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Thermoplastic extrusion trials of some oil-seed, legume and cereal proteins

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In 1974 a project was initiated at the Food Research Laboratory on the application of thermoplastic extrusion to the production of textured protein foods from vegetable protein materials of Australian origin (Kefford 1974). Since the economic incentive of high meat prices has largely disappeared the project has been judged to have a low priority and has been terminated.

In the course of the project observations were made on the texturing properties of the 'flours' from a number of legume and oil-seed crops grown in Australia, and of some other protein and cereal mixtures; these results are here reported.

On the world scene soybeans are the most widely used protein source for the textured products. However, in Australia, experience has shown that for reliable yields soybeans must be irrigated. The main purpose of the project described was to assess the suitability for texturing by thermoplastic extrusion of high-protein crops which can be successfully grown, preferably without irrigation, in Australia.

Materials

If the required dehulled ground flour was unobtainable, seeds were cracked, either in a Fitzmill Model M or a coffee grinder, and the hulls removed by air classification. The meats were then ground in either the Fitzmill or an Alpine Kolloplex Laboratory Mill 160Z. The resultant full-fat flour was extracted with hexane, to yield a flour containing less than 0.5% oil. After drying, the flour was reground, in either the Fitzmill or the Alpine Mill, to the desired particle size.

Equipment

A Wenger X-5 Laboratory Extruder (Figs 1 and 6) was used to examine the suitability of the materials for the production of textured protein products or expanded cereal-based foods.

The extruder screw was driven at 450–950 r.p.m. by a 5 horse-power motor through a variable speed expansion pulley. Interchangeable extruder screws (Fig. 2) permitted operation of the unit with a barrel of either five or eight tandem-stacked extrusion heads (Figs 1 and 6). The first (or feed) head and four others had internal spiral grooves (Fig. 3). The three remaining heads had internal straight grooves (Fig. 4). For the production of textured vegetable protein (TVP) the eight-head barrel was used (see Fig. 5); the configuration of the barrel placed the spiral grooved heads in positions 1, 2, 3, 4 and 8. For the production of expanded cereal-based foods the five-head barrel was used, the configuration placing the spiral

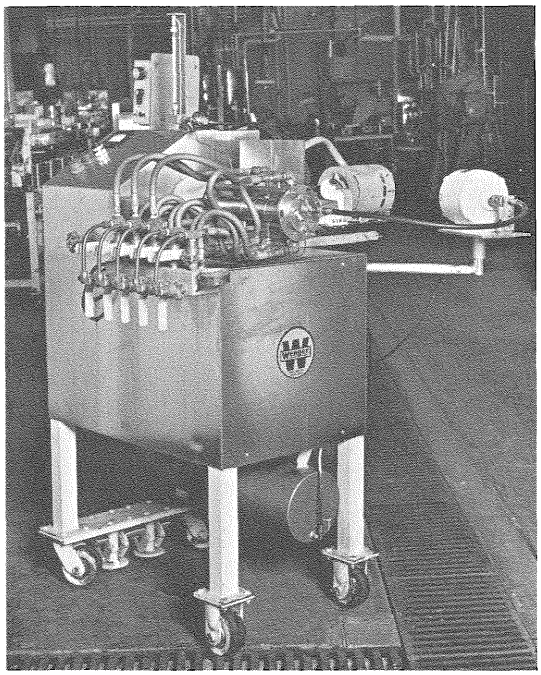

Fig. 1. Wenger X-5 extruder fitted with five-head barrel and outlet die for cereal-based snack food production.

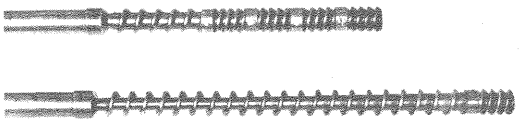

Fig. 2. Top: Cereal screw; Bottom: TVP screw.

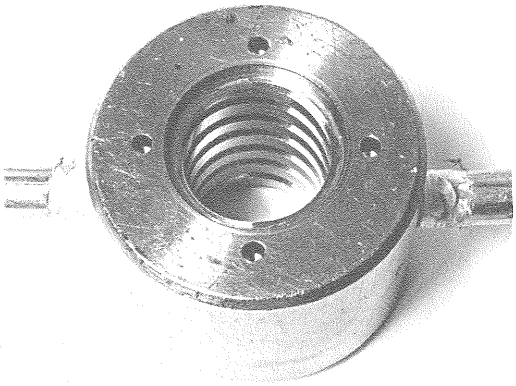

Fig. 3. Head with internal spiral grooves.

grooved heads in the positions 1, 2 and 5.

Each head was jacketed to permit either steam-heating or water-cooling of that particular head. This provided some control of head temperature and, hence, gelatinization, texturization, extrusion temperature, cell structure, product density and other parameters, that influenced the texture of the final product. When the eight-head barrel was used the temperature of the first six heads, in accordance with the dictates of the machine design, was controlled in pairs while the final two heads were controlled independently. When the five-head barrel was used each head could be controlled independently.

The outlet die supplied for textured protein production gave extremely variable and spasmodic extrusion and was replaced by a conical die chamber with an 8 : 1 taper terminating in a single 0.71-cm diameter outlet (see Fig. 6). The output from this die proved to be much more uniform than the original die, with no apparent detriment to product texture.

The cereal die plate had provision for the insertion of one of a number of dies of various diameters. The outlet side of this die plate was equipped with a variable-speed knife driven by a 1/8 horse-power motor.

The raw material was fed into the extruder by a variable-speed Archimedian screw from a hopper fitted with a belt-driven agitator. The screw was driven by a 1/3 horse-power motor.

Mains water was fed through a flowmeter and a fine control valve into the raw material in the first extruder head.

As delivered, the extruder could not be operated without extremely rapid wear of the internal surfaces of the heads; the alignment of the screw altered with change of screw

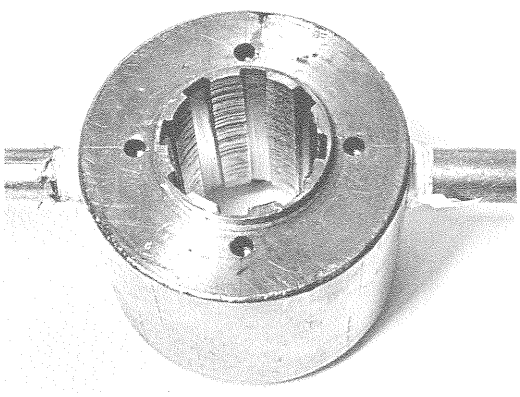

Fig. 4. Head with internal straight grooves.

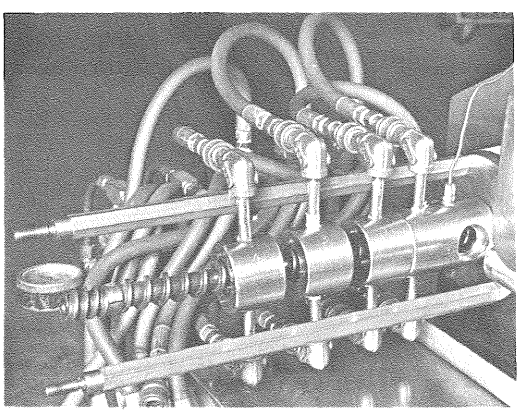

Fig. 5. TVP screw in position with heads being assembled.

speed, and it was impossible to maintain correct alignment of the screw in the barrel. Even after extensive modifications to remedy these faults, excessive wear persisted, presumably due to the ease with which the screw could be displaced by variable material density or by uneven distribution of the material in the barrel. At times, substantial wear of the distal heads occurred during a single operational period of less than 1 h.

The relatively dry conditions used in the extrusion of cereals to obtain highly expanded products caused more rapid wear of the extruder heads than did the texturing of vegetable proteins. Therefore the cereal products tested were expanded using the shorter screw (five heads) which is less susceptible to 'whipping' than the longer screw (eight heads).

Since the extrusion process has no established theoretical basis, the operating conditions for a particular product are determined empirically and subsequently reproduced for manufacturing runs. With the Wenger X-5 extruder this was not possible as its behaviour was unpredictable. Even when identical conditions were reproduced, product quality varied widely.

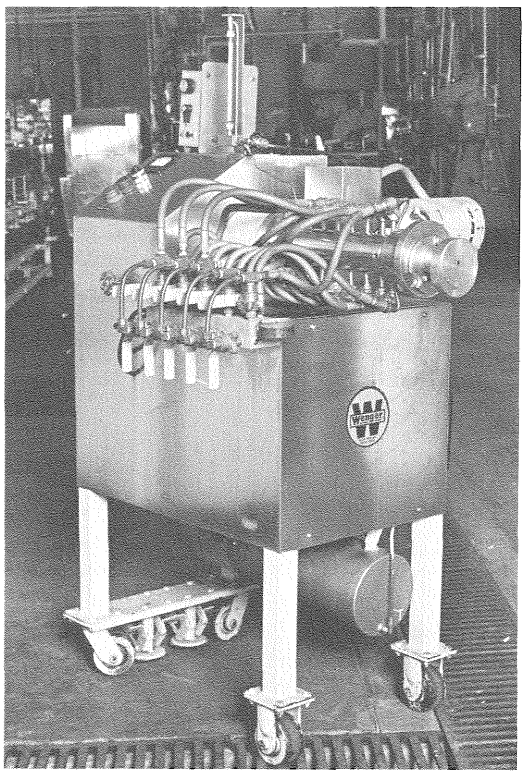

Fig. 6. Wenger X-5 extruder fitted with modified outlet die for TVP production.

Within the limitations imposed by the machine, the suitability for texturing by extrusion processing of each flour or combination of flours was determined under a variety of processing conditions. These conditions were achieved by separately varying the extruder speed, water flow, feed rate, and head temperatures.

