).

It has been noted that 18–46 μg biotin are excreted in urine each 24 hours (Bonjour 1977), but the amount of biotin, if any, that has been synthesized by micro-organisms and absorbed is not known. In general, a recommended daily allowance (RDA) for biotin is not set due to uncertainty as to the level of contribution of biotin by intestinal micro-organisms. Rather than provide a RDA, the United States Food and Drug Administration has published an estimated safe and adequate daily dietary intake of 100–200 μg of biotin for adults and adolescents over 11 years of age. Therefore, breakfast cereals provide approximately one percent of the estimated daily requirement for biotin.

Although biotin is found in most foods, cereals are considered to be good sources of the vitamin. Only liver, soy products and egg yolk are major food items which have more biotin than cereals. Therefore, it seems unlikely that the average Australian would achieve a daily intake approaching 100–200 μg of biotin. In view of the lack of biotin deficiency symptoms in man, the estimated safe and adequate daily dietary intake has probably been set too high, so that fortification at a level to provide 100–200 μg per day may not be necessary. However, since manufacturers of breakfast foods supplement their products with other B group vitamins, the daily intake of biotin could be increased by fortifying breakfast cereals with biotin. Fortification is more important for breakfast cereals using wheat as a major ingredient since only a small proportion of biotin in wheat is biologically available.

Summary

Using a radiochemical assay, biotin contents of 20 breakfast cereals were within the range of 7.6 to 167 ng/g of product. Variation in biotin content was due largely to the fraction of the grain used in manufacture of the breakfast cereal. Excepting breakfast cereals with rolled oats or bran as a major ingredient, most cereals are low in biotin because endosperm is the major cereal ingredient. The contribution of breakfast cereals is only 1–2% of the estimated adequate daily requirement for biotin.

References

Anderson, P. A., Baker, D. H. and Mistry, S. P. (1978). Bioassay determination of biotin content of corn, barley, sorghum and wheat. *J. Anim. Sci.* **47**, 654-9.

Australian Bureau of Statistics. (1983). Apparent consumption of foodstuffs and nutrients. Australia 1981-82.

Bonjour, J. P. (1977). Biotin in man's nutrition and therapy – a review. *Int. J. Vitam. Nutr. Res.* **47**, 107-18.

Frigg, M. (1976). Bio-availability of biotin in cereals. *Poult. Sci.* **55**, 2310-8.

Hardinge, M. G., and Crooks, H. (1961). Lesser known vitamins in foods. *J. Am. Diet. Assoc.* **38**, 240-5.

Hinton, J. J. C., Peers, F. G., and Shaw, B. (1953). The B vitamins in wheat: the unique aleurone layer. *Nature* **172**, 993-5.

Hood, R. L. (1975). A radiochemical assay for biotin in biological materials. *J. Sci. Food Agric.* **26**, 1847-52.

Hood, R. L. (1977). The use of linear regression analysis in the isotope dilution assay of biotin. *Anal. Biochem.* **79**, 635-8.

Hood, R. L., Johnson, A. R., Fogerty, A. C., and Pearson, J. A. (1976). Fatty liver and kidney syndrome in chicks. II Biochemical role of biotin. *Aust. J. Biol. Sci.* **29**, 429-41.

Hoppner, K., and Lampi, B. (1983). The biotin content of breakfast cereals. *Nutr. Rep. Int.* **28**, 793-8.

Johnson, A. R., Hood, R. L., and Emery, J. L. (1980). Biotin and the sudden infant death syndrome. *Nature* **285**, 159-60.

Nutrition Canada (1977). Food consumption patterns report. (Bureau of Nutritional Sciences, Health Protection Branch, Department of National Health and Welfare: Ottawa.)

Oura, E., and Suomalainen, H. (1978). Biotin and the metabolism of baker's yeast. *J. Inst. Brew. (London)* **84**, 283-7.

Pearson, J. A., Johnson A. R., Hood, R. L., and Fogerty, A. C. (1976). Fatty liver and kidney syndrome in chicks. I. Effect of biotin in diet. *Aust. J. Biol. Sci.* **29**, 419-28.

Prolongation of storage life of vacuum-packaged lamb

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Introduction

Exports of Australian lamb to the Middle East Region for the year ended 30 June 1983 were nearly 21 000 tonnes. Much of this was exported as chilled carcasses because chilled meat is still preferred to the frozen product. Also, carcasses are preferred to cuts, particularly in Saudi Arabia.

Transport of chilled carcasses by air has led to the development of a substantial market. However, further expansion of Australia's chilled lamb exports by air will be restricted by the limited space available on scheduled passenger and chartered aircraft, by the high cost, and by the limited number of airports able to accept international aircraft.

Extension of the storage life of chilled lamb carcasses by up to six weeks may be achieved by vacuum packaging provided a temperature of $0°C \pm 1°C$ is maintained, thus making possible the use of refrigerated sea transport. However, for some markets this storage life has been found to be inadequate, particularly if delays occur in shipping or in subsequent distribution.

The storage life of vacuum-packed carcasses is generally less than that of sheepmeat cuts. Roberts (1973) found that cuts of yearling merino sheepmeat could be stored for at least 70 days at $0°C$ in vacuum packs. On the other hand he reported (Roberts 1975) that reliable storage life of sheep carcasses in vacuum packs was only about 55 days.

One of the potential disadvantages of vacuum packaging lambs as carcasses is the large headspace, or void volume, of the abdominal and chest cavity. There will generally be a larger quantity of residual oxygen than is normally found in vacuum-packaged cuts of meat and it is likely that the build-up of carbon dioxide to concentrations inhibitory to spoilage microorganisms will be slower. Shelf life will be shorter in consequence. The stowage rates of lamb carcasses can be improved by nearly 45% by telescoping the carcass (Roberts 1982). In this procedure the spinal column is cut and the hind legs are folded into the chest cavity. It has the added advantage of reducing the volume of the body cavity, but a headspace of around two litres remains.

Consideration of possible processes for extending the storage life of sheep carcasses in vacuum packs included a hot water treatment before packaging. Graham *et al.* (1978) showed that this process significantly reduces the number of bacteria contaminating the surfaces of the lamb and beef carcasses. However, the storage life of beef primal cuts in vacuum packs was not extended when the meat was treated with hot water immediately before packing (Bensink *et al.* 1973; Eustace and Powell unpublished data). The hot water treatment of sheep carcasses to extend vacuum-packaged storage life has therefore not been pursued.

