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Preservative resistance of *Zygosaccharomyces bailii* and other yeasts

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Zygosaccharomyces bailii, a major cause of spoilage of acid foods in Australia, is extremely tolerant not only to preservatives, but to acid, ethanol and high sugar concentrations. At pH 3—4.5 it can grow and produce large amounts of carbon dioxide in the presence of 1400 mg benzoic acid/l, much more than the allowable concentration. Some aspects of the mechanism of uptake of preservatives and their possible effects on microorganisms growing in an acid environment are discussed. Data are given for the effects of environmental conditions on the growth and benzoic acid resistance of *Z. bailii.*

Introduction

Spoilage of preservatized beverages and other acid foods in Australia is caused largely by the yeast *Zygosaccharomyces bailii* (Pitt and Richardson 1973; Rankine and Pilone 1973), but several other species may cause problems (Table 1). Products affected are acid, pH 2.5—4.5, and may contain high levels of sugars, ethanol or carbon dioxide (Table 2). The yeasts are not known to be toxic or to produce serious off-flavours, but spoil the product either by carbon dioxide production, which may distort or explode the container, or by visible haze or sediment, which is unacceptable in wines and clear drinks.

What constitutes a preservative?

The properties desired are shown in Table 3. A weak acid with a moderately lipophilic undissociated form is required for certain important effects on the micro-organism. Expense and water solubility are practical considerations and the other factors pertain to safety and acceptability. In practice, only a few substances sufficiently fulfil these criteria. They are: acetic, propionic, sorbic and benzoic acids and sulphur dioxide. These compounds have been very extensively tested and widely used. Recently it has been found that a few individuals can be sensitive to one or other of these preservatives, but generally their toxicity is very low and they are free from the major intolerance, allergic, toxic and nutritional problems that sometimes occur with nearly all of our normal foods. All these compounds occur naturally, sometimes in high concentrations.

TABLE 1

Yeasts that spoil preservatized acid foods

Zygosaccharomyces bailii
Zygosaccharomyces bisporus
Zygosaccharomyces rouxii
Pichia membranaefaciens
Candida krusei
Brettanomyces spp
Torulopsis spp
Schizosaccharomyces pombe

TABLE 2

Foods spoiled by preservative-resistant yeasts

Cordial	Salad dressing
Soft drink	Mayonnaise
Wine	Tomato sauce
Cider	Soy sauce
Fruit concentrate	Cocktail mix

TABLE 3

Properties required for weak acid type preservatives

Weak acid
Lipophilic form
Water soluble
Non-toxic
Tasteless
Odourless
Inexpensive

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Although there are important individual differences between each preservative, their major physiological effects are similar. Also, yeast species that are resistant to one tend to be resistant to the others as well (Table 4, from Warth 1985). The first four species in Table 4 are common yeasts and were found to be sensitive to each preservative. *S. cerevisiae* is of interest in that the bakers strain was sensitive, but the wine strain, which would have been exposed to sulphur dioxide was more resistant, not just to sulphur dioxide but to benzoic and sorbic acids also. The other species were isolated from foods spoiled in the presence of benzoic acid or sulphur dioxide, and are generally resistant.

Mode of action and effects of preservatives

These preservatives owe their effectiveness to the undissociated form which, being lipophilic, readily penetrates the cell membrane. Once inside the cell it dissociates in accordance with the internal pH (Fig. 1). Assuming that the cell membrane is not permeable to the preservative anion, as is typical for most cells, it is readily calculated that, at equilibrium, the ratio of the

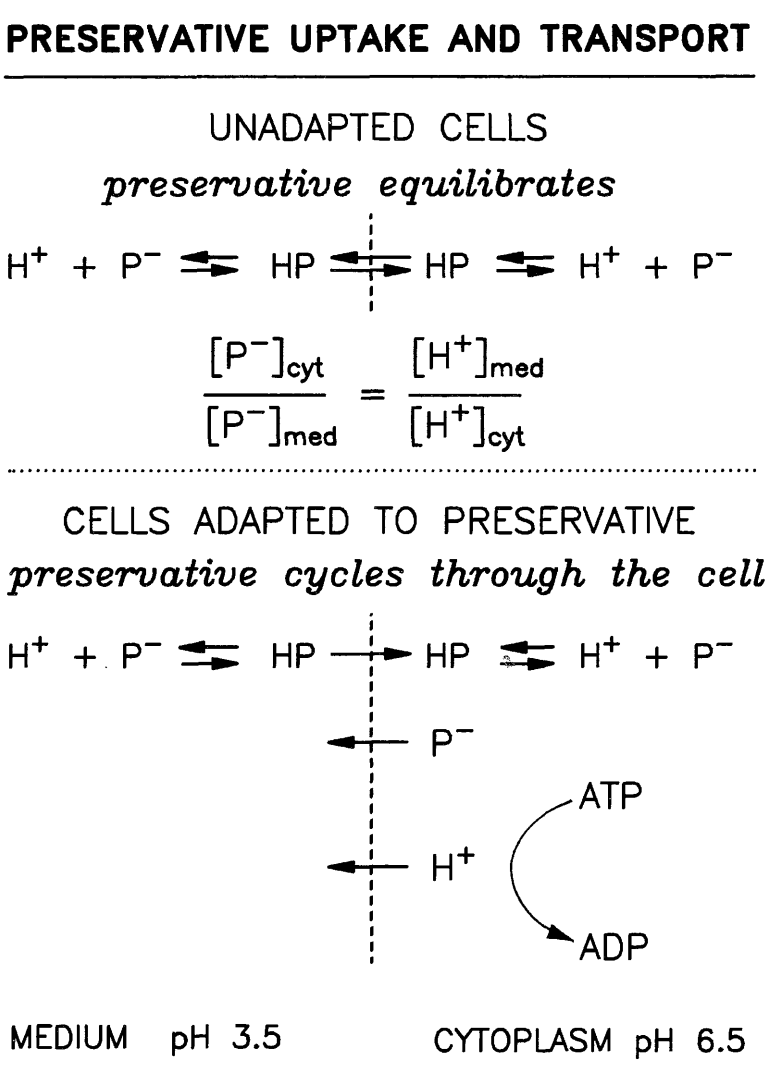

Fig. 1. Preservative accumulation and transport by cells. Unadapted cells are permeable to undissociated preservative, but not to the anion. At equilibrium, preservative again accumulates in the cytoplasm because of the pH difference. Cells grown in the presence of preservative adapt by forming a transport system for preservative anion. This enables the cell to reduce the internal concentration of preservative, but requires energy to maintain cytoplasmic pH.

TABLE 4

Maximum concentrations of preservative permitting growth[A]

Species	Sorbic acid (mM)	Benzoic acid (mM)	Free SO_2 (mM)
Klockera apiculata	1	1.5	0.05
Hansenula anomala	1	0.5	0.48
Kluveromyces fragilis	1	1	0.34
Saccharomyces cerevisiae	1	0.7	0.14
S. cerevisiae (wine)	2	2	0.51
Candida krusei	3	3	0.48
Saccharomycodes ludwigii	3	3	2.2
Schizosaccharomyces pombe	4	4	1.9
Zygosaccharomyces bailii	2	2	2.8
Z. bailii FRR1292	4	4	2.6

[A]Anaerobic growth from unadapted cultures at pH 3.5.

concentration of preservative anion inside to that outside the cell is inversely proportional to the concentrations of hydrogen ion (Fig. 1). In a medium of pH 3.5, a cell with a cytoplasmic pH of 6.5 would concentrate preservative anion 1000-fold, e.g. 3mM (363 mg/l) total benzoic acid in the medium at pH 3.5 would be in equilibrium with 0.5 mM benzoate anion in the medium and 0.5 M in the cytoplasm. In practice, such high concentrations are not realized. The cytoplasmic pH may drop because of dissociation of the preservative. The anion may leak from the cell. This displaces the equilibrium, allowing a further influx of preservative, which when it reaches the cytoplasm dissociates, releasing a proton which acidifies the cytoplasm further.

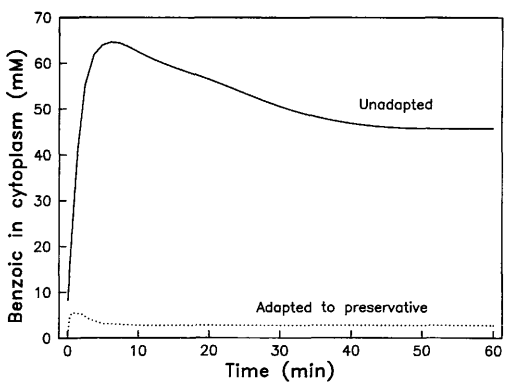

Fig. 2. Unadapted cells of *Z. bailii* accumulate benzoic acid rapidly. Adapted cells grown in sorbic acid maintain a low content of benzoate. The medium contained 1.65 mM total benzoic acid (0.38 mM benzoate anion), pH 3.67, and 5% glucose.

Preservative anion transport

In *Z. bailii* permeability to preservative anion appears as a specific adaptation to the presence of preservatives.

Benzoic acid rapidly enters *Z. bailii* and becomes concentrated in the cytoplasm (Fig. 2), consistent with a high permeability to the undissociated preservative and a low permeability to the anion. However, it was found that growth in the presence of a preservative induced the formation of a system for transporting preservative anion (Warth 1977). A number of weak organic acids both induced the system, and are transported by it. Cells so adapted were able to avoid a high intracellular concentration of preservative by allowing the anion to escape from the cell (Fig. 2) but at the expense of using energy to eliminate the hydrogen ions that continuously enter the cell in the form of undissociated preservative (Fig. 1). When the energy supply is low, benzoate is accumulated (Fig. 3), thus saving energy. On addition of glucose, benzoate is eliminated.

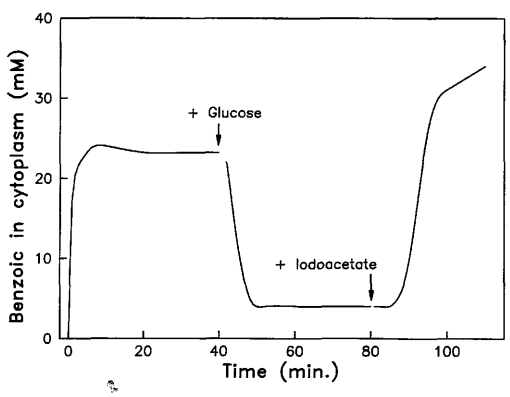

Fig. 3. Energy requirement for elimination of preservative. Starved cells of *Z. bailii* accumulate benzoate. Addition of glucose reduces the cytoplasmic benzoate concentration greatly. Addition of iodoacetate, an inhibitor of glycolysis, caused further accumulation. Medium contained 1.5 mM benzoic acid pH 3.67.

