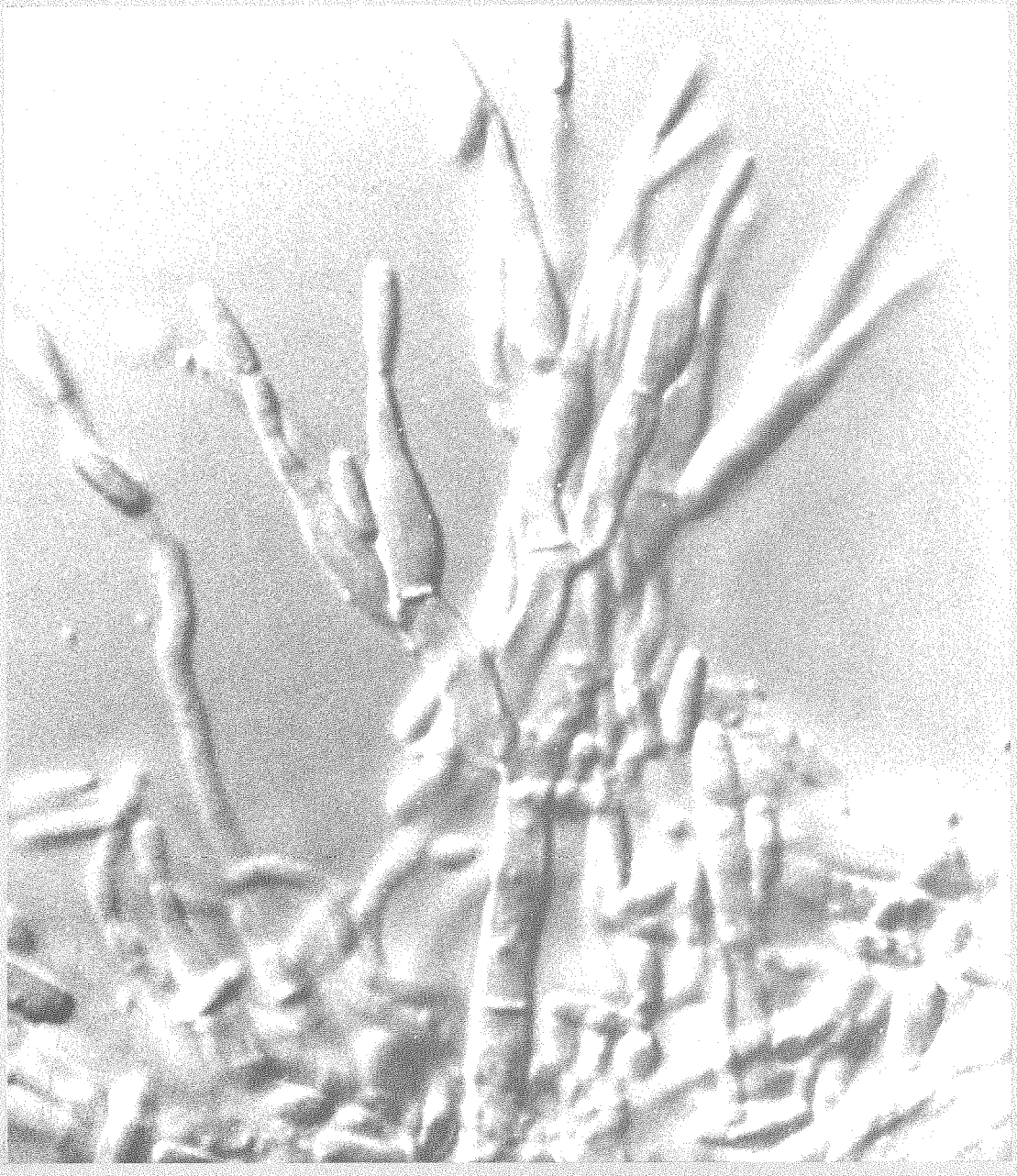
Vol. 44 No. 4 December 1984

CSIRO Food Research Quarterly



Food spoilage fungi. II. Heat-resistant fungi

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Spoilage problems caused by heat-resistant fungi have been reported frequently in Australia in the past few years. New developments in packaging technology, along with increased consumer demand for fruit juices and other fruit-based products, have led to a large increase in the marketed volume of these products. A trend towards pasteurized preservative-free juices has increased the risk of spoilage caused by heat-resistant fungi. The species capable of causing this type of spoilage are described in this paper.

Introduction

It is probable that spoilage problems caused by heat-resistant moulds have been with us since man began to preserve fruit by heat processing. The problem was first recognized in canned strawberries in the early 1930s at the Campden Fruit and Vegetable Preservation Station, England. Olliver and Rendle (1934) showed that *Byssochlamys fulva* was responsible for the spoilage. They conducted extensive investigations on the incidence of *B. fulva* in fruits, in sugar and water, and in packing materials. Although wooden trays, baskets, glass bottles and jars were found to be contaminated with the fungus, the initial source was shown to be the fruits coming in from the fields and orchards. Strawberries and plums were infected more heavily than most other fruits, but Olliver and Rendle (1934) also found *B. fulva* on gooseberries, loganberries, blackberries, black currants and apples. Since all positive samples of stone fruits were taken from the ground, and strawberries, too, are easily contaminated with soil, Olliver and Rendle suggested that *B. fulva* may be a soil-borne fungus.

Hull (1939), investigating the sources of infection by *Byssochlamys*, found the fungus on leaves, fruits and straw from the strawberry fields, and also on mummified plums, raspberry refuse, and in the baskets used to collect fruit. He concluded that *Byssochlamys* was a saprophytic rather than a parasitic fungus, and that the ascospores could survive in orchard refuse over winter, to contaminate the following season's fruit. Although *Byssochlamys* did not actually grow in soil, the soil acted as an important reservoir for the ascospores, and fruits which came into contact with soil were particularly susceptible to contamination by *Byssochlamys* and other heat-resistant fungi.

The Australian situation

In Australia, spoilage problems caused by *Byssochlamys* were first recognized in the early 1960s, again in canned strawberries (Spurgin 1964; Richardson 1965). In recent years, there has been an increase in the volume and variety of heat-processed fruit-based products on the market. New packaging technology, and an increase in consumer demand, have seen a huge increase in the marketed quantity of fruit juices and fruit-juice drinks. Preservation often has relied on either pasteurization or ultra high temperature (UHT) processing, rather than the use of preservatives. Spoilage problems due to heat-resistant moulds have occurred in these products, and other products of a similar type, such as fruit-gel baby foods.

In Australia, in our experience, the raw materials most likely to be contaminated with heat-resistant fungal spores are passionfruit and strawberries. Pineapple and mango juices and pulps may also be a source of contamination, but orange and other citrus products have rarely been implicated. Passionfruit are particularly susceptible to contamination because the fruit are usually harvested after they have fallen, and have thus been in contact with the soil. Strawberries may also come in contact with soil, and both strawberries and pineapples may be contaminated with soil by rain splash.

The best way to ensure that heat-resistant moulds will not cause spoilage of susceptible products is careful selection and handling of raw materials, in conjunction with an effective screening procedure for heat-resistant fungal

spores. For example, passionfruit crops consist of mostly smooth, sound fruit, together with a proportion which have wrinkled skins. The smooth fruit will generally have a lower mould spore count than the wrinkled fruit, and are much easier to decontaminate. Smooth fruit should be thoroughly washed, preferably in hypochlorite solution (approximately 100 ppm), then rinsed before processing. Wrinkled fruit should be processed separately, and the juice or pulp used in products such as refrigerated or frozen desserts, that will not be spoiled by heat-resistant moulds.

Screening for heat-resistant fungi

A number of methods have been developed for the enumeration of heat-resistant fungal spores. All include a heat treatment, ranging from 5 minutes at 75°C to 35 minutes at 80°C. Most of these methods are outlined by Beuchat and Rice (1979). One of these, developed specifically for screening fruit juices and concentrates by Murdock and Hatcher (1978), has been adapted by Budnik (unpublished) to enable the screening of a larger sample (100 ml) of raw material. The method is described below, and outlined diagrammatically in Fig. 1.

If the sample to be tested is greater than 35° Brix, it should first be diluted 1 : 1 with 0.1% peptone or similar diluent. Two 50 ml samples are taken for examination. For passionfruit juice, which normally has a pH of about 2.0, the pH should be adjusted to 3.4–3.6. The two samples are heated in 200 × 30 mm test tubes in a closed water bath at 80°C for 30 minutes, then rapidly cooled. Each 50 ml sample is then distributed over four 150 mm Petri dishes and mixed with 1½ strength potato dextrose agar. The Petri dishes are loosely sealed in a plastic bag to prevent drying, and incubated at 30°C for up to 30 days. Plates are examined weekly for growth. Most surviving moulds produce visible colonies within ten days, but incubation for up to 30 days allows for the possible presence of badly heat-damaged spores, which may germinate very slowly. This long incubation time also allows most moulds to mature and sporulate, aiding their identification.

The main problem associated with this dilution technique is the possibility of aerial contamination of the plates with common mould spores which will give false positive results. The appearance of green *Penicillium* colonies, or colonies of common *Aspergillus* species such as *A. flavus* and *A. niger*, is a clear indication of contamination, as these fungi are not heat-resistant, and their spores will not survive the 75° to 80°C heat treatment. To minimize this problem, plates should be poured in clean, still air, or a laminar flow cabinet if possible. If a product contains large numbers of heat-resistant bacterial spores (e.g. *Bacillus* species), antibiotics can be added to the potato dextrose agar. The addition of 100 mg of chloramphenicol per litre of medium will prevent the growth of these bacteria.

A more direct method can be used for screening fruit pulps and other semisolid materials which avoids the problems of aerial contamination. Place approximately 30 ml of pulp in a flat bottle such as a 100 ml medicine flat. Heat the bottle in the upright position for

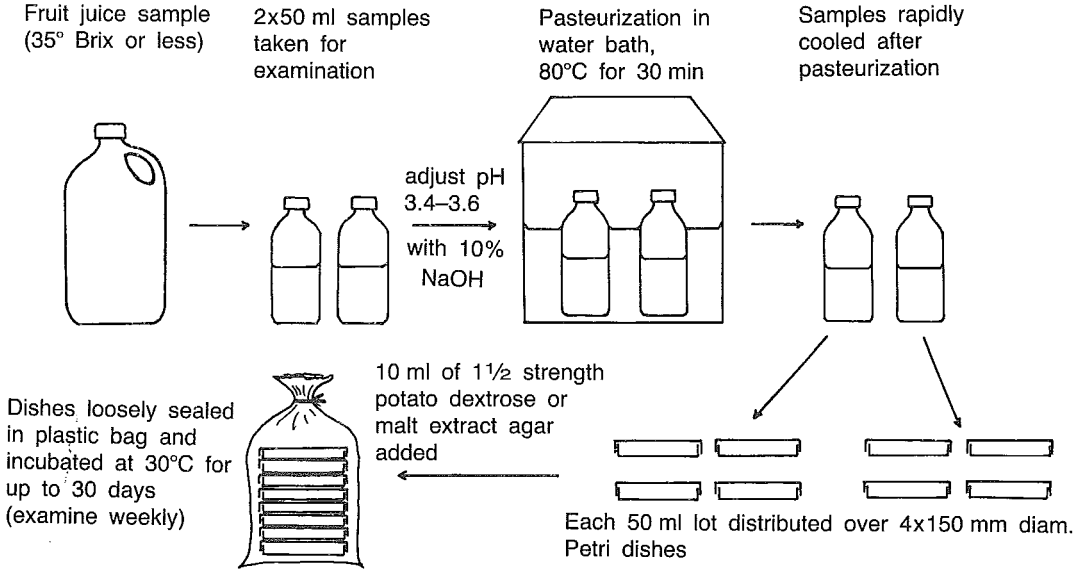

Fig. 1. Procedure for detection and enumeration of heat-resistant mould spores.