Eustace and Powell (unpublished data) demonstrated that the acceptable storage life of beef striploins in vacuum packs was increased by treatment with a dilute solution of acetic acid immediately before they were packed.

Work was undertaken to establish whether an acetic acid treatment would extend the storage life of vacuum-packaged sheep carcasses.

An initial investigation (Eustace *et al.* 1979) showed that treatment of lamb carcasses with dilute acetic acid before they were vacuum-packaged extended the storage life by at least four weeks. The treatment given the carcasses was a spray for 10 seconds with 1.5% or 3.0% (w/v) acid immediately after dressing and inspection. After an overnight chill the carcasses were vacuum-packaged and stored at 0°C. This preliminary work created considerable interest within the Australian lamb export industry. As the findings from this work were based on a very limited number of carcasses a larger follow-up trial was undertaken. Details are given below.

Experimental procedure

Carcass treatment and packaging

Second-quality lamb carcasses, weight 12 to 16 kg, were selected from a commercial consignment that had been slaughtered and dressed as part of a normal kill. They were assigned at random to one of the treatment groups below, before they were chilled.

Group FS: Frozen stored. 'Fresh' controls (18 carcasses). No treatment.

Group CON: Vacuum-packaged controls (42 carcasses). No treatment.

Group AT: Treated with acetic acid (54 carcasses). Immersed in hot (55°C), 1.5% (w/v) acetic acid for 10 seconds.

The latter carcasses were manually immersed in a tank containing 200 litres of acetic acid adjusted to 55°C. The 1.5% acid solution was prepared by the addition of 3125 ml of 90% (w/v) acetic acid to hot water. The acid solution was changed after every sixth carcass was treated to ensure that the acid strength was maintained. The concentration of the acid in the tank was checked before and after the six carcasses were dipped.

A further six carcasses were treated with hot water. They were immersed for 10 seconds in a tank containing 200 litres of water at 55°C.

After treatment, all carcasses were transferred to a chiller (0°–2°C) for overnight chilling. Next day, Group FS carcasses were enclosed in polyethylene and stockinette and transferred to a carcass freezer. Frozen carcasses were stored at −30°C.

The carcasses of Groups CON and AT were prepared for vacuum packaging in a room held at 10°C in the following manner. Forelegs were sawn off close to the rib cage and discarded. Parts of the hind shanks and the whole necks were also sawn off and discarded to improve the compactness of the vacuum pack and because Roberts (1975) suggested that their inclusion might encourage more rapid spoilage. The carcasses were cut through the spinal vertebrae at the anterior end of the sacrum and the hind legs were folded into the chest cavity. Before the carcasses were put in bags, exposed bones at the butt of the neck, the fore-leg, the brisket and over the exposed vertebrae column were covered with a protective material ('Bonegard'*) to reduce the incidence of bone-induced puncturing of the bags.

The bags used were 'W' gauge 'Cryovac'* barrier bags of average thickness (preshrunk) 0.09 mm. The oxygen transmission rate was determined using the method described by Davis and Huntington (1977), as 28 ml (STP) $\times$ m^{-2} $\times$ 24 h^{-1} $\times$ 76 cm^{-1} Hg at 25°C and 75% R.H.

Packs were evacuated and clipped using Cryovac 8200 equipment. The clipped bags were heat-shrunk by immersion for one second in a water bath maintained at 90°C. The carcasses were placed in cartons that were specially designed to hold two telescoped vacuum-packed lamb carcasses. They were stored at 0° to 1°C until required for sampling.

Panel appraisal after storage

Six carcasses from each of the two vacuum-packaged groups were evaluated after 4, 8, 10, 12, 14 and 16 weeks of chilled storage. Gas samples (1 ml) were withdrawn with a gas-tight syringe via a seal of cured silicone sealing compound, from each vacuum pack just prior to opening. A Fisher-Hamilton Model 29 gas partitioner was used for the estimation of the concentrations of N_2, CO_2 and O_2 present.

An assessment panel of eight persons rated the appearance of the intact packs, and the odour and appearance of the carcasses when the packs were opened. The panel recorded their assessments according to nine-point hedonic scales (Appearance: 0 = severe discoloration, 2 = poor, 4 = good, normal, 6 = fresh, slight discoloration, 8 = very fresh, no discoloration. Odour: 0 = extreme off odour, 2 = strong off

*Registered trade name of W. R. Grace & Co.

odour, 4 = moderate off odour, 6 = slight off odour, 8 = fresh, no off odour) and also recorded relevant comments.

After the above assessment and after tissue samples were taken for microbiological analysis, the hindquarter pair of each carcass was detached, placed in a polyethylene bag, transferred to a freezer, and stored at −30°C until required for testing.

The frozen hindquarter pairs, including those from Group FS carcasses, were sawn into chops. The chops were thawed for 24 hours at 5°C. They were grilled on open racks at 230°C for 20 min and presented hot to a taste panel of 18 laboratory personnel.

The panellists were asked to evaluate them for foreign or 'off' odours and flavours, 'meat' aromas and flavours, and also overall acceptability. Aromas and flavours were rated on nine-point intensity scales: 0 = none, 2 = slight, 4 = moderate, 6 = strong, 8 = very strong. Acceptability was rated on a nine-point hedonic scale: 0 = very poor, 2 = poor, 4 = moderate, 6 = good, 8 = very good.

For investigation of aerobic storage life of lamb subsequent to vacuum-packaged storage, a shortloin was cut from each carcass after the pack was opened, placed on a polystyrene tray and overwrapped with polyvinyl chloride film. These packs were stored at 5°C for two days at a light intensity of approximately 500 lux to simulate the light conditions in retail display cabinets. The odour and appearance of the shortloins were assessed by a four member panel, which used the scales defined above. The appearance was assessed at three separate sites: the external surface, the internal surface (i.e. within the body cavity), and the eye muscle at the forward end of the loin.

