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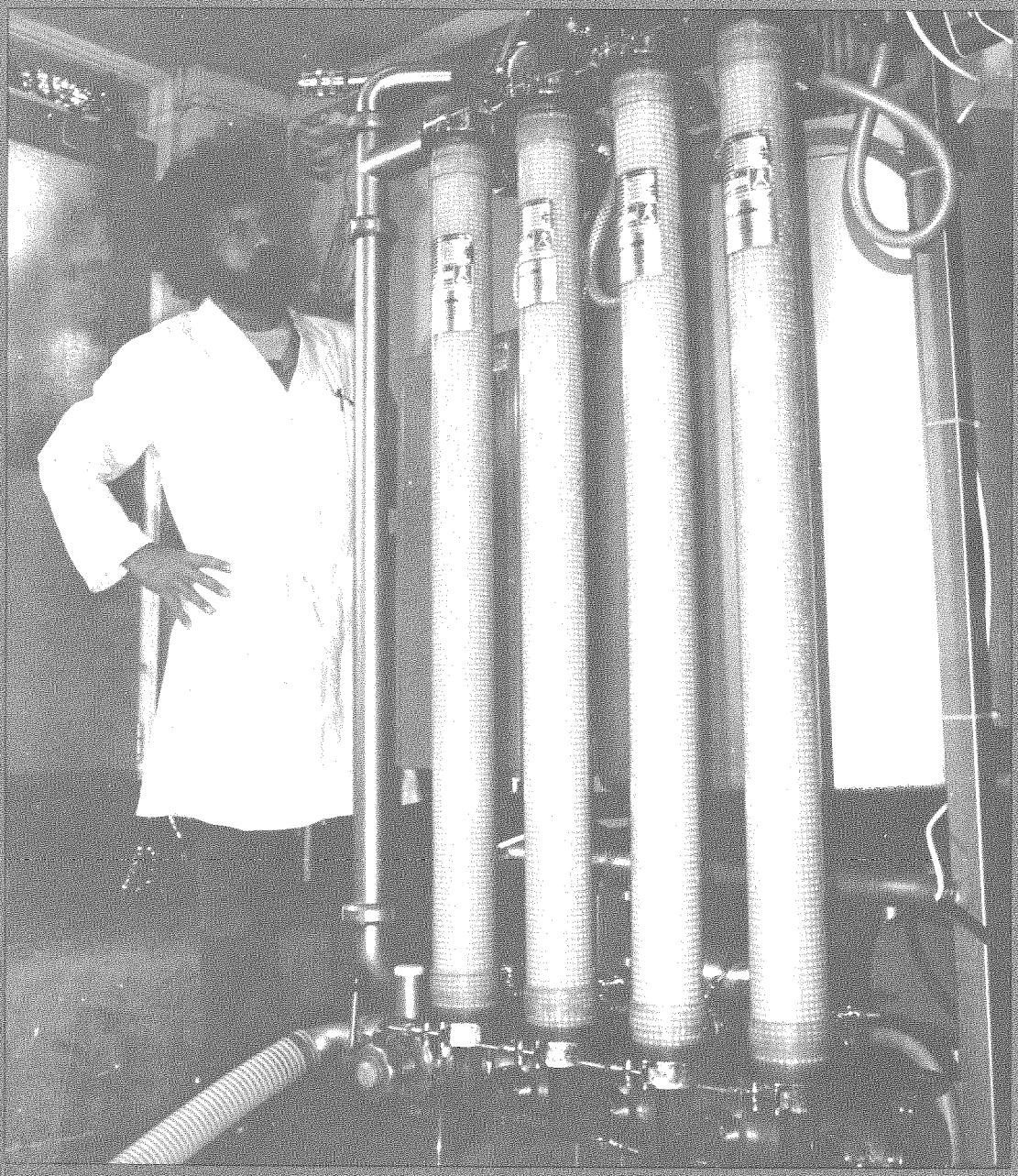

Food spoilage fungi. I. *Xeromyces bisporus* Fraser

By J. I. Pitt and Ailsa D. Hocking

CSIRO Division of Food Research, North Ryde, N.S.W. 2113

The spoilage mould *Xeromyces bisporus* Fraser is able to grow at a lower water activity than any other known organism. As a consequence, it is capable of growth in dried and concentrated foods usually regarded as safe from microbial attack, and has been the cause of significant spoilage of such products. Cultural and microscopic features of this unique fungus are described in this paper, together with its physiological properties and techniques for its isolation and maintenance.

Introduction

Xeromyces bisporus Fraser was originally isolated from mouldy Australian licorice by W. J. Scott in this laboratory, and was described by Fraser (1953). It was later found to be a major cause of spoilage in Australian dried and high moisture prunes (Pitt and Christian 1968), and as an uncommon but widespread spoilage agent in England, where Dallyn and Everton (1969) isolated it from table jellies, dried prunes, tobacco, currants and chocolate sauce. In the past few years, we have isolated *X. bisporus* from spice powders, imported Chinese dates, fruit cakes and its first-known habitat, licorice.

Spoilage losses of Chinese dates and fruit cakes were high. The dates had been packed in 10-kg cartons, and were extensively damaged by mould growth (Fig. 1*a*). At the time of our examination, the water activity (a_w) of the dates averaged 0.72, with little point to point variation within the cartons, indicating a stable a_w since packing. In the absence of this particular fungus, Chinese dates at 0.72 a_w would have been safe from microbial attack for at least 12 months.

The fruit cakes (Fig. 1*b*) were manufactured locally to a standard recipe which over a 10-year-period had provided trouble-free storage. The a_w of the product was 0.75, a figure considered to ensure a shelf life of six months. In this outbreak, spoilage often occurred within two months, again

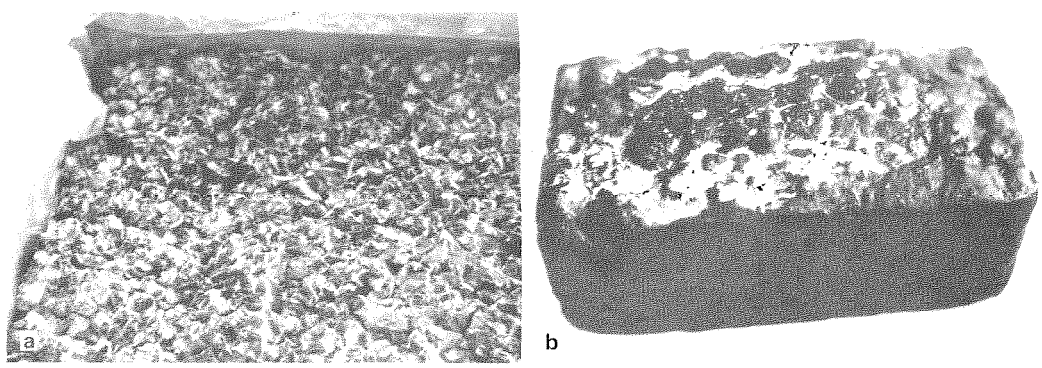

Fig. 1. Spoilage of foods by *Xeromyces bisporus*: (*a*) Chinese dates, packed for several months, with mould growth extending throughout the carton; (*b*) fruit cake, two months old, with mycelial penetration of 2 cm or more in places.

emphasizing that *X. bisporus* is capable of destructive growth at what otherwise would be safe water contents.

Isolation techniques

Because *X. bisporus* will not grow above 0.97 a_w, isolation on standard laboratory media is impossible. Moreover, unless mature ascospores are present, isolation by dilution plating will be unsuccessful because commonly used diluents such as peptone water will damage hyphae by osmotic shock. Dilution plating with low a_w diluents such as 50% glucose is possible but impractical because of their high viscosity. It is important therefore to attempt isolation of *X. bisporus* by direct plating.

Provided this species is present in effectively pure culture (as is often the case when it is growing on substrates of low a_w), the most suitable isolation medium is malt extract yeast extract 50% glucose agar (MY50G; see formula below). Inoculate this medium with small masses of mycelium or small pieces of substrate cut off with a sterile blade. Sprinkle small amounts of powdered substrates directly on the agar. Enclose Petri dishes in a polyethylene bag to stabilize the a_w of the medium.