The results of the processing trials are presented in Table 1. As far as prediction of performance in commercial scale extrusion equipment is concerned, these results must be interpreted with caution. However, since defatted soybean flour showed consistently good texturing properties, even in the laboratory extruder, the results may be taken as indicating the texturing properties of the other materials in comparison with soybean flour.

Dried whole blood

Dried whole blood, prepared under conditions of hygiene appropriate to human foods, was not directly amenable to extrusion processing, but it may be a useful adjunct to other raw materials for increasing total protein content or improving the nutritional value of the protein. The addition of up to 20% dried whole blood to soybean flour did not hinder its texturizing ability, but the extruded product had an unpleasant odour and very unattractive colour.

Expanded cereal-based foods

Expanded cereal-based snack foods were easier to produce in the laboratory extruder than textured protein foods. A number of extrusion trials were made for the production of protein-enriched products.

Rice was the basis of most of these snack foods. Good products were obtained from rice blended with 30% defatted soybean flour, up to 20% vital wheat gluten, 40% wholemeal wheat flour, 20% wholemeal wheat flour plus 20% defatted soybean flour, 20% defatted soybean flour plus 20% oatmeal flour, 20% defatted lupin flour, or 20% full-fat lupin flour.

Wheat flours gave satisfactory expanded products.

Conclusions

The fact that most of the flours tested needed to be blended with defatted soybean

Table 1. Results of thermoplastic extrusion trials

Raw material	Approximate protein content Wet basis (%)	Texturing properties
Soybean *(Glycine max)*		
Straight flour	50	Very good
Lupin *(Lupinus angustifolius)*[A]		
Straight flour	40	Nil
Blends		
1 Lupin : 1 Soybean		Poor
1 Lupin : 1 Gluten		Good
3 Lupin : 5 Gluten		Good
3 Lupin : 2 Gluten		Fair–good
3 Lupin : 3 Gluten : 2 Soybean		Good
2 Lupin : 2 Gluten : 1 Soybean		Good
2 Lupin : 1 Gluten : 1 Soybean		Good
4 Lupin : 5 Gluten : 1 Soybean		Good
4 Lupin : 1 Full-fat lupin : 5 Gluten		Poor
3 Lupin : 2 Full-fat lupin : 5 Gluten		Poor
Vital wheat gluten		
Straight flour (not extracted with hexane)	72	Nil
Blends containing up to 40% gluten, remainder soybean		Very good
Peanut *(Arachis hypogaea)*		
Straight flour	56	Nil
Blends		
1 Peanut : 1 Soybean		Very good
2 Peanut : 1 Soybean		Good
1 Peanut : 1 Soybean : 1 Gluten		Very good
2 Peanut : 1 Soybean : 2 Gluten		Good
1 Peanut : 1 Lupin		Nil
White mustard *(Sinapis alba)*[B]		
Straight flour	55	Nil
Blends		
1 White mustard : 1 Soybean		Good
3 White mustard : 1 Soybean		Poor
Brown mustard *(Brassica juncea)*[C]		
Straight flour	56	Nil
Blend		
1 Brown mustard : 1 Soybean		Good
Subterranean clover *(Trifolium subterraneum)* Var. Woogenellup[D]		
Straight flour	52	Nil
Blends		
1 Sub. clover : 1 Gluten		Poor
1 Sub. clover : 1 Soybean		Poor
Chick pea *(Cicer arientinum)*		
Straight flour	27	Nil
Blends		
1 Chick pea : 1 Soybean		Nil
1 Chick pea : 1 Gluten		Nil
1 Chick pea : 3 Soybean		Poor
2 Chick pea : 3 Soybean : 3 Gluten		Nil
Split pea *(Pisum sativum)*		
Straight flour	24	Nil

Table 1. Results of thermoplastic extrusion trials *(continued)*

Raw material	Approximate protein content Wet basis (%)	Texturing properties
Blends		
3 Split pea : 1 Gluten		Nil
1 Split pea : 1 Gluten		Nil
1 Split pea : 1 Soybean		Nil
Broad bean *(Vicia faba)*		
Straight flour	24	Nil
Blends		
1 Broad bean : 1 Soybean		Poor
1 Broad bean : 1 Gluten		Nil
1 Broad bean : 1 Lupin		Nil

[A]The lupin flour even after hexane extraction and extrusion, retained its strong bean-like flavour and yellow colour. Other lupin species may be better. Slight texturing was achieved with lupin flour which had been extracted with 50% aqueous ethanol. This process removed about 10% soluble material, believed to be mainly carbohydrate.
[B]The extruded product retained the distinctive bitter flavour of the flour.
[C]Brown mustard flour retains its extremely 'hot' principle after hexane extraction and does not seem to be suitable for commercial application.
[D]The extruded product had a very strong, unpleasant, flavour and was pale green in colour.

flour to enable them to be textured suggests that it may be more profitable to breed soybean varieties that can be economically produced in Australia than it would be to use established crops, with the probable exception of wheat (vital gluten) and peanuts.

An economic difficulty with some of the flours examined is that they have an oil content (2–15%) that is too low to permit economic extraction and yet too high to allow satisfactory texturing by thermoplastic extrusion. It may be feasible, by plant breeding investigations, to overcome this problem. The commercial value of some of these oils is also unknown.

Expanded cereal-based snack and breakfast foods were successfully produced by extrusion-cooking. The Wenger X-5 Laboratory Extruder in the Food Processing Laboratory at FRL is available to the food industry for processing trials under agreed conditions.

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Adhesion of fruit cakes to aluminium trays

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Trays used as moulds in the production of bakery products are often made from light gauge aluminium alloy because this material is cheap and may be recycled. Sometimes a release agent is applied to the internal surfaces of the trays to prevent adhesion of the product. In commercial practice the incidence of 'stickers' is low and in most cases can be avoided by the application of a suitable release agent. However, one manufacturer, who applied a lecithin/white oil mixture, recently experienced a high incidence of 'stickers' with a batch of cake trays. Other batches of trays made from alloys of the same nominal composition performed satisfactorily.

This paper describes a mechanism of adhesion of bakery products to their moulds and the experiments undertaken to determine the cause of product adhesion in a batch of trays.

Adhesion theory

Bakery products tend to stick to trays when the water in the dough wets the metal surface and then dries during baking so that dissolved materials are deposited to form a bond between the metal and the product (Fig. 1). Oiling the surface of the tray is intended to prevent wetting so that the adhesive bond cannot form.

The degree of wetting that occurs between a liquid and the plain or oiled surface of a solid may be gauged from the angle that forms where the liquid contacts the surface. (Fig. 2). The degree of wetting of a solid by a liquid is related to the surface free energy of both the liquid and the solid (Birnbaum 1965). The surface free energy is a measure of the tendency a surface has to attract molecules or atoms.

Metals are characterized by high surface free energies ranging from 0.5 to 5 J m^{-2}. Any liquid with a surface free energy less than that of the metal will be more attracted to the metal than to itself and will spread across the metal and thus wet it. When the metal is coated with an oil, usually with a surface free energy of about 0.02 J m^{-2}, the high energy surface of the metal is covered with the low energy surface of the oil. When water with a surface free energy of 0.07 J m^{-2} comes in contact with the oil, therefore, the larger surface free energy of the water prevents the water from spreading across the oil, resulting in reduced wetting of the oiled tray surface.

Therefore, to minimize adhesion of cake to aluminium trays, there should be a high degree of wetting of the aluminium surface by the lecithin/white oil mixture and a low degree of wetting of the oil surface on the tray by the water in the dough.

The surface roughness of the tray also influences adhesion. Lebedev *et al.* (1975), for example, found that adhesion of spaghetti dough to moulds was related to the degree of unevenness of the metal surface.

Tray manufacture

The first step in the manufacture of aluminium trays is to hot roll an ingot of the required alloy to a thickness of about 5 mm.

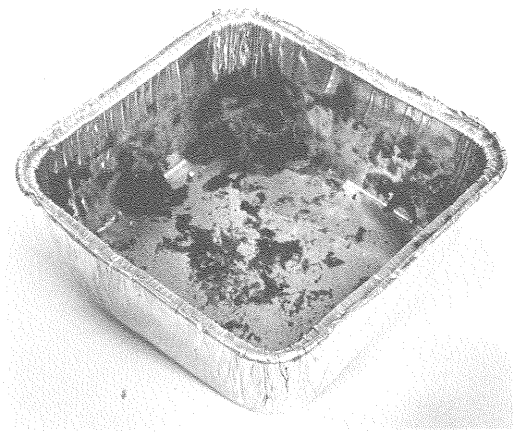

Fig. 1. Example of cake adhering to an aluminium tray.

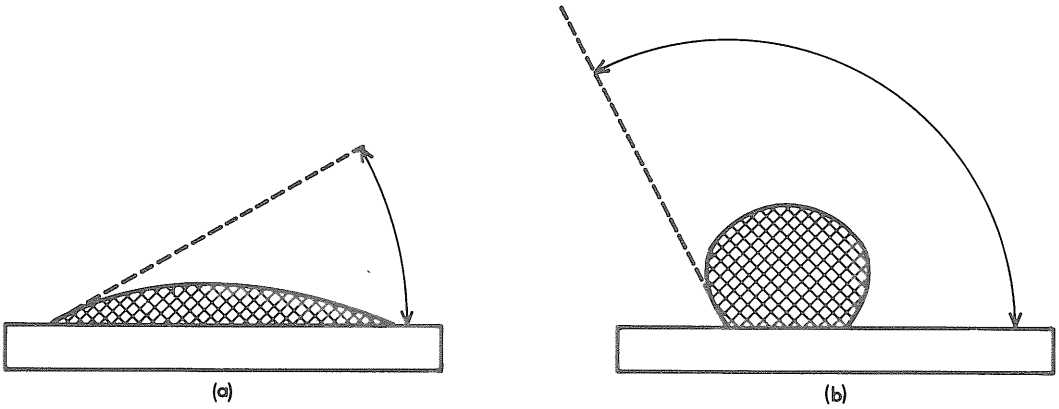

Fig. 2. Contact angles of a liquid on a solid surface. (a) Small contact angle associated with a high degree of wetting; (b) large contact angle associated with a low degree of wetting.