Microbiological assessment

Six carcasses from the group treated with acetic acid were sampled before immersion, and again approximately five minutes after the treatment. The carcasses that had been subjected to the hot water treatment were also sampled before and after immersion. Samples were obtained by excision of 50 cm^2 of surface tissue from each of two selected sites: rectal area of the rump and brisket. The samples were combined in a sterile polyethylene bag. To each bag 90 ml of 0.1% peptone solution was added and the samples were treated for 1 min with a Colworth Stomacher, Model 400 (A. J. Seward Co. Ltd., London). Appropriate dilutions were spread-plated on tryptone soya agar (Oxoid) supplemented with 0.2% (w/w) yeast extract (Oxoid) and 0.2% glucose (TSYG agar) and incubated at 25°C for 3 days for estimation of the total aerobic plate count. Appropriate dilutions were also pour-plated with violet red bile agar (VRB, Oxoid) and incubated at 37°C for 24 hours to give counts of coliform bacteria.

The effect of chilled storage on the microbiological status of the carcasses was determined by analysis of samples taken immediately before vacuum packaging (designated as zero time) and when carcasses were opened for organoleptic appraisal after chilled storage.

Tissue samples, each of 5 cm^2, were taken from the rump, mid-back, brisket, a site adjacent to the butt of the neck, and from the abdominal flap. These five samples were combined to give a composite sample for the exterior of the carcass. In addition, a separate tissue sample, 10 cm^2, was taken from the kidney region within the body cavity. Samples were prepared for microbiological testing as described above.

Appropriate dilutions of tissue suspension were spread-plated on TSYG agar, TSYG agar containing 2.0 mg/l penicillin, G, VRB agar, MRS (de Man, Rogosa, Sharpe), agar (Oxoid) and streptoymycin-thallous acetate agar (STAA, Gardner 1966). Total aerobic counts were obtained from TSYG plates incubated at 25°C for 3 days and psychrotrophic counts from TSYG plates incubated at 0°C for 21 days. Counts of gram-negative organisms were obtained from plates of TSYG + penicillin G agar and VRB agar, each incubated at 25°C for 3 days. Twenty colonies from each of the countable plates of these two media were randomly selected and tested for oxidase (N, N-dimethyl-*p*-phenylene diamine hydrochloride, 1% w/v) to give estimates of the numbers of oxidase-negative (Enterobacteriaceae) and oxidase-positive organisms. Plates of MRS and STAA agars were incubated at 25°C for four days to give counts of lactobacilli and *Brochothrix thermosphacta* respectively.

Samples were taken from the shortloins subjected to aerobic storage after the vacuum packs were opened. Tissue (5 cm^2) was excised from each of three sites (external surface, internal surface and exposed longissimus dorsi muscle at the forward end of loin), combined and treated as described earlier. Aliquots of appropriate dilutions were spread-plated on TSYG agar and on TSYG agar with added penicillin.

Results and discussion

Organoleptic assessment

The treatment of lamb carcasses with acetic acid or hot water caused negligible permanent discoloration. Immediately after the treatment the carcasses appeared slightly cooked and bleached, but the colour was completely restored after the carcasses had been stored overnight in a chiller at 0°–2°C. In contrast, use of more concentrated acetic acid (3.0%, 40°C) by Anderson *et al.* (1980) resulted in development of slight grey discoloration of the top one millimetre of fat on beef carcasses. From the appearance of either the intact (vacuum-packaged) lamb packs or the carcasses when they were removed from the packs after chilled storage no adverse effect could be attributed to the acid. Differences in gaseous composition (oxygen, nitrogen and carbon dioxide) between groups were small and not statistically significant. Oxygen concentrations, which ranged between 1% and 3%, showed no trend during the storage period. Carbon dioxide concentrations gradually increased from around 12% at four weeks to around 35% at 16 weeks.

Both the appearance and odour of carcasses deteriorated progressively during vacuum-packaged storage. Green discoloration was apparent on several carcasses stored for 10 weeks or longer (Table 1). During the period from 10 to 16 weeks inclusive, 17 of 24 control carcasses had obvious areas of greening. Only 4 of 24 acid-treated (AT) carcasses were affected by any greening. Panel assessments of odour are summarized in Fig. 1. Off-odours for AT carcasses were less ($P < 0.05$) than those of the controls at 4, 12 and 14 weeks. At 16 weeks all control carcasses were rejected as unfit for consumption, whereas 4 of the 6 AT carcasses could still be submitted for taste testing.

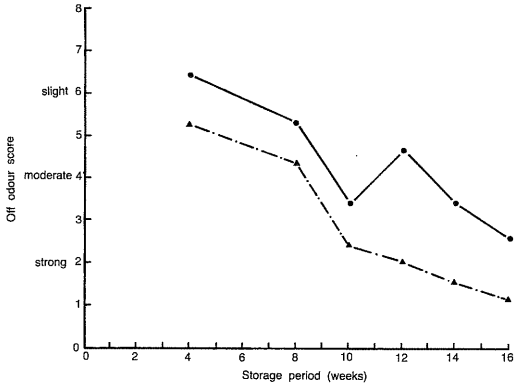

Fig. 1. Confinement odour of lamb carcasses at the time of opening vacuum packs.
(▲ controls; ● acetic acid treated).

TABLE 1

Number of vacuum-packaged lamb carcasses unfit for consumption

Storage time (Weeks)	Group	Number rejected[A]	Reason for rejection
10	CON	3	Greening/odour
	AT	1	Slight greening/odour
12	CON	3	Greening/odour
	AT	0	—
14	CON	5	Greening/putrid odour/visible colonies
	AT	1	Greening
16	CON	6	Greening/odour/visible colonies
	AT	2	Greening/putrid odour

[A]Six carcasses assessed at each time from each group.

Cooked leg chops from all carcasses stored for 4 and 8 weeks were considered satisfactory by the taste panel. After 10 weeks storage three control carcasses were of poor quality due to objectionable odours and/or obvious green discoloration (Table 1). Chops from these carcasses were not submitted to the panel. Consequently, comments below on taste panel assessments are based on mean scores for those leg chops submitted. The data presented in Table 2 for 10 and 12 weeks' storage are the mean scores for only three lambs for the control group. At 10 weeks one of the AT carcasses was not presented. The data presented for Groups AT and FS are the mean scores for five carcasses. Lack of sufficient carcasses for satisfactory statistical analysis resulted in formal taste testing being abandoned after 12 weeks' storage.