Isolation of *X. bisporus* is further complicated by the fact that it will not compete against the vigorous growth of *Eurotium* species (members of the *Aspergillus glaucus* series) at a_w levels above about 0.75. In the presence of such fungi, identifiable by grey-green or yellow growth and the presence of *Aspergillus* heads, the most satisfactory isolation medium for *X. bisporus* is malt extract yeast extract 70% glucose fructose agar (MY70GF; see formula below), which is near 0.75 a_w. To prevent drying of this medium, Petri dishes should be incubated in the presence of a saturated solution of NaCl, in a sealed container such as a desiccator or plastic food box. Again, inoculation should be by direct plating.

Incubation should be at 25° to 30°C: on MY50G agar, good growth should occur within a week to 10 days; on MY70GF, incubation times of three weeks or more are necessary. On both media, *Xeromyces* colonies are low and spreading: transfer peripheral hyphae to MY50G slants and again incubate at 25° to 30°C. Colonies of *Xeromyces* will remain low and white or translucent. Identity is confirmed by development of the characteristic cleistothecia (see description below.)

Media

MY50G agar has the following composition: malt extract, 10 g; yeast extract, 2.5 g; glucose, 500 g; agar, 10 g; water, 500 g: sterilize by steaming for 30 min. Final medium is about pH 5.5 and 0.89 a_w. If glucose monohydrate is used, add 550 g and reduce water to 450 g.

MY70GF agar has the following composition: malt extract, 6 g; yeast extract, 1.5 g; glucose, 350 g; fructose, 350 g; agar, 6 g; water, 300 g: sterilize by steaming for 30 min. Final medium is about pH 5.5 and 0.75 a_w. If glucose monohydrate is used, add 385 g and reduce water to 265 g.

In our experience, powdered light malt extract sold for home brewing is entirely satisfactory for the preparation of media such as these. Food grade glucose monohydrate may be substituted for anhydrous glucose, provided that allowance is made for the water of hydration (see formulae). In the preparation of such concentrated media, it is desirable to melt the agar before adding the sugars, because in lowering a_w, they also reduce agar solubility.

Maintenance

The most satisfactory medium for the

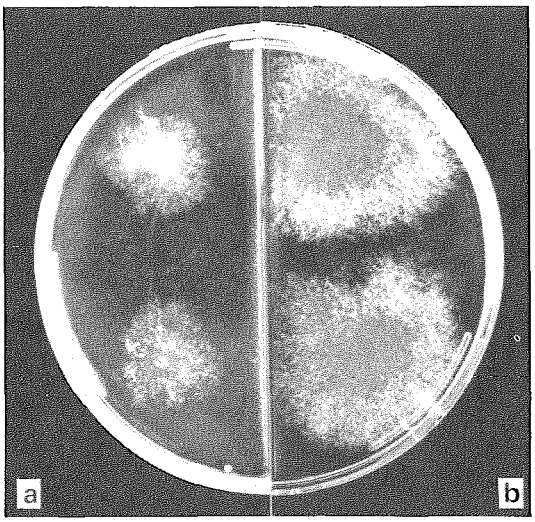

Fig. 2. Colonies of *Xeromyces bisporus* on MY50G agar at 25°C (*a*) after two weeks; (*b*) after four weeks incubation.

maintenance of *Xeromyces* is MY50G agar. Allow 4–6 weeks' incubation at 25° to 30°C for ascospores to mature, and check for their presence by microscopy before storing cultures, which may be held at 5° to 25°C for several months.

Subculturing of *Xeromyces* isolates over long periods may cause delayed or diminished ascospore production. The most satisfactory method for long-term maintenance is lyophilization, provided adequate numbers of mature ascospores are present.
An alternative method is to grow *Xeromyces* on commercially prepared fruit cake, which is commonly about 0.75 a_w. Place slices of cake in Petri dishes, enclose in foil or an oven-proof plastic bag, sterilize by steaming for 30 min, and then inoculate the upper surface of the cake with *Xeromyces*. Wrap the Petri dishes in polyethylene and store them at about 25°C. Cultures will be ready for use in about two months, and will provide inocula of viable ascospores for a long time.

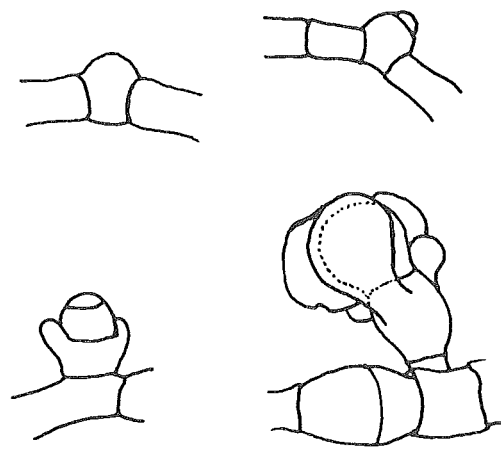

Fig. 4. Stages in the development of cleistothecial initials of *Xeromyces bisporus*.

Description

After incubation for seven days on MY50G agar at 25°C, colonies are 3–6 mm in diameter, low and sparse. After 14 days, colonies are 15–20 mm in diameter, with a low and dense texture, translucent with a glistening surface, and colourless or very pale red brown; the reverse is uncoloured (Fig. 2*a*). After four weeks' incubation, colonies are 50–70+ mm in diameter, low, translucent and sometimes glistening, colourless or faintly red brown, with contiguous layers of colourless cleistothecia visible under the low power microscope; the reverse remains uncoloured (Fig. 2*b*).

Reproductive structures are cleistothecia and aleurioconidia, the latter solitary, developing only below 0.9 a_w, and usually measuring 15–20 x 12–15 μm (Fig. 3*a*). Cleistothecial initials are evident on MY50G agar at two weeks. They commence as three short cells, then develop finger-like processes from the bottom cell, which envelop the second and enlarge to form the cleistothecium (Fig. 4). Mature cleistothecia, often outnumbered by abortive ones, form within 4–6 weeks on MY50G agar. They measure 40–120 μm in diameter, and have thin, structureless walls (Figs 3*b*, *c*). Asci are inconspicuous and evanescent, and contain two ascospores only; ascospores are ellipsoidal, flattened on one side ('D'-shaped), measure 10–12 x 4–5 μm, and have smooth walls (Fig. 3*d*).

Water relations

Pitt and Christian (1968) reported germination of *X. bisporus* at 0.61 a_w after 120 days' incubation; aleurioconidia were produced in 80 days at 0.66 a_w and ascospores at 0.67 a_w within a similar time interval. The ability to complete a sexual life cycle below 0.7 a_w is a remarkable attribute, shared only with the xerophilic yeast *Saccharomyces rouxii*.

The effect of a_w and solute on the germination of *X. bisporus* is shown in Fig. 5 (Pitt and Hocking 1977). Ascospores of *X. bisporus* germinate remarkably slowly, taking at least three days even under optimal conditions such as a glucose/fructose medium at 0.9 a_w. More remarkable is the fact that germination on the same substrate at 0.75 a_w requires less than twice this time. This plateau in the germination curve for *X. bisporus* over the range 0.95 to 0.75 a_w is unique among microorganisms.

As illustrated in Fig. 6, *X. bisporus* grows much more rapidly in media containing high concentrations of glucose or fructose than of glycerol or NaCl at the same a_w. Pitt and Hocking (1977) reported that this species failed to germinate in NaCl-based media of pH 6.5 at any a_w. It is thus to be expected that spoilage by *X. bisporus* will occur most often in products containing sugars as the major solute.