The 5 mm strip is then cold rolled to a final thickness of about 0.1 mm which is required for foil. A lubricant consisting of a hydrocarbon-based mineral oil containing proprietary additives is applied to the strip during cold rolling. The foil is then coiled and annealed at about 350 °C for 2–3 h. Most of the rolling oils are removed by the annealing process and the foil is usually examined for residues of the rolling oil by a wettability test which is described by the Aluminium Development Council of Australia (1978).

Trays are then stamped from the foil using a lubricant, such as peanut oil or butyl stearate in white oil, to prevent the trays sticking to the dies. This lubricant and any residues of rolling oil may affect subsequent adhesion of cakes to the trays. Marston and Howard (1977) state that the build-up of polymerized oil on bread-baking pans is a major problem as it reduces their life and increases the incidence of sticking loaves and stained bread.

Alloy composition

Samples of trays from the complaint batch (Code A7) and from two satisfactory batches (Codes A8 and A9) were analysed in duplicate by atomic absorption spectroscopy for manganese, iron, silicon, copper, and magnesium. The results presented in the table show that there were no marked differences between the alloys. It seems unlikely, therefore, that differences in the composition of the alloys were responsible for the poor performance of the batch coded A7.

Composition of aluminium alloy trays

Batch code	Manganese %	Iron %	Silicon %	Copper %	Magnesium %
A9	1.1	0.68	0.25	0.13	<0.002
A8	1.1	0.75	0.25	0.13	<0.002
A7	1.0	0.70	0.25	0.13	<0.002

Properties of the oxide layer

A thin oxide layer rapidly forms on the surface of all aluminium alloys on exposure to air. The chemical composition of the alloy and subsequent manufacturing treatments affect the nature and composition of the oxide layer, the presence of which plays an important role in the final surface properties of the alloy, including its corrosion resistance.

The thickness and nature of the oxide layer could not be determined directly. However, if the oxide layers on the different batches were sufficiently different to cause the observed differences in adhesion, it is likely that the corrosion resistance of the various batches would also differ markedly.

Corrosion rate experiments were therefore carried out at 25 °C on the batches of trays by immersing defined areas of the trays in 4 M HCl and measuring the rates of hydrogen evolution from the surfaces. The results of these tests provided no evidence of a difference between the oxide layers.

Wetting properties of the lecithin/white oil mixture

Attempts were made to measure the contact angles, and hence the wettability, of the lecithin/white oil mixture on the unused

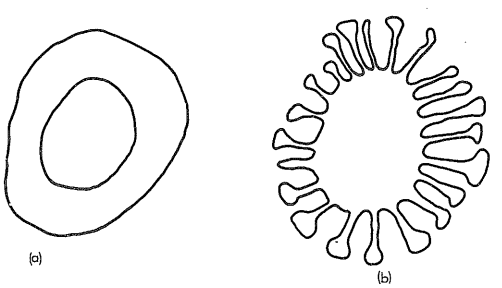

Fig. 3. Pattern of Lecithin/white oil drops placed on surfaces of trays made from (a) alloy A9; (b) complaint alloy A7.

tray surfaces. Drops (20 μl) of the mixture were placed on uncleaned trays from each of the three batches. However, the diameters of the oil drops increased continuously and, as shown in Fig. 3, they sometimes formed two distinct regions, a centre and an outer one. In addition, the pattern of the drops on alloy A9 (Fig. 3a.) differed from those on alloy A7 (Fig. 3b.). The pattern for A8 was intermediate between the patterns for A9 and A7, but more closely resembled that of A9. After cleaning the trays with tissues soaked in trichloroethylene the patterns of the drops on all trays resembled those on the A9 trays.

The wettability of the oiled surface was then assessed by spraying uncleaned trays with the lecithin/white oil mixture and placing a drop (20 μl) of water on the oiled surface. Water drops on the surface of the trays coded A9 and A8 were 5 mm in diameter while water drops on the surface of the trays coded A7 were 6 mm in diameter.

The main difference, however, was that the sprayed films of lecithin/white oil mixture on the trays coded A7 had a definite mottled or 'orange peel' appearance which was less pronounced on samples coded A8 and A9.

These results suggest strongly that the surfaces of the code A7 trays differed from those of the other trays.

Microscopic examination

Contamination of the metal surfaces during manufacture of the foil or tray was considered to be a likely cause of the observed difference in adhesion between batches of trays. We explored this possibility with the help of Mr L. B. Brunckhorst, Fuel Geoscience Unit CSIRO, who examined the surfaces of the uncleaned trays for contaminants with a Cambridge scanning electron microscope. Trays coded A7 had a large number of dark areas (Fig. 4) about 20 μm in diameter, that were concentrated along the rolling lines. Trays coded A8 and A9 had considerably fewer dark areas (Fig. 5).

The code A7 trays were scrubbed with a tissue soaked in trichloroethylene and almost all of the dark areas were removed (Fig. 6.). When the trays were merely rinsed in trichloroethylene the dark areas remained; this treatment would have removed any die lubricant from the surface. X-ray fluorescence examination of the dark areas indicated that they consisted of organic material.

Conclusions

Microscopic examinations showed that the trays from the complaint batch coded A7 had

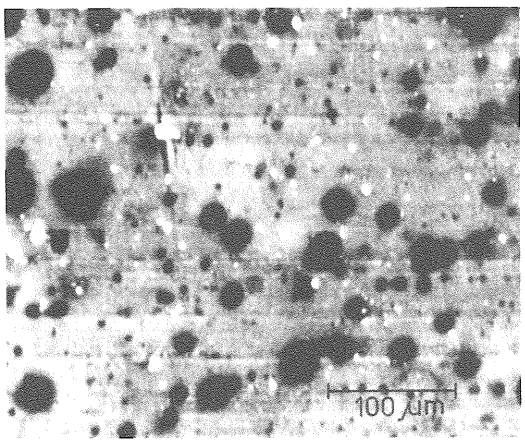

Fig. 4. Scanning electron micrograph of uncleaned code A7 tray. Note dark areas along rolling lines.

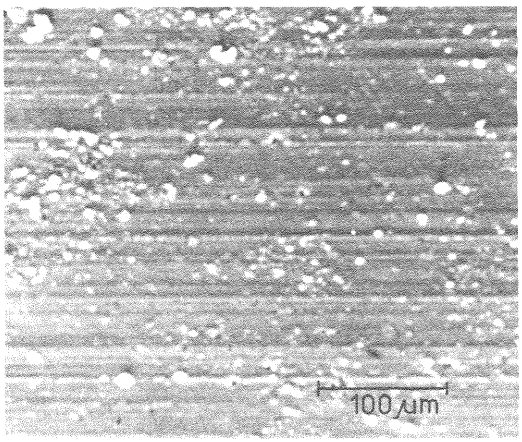

Fig. 5. Scanning electron micrograph of uncleaned code A9 tray surface. The white areas are dust particles.

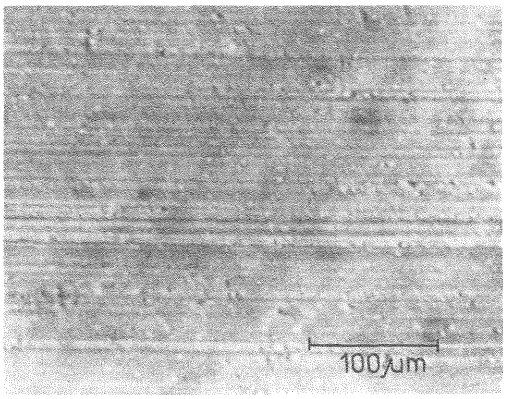

Fig. 6. Scanning electron micrograph of code A7 tray after cleaning with trichloroethylene-soaked tissue.

a high concentration of surface contaminants whereas those coded A8 and A9 in which adhesion was not a problem had a low concentration of surface contaminants. It is evident that these surface contaminants caused de-wetting or incomplete wetting on the complaint trays when they were sprayed with the lecithin/white oil mixture and this in turn allowed the cakes to adhere to the trays during baking.

Although the nature and origin of the surface contaminant were not determined it is likely that it was an oil, used as a die or rolling lubricant, that had polymerized during the annealing process or subsequent storage.

Where possible, therefore, cake manufacturers should specify foil with excellent wetting properties towards their release agent, in this instance, the lecithin/white oil mixture. The results of the drop test, shown in Fig. 3, suggest that this test may be suitable for quality control. An alternative would be to coat the foil with a lacquer containing release agents as described by Pikalova *et al.* (1976).

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Oxidative enzymes and oxidative processes in milk

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There are many different types of enzymes in milk; Shahani *et al.* (1973) listed 35 enzymes that had either been isolated from, or their activity detected in milk, and since then more have been reported. Most of these are present in very small quantities and can be regarded as products of leakage from the mammary cells. Two enzymes, however, are present in quite high concentrations; these are the oxidative enzymes lactoperoxidase and xanthine oxidase. Typical concentrations for these enzymes are 30 mg l^{-1} and 120 mg l^{-1} respectively (Groves 1971). Usually, quite small amounts of enzyme are sufficient for effective action. For example, in cheese manufacture, only about 0.04 mg l^{-1} of pure rennin is sufficient to coagulate the milk. The quantities of lactoperoxidase and xanthine oxidase are several orders greater than this, and these enzymes therefore are unlikely to be leakage products but should be viewed as having a definite function in the milk. This

function, most probably, is to inhibit the growth of bacteria while milk is in the udder.