For those leg chops from Groups CON and AT presented to the panel, little 'off' or foreign aroma was detected. They compared favourably with chops from FS carcasses. No strong 'off' flavours were detected, although slight 'off' flavours (viz. mean 'off' flavour scores greater than 2) were frequently detected after 10 weeks, particularly in chops from the FS and CON groups. Data were obtained also for meat aroma and meat flavour. There were no differences between the groups for either attribute.

TABLE 2

Results of evaluation by taste panel of leg chops from vacuum-packaged lamb carcasses

Storage time (weeks)	Treatment	Parameters measured			l.s.d.[A]		
		Off[B] aroma	Off[B] flavour	Accept-[C] ability	Off aroma	Off flavour	Accept-ability
4	Frozen stored (FS)	1.25	1.26	4.46			
	Control (CON)	1.00	1.25	4.22			
	Acetic acid (AT)	0.96	1.12	4.45	0.32	0.42	0.40
8	FS	1.90	1.70	3.77			
	CON	1.71	2.42	3.14			
	AT	1.06	1.58	3.96	0.63	1.05	0.76
10	FS	2.40	2.17	3.23			
	CON	1.85	2.66	2.59			
	AT	1.77	2.01	3.34	1.75	2.04	1.25
12	FS	2.45	2.22	2.65			
	CON	1.70	2.60	2.47			
	AT	0.94	1.47	3.71	1.88	1.21	1.19

[A]Least significant differences between carcass treatment, P = 0.05

[B]0-8 None to very strong.

[C]0-8 Very poor to very good.

The overall acceptability of chops from all groups declined with storage time. At 8 and 12 weeks, chops from Group AT were considered more acceptable ($P < 0.05$) than chops from the control group.

Neither the panel which assessed the carcasses nor the taste panel detected any acetic acid odour or flavour.

The appearance, odour and flavour data indicate that lamb carcasses treated with acetic acid were still acceptable at 12 weeks, whereas 50% of the carcasses vacuum-packaged with no other treatment were unacceptable if stored longer than eight weeks.

Microbiological assessment

Treatment of carcasses with 1.5% acetic acid resulted in an immediate reduction in the total number of aerobic bacteria of 1.3 $\log_{10}$ units (95%). The number of coliform bacteria was reduced by 2.0 $\log_{10}$ units (99%). These reductions compared closely with reductions of 1.4 and 1.9 $\log_{10}$ units respectively, obtained in a preliminary trial. In contrast, the hot water treatment resulted in only small reductions (0.1 and 0.4 $\log_{10}$ units for total, and coliform bacteria respectively). Clearly, the immediate action of the acetic acid solution on bacteria is not simply a temperature effect.

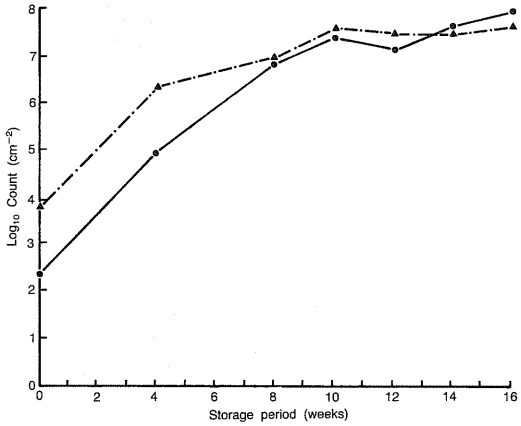

Fig. 2. Changes in numbers of aerobic bacteria with time of storage at 0°C of vacuum packs. (▲ controls; ● acid treated).

The effect of time of storage upon total aerobic plate counts on the exterior of carcasses is shown in Fig. 2. At four weeks the counts on AT carcasses were significantly lower ($P < 0.05$) than counts on control carcasses. The increases in bacterial counts on the interior of carcasses followed similar patterns to those shown in Fig. 2 for external surfaces. For

carcasses stored for 8 weeks or more, the counts on internal surfaces were around 0.5 $\log_{10}$ units higher than counts on external surfaces.

For carcasses sampled after storage in vacuum packs, the counts obtained after incubation at 0°C and at 25°C were similar. For samples taken at the beginning of the trial, after the acid treatment and overnight chilling, but before packaging the carcasses, the counts obtained after incubation at 0°C were 7% and 26% of the counts obtained after incubation at 25°C for the control and acid-treated carcasses respectively.

The number of lactobacilli present increased by at least 5 $\log_{10}$ units during the first eight weeks of storage. From eight weeks on, lactobacilli represented a high proportion (95% or higher) of the total bacterial population. Counts of lactobacilli on AT carcasses were significantly lower ($P < 0.05$) than on control carcasses at four weeks.

Mean numbers of *B. thermosphacta* increased 4.5 to 5 $\log_{10}$ units during the first eight weeks of storage. Thereafter, further increases were small (Fig. 3). At 4, 10, 12 and 14 weeks, the numbers of *B. thermosphacta* were lower ($P < 0.05$) on AT carcasses than on control carcasses. It is not known whether *B. thermosphacta* at numbers of $1 \times 10^6 - 3 \times 10^6/cm^2$ (the numbers on the control carcasses) would have a definite deleterious effect on the organoleptic quality of vacuum-packaged lamb. Egan and Grau (1981) found that vacuum-packaged beef is spoiled when the population of *B. thermosphacta* reaches $10^8/cm^2$. However, they found that a population of this organism of between 3×10^5 and 1×10^6 cells did not cause spoilage at 5°C over a period of five weeks.

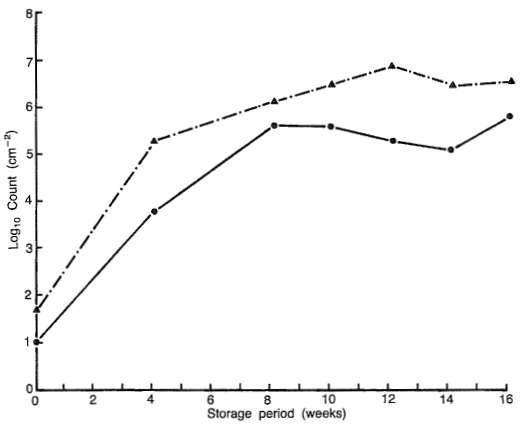

Fig. 3. Growth of *B. thermosphacta* on vacuum-packaged lamb carcasses stored at 0°C. (▲ controls; ● acid treated).