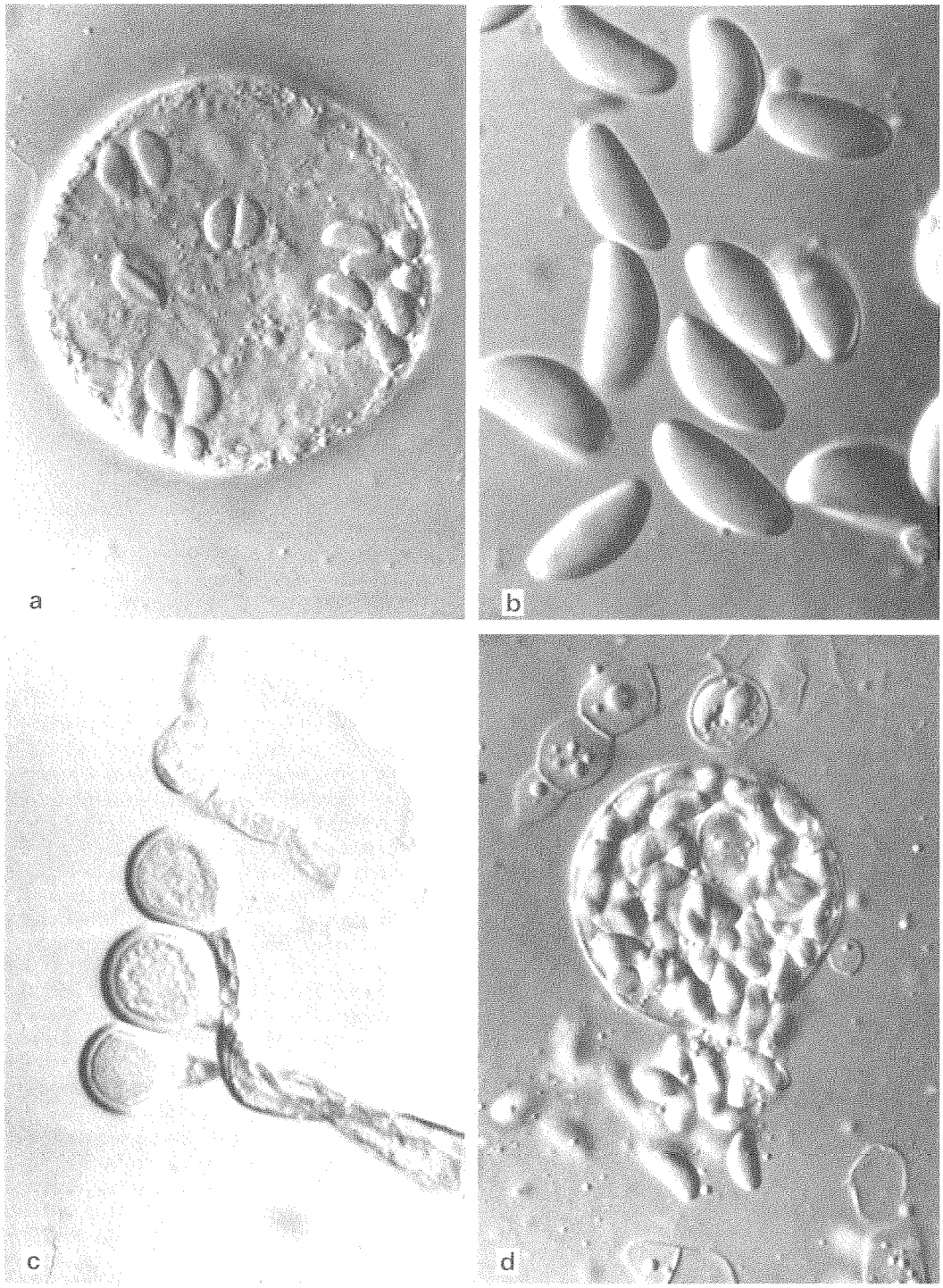

Fig. 3. Reproductive structures, as seen in wet mounts by interference contrast light microscopy: (*a*) aleurioconidia, x750; (*b*) developing cleistothecium showing ascospores borne in pairs, x750; (*c*) mature cleistothecium discharging ascospores, x750; (*d*) ascospores, x 1875.

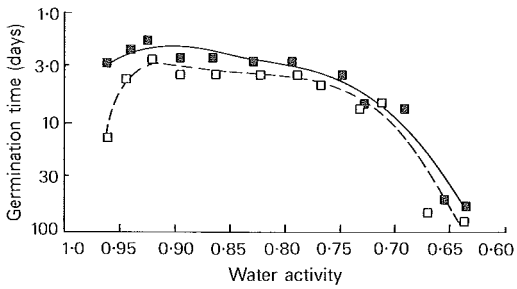

Fig. 5. Effect of a_W on the time taken (t) for germination of spores of *Xeromyces bisporus*. ■, glucose/fructose, pH 4.0; □, glucose/fructose, pH 6.5.

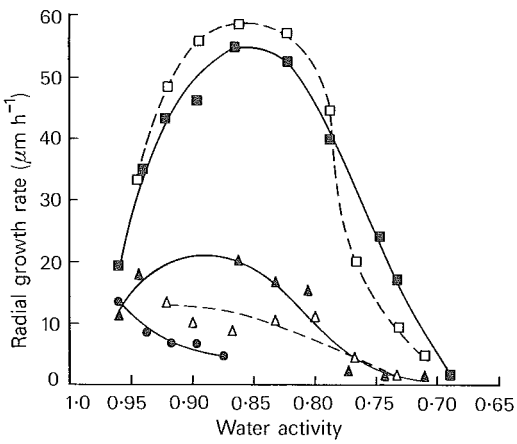

Fig. 6. Effect of a_W and solute on the radial growth rate of *Xeromyces bisporus*. ■, glucose/fructose, pH 4.0; □, glucose/fructose, pH 6.5; ▲, glycerol, pH 4.0; △, glycerol, pH 6.5; ●, NaCl, pH 4.0.

Heat resistance

Dallyn and Everton (1969) and Pitt and Christian (1970) independently studied the heat resistance of *X. bisporus* ascospores, with comparable results. Pitt and Christian (1970) reported 0.1% survivors when 1000 ascospores were heated at 80 °C for 10 min; Dallyn and Everton (1969) reported that when 2000 ascospores per ml were heated at 80 °C, some survived after 9 min but none after 12 min.

By assuming survival of one spore per ml at the mean of the survival and death times given by Dallyn and Everton (1969; Table 1), decimal reduction times (DRT) can be estimated graphically, and these are plotted in Fig. 7. Points at temperatures above 75 °C can be connected by a straight line which, under the test conditions of Dallyn and Everton (1969), is defined by a z value of 16.0 C° (= 28.8 F°) and a DRT at 82.2 °C (F_{180}) of 2.3 min (Fig. 7). Their test conditions (50% sucrose medium, 0.94 a_w) would be similar to a fruit cake mix, and the reduction in a_w as the cake dries during baking would confer increased heat resistance on the ascospores. From these data, the heat resistance of *X. bisporus* is sufficiently high to account for its presence in fruit cake as being due to survival of ascospores during baking.

Influence of oxygen and carbon dioxide tension

Dallyn and Everton (1969) showed that *Xeromyces* was capable of growth in an atmosphere containing only 1% oxygen, even in the presence of 70–95% carbon dioxide, so vacuum- or gas-packing of foods may not completely prevent growth of this mould.

Ecology

The natural habitat of *Xeromyces* is not known. It has never been isolated from soil or decaying vegetation, and its water relations would appear to preclude it from such habitats. Indeed, its physiological properties

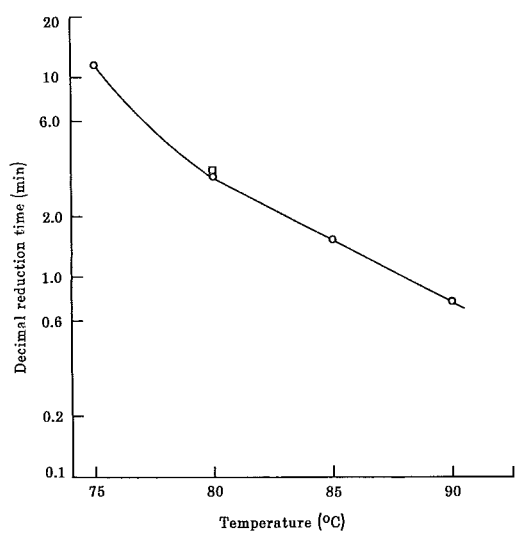

Fig. 7. Relationship of decimal reduction time and temperature for *Xeromyces bisporus*. ○, data of Dallyn and Everton (1969); □, data of Pitt and Christian (1970).

indicate that *Xeromyces* could grow in nature only on sugary materials such as drying, or dry, fruits and berries, honey or sugary exudates. All records to date have come from foods or dried tobacco, or food-processing equipment.

Prevention of spoilage by Xeromyces

From ecological considerations and isolation data, the most common sources of *Xeromyces* infections in foods or food plants appear likely to be dried fruits or spices. It is fortunate that such infections are rare: monitoring for *Xeromyces* is ineffective both because of its slow growth rate and because of its inability to compete with *Eurotium* species even on media specifically designed for isolation of xerophiles, such as DG18 (Hocking and Pitt 1980).