This system has been observed in a number of yeast species (Warth unpublished), and appears to be a major factor in the resistance of yeasts to preservatives.

Physiological effects on the cell

A major consequence of the anion carrier is the energy demand: preservatives stimulate fermentation (Fig. 4). At the same time cell growth is reduced, giving greatly reduced growth yields. Yeasts growing in the presence of high concentrations of preservative and

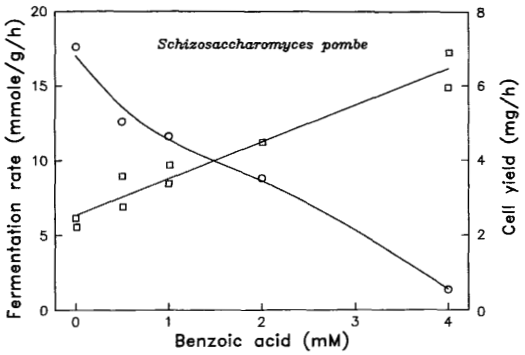

Fig. 4. Effect of benzoic acid on fermentation rate and on cell production rate in chemostat cultures of *Schizosaccharomyces pombe:* □, Rate of CO_2 and ethanol production; O, cell production.

with an adequate energy supply produce copious amounts of CO_2, yet may scarcely grow to visible cell densities. This uncoupling of respiration from nett energy production is not unique to preservatives and is well understood by biochemists. Preservatives also inhibit the active transport of nutrients into the cell (Freese and Levin 1978). In *Z. bailii,* benzoate inhibited proline transport (Warth 1977). Inhibition of transport could be a factor in preservative inhibition of growth of yeasts under some conditions.

Under energy starvation conditions and in species not induced for or lacking the preservative transport system, preservative anion will accumulate to high levels in the cell. The concentration of anion together with associated cations could be a significant osmotic stress on the cell, irrespective of any specific action of the preservative on enzymes, metabolites or membranes. Any direct action of preservative will depend upon the intracellular concentration and therefore will depend upon pH in a similar manner to the energy and osmotic stresses.

Sorbic and benzoic acids have been reported to inhibit a number of enzymes (Liewen and Marth 1985) and may compete with acetate for coenzyme A. However, the simple chemical nature of the preservatives suggests that highly specific inhibitions are unlikely. Little has been done to show that any particular inhibition is critical to growth or survival under ordinary conditions. Typically, the organic preservatives do not cause rapid death, and their effect may be similar to the normal loss of viability following the stationary phase of growth. On the other hand sulphur dioxide is reactive with numerous cell components (Wedzicha 1984) and can cause rapid loss in viability (Hammond and Carr 1976; Schimz 1980). Its major chemical effect is to react with acetaldehyde, formed during glycolysis, thus blocking the regeneration of NADH required for continuing glycolysis. It is therefore useless in the presence of an appreciable number of yeast cells as all the sulphur dioxide reacts with acetaldehyde and other metabolites and is then ineffective as a preservative.

The major stress of preservatives on yeasts under acid conditions appears to be maintenance of intracellular pH. At present little is known about the mechanisms by which cells maintain viability during starvation in an acid medium, although progress is being made with acidophilic bacteria (Cobley and Cox 1983). Krebs *et al.* (1983) reported that, in *S. cerevisiae,* benzoic acid lowered the intracellular pH and inhibited glycolysis by specifically inhibiting phosphofructokinase, which was particularly sensitive to low pH. However, in *Z. bailii* and *S. cerevisiae,* metabolite levels during benzoate inhibition of glycolysis did not show that phosphofructokinase or any single enzyme was uniquely responsible for the inhibition (Warth unpublished).

How do resistant species tolerate preservative?

There are many ways in which the most resistant species may need to adjust in order to cope with high preservative concentrations. Comparison of the physiology of the species in Table 4 has shown that resistant species all have the preservative transport system, and tend to have lower fermentation rates at given benzoic acid concentrations, suggesting lower permeability to benzoic acid (Warth unpublished). There was no difference in the maximum rate of fermentation between sensitive and resistant species, showing that energy-generating capacity is not a limiting factor.

Growth properties of Z. bailii

The foregoing considerations suggested that preservative resistance in yeasts may depend critically on energy supply, concentration of minor nutrients, pH, and adaptation to preservative. The following is a summary of the main growth requirements of *Z. bailii* and the effect of conditions on its resistance to benzoic acid. Most of the data are for *Z. bailii* FRR 1292, but the benzoic resistance of a number of other isolates from preserved foods was similar.

Growth was determined in microwell

plates under aerobic conditions at 25°C in 5% fructose-yeast extract medium (Warth 1977) at pH 3.5. Inocula were actively growing cells, adapted to 242 mg/l benzoic acid.

Sugar concentration

Z. bailii grows better on fructose than glucose and strains vary in their ability to ferment sucrose. Growth occurs in media containing up to 60% w/w glucose, with a water activity of 0.85. Surprisingly, sugar type and concentrations up to 30%, had little effect on resistance to benzoate (Fig. 5). Only at high sugar concentrations where growth was inhibited by reduced water activity, was benzoate tolerance lowered. Although in the absence of an added carbohydrate source growth was limited, benzoate resistance was little affected and it appears that under aerobic conditions, sufficient energy to cope with the preservative can be obtained from substrates in the yeast extract. Under anaerobic conditions in the range of 5-50% sugar, benzoate resistance was only slightly reduced.

Minor nutrients

Reduction in the concentration of yeast extract plus the inorganic nutrients to 3% of the normal concentration in the medium limited growth, but had no effect on sensitivity to benzoate. At greater dilutions, both maximum growth and resistance were considerably reduced (Fig. 6). These results show that, at concentrations of nutrients usually present in acid foods, inhibition of active transport is not an important factor in the mechanism of preservative resistance. At low nutrient levels, inhibition of transport may critically reduce growth and survival. Benzoic acid may therefore be more effective in foods deficient in minor nutrients.

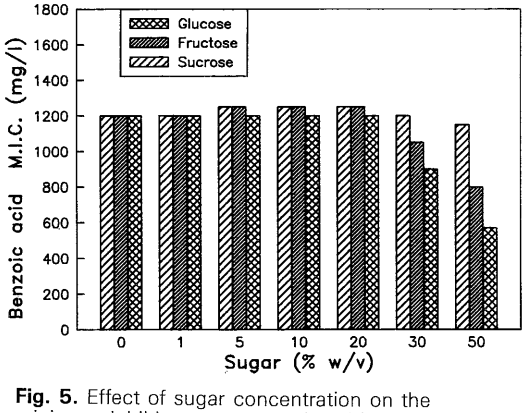

Fig. 5. Effect of sugar concentration on the minimum inhibitory concentration (M.I.C.) of benzoic acid to *Z. bailii*.

pH

The pH range for growth was 2.2—5.5 (Fig. 7) with a broad optimum between pH 3 and 4.5. The minimum inhibitory concentration (M.I.C.) of benzoic acid

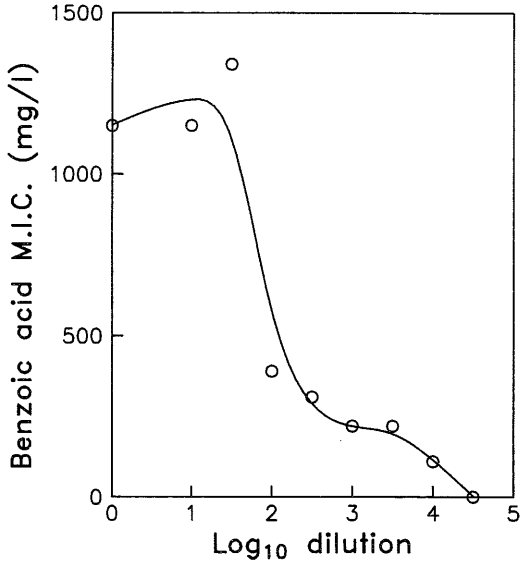

Fig. 6. Effect of concentration of minor nutrients on the M.I.C. of benzoic acid to *Z. bailii*. Initial concentrations were: yeast extract, 2 g/l; $(NH_4)_2SO_4$, 2 g/l; KH_2PO_4, 1 g/l; $CaCl_2$ and $MgCl_2$, 25 mg/l.

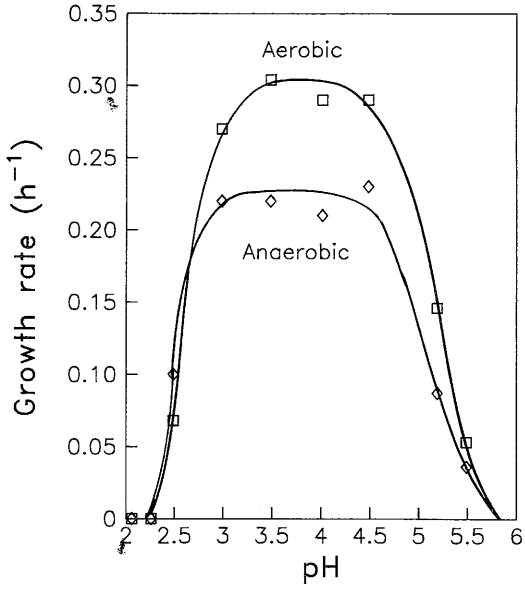

Fig. 7. pH response of *Z. bailii*.

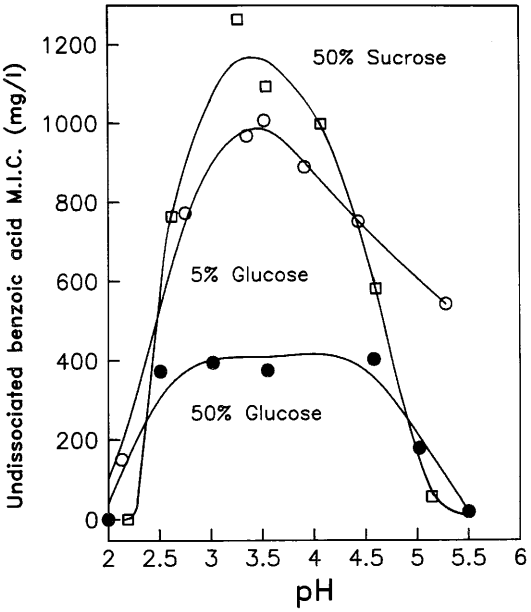

Fig. 8. Effect of pH on the M.I.C. of benzoic acid expressed as the concentration of undissociated acid.