30 minutes at 80°C and cool, as described previously. The bottles of pulp can then be incubated directly, without opening and without the addition of agar. They should be incubated flat, allowing as large a surface area as possible, at 30°C, for up to 30 days. Any mould colonies which develop will need to be sub-cultured onto a suitable medium for identification. If suitable containers such as Roux bottles are available, larger samples can be examined by this technique.

Acceptable limits for heat-resistant moulds

The acceptable level of contamination of a raw material with heat-resistant ascospores will depend very much on the end product. Considerations such as whether the raw material is a major or a minor ingredient, whether the final product will contain preservative, and the heat process to which the product or the raw material will be subjected, all must be taken into account. Practical experience has shown that, for passionfruit juice, a contamination level of less than 2 spores per 100 ml should give a negligible spoilage rate in most finished products. Contamination levels of more than 2 spores per 100 ml sample are often unacceptable. However, for some products, such as UHT processed fruit juice blends which contain a high proportion of passionfruit juice and do not contain preservative, an even lower level of contamination is required. One manufacturer of this type of product specifies that heat-resistant mould spores should be absent from a 100ml sample of passionfruit juice.

Types of spoilage

In canned or bottled fruits affected by *Byssochlamys* species, the first sign of fungal spoilage is usually a slight softening of the fruit. This progresses until total disintegration takes place, due to the production of a powerful pectinase by the fungus (Hull 1939; Beuchat and Rice 1979). Off-odours, and a slightly sour taste may develop, and there may be gas production. It is rare for species other than *Byssochlamys* to be responsible for spoilage of canned fruits.

Byssochlamys species are capable of growth at extremely low oxygen tensions. In liquid products under these conditions, fermentation apparently occurs, with the production of CO_2. The production of gas then causes visible swelling and spoilage of the product. Even small amounts of oxygen in the headspace of a jar or bottle, or slow leakage of oxygen through a package such as a Tetra-Brik, can provide sufficient oxygen for these fungi to grow. In solidified products, like fruit gels, heat-resistant moulds cause spoilage by growing as visible colonies on the surface of the product.

Heat resistance

Studies on the heat resistance of fungal spores have concentrated on *Byssochlamys* species, no doubt because of their dominant role in the spoilage of heat-processed foods. Moreover, ascospores of *Byssochlamys* species appear to be among the most heat-resistant fungal spores known.

Many variables can affect heat resistance. For *Byssochlamys* species, information on this topic has been comprehensively reviewed by Beuchat and Rice (1979). Heat resistance can vary markedly from isolate to isolate (Bayne and Michener 1979; Hatcher *et al.* 1979). Factors such as pH, water activity, and the presence of preservatives also have an effect. Ascospores are more susceptible to heat if the pH is low (Bayne and Michener 1979), and/or if preservatives such as SO_2 are present (King, Michener and Ito 1969). On the other hand, high levels of sugar have a protective effect (Beuchat and Toledo 1977). For *Byssochlamys fulva*, a D value between 1 and 12 minutes at 90°C (Bayne and Michener 1979) and a z value of 6 to 7 minutes (King *et al.* 1969) are practical working values.

The heat resistance of *Byssochlamys nivea* is marginally lower than that of *B. fulva* (Put and Kruiswijk 1964; Beuchat and Rice 1979).

The most heat resistant of other moulds isolated from heat-processed foods appears to be the ascomycete *Neosartorya fischeri*, which is more commonly known by the name of its conidial state, *Aspergillus fischeri*. Kavanagh *et al.* (1963) reported that ascospores of an isolate more recently identified as *N. fischeri* withstood boiling in distilled water for 60 min. They reported that spore age, pH and sugar concentration affected heat resistance, but no details of spore numbers heated or experimental procedures were given. McEvoy and Stuart (1970) also heated ascospores of *N. fischeri* in distilled water: they reported 100% survival after 20 min at 80°C, and 0.002% survival after 5 min at 100°C. Splittstoesser and Splittstoesser (1977), however, reported that the heat resistance of a *Neosartorya* isolate, probably *N. fischeri*, was comparable to that of *B. fulva*.

Taxonomy of heat-resistant fungi

The basis for high heat resistance in these fungi is the production of a teleomorphic state, that is, they form *ascospores*. Ascospores are produced, generally in groups of eight, within a closed sac, the *ascus* (pl. *asci*); ascospores are the prime characteristic of the class of fungi called

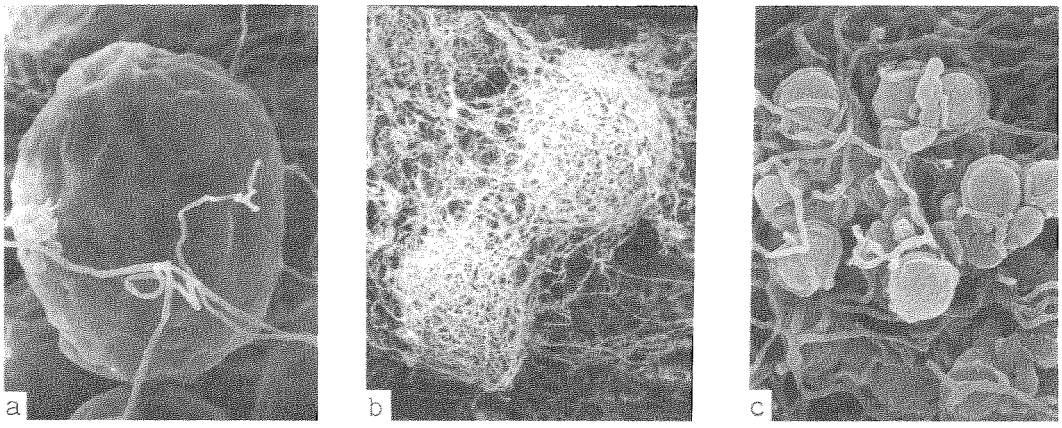

Fig. 2. Different bodies in which ascospores may be formed: (a) cleistothecium (*Neosartorya, Eupenicillium*); (b) gymnothecium (*Talaromyces*); (c) unenclosed asci (*Byssochlamys*).

Ascomycetes. In nearly all ascomycete genera, asci are in turn enclosed, in large numbers, within larger bodies. In genera of interest here, these bodies may have a solid, totally enclosed wall (a cleistothecium, Fig. 2a) or be composed of fine, interwoven hyphae (a gymnothecium, Fig. 2b). Only in *Byssochlamys* are asci borne singly and unenclosed (Fig. 2c).

As well as ascospores, ascomycetes generally produce an anamorphic state with asexual spores, that is they produce *conidia* (sing. *conidium*). Conidia are not very heat resistant, and are usually readily destroyed by pasteurizing heat processes or the screening techniques outlined above. The fungi of interest here produce conidial states characteristic of the genera *Aspergillus* (Fig. 3a), *Geosmithia* (Fig. 3b), *Paecilomyces* (Fig. 3c), and *Penicillium* (Fig. 3d).

Common heat-resistant fungi

It is probably true that most fungal ascospores possess relatively high heat resistance and could in theory cause food spoilage. In practice only a few species have been encountered in our work or reported in the literature. Principal among these are *Byssochlamys* species: *B. fulva*, which is commonly associated with spoilage problems in Australia, and *B. nivea*, which appears to be much less common here than in Europe. Other fungi which less frequently cause spoilage in processed fruit products are *Talaramyces flavus*, *T. bacillisporus* and *Neosartorya fischeri* (= *Aspergillus fischeri*). Species from the genus *Eupenicillium* also form heat-resistant ascospores, but do not commonly cause spoilage. Some *Penicillium* species form hard *sclerotia*, really undeveloped cleistothecia similar to those in *Eupenicillium*. These too appear to be highly heat resistant, and have occasionally caused food spoilage (Williams *et al.* 1941).

In practical terms, recognition of these species of fungi relies on four principal factors; first, isolation from a heat screening process; second, observation of colony growth and appearance on standard media; third, observation of cleistothecia, gymnothecia or unenclosed asci; fourth, microscopic observation of conidial structures. The first of these has been outlined above, the others are incorporated in the descriptions given below.

Isolation and culture of heat-resistant fungi

In general, heat-resistant fungi do not require special media or cultural conditions. Suspected heat-resistant colonies can be isolated by picking spores or mycelium with a sterile needle from colonies which have grown on heated product and transferring as a single point to a slant of suitable medium. Either Czapek yeast extract agar or malt extract agar is suitable (see formulae below). Transfer by using a needle which has been flamed, and then cooled in agar, leaving a moist tip to which spores will adhere. Incubate slants at 25° to 30°C for a week or more, until fully grown.

Media

Czapek yeast extract agar (CYA) has the following composition: $NaNO_3$, 3.0 g; KH_2PO_4, 1.0 g; KCl, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; $FeSO_4.5H_2O$, 0.01 g; yeast extract, 5.0 g; sucrose, 30 g; agar, 15 g; water to 1 l. Malt extract agar (MEA) has the following composition: malt extract, 20 g; glucose, 20 g; bacteriological peptone, 1.0 g; agar, 20 g; distilled water to 1 l. We have found light malt extract sold for home brewing to be as effective for making media as malt extract sold

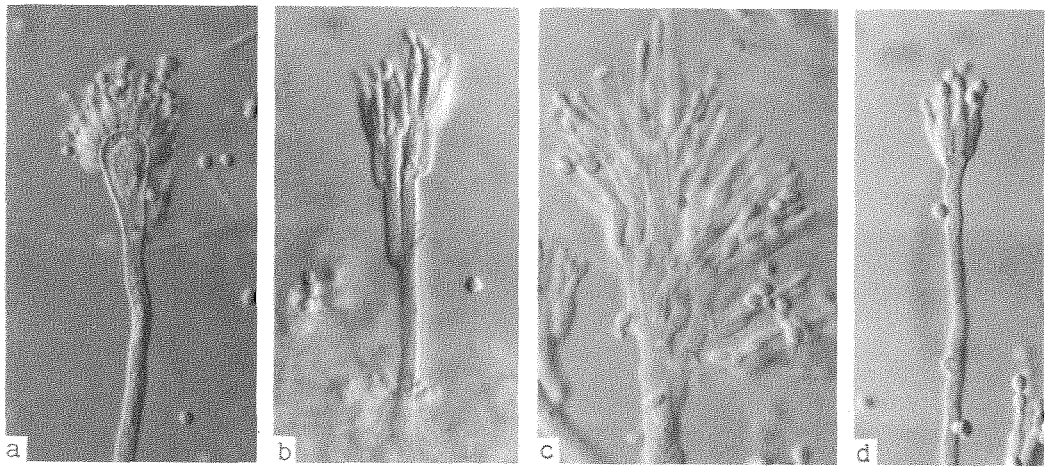

Fig. 3. Anamorphic states produced by heat-resistant fungi: (a) *Aspergillus* anamorph of *Neosartorya;* (b) *Geosmithia* anamorph of *Talaromyces;* (c) *Paecilomyces* anamorph of *Byssochlamys;* (d) *Penicillium* anamorph of *Eupenicillium.*

specifically for that purpose.

Detergent agar, optional for inoculating Petri dishes (see below), consists of one drop per 100 ml (0.05%) of a food grade detergent such as polysorbitan 80 (Tween 80) in 0.2% agar. Dispense this medium in *c.* 0.25 ml quantities in small vials.

All media should be sterilized by autoclaving at 121°C for 15 min.

Identification of isolates

To identify heat-resistant fungal isolates, proceed as follows. Inoculate each isolate onto four Petri dishes, two each of CYA and MEA. Inoculate each plate at three equally spaced points. Avoid chance inoculation of stray spores as far as possible, by one of two techniques. Either hold plates upside down and inoculate with a needle point of spores (wet needle), or mix a needle point of spores with detergent agar in a small vial, and inoculate plates with a loop. Vials may be steamed or autoclaved and reused several times before cleaning.