Xanthine oxidase catalyses the oxidation of a variety of substrates (xanthine, aldehydes) with the concomitant reduction of oxygen to hydrogen peroxide. Lactoperoxidase similarly catalyses the oxidation of various substrates (ascorbate, adrenalin, phenols, thiocyanate) and is activated by hydrogen peroxide which is reduced to water in the course of the reaction. Therefore, when xanthine oxidase acts on a suitable substrate in milk, it provides the peroxide required to activate the lactoperoxidase. The latter can then catalyse the oxidation of thiocyanate to a substance that is inhibitory to the growth of bacteria (Oram and Reiter 1966). The xanthine oxidase-lactoperoxidase system in milk is thus quite similar to the xanthine oxidase-myeloperoxidase antimicrobial system investigated by Klebanoff (1974). In a survey of Victorian milks, Lawrence (1970) found thiocyanate contents to range from about 2.5×10^{-5} M to 1.8×10^{-4} M, concentrations that are adequate for effective bacterial inhibition (Hogg and Jago 1970). In the absence of such an inhibitory system, the udder would provide ideal conditions for growth of foreign organisms.

Both lactoperoxidase and xanthine oxidase generate the superoxide radical O_2^-, as an intermediate in their reactions (Misra and Fridovich 1972; Hill 1977). As this radical can act either as an oxidant or as a reductant, and in addition can be simply transformed to the powerful oxidant, singlet oxygen, it is obvious that in milk stored for use by man there is considerable potential for oxidative damage to lipids from this source. In fact, it has been shown in model systems based on other lipids that xanthine oxidase acting on xanthine (Pederson and Aust 1973) or on acetaldehyde (Kellogg and Fridovich 1975) can initiate lipid oxidation in this way. Their evidence showed that the agent finally acting on the lipid was singlet oxygen derived from the superoxide radical.

Oxidation of lipids in conventional milk

In milk itself, the effect of the oxidative enzymes was less clear. Although Aurand and Woods had produced evidence in 1959 that the spontaneous occurrence of oxidized flavour in milks was caused by xanthine oxidase activity, other workers were unable to reproduce their results, and Smith and Dunkley (1962) concluded that in milk the effective catalyst for the oxidation of the lipids was not xanthine oxidase but copper. Their conclusion was also based on the failure to demonstrate that there was a suitable substrate for xanthine oxidase in milk. On the other hand, there was little difficulty in demonstrating the effect of added copper in promoting oxidation of the milk lipids, and it had been fairly clearly shown that the reaction of copper ions with ascorbic acid provided free radicals that initiated oxidation (Haase and Dunkley 1969). The type of reactant involved, whether superoxide or hydroxyl radical, or singlet oxygen, was not established.

Oxidation of lipids in milks rich in linoleic acid — Hi-Lin milks

Oxidation in milk fat occurs mainly at the expense of the polyunsaturated fatty acids, *viz.* mainly linoleic, with relatively small amounts of linolenic and arachidonic acids that contain respectively 2, 3 and 4 unsaturated linkages. In the past, the task of demonstrating effects by either copper or enzymes on the oxidation of the fats was made more difficult by the relatively low content (about 2%) of these polyunsaturated fatty acids in the fat of normal bovine milk. This situation was changed recently with the development of a method for producing milk with a much higher content of polyunsaturated acids. Normally, any polyunsaturated fatty acids in the feed of the cow are hydrogenated by the bacteria in the rumen, so that the fats passing through the rumen for use by the animal are, largely, saturated. By feeding cows a supplement in which the oil was protected by encapsulation in formaldehyde-treated protein, the polyunsaturated acids could pass through the rumen unaltered and become available for use by the cow (Scott *et al.* 1970). By this means, milks containing up to 35% of polyunsaturated fatty acid could be produced. This development produced both a new challenge and a new opportunity. The challenge was to find practical ways of preventing oxidation in these milks, which could be much more susceptible to oxidation than normal milks, with accompanying severe flavour defects (Sidhu, Brown and Johnson 1975). The opportunity arose because with greater quantities of oxidizable material present, the effects of oxidation were

quantitatively greater and hence were more easily investigated.

Control of oxidation by antioxidants

Oxidation in conventional milks could be controlled effectively by antioxidants such as butylated hydroxyanisole (BHA) and less effectively by tocopherol. Dunkley and his coworkers had shown that to include the antioxidants in the diet of the cow was not effective, as only a small percentage of the ingested antioxidant was transferred to the milk (Dunkley, Franke and Robb 1968). The antioxidants, therefore, must be added directly to the milk. It was important to determine whether the more serious problem of oxidation in the new type of milk — 'Hi-Lin' milk — could be similarly controlled. Sidhu *et al.* (1975) showed that the oxidation could be controlled by adding emulsified butylated hydroxyanisole, sesamol, nordihydroguaretic acid or ethoxyquin in amounts of 10–15 mg l^{-1}. Tocopherols were, again, less effective. The same authors later showed that oxidation was also controlled by adding hydrogen peroxide, before pasteurizing the milk, in amounts sufficient to destroy the ascorbic acid (Sidhu *et al.* 1976). This effect demonstrated the importance of the copper–ascorbic acid system in inducing oxidation in these milks. The treatment with peroxide would also have inactivated the lactoperoxidase which is susceptible, even at room temperature, to peroxide in the concentrations used (Hill 1977). This effect would have been enhanced at the temperature of pasteurization. Thus the oxidative enzymes may also play a part in the oxidation of the lipids.

Effects of oxidative enzymes in Hi-Lin milk

In order to study the roles of the enzymes, it would have been desirable to compare the behaviour of milks containing controlled amounts of enzyme with that of milks without any. In practice the selective removal of the oxidative enzymes would be difficult to achieve. Instead, in our experiments the enzymes were inactivated by pasteurizing the milk at higher temperatures than the normal 72 °C for 15s. Both lactoperoxidase and xanthine oxidase survive the normal pasteurization with relatively little loss of activity. However, lactoperoxidase in particular loses 80–90% of its activity as a result of pasteurization at 80 °C for 15s, and xantine oxidase about 50%. Although the enzymes were more completely inactivated by pasteurization at temperatures above 80 °C, this treatment caused a persistent cooked flavour in the milk, which was quite marked when the temperature of pasteurization was 85 °C. Most of the pasteurization was therefore carried out at temperatures of 80 °C or lower.

Effects of temperature of pasteurization on oxidative stability of Hi-Lin milk

If oxidative enzymes accelerate the oxidation of milk lipids, then milks pasteurized at 80 °C should be more stable toward oxidation than those pasteurized at 72 °C. The rates of oxidation in the milks were assessed in three ways:

- by tasting the milks for oxidized flavour using a flavour panel
- by measuring the concentration of malonaldehyde — a product of lipid oxidation — using the thiobarbituric acid (TBA) test
- by measuring ascorbic acid which is quite readily oxidizable.

All these measures showed a greatly increased resistance to oxidation in the milks pasteurized at 80 °C. Flavour scores for a series of eight milks showed that after storage at 5 °C for 120 h, oxidized flavour in the milks pasteurized at 80 °C was barely detectable (score 0.4), whereas it was quite marked (score 2.4) in those pasteurized at 72 °C. TBA scores for the 80 °C milks were less than half those for the 72 °C controls at 120 h age, while the rate of destruction of ascorbic acid was similarly reduced (Hill *et al.* 1977). Further insight into the roles of these enzymes might therefore be gained by adding them to the milks pasteurized at 80 °C in quantities sufficient to restore activity to about the original level.

Effect of added oxidative enzymes

Although all the Hi-Lin milks used in these experiments contained about 20% of polyunsaturated acids in their fat — and frequently rather more than this — there were considerable differences in oxidative stability when these milks were pasteurized at 72 °C. After pasteurizing at 80 °C, the addition of lactoperoxidase or xanthine oxidase to the more readily oxidizable milks caused a more rapid development of oxidized flavours. However, when the more stable milks were pasteurized at 80 °C and the enzymes were

added, lipid oxidation and destruction of ascorbic acid were not accelerated, but these processes were accelerated when a substrate for xanthine oxidase was also added (Hill *et al.* 1977). Krukovsky and Guthrie (1946) showed that ascorbic acid in milk was destroyed by peroxidase if hydrogen peroxide were present. Thus, it appears that in the unstable milks there was a substrate on which xanthine oxidase could act and so generate hydrogen peroxide, while such a substrate was lacking in the more stable milks.

Effect of added superoxide dismutase

Both lactoperoxidase and xanthine oxidase generate, as an intermediate in their reactions, superoxide radicals that can initiate directly or indirectly lipid oxidation. It was therefore of interest to determine if the stability of the milks pasteurized at 80 °C could be further improved by adding superoxide dismutase, which scavenges superoxide radicals by converting them to peroxide and oxygen (McCord and Fridovich 1969). In fact, the addition of as little as 1 mg l^{-1} of superoxide dismutase (and a similar amount of catalase to remove the peroxide produced by the dismutase) improved the stability of these milks considerably. Thus superoxide radicals appear to initiate oxidation in these milks.