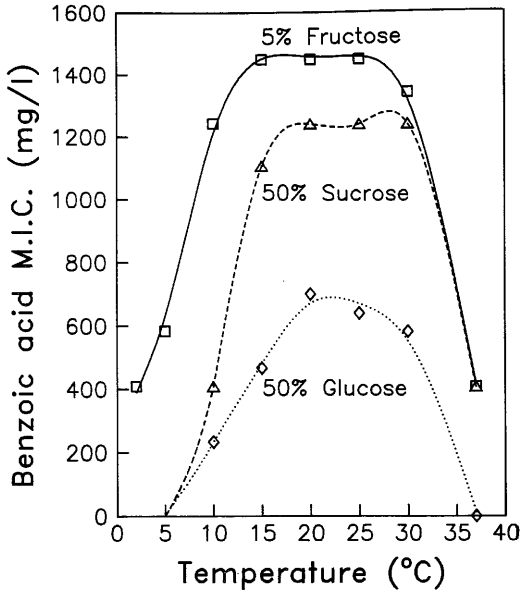

Fig. 9. Effect of temperature on the M.I.C. of benzoic acid to *Z. bailii*.

increased greatly with pH. When plotted in terms of the concentration of undissociated acid (Fig. 8), M.I.C. dependence on pH was similar to the growth response. This confirms that the concentration of undissociated preservative is strongly related to the stress on the cell due to preservative. The greater sensitivity at lower and higher pH probably reflect the reduced fitness of the organism outside its optimum pH range.

Adaptation

The maximum levels of benzoic acid permitting growth found in this laboratory (1000—1400 mg/l at pH 3.5) are considerably above those previously reported (Pitt 1974; Warth 1985). The difference is due to the use of actively growing cultures, adapted to preservative. Cultures grown in the absence of added preservative had maximum tolerances of 500—600 mg/l, and stationary phase cultures were even less resistant.

Temperature

Growth was detected over the range 2°—37°C (Fig. 9). Resistance to benzoic acid was maximal between 15° and 30°C. In the presence of less than the M.I.C. of benzoic acid, growth above 25°C was rapid, but cells quickly died. At low temperatures, cells remained viable in the presence of preservative and grew when the temperature was raised.

Ethanol

Z. bailii grew in ethanol concentrations up to 14% v/v. This is high for a wild yeast and explains the ability of *Z. bailii* to spoil finished wines. At 10% ethanol, M.I.C. to benzoic was 800 mg/l showing that ethanol reduced benzoic resistance, but not greatly. Concentrations of fructose up to 5% had little effect on the tolerance to ethanol, but in 20% and 50% fructose, the maximum ethanol concentration tolerated was reduced to 10% and 4% respectively.

Heat resistance

Ascospores had D60° values of 7—10 min in buffer at pH 4.5, and 20 min in sucrose

TABLE 5

Minimum inhibitory concentrations (M.I.C.) of preservatives to *Z. bailii*

	Unadapted M.I.C. (mg/l)	Adapted[A] M.I.C. (mg/l)
Acetic acid	12 000	20 000
Propionic acid	7 500	15 000
Hexanoic acid	800	1 400
Sorbic acid	600	1 400
Benzoic acid	600	1 200
Sorbic + benzoic acids	800	1 400
Methyl paraben	600	600

[A]Cultures adapted to 242 mg/l benzoic acid, pH 3.5.

at 0.95 a_w (Put and De Jong 1980). Vegetative cells appear to be less heat resistant.

Other preservatives

The effectiveness of other common preservatives against *Z. bailii* is shown in Table 5. Hexanoic, sorbic and benzoic acids were similar in their potency. Sorbic and benzoic appear to act additively. Adaptation increased the resistance to the weak acids, but not to methyl paraben, suggesting that induction of the anion transport system is the main feature in adaptation that is responsible for increased preservative resistance.

Recognition

Pitt and Richardson (1973) and Pitt and Hocking (1985) give convenient guides to the isolation and recognition of *Z. bailii* and other common spoilage yeasts. On malt extract agar, 2—3 mm smooth elevated round cream-white colonies are formed after 3 days at 25°C. Benzoic acid (220 mg/l) or acetic acid (0.5%) may be added. Under phase-contrast microscopy, the cells appear large and ellipsoidal with a tendency to bud near the bud scar of the mother cell. Chains and clumps are rare. After 1 week on malt agar distinctive asci are formed. Growth on 50 and 60% w/w glucose agar, and growth in media containing 440 mg benzoic acid/l at pH 3.5 are useful tests. Yeasts may be identified systematically using the methods and keys of Barnett *et al.* (1983) or Yarrow (1984).

Conclusion

In an acid environment, preservatives stress cells by making them effectively permeable to acid. This stresses the cell's mechanisms for maintenance of cytoplasmic pH and causes increased fermentation, decreased growth yield, inhibition of active transport, accumulation of preservative anion and reduction of cytoplasmic pH.

Generally, *Z. bailii* is more sensitive to benzoic acid near the limits to growth imposed by each other factor, as would be expected by a general additive effect of stresses on the cell. No strong synergisms or interactions were found. The results suggest that, under most conditions, energy substrates and minor nutrient uptake are not limiting factors for growth or survival. On the other hand the physiological fitness and adaptation to preservative of the inoculum significantly increase survival. Within the pH range 2.5—4.5, *Z. bailii* is extremely tolerant of benzoic acid over a wide range of media compositions. In addition, it is very resistant to sulphur dioxide, acetic and sorbic acids, high sugar concentrations, ethanol and to carbon dioxide.

Note: A recent paper by Thomas and Davenport (1985) discusses the spoilage activities and characteristics of *Z. bailii.*

References

Barnett, J.A., Payne, R.W., and Yarrow, D. (1983). 'Yeasts: characteristics and identification'. (Cambridge University Press: Cambridge).

Cobley, J.G., and Cox, J.C. (1983). Energy conservation in acidophilic bacteria. *Microbiological Reviews* **47,** 579-95.

Freese, E., and Levin, B.C. (1978). Action mechanisms of preservatives and antiseptics. *Developmental and Industrial Microbiology* **19,** 207-27.

Hammond, S.M., and Carr, J.G. (1976). In 'Inhibition and inactivation of vegetative microbes' (Eds. F.A. Skinner, and W.B. Hugo). (Academic Press: London).

Krebs, H.A., Wiggins, D., Stubbs, M., Sols, A., and Bedoya, F. (1983). Studies on the mechanism of the antifungal action of benzoate. *Biochemical Journal* **214,** 657-63.

Liewen, M.B., and Marth, E.H. (1985). Growth and inhibition of microorganisms in the presence of sorbic acid: A review. *Journal of Food Protection* **48,** 364-75.

Pitt, J.I. (1974). Resistance of some food spoilage yeasts to preservatives. *Food Technology in Australia* **26,** 238-41.

Pitt, J.I., and Hocking, A.D. (1985). 'Fungi and food spoilage'. (Academic Press: Sydney).

Pitt, J.I., and Richardson, K.C. (1973). Spoilage by preservative-resistant yeasts. *CSIRO Food Research Quarterly* **33,** 80-5.

Put, H.M.C., and De Jong, J. (1980). In 'Biology and activity of yeasts'. (Eds. F.A. Skinner, S.M. Passmore, and R.R. Davenport) (Academic Press: London).

Rankine, B.C., and Pilone, D.A. (1973). *Saccharomyces bailii,* a resistant yeast causing serious spoilage of bottled table wine. *American Journal of Enology and Viticulture* **23,** 55-8.

Schimz, K-L. (1980). The effect of sulfite on the yeast *Saccharomyces cerevisiae. Archives of Microbiology* **125,** 89-95.

Thomas, D.S., and Davenport, R.R. (1985). *Zygosaccharomyces bailii* — a profile of characteristics and spoilage activities. *Food Microbiology* **2,** 157-69.

Warth, A.D. (1977). Mechanism of resistance of *Saccharomyces bailii* to benzoic, sorbic and other weak acids used as food preservatives. *Journal of Applied Bacteriology* **43,** 215-30.

Warth, A.D. (1985). Resistance of yeast species to benzoic and sorbic acids and to sulfur dioxide. *Journal of Food Protection* **48**, 564-9.

Wedzicha, B.L. (1984). 'Chemistry of sulphur dioxide in foods'. (Elsevier: London.)

Yarrow, D. (1984). In 'The yeasts, a taxonomic study'. (Ed. N.J.W. Kreger-van Rij) (Elsevier: Amsterdam).

Microbiological hazards associated with fishery products*

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Introduction

In those countries that maintain useful records of food-borne disease, fishery products account for a significant proportion of the outbreaks reported. The proportion varies from one country to another, depending on climate, dietary customs and other social differences. In the USA about 11% (233) of food-borne disease outbreaks reported between 1970 and 1978 were transmitted by fish, shellfish or marine crustacean products (Bryan 1980). In Japan, where dishes based on raw seafood are extremely popular, about 70% of the cases of food poisoning that occur in summer months are caused by a single bacterial pathogen derived from fishery products, *Vibrio parahaemolyticus* (Joseph *et al.* 1982). The limited information available on food-borne disease in Australia indicates that seafoods cause about 20% of confirmed food poisoning incidents (Sutton 1973; Davey 1985).

Many substances or organisms hazardous to health can be ingested with fishery products. These include various parasites (e.g. *Anisakis*), toxic chemical pollutants (e.g. mercury, pesticides) and a variety of toxins found in fish (e.g. tetrodotoxin in puffer fish). However, diseases caused by micro-organisms, especially bacteria, constitute the largest proportion of fish and shellfish-borne diseases.

This paper will consider some of the more significant diseases caused by micro-organisms that can be transmitted by fishery products. Fish-borne and shellfish-borne diseases are frequently divided into three categories on the basis of the major source of the responsible agent (Bryan 1980): 1. agents naturally present in aquatic environments, 2. agents derived from pollution of aquatic environments, 3. agents derived from workers, equipment or the environment of food handling, processing or service establishments.

* Based on a paper presented at the International Atomic Energy Agency Regional Workshop on Commercialisation of Ionising Energy Treatment of Food held at Sydney, 1985.

Agents native to aquatic environments

Clostridium botulinum

Toxins produced by the various types of the bacterium *Clostridium botulinum* cause botulism, the well-known neuroparalytic disease affecting humans and animals. Although modern medical techniques have brought about a marked decline in mortality in outbreaks of botulism, it remains a very serious, potentially fatal disease. Its prevention is one of the most important considerations in assuring the microbiological safety of a wide range of foods. Many procedures used for the processing and storage of fishery products are designed specifically to prevent the growth of *C. botulinum.* Food-borne botulism is now relatively rare, but history has shown repeatedly that food processors who use improper procedures and cause an outbreak of botulism face severe, often ruinous, economic problems. These problems are frequently not restricted to the responsible processor, but affect a whole sector of the food industry.