Incubate plates for one week: one each of MEA and CYA at 25°C or near, and the others at 30°C.

After incubation, examine plates by eye, measuring colony diameters with a ruler, and make wet mounts to examine small pieces of fungus under the compound microscope.

To make wet mounts, cut a small piece of fungus from the colony using a chisel-pointed wire or a steel sewing needle, and transfer to a clean slide, adding a single drop of 70% ethanol to aid transfer and to wet the spores. After most of the ethanol has evaporated, add a single drop of a suitable stain, such as lactofuchsin (0.1% acid fuchsin in pure lactic acid), add a cover slip, and gently blot away surplus stain. Examine with 10×, 40× and 100× objectives. Pieces of freshly sporing areas from the peripheries of colonies make the best wet mounts for conidial structures; areas near the centres of colonies are usually the first to produce mature ascospores.

The following key will assist in identification.

Key to common heat-resistant fungi

1. Asci produced in discrete bodies with totally enclosed walls (cleistothecia) 5
   Asci produced in bodies with walls of woven hyphae (gymnothecia) or openly 2
2. Asci enclosed in gymnothecia (*Talaromyces*) 3
   Asci produced openly; fine hyphae may be present, but asci not enclosed (*Byssochlamys*) 4
3. Colonies on MEA at 25° C exceeding 25 mm diam.; conidial state *Penicillium* *Talaromyces flavus*
   Colonies on MEA at 25°C not exceeding 25 mm diam.; conidial state *Geosmithia* *Talaromyces bacillisporus*
4. Colonies on CYA and MEA predominantly buff or brown *Byssochlamys fulva*
   Colonies on CYA and MEA persistently white or cream *Byssochlamys nivea*
5. Colonies on CYA and MEA at 25°C exceeding 50 mm diam. in 7 days; predominantly coloured white or cream *Neosartorya fischeri*
   Colonies on CYA and MEA at 25°C otherwise *Eupenicillium* spp.

Genus *Byssochlamys* Westling

Byssochlamys has the distinction of being

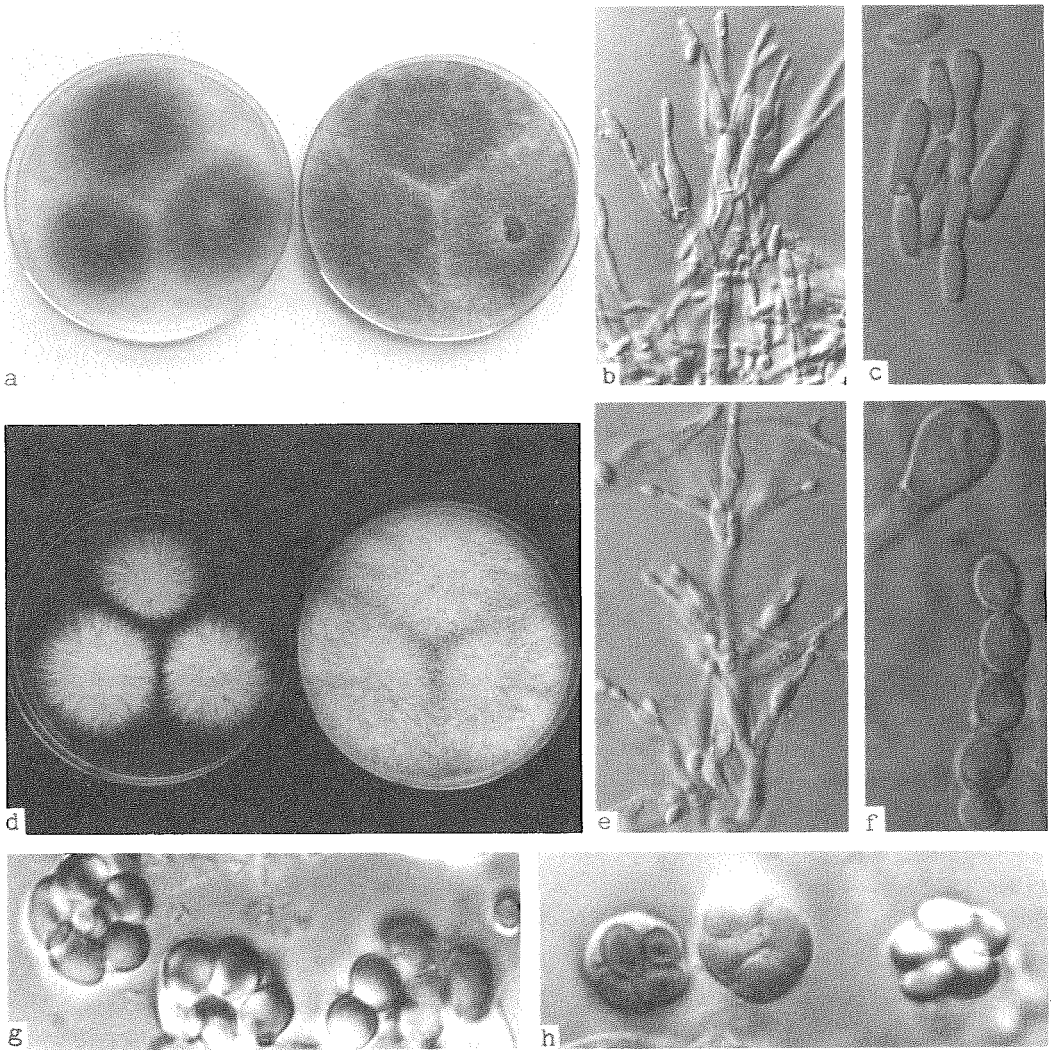

Fig. 4. *Byssochlamys* species: (a)-(c), (g) *B. fulva*; (d)-(f), (h); *B. nivea*. *B. fulva*: (a) colonies on CYA and MEA, 25°C, 7d.; (b) penicillus; (c) conidia; (g) ascospores. *B. nivea*: (d) colonies on CYA and MEA, 25° C, 7d.; (e) penicillus; (f) conidia; (h) ascospores.

almost uniquely associated with food spoilage, and in particular with the spoilage of heat-processed acid foods. Apart from the original isolation and description of *B. nivea* from soil by Westling in 1911, *Byssochlamys* has been reported only rarely from sources other than heat-processed foods. Its natural habitat appears to be soils, but the genus is mentioned very seldom in lists of fungi from soils other than those used for the cultivation of fruits.

Byssochlamys is an ascomycete genus characterized by the absence of cleistothecia, gymnothecia or other bodies which in most ascomycetes envelop asci during development. Asci in *Byssochlamys* are borne in open clusters, in association with, but not surrounded by, unstructured wefts of fine, white hyphae.

In our experience, the temperature range for observation of *Byssochlamys* asci and ascospores in the laboratory is sometimes very narrow. Cultures need to be incubated at 30°C as some isolates do not produce asci at 25°C or 37°C. However, presumptive evidence of the presence of *Byssochlamys* can be made from plates at 25° or 37°C if the isolate has come from heat-processed foods or raw materials.

Byssochlamys fulva Olliver and G. Smith (Fig. 4, a-c,g)
Anamorph: *Paecilomyces fulvus* Stolk and Samson

At 25°C, colonies on CYA and MEA at least 60 mm diam., often covering the whole Petri dish, relatively sparse, low or somewhat floccose; conidial production heavy, uniformly coloured olive brown; reverse in similar colours or pale. At 30°C, colonies on CYA and MEA usually covering the whole Petri dish, low to moderately deep, sparse, with moderate conidial production, coloured brown, overlaid by white hyphae from which asci produced; reverse olive brown to deep brown.

Teleomorphic state single asci borne from, but not enveloped by, wefts of contorted white hyphae, best developed at 30°C, maturing in 7-12 days, occasionally formed at 25°C in fresh isolates but maturing slowly if at all; asci spherical to subspheroidal, 9-12 μm diam.; ascospores ellipsoidal, hyaline or straw coloured, 5-7 μm long, smooth walled.

Anamorphic state best observed at 25° C, consisting of penicilli borne from surface hyphae or long, trailing, aerial hyphae; stipes 10-30 μm long; phialides of nonuniform appearance, flask-shaped or narrowing gradually to the apices, 12-20 μm long; conidia mostly cylindrical or barrel-shaped, narrow and 7-10 μm long, but sometimes longer, wider or ellipsoidal from particular phialides, smooth walled.

Distinguishing characteristics. – In culture at 25°C, *Byssochlamys fulva* closely resembles the common fungus *Paecilomyces variotii.* The simplest microscopic distinguishing feature is that *P. variotii* produces ellipsoidal not cylindrical conidia. At 30°C, *B. fulva* is distinguished by rapidly growing olive brown colonies with areas of fine white hyphae, in which asci are produced in open clusters.

Byssochlamys nivea Westling (Fig. 4, d-f,h)
Anamorph: *Paecilomyces niveus* Stolk and Samson

At 25°C, colonies on CYA 40-50 mm diam., low and quite sparse, white to slightly grey; reverse pale to mid-brown. Colonies on MEA covering the whole Petri dish, low and sparse, white to creamish, with small knots of dense hyphae; reverse pale to brownish. At 30°C on CYA, colonies covering the whole Petri dish, similar to those on MEA at 25°C, but often more dense, enveloping distinct knots of dense hyphae.

Teleomorphic state similar to that of *B. fulva* except for slightly smaller asci (8-11 μm diam.), and ascospores (4-6 μm diam.), maturing in 10-14 days at 25°C and in 7-10 days at 30°C but rarely found at 37°C.

Anamorphs of two kinds produced, aleurioconidia and penicilli; aleurioconidia borne singly, common at 30°C and 37°C, spherical to pear-shaped, 7-10 μm diam.; irregular penicilli sparsely produced, and phialides sometimes borne solitarily from hyphae as well; phialides 12-20 μm long, cylindrical then gradually tapering; conidia ellipsoidal to pear-shaped, 3-6 μm long, smooth walled.

Distinguishing characteristics. – *Byssochlamys nivea* is readily distinguished from *B. fulva* by its persistently white to cream colonies. It differs from other fungi by forming three characteristic types of reproductive structures: aleurioconidia, sparse penicilli, and solitary asci as well.

Genus *Eupenicillium* Ludwig

Eupenicillium is characterized by the production of macroscopic (100-500 μm diam.), smooth walled, often brightly coloured cleistothecia, in association with a *Penicillium* anamorph. In many species cleistothecia become rock hard as they develop, and may remain so for many weeks or months, finally maturing from the centre to yield numerous eight-spored asci.

Most *Eupenicillium* species are soil fungi, and of little interest to the food microbiologist. However, they do occur from time to time as survivors of heat processing. Williams *et al.* (1941) recorded that a new species, *Penicillium lapidosum* (stone-like, an apt name) was causing spoilage of canned blueberries. It possessed highly heat-resistant sclerotia (immature cleistothecia). This fungus was later shown to produce a *Eupenicillium* state. Two points are worth noting: first, it was the immature cleistothecium itself which was acting as the heat-resistant body, and second, most *Eupenicillium* species produce heat-resistant ascospores. Fortunately, they rarely find their way into heat-processed foods.

We and others (Anon. 1967) have isolated *Eupenicillium* species as heat-resistant contaminants of fruit juices on several occasions. No particular species appears to be significant, and growth of the fungus has occurred in the product only rarely. As a cause of food spoilage, *Eupenicillium* ascospores can be safely ignored unless an unusual set of circumstances leads to excessive contamination of some raw material or product.

As an example of this genus, one species, *Eupenicillium brefeldianum*, is described below. This species was isolated from spoiled fruit juice in South Africa (Anon. 1967).