Effect of added copper

Rapid oxidation of the lipids in most of these milks was also caused by adding *c.* 0.1 ppm of copper after pasteurizing the milk at 80 °C. If this oxidation, also, depended upon the superoxide radical, it too should be inhibited by adding superoxide dismutase and catalase in quantities similar to those of the previous experiment. However, there was no inhibition in these circumstances but there was a substantial inhibition when formate, an OH.radical scavenger, was added as well as the enzymes. Formate transforms the OH.radical to superoxide, which can then be removed by the added superoxide dismutase. The copper-induced oxidation in these milks therefore depends upon OH.radical, and in this respect differs from the enzyme-catalysed oxidation. The levels of copper used in these experiments were low (*c.* 0.1 mg l^{-1}), about the level of contamination that might be expected in milk when copper hygiene is somewhat inadequate. At this level practically all of the copper is bound to the casein fraction (Hill *et al.* 1977). At higher levels of added copper, such as those used by Aurand *et al.* (1977), a significant portion may be bound elsewhere so that radicals other than OH. may be produced. In these circumstances, superoxide dismutase might inhibit oxidation.

One interesting result of our work is that the effect of copper depends greatly upon whether it is added before or after the pasteurization at 80 °C. When copper was added after pasteurization at 80 °C, rapid oxidation occurred with accompanying flavour defects. In contrast, when the copper was added before the 80 °C pasteurization there was little or no acceleration of the oxidation. When the milk was pasteurized at 72 °C, copper caused rapid oxidation irrespective of when it was added. Raising the temperature of pasteurization from 72 ° to 80 °C therefore provides protection against the effects of copper that has contaminated the milk before pasteurization. This effect may be due to an unfolding of the casein at the higher temperature, exposing sites at which the copper is more firmly bound (Hill *et al.* 1977). This protective action is effective up to levels of copper of about 0.15 ppm, which is several times the amount that is likely to contaminate the milk in reasonably hygienic plants.

Summation

Thus, there are two systems for catalysing oxidation in milk: one depends upon copper and OH. radical while the other is activated via oxidative enzymes which generate O_2^- radicals and singlet oxygen. For simplicity these systems have been treated separately. It is important to realize that in milk these systems are coupled, as the aldehydes produced via a copper-catalysed oxidation are themselves a suitable substrate for xanthine oxidase, and they therefore activate the enzymic oxidation system. Pasteurizing the milk at 80 °C instead of 72 °C can therefore be seen to have several useful advantages. Firstly, it protects the milk against the effects of copper (up to 0.15 ppm) which may be present in the milk before pasteurization. Secondly, it reduces considerably the activities of the oxidative enzymes. These two effects are not only beneficial in themselves, but together they reduce the magnitude of the 'coupling effect' mentioned earlier. In addition, the milk is in a condition in which the stability of the milk

may be further enhanced by adding relatively minute amounts of superoxide dismutase and catalase. Thirdly, the higher pasteurization temperature ensures a greater destruction of microorganisms.

These effects add up in practice to a longer shelf life for milks pasteurized at 80 °C because of reduced fat oxidation, better survival of ascorbic acid and better bacteriological quality. These qualities are clearly important for milks rich in linoleic acid. Because of present trends in the marketing of milk which lead to an increasing length of time between milking and consumption, the improvements obtained by pasteurizing at 80 °C should also have practical value for conventional milk.

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Training program for canners

A recent activity undertaken by the Food Research Laboratory was an in-plant training program for retort operators in the canning industry. Some overseas countries importing Australian canned foods now require that operators and supervisors responsible for the heat processing of low-acid canned foods undergo specialized training.

At the request of the Department of Primary Industry and at the invitation of the canners, V. M. Stekly of FRL visited 20 canneries in N.S.W., Victoria, Queensland, and Western Australia during the period March to November 1978.

The training program at each cannery typically involved not only the retort operators but also supervising and management personnel and the resident inspectors of the Department of Primary Industry. They received theoretical and practical instruction in correct retort operation, particular attention being paid to common errors that may occur during venting, processing, and cooling.

In addition, Mr Stekly inspected all retorts and ancillary equipment, and checked the accuracy and general condition of retort thermometers and recorder-controllers.

The in-plant training program concluded with an audio-visual aid 'For the Retort Operator' produced by the National Food Processors Association (U.S.A.).

Composition of some Australian table margarines

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The role of dietary fats in human nutrition has been the subject of much speculation in the last two decades (for reviews see Vergroesen 1975, and the report of the FAO/WHO Expert Consultation Group 1977). Epidemiological studies have suggested that a number of diseases, such as heart disease, arteriosclerosis, and certain types of cancer, that are prevalent in affluent societies, may be related to the type of diet consumed within such societies. Typically this type of diet is rich in fats and protein and low in starches and fibre. In recent years various medical authorities have suggested that a reduction in the amount of fat consumed per capita would help to lower the incidence of heart disease in the community. In this connection it has also been recommended that the intake of saturated fats, such as those derived from beef, lamb and dairy products, be reduced, while the intake of polyunsaturated fats, derived from vegetable oils, be increased. Typical of such recommendations are those embodied in the report entitled 'Dietary Goals for the United States' (U.S. Senate; Select Committee on Nutrition and Human Needs, 1977). Goal 4 of this report suggests that overall fat consumption should be reduced from about 40% of energy intake to about 30%, and Goal 5 suggests that the fat intake be divided equally between saturated fat, monounsaturated fat and polyunsaturated fat, i.e. each of these three to contribute 10% of the energy intake. (In 1976 in the U.S.A. saturated fat comprised about 16% of the energy intake, monounsaturated fat about 19%, and polyunsaturated fat about 7% (U.S. Senate; Select Committee on Nutrition and Human Needs, 1977).) In Australia, as well as overseas, partly as a result of these pronouncements, there has been a considerable increase in the use of edible vegetable oils in recent years. In particular there has been a marked increase in the market for table margarines (tub margarines) containing no cholesterol and a high content of polyunsaturated fats. In Australia the term 'table margarine' generally refers to margarines containing vegetable and hydrogenated vegetable oils as the sole or major component. The National Health and Medical Research Council of Australia has proposed a number of standards for margarine (NHMRC 1976), and margarine sold as table margarine is required to contain vitamin A in an amount equivalent to not less than 8.5 mg of retinol activity per kg, and vitamin D in an amount equivalent to not less than 55 μg of cholecalciferol per kg. A polyunsaturated margarine is defined as a table margarine in which the total fatty acids present contain not less than 40% *cis*, methylene-interrupted polyunsaturated fatty acids and not more than 20% saturated fatty acids. The NHMRC standards also set out the specific requirements for the correct labelling of cooking, table and polyunsaturated margarines.

Vegetable oils used in the manufacture of foodstuffs such as table margarines and cooking oils are usually subjected to a number of refining processes during manufacture, such as alkali-refining, bleaching, and deodorization. (For brief review, see Hayes 1979.) Partial hydrogenation of some oils is often undertaken to improve their stability or to obtain the correct consistency required for a particular end-use. During these processes some of the unsaturated fatty acids in the oils undergo isomerization with changes in the positional and geometric configuration of their double bonds. For example, hydrogenation of vegetable oils containing the polyunsaturated fatty acids linoleic acid or linolenic acid will yield, as the main products, compounds in which some of the double bonds have simply been hydrogenated (Fig. 1), but may also produce isomers in which the natural *cis*, *cis*, methylene-interrupted double bond configuration of the polyunsaturated acid has been altered by a double bond shifting one place closer to the adjacent double bond (Fig. 2). Such isomers are said to have a

conjugated double bond system. The geometric configuration of the shifted bond usually alters from *cis*- to *trans*- during the double bond rearrangement. Thus linoleic acid (*cis*-9, *cis*-12-octadecadienoic acid) may form *cis*-9, *trans*-11- and *trans*-10, *cis*-12-octadecadienoic acids. Further hydrogenation of such conjugated dienoic acids may produce monoenoic fatty acids in which the remaining double bond has the *trans* configuration. Moreover, the conditions of hydrogenation often cause double bond migration with a change from the *cis* to the *trans* configuration, so that a number of positional isomers of the unsaturated fatty acids may be formed. Parodi (1976) has analysed the geometrical and positional isomers of the unsaturated fatty acids in some Australian edible oils, and found *cis*- and *trans*-octadecenoic acids with the double bond located at any position between C-6 and C-14. In natural vegetable oils the octadecenoic acid would be almost exclusively *cis*-9, i.e. oleic acid.

The presence of fatty acid artefacts, such as those described above, in processed vegetable oils has been the subject of some controversy. While it is true that fat derived from ruminant animals (as in beef, lamb and dairy products) also contains conjugated and *trans* unsaturated fatty acids (arising from biohydrogenation of unsaturated fatty acids in the rumen), these are present only in small amounts. *Trans* fatty acids in processed oils such as shortenings, however, may reach levels of over 50% (Heckers and Melcher 1978). *Trans* fatty acids ingested by monogastric animals, including humans, are deposited in the depot and organ fat of those animals; but they appear to have no deleterious effects provided an adequate intake of essential fatty acids, such as linoleic acid, is maintained (FAO/WHO 1977).

This report presents data on the composition of various brands of table margarine obtained at retail outlets in Sydney in 1975 and 1978. Similar analyses on various edible oils purchased in Brisbane in 1973/74 have been presented by Parodi (1976). Heckers and Melcher (1978) provide data on German margarines in 1973/74 and in 1976.