The species *C. botulinum* includes a heterogeneous collection of anaerobic, spore-

forming bacteria which have in common the production of one of the characteristic protein neurotoxins that cause botulism (Hobbs 1981). The species is divided into types A to G on the basis of the antigenic specificity of the toxins. Types A, B, E, F and G cause human botulism. These types are divided into two groups, proteolytic (A,B,F) and non-proteolytic (B,E,F) on the basis of physiological characteristics. The non-proteolytic types are of particular interest in the present context because of their low minimum growth temperatures (3^0-4^0C). Proteolytic types will not grow below 10^0-15^0C. *C. botulinum* has been shown to grow well in a variety of foods. Toxin production usually accompanies growth. The toxins are heat labile and destroyed readily by many cooking processes.

C. botulinum is widely distributed in soil, aquatic environments and other habitats. It is a natural contaminant of fish, including shellfish, and may be found in both ocean and freshwater fish at the time of catching or harvest (Hobbs 1976). All *C. botulinum* types may be present in aquatic environments, but type E has been isolated from aquatic sources most frequently and is most commonly implicated in outbreaks of botulism caused by fishery products. The number of *C. botulinum* spores or vegetative cells present in freshly harvested fish is believed to be low, no more than a few per gram of flesh, although few studies have been quantitative.

The incidence of *C. botulinum* and the types present in aquatic environments depend on a variety of ecological factors and vary markedly in different geographical regions (Hobbs 1981). The cold-tolerant (psychrotrophic) type E is present in a high proportion of samples collected from some cooler areas, such as parts of the North Sea, the US Great Lakes and around the Northern Japanese Islands, but is found infrequently in tropical and sub-tropical waters. Tanasugarn (1979) examined over 2000 fish samples from the Gulf of Thailand and found type E in only 5 samples and type D in 10 samples. *C. botulinum* was found in 2.4% of 3433 sediment and seafood samples from Indonesian waters (Suhadi *et al.* 1981). Type E was not detected. The levels of contamination of Australian soils and waters with *C. botulinum* are also relatively low (Murrell and Stewart 1983) and type E appears to be quite uncommon. This may be one reason why Australia has experienced only a handful of food-borne botulism outbreaks. Although the risk that fishery products will cause botulism varies markedly from place to place, it can never be assumed that *C. botulinum* spores are not present in raw fishery products.

For food-borne botulism to occur, the following events must coincide (Eklund 1982). 1. A food must become contaminated with *C. botulinum,* usually from the environment. 2. The processing to which the food is subjected must be inadequate to inactivate the *C. botulinum* present, or the product must be recontaminated after processing. 3. The food must be held under conditions which allow *C. botulinum* to grow and produce toxin. 4. The toxic food must be acceptable to the consumer and must be eaten without cooking or after insufficient heating to inactivate the toxin. The non-proteolytic types in particular have little effect on the odour or flavour of food.

The techniques used to prevent food-borne botulism fall into two broad categories:

- The complete destruction of the spores, usually by heating, accompanied by measures to prevent recontamination of the product (e.g. canning of low-acid foods).
- Inhibition of the growth of *C. botulinum* by physical or chemical means or a combination of these (e.g. salting, smoking, etc.).

The historical record shows that fresh or frozen fishery products are low risk foods with respect to botulism, largely because of two important safety factors. The first of these is the activity of the spoilage microflora. Although some *C. botulinum* types can grow and produce toxin at refrigeration temperatures, toxin production in unprocessed fish is usually so slow at temperatures below 10^0C that the fish is rejected because of spoilage before detectable amounts of toxin are produced. In general, there is believed to be an increased safety margin between spoilage and toxin production as temperatures are reduced below 10^0C. The second safety factor is cooking, since normal cooking of raw fish substantially inactivates botulinum toxins. Nearly all of the outbreaks in which fishery products have been implicated have been due to preserved products, i.e. smoked, salted, canned or fermented, usually eaten without further cooking.

Clearly the risk of botulism can be increased by preservation processes that selectively destroy or inhibit the spoilage flora while having little lethal effect on *C. botulinum* spores and possibly enhancing future growth of *C. botulinum.* Treatments such as smoking, irradiation or modified

atmosphere storage eliminate or modify the type of spoilage typical to raw products, extend the refrigerated shelf-life of the product, and interfere with the first of the safety factors mentioned above (Eyles and Warth 1981; Eklund 1982; Genigeorgis 1985). For example, experiments performed using fish artificially inoculated with *C. botulinum* spores have shown that the radurization process can increase the potential hazard from *C. botulinum* if irradiation doses over 100 Krad (1 kGy) are employed and products are stored above 3.3°C (Eklund 1982). Toxin production in advance of spoilage has been demonstrated experimentally in several species of fish from temperate waters.

There has been considerable debate in recent years over whether this increased hazard is sufficient to preclude the use of some potentially valuable techniques, particularly radurization and modified atmosphere storage, for extension of the storage life of fresh fish. A decision on whether new techniques for preservation of fishery products create an unacceptable botulism hazard will vary with the circumstances surrounding each application. The degree of contamination of raw materials with *C. botulinum,* the nature of the product, the integrity of the cold chain, and many other factors must be considered. There is certainly no hazard if fish are held at or below 3°C, but the integrity of the cold chain cannot be assured, particularly at the retail and domestic levels of distribution and storage. Similarly, consumers cannot always be relied upon to use sensible cooking procedures.

Vibrio parahaemolyticus and other vibrios

Vibrio parahaemolyticus was first isolated from cases of gastroenteritis in Japan in the 1950s. Since then it has become widely recognized as a food-borne enteric pathogen, with reports of infection coming from all over the world. Diarrhoea is the main clinical sign of infection. The incubation period may be between a few hours and one or two days. The illness usually subsides within a few days, but in a proportion of cases persists for a week or more. Some victims can be affected quite severely.

V. parahaemolyticus infections of man usually occur as a result of the consumption of contaminated and incorrectly handled fishery products. Some outbreaks caused by commercially prepared fishery products have been very large, involving hundreds of cases. Other types of food which have become contaminated from seafood or aquatic sources have also acted as a vehicle for the illness. The organism is a part of the normal microflora of estuarine and coastal waters throughout the world. It is found in water, sediment, plankton, fish and shellfish. The level of contamination usually follows a seasonal cycle. In cooler areas the highest counts are recorded in summer and autumn and the lowest counts in winter. Several investigators have observed such a cycle in New South Wales waters. In tropical countries the seasonal cycle has been correlated with rainy and dry seasons. *V. parahaemolyticus* is found infrequently in fresh water or the open ocean (Joseph *et al.* 1982).

Since it is impossible to prevent the presence of *V. parahaemolyticus* on raw fish and shellfish, various measures must be taken by those who handle and process fishery products to prevent outbreaks of food-borne illness. Fortunately, the number of *V. parahaemolyticus* cells present in freshly harvested animals is usually well below the large number required to cause illness. In addition, the organism is readily destroyed by cooking. Therefore, the most important means of controlling infection of man is the use of appropriate hygienic procedures to prevent growth of the organism in seafoods and to prevent recontamination of cooked foods from raw seafoods. Refrigeration and freezing are the most important methods of preventing growth of *V. parahaemolyticus.* Growth ceases at about 9°-10°C and below. At higher temperatures, particularly between about 20° and 40°C, it grows very rapidly in suitable foods and can reach an infective dose within a few hours (Fig. 1). Cross-contamination between raw fishery products and cooked foods must be avoided by good sanitation in food handling establishments and strict separation of raw and cooked products. Cooling, rinsing or thawing of cooked products with sea water is also hazardous.

The incidence and pattern of infection with *V. parahaemolyticus* in different parts of the world reflects the ecology of the micro-organism and national dietary habits (Joseph *et al.* 1982). In Japan, 24% of many thousands of cases of food poisoning reported in a large survey were attributed to *V. parahaemolyticus* (Blake *et al.* 1980). Most of the cases in Japan occur in the warmer months, when the level of contamination of seafood with *V. parahaemolyticus* is highest. The high incidence of the illness in Japan is

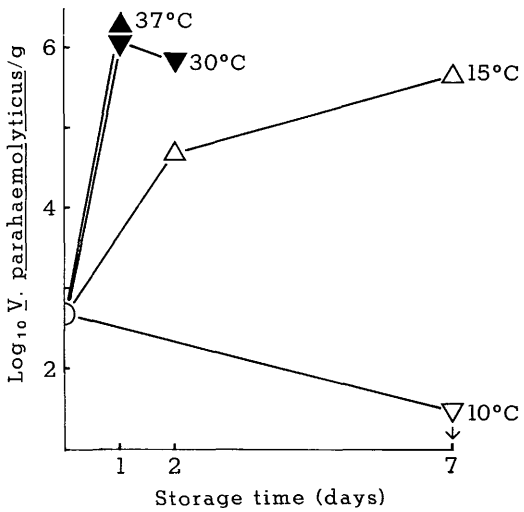

Fig. 1. Growth of *V. parahaemolyticus* during storage of opened Sydney rock oysters.

due to the national preference for raw fish and shellfish (Sakazaki 1979).
V. parahaemolyticus is known to be a common cause of gastroenteritis in several other Asian countries, such as Thailand and the Philippines (Sakazaki 1979). In countries like the USA and Australia, *V. parahaemolyticus* infections are much less frequent and constitute a minor proportion of food-borne disease. Infection is most often caused by cooked fishery products, especially shellfish such as crab, shrimp and lobster, which have been improperly cooked or recontaminated after cooking, then held at temperatures allowing growth of *V. parahaemolyticus* (Joseph *et al.* 1982).

Various other members of the genus *Vibrio* that are native to aquatic environments also appear to cause human illness when ingested with food, especially fishery products. Research on several of these organisms is at an early stage and our understanding of their ecology and pathogenicity is far from complete. *V. cholerae* is a common part of the normal microflora in brackish surface waters and may be present in oysters and other foods harvested from estuaries, etc. Gastrointestinal illness caused by *V. cholerae* has been associated with the consumption of raw seafoods and other foods. *V. cholerae* is well known, as the species includes the strains that produce cholera toxin and cause cholera. The distinction between pathogenic and non-pathogenic strains of *V. cholerae,* as with *V. parahaemolyticus*, is not clear. The public health significance of strains of both of these species isolated from environmental or food sources is often difficult to establish. Infections with *V. vulnificus* have been attributed to consumption of raw seafoods, especially oysters. *V. vulnificus* infections are not common but can be particularly dangerous. In persons with pre-existing illnesses (e.g. liver disease) the infection may result in an often fatal septicaemia. *V. vulnificus* appears to be relatively common in the estuarine and coastal environments that have been studied. These and other vibrios capable of causing enteric infections (e.g. *V. mimicus, V. fluvialis*) are present in Australian waters and cause human infections in Australia on occasions (Desmarchelier 1984). Recent reviews (Blake *et al.* 1980; Joseph *et al.* 1982; Blake 1983; Desmarchelier 1984) have discussed the characteristics and ecology of the pathogenic vibrios in detail.