Eupenicillium brefeldianum (B. Dodge) Stolk and Scott

Anamorph: *Penicillium dodgei* Pitt

At 25°C, colonies on CYA 30-40 mm diam., radially furrowed, consisting of dense, velvety, pale yellow to greyish orange mycelium; cleistothecia abundant, enveloped by the mycelium; conidia sparse; exudate limited to copious, clear; reverse pale to bright yellow, more usually ochre to umber. Colonies on MEA 30-50 mm diam., low, plane and relatively sparse, otherwise similar to those on CYA but reverse usually yellow to yellow brown. At 30°C, colonies similar to those at 25°C, except reverse pale to bright orange.

Cleistothecia buff to brown, 150-250 μm diam., relatively soft, maturing in 2 weeks at 25°C; ascospores ellipsoidal, 3.0-4.0 μm long, with roughened walls and a faint longitudinal furrow. Conidiophores borne from aerial hyphae, long and slender, 50-200 μm long, smooth walled, bearing simple penicilli of phialides only; conidia subspheroidal to ellipsoidal, 2.5-4.0 μm long.

Distinguishing characteristics - *Eupenicillium* species are distinguished in general terms by relatively slowly growing, compact, brightly coloured colonies which produce hard, spherical to ovoid cleistothecia in 1-2 weeks, and usually a sparsely-produced *Penicillium* anamorphic state.

Genus *Neosartorya* C. R. Benjamin

A genus of soil fungi, *Neosartorya* is of interest here because of its highly heat-resistant ascospores. It occurs from time to time in heat-processed foods and has occasionally been reported as a cause of spoilage. Kavanagh *et al.* (1963) isolated it from spoiled canned strawberries; McEvoy and Stuart (1970) reported that strawberry canneries in Ireland experienced problems with *N. fischeri* in all but one season between 1958 and 1968, despite increases in process severity. Splittstoesser and Splittstoesser (1977) studied a *Neosartorya*, probably *N. fischeri*, from a spoiled fruit drink. We have isolated this species quite frequently from passionfruit juice, and although it does not appear to be as troublesome in finished product as *Byssochlamys fulva*, its presence on screening plates is an indication of potential problems.

Neosartorya includes several species (see Raper and Fennell 1965, under *Aspergillus fumigatus* group), but only *N. fishceri* is at all common.

Neosartorya fischeri (Wehmer) Malloch and Cain (Fig. 5)

Anamorph: *Aspergillus fischeri* Wehmer

On CYA and MEA at 25°C, colonies 50-65 mm or more in diam., of low and sparse to moderately deep and cottony white to cream mycelium, surrounding abundant white developing cleistothecia and overlaid by scattered, usually inconspicuous blue to green conidial heads; reverse pale to yellow. At 30°C, colonies covering the whole Petri dish, similar to those at 25°C, but often deeper and more luxuriant.

Cleistothecia white, 300-400 μm diam., maturing in 1-2 weeks at 25°C; ascospores ellipsoidal, overall 6-7 × 4-5 μm, ornamented with two prominent, sinuous, longitudinal ridges and usually with other irregular ridges as well. Anamorph *Aspergillus*, with sparse conidiophores, 300-1000 μm long, terminating in small swellings, 12-18 μm diam.; phialides crowded, 5-7 μm long; conidia spheroidal, 2.0-2.5 μm diam., with finely roughened walls.

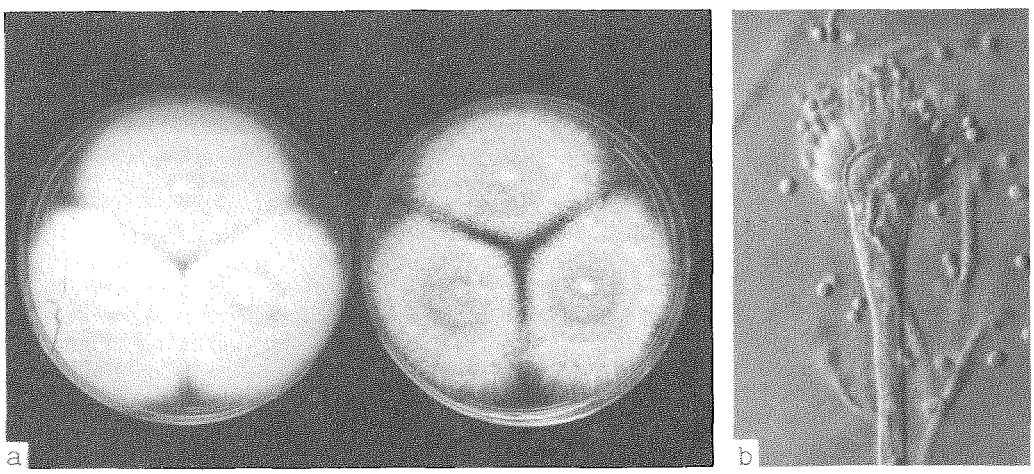

Fig. 5. *Neosartorya fischeri.* (a) colonies on CYA and MEA, 25°C, 7d.; (b) conidiophore.

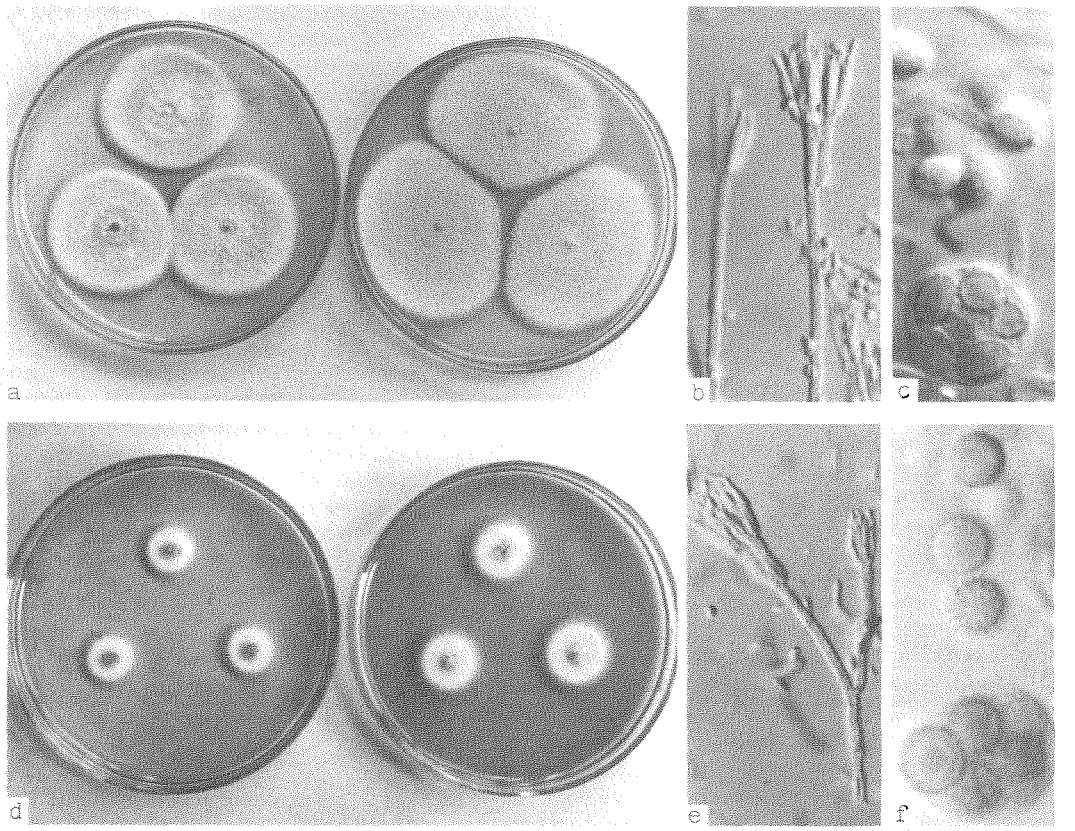

Fig. 6. *Talaromyces* species: (a)-(c) *T. flavus*; (d)-(f) *T. bacillisporus*.
T. flavus: (a) colonies on CYA and MEA, at 25°C, 7d.; (b) penicillus; (c) ascospores.
T. bacillisporus: (d) colonies on CYA and MEA, 25°C, 7d; (e) penicillus; (f) ascospores.

Distinguishing characteristics. – This is the only common heat-resistant fungus with an *Aspergillus* anamorph. Colonies grow rapidly and are persistently white; cleistothecia are abundant and produce highly-ornamented ascospores.

Genus *Talaromyces* C. R. Benjamin

The name *Talaromyces* is derived from the Greek word for a basket, and aptly describes the body in which this teleomorphic genus produces its asci. Known as a *gymnothecium*, this body is composed of fine hyphae woven into a more or less closed structure of indeterminate size (Fig. 2b).

Talaromyces is characterized by the production of yellow or white gymnothecia in association with an anamorphic state characteristic of *Penicillium*, *Paecilomyces* or *Geosmithia*. It is a genus of about 25 species, mostly soil inhabiting. By far the most commonly encountered species is *T. flavus*, and we have isolated it as a heat-resistant species quite frequently from fruit juices and fruit-based products. No significant studies on the heat resistance of ascospores of *T. flavus* or other *Talaromyces* species appear to have been published, but it is significant that all known heat-resistant isolates have possessed larger than normal ascospores. The more common, smaller-spored isolates of *T. flavus* presumably possess a much lower heat resistance.

A second species of interest here is *T. bacillisporus*, a rare fungus with a *Geosmithia* anamorph which we have isolated on several occasions in screening fruit juices for heat-resistant fungi.

Talaromyces flavus (Klöcker) Stolk and Samson (Fig. 6, a-c)

Anamorph: *Penicillium dangeardii* Pitt

At 25°C, colonies on CYA 18-30 mm diam., plane, low and quite sparse to moderately deep and cottony; mycelium bright yellow, less commonly buff or reddish brown, in most isolates concealing developing gymnothecia; clear to reddish exudate present occasionally; reverse sometimes yellow, more usually orange,

reddish or brown. Colonies on MEA 30-50 mm diam, generally similar to those on CYA but gymnothecia more abundant; reverse usually dull orange or brown, but sometimes deep brown or deep red. At 30°C on CYA, colonies 30-45 mm diam., generally similar to those at 25°C, but sometimes with white or brown mycelium or overlaid with grey conidia or with conspicuous red soluble pigment and reverse colour. At 30°C on MEA, similar to those at 25°C, usually producing abundant gymnothecia; reverse sometimes also red or olive.

Gymnothecia of tightly interwoven mycelium, bright yellow, about 200-500 μm diam., closely packed, maturing within 2 weeks; ascospores yellow, ellipsoidal, 3.5-5.0 μm long, with spinose walls. Anamorph *Penicillium*, with conidiophores borne from aerial hyphae, stipes 20-80 μm long, bearing terminal biverticillate or less commonly monoverticillate penicilli; phialides needle-shaped, 10-16 μm long; conidia ellipsoidal, 2.5-4.0 μm long, with smooth to spinulose walls.

Distinguishing characteristics. - Relatively rapidly growing, bright yellow colonies at both 25° and 30°C, and the presence of abundant yellow gymnothecia make *Talaromyces flavus* a distinctive species.

Talaromyces bacillisporus (Swift) C. R. Benjamin (Fig. 6, d-f)

Anamorph: *Geosmithia swiftii* Pitt

At 25°C, colonies on CYA 18-25 mm diam., plane, sparse, cottony; mycelium white to very pale yellow, surrounding abundant developing yellow gymnothecia; conidia sparse, greyish; reverse characteristically very dark green, but occasionally pale or brown. Colonies on MEA 18-25 mm diam., similar to those on CYA except reverse very dark greyish orange. At 30°C, colonies 20-40 mm diam., similar to those at 25°C.