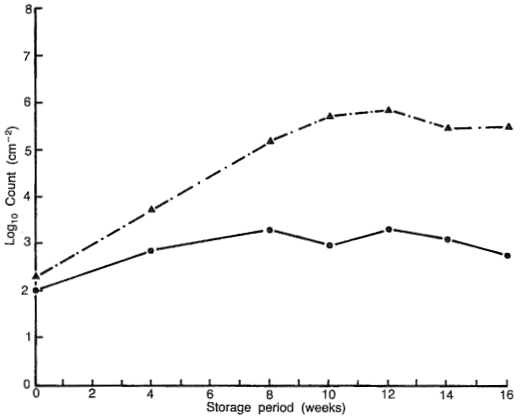

Fig. 4. Growth of gram-negative bacteria on vacuum-packaged lamb carcasses. (▲ controls; ● acid treated).

The patterns of growth of gram-negative bacteria are presented in Fig. 4. Counts on control carcasses increased by approximately 3 $\log_{10}$ units during the first eight weeks of storage. On CON carcasses stored eight weeks or longer, the gram-negative flora was generally dominated (around 80%) by Enterobacteriaceae. In contrast, growth of gram-negative organisms, particularly Enterobacteriaceae, was strongly inhibited on the carcasses treated with acetic acid. After storage, counts on AT carcasses were lower ($P < 0.05$) than the counts on control carcasses on all occasions.

The effectiveness of the acetic acid treatment in extending the storage life of treated lamb carcasses after removal from the vacuum packs is indicated by the odour and appearance of shortloins after two days' aerobic storage (Table 3). The off-odours for loins from AT carcasses were less ($P < 0.05$) than for those from the control carcasses on all occasions. The panel ratings for appearance given in the table are for interior surfaces. The appearance of the interior surfaces of loins from the control carcasses rated worse than other surfaces. This was not so for cuts from AT carcasses which were considered to have superior appearance ($P < 0.05$) to that of the control group on the four occasions.

The effect of the acetic acid treatment on numbers of gram-negative bacteria at opening (Fig. 4) was still evident after aerobic storage (Table 3). There was a consistent difference of at least 2 log units between the CON and AT groups. The inhibition of growth of gram-negative bacteria, which includes the aerobic

TABLE 3

Evaluation of overwrapped shortloins stored aerobically at 5°C for two days

Storage[A] time (weeks)	Treatment group	Panel ratings[B]		Mean number gram negative bacteria[C]
		Appearance	Odour	
4	CON	2.2	3.6	7.4[D]
	AT	4.0	5.3	4.0[D]
8	CON	2.1	2.7	6.7*
	AT	3.4	4.4	4.7
10	CON	1.6	2.0	7.4
	AT	2.6	3.6	4.9
12	CON	1.4	1.8	7.0
	AT	3.1	4.2	4.3

[A]Shortloins removed from carcasses stored in vacuum packs at 0°C for specified periods.
[B]Appearance: 0-8 Severe discoloration to very fresh, no discoloration.
Odour : 0-8 Extreme off-odour to fresh, no off-odours
[C]Log_{10} units per cm^2
[D]Three days' aerobic storage, not two days.

spoilage organisms of the *Pseudomonas* group, will contribute to the extension of aerobic storage life, which is generally considered to be limited by the time taken for the gram-negative bacteria to reach spoilage numbers (7 to 8 log_{10} units per cm^2).

The potential of acetic acid to extend the storage life of lamb carcasses once the vacuum packs are opened is worthy of further attention.

Conclusion

The data obtained from this experiment indicate that treatment of lamb carcasses with dilute solutions of acetic acid will significantly increase their acceptable storage life in vacuum packs.

After acetic acid treatment, the vacuum-packaged carcasses were still visually and organoleptically acceptable after at least 12 weeks' storage. Carcasses packaged without the acid treatment were barely acceptable at eight weeks. At 10 weeks, 50% were completely unacceptable. The acid causes negligible discoloration of carcasses, and no odour of the acid was evident from either the freshly-opened packs or cooked chops.

The acetic acid treatment results in initial reductions in numbers of contaminating bacteria, and inhibits further microbial growth within the vacuum pack, at least for certain types of bacteria. The suppression of growth within the packs of those bacteria that contribute to aerobic spoilage when the packs are opened, e.g. *Pseudomonas*, means that acceptable storage life of the lamb once the pack is opened is also extended.

References

Anderson, M. E., Marshall, R. T., Stringer, W. C., and Naumann, H. D. (1980). In-plant evaluation of a prototype carcass cleaning and sanitizing unit. *J. Food Prot.* **43**, 568-70.

Bensink, J. C., Ford, Anne L., and Yates, J. R. (1973). Properties and performance of a range of commercial vacuum-packaging films used for packing chilled beef. CSIRO Div. Food Res. Meat Res. Rept No. 4/73.

Davis, E. G., and Huntington, J. N. (1977). New cell for measuring the permeability of film materials. *CSIRO Food Res. Q.* **37**, 55-9.

Egan, A. F., and Grau, F. H. (1981). Environmental conditions and the role of *Brochothrix thermosphacta* in the spoilage of fresh and processed meat. *In* 'Psychrotrophic organisms in spoilage and pathogenicity', ed. T. A. Roberts, G. Hobb, J. H. B. Christian and N. Skovgaard (Academic Press: London)

Eustace, I. J., Powell, V. H., and Bill, B. A. (1979). Vacuum packaging of lamb carcasses: Use of acetic acid to extend chilled storage life. A preliminary investigation. CSIRO Div. Food Res. Meat Res. Rept No. 3/79.

Gardner, G. A. (1966). A selective medium for the enumeration of *Microbacterium thermosphactum* in meat and meat products. *J. Appl. Bacteriol.* **29**, 455.