Other pathogenic members of the family Vibrionaceae that are present in aquatic environments, fish and shellfish include *Aeromonas hydrophila, A. sobria* and *Plesiomonas shigelloides.* These organisms are receiving increasing attention as causative agents of gastroenteritis, including food-borne gastroenteritis. *P. shigelloides,* for example, appears to have caused several recent outbreaks of oyster-borne gastroenteritis in North America. The importance of these organisms as food-borne pathogens is unresolved.

Scombroid poisoning

Scombroid poisoning occurs world-wide and has been reported on several occasions in Australia and New Zealand (Taylor *et al.* 1984). Fresh, canned, dried, smoked and salted fish have all been implicated. The symptoms of scombroid poisoning mimic those of allergic reactions and histamine toxicity. They may include an oral burning sensation, flushing of the face and neck, headache, palpitations, rash, itching, gastrointestinal symptoms, and others. Both the incubation period (several minutes to a few hours) and the duration of the illness (<1 day) are usually brief.

Scombroid poisoning is caused by the consumption of scombroid fish (tuna, bonito, mackerel, saury, etc.) and some non-scombroid fish in which bacterial growth has taken place. The flesh of these fish contains large amounts of the free amino-acid histidine, which can be decarboxylated to histamine by certain bacteria (e.g. several members of the Enterobacteriaceae). The physiological basis for scombroid poisoning is still unclear. There is strong evidence that

histamine is important in producing the symptoms, although other compounds are also involved (e.g. cadaverine), possibly as potentiators of histamine action (Taylor *et al.* 1984). Fish incriminated in outbreaks usually contain high levels of histamine, up to several hundred mg/100 g of flesh. A level of 50 mg/100 g is considered hazardous by authorities in the USA (Taylor *et al.* 1984). Storage temperature is the critical factor influencing histamine formation (Sun Pan and James 1985). Scombroid poisoning can be prevented by rapid and uninterrupted refrigeration of susceptible fish after catching.

Dinoflagellate toxins

Certain marine dinoflagellates (photosynthetic microorganisms) produce toxins that cause disease in man. The dinoflagellates (Fig. 2) form part of the food supply of higher marine organisms and cause human disease when fish or shellfish containing hazardous quantities of the toxins are consumed. Ciguatera and some types of shellfish poisoning are diseases caused by dinoflagellate toxins that have significant public health and economic consequences. Many features of these diseases and their aetiology are still poorly understood.

The symptoms of ciguatera poisoning are complex and may appear in various combinations, with involvement of the digestive, cardiovascular and nervous systems (Ragelis 1984). Distinctive features of the illness include reversal of hot and cold sensations, tingling and numbness of the extremities, and severe pruritis. The neurological symptoms may persist for months or years. Fatalities occur in severe cases.

Ciguatera is caused by the consumption of toxic individuals of over 400 species of tropical and sub-tropical fish, predominantly from waters around coral reefs and islands. The dinoflagellate *Gambierdiscus toxicus* has been identified as an important source of ciguatoxin, the toxin believed to be the principal agent of ciguatera (Ragelis 1984). In general, outbreaks of ciguatera are sporadic and unpredictable, both in geographic distribution and time. Not all fish of the same species caught at the same time and place are toxic.

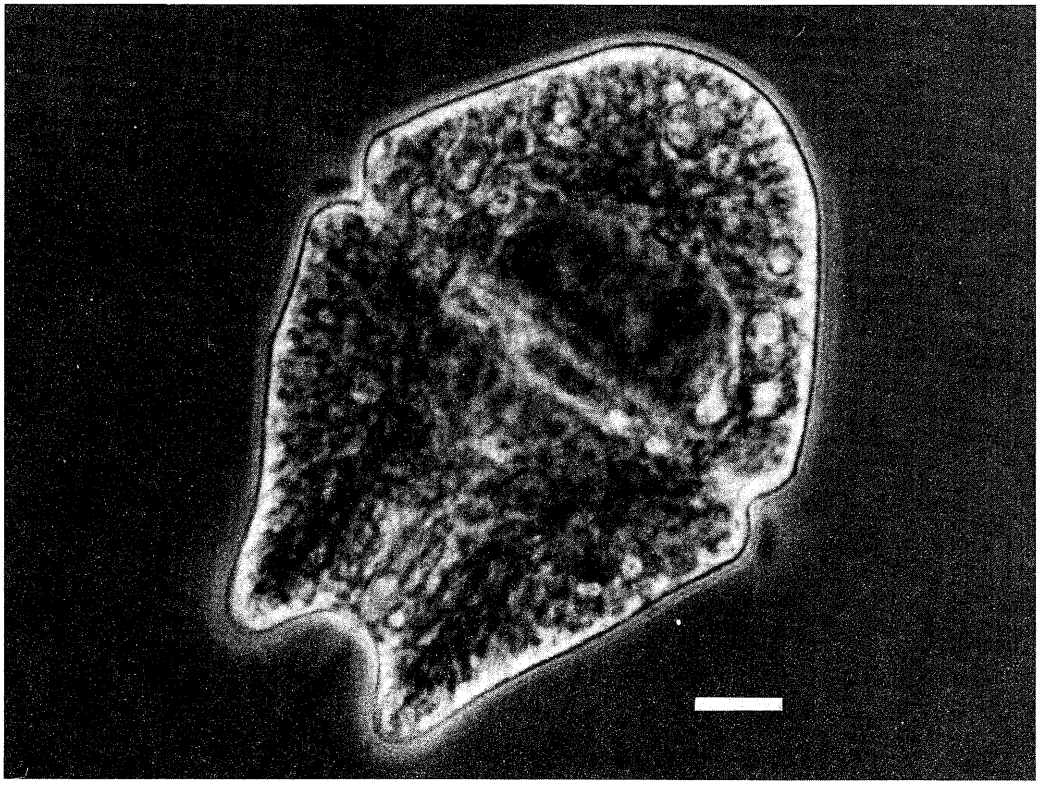

Fig. 2. A marine dinoflagellate. Dinoflagellates show considerable morphological variation from species to species. This species is not known to be toxic. (Photo J.C. Eyles). (Bar = 10 μm).

In Australia, toxic fish are frequently caught on the Great Barrier Reef (Gillespie 1980). Ciguatera has also been caused by fish from further south, principally by Spanish mackerel and other species from the Hervey Bay area. From 1976-80, at least 38 toxic Spanish mackerel, which caused 217 poisonings, came from this area (Lewis and Endean 1983). Ciguatera has also been reported in New South Wales, caused by fish from Queensland waters (Davey 1985). At present, control can be achieved only by preventing the marketing of fish likely to be hazardous. However, the value and practicality of this approach are limited in Australia and elsewhere (Gillespie 1980). There is no convenient test for identifying toxic fish, nor is the toxin destroyed by cooking, freezing, smoking, salting or drying.

Many species of shellfish may become poisonous through feeding on toxic dinoflagellates. A number of dinoflagellate species may cause illness in this manner, creating a significant public health problem in some parts of the world. Paralytic shellfish poisoning, often associated with "red tides" caused by blooms of dinoflagellates, is an important example. These toxins will not be discussed in detail in this presentation (see Bryan 1980; Ragelis 1984). Prevention of the illnesses caused by these toxins is usually achieved, where possible, by monitoring waters known to present a hazard and preventing the harvesting of shellfish at times when the toxic algae are present in the water in high concentrations.

Agents derived from pollution of aquatic habitats

Persons suffering from enteric infections excrete pathogenic microorganisms in their faeces in vast numbers. Thus, as a result of pollution of rivers, lakes and coastal waters by human wastes, aquatic animals harvested for food may become contaminated with a wide range of pathogenic microorganisms. Pathogens are frequently introduced into watercourses or coastal waters by the intentional or accidental discharge of treated or untreated sewage and by runoff from the land during rain. Although sewage treatment processes reduce pathogen concentrations in domestic sewage by varying degrees, very few of the processes in current use produce pathogen-free effluent. Agricultural wastes can also be a source of hazardous micro-organisms, for example *Salmonella* species. Many outbreaks of hepatitis A, typhoid fever, cholera and viral or bacterial gastro-enteritis, some involving hundreds of cases, have been attributed to the consumption of fish or shellfish from polluted waters (Bryan 1977, 1980; Eyles 1986). Hepatitis and several large outbreaks of viral gastroenteritis have been transmitted by oysters or mussels from Australian waters (Eyles 1986).

Several factors have made shellfish, especially bivalves, the major source of problems of this nature. Shellfish are frequently cultivated in or harvested from estuarine or coastal waters subject to pollution. Bivalves (oysters, mussels, clams) collect their food by filtration of large volumes of water, so that the contents of the bivalve digestive tract closely reflect the material suspended in the water. Bivalves may accumulate human pathogens to concentrations well above those in the surrounding water. This problem is exacerbated by the common custom of eating bivalves raw. Even when they are cooked, the cooking procedures are often too mild to inactivate all the pathogens that might be present. Cooked bivalves have transmitted both hepatitis A and gastroenteritis viruses.

Mis-handling of food after harvest is not necessarily required for disease transmission to occur by this route. The enteric viruses, such as those which cause hepatitis and gastroenteritis, do not multiply outside their human host. The dose of these agents necessary to initiate an infection is very small and the contamination acquired initially from polluted water is sufficient to cause disease. Some of the bacterial pathogens mentioned above (e.g. *Salmonella*) usually have a much larger infective dose and disease can occur only if contaminated fishery products are subjected to temperature abuse between harvest and consumption, thereby allowing growth of the pathogen.

Control of disease transmission by this route can be achieved by harvesting only from waters which are not unacceptably polluted or by subjecting shellfish or fish harvested from doubtful waters to a process that will inactivate or remote pathogens (e.g. effective heat treatment). Many countries have established procedures for controlling the quality of waters from which bivalves are harvested. These procedures involve sanitary surveys of waterways to identify sources of pollution, followed by classification of areas into various categories of acceptability. These surveys should be supported by continued monitoring of the bacteriological quality of water and shellfish.

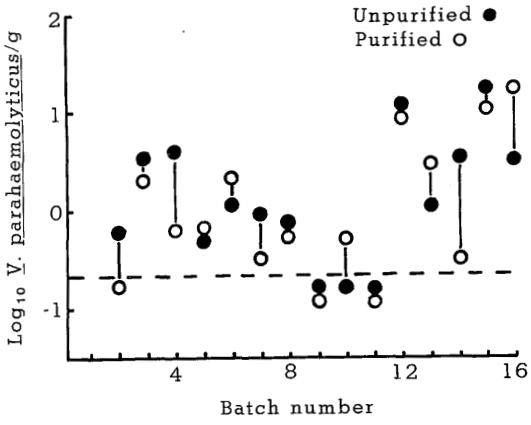

Fig. 3. *V. parahaemolyticus* in unpurified and purified Sydney rock oysters (Eyles and Davey 1984).