Gymnothecia yellow, 80-150 μm diam., of fine, closely interwoven hyphae, maturing in 2 weeks at 30°C, but only sporadically after long intervals at 25° or 37°C; ascospores spherical, 3.5-4.5 μm diam., with spinose walls. Anamorph *Geosmithia*, with condiophores borne from aerial hyphae, stipes 20-50 μm long, with thick, smooth to rough walls; penicilli monoverticillate or biverticillate, with elements rough walled; phialides needle-shaped, 10-12 μm long and occasionally up to 15 μm, often with finely roughened walls; conidia mostly cylindrical and very narrow, 4.0-5.0 × 1.0-1.5 μm, smooth walled.

Distinguishing characteristics. - More rapid growth at 30°C than 25°C, dark green reverse colours and very narrow, cylindrical conidia make *Talaromyces bacillisporus* a readily recognized species.

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Off-flavour in packaged foods*

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Introduction

As one of the most important attributes that determine the acceptance of a foodstuff, flavour deserves more than a passing consideration because the absence of desirable flavour or the presence of an undesirable off-flavour may be the factor that determines the continued prosperity of any sector of the food industry.

Chemicals are responsible for flavour when they are present in a foodstuff above a limiting concentration – the threshold concentration (TC) – and the intensity of the flavour can be represented by the ratio of the actual concentration in the foodstuff to the threshold concentration in that foodstuff – sometimes called an 'odour unit' or 'flavour unit' (Guadagni *et al.* 1966). The TC of a chemical will be different in various foodstuffs which have different proportions of fat, water and protein. It is necessary to remember that an off-flavour, by definition, is a flavour that should not be present in a specified food, and a component which may be a desirable flavour in one matrix may be unacceptable and an off-flavour in a different one, or at a different concentration. For example, 'contents of *o*-, *m*- and *p*-cresol form the main difference between whiskies produced from peated malt and other whiskies' and 'it is possible that phenols contribute in a subliminal manner to the aroma of Scotch Whisky' (Lehtonen 1982) but the occurrence of phenols or cresols in a dairy product at levels much above their TC causes an unacceptable off-flavour defect.

This paper considers two types of off-flavour that have been observed many times in many foodstuffs in Australia during the last twenty years: styrene contamination and phenolic contamination. The examples are used to demonstrate that off-flavours can be avoided by a combination of awareness and careful practice.

*This paper is based on a talk presented to 'Horizon 90' the 16th Annual Convention of the Australian Institute of Food Science and Technology on 1 June 1983 in Melbourne. Readers should also refer to the paper 'Some flavours which industry could well do without: Case studies of industrial problems' by Dr F. B. Whitfield, which appeared on pages 96-106 of the December 1983 issue of *Food Research Quarterly*.

Techniques

As compounds responsible for flavour are usually present at concentrations in the part per billion (ppb) to part per million (ppm) range and the amount of foodstuff available for analysis is often restricted to a few grams, methods have been developed for the analysis of small amounts of material of the order of nanograms to micrograms. Typically, an analysis includes collection of a flavour extract by high vacuum distillation, vacuum-steam-distillation or headspace flushing; isolation of a flavour essence from the aqueous phase by solvent extraction or acid/base extraction; separation of and identification of the individual components by combined gas chromatography and mass spectrometry or gas chromatography with a selective detector (e.g. electron capture for chlorophenols). The choice of the method of analysis depends on the compound and the food matrix and is dictated by the flavour chemists' experience of similar taints and knowledge of the advantages and disadvantages of each method for various foodstuffs. Thus, styrene may be collected from dairy products by vacuum distillation or by headspace flushing whereas phenolic contaminants are removed from dairy products more easily by vacuum-steam-distillation using the apparatus shown in Fig. 1 (Forss and Holloway 1967). Typically, 2 l of distillate is collected in D by passing steam through 0.5-1 l of an aqueous solution or slurry of the dairy product. Refluxing the distillate in D through the vertical condenser at 0 °C allows the more volatile components to be taken off through capillary E and concentrated in 2-20 ml of water collected in trap G. Acidic and phenolic compounds remain with the less volatile or more hydrophilic components in D. The chlorophenols may be isolated by solvent extraction of the aqueous phase at high pH to remove non-acidic components followed by solvent extraction at low pH to collect the chlorophenols. Difficulties arise frequently

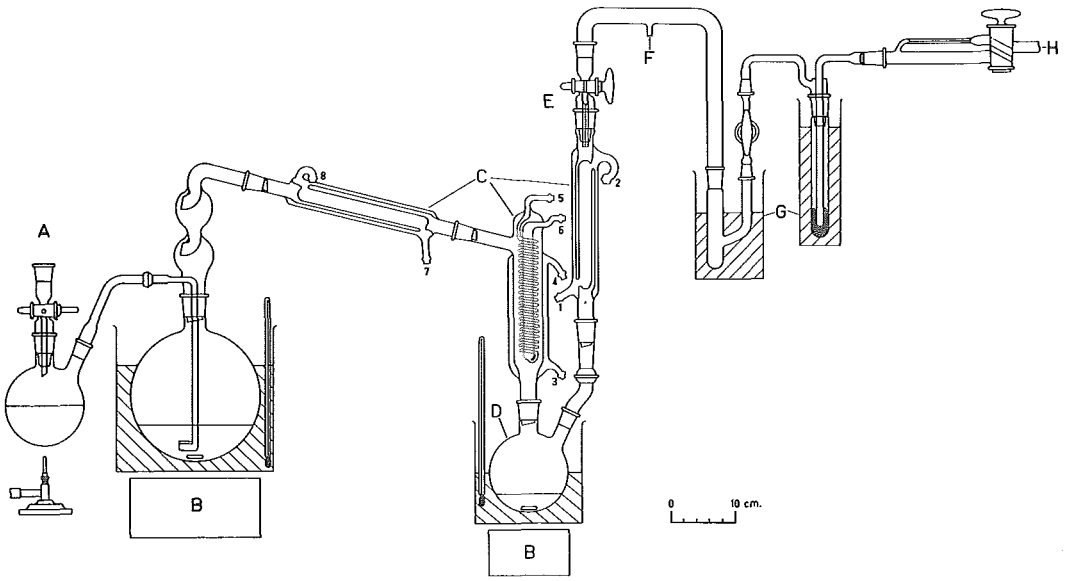

Fig. 1. Reduced pressure steam distillation of volatiles from dairy products and concentration of distillate.

when compounds which play no part in the off-flavour are isolated at the same time as the flavour-active compounds due to their chemical similarities. Thus styrene collected by vacuum distillation or headspace flushing may be accompanied by large amounts of other hydrocarbons, while phenolic compounds isolated from dairy products by steam distillation and acid/base extraction will be accompanied by large amounts of alkanoic acids. Determination by gas chromatography with an electron capture detector can be complicated by the presence of large amounts of compounds that show relatively high electron capture response (e.g. plasticizers).

As in all flavour research, it is prudent to monitor the analysis by organoleptic evaluation of the isolated extract and the residue to ensure that the off-flavour has been removed from the food and collected unaltered. For final confirmation of the identity of the flavour, the pure compound is added to the uncontaminated foodstuff to confirm qualitatively the nature of the off-flavour and quantitatively its occurrence above the TC.

Styrene contamination

Styrene has a characteristic unpleasant, plastic-like chemical odour or taste and has been blamed for several adverse health effects (see, for example, Varner *et al.* 1983). Occupational exposure to high concentrations of styrene vapour has caused eye, nose, throat and skin irritation, toxic effects on the liver, depression of the central nervous system and increase in the frequency of lymphocytic chromosomal aberrations. Polystyrene is the second most frequently used polymer for packaging foods and it may contain a residue of unpolymerized styrene monomer. At levels below a toxicological threshold, styrene may be safe for human consumption but the contamination will cause an off-flavour that will result in rejection of the foodstuff by the consumer. In the sixties, styrene was a troublesome contaminant that was isolated at the Dairy Research Laboratory (DRL) from tins of shortbread biscuits (source: plastic wrappers), fish fingers (source: transport containers) and butter (source: shipping containers). Characteristically, it was found in the corners of the surface layer of the 56 lb blocks of butter at higher levels than in the centre of the butter block (CSIRO Division of Dairy Research 1969). The off-flavour defect caused rejection of the butter by the customer.

Many studies have shown that styrene, present in packaging polymer as unreacted residual monomer, migrates into the foodstuff during storage. The strength of the off-flavour that develops depends on the concentration in the packaging polymer, type of food matrix, time of contact and TC in the foodstuff. Reported TC vary from 0.005 ppm in sour cream (Miltz *et al.* 1980) to about 5 ppm in butter (CSIRO Division of Dairy Research 1969) and in one study varied from 0.2 ppm for tea through 0.5 ppm for yoghurt to 1.2 ppm for whole milk (Jenne 1980). These results imply that the TC is higher in higher fat products

making styrene defects less noticeable in high fat products. On the other hand, styrene migrates faster from the package polymer into higher fat products. These two opposing influences prevent accurate prediction of the likelihood or strength of off-flavour development in any system except by experiment. A more useful approach is to limit the level of free styrene in the polymer: Rösli and Marek (1977) concluded that the level of styrene in dairy products rarely exceeded 0.05 ppm if the level in the polymer was below 1000 ppm. In practice, empirical standard methods of assessment have been devised to simulate storage and consumer reaction (British Standards Institution 1964, German Democratic Republic 1979, Standards Association of Australia 1983). For example, to assess the danger of the taste being transmitted in the vapour phase to a fatty food of low water content, a 10 dm^2 specimen of the packaging material is placed in a closed glass container with 50 g of grated milk chocolate. This is stored under ambient conditions for at least 48 hours together with an identical test container of a reference material free from foreign tastes and aromas. The deviation of the experimental chocolate from that of the reference is determined by a panel of at least three assessors using a scale from 0 (none) to 4 (pronounced). Such tests should become part of a code of practice agreed on between package suppliers and food manufacturers and can reduce greatly, if not eliminate, complaints of off-flavours due to packaging materials (Goldenberg and Matheson 1975).

The causes and occurrence of styrene contamination have become well-known to package and food manufacturers and the food industry has learnt to avoid them. Polymers used in packaging now contain less residual styrene monomer and shipping containers are cured more completely. Food manufacturers can select packaging materials by using the methods described in the standards referred to above or specify that the packaging material must meet the relevant standard. Nevertheless, in an innovative world where new manufacturers, new foodstuffs and new packaging ideas abound, constant vigilance is necessary to avoid styrene off-flavours and the economic loss they cause.

Phenolic contamination

Free phenols have a characteristic chemical, medicinal, or antiseptic-like odour often perceived more readily as a taste associated with a tingling sensation on the tongue. They are responsible only rarely for flavour defects in foodstuffs as they have a relatively high TC. The potential for off-flavour occurrence is increased greatly when they are chlorinated to form chlorophenols, which have a similar medicinal flavour at a much lower TC. Further reaction by methylation results in chloroanisoles which are among the most potent flavouring compounds known but which are quite different qualitatively, having musty, mouldy, earthy odours. Thus an acceptable level of free phenols (i.e. non-tainting, below the TC) may be changed during processing (e.g. chlorination by residues of in-plant sanitizers) into a potent off-flavour, causing a severe problem. Similarly, chlorophenols may be changed by fungal methylation during storage into chloroanisoles resulting in a musty, mouldy off-flavour. Although the dairy industry has learnt to avoid direct contamination from causes such as phenols in the water supply, chlorophenols in teat-dips, phenols or chlorophenols in boiler-water algicides, the food industry has not learnt to recognize the cause of musty, mouldy off-flavours as trace amounts of chloroanisoles. Indeed, it is only in recent times that sufficiently sensitive and selective methods have been available to identify positively the chloroanisoles causing musty taints in foodstuffs such as wine or milk powder.