Graham, A., Cain, B. P., and Eustace, I. J. (1978). An enclosed hot water spray cabinet for improved hygiene of

carcass meat. CSIRO Div. Food Res. Meat Res. Rept No. 11/78.
Roberts, D. W. (1973). The vacuum packing of chilled yearling sheepmeat. CSIRO Div. Food Res. Meat Res. Rept No. 3/73.
Roberts, D. W. (1975). The preparation and outturn of a trial consignment of chilled meat to Kuwait and Iran. CSIRO Div. Food Res. Meat Res. Rept No. 2/75.
Roberts, D. W. (1982). Yield in sheepmeat processing. *In* 'Proc. Adv. in Meat Technol.' III Conf. CSIRO Div. Food Res. Meat Res. Lab.

New food journal: ASEAN Food Journal

The first issue of a new journal, the *ASEAN Food Journal*, will appear early in 1985. The Journal will provide a forum for food scientists and technologists to publish the results of their research in the fields of:

- Food science and technology
- Food waste management
- Postharvest food handling
- Food habits and nutrition.

The Journal, which will appear four times a year, is the joint undertaking of the food-related projects under the ASEAN-Australia Economic Cooperation Program: the ASEAN Sub-Committee on Protein, the ASEAN Food Waste Materials Working Group, the ASEAN Food Technology Research and Development Working Group and the ASEAN Sub-Committee on Food Handling. The ASEAN Food Handling Bureau based in Kuala Lumpur will publish the Journal on behalf of the four ASEAN groups. Initially, 2000 copies of each number will be printed.

Eminent scientists and professionals working in the food sciences and related disciplines in the ASEAN region are among those represented on the Editorial Board of the Journal. The Chairman of the Board is Professor Oei Ban Liang and the members are: Dr Estrella Albastro, Professor Amara Bhumiratana, Dr Alex Buchanan, Dr Chua Sin Bin, Dr Rudy Florentino, Mr Mohd Hashim Hassan and Dr Nga Been Hen.

The Board perceives the Journal as being designed to serve ASEAN's needs with the following aims:

- To facilitate the exchange of useful scientific and technological findings in the food discipline.
- To serve as a medium for ASEAN researchers, development workers and professsionals in the food and allied industries to keep in close touch with one another.
- To help focus attention from within ASEAN on research results originating outside the ASEAN region but which are relevant to ASEAN's research and development efforts in the food discipline.
- To cater for various other groups who have technical interests relevant to topics covered by the Journal.

The Editorial Board is now accepting contributions for Volume 1, Numbers 1, 2 and 3. These contributions should, in the main, be articles reporting the results of original research. One or two review articles will also be published in each issue. In addition, contributors are welcome to submit technical information in the form of notes and short communications. Book reviews may be arranged with the Editor.
Further details on submission of manuscripts, referees and style may be obtained from the Editor, ASEAN Food Journal, ASEAN Food Handling Bureau, 8th Floor Syed Kechik Foundation Building, Bangsar, Kuala Lumpur, Malaysia.

News from the Division

Technology transfer: Counter-current extraction

In his paper 'Counter current extraction of soluble solids from foods' *(CSIRO Food Res. Q.* **43**, 38-43, June 1983) Dr Don Casimir of FRL described an improved process of extracting solubles from a range of foods. His work has now led to the formation of Bioquip Australia Pty Ltd, a company set up to build and advance food-processing equipment.

The Australian Industry Development Corporation (AIDC) has invested $1.3 million in the venture and will hold 24% of the company's ordinary equity. The other two major shareholders are the Howden Group Australia Pty Ltd and Alfa Laval Pty Ltd who will provide marketing support. Mr Tim Lang, formerly of Howden Equipment Services and who collaborated with Dr Casimir in the developmental work, has become Bioquip's managing director. CSIRO will retain a small interest in the company and receive royalties on sales.

Further details are contained in CSIRO's 'Industrial Research News' No. 165 (July 1984) or can be obtained by contacting the Division.

Awards

At the Seventeenth Convention of the Australian Institute of Food Science and Technology Ltd (AIFST) in April 1984, Dr J. H. B. Christian, Chief of the Division of Food Research, was presented with the Institute's Award of Merit, which is made for achievements within food science and technology in the wide areas of research, industry and education, and for contributions to further the aims and objectives of the Institute. The award citation made reference to three fields of particular concern to Dr Christian: microbiological reseach, national and international food regulation and the encouragement of the application of research results in industry.

Retirements

M. B. Smith

Dr Malcolm B. Smith, a Principal Research Scientist with the CSIRO Division of Food Research, retired from CSIRO on 29 February 1984 after 37 years' service. Malcolm obtained a Diploma in Industrial Chemistry from the South Australian School of Mines in 1945 after working first with the Department of Munitions in Salisbury, South Australia, and then with a firm of Consulting Industrial Chemists in Adelaide. In 1947 he joined the then CSIR Division of Food Preservation and Transport at Homebush and commenced work in the Physics Section as a Technical Officer. He worked with E. W. Hicks measuring temperatures and humidity in cool stores and in rail cars used for the transport of perishable foods. This often involved 'sleeping on the job', sometimes in very uncomfortable conditions and taking measurements throughout the night. Some train trips lasted several days during which one small compartment served as laboratory, kitchen and bedroom. They found that there was a need for instruments which would function in a moving train and for instruments which could measure and record temperatures and humidity in hundreds of remote points in cool stores. This developmental instrumentation immediately appealed to Malcolm and set the pattern for a continued interest throughout his career. Other Sections in the Division were also finding a need for specialized instruments and in 1950 Malcolm proposed that an instrument workshop be set up. Undaunted by a lack of money and facilities, he and Stan Rose scoured disposal yards for building materials with which they furnished a small store-room where they set up a limited collection of machine and hand tools. Immediately, requests for large and small instruments poured in, culminating in a request from Hugh McKenzie to design and build a massive moving boundary electrophoresis apparatus.

In 1953, having accomplished this last task, Malcolm joined Hugh McKenzie in the Division's Physical Chemistry Section which was housed in the Biochemistry Department of the University of Sydney. There he was given responsibility for installing not only the electrophoresis apparatus but also a new analytical ultracentrifuge, the first of its kind in Australia. Once again, Malcolm showed his

inclination to work from the ground up by first pouring solid concrete foundations for the two instruments.