Control measures of this type have been introduced recently in the Tasmanian oyster industry.

Purification is a process used to remove human pathogens from bivalves. Human pathogens derived from pollution are believed to be associated transiently with bivalves. Thus, in several parts of the world, including New South Wales, live shellfish are held in unpolluted water for a short time before marketing to allow any pathogens present to be excreted by the animals. The purification process is referred to as depuration if carried out in man-made tanks of water, or relaying if an unpolluted natural waterway is used. The process does not remove some pathogens (e.g. *V. parahaemolyticus*) that are native to aquatic environments (Fig. 3) (Eyles and Davey 1984) and its ability to remove viruses reliably is doubtful (Eyles 1986).

Agents derived from contamination after harvest or catching

During processing, storage, distribution and preparation for consumption, fish or shellfish may become contaminated with a number of potentially pathogenic micro-organisms. Pathogens which cause some serious diseases, such as typhoid fever, hepatitis and cholera, can be introduced to fishery products by infected workers or polluted water in food processing or preparation establishments. Such contamination is not acceptable and, fortunately, is not common. Fishery products become contaminated much more frequently with bacteria capable of causing food poisoning, i.e. *Staphylococcus aureus, Salmonella, Clostridium perfringens, Bacillus cereus,* etc.

Bacterial food poisoning is characterized by a sudden onset of gastrointestinal symptoms, such as vomiting, nausea, diarrhoea and stomach cramps, usually within a day or two of eating contaminated food. The illness is usually caused by the consumption of food in which food poisoning bacteria have been permitted to grow to large numbers. Moist, high protein foods that do not contain excessive concentrations of acid, salt or other inhibitors (i.e. many fishery products and dishes prepared from them), provide ideal conditions for growth of food poisoning bacteria. Some contamination with food poisoning bacteria is almost inevitable during the handling, processing and preparation of fishery products for consumption. A substantial proportion of the food poisoning outbreaks attributed to fishery products is caused by food poisoning bacteria derived from utensils or equipment, the bodies of workers, food ingredients, etc. Nevertheless, good hygienic practices and temperature control can keep the degree of contamination within acceptable limits, prevent the growth of pathogens, and prevent outbreaks of illness (Davey 1985).

Staphylococcus aureus is a food poisoning organism of particular interest in the present context, since it has been one of the main causes of rejection of crustacean shellfish products by the health authorities of importing countries. *S. aureus* is a part of the normal microbial flora of the skin, nose and throat of man and can be found in particularly large numbers in skin eruptions and inflammations (e.g. boils, acne) and wounds. Staphylococci most commonly enter fishery products during processing and preparation from the bodies of workers. The small numbers of staphylococci found in food as a result of the initial contamination cannot cause illness. Staphylococcal food poisoning is caused by enterotoxins produced by the microorganisms during growth in food. Thus, illness can occur only if contaminated food is held under conditions which allow the staphylococci to grow to large numbers and produce harmful quantities of toxin.

S. aureus may be found on some raw seafoods at the time of catching or may enter raw products during primary handling and processing, e.g. filleting. Nevertheless, the staphylococci compete poorly with the normal spoilage flora of raw products and it is only if they are allowed to contaminate cooked products that they usually present a hazard. Because such a high proportion of

humans carry staphylococci on their bodies, heat-processed seafoods are very likely to become contaminated during various handling operations and final preparation for consumption. Contamination of pre-cooked crustacean products (e.g. frozen prawns) is considered to present a particularly significant hazard because these products are commonly eaten without further cooking in salads, cocktails, etc., that may be exposed to elevated temperatures before and during serving. They also provide an excellent environment for staphylococcal growth.

Control of staphylococcal contamination of food requires a high standard of personal hygiene among processing personnel, the use of procedures designed to minimize direct or indirect human contact with food, and stringent control of the temperature of the product at all stages of processing. *S. aureus* will not grow at temperatures below 6°C or above 46°C.

Conclusion

The preceding overview has demonstrated that a variety of public health problems can be caused by fishery products produced under unsatisfactory conditions. The key element in control of all these illnesses is effective education and training. Managers and supervisors must be aware of the food-borne disease problems that confront the industry and must realize that the implementation of effective hygiene and other control measures makes good economic sense. Examples of economic benefits derived from good sanitation and hygiene include: (1) prevention of the considerable losses incurred by processors who cause food-borne disease outbreaks, (2) production of a high quality product with good storage life and minimal spoilage losses, (3) elimination of rejections of unsatisfactory products by importing countries and customers, thereby expanding potential markets. Managers and supervisors must be capable of recognizing unsatisfactory situations and must be able to take appropriate remedial action or obtain professional assistance.

Workers should be trained in safe procedures for handling and processing fishery products and in proper sanitation of equipment. They should understand that fishery products can be a source of hazardous microorganisms and should know which steps in the operations they perform are critical in controlling pathogens. Managers and supervisors must motivate their employees to follow the procedures laid down. Fishermen should be able to identify potentially toxic fish in their areas and should be aware of the hazards associated with fish or shellfish taken from waters subject to pollution or growth of toxic micro-algae. Regulatory personnel often have an important part to play in the education and training of those in the industry. They must be given sufficient knowledge to enable them to carry out this role effectively and to perform their regulatory functions intelligently.

References

Blake, P.A. (1983). Vibrios on the half shell: what the walrus and the carpenter didn't know. *Annals of Internal Medicine.* **99,** 558-9.

Blake, P.A., Weaver, R.E., and Hollis, D.G. (1980). Diseases of humans (other than cholera) caused by vibrios. *Annual Review of Microbiology.* **34,** 341-67.

Bryan, F.L. (1977). Diseases transmitted by foods contaminated by wastewater. *Journal of Food Protection.* **40,** 45-56.

Bryan, F.L. (1980). Epidemiology of foodborne diseases transmitted by fish, shellfish and marine crustaceans in the United States, 1970-1978. *Journal of Food Protection.* **43,** 859-76.

Davey, G.R. (1985). Food poisoning in New South Wales 1977-84. *Food Technology in Australia.* **37,** 453-6.

Desmarchelier, P.M. (1984). Significance of *Vibrio* spp. in foods. *Food Technology in Australia.* **36,** 220-2.

Eklund, M.W. (1982). Significance of *Clostridium botulinum* in fishery products preserved short of sterilization. *Food Technology.* **36** (12), 107-12, 115.

Eyles, M.J. (1986). Transmission of viral disease by food — an update. *Food Technology in Australia.* **38,** in press.

Eyles, M.J., and Davey, G.R. (1984). Microbiology of commercial depuration of the Sydney rock oyster, *Crassostrea commercialis. Journal of Food Protection.* **47,** 703-6.

Eyles, M.J., and Warth, A.D. (1981). Assessment of the risk of botulism from vacuum-packaged raw fish: a review. *Food Technology in Australia.* **33,** 574-80.

Genigeorgis, C.A. (1985). Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish. *International Journal of Food Microbiology.* **1,** 237-51.

Gillespie, N.C. (1980). Fish poisoning causes concern in Queensland. *Australian Fisheries.* **39** (5), 28-30.

Hobbs, G. (1976). *Clostridium botulinum* and its importance in fishery products. *Advances in Food Research.* **22,** 135-85.

Hobbs, G. (1981). The ecology and taxonomy of psychrotrophic strains of *Clostridium botulinum.* In 'Psychrotrophic Microorganisms in Spoilage and Pathogenicity', (Eds. T.A. Roberts, G. Hobbs, J.H.B. Christian and N. Skovgaard) (Academic Press, London).

Joseph, S.W., Colwell, R.R., and Kaper, J.B. (1982). *Vibrio parahaemolyticus* and related halophilic vibrios. *Critical Reviews in Microbiology.* **10,** 77-124.
Lewis, R.J., and Endean, R. (1983). Occurrence of a ciguatoxin-like substance in the Spanish mackerel *(Scomberomorus commersoni). Toxicon.* **21,** 19-24.
Murrell, W.G., and Stewart, B.J. (1983). Botulism in New South Wales, 1980-1981. *Medical Journal of Australia.* **1,** 13-15.
Ragelis, E.P. (1984). 'Seafood Toxins'. (American Chemical Society: Washington).
Sakazaki, R. (1979). *Vibrio* infections. In 'Food-borne Infections and Intoxications', (Eds. H. Riemann, and F.L. Bryan) (Academic Press: New York.).
Suhadi, F., Thayib, S.S., and Sumarsono, N. (1981). Distribution of *Clostridium botulinum* around fishing areas of the Western part of Indonesian waters. *Applied and Environmental Microbiology.* **41,** 1468-71.
Sun Pan, B., and James, D. (1985). Histamine in marine products: production by bacteria, measurement and prediction of formation. FAO Fisheries Technical Paper 252.
Sutton, R.G.A. (1973). Food poisoning and *Salmonella* infections in Australia. In 'The Microbiological Safety of Foods', (Eds. B.C. Hobbs, and J.H.B. Christian) (Academic Press: London.).
Tanasugarn, L. (1979). *Clostridium botulinum* in the Gulf of Thailand. *Applied and Environmental Microbiology.* **37,** 194-7.
Taylor, S.L., Hui, J.L., and Lyons, D.E. (1984). Toxicology of scombroid poisoning. In 'Seafood Toxins', (Ed. E.P. Ragelis) (American Chemical Society: Washington.).

Recommendations for the export of onions in Fantainers

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This paper is intended to be a guide to exporters and shipping companies. It is based on research conducted by the CSIRO Division of Food Research and on past experience of the industry, and incorporates material from the earlier document, "Onions: mechanically ventilated container exports", circulated for the 1985 export season by the Australia-Europe Shipping Conference.

Introduction

Well-cured onions can be shipped to distant markets without refrigeration provided that they are mechanically ventilated with ambient air throughout the journey. Onions can be ventilated satisfactorily in the ventilated decks of roll-on-roll-off ships or in containers. Both porthole insulated containers and general-purpose containers can be ventilated by fitting them with exhaust fans; such containers have become known as 'Fantainers'. In porthole insulated containers the fan is fitted into the upper porthole, the lower porthole becomes the inlet vent (see Fig. 1), and air is distributed throughout the container through the standard 50 mm-deep T-bar floor. General-purpose containers require more extensive modifications; the fan is fitted into a hole

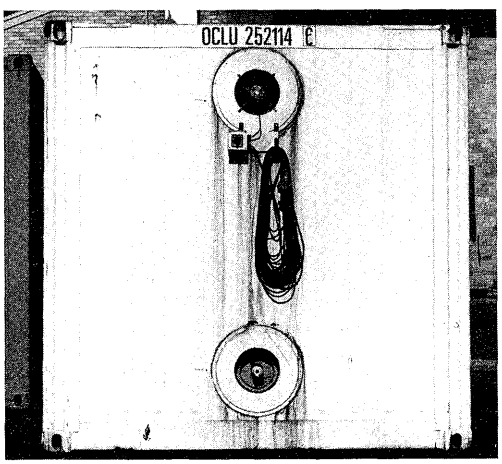


Fig. 1. Insulated porthole container fitted with exhaust fan for use as Fantainer.