Pentachlorophenol usage

Many of the recent problems may be traced to the use of pentachlorophenol as a biocide. As supplied, it contains up to 20% tetrachlorophenol with smaller amounts of trichlorophenol and hence, if methylated, may cause contamination with pentachloroanisole, tetrachloroanisole and trichloroanisole. PCP* is registered in the U.S.A. and the U.K. for use as a preservative, insecticide, herbicide, microbiocide, fumigant, anti-mildew agent, moth-proofing agent, paint preservative, anti-fungal agent, and slimicide (Crosby 1981). Crosby (1981) suggests that the total world production of PCP rose to 5×10^7 kg per annum with about 23×10^6 kg per annum used in the U.S.A. where 194 of the 468 wood-treatment plants used PCP (Cirelli 1978). Similarly, most of the 5-6 $\times 10^6$ lb PCP per year used in Canada were applied to the protection and preservation of wood products (Hoos 1978). In Japan, PCP has been an important herbicide and about 15×10^3 tons were used on the rice crop in 1970 but usage has decreased because of its toxicity to fish (Kuwatsuka and Igarashi 1975).

Although usage has dropped in the last few

*In this paper PCP denotes the mixture of penta-, tetra- and trichlorophenols.

years PCP does not degrade readily and sufficient is present in the environment to cause concern because of such undesirable effects as toxicity to fish. Similarly, it is being recognized slowly that PCP can be recycled, for example in PCP-treated wood or waste paper converted into packages and animal hides, scraps or waste converted into animal foodstuffs. The residues of PCP do not cause off-flavours but their conversion into anisoles, with or without dechlorination, could produce the potent musty off-flavours that cause much commercial heartache and significant loss of revenue – without a clear culprit on whom to pin the commercial loss.

Occurrence of phenolic contamination

In order to demonstrate the breadth of the problem and draw attention to the need to control all facets of the production process, the following examples have been drawn from the literature, as well as DRL experience.

Raw materials

Chicken meat and eggs developed chloroanisole-type musty flavours as a result of using litter of wood shavings contaminated with PCP and in one study 32 broiler houses had litters with mean concentrations of 53 ppm tetrachlorophenol and 12 ppm pentachlorophenol (Parr *et al.* 1974). Similar flavours were caused by feeding a proprietary feed which included fleshings from hides treated with PCP (Scott *et al.* 1981).

Gouda cheese manufactured in Holland using rennet contaminated with lactobacilli had a musty, stockyard flavour. This flavour was attributed to the production of *p*-cresol by the lactobacilli (Badings *et al.* 1968). A similar defect in Australian Cheddar cheese was analysed at DRL and in both cases *p*-cresol was isolated and was found to be above its TC of 0.3 ppm.

Mushrooms contained up to 0.2 ppm pentachlorophenol after growth in wooden containers which were found to contain up to 3400 ppm of pentachlorophenol (Meemken *et al.* 1982).

Environment

In a classic study of the cause of an off-flavour in biscuits, Goldenberg and Matheson (1975) traced a disinfectant, antiseptic soapy taste to airborne contamination from a herbicide factory several miles distant. The case history showed more than 300 complaints had been received intermittently over 11 years. Baking tests did not implicate the raw ingredients, the water supply or contamination during manufacture and processing. Eventually, the dates when the external air had a strong taint were compared with the factory records and this showed that complaints were received only for biscuits that had been actually baked, processed or handled on those days, and were unrelated to the type or age of the biscuit. The contaminant, 6-chloro-*o*-cresol, caused a clear, disinfectant-like off-flavour at a concentration of only 0.0001 ppm. This report shows how difficult it is to determine the causes of off-flavours that occur intermittently unless good factory records and a representative selection of 'library' samples are available. Chemical identification of the off-flavour component may be only one clue among many in the detective work necessary to pin-point the cause or origin of a defect.

The environment was blamed in a similar manner when cheese was matured in a room whose floors had been treated with a phenol-based resin. The cheese developed a characteristic off-flavour and was found to contain up to 30 times the TC of phenol (Toppino *et al.* 1977).

It is interesting to note an experimental study in which a piece of wood treated with preservative was placed in a closed vessel with 10 g of flour but out of physical contact (Kroyer *et al.* 1982). Over a period of 24 hours the flour picked up as much as a microgram of pentachlorophenol – a solid having a melting point of 190 °C, and a boiling point of 300 °C, but having a significant vapour pressure at room temperature (Crosby 1981). Of special importance to the food chemist is the fact that the flour picked up more pentachlorophenol than did aluminium oxide in similar conditions.

Manufacturing

Phenols or chlorophenols may contaminate a pure product by accident during manufacture when contaminated ingredients are used, e.g. gelatin may contain PCP at ppm levels (Stijve 1981). In one problem examined at DRL a large batch of product was ruined by the use of an ingredient packed in multiwall bags that had been standing on a pallet contaminated with phenol. Another cause of frequent problems in the past has been the use of phenol compounds as algicides or mould inhibitors in boiler water. A slight leak from boiler condensate or even the direct use of live steam from such boiler water has been responsible for medicinal off-flavours in the product.

Packaging

It might be expected that the purest and safest package, from a flavour viewpoint, would be a glass jar but in one investigation the lids of

preserving (Mason) jars were found to contain up to 100 μg pentachlorophenol, presumably from biocide treatment. The preserved vegetables acquired up to 40 ppm pentachlorophenol (Heikes and Griffitt 1980).

Care is required with paper bags and cartons since recycled paper may contain PCP and bags made from virgin paper were found to contain significant amounts of PCP. The PCP was concentrated along the seam. It had been added to the glue as a mould inhibitor. While PCP, as such, may not cause off-flavour in these instances, there is circumstantial evidence suggesting that it could be converted biologically to chloroanisole and cause musty flavours.

Presentation

Many studies have emphasized the dangers of off-flavours from the solvents or printing inks associated with labelling and presenting the product. These off-flavours may be avoided quite easily by prudent management and they should not cause problems. However, DRL has examined icecream that had a medicinal flavour caused by phenol. Phenol was present in the glue used to stick the label onto a plastic ice cream container and it migrated rapidly through the plastic.

Storage

Several occurrences of musty flavour in products during storage and/or transport have proved puzzling in recent years. In two instances examined at DRL products were rejected for musty flavour on arrival at their destinations while samples of the same production run, either held as 'library' samples or transported to different markets were found to be good quality and not musty. In both cases 2,4,6-trichloroanisole could be isolated from the musty-flavoured product but not from the product of good quality. In the one case followed through, the package was found to be contaminated with PCP and the problem disappeared when packages not contaminated with PCP were used. Coupled with the investigations of many workers into the formation of chloroanisole from chlorophenol by fungal action, this suggests that the chloroanisole has been formed during storage and/or transport.

It is difficult to prove the total chain of evidence unequivocally since the amounts of chloroanisole are so small that it is difficult to rule out alternative sources – probably experiments with radioactive tracers would be necessary. Nevertheless, DRL has made some progress by showing that organisms capable of metabolizing and existing on PCP could be isolated from both the products mentioned above.

Conclusions

This short presentation by no means exhausts the list of known pathways of contamination. It demonstrates that there is the potential of a very serious problem, which could cost millions of dollars. Food manufacturers must become aware of possible problems and must appreciate the value of good factory records. Modern methods of analysis are very powerful and a contaminant may be identified within days, but its identity may be of no value if the history of the product, the ingredients or the packaging are not known. In these circumstances it may not be possible even to hazard a guess at the origin of the taint or to make recommendations for its prevention, until it has occurred again. Whitfield (1983) in a recent issue of this Journal has discussed some similar off-flavours and recommended precautions to avoid phenol, chlorophenol or chloroanisole contamination. In summary, they demand that the food industry take positive steps to ensure that such compounds are not added to foodstuffs during their processing, packaging and storage. A number of products have maximum limits for PCP and it may be necessary for the food industry to enforce similar limits on raw materials from its suppliers.

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News from the Division

Review of the CSIRO Division of Food Research

It is the practice in CSIRO for Divisions to be 'reviewed' by an external committee in anticipation of the completion of a Chief's term. Accordingly the CSIRO Executive has decided that the Division of Food Research will be reviewed early in 1985, by a committee comprising the following:

Dr A. Skulberg, Director, Norwegian Food Research Institute, Ås, Norway – a food technologist and ex-Member of Parliament

Dr A. J. Bailey, Director, AFRC Meat Research Institute, Langford, UK – a biochemist and meat scientist

Mr P. Seale, Chief Chemist, Golden Circle Cannery, Brisbane – a food technologist and fruit and vegetable processor

Professor B. A. Stone, Foundation Professor and Chairman, Department of Biochemistry, La Trobe University, Victoria

Mr E. W. Barr, Chairman, Australian Dried Fruits Corporation and a former senior executive with H. J. Heinz & Co.

Dr K. A. Ferguson and Mr A. W. Charles, Director and Secretary respectively of the CSIRO Institute of Animal and Food Sciences, will be Chairman and Secretary of the Committee.

The Review Committee's terms of reference are shown on page 96.

CSIRO has invited interested organizations and individuals to send comments relevant to the review to the Committee's Secretary; the Committee itself is likely to visit all of the Division's laboratories during March, 1985.

New IIR Award

The Australian National Committee of the International Institute of Refrigeration has created an annual award to be called the James R. Vickery Award for outstanding achievements in the application of refrigeration to the preservation of foods and food products.

The use of food additives in Australia*

By K. C. Richardson

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Many social and economic changes in Australia in the last few decades have mirrored those occurring in other developed countries. These changes have led to an increasing role for manufactured and processed foods in the diet.

With this increase in consumption of foods that have been partly prepared outside the home, there has been a growing interest at many levels in the nutritional value and safety of foods, albeit accompanied by some unease about manufactured products. At times this unease develops into distrust of and even hostility towards these foods and the industry that produces them. Often, it seems that the more remote that people are from food production and preparation, the more suspicious they are about how it is done.

One aspect of this interest in and concern about the food supply has been concern over the use of food additives. This is not to suggest that the use of food additives or concern about them is new. Food additives, including some used today, have an ancient and, as critics are quick to point out, not always honourable history. If we include such substances as salt and vinegar, which are not included in the definition of preservatives in Australian food law, this history goes back still further.

It is true that with the growth in sales of convenience foods, i.e. those that require little or no preparation in the home, a greater range of foods contains additives. Without certain classes of additives many convenience foods could not be offered for sale in their present form and would never have appeared on the market.

It is also evident that the introduction of open date marking of foods has led to applications, many of which have been approved, for use of preservatives and antioxidants in foods once accepted as having a life of only a few days e.g. cottage cheese, fruit yoghurt, and 'fresh' fruit salad. Retailers and consumers now look for, and demand, shelf life of some weeks in these and similar products. Manufacturers can answer these demands but they frequently require additives to do so.

*This paper was presented to the 11th National Conference of the Australian Institute of Health Surveyors, Launceston, Tasmania, 7-12 October, 1984.

Principles governing the use of food additives

Australia uses as the basic element of its food law the system of prohibition. In this system anything that is not expressly authorized is prohibited, and the Model Food Act and Model Food Regulations developed by the National Health and Medical Research Council (1983) state: 'Save where it is expressly permitted by these regulations, the addition of a food additive ... is prohibited'. The system involves the preparation of lists of permitted food additives in order that approved substances may be added legally to specified foods.

The classes of food additives described in the Model Food Regulations are shown in Table 1.