Using these two sophisticated instruments, Malcolm turned his attention to a study of protein denaturation. The first step was to master the art of preparing proteins from egg white, milk, blood serum and fish muscle. A graduate student, R. G. Wake, now Professor of Biochemistry, University of Sydney, was also working in the laboratory, developing techniques for studying these proteins. The laboratory was already gaining a reputation for protein research. In collaboration with Professor Alexander from the N.S.W. University of Technology, Malcolm also made a study of the properties of soap micelles using the new ultracentrifuge. This work was to prove useful in later years in studies of proteins and detergents.

Malcolm's interest then focused on ovalbumin, the main protein of egg white and his studies led first to the award of an M.Sc. degree from the University of N.S.W., where he had previously done a part-time B.Sc. Next came his more notable discovery that a second form of ovalbumin occurs in egg white and that this form is more stable to heat and other denaturing agents than ordinary ovalbumin. He called this ovalbumin-X and later S-ovalbumin. He devised a method for determining the age of an egg based on his observation that the amount of S-ovalbumin in egg white increases with time after laying. Investigation into the chemical mechanism of the conversion of ovalbumin to S-ovalbumin and the reason for the increased stability of S-ovalbumin occupied Malcolm and his colleague Joan Back for many years. His published work on this subject brought him recognition as an authority on the chemistry of ovalbumin. During that time, Ralph Burley, who had joined the Section, had become an authority on the lipoproteins of egg yolk. Their joint expertize on the proteins of egg white and yolk gave the laboratory an international reputation.

The nature of the ovalbumin conversion to S-ovalbumin remains unknown today despite the efforts of workers both here and overseas. Although Malcolm returned to this problem many times from different points of view, he was also working on ovomucin, another protein from egg white, ovalbumin from duck and turkey eggs, and also on the effects of sugars and polyols on the stability of proteins.

In 1964 a major upheaval occurred when the Section and its equipment were uprooted and then settled into the basement at North Ryde to continue its work.

All through his research career, whenever his work required measurements which could not be carried out with the desired sensitivity by available apparatus, Malcolm set about designing one to his own specifications. Many of these custom-built instruments were built in Physical Chemistry's own workshop. One of the most useful was the differential scanning calorimeter which was used to study, amongst other things, the thermal transitions in low density lipoproteins of egg yolk.

In 1980 Malcolm was awarded a D.Sc. degree by the University of N.S.W. for his studies on protein, in particular the stability and structure of egg proteins.

Another interest of Malcolm's is communication and he has produced an audio visual presentation of some of the work in his Section which has received much acclaim.

His activities in the social life of the Division have included a term as President of the Staff Club when the first family Christmas party was held. This has been continued as a tradition in much the same form ever since. His long interest in amateur theatrical productions has also extended into two Divisional Christmas presentations.

Malcolm will continue his association with the Division through a post-retirement fellowship to work part-time developing a system for automatically photographing seedlings growing under a controlled environment.

His retirement was marked by a Divisional dinner attended by his family and past and present colleagues.

JFB.

E. G. Davis and P. M. Moy

Eric Davis, a pioneer in the science of food packaging in Australia, retired from the Division of Food Research on 4 July 1984 after 34 years' service.

Eric served for three years in the Royal Australian Air Force during World War II, then enrolled in a chemistry course at Sydney University under the Commonwealth Reconstruction Training Scheme, graduating BSc with Honours in Organic Chemistry in 1949. His appointment to the Division in 1950

was virtually his first research job and he was assigned to investigations on organic protective coatings for tinplate containers. The results of his work were of immediate practical importance to the canning industry and provided a sound foundation for the selection of protective lacquers for specific foods.

In 1958 the late L. J. Lynch, Leader of the Canning Section of the Division, foresaw a need in the Australia food industry for technical guidance in flexible film packaging. He persuaded Eric Davis to spend a year at the Massachusetts Institute of Technology in the laboratory of Professor Bernard Proctor to gain research experience in this field. On his return to the Division Eric equipped a laboratory and initiated a research program on the flexible film packaging of foods.

In entering this new field he was perceptive in the selection of projects providing the information most needed in the current state of the industry. It was essential to build up quickly a body of reliable knowledge about the properties of the extensive range of flexible films offered to the food industry, notably about permeabilities to oxygen and water vapour. Eric explored the effects on flexible film properties of humidity and of temperatures below 0 °C where there was a notable lack of information to guide the frozen food industry. He demonstrated how to predict package performance in terms of the limits of acceptability of a packaged food, and he assisted the industry with many serious problems of tainting of foods by packaging materials. The development of that unique Australian retail container, the wine cask, was helped along its path to international acceptance by Eric's measurements of the permeation of oxygen through the plastic barrier bag and valve.

When the food packaging industry became more self-sufficient technically Eric Davis was able to extend his research interests to basic questions about the molecular transport of gases in the polymers used for packaging films, and by imaginative use of new techniques and apparatus of his own design he made important contributions in this field. His research on the permeation of sulphur dioxide (SO_2) through polymer films had immediate practical usefulness in the packaging of preservatized foods and also led to studies of SO_2 equilibria in foods. Arising from this work Eric developed a new and sensitive method for the determination of SO_2 in foods.

Eric Davis accepted readily the responsibility of passing on his research findings to industry and he was always well-received as a speaker, and as a writer of survey and extension papers. He found he had to work with an industry which initially lacked technical leadership but he quickly gained the confidence of manufacturers, fabricators and users of flexible packaging films. From the first stages of supplying urgent needs for reliable data about the permeability properties of films, Eric slowly but very significantly raised the level of packaging science throughout the industry. He was active in the formation of the professional institute, the Australian Institute of Packaging, in 1973 he was elected a Fellow and served as President, and in 1984 the Institute conferred upon him its Distinguished Service Award.

Eric Davis's special achievement was the bringing of sound science into food packaging in Australia, a field that was almost wholly empirical but is now firmly based on physico-chemical principles. His reputation in the industry is epitomized in a remark by Mr Norman Law, then Director of the Meat Industry Research Institute of New Zealand, who said on the occasion of a visit to the Division 'Eric Davis is the first man I have encountered who talks sense about plastic packages for foods'.

During almost the whole of his career with the Division Eric Davis had as his laboratory assistant Mrs Phyl Moy, and she elected to retire from the Division on the same day as Eric, after 33 years' service.