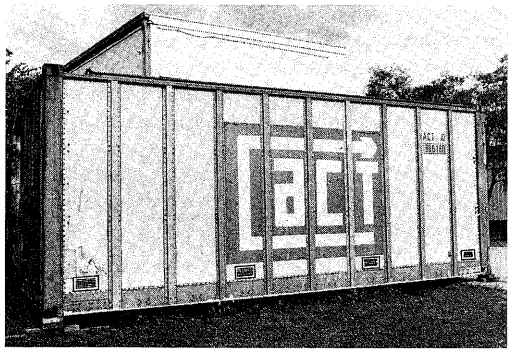


Fig. 2. General-purpose container fitted with side inlet vents.

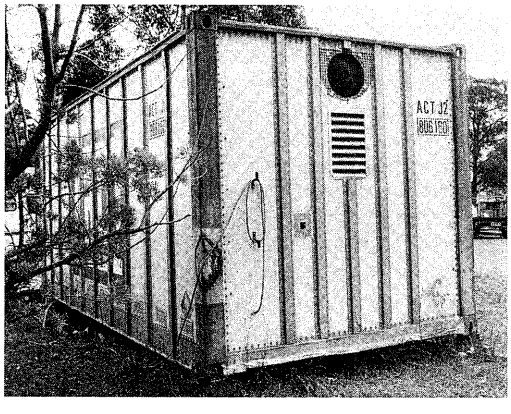


Fig. 3. General-purpose container fitted with end inlet vent.

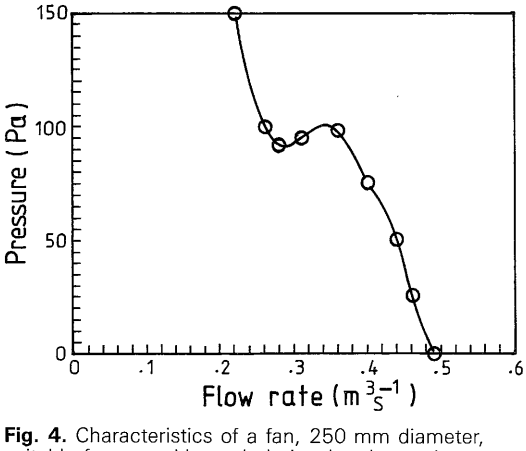


Fig. 4. Characteristics of a fan, 250 mm diameter, suitable for use with porthole insulated containers. This fan fits within the upper porthole after the internal inspection cover is hinged down. Fan type: Ebm S2D 250

cut in the upper part of the end wall, inlet vents are cut into the lower part of both side walls (see Fig. 2), and a one-trip false floor is fitted to distribute the air beneath the load. Alternatively, a single inlet can be cut into the end walls, beneath the fan (see Fig. 3), in which case the air is distributed beneath a layer of pallets on which the produce is stowed. The shipment of onions at ambient temperatures is described by Sharp and Irving (1984) and by Irving and Sharp (1986). From one experimental container, shipped in 1982, Australian onion exporters have adopted Fantainers rapidly, shipping 100 Fantainers in 1983, 173 in 1984 and 710 in 1985. Each Fantainer carries between 10 and 14 tonne of onions.

Fans

The fan should be fitted inside the container to provide it with mechanical and environmental protection, but it must be fitted in such a way that the motor assembly can be replaced from outside. The outlet must be fitted with a protective grille and telltale to indicate that the fan is operating. (Strips of brightly-coloured plastic film knotted to the grille act as a very effective telltale).

A ventilation rate of 35 air changes per hour is required (i.e. 1000 m^3/h, 0.30 m^3/s). The ventilation rate produced by the fan depends on the resistance to air flow of the inlet vents. The characteristics of a fan suitable for use with porthole containers are given in Fig. 4. The characteristics of two fans suitable for general-purpose containers are shown in Fig. 5. With the fans suggested for general-purpose containers, the inlet vents must have a total free area such that a negative pressure of about 50 Pa is obtained in the container. Baffles or louvres, fitted to the inlet vents to help exclude rain and spray, will reduce the free area for air flow, and the inlet vents must be sized to compensate for this; normally, baffles are not fitted to the inlet of porthole containers.

Electrical

The fan motors should operate on 415/440 volt, 50/60 Hz, three phase power (to suit the power supplies most commonly available in terminals and at sea).

Each container should be fitted with a suitable length of power cable (30 m is suggested), terminated in a Wilco WIP 430 power plug, or equivalent. A device to secure unused cable should be fitted to the end wall of the container. Transformers and

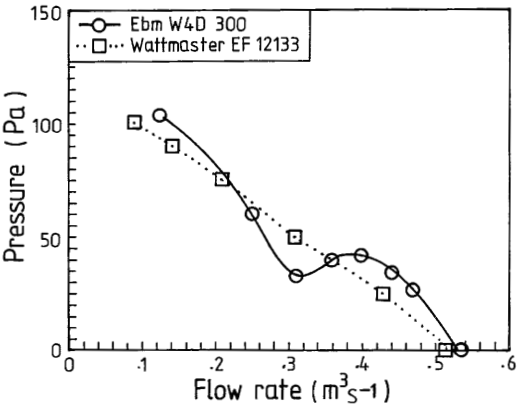


Fig. 5. Characteristics of two fans, 300 mm diameter, suitable for use with general-purpose containers.

adaptors should be provided to suit voltages and power connectors in use in other parts of the world.

Each container should be provided with a three-position reversing switch to ensure the fan exhausts air from the container to atmosphere. (Note: when reversing the direction of fan rotation, leave the switch in the central 'off' position until the blades come to rest.) The telltale attached to the grille indicates the direction of air flow.

Power distribution boards may be used to allow several fans to be connected to a single power outlet; the power requirement of each fan is less than 200 W.

At least two spare fan motor assemblies of each type of fan in use should be carried on any vessel carrying Fantainers.

Supervision

Each Fantainer should carry a label not smaller than 250 mm square indicating that it requires electrical power; a suitable label is shown in Fig. 6.

The fan should run whenever practicable, starting as soon as the container is loaded. Each container should be inspected twice each day to confirm that power is connected and the fan is operating correctly. A record of inspections should accompany the container.

Between the time of receival and loading aboard the overseas vessel, and after discharge but before delivery, the *container* operator is responsible for making and recording these inspections and for repairing or replacing defective equipment. Whilst on

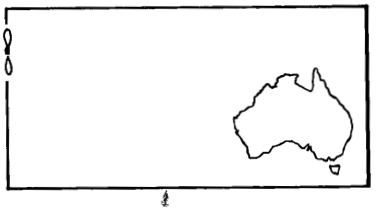
FANTAINER

AUSTRALIAN ONIONS

MUST BE CONNECTED TO POWER

(415/440 VOLT 50/60 HZ 3 PHASE)
EXCEPT WHEN BEING HANDLED

ENSURE CORRECT
FAN ROTATION



WHEN REVERSING FAN LEAVE
PHASE SWITCH IN CENTRAL OR OFF
POSITION UNTIL BLADES COME TO REST

FAN MUST EXHAUST
AIR FROM THE CONTAINER TO ATMOSPHERE

Fig. 6. Label suitable for the identification of a Fantainer.

board the export vessel, and during transshipment, the *vessel* operator is responsible for inspection, logging and repairs.

Converting containers to Fantainers

Porthole insulated containers

The fan should fit entirely within the diameter and depth of the porthole (250 mm diameter, 75 mm deep) so that it is physically protected from inside the container as well as outside. The cowling of the fan characterized in Fig. 4 can be modified to allow the fan to be fixed by expanding the cowling into the porthole. Alternatively the cowling can be discarded and the fan attached from the outside using self-tapping screws. The walls of some containers are too thin to hold such screws securely, and anchor bolts should be substituted.

The inspection cover, providing access to the upper porthole from the interior of the container, *must* be hinged down to ensure unobstructed air flow from the interior to the fan.

General-purpose containers

The fan should be fitted in the end wall of the container, at half width, and as close to the ceiling as practicable.

Two inlet vent systems may be used; both give adequately uniform distribution of air through the load.

General-purpose containers with side-inlet vents:— Equal numbers of inlet vents should be fitted in each side wall, as close to the lower side rail as is practicable, and uniformly spaced along the length of the container (Note: this supersedes a previous recommendation that the inlet vents be positioned towards the door end of the container). The required ventilation rate can be obtained using moulded plastic building vents, 200 mm x 100 mm; suitable vents are manufactured in Australia by Streamlux (model 8 3/4 x 4 1/2) and Torco (model 8-4). *The interior mesh must be removed from these vents* to ensure free air flow. The free area for ventilation is then 0.008 m^2 per vent, and eight such vents are required per container.

A false floor must be fitted, to create a free space of 100 mm beneath the stow to distribute the air. The false floor must be sufficiently strong to withstand the weight of the loading gang, as well as the dead weight of the stow.

The floor-decking should be constructed of longitudinal planks spaced no more than 40 mm from each other; timber planks of 150 x 12 mm are suitable. The outer planks should lie as close to the side walls as practicable. If the underside of these planks were lower than the top edge of the inlet vents, the planks should be angled up to allow free air flow from the vents to the under-floor space (see Fig. 7).

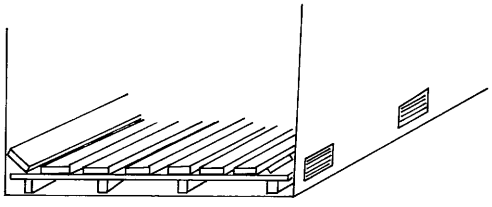


Fig. 7. General-purpose container with side-inlet vents: suggested arrangement of false floor showing side plank inclined to allow free air flow from inlet vent into floor space.

The decking should be supported on transverse joists spaced 600 mm apart, extending the full width of the container (not necessarily in one length); timber 50 x 40 mm, laid on edge, is suitable for the joists. The transverse joists should be supported on blocks or longitudinal bearers, at least four blocks or bearers across the width of the container with the outermost no closer to the walls than 200 mm; timber 50 x 40 mm is suitable, laid on edge.

A layer of pallets is not an acceptable alternative to a false floor in a container fitted with side-inlet vents, because the side rails of the pallets would restrict air flow.

General-purpose containers with an end-inlet vent:— A single inlet vent should be fitted into the end wall, beneath the fan. The total free area of this vent depends on the type of duct used inside the containers.