TABLE 1
Classes of food additives described in the Model Food Regulations

Class of additive	Property of food influenced
Preservatives	Shelf life
Colourings	Appearance
Flavourings	Flavour
Antioxidants	Shelf life
Artificial sweeteners	Flavour, energy value
Vitamins and minerals	Nutritive value
Modifying agents	
vegetable gums	Texture, appearance
mineral salts	Texture, appearance
food acids	Shelf life, flavour, texture
emulsifiers	Texture, appearance
humectants	Texture, shelf life
thickeners	Texture, appearance

For many years the Commonwealth and State Governments have cooperated in health matters, including food legislation, through the National Health and Medical Research Council (NHMRC). The NHMRC relies on its Food Science and Technology Subcommittee (FST) which reports to the Food Standards Committee (FSC) for an evaluation of potential new additives and also for a continuing review

of the use of currently permitted additives.

Australian authorities, in common with similar bodies in other countries, are guided by the reports of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Although they are independent of decisions taken in other countries on the use of food additives, in no sense are local authorities isolated from what is happening at a legislative or scientific level overseas. They must, however, take into account patterns of food production and usage which may be peculiar to this country.

At the first meeting of JECFA in 1956 a number of principles were elaborated to be observed in evaluating any substance to be used as a food additive (FAO/WHO 1957). These principles can be summarized briefly as:
1) a technological need must be established for the use of an additive
2) the safety in use of the additive must be established.

Protection of the nutritive value of a foodstuff is inherent in the second principle.

Food additive legislation has been attacked on both scores. Critics argue that technological need has been interpreted far too widely, particularly with regard to colours and flavours. The same critics also argue that the results of animal feeding trials cannot be directly translated to consumption by humans, and that interactions between different dietary components are not studied adequately. Epidemiological studies are used to supplement feeding trials where additives have been in use for long periods in defined populations, e.g. saccharin, but results of these studies are often equivocal. (Fairweather and Swann 1981.)

Assessment of a food additive

With this background in mind, the FST examines applications for the use of a food additive in a specified food. The application may be for a new food additive not examined previously – these are now comparatively rare except in the artificial sweetener field – or for the use of an approved additive in a food in which it has not previously been permitted, e.g. potassium sorbate is widely used as a preservative in fruit juices, cheese in flexible packages and baked goods and its use has recently been extended to cottage cheese.

Members of the FST committee have to evaluate
i) information supplied in support of a food additive petition by the applicant
ii) the reports of JECFA
iii) the scientific literature on relevant areas of research particularly that published since the latest JECFA report, and in many instances
iv) the results of their own investigations.

An essential role of the toxicologists on the FST is to identify poor data and unsound judgments, i.e. data derived from poorly constructed or executed experiments and judgments based on such data. The banning in the U.S.A. in 1976 of the red food colour amaranth is an illustration of a decision being taken on the basis of poorly designed and conducted studies (Fairweather and Swann 1981). The study on which the ban was based was faulty in two important respects: (1) post-mortems of dead rats were not carried out in sufficient time to prevent serious autolysis, (2) at one stage in the study several rats were placed in the wrong cages thus producing interchange of some treatment and control animals.

A revised statistical analysis, which has been challenged, revealed that although there was no increase in total number of tumours in test animals, the ratio of malignant to benign tumours did increase in female rats given diets containing 3% amaranth (the highest dose). Few countries followed the American action yet this particular trial is still used to suggest that Australian authorities were less than diligent in not removing amaranth from the permitted listings (Anon 1984).

Establishing a technological need

When considering an application to use a food additive, it is usual for the FST to consider first the question of technological need. To establish a technological need, a petitioner has to demonstrate that a satisfactory product cannot be presented to the consumer by the use of alternative technology. This term is used to include physical means of processing, the use of new or more modern equipment or improved quality control.

The use of potassium sorbate as a preservative in cottage cheese, already referred to as a recent change in regulations, can be used to illustrate some of these points.

Potassium sorbate, at relatively low concentrations, is an effective preservative against mould growth. It is widely used throughout the world for this purpose and it has been allocated a relatively high acceptable daily intake (ADI*) by JECFA because of its very low toxicity in animal studies. There is no immediate concern that the potential daily intake of the additive will exceed the acceptable daily intake.

*ADI expressed as mg/kg body weight is defined as the amount of a chemical which might be ingested daily, even over a lifetime, without appreciable risk to the consumer.

An applicant who wished to use potassium sorbate in cottage cheese made reference to a number of features of modern food manufacture and distribution that were relevant to the request for the use of the preservative in a product the name of which indicates its humble origins.

Manufacture of cottage cheese is now concentrated in a few large manufacturing plants that supply one or more States. Direct deliveries from manufacturer to store are becoming uncommon as supermarket chains move to bulk warehousing. When the supermarket takes delivery of the product it requires a substantial proportion of the nominated shelf life to be intact to suit its own distribution and retailing practices. The consumer, not surprisingly, also has certain expectations about how long the product may be kept before its 'use by' date expires.

Cottage cheese is therefore anything but 'cottage' cheese in a manufacturing sense and to reach the urban shopper and consumer in consistently sound condition, some preservative is now necessary. The applicant made available details of the process, including the quality control procedures used and the steps that had been taken to minimize the initial level of mould contamination. The nature of the product precluded the use of a pasteurization step to inactivate contaminants introduced after the initial heat treatment of the ingredients.

The option of nominating a lesser shelf life, for a product free from preservative, was also addressed. It was claimed that this would lead

a) to the major distributors declining to handle the product,

or b) the eventual total rejection of the product by consumers because of its unreliable quality

The application was duly approved and in some respects typifies the changes in food production and marketing that have resulted in the increased use of food additives.

Colours and flavours

No comparable technological justification can be made for the use of colours and flavours. This does not mean that to obtain permission to use a prescribed colouring in a food a manufacturer has to demonstrate only the safety in use of that colouring.

When JECFA laid down its principles governing the use of food additives, it regarded as reasonable the use of additives to make sound food more attractive to the consumer. However, in describing situations in which the use of a food additive was not justified, the Committee set down a number of constraints that are relevant to the use of food colourings. The most important of these – and the one about which the others revolve – is that food additives may not be used to deceive the consumer. When one studies the list of standards for foods to which prescribed colourings may be added (National Health and Medical Research Council 1983), it is clear that the majority of foods listed are such that a serious question of deception does not arise, e.g. with confectionery, cordials, dessert and custard mixes, flavoured milk, ice cream and soft drinks.

However, direct applications for the use of colour (and flavour) in foods are rare indeed. Most new products containing these additives find their way on to the market under the Standard for Foods not Elsewhere Standardized (National Health and Medical Research Council 1983). This is a catch-all Standard designed to facilitate the marketing of new products without forcing manufacturers to wait for an indefinite period before gaining specific approval to market each new product. Some which survive in the market place will ultimately have a standard described for them but many more never achieve this status. The Standard for Foods not Elsewhere Standardized permits the addition of permitted colouring, flavouring and modifying agents. A manufacturer seeking to use an additive outside these classes would have to seek specific permission to do so.

Some of the objections to this broad brush approach are overcome by the requirement to list all ingredients on the labels of packaged foods. However, some foods are exempted from this requirement, e.g. alcoholic beverages and most cheeses, and the listing of food additives need not be specific. This means that class names such as 'colouring' and 'flavouring' may be used. This approach is clearly regarded as unsatisfactory for some consumers.

While added colours are examined toxicologically in the same way as other less controversial food additives, flavours, in general, are not. There is no list of permitted flavours that may be added to food although in New South Wales, for example, there is a short list of prohibited flavours containing safrole and some related compounds (N.S.W. Pure Food Act No. 31, 1908 and Regulations Thereunder, Revised Issue 1978).

The food industry defends its use of flavours and colours by arguing that, when used in a reasonable manner, they:

1. restore desirable properties lost during processing
2. overcome undesirable properties resulting from processing
3. overcome variations in properties to ensure

a consistent product
4. introduce desirable sensory properties into products that are not intrinsically appetizing (Coulson 1979).

It is this fourth use listed above that has probably caused the strongest reaction to food colours. They have become recognized as an intrinsic part of many foods regarded as less nutritionally desirable, e.g. snack foods. Concern has been expressed by some groups (Commission of the European Communities 1980) that the use of additives is permitting the manufacture of foods that encourage poor dietary habits. This would seem, however, to be a matter for education of the public rather than for regulatory control.

Adverse reactions to food additives

Cancer has always been a relatively common disease and despite public concern there is no sound evidence that its total incidence is increasing. There is an overall decline in the incidence of cancers associated with the alimentary tract in England and Wales (Alderson 1980), the United States (Shubick 1980) and Australia (Rohan and McMichael 1981). However, such trends conceal changes in the death rate from individual cancers within this group (Rohan and McMichael 1981) and epidemiological studies are being pursued in many countries (Joossens and Geboers 1981; McMichael 1980) in relation to diet and cancer.

A number of major dietary components (e.g. fat and alcohol) have been examined and associations have been found between some of them and certain types of cancer. At least one major study (Joosens and Geboers 1981) has also found an association between consumption of traditional salted foods and gastric cancer. Food contaminants of natural origin, particularly the aflatoxins, have been associated with potent carcinogenicity in man, in this case reinforcing the conclusions of many laboratory studies. The contribution to the total cancer incidence of intentional food additives, with the possible exception of some component of traditional salted foods, appears to be nil (Shubick 1980).

These findings will, however, do little to allay the fears of that section of the community that is influenced by the questionable public reporting of food safety matters.

There have, of course, been a few instances where a permitted additive has been determined to be a potential carcinogen and on these occasions permission to use the additive has been immediately withdrawn. One which comes to mind in Australia is the preservative diethyl pyrocarbonate. This was thought to break down completely to carbon dioxide and alcohol in alcoholic beverages. However, it was subsequently demonstrated that, under certain conditions, the carcinogen urethane could be formed as a reaction product and permission to use the preservative in beverages was withdrawn in 1974. With improvements in testing methodology, the possibility of other current additives being removed from permitted listing cannot be ruled out. However, since they have already met stringent requirements the possibility that, in practice, they represent a hazard is extremely remote.

Of more concern to some people is the problem posed by immediate adverse reaction to a specific food component or additive. Adverse reaction to food components may be a cause of chronic or recurrent symptoms and has been reported to affect several systems, e.g. skin, gastrointestinal tract and upper respiratory tract. Some of these reactions are true allergies, i.e. there is a demonstrable immunological response. Many are more accurately termed food sensitivity which implies there is an abnormal but reproducible reaction to the ingestion of a specific food or compound but to which there is no immunological response. Concern with food additives falls mainly into this area of sensitivity.

Recent reports (Baker, Collett and Allen 1981; Hill 1982; MacGibbon 1983) give some idea of the nature and extent of the problem and also estimates of the frequency of these adverse reactions. Knowledge of these reactions is not new (Hill 1982) but response at the regulatory level has probably been delayed for a number of reasons:

- reaction to food additives appears to be much less frequent than allergy to foods (MacGibbon 1983)
- there is no good animal model in which to study these reactions and human studies have been restricted.
- it is only recently that the severity of some of these responses, particularly asthma symptoms, have been adequately reported.

Labelling

It is now generally accepted that comprehensive listing of ingredients on the labels of packaged foods, which would include all additives as well as major components, is desirable and necessary. Since early this century State food laws in Australia have required that foods to which colour, flavour or preservative have been added must carry an appropriate declaration, e.g. preservative added. In 1980 the NHMRC produced a model ingredient labelling standard which was written into law by the individual States.

However, as noted previously, this standard permitted the use of class names such as colour or antioxidant rather than requiring the specific name, e.g. tartrazine or sodium metabisulphite.

This system is considered inadequate by those health professionals who have the responsibility for compiling diets for people suffering from food sensitivities. Consumer organizations also complain.

The food industry, however, has been against labels showing the specific name of food additives. Some objections by the industry have been based on practical grounds, notably that the formulation of a product will vary depending on availability and cost of ingredients. However, the main industry objection has been that specific disclosure of additives on the label does not acknowledge the essential chemical composition of all foods. This is seen as promoting undue concern about additives in the minds of consumers, particularly as many chemicals used as additives occur naturally, and some of these e.g. benzoates, are acknowledged as a cause of adverse reations. Functional, added concentrations of these naturally occurring additives are usually higher than would otherwise be present, however.