Phyl had no formal training in science but by her intelligent approach, neat and accurate recording, and natural skills in the laboratory she was able to rise through the grades of Assistant and Technical Assistant to Technical Officer. At this level she assisted Eric Davis with many specialized laboratory operations, such as measurement of the barrier properties of packaging film, investigation of tainting problems associated with packaging systems, and studies of SO_2 in foods.

Ancillary to her technical duties was Phyl Moy's role for many years as 'little mother' to the inhabitants of the Food Technology Building at FRL; her unending thoughtfulness and concern for the welfare of her colleagues will be sorely missed.

JFK

D. E. Fenwick

Dorothy Fenwick, Experimental Officer, retired from CSIRO in August 1984. She was appointed to the Division in July 1958, having

transferred from the Department of Customs and Excise. Her first assignment was with Dr Thelma Reynolds, working on non-enzymic browning reactions. Then, when this work was wound down, Dorothy became part of a small group working on the composition of Australian honeys. When Dr Reynolds retired in 1970 Dorothy came to work with me.

Dorothy is a graduate of what was then Sydney Technical College and having had such an excellent scientific education was able to turn her hand to almost anything from physical chemistry to the care of laboratory animals. When she started with me we were interested in the structure of water and the hydrophobic effect. Dorothy measured reaction rates and made endless careful and painstaking electrical conductivity measurements on electrolyte solutions. Later, when we moved from such esoteric interests to dietary fibre and nutrition, she was equally competent at analysing foods for saponins, measuring plasma cholesterol concentrations and preparing diets for laboratory rats.

Dorothy joined CSIRO when the Division was still at Homebush and has a fund of interesting stories about the youthful frivolities of now very senior members of the organization! Real science was done in those days with fires and explosions apparently frequent occurrences in certain laboratories. Modern methods (and safety officers) have deprived us of a lot of fun.

Dorothy is a keen gardener and is considering moving to the kinder climate of the North Coast of New South Wales. Her scientific skills will be greatly missed — as also will be her contributions to the deliberations of the outdoors morning tea group.

DGO

P.L. Conway

Patricia L. Conway, Experimental Officer, resigned from the Division in May, 1984, after 10 years' service. Patricia began her career with CSIRO at MRL where she worked on the use of the ruminant paunch fermentation system to utilize wastes from the food industry to produce a nutritionally valuable protein supplement for stock food. After three years, Patricia joined FRL as a microbiologist participating in studies on the effect of food components on the gut microflora.

Her considerable skills as a microbiologist contributed significantly to the progress of the project. As different techniques were utilized, Patricia readily acquired expertise in anaerobic microbiology and in the application of scanning electron microscopy to gut microflora studies. Patricia participated in research involving the inhibition and promotion of the association of bacteria with the gut surface. This work developed into her main interest at FRL: the mechanism of the association of bacteria with the gut surface. More recently, her interest had focussed on the adhesion of lactobacilli to gut epithelial cells and the role of bacterial adhesion on the colonization of lactobacilli in the gut.

Patricia showed considerable enthusiasm for collaborative research projects and made meaningful contributions to several studies. These included the project at FRL on botulism. This project researched the production *in vivo* of *Clostridium botulinum* Type A toxin and the effect of the toxin on gut physiology.

Patricia was awarded the Australian Society for Microbiology Sherris scholarship in 1982 allowing her to visit the Tufts University Medical School, Boston, U.S.A., to study aspects of the gut microflora.

Patricia took an active role in the N.S.W. branch of the Australian Society for Microbiology and served on the committees of two ASM national conferences.

RFA

R. Beeby

Ralph Beeby was appointed to the staff of the Dairy Research Section as a Laboratory Assistant in 1946. In 1952 he was reclassified as Technical Officer, later Experimental Officer. From 1954-8 he worked with Dr K. Kumetat on problems associated with the use of milk proteins as egg substitutes and in the baking industry. After a year spent (at his own expense) with Professor As. Nitschmann in Berne, Switzerland, studying aspects of casein chemistry, Ralph returned in 1960 to the newly formed group at Highett studying the chemistry of the milk proteins. On the basis of this work he was later reclassified to SRS (1965). The work was concerned with the isolation from κ-casein, of some naturally occurring peptides that contained the rennin-sensitive bond or that were rich in sulphur-containing amino-acids. At that stage Ralph Beeby's working hypothesis was that κ-casein itself was an equimolar complex of these peptides. This was later shown not to be so, and the mode of origin of these peptides is still uncertain. Nonetheless much useful information was obtained on such matters as

the sulphur chemistry of κ-casein, the structures that confer sensitivity on the rennin-sensitive bond, and methods for preparing the sulphur-rich $\alpha_{s,2}$-casein and peptides from κ-casein.

During the last few years Ralph has been studying the possibilities for using immobilized enzymes for coagulating the milk in cheese-making. His work has shown up some unsuspected defects in currently accepted methods for attaching enzymes to solid supports. In fact, these methods frequently resulted in a preparation in which the enzyme was not covalently attached to the support but merely held there very firmly as a result of strong ionic interactions. Enzyme molecules could, however, be displaced by substrate into the liquid phase, causing loss of activity. In other methods, 'spacers' (e.g. hexamethylene-diamine) are used to attach the enzyme to the support to allow the enzyme to have a limited mobility and hence greater activity. However, interaction between the hydrophobic chains in the commonly used 'spacers' and the hydrophobic surface of the supports tended to defeat this purpose. To overcome these defects Beeby developed a new support based on a coating of ethylene-maleic anhydride copolymer, to which the enzyme molecules were attached via long, non-degradable, hydrophilic chains such as poly DL-alanine. By these means the activity of the immobilized enzymes was improved and their working life extended. He also contributed to the book 'Milk Proteins', edited by H. A. McKenzie (Academic Press, N.Y., 1971).

Ralph Beeby's particular qualities, which have been the subject of comment by several independent referees during his career, are the ability to take an independent view, a broad-ranging interest and experience – to which his publications testify – and a wide knowledge of relevant literature as well as a considerable capacity for work. In his interaction with others he is tolerant of, and sensitive to, other opinion.

Ralph was also a poet of note at DRL and many a social gathering bore witness to yet another 'Beeby gem'. He is missed at DRL, but we wish him a long and happy retirement.

HD