Experimental procedure

Polyunsaturated table margarines (500 g packs) were purchased in Sydney in 1974 and a wider range of table margarines was purchased in 1978. The fat was extracted from 5 g of each margarine by the method of Bligh and Dyer (1959), and in each case the

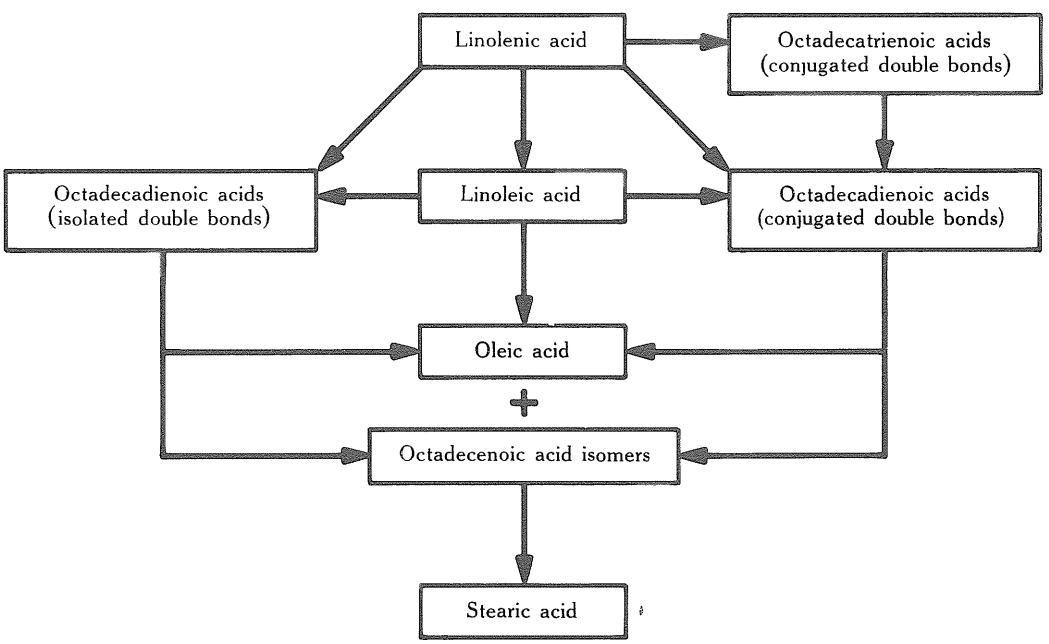

Fig. 1. Fatty acids formed during hydrogenation of vegetable oils containing linolenic and linoleic acids.

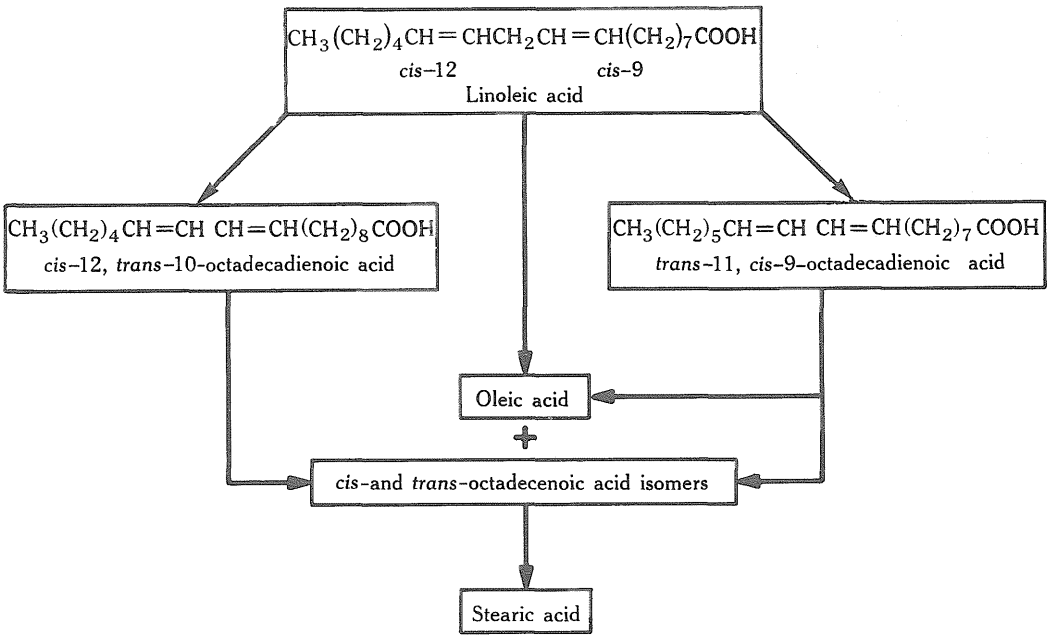

Fig. 2. Formation of conjugated double bond system during hydrogenation of linoleic acid.

fat content of the margarine was calculated from the weight of the recovered fat.

A small sample (10 mg) of each extracted fat was transesterified by the method of Glass and Christopherson (1969) and the resulting methyl esters were analysed by gas–liquid chromatography (g.l.c.). The 1975 samples were analysed by means of a Packard gas chromatograph (Model 7401) fitted with a flame ionization detector and a 180 cm by 0.2 cm I.D. U-shaped glass column containing 10% SP-222-PS on 100/120 support (Supelco, Bellefonte, Pa.) operated at 170 °C. The 1978 samples were analysed with a Pye chromatograph (Model 104) using a flame ionization detector and a 360 cm by 0.4 cm coiled glass column containing 10% Silar 10C on 100/120 Gas-Chrom Q (Applied Science Laboratories, State College, Pa.), operated at 175 °C. The highly polar Silar 10C column used for the 1978 series was able to effect partial resolution of *cis*- and *trans*-isomers, so that an estimate of *trans*-fatty acid content could be obtained by g.l.c. as well as by infrared analysis (see below).

Cis, cis, methylene-interrupted fatty acids were determined by the lipoxidase method, using the extracted fat and following the procedure suggested by the IUPAC Commission on Oils and Fats, Working Party 5 (1974). This assay is specific for the naturally-occurring polyunsaturated fatty acids, since only these possess the characteristic *cis, cis*, methylene-interrupted double bond systems; isomers with conjugated or isolated double bonds do not react with lipoxidase and are not estimated in this assay.

The percentage of fatty acids containing conjugated dienoic double bonds in each margarine was determined spectrophotometrically by measuring the absorption due to conjugated diene at 233 nm and calculating the result on the basis of a molar extinction coefficient (ε_{max}) of 28000 for conjugated dienes. *Trans*-unsaturation was determined by infrared spectroscopy of the methyl esters using the AOCS Tentative Method Cd 14-61 (American Oil Chemists' Society 1977). An estimate of *trans*-unsaturation for the samples in the 1978 series was also obtained by g.l.c. (see above).

Results and discussion

The results obtained for the analyses of the table margarines are shown in Tables 1, 2 and 3, the samples being identified not by brand-name but by code. (Brand names attached to the code initials may be obtained from the authors on written request and at the discretion of the authors.)

In 1975 (Table 1) two separate samples of

Table 1. Composition of polyunsaturated table margarines, 1975

Component	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
% fat	—	77.2	79.6	—	80.6	83.1	78.4	74.1	77.3
	82.6	81.4	81.0	82.8	84.5	81.5	84.1	79.8	80.1
P/S ratio[A]	—	3.6	3.1	—	2.2	2.3	2.4	2.4	2.2
	2.8	3.1	2.8	2.9	2.1	2.5	2.3	2.2	2.3
cis, *cis* methylene interrupted[B]	—	49.8	47.8	—	33.6	36.9	41.9	39.4	39.3
fatty acids (% fatty acids)	59.9	53.6	51.3	48.4	42.9	48.5	44.4	47.7	—
Polyunsaturated fatty acids	—	53.2	54.0	—	44.7	43.0	44.9	42.7	44.0
(% by g.l.c.)	58.8	52.2	51.8	47.8	43.7	47.0	43.6	45.8	46.4
Monounsaturated fatty acids	—	31.0	28.7	—	35.4	38.4	36.2	39.6	35.8
(% by g.l.c.)	20.1	31.1	29.3	35.5	35.2	33.8	37.4	33.1	33.6
Saturated fatty acids	—	15.8	17.3	—	19.9	18.6	18.9	17.6	20.2
(% by g.l.c.)	21.1	16.7	18.7	16.4	20.9	19.0	18.9	21.1	19.9
Polyunsaturated fatty acids	—	39.3	41.1	—	34.5	34.2	33.7	30.3	32.5
(g per 100 g product)	46.5	40.7	40.1	37.9	35.3	36.6	35.1	35.0	35.6
Conjugated dienes (% fatty acids)	0.61	0.41	0.75	0.57	0.53	0.32	0.34	0.50	0.66
trans-fatty acids % (i.r.)	—	10.0	11.6	—	—	10.7	12.6	16.9	14.3
	5.5	12.5	11.9	14.7	14.5	12.9	17.2	11.0	—

[A] P/S ratio values to be read as '3.6 to 1', '2.8 to 1' etc.

[B] Estimated by the lipoxidase method and expressed as % fatty acids (comparable with the g.l.c. figures).

most of the table margarines *A* to *I* were analysed. In 1978 the same brands *A* to *I* were again purchased and analysed (Table 2), together with a number of the tub margarines which have become available (*J* to *Q*, Table 3). One of these, *M*, was actually a cooking margarine, although this was not immediately apparent from the design of the label on the tub lid; it is included in Table 3 because non-discerning consumers would probably use it in the same way as a table margarine. All of the other tub margarines were clearly labelled as table margarines.

Comparison of the analyses of the margarines *A* to *I* for 1975 and for 1978 (Tables 1 and 2) shows no marked differences between the samples purchased in 1975 and those purchased in 1978.

It is clear from the data in the tables that tub margarines vary considerably in composition. The *trans*-fatty acid contents range from nil to 18.2% but the values are generally lower than those obtained for polyunsaturated margarines analysed in Brisbane in 1973–74 (Parodi 1976). The values obtained for *cis*, *cis*, methylene-interrupted polyunsaturated fatty acids by the lipoxidase method are fairly close to the values for polyunsaturated fatty acids obtained by g.l.c. Since the latter values include fatty acids with conjugated and isolated double bond systems as well as those with methylene-interrupted double bond systems, this suggests that the amounts of polyunsaturated fatty acids with such conjugated or isolated double bonds are fairly small. This is partly corroborated by the low values (less than 1% of fatty acids) obtained for conjugated dienes. The highest conjugated diene values were found in samples *L*, *M* and *Q*. The labels on samples *L* and *Q*, indicated that they contained some animal fat, and presumably *M* (the cooking margarine) did also. The animal fat in these margarines is probably beef or mutton tallow containing about 1% conjugated diene (Parodi 1976), which may account for these samples having somewhat higher conjugated diene values than the other vegetable oil-derived margarines.