If a processing or packaging plant does become infected with *Xeromyces*, normal cleaning and sanitation procedures will usually be effective in removing it, as ascospores and aleurioconidia are easily wetted. For the same reason, the spores are apparently not aerially dispersed and recontamination is unlikely.

Summary

X. bisporus is a seldom recognized but very significant cause of food spoilage, capable of destructive growth at exceptionally low levels of a_w and oxygen tension. Its ascospores possess a high heat resistance, probably sufficient to withstand baking processes. Isolation and identification of *Xeromyces* are not difficult once it is realized that standard laboratory media and isolation techniques are completely ineffective.

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Treating food wastes for profit. Physicochemical separations.

Ultrafiltration and reverse osmosis*

By. K. R. Marshall

New Zealand Dairy Research Institute, Palmerston North, N.Z.

Ultrafiltration and reverse osmosis are membrane separation processes driven by a pressure gradient. Whilst many laboratory and pilot-scale studies of the treatment of food wastes by membrane processes have been published, only the dairy industry has made extensive commercial use of these unit operations. Ultrafiltration is being used to recover from whey a high-value protein material with unique properties. Reverse osmosis is being used to concentrate whey before transport and to increase the capacity of evaporators. In other industries, commercial uses include the treatment of blood and sugar and fermentation wastes. Many food industries could use membrane processes to recover waste materials, but high investments in research and equipment are required.

Introduction

Ultrafiltration and reverse osmosis are relatively new fractionation processes that could prove commercially attractive for treating materials previously considered to be waste products from the food industry (Goodall 1972). The dairy industry, in particular, has made considerable progress in the use of these techniques over the last decade (Glover *et al.* 1978).

This paper reviews the theory of the two unit operations, outlines the range of commercial equipment available, summarizes the food waste materials that have been treated either commercially or experimentally and considers some relevant aspects of membrane processing, including the pretreatment of raw materials, the effect of membrane processing on the polluting strength of the waste, and the costs of membrane processes. Much of the material presented relates to wastes from the dairy industry, but the principles should be applicable to other food wastes.

*Abbreviated version of a paper presented at the Australian Academy of Technological Sciences workshop on 'Treating food wastes for profit' held in Melbourne in October 1980.

Ultrafiltration and reverse osmosis

The basic principles of reverse osmosis (also known as hyperfiltration) and ultrafiltration have been well documented (e.g. Sourirajan 1970; Lonsdale 1972; Sourirajan 1977). Reverse osmosis and ultrafiltration are general physico-chemical separation techniques in which a pressurized solution flows over a porous membrane. The membrane allows the passage of only relatively small molecules. Thus the solvent, sometimes together with low molecular weight solutes, flows through the membrane and is withdrawn as permeate. The remaining material, known as the retentate, is relatively more concentrated in one or more components compared with the feed material.

In theory, at least, there is little to distinguish between ultrafiltration and reverse osmosis. Sourirajan (1977) considers

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ultrafiltration membranes to be simply reverse osmosis membranes with relatively large average size pores in the membrane surface. In practice, however, the separation achieved, and the membranes and operating conditions used, are usually quite different for the two unit operations. Reverse osmosis membranes are generally considered to be those that will restrict the passage of low molecular weight solutes, e.g. salts like sodium chloride will be rejected by the membrane and will not pass through. Ultrafiltration membranes have little or no selectivity for low molecular weight solutes – colloids and macromolecules only are rejected.

Hence molecular size of the solute rejected by the membrane is one criterion used to distinguish between reverse osmosis and ultrafiltration. Another related difference is that in reverse osmosis there is usually a significant osmotic pressure to overcome, whereas in ultrafiltration the osmotic pressure exerted by the high molecular weight solutes which do not permeate the membrane is low enough to be ignored. Reverse osmosis, therefore, is undertaken at high pressure ($>$ 2.5 MPa) with 'tight' membranes and the permeate consists essentially of solvent only. In practice, membranes will allow some solute to pass through; rejection is not 100%. Ultrafiltration is undertaken at low pressure ($<$ 1 MPa) with 'loose' membranes and only macromolecules (molecular weight $>$ 10 000), colloids and suspended particles do not pass through the membrane; the permeate consists of the solvent and low molecular weight solutes. In practice, the membrane will reject some of the low molecular weight solutes and rejection is greater than zero.

Membranes

Commercial reverse osmosis membranes are anisotropic, i.e. the membrane is essentially a laminate comprising a thin (*c.* 0.1 μm) film of dense polymer overlaying a substantially thicker layer (100–250 μm) of porous polymer. The thicker, porous layer supports the thin effective surface. The membrane rejects solutes only when the dense surface layer is exposed to the feed solution. Ultrafiltration membranes are asymmetric microporous structures, the effective layers of which appear to contain pores of diameter ranged from 1–20 nm.

Most reverse osmosis membranes and the earlier versions of ultrafiltration membranes were manufactured from cellulose acetate. More recently, ultrafiltration membranes have been manufactured from synthetic polymers (e.g. polysulphone or polyamide) characterized by resistance to high temperature (up to 100 °C) and a wide pH range (pH 1–13). Some have a very high resistance to chlorine (widely used for sanitizing) and can be cleaned with detergents normally used in the food industry (e.g. nitric acid and sodium hydroxide).

Whilst non-cellulosic reverse osmosis membranes (such as polyamide) have been produced, they are very sensitive to chlorine and, so far, have been unsuitable for use by the food industry.

Design of membrane equipment

The design of commercial membrane equipment has been directed at minimizing the resistance to flux. Flux is the main determinant of the capital cost of membrane equipment and should be maximized.

Membranes may be produced in the form of tubes, flat sheets or hollow fibres and these shapes form the basis of the equipment commercially available. The basic configurations and the principal manufacturers providing ultrafiltration equipment to the food industry are:

- open tubular (4–25 mm diam.): Abcor, Paterson Candy International (PCI)
- plate and frame (0.7–1.5 mm channel spacing): De Danske Sukkerfabrikker (DDS); Rhone Poulenc
- flat leaf (1.0–2.3 mm spacing): Dorr Oliver
- hollow fibre (0.5–1.5 mm diam.) Romicon
- spiral wound (0.9–1.2 mm spacing): Abcor, Ladish-Triclover.

For reverse osmosis, tubular (Abcor, PCI, Wafilin) and plate and frame (DDS) equipment are the most common. A detailed survey of equipment available for membrane processing has been published by the International Dairy Federation (1979). Equipment for reverse osmosis was reviewed by Short and Doughty (1976). Horton (1975, 1979) has outlined the types of equipment available and discussed their advantages and disadvantages.

Membrane processing of food wastes

Wastes from food-processing industries are

generally solutions containing relatively low concentrations of raw material that cannot be recovered economically. Reverse osmosis or ultrafiltration may be applied to such wastes to recover any valuable materials dissolved in the waste stream, recover good-quality water for re-use, or to concentrate the potential pollutants in order to facilitate disposal.

Although the number of potential applications for reverse osmosis and ultrafiltration is very large, few have been applied on a commercial scale. In food-waste treatment, only the processing of whey from the dairy industry has been widely adopted. The limited commercial use to date may be because the technology is still undergoing development, pilot-scale studies are relatively expensive, or long-term performance of available equipment has yet to be proven. Profitable uses for the products may also be proving difficult to develop. Maubois (1980) estimated that ultrafiltration was being used to treat 8000 m^3 of cheese whey per day in the world, i.e. *c.* 3% of the total. He attributed the rapid increase in commercial adoption of whey processing by ultrafiltration over the last few years to the progress in membrane technology, particularly the advent of membranes more resistant to the operating conditions than cellulose acetate, and to a better knowledge of the behaviour and properties of whey components. Suitable markets for concentrated whey and whey protein concentrates have also been developed. Whey processing on this scale has transformed whey from being regarded largely as a waste, and membrane processing is enhancing the fuller utilization of the solids in the milk used for cheese and casein manufacture.

Commercial uses

The use of membrane processing in the dairy industry has been reviewed by Glover *et al.* (1978), Matthews (1979) and Zall *et al.* (1979).