Furthermore, a group such as the salicylates which in at least one study (Allen *et al.* 1984) was the most common cause of adverse reactions is widely distributed in plants and vegetables.

The Food Standards Committee of the National Health and Medical Research Council in 1984 recommended that to help overcome some of the problems posed, additives should be identified on the label of the packaged foods by either code numbers or specific names in addition to class names. It was suggested that the code numbers be based on the food additive numbering system published in directives of the European Economic Community.

If these recommendations are implemented in all States, it will permit consumers to identify and avoid foods containing additives to which they believe they are sensitive. Charts listing food additives and their codes will, of course, have to be freely available. While this system will not overcome identification problems completely, it should certainly make the task of health professionals simpler than it is at the moment.

Negative labelling

Unfortunately, one response to the criticism to which the food industry has been subjected over its use of additives has been the negative claim on the label. This claim takes the form of statements such as 'No preservatives' or 'No artificial colour or flavour'. All too frequently these claims appear on the labels of foods not permitted to contain the nominated additives. By implication, products of other companies, operating under the same regulations, which do no make such a claim could contain these additives. The vast majority of canned and frozen foods are not permitted to contain additives because there is simply no need for them. Their stability and palatability is ensured by the physical processing they receive.

Even when a product is permitted to contain an additive, it is not unusual to find certain brands of the product manufactured and marketed without the additive. An example of this type of product is fruit juice. High temperature short time processing of juice and filling the cooled juice aseptically into pre-sterilized containers has eliminated the need for preservative in this type of pack. However, the equipment to execute this process is extremely expensive and it is not reasonable to require every processor of juice to avail himself of this technology. Permission to use preservative in fruit juice, where required, continues.

However, for the manufacturer not using preservative to imply, by negative labelling, that his product is perhaps safer than the opposition product damages the credibility of the entire food industry including, ultimately, his own company.

Conclusion

Providing an urbanized society with an adequate and safe food supply is not a simple matter but the food processing industry has responded well to the challenge. When individuals in such a society elect to follow different lifestyles in which food preparation and consumption patterns vary considerably the task becomes more complex. Food additives have come to play an important, but not dominant, role in this situation.

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Measuring the age of stored eggs

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Although eggs are classed as a perishable food, there is often little attention given to their storage conditions in retail outlets. Unlike meat, fish and dairy products, which have had some form of preparation for sale, eggs are sold in their natural 'package' and their condition is only evident to the purchaser at the time of use.

Egg quality

It is generally agreed that fresh eggs are more attractive in appearance, have a better flavour, and perform better in cakes and custards than eggs that have been kept for prolonged periods at room temperature. Most cooks also know that eggs deteriorate after laying and that their fresh qualities may be retained longer by means of cool storage. However, the state of freshness of eggs at the time of purchase is uncertain; it is not evident by inspection, and any 'use by' date is based on unspecified storage conditions.

This state of uncertainty about the quality of the product begins with the packer and wholesaler. 'Egg quality' may be assessed by examining a reasonably large sample of broken-out eggs, e.g. by measuring Haugh units. However, quality may vary quite widely even in fresh eggs, depending on the age and breeding of the hens (Shenstone 1967), so that these measurements will only indicate the deterioration of a stored sample if the initial quality is known. Even then it is not possible to estimate the 'equivalent age' (see below) without taking into account other factors such as egg size and water lost by evaporation.

S-ovalbumin in eggs

A unique solution to the problem of measuring age is to make use of a built-in 'biological clock' in eggs, which was discovered in this laboratory 20 years ago (Smith and Back 1962); the reaction in which ovalbumin, the main protein in egg white, is converted to a more heat-stable form, S-ovalbumin. This reaction occurs when the pH of the egg white rises above 8.5, which it normally does in about three days after laying, reaching a steady value of 9.0 to 9.3. After this, the rate of conversion depends only on temperature, and the rates at different temperatures may be compared by specifying the times taken for half the ovalbumin to be converted to S-ovalbumin. These half-times are shown in Table 1.

The times at 20°C and 30°C are similar in magnitude to the 'half-times' of acceptable quality in eggs kept at different temperatures (Coote *et al.* 1966). It was at one time considered that there was a connection between storage changes such as the thinning of the thick white and the weakening of the yolk membrane, and the conversion to S-ovalbumin (Smith and Back 1965). This theory has not been supported by later investigations, and the only effect of

TABLE 1
Rates of conversion of ovalbumin to S-ovalbumin *in vitro*

Temperature (°C)	Time for half conversion at	
	pH9.0	pH 9.5
20	13 d	9 d
30	89 h	47h
40	27 h	11 h
50	8.8 h	2.6 h

increased proportions of S-ovalbumin on the culinary properties of eggs is now considered to be a loss of gelling or thickening properties (Smith and Nguyen 1983).

If we measure the proportions of ovalbumin and S-ovalbumin in a sample of egg white, we may calculate the time required at a particular temperature to give this amount of conversion. We can call this the equivalent age at, say, 20°C. (We assume that the pH of the white has risen normally; however, treatments such as oiling or refrigeration, which slow deterioration, also reduce the equivalent age.)

In the early work (Smith and Back 1965) the amount of conversion was calculated from the change in solubility of the isolated ovalbumin after heating at 73.5°C and pH 7.0 for 60 mins. Since S-ovalbumin is more heat-resistant than ovalbumin, the solubility of the protein in a heated mixture increases (from 12% to 84% under the above heating conditions) as the proportion of S-ovalbumin increases and less protein is denatured on heating. More recently, the proportions of ovalbumin and S-ovalbumin in egg white have been obtained by differential scanning calorimetry. Using this technique Donovan and Mapes (1976) found that S-ovalbumin is itself two components with different heat stabilities. This has not changed the calculation of reaction times based on the decrease in ovalbumin content. Scanning calorimetry has confirmed the choice of heating temperature at which the difference between ovalbumin and S-ovalbumin may be best resolved, but the method is unsuitable for routine examination of large numbers of samples and requires expensive instruments.

We have developed a method based on the earlier determination of solubility change but applicable to egg white without prior separation of the ovalbumins. It requires only simple apparatus and allows one person in a day to analyse 40–50 samples, each of which may be taken from a single egg or from bulked whites. Since the method does not require knowledge of the initial quality or of the egg size, and is not affected by changes in water content, much more representative results are obtained than by Haugh unit measurements.

This project was supported by a grant from the Australian Poultry Research and Advisory Committee.

Analytical method

Reagents

Phosphate buffer. – 13.7 g $NaH_2PO_4.2H_2O$ and 43.1 g Na_2HPO_4 in 1 l. Dilute 20 times to give pH 7.5, ionic strength 0.05.

Precipitating solution. – 0.5 M NaCl plus 0.1 M Na acetate adjusted to pH 4.7 with acetic acid.

Biuret solution. – dissolve 1.8 g $CuSO_4.5H_2O$ and 7.2 g NaK tartrate. $4H_2O$ in 500 ml water, add 36 g NaOH in 360 ml H_2O, make up to 1 l.

Method

Mix the egg whites thoroughly in a food blender at low speed, taking care not to beat in air. Weigh 5 g (± 0.5 g) and add 25 ml diluted phosphate buffer. Stir for 5 min with a magnetic stirrer. Pipette 5.0 ml lots of the diluted white into two 20 ml capped tubes, and heat one tube for 30 min in a water bath kept at 75°C (± 0.5°C). Cool the tube and add 5.0 ml precipitating solution to each tube, mix, tip contents into centrifuge tubes and rinse with a further 5.0 ml precipitating solution, transferring the liquid as quantitatively as possible to the respective tubes.

Mix the contents of the centrifuge tubes and let stand for 10 min. The protein denatured during the heat treatment will appear as a flocculent precipitate, along with some other proteins not soluble at pH 4.7 which appear in both tubes.

Centrifuge the tubes (e.g. 5 min at 10 000 rpm) and carefully pipette 2.0 ml of clear solution from the top of each tube and mix it with 4.0 ml of biuret solution. Let the mixture stand for 30 min and then measure the absorbance at 540 nm. A 'blank' of 2.0 ml water and 4.0 ml biuret should be employed. Calculate the percentage soluble (S) from the absorbances of the heated and unheated solutions.

Treatment of results

With white from fresh eggs (less than 3 days old) we found that 22% of the protein remained soluble after the heat treatment, whereas with white from eggs that had been stored for longer than 6 weeks at 20°C the soluble protein was 86% of the total. Thus, at intermediate storage times, with S% soluble, the fraction A converted to S-ovalbumin is $(86 - S)/(86 - 22)$. Since the relationship between A and time, t, for a first-order reaction is $-\log A = kt$, plotting

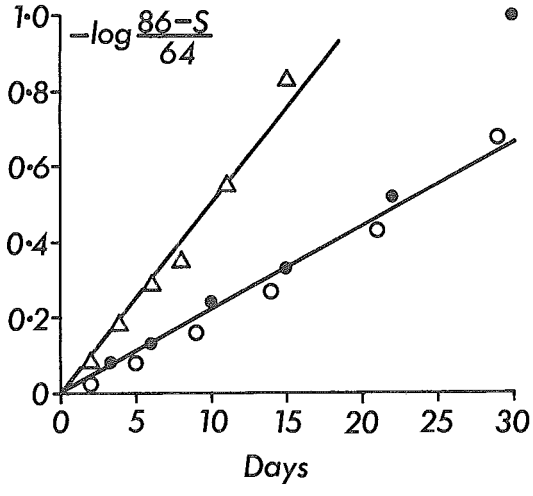

Fig. 1. Solubility change in egg white proteins after heat treatment *versus* storage time at two temperatures. △, Stored at 30°C. ● and ○, two storage experiments at 20°C.

– log A against t should give a straight line with a slope k which will vary with temperature. The results of storage experiments carried out to test this method are shown in Fig. 1. The straight-line relationship is followed up to about 30 days at 20° C and 15 days at 30°C, after which the term (86 – S) becomes too small to measure accurately. There is also some uncertainty about the zero time for the experiments, because the reaction is slow until the pH of the egg white rises to 9 (as carbon dioxide is lost through the shell). However, the half-times for the reaction (i.e. at $-\log 0.5$) are in reasonable accord with the half-times for the *in vitro* conversion at pH 9 shown in Table 1.

For a practical unit of egg deterioration we suggest that 'equivalent age at 20°C' be used, calculated, on the basis of 13 days half-life at pH 9, by means of the expression:

$$\text{Equivalent age at } 20°\text{C} = -43 \log \frac{86-S}{64} \text{ days}$$

where S is the percent soluble determined by the above method.

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Review of the CSIRO Division of Food Research

The Review Committee's terms of reference are:

(i) to assess the appropriateness of the Division's program objectives and its progress towards attaining them, the quality of the research and the Division's ordering of research priorities, and the effectiveness of its efforts to transfer the results to potential users;

(ii) to comment on the resources available to the Division (i.e. Appropriation/ contributory funding, staff, facilities, buildings), the effectiveness with which these are utilized and any additional opportunities for optimizing their use through collaboration or sharing, either internally or externally;

(iii) to review the management and internal organization of the Division, including the research leadership provided by senior staff;

(iv) to comment on any other matters peculiar to the Division that may warrant special attention, as decided by the Executive or identified by the Committee;

(v) with due regard to the responsibilities and activities of other groups in CSIRO and other organizations in Australia and overseas, to comment on the future direction and appropriate institutional arrangements for those activities for which a continuing need is identified; this may include comment on the continued existence of the Division, and the assessment and, if necessary, redefinition of the Division's role in contributing to the research needs of relevant industry sectors/ community interests appropriate to CSIRO.