The low levels of conjugated diene fatty acids in the margarines indicate that *trans*-unsaturation, where present, is largely due to monounsaturated fatty acids with *trans*-bonds, rather than to *cis*-, *trans*-conjugated dienes. This was confirmed by g.l.c. which showed that *trans*-octadecenoic acid isomers contributed most of the *trans*-fatty acid content, with *trans*-hexadecenoic acids making a small contribution. Parodi (1976) and Heckers and Melcher (1978) also concluded that *trans*-octadecenoic acids were the main *trans*-fatty acids in margarines.

The amount of polyunsaturated fatty acids ranged from 8.2 g per 100 g product to 47.6 g per 100 g product (based on g.l.c. values).

Table 2. Composition of table margarines, 1978

Component	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
% fat	81.2	78.3	80.9	76.4	79.7	77.0	76.7	80.7	81.0
P/S ratio[A] – claimed	>3.0	n.s[D]	n.s	>3.0	>2.0	>2.0	>2.0	n.s	>2.0
– found	3.4	2.6	1.9	3.2	2.2	2.4	2.6	2.1	2.6
cis, *cis* methylene interrupted[B] fatty acids (% fatty acids)	60.4	49.0	44.9	48.0	42.2	46.4	45.8	43.2	43.4
Polyunsaturated fatty acids (% by g.l.c.)	61.3	49.4	43.1	54.0	44.5	47.9	47.0	42.5	46.8
Monounsaturated fatty acids (% by g.l.c.)	20.5	31.7	34.3	29.1	34.9	32.0	35.1	37.6	35.5
Saturated fatty acids (% by g.l.c.)	18.1	18.9	22.6	16.9	20.6	20.1	17.9	19.9	17.6
Polyunsaturated fatty acids (g per 100 g product)	47.6	37.0	33.4	39.5	33.9	35.3	34.5	32.8	36.3
Conjugated dienes (% fatty acids)	0.54	0.29	0.33	0.59	0.36	0.32	0.37	0.40	0.47
trans-fatty acids – (% by i.r.)	3.8	11.9	14.1	10.8	13.6	9.2	18.2	11.9	17.0
– (% by g.l.c.[C])	6.2	12.4	14.5	11.1	15.0	11.9	18.5	13.4	15.5

[A] P/S ratio values to be read as '3.0 to 1' etc.

[B] Estimated by the lipoxidase method and expressed as % fatty acids (comparable with the g.l.c. figures).

[C] % *trans*-fatty acids by g.l.c. was calculated by summing 16:1*t*, 18:1*t*, 18:2*tt*, plus 18:2*ct*(*tc*).

[D] n.s.: not stated on label.

Table 3. Composition of table margarines, 1978

Component	*J*	*K*	*L*	*M*	*N*	*O*	*P*	*Q*
% fat	78.2	81.0	80.9	76.2	81.8	78.0	76.7	77.1
P/S ratio[A] – claimed	n.s[D]	n.s.	n.s.	n.s.	n.s.	>2.0	n.s.	n.s.
– found	1.0	1.0	0.4	0.3	1.0	2.4	1.0	0.3
cis, *cis* methylene interrupted[B] fatty acids (% fatty acids)	35.0	33.8	14.3	10.5	35.2	43.5	38.2	9.6
Polyunsaturated fatty acids (% by g.l.c.)	36.3	34.5	16.3	11.8	35.4	46.7	33.3	11.1
Monounsaturated fatty acids (% by g.l.c.)	26.5	29.4	43.8	45.9	30.8	33.5	32.7	45.6
Saturated fatty acids (% by g.l.c.)	37.2	36.1	39.7	42.3	33.8	19.8	34.0	43.3
Polyunsaturated fatty acids (g per 100 g product)	27.2	26.7	12.6	8.6	27.7	34.8	24.4	8.2
Conjugated dienes (% fatty acids)	0.54	0.33	0.79	0.90	0.46	0.28	0.41	0.94
trans-fatty acids – (% by i.r.)	6.0	nil	4.9	3.9	10.8	13.8	1.4	2.6
– (% by g.l.c.[C])	6.7	nil	2.9	3.1	12.3	14.8	0.6	3.6

[A] P/S ratio values to be read as '1.0 to 1', '2.4 to 1', etc.

[B] Estimated by the lipoxidase method and expressed as % fatty acids (comparable with the g.l.c. figures).

[C] % *trans*-fatty acids by g.l.c. was calculated by summing 16:1*t*, 18:1*t*, 18:2*tt*, plus 18:2*ct*(*tc*).

[D] n.s.: not stated on label.

One product, *A*, had a polyunsaturated fatty acid content of more than 40 g per 100 g product coupled with a relatively low level of *trans*-fatty acids (less than 5% by i.r.), which indicated that isomerization due to processing has been minimized. Another product, *K*, had no *trans*-fatty acid content at all, but a lower polyunsaturated fat content than *A*.

In view of the wide range of compositions found in the table margarines it is not possible to make any dietary recommendations for their use. Most dietitians would be mainly concerned with

the amount of polyunsaturated fat available from each brand, and several of those listed provide more than 35 g polyunsaturated fat per 100 g of product. The level of *trans*-fatty acids in these products does not appear to be of dietary significance in view of the findings of FAO/WHO (1977). The competition between the manufacturers of margarines to promote their products has placed the consumer in the fortunate position of having a wide variety of table margarines to choose from at reasonable prices, and the technological sophistication of the manufacturing processes provides assurance of a high quality product.

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IIR Publications

The International Institute of Refrigeration has published three further volumes of interest to food scientists and technologists in the series *Refrigeration Science and Technology*.

'Freezing, Frozen Storage, Freeze-drying', 1977, 493 pp., hard cover, 80 French francs. This volume contains 47 papers in English and 6 in French providing up-to-date reports on world-wide research in the following subject areas: basic phenomena of freezing biological materials; physico-chemical phenomena of the freezing process; effects of the freezing process and frozen storage on animal tissue and blood; effects of the freezing process and frozen storage on vegetable materials and prepared foods; mathematical treatment of the freezing and thawing processes; industrial freezing of foods and distribution of quick frozen foods; and freeze-drying.

'Container Ships: Reliability and Automation of Shipboard Refrigerating Systems', 1977, 151 pp., hard cover, 30 French francs. Modern developments in refrigerated sea transport are covered in 13 papers and panel discussions in English, presented under four main topics: insulation and refrigeration for container ships; reliability of shipboard refrigerating systems; temperature monitoring and control; and container transport and ship design.

'Table Grapes and Refrigeration', 1977, 248 pp., hard cover, 40 French francs. The refrigerated storage of grapes, one of the most fragile foodstuffs to maintain in good condition from harvest to consumption, is covered in this collection of 28 papers, 21 in French and 7 in English, ranging in subject from product characteristics and pre-harvest processes to packaging techniques, precooling, storage and transport.

These volumes are available post-free if remittance with order, from Institut International du Froid – Editeur – 177 bd Malesherbes, 75017 – Paris.

Treatment of food industry effluents by trickling filtration

By S. P. Moodie

CSIRO Division of Food Research, Cannon Hill, Qld.

Introduction

Although food industry effluents rarely contain toxic materials they can cause pollution problems. Abattoir effluents usually have a biochemical oxygen demand (BOD) of about 2000 mg l^{-1}, and waste whey from dairy processing has a BOD of approximately 40 000 mg l^{-1}. In comparison, the BOD of domestic sewage is usually between 200 and 300 mg l^{-1}.

Water pollution in most states is controlled by strict legislation, and although the regulations have not always been stringently enforced, community pressure on industry to minimize water pollution is increasing.

In metropolitan areas, untreated effluent is often discharged into municipal sewers. However, the cost of treating effluent in sewage treatment plants is increasing and pre-treatment of effluent before discharge is being encouraged. One method for pre-treatment is trickling filtration, and this method has been used successfully for the treatment of wastes from abattoirs, dairy processing and fruit and vegetable processing.

Description of the trickling filtration process

After all the gross solids and fats or oils are removed, the wastewater trickles through inert packing material contained within a tower. A wide variety of microorganisms grows on the packing, and forms a slime layer. The microorganisms consume the organic matter in the wastewater, reducing the organic content (and therefore the BOD) of the wastewater. Oxygen required by the microorganisms is supplied by natural convection of air through the packing, and diffusion of oxygen through the liquid and biological films. The word 'filter' is a misnomer, as there is no filtering or straining mechanism. A better name would be 'fixed-film biological reactor'.

The microorganisms in the slime layer are in a continual state of growth and decay, and parts of the slime layer are washed off or sloughed off the packing periodically. A clarification step, to settle out the solid material, is therefore a necessary part of the process.

Advantages and disadvantages of trickling filters

In recent years, fabricated plastic packings for trickling filters have become more popular than conventional stone packing because they are light and have a high surface area per unit volume of packing. Some of the advantages claimed for plastic media trickling filters are:

- low maintenance — pumps and distributors are the only moving parts, and pumps may not be necessary if gravity feed is possible. Reaction-driven rotary distributors are commonly used and are generally reliable
- low operating costs — trickling filters usually need little attention and labour costs are small. The only other operating cost is for pumping
- large areas of land are not required — because plastic packing is light and strong, trickling filters can have structures with packing depths of up to 10 m. Requirements for space are therefore small
- suitable for treatment of wastes with high BOD — at a given flow rate, the percentage removal of BOD is constant over a wide range of influent BOD. Thus the greater the BOD of the waste to be treated, the greater the absolute removal of BOD. Trickling filters can cope with the rapid and wide fluctuations in BOD typical of food processing effluents, with only slight decreases in efficiency.