Reverse osmosis can be a competitive alternative to evaporation for concentration of whey to 12–18% total solids (de Boer *et al.* 1977; Eriksson 1977) and commercial plants are operating in Europe and the U.S.A. In some parts of Europe, particularly the Netherlands (Eriksson 1977), but also in Denmark and France, cheese whey is concentrated to about half its original volume before transport to a central whey-processing plant. In the U.S.A., the capacity of existing whey evaporators is the main bottleneck to expanding cheese production in many plants, and reverse osmosis is being introduced to pre-concentrate the whey to increase evaporation capacity (Zall *et al.* 1979). In the dairy industry reverse osmosis may also be used to concentrate the permeate from ultrafiltration.

Ultrafiltration is being used in many countries to produce whey protein concentrate powders with protein contents ranging from 35–85% (Glover *et al.* 1978; Matthews 1979; Maubois 1980).

In other industries, commercial membrane processes are being used to recover protein from potato-starch wastes, extract grape-skin colour from grape juice, treat blood from meat works, remove solutes from the effluent from a food fermentation process and to concentrate dilute sugar streams.

Small-scale studies

Many pilot-scale experiments and potential applications have been reported. Thus the upgrading of potato-starch manufacturing wastes by a process incorporating reverse osmosis was described by Porter *et al.* (1970) and Rosenau *et al.* (1978). Eriksson (1974) described pilot-plant trials on potato-starch waste water, blood, raw lucerne juice, fish protein solutions and waste waters from beetroot processing. Peri and Baccioni (1974) reported that pollution problems associated with distillery wastes were overcome by reverse osmosis treatment of the fermented raw materials before distillation. Pepper (1975) reviewed the use of membranes in the treatment of beverage- and food-plant wastes, including the concentration of effluents from the extraction of vegetable protein, production of starch-reduced products, barley steeping, fish processing and whisky production; recovery of sugar from biscuit and confectionery manufacture; recovery of protein from potato processing; gelatine recovery from abattoir effluent; and recycling coffee from the effluent from coffee extraction. Spatz and Trauberman (1975) outlined the use of reverse osmosis and ultrafiltration to recover sugars and proteins from liquid wastes with specific reference to candy manufacture and marachino cherry production. Rothenberger (1977) described the use of reverse osmosis for

the recovery of a sugar concentrate from process water from a coconut cutting room. The recovered material was further concentrated by evaporation and used in candy manufacture. The concentration and recycling of sugar in candy manufacturing were discussed by Sourirajan (1977) and fractionation and concentration of waste streams in the processing of sugar were outlined by Henschied *et al.* (1977). Stana (1977) presented data on treatment of sugarbeet effluent, pickle brine, nut-processing brine, soybean whey and brewery spent-grain effluent. The treatment of orange-peel waste was discussed by Cohen and Loeb (1977). Howe (1977) discussed the use of reverse osmosis and ultrafiltration to treat meat wastes, and Fane and Friend (1977) described the concentration of gelatin process liquors to *c.* 25% total solids by ultrafiltration. Cottonseed whey constituents were fractionated and recovered in a process which incorporated ultrafiltration and reverse osmosis (Lawhon *et al.* 1975; Lawhon *et al.* 1976). Soy isolate production (Hensley and Lawhon 1979), processing soybean water extracts (Cheryan and Schlesser 1978) and production of protein isolates from fababeans (Olsen 1978), all incorporating membrane processing, have been described. Other waste streams treated by reverse osmosis and mentioned in the review by Goodall (1972) include those from olive and corn processing, as well as from a mobile kitchen.

Thus a wide range of materials has been treated by membrane processes. Wastes containing proteins or carbohydrates are particularly prominent in the list.

Pretreatment for membrane processing

Many materials require pretreatment before membrane processing in order to increase flux, reduce fouling (and thereby maintain the high initial flux) or prevent damage to the membrane.

The pretreatment of whey for reverse osmosis or ultrafiltration has been reviewed by Matthews (1979) and Muller and Harper (1979). Whey, particularly sweet cheese whey, is separated (to remove milk fat as whey cream) and frequently pasteurized before membrane processing. Holding sweet whey at elevated temperatures (55 °C) for 1 to 2 h before processing has been claimed to improve membrane processing rates. However, this treatment did not affect the processing of acid whey (Matthews *et al.* 1978).

Treatments found to improve the ultrafiltration of whey include clarification (gravity settling, centrifugation or filtration) sometimes preceded by calcium addition, pH adjustment or demineralization; heating also sometimes preceded by demineralization, calcium addition or pH adjustment; and the addition of calcium sequestrants. Preconcentration of whey before ultrafiltration also improves the productivity of the process in some circumstances, although this pretreatment does not necessarily affect fouling (Goudedranche *et al.* 1976, de Boer *et al.* 1977).

Some of the pretreatments found useful for ultrafiltration lead to lower flux for reverse osmosis. For reverse osmosis of whey, demineralization and pH adjustment were effective in improving flux (Muller and Harper 1979).

Few pretreatments of other food-waste streams have been reported, although Henschied *et al.* (1977) pointed out that successful reverse osmosis concentration of sugarbeet wastes required meticulous attention to such operating parameters as the concentration of lime salts and suspended solids, pH and temperature, if membrane life is not to be adversely affected. Before reverse osmosis concentration of the process water from a coconut cutting room, coconut solids were removed by screening, and fats and oils by centrifugation and filtration (Rothenberger 1977).

In general, pretreatment for membrane processes would require removal of suspended solids to prevent blocking of flow passages or damage to the membrane, and removal of fats and oils which may foul membrane surfaces. Other physical and chemical pretreatments may be justified, but require careful study, particularly the effects on the composition and properties of the product.

Removal of biochemical oxygen demand

Food-plant wastes often contain materials with high values of biochemical oxygen demand (BOD) which could have an adverse effect on fish and plant life if discharged to a natural waterway. Expensive treatment processes are necessary to reduce the BOD to a concentration that will allow the waste to be discharged without harm to the

environment. For the food industries such treatment processes can be expensive, particularly because of the relatively high concentrations of rapidly biodegradable components (thus necessitating high oxygen transfer rates) and the seasonal variation in the supply of raw materials. Membrane processes may assist in the treatment of such effluents. However, membrane processes do not destroy biochemical or chemical oxygen demands; they concentrate the potential pollutants into a smaller volume and thereby, perhaps, facilitate utilization (by recycling or development as by-products) or disposal.

Whey

There is little reduction of the BOD of whey (initial value, 35 000–40 000 mg l^{-1}) by ultrafiltration. Permeate has a BOD concentration *c.* 90% of that of the initial whey. Suitable processes for the use or treatment of this permeate must be found if whey ultrafiltration is to be useful in solving an effluent problem.

The permeate from the reverse osmosis of whey or whey ultrafiltration permeates has a BOD that is a function of membrane type and operating conditions, particularly pH. Values reported are generally in the range 100–500 mg l^{-1}

Other food wastes

In pilot-scale trials, Lawhon *et al.* (1975) reported the reduction of the chemical oxygen demand of cottonseed whey from 12 000 mg l^{-1} to 65 mg l^{-1} by a combined ultrafiltration and reverse osmosis process. Pepper (1975) has cited data which showed 85.1–99.7% removal of BOD from the effluents from various food-processing operations.

Costs of membrane processes

A full technical and economic analysis needs to be carried out to assess the viability of any membrane process. A membrane with appropriate selectivity to bring about the required separation must be available. A key parameter is the flux, which must be sufficiently high to make plant capital and operating costs attractive in comparison with competing processes. A cleaning regime sufficient to maintain adequate hygiene without adversely affecting the membrane or the product must be determined. Pilot-plant studies may be needed to determine the above factors.

The economic analysis would include consideration of the following:

- the capital cost of the system, including the ancilliary equipment required for other than membrane processing (e.g. for pre- and post-treatment);
- anticipated membrane life;
- membrane replacement costs;
- labour costs;
- energy costs;
- cleaning costs;
- maintenance costs;
- treatment or disposal costs associated with the unwanted stream;
- the availability of raw material, particularly the effect of seasonal variations; and
- the value and yield of the product.

It is axiomatic that the total cost of producing the product(s) must be sufficiently attractive in comparison with the selling price to justify the investment.

At present, insufficient published data are available to reach generalizations about the costs of membrane operations. Accurate capital and operating costs cannot be determined without the assistance of commercial equipment suppliers to provide up-to-date costs and service requirements.