Either of two types of duct may be used to direct the inlet air stream into the floor space. A box-duct leaves greater usable volume within the container and may be preferred for bulk stows or hand-stacked bags. For a unitized stow, however, the space each side of a box-duct cannot be used, and a bulkhead duct, which will help stabilize the stow, may be preferred.

Australian standard pallets (1165 mm x 1165 mm) are too large to fit into a general-purpose container but pallets 1100 mm square (a standard size in Europe) are suitable. Ten such pallets stowed from the door end of the container leave approximately 300 mm, which is sufficient depth for the inlet duct.

i) Box duct:— A box duct (dimensions 630 mm wide and 265 mm deep) should be attached and sealed to the interior of the end wall. The duct must end above the underside of the floor decking to ensure free air flow. This duct can be made from a single sheet of galvanized steel, 1830 x 1220 mm (see Fig. 8).

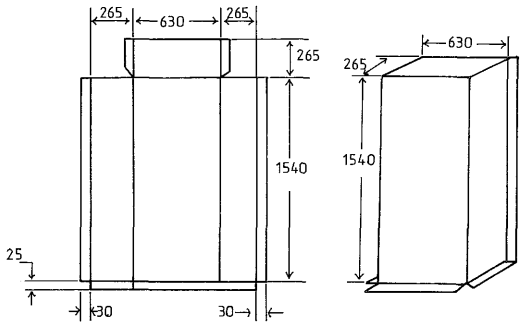


Fig. 8. General-purpose container: sheet metal duct for end-inlet vent.

The false floor should be constructed as described for the side-inlet container. Alternatively, a layer of pallets may be used as a false floor provided that gaps between the duct, the end wall and the end pallets are covered to direct the air stream without obstruction into the ends of the pallets (see Fig. 9). It is not necessary to seal gaps between pallets or between the pallets and the side walls or doors.

When carrying unitized loads in such a container, the two spaces between the end wall, the duct, and the side wall should be closed without obstructing air flow to the

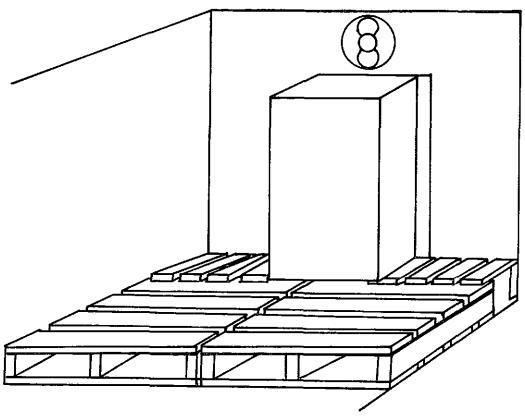


Fig. 9. General-purpose container with end inlet vent: suggested arrangement of floor using pallets over which produce will be bulk or hand stowed.

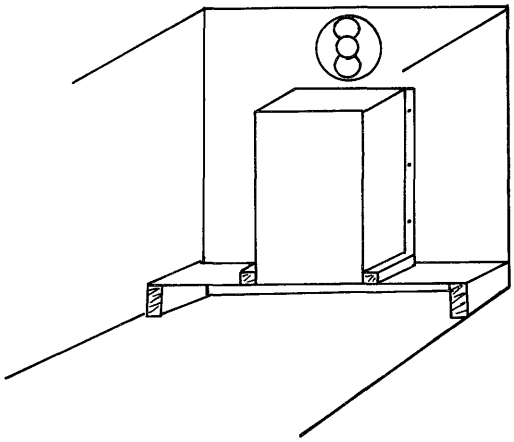


Fig. 10. General-purpose container with end inlet vent: suggested arrangement of duct and duct-wall seals to ensure unobstructed air flow beneath a unitized stow.

under-floor region. The arrangement illustrated in Fig. 10 achieves this, and also acts as an end-stop to prevent the pallets from crushing the duct. Additional bags may be stowed on this sealing plate only if it is made with appropriate strength. It is not necessary to seal gaps between pallets or between the pallets and the side walls or doors.

The free area of the inlet vent needed in conjunction with this duct and the fans shown in Fig. 5 is 0.064 m^2. If the vent is screened with light mesh, which reduces the free area by 10%, then an inlet opening of

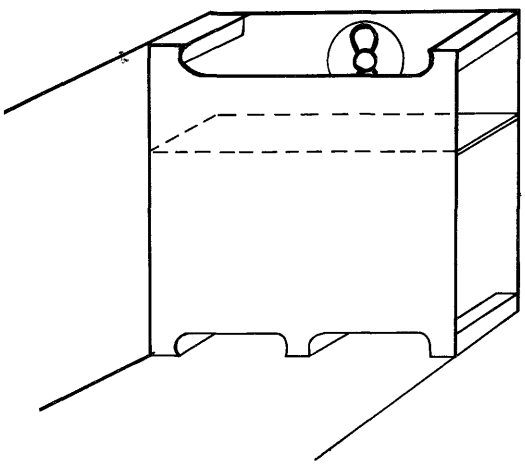


Fig. 11. General-purpose container with end-inlet vent and bulkhead-duct: note baffle, which prevents air from short-circuiting from inlet to fan. Note also braces, which together with baffle, help stabilize stow.

0.07 m^2 (i.e. 265 mm square) is needed.

ii) Bulkhead duct:— A full-sized bulkhead approximately 300 mm from the end wall, with a baffle to prevent air from short-circuiting, may be used to create a duct, as shown in Fig. 11. Cut-outs, 125 mm high and as wide as practicable, should be provided at floor height and at ceiling height to permit free movement of air into the floor-space, and from the load-space to the fan. The baffle will act as a brace between the bulkhead and the end wall, and additional braces should be provided at corners and half-way across the floor.

The free area of the inlet vent needed in conjunction with this duct and the fans shown in Fig. 5 is 0.042 m^2. If the vent is screened with light mesh which reduces the free area by 10%, then an inlet opening of 0.046 m^2 (i.e. 220 mm square) is needed.

Stowage

Onions may be hand-stacked into the container in net bags, or bulk loaded after fitting a restraining bulkhead at the door end. They may also be carried as unitized modules (wrapped pallets or bins) provided that air can flow freely through the stow, and that spaces between the pallets, and between pallets and container walls, are sealed to prevent the air-stream short-circuiting past the load.

Hand-stacked bag-stows may be packed to the ceiling, as these stows have been found to settle by approximately 100 mm. A head-space of 100 mm should be left over other stows.

All parts of the stow are sufficiently well-ventilated, without the need for any modification such as a perforated floor overlay, to maintain the quality of properly-cured onions. The floor of a porthole container, however, may be covered partially if desired, to prevent mechanical damage to the product. A suitable method is to place strips of fibreboard or polystyrene foam across the floor, leaving at least 10% of the total floor area, evenly distributed along the container, open for air flow.

References

Sharp, A.K., and Irving, A.R. (1984). The transport of onions in general-purpose containers force-ventilated with ambient air. Proceedings of the XVI International Congress of Refrigeration, Paris 1983, **4**, 383-9.

Irving, A.R., and Sharp, A.K. (1986). Transport of onions in fan-ventilated containers. Proceedings of the Postharvest Horticultural Workshop, Melbourne 1985. (In press).

Oxygen transmission rates of a plastic laminate for wrapping cheese

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Introduction

For the last few years we have used a simple spectrophotometric method for measuring the oxygen transmission rates of packaging films of various kinds and under a range of conditions (Holland *et al.* 1980; Holland *et al.* 1981). The film under test is placed above and below a photosensitive detector film, and the three films are sealed in a cell, as shown in Fig. 1. Atmospheric oxygen diffuses through the test films and reacts with constituents of the detector film; the rate of diffusion of oxygen is measured (after illumination of the cell) by the absorbance loss in the detector film.

Oxygen permeability at high humidity

Oxygen permeation values obtained recently by coulometric, gas chromatographic, and spectrophotometric methods at 100% R.H. gave good agreement for two films used for wrapping cheese, but with a third film the spectrophotometric method gave values which were up to eight times

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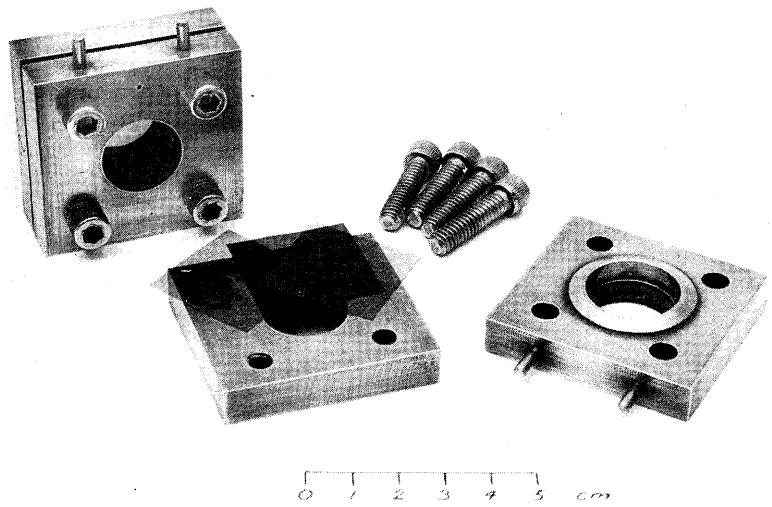

Fig. 1. Cell components and films together with an assembled cell in top left hand corner of the figure. The cell has positioning dowels for accurate placement in spectrophotometer.

higher than those obtained using the other methods. We noted that the detector films giving the high results became blotchy and clouded. This effect would give low estimates of oxygen permeability. Clearly, another factor was responsible for the overestimates, one that would require an abnormally rapid disappearance of the detector material dimethylanthracene (DMA).

The packaging material giving the abnormal results was a cellulosic film, a symmetrical laminate of cellulose/ polypropylene/cellulose coated with polyvinyl dichloride (PVDC). The cellulose layers in such films usually contain glycerol, propylene glycol or other polyhydroxy compounds. At a R.H. of, say, 75%, these deliquescent materials absorb less than their own weight of water, at 95% R.H. they absorb over six times their own weight, and the ratio increases rapidly as the R.H. approaches 100%. Even when the cellulose is coated with a highly impervious layer, such as PVDC, the film still absorbs water depending on the humidity and conditioning time. At high R.H. (97-100%), a film of liquid and small droplets form on the surface of the laminate, but not at lower R.H. The wet laminate causes the detector film eventually to become blotchy probably because the DMA becomes less soluble in the detector film with increasing wetness.

Oxygen is absorbed by the detector film only when the film is illuminated. The DMA molecules are imprisoned in the polymer matrix of the dry detector film, but in the presence of liquid they become mobile, and in the presence of light they may interact with each other, as well as with oxygen. This interaction of DMA molecules (photodimerization) occurs in solution, and leads to the loss of the characteristic U