Plastic media filters have a number of disadvantages. These are:

- low efficiency — unlike alternative

processes, such as activated sludge and extended aeration, the reduction in the BOD of the effluent is comparatively small

- cost – in some instances the capital cost can be high, but this may be largely offset by low operating costs.
- a uniform flow rate is required – trickling filters operate most efficiently at uniform flow rates. If the packing is not kept wet at all times, the microorganisms will start to die. A system of flow equalization ahead of the filter or a provision for recycling of filter effluent is necessary if the flow rate of liquid over the packing is to be maintained in times of low influent flow.

For a combination of technical and economical reasons, plastic media filters are best suited for 'roughing', or partial treatment. 'Roughing' filters are designed to remove only part of the influent BOD, usually between 40% and 75%, before further treatment by processes such as activated sludge, or before discharge to municipal sewers.

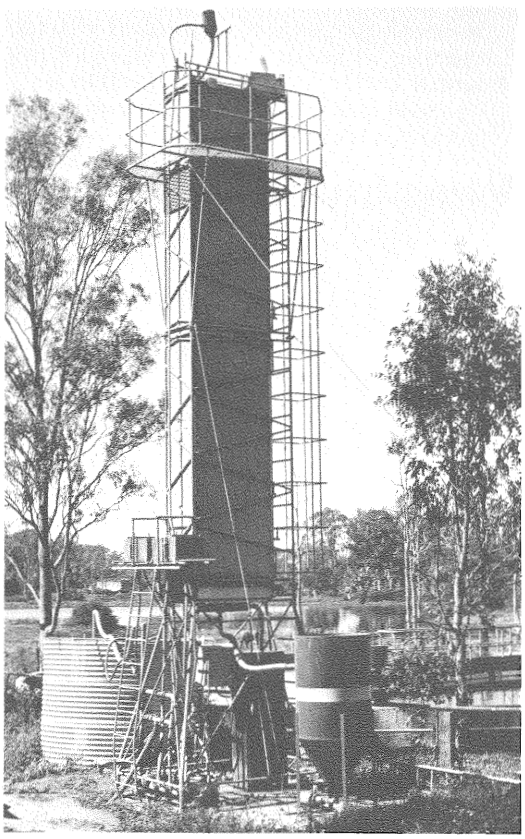
Difficulties in design and theory

Although the process of trickling filtration is more than 80 years old, it is not yet well understood, and there is considerable disagreement about the effects of a number of important design variables. Even design methods based on empirical studies with domestic sewage, the most widely studied system, give markedly different predictions. For a given set of design conditions, Baker and Graves (1968) showed that three of the most popular design equations predicted volumes of packing that varied by as much as 13 to 1.

The microbiology of the process is complicated. Food industry wastewaters can contain sugars, starches, fats, proteins and fatty acids in varying amounts, and also show significant diurnal and seasonal variations. There are also many different environments within the filter. At the bottom, for example, the substrate concentrations and temperatures are usually lower, the pH is more neutral, and dissolved oxygen concentration higher than at the top of the filter. The diversity of available substrates and environments is responsible for the diversity of microbial populations found within trickling filters, and it is often suggested that this is the main reason for their ability to withstand shock loads. The main microorganisms found in trickling filters are gram-negative, motile, capsulated rods, but considerable populations of algae (at the top of the filter), fungi and protozoa are also present. Macrofauna, such as flies and worms, are also commonly found in trickling filters, and play a part in the process.

Owing to this complexity, the kinetics of microbial growth and substrate removal are not well established. Monod kinetics appear to apply reasonably closely to laboratory-scale filters using simple substrates such as glucose, but not so well to large filters using real wastes.

The most commonly proposed mechanism for the trickling filter process is the biological oxidation of soluble material by bacterial cells in the slime layer on the filter packing. However, many wastewaters contain a significant proportion of suspended and colloidal matter. For example, about half the



A trickling filter tower installed at the Metropolitan Public Abattoir Board Works, Brisbane.

BOD of abattoir effluent is due to insoluble matter. The percentage of insoluble matter that is removed is usually of the same order as that of soluble matter, but no theoretical model for trickling filtration includes any consideration of the removal mechanisms for insoluble matter. The models ignore the effects of the settling stage that usually follows trickling filtration. One possible mechanism for the removal of insoluble matter is by flocculation during passage through the filter. Possible agents for flocculation are exocellular polymers secreted during the endogenous phase of bacterial growth. Pavoni *et al.* (1972) have shown the existence of such polymers in activated sludge systems.

Conclusions

Plastic media trickling filters are particularly suited to the partial treatment of food industry effluents. Typical applications are for the treatment of effluent before discharge into the sewer, or to relieve overloading on existing wastewater treatment plants, especially where available land is limited

Many complicated theoretical models have been developed for trickling filtration, but their application to the design and characterization of full scale systems has been limited. One reason for this is that trickling filter systems are so complex. Any model has to be simplified, and unrealistic assumptions have to be made. There is a need for more work to be done on full and pilot scale trickling filters so that theoretical models can be based on firm empirical evidence. Useful data on trickling filter design would also be obtained from such work

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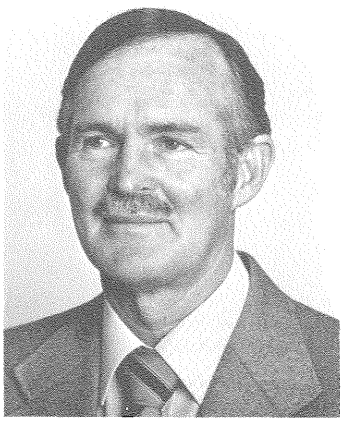
News from the Division

Book published

Mr J. D. Mellor's book 'Fundamentals of Freeze-drying' has been published by Academic Press, London. The book is divided into three sections. The first deals with the classical theory of ice sublimation; the second with cyclic-pressure operation, a freeze-drying process newly developed by the author which gives up to 50% reduction in the long drying runs normally encountered; and the third with applications including the design of equipment and laboratory apparatus for foods, vaccines and microorganisms, histological specimens and other novel uses.



Appointments

Miss Judy T. McAvoy, B.Sc. (Biochem.) has been appointed as an Experimental Officer in the Microbiology Section at MRL, to assist in studies on *Microbacterium thermosphactum*, and Miss Heather C. Morton, B.Sc. (Biochem.) has been appointed to a similar position in MRL's Biochemistry Section, to work on post-mortem muscle metabolism. Both appointments were made

in February and are for a duration of two and three years respectively.

Also in February, Dr A. J. G. Pirie of the Riverina C.A.E., joined FRL's Plant Physiology Group for one year as a Research Fellow. The Fellowship is supported by the Rural Credit Development Fund of the Reserve Bank. Dr Pirie is working on pH problems in wine grapes.

Visiting workers

The Food Structure Group was host to Dr P. J. Quinn of the University of London, for a period of 12 weeks. Dr Quinn who has particular interests in the structure and function of biological membranes was involved in characterizing the properties of the lipid molecules, when dispersed or aligned in aqueous systems, using n.m.r. spectrometry.

Professor P. Gerhardt, Michigan State University, East Lansing, U.S.A. spent three weeks from mid-March in FRL's Food Safety and Nutritional Quality Group. He gave talks at Hawkesbury Agricultural College and at a joint meeting of AIFST and the Australian Society for Microbiology, as well as participating in an informal workshop/seminar on 'Resistance mechanisms of bacterial spores'. This meeting was held at FRL on 29–30 March under the U.S.–Australia Cooperative Science Program, of which Professor Gerhardt is the U.S. Coordinator.

Associate Professor E. C. Tigchelaar, Department of Horticulture Purdue University, West Lafayette, Indiana, U.S.A., returned to FRL for two weeks in February 1979 to continue a collaborative program, with Dr W. B. McGlasson, on processing tomato variety improvement and on Fl hybrids of *nor* tomatoes.

Dr Hans-Rolf Schulten of the Institute of Physical Chemistry, University of Bonn, West Germany, spent four weeks at FRL on the application of field desorption mass spectrometry to problems in food chemistry.

Mr R. Jansen, a Colombo Plan Fellow from Sri Lanka, spent some time with the Industry Section at MRL studying hide preservation techniques.

Government visitors

On 29 March 1979 Dr Bacharaddin Habibie, Indonesian Minister for Research and Technology, accompanied by members of his staff, visited the CSIRO Food Research Laboratory, North Ryde. He is shown observing extrusion of a textured soy bean protein food.

Left to right: Dr A. R. Johnson, Officer-in-Charge, Food Research Laboratory, Dr Harsono D. Puspongoro, Assistant to the Minister, Mr J. F. Kefford, Assistant Chief, Division of Food Research, Dr Habibie, Mr Burhan Napitupulu, Mr Graham Warden, CSIRO Head Office, Mr J. H. Last, Food Research Laboratory.

An eight-member Food Industry Survey Mission from China visited the Food Research Laboratory on 10 and 11 April 1979. The leader of the Mission was Mr Yin Zong-Lun, Vice-President of the Food Research Institute, Peking; he was accompanied by engineers and technologists from other institutes and the canning industry. The Mission showed particular interest in the flame sterilizer for canned foods demonstrated by Dr D. J. Casimir.

Award

Professor M. R. J. Salton who was formerly a microbiologist in the Division's laboratories at Homebush has been elected a Fellow of the Royal Society, for his fundamental research in microbiology. He was the first professor of Microbiology at the University of N.S.W. and is now Chairman of the Department of Microbiology at the New York University Medical School.