Ultrafiltration

A comparison of estimated operating costs for a number of commercial plants processing whey, undertaken by the New Zealand Dairy Research Institute, shows that capital related costs (depreciation, interest and maintenance) averaged 40% of the total ultrafiltration operating costs (excluding raw material costs, the cost of processes preceding and subsequent to ultrafiltration and costs of marketing, research and development).

Membrane replacement costs (average 16% of operating costs) are a major cost item. Projected costs may be based on the life guaranteed by the equipment supplier but actual costs will vary in practice. For whey processing most manufacturers guarantee a membrane life of 12 to 18 months. Actual membrane life has varied from 3 months to 3 years.

Energy costs (average 14%) can be significant and, in this regard, spiral wound modules have a distinct advantage. Comparison of commercial systems by the Institute revealed that the installed power for one type of ultrafiltration plant was nearly

three times that for a plant with the same daily throughput fitted with spiral wound modules. The installed power per unit of membrane area (the plants each had a different total membrane area) ranged from 0.19 kW m^{-2} to 0.84 kW m^{-2}.

The costs of cleaning and sanitizing chemicals (4%) are comparatively low and have been reduced in latter years with the introduction of non-cellulosic membranes. These membranes will withstand conventional cleaning materials whereas expensive enzyme detergents were necessary for the cellulose acetate membranes.

Yield of product has a significant effect on the manufacturing cost per unit mass of product. In the production of whey protein concentrates from a given volume of whey with a specified pretreatment, yield is mainly affected by the desired protein content of the product – the higher the protein content the lower the yield.

Whey concentration

Donnelly *et al.* (1974) concluded that, in general, a reverse osmosis plant compared with a thermal evaporator has higher capital costs and lower operating costs. Detailed costs have been presented by de Boer *et al.* (1977) comparing reverse osmosis concentration to concentration factors of 2 and 4 (*c.* 12% and 24% total solids) with evaporation to a factor of 4. Capital and membrane costs per volume of water removed represented *c.* 50% of the costs of reverse osmosis and increased as the whey was processed to a higher concentration. Energy costs (steam, electricity and cooling) were *c.* 25% of the total costs of reverse osmosis and 55% of the costs of evaporation, a difference which will alter as the relative prices of fuel (oil, gas or coal) and electricity change. It was concluded that the costs of reverse osmosis to a concentration factor of 2 were favourable compared with those for evaporation, but evaporation would be preferred at a concentration factor of 4. Stirland (1978) showed that reverse osmosis was 25–50% more economical than evaporation for concentrating whey at various solids concentrations up to 17%.

Eriksson (1977) presented an analysis of actual operating costs of reverse osmosis based on a study of commercial plants in the Netherlands concentrating whey to *c.* 12% total solids. For two different makes of plant (PCI and DDS) the capital related costs represented 61–51% of the total costs; membranes 13–11%, cleaning 8–7%, labour 12–7%, and energy 6–25% respectively.

It is apparent that whey concentration to 12–18% total solids can be cheaper by reverse osmosis than by thermal evaporation in many instances.

Concentration before transport

Hiddink *et al.* (1976) concluded that it was not economically viable to preconcentrate whey by reverse osmosis to 11–20% total solids before transport to a central ultrafiltration plant. Total processing costs might, however, be reduced by concentration of whey before transport in cases where the whey was to be further processed. Muller (1979), using 1978 Australian costs, also concluded, for a specific case study, that costs of preconcentrating whey by reverse osmosis exceeded the savings in transport costs.

Despite these cost estimates, reverse osmosis is being used commercially to concentrate whey before transport to central processing facilities (Eriksson 1977).

Other food wastes

A detailed economic evaluation of soy-isolate production by a membrane isolation process was presented by Hensley and Lawhon (1979). The study was based on pilot-scale data using mid-1978 costs and factor-costing techniques. The process involved protein extraction from soy flour, separation and concentration of protein by ultrafiltration and recovery of residues by reverse osmosis. Both the concentrates from ultrafiltration and reverse osmosis were spray dried. The study included an evaluation of the effects of scale (5, 15 and 25 million lb per year of soy isolate) and an analysis of the sensitivity of the isolate price to variations in the assumptions made.

The capital costs of the membrane-processing equipment per unit of isolate production remained essentially constant, a reflection of the modular nature of these processes. Similarly, the unit costs for membrane processing showed little change with scale. Membrane-processing production costs represented 12–15% of the total production costs. It was concluded that the membrane-isolation process was economically feasible, producing a product of improved functionality and yields compared with the traditional process and eliminated a major waste-treatment problem.

Conclusion

Michaels (1974) predicted that dairy applications held the greatest promise for the growth of the membrane-processing industry. He believed that the dairy industry could make the best use of ultrafiltration. Since 1974, the dairy industry has in fact made considerable progress in the use of reverse osmosis and ultrafiltration to treat materials previously considered to be wastes. Economic considerations have led to the use of reverse osmosis to concentrate whey to 12–18% total solids before transport to a central plant or as a means of increasing the throughput of thermal evaporators. Ultrafiltration has been used to recover a valuable protein with its unique properties intact, thus making available to the food technologist a versatile ingredient.

Few others in the food industry have followed the dairy industry's lead despite many studies on a wide variety of waste materials. Whilst the investment required for research is high, many pilot-plant studies have indicated that the potential returns from some waste streams are attractive enough to justify further studies and commercial exploitation of these membrane processes. Food manufacturers faced with expensive treatment for wastes containing sugars or proteins which could be recovered for re-use or sale in their own right should consider the use of reverse osmosis or ultrafiltration. The claim that membranes can be tailor-made for any required separation should be investigated for each waste stream being considered. These studies will require close collaboration with membrane manufacturers.

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'Contestor', an automatic pressure-decay timer

By A. K. Sharp[A] and E. R. Cousins[B]

[A]CSIRO Division of Food Research, North Ryde, NSW 2113.
[B]RMB 128E, Martins Lane, Piallamore via Tamworth, NSW 2340.

Introduction

Gas-tightness is an important property of any enclosure in which an atmosphere is to be maintained in a state different from that surrounding the enclosure, whether the difference is one of temperature, humidity, gas composition or a combination of these parameters.

The rate of gas interchange that occurs between an enclosure and its surroundings depends not only on the gas-tightness of the enclosure, but also on many environmental factors, including the strength and direction of the wind, changes in atmospheric temperature and pressure, as well as changes in the internal temperature. The actual rate of gas interchange can be measured by means of a tracer gas, but in order to characterize the enclosure a measure of gas-tightness is required that is repeatable and independent of ambient conditions. To satisfy these requirements, gas-tightness usually is measured with a pressure test. Two types of pressure test are in common use. One, the steady-state test, measures the flow rate of air required to maintain a specified internal over- or under-pressure (often 250 Pa), whereas the other, the pressure-decay test, measures the time for an imposed pressure difference to decay from one value to another (often to 50% of the initial value). Both types of test require measurement of pressure, for which an inclined manometer is often used. However, the steady-state test requires a regulated supply of air to produce a constant flow rate, and a meter to measure this rate, whereas with the pressure-decay test because the air flow rate is not measured, an unregulated air supply will suffice for the initial pressurization of the enclosure, and a stopwatch can be used to measure the decay time. Thus, the steady-state test suffers two disadvantages in comparison with the pressure-decay test; it requires more complicated equipment and, because of the requirement for steady-state conditions, it takes longer to perform.

In refrigerated enclosures such as road and rail vehicles, freight containers, and refrigerated warehouses, air interchange is well known as a potential source of additional load on the refrigeration unit. Indeed, air leakage into refrigerated road vehicles is the subject of a recent report to the U.S. Department of Energy (Bodenheimer 1978). Additional to the sensible heat load of infiltrating air is the latent heat of the water vapour associated with this air, and the need for more frequent defrosting of the evaporator unit. In recognition of this an air leakage test is specified in most standards for testing the thermal performance of refrigerated vehicles and containers (Guilfoy 1973). In larger structures such as cool stores, gas-tightness may not be specified explicitly

since generally it is less important because of the smaller ratio of surface to volume, but in Controlled Atmosphere stores stringent gas-tightness levels must be specified (Atkins and Holligan 1972; van de Plasse 1979).

The gas-tightness of certain unrefrigerated structures must also be regulated if they are to be used to contain a modified gas compostion, as in the case of in-transit disinfestation of grain in containers (Sharp and Banks 1980) and the inert-gas storage of grain (Bailey and Banks 1980). These techniques require levels of gas-tightness similar to those specified for refrigerated enclosures, but because their commercial use is quite recent, the gas-tightness levels have not yet been incorporated into standards.

Correlation between pressure-decay and steady-state tests

Required levels of gas-tightness sometimes are specified in terms of the steady-state and sometimes in terms of the pressure-decay test, but in no standards for gas-tightness is the correlation between these two types of test discussed. However, studies by this laboratory have revealed good experimental correlations for freight containers having the same form of construction, and a theoretical basis for the relationship between the two types of test is given elsewhere (Sharp 1982).

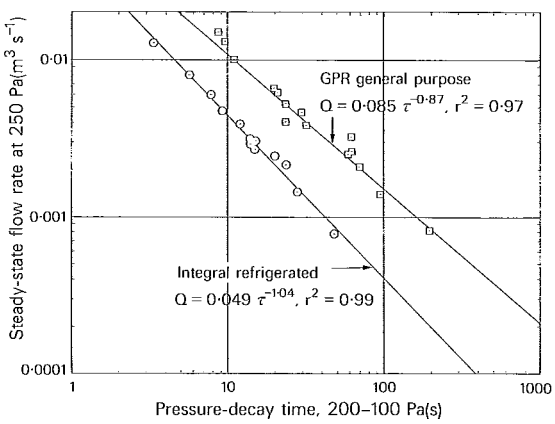

Fig. 1. Correlation between steady-state and pressure-decay tests of gas-tightness of empty 20-ft GRP, general-purpose freight containers.
$Q_{250} = 0.085\,\tau^{-0.87}$ $r^2 = 0.97$
and empty 20-ft integral refrigerated freight containers.
$Q_{250} = 0.0486\,\tau^{-1.04}$ $r^2 = 0.986$

The results of steady-state and pressure-decay tests performed on empty 20-ft GRP (glass-reinforced plywood), general-purpose containers (Sharp, unpublished results) are shown in Fig. 1. These results include containers of similar construction built by several different European manufacturers. The results of a second series of tests performed on empty, 20-ft, integral, refrigerated containers (Sharp 1982), which were all of similar construction but from several manufacturers, are also shown in Fig. 1. For each type of construction the relationship between the two types of test is well fitted by a function of the form

$$Q = a\tau^{b},$$

where Q is the steady-state flow rate at a specified applied pressure, τ is the time for the applied pressure to decay from one specified pressure to a lower one, and a and b are constants.

The values of the latter, however, differed between the two types of container. Thus for containers of a particular form of construction the two measures of gas-tightness are interchangeable and once the correlation has been determined a pressure-decay test can be used in place of a steady-state test, or vice versa.

Description of 'Contestor'

A device has been developed to simplify the performance of the pressure-decay test by replacing the inclined manometer and stopwatch with an automatic timer operated by pressure sensors (see Fig. 2). 'Contestor', which is built in a weatherproof case as a self-contained battery-operated unit, can be used in wet or dusty conditions. The device incorporates three diaphragm pressure switches which generate 'reset', 'start' and 'stop' signals to activate an electronic timer coupled to a digital display. The state of each switch is indicated by a LED (light emitting diode) fitted to the front panel. An optional pressure gauge indicates the pressure within the test enclosure and can be used to check the settings of the pressure switches. The complete unit, with batteries fitted, weighs 4 kg. This device is not appreciably sensitive to level (levelling screws are unnecessary) and is operated with a single 'power on' switch. Also shown in Fig. 2 are the other items used in the test — an air hose fitted with a shut-off valve and a 'finger device' used to admit compressed air to pressurize the enclosure

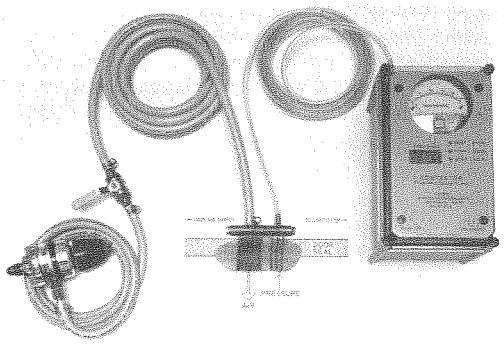

Fig. 2. Automatic pressure-decay timer, 'Contestor', together with air hose and ball valve, and the 'finger' device used to admit air to the enclosure being tested and to sense the internal pressure.

under test and also to sense the pressure developed. The 'fingers' of annealed copper tubing are shaped to fit the door jamb and then are embedded in a wedge of silicone rubber. These 'finger' devices have been found to seal well against container doors of many different shapes.

To perform the pressure-decay test, the door of the container, vehicle or room is closed over an appropriately shaped 'finger' device. Air is then introduced into the enclosure from a compressor or cylinder of compressed air. When the 'reset' LED lights up, showing that a starting pressure of, say, 220 Pa has been reached, the operator cuts off the air supply. The timer then automatically measures the time taken for the internal pressure to fall from one level to another, say from 200 Pa to 100 Pa. This time, which characterizes the gas-tightness of the enclosure, is then shown by the digital display until reset by the start of the next test. The timing circuit incorporates various logic interlocks to ensure that it is not affected by invalid sequences of signals from the pressure switches such as those caused by pressure fluctuations.

Operating experience

Prototype pressure-decay timers have now been used for two seasons for the selection of containers suitably gas-tight for the in-transit disinfestation of wheat using carbon dioxide generated from dry ice. After initial evaluation by this Laboratory the timers were used by repair contractors at a Sydney container terminal. The device has proved to be sufficiently reliable to survive use by repair workers operating in the open under hot, dusty or wet conditions. After each season of use (*c.* 2 months) the timers were returned to the laboratory for checking. Only minor adjustments to the settings of the pressure switches were necessary, and no electronic failures have been experienced. The device was also used to evaluate ways of improving the gas-tightness of containers which only narrowly failed to meet the required level. The repairmen who operated the devices commented that they found them simple to use and that they also provided useful feedback on the success of repairs made to containers.

A prototype timer has also been tested at the wharf-side container terminal in Papua New Guinea (see Fig. 3). Again, it performed well under arduous conditions, and was considered particularly suitable for use by semi-skilled personnel because it eliminated errors associated with setting up and reading an inclined manometer and with using a stop-watch.

Conclusions

- An automatic pressure-decay timer provides a rapid, simple way to measure gas-tightness and prevents errors arising from incorrect set-up and reading of the instruments normally used.
- For containers of certain types of construction the pressure-decay measure of

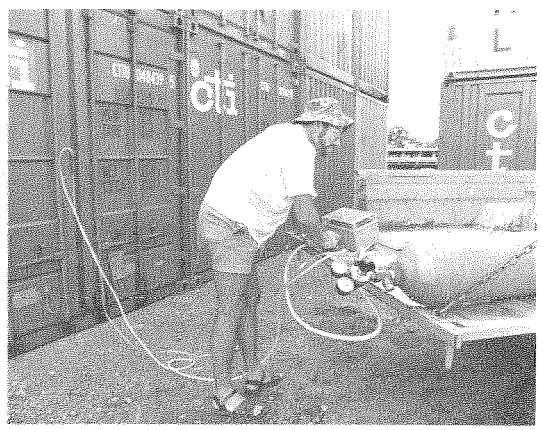

Fig. 3. 'Contestor' being used on the wharf in Lae, PNG, to select containers suitably gas-tight for the in-container disinfestation of green coffee beans intended for export.

gas-tightness is related directly to the steady-state measure that is specified in some standards.

- ▶ Prototype automatic pressure-decay timers are easily understood and operate reliably when used by semi-skilled personnel under arduous field conditions.
- ▶ Ready access to a simply operated instrument to measure gas-tightness encourages repairmen to monitor the success of their repair work.

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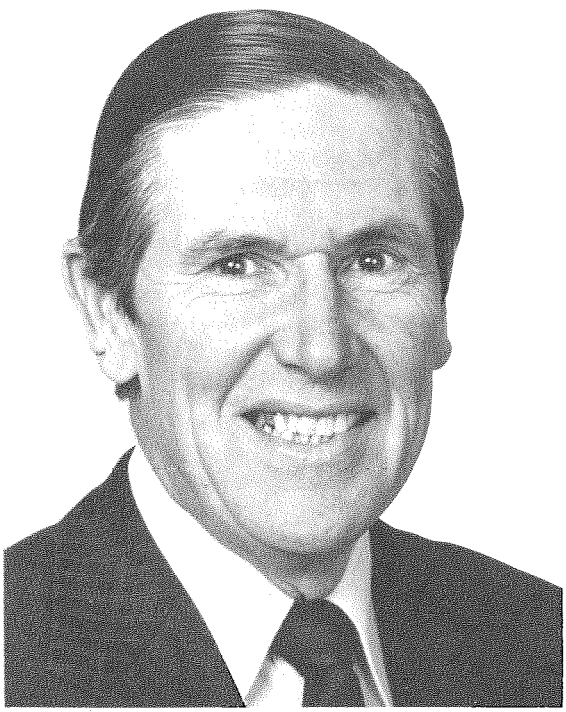
Two distinguished scientists retire



J. F. Kefford

Mr J. F. Kefford retired from the CSIRO Division of Food Research on 5 February 1982, after more than 43 years' service. He joined the Food Preservation Research Laboratory as an Assistant Research Officer in 1938 with Bachelor's and Master's degrees in Science from the University of Melbourne and left the Division as a Chief Research Scientist with a most enviable reputation in food science and technology.

His career had many facets — science, technology, industry relations and administration. His initial research, on the effect of ozone on microbial growth on beef muscle and on the flavour of beef fat, was not published until 1948 because the exigencies of World War II turned the Laboratory's attention to more immediate problems. One of these was the upgrading and expansion of the food processing industry to help provision Allied Forces in the South-West Pacific. Jack Kefford, with others in L. J. Lynch's Canning and Fruit Products Section, was soon plunged into this work. One of the group's many activities in 1943 was to assist in the setting up of 20 citrus juice plants. The

problems of orange juice processing, particularly in relation to bitterness, remained major research interests throughout Kefford's career and he is an acknowledged world authority in this field.

It was during this period that Kefford demonstrated his talents as a teacher, becoming heavily involved in the first extension courses to be given at the Laboratory at Homebush. He has continued to be in great demand as a highly skilled lecturer to such diverse audiences as service clubs and Industrial Mobilization Courses.

Post war, Jack Kefford continued his research interests in food technology, concentrating on canning, but expanding them to include many other aspects, among them condensation problems in food shipments and the biodegradation of food processing wastes.

In 1965, the Division's canning, freezing and drying investigations were combined to form a Food Technology Section, with Kefford as its leader. He became an Assistant Chief of the Division in 1967. In 1970, the CSIRO Divisions of Food Preservation and of Dairy Research merged and Jack Kefford became Officer-in-Charge of the Food Research Laboratory of the new Division at North Ryde, N.S.W. His term in this position was particularly notable for his qualities of leadership and, or perhaps because of, his great concern for the well-being of his staff.

In 1976, after distinguished service with FRL, he joined the Headquarters group as Assistant Chief (External Relations), a position he held until his retirement.

Besides his skills as a researcher and administrator, Jack Kefford has unique gifts of communication with colleagues at all levels in the Australian food industry and in many other countries. His unbiased counsel is greatly valued by industry, as evidenced by the many committees, some otherwise exclusively industry bodies, on which he has been invited to serve. He is also a most accomplished author and editor, and has served as Chairman of the Editorial Committee of the Food Research Quarterly since 1977.

He was a member of the Australian delegation to the inaugural meeting of the Codex Alimentarius Commission and has always maintained the strongest international connections. His service to the international community and his professional reputation were recognized in 1978 by his election as Secretary-General of the International Union of Food Science and Technology. This commitment does not cease with Jack's retirement from CSIRO and he will continue as an Honorary Research Fellow at the Food Research Laboratory, to the great benefit of both the Division and IUFoST.

More adequate appreciations of J. F. Kefford's career appear in Food Technology in Australia, Volume 34.

J. H. B. Christian

J. F. Kefford: List of publications

Lynch, L. J., and Kefford, J. F. (1939). Internal lacquering of tinplate containers for foods. I. Determination of tin in foods and a survey of the tin contents of some canned foods. *J. Counc. Sci. Ind. Res.* **12**, 303-10.

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J. D. Mellor

J. D. Mellor, Principal Research Scientist in the Applied Food Science Group, who retired from the Division of Food Research on 12 February 1982 was one of the longest serving officers of CSIRO having worked for the Organization and its predecessor CSIR for almost 44 years. Jack commenced duty in 1938 in the Physics Section of the Food Preservation Research Laboratory as a Junior Assistant, at the princely salary of $5.90 per fortnight.

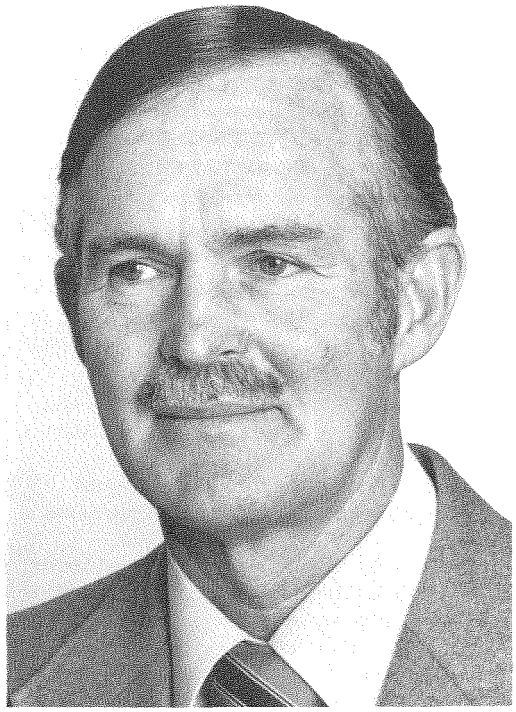
In 1941, Jack Mellor enlisted in the RAAF and served until 1946 in No. 10 Squadron. Most of his service was spent in Britain where he not only married but also completed two years of an Intermediate B.Sc. degree in Mathematics and Physics. After demobilization he returned to the Division as a Technical Officer to work on physical measurements in connection with refrigerated rail transport and cool stores.

Jack Mellor never completed a formal professional qualification but he is an excellent example of an officer who moved from the technical to the research scientist grades by acquiring professional status through his published work, his international reputation and his outstanding contribution to his particular studies.

Jack Mellor has been the pivot of the Division's extensive freeze-drying research spanning almost 20 years. This culminated in the publication, in 1978, of his book 'Fundamentals of Freeze-Drying'. The reviews of this book established it as the definitive text in the area and confirmed his international reputation in the area of heat and mass transfer as related to the freeze-drying of foods and other biological materials.

The research carried out by Jack Mellor did much to change freeze-drying from an empirical process to one with a sound theoretical basis — the moving boundary theory of ice sublimation. His major contribution was the development of the theory and practice of cyclic-pressure freeze-drying, which constituted the most significant advance in freeze-drying in recent years.

Jack Mellor developed his discoveries to the stage of commercial application which involved extensive collaboration with engineering firms. His concepts were incorporated in the design of a large-capacity



freeze-dryer which is used by the Armed Forces Food Science Establishment, Scottsdale, Tasmania, to produce components for combat and survival rations for the Australian Armed Forces.

In Australia, Jack's work in freeze-drying was recognized when he was elected President of the Vacuum Physics group of the Australian Institute of Physics (1969–71).

More recently, Jack Mellor became aware of deficiencies in the availability of reliable thermophysical data for foodstuffs, and in the methods of obtaining such data. In collaboration with the International Institute of Refrigeration (IIR), for which he is the Australian representative on Commission C1, and EEC-COST*, he initiated a project to collate, interpret and evaluate the published data and methods. This collaboration has involved several visits to Europe and has already resulted in the publication of a number of tables of reliable data. Others are presently being prepared. Although Jack will

*European Economic Community — Cooperation on Science and Technology

be retiring officially, he intends to maintain his science affiliations, especially with IIR, and has accepted a position of Honorary Research Fellow at the Food Research Laboratory.

Jack will undoubtedly plan to visit his two married daughters, one of whom lives in Zürich, Switzerland, and the other in Wellington, New Zealand.

A. R. Johnson

J. D. Mellor — List of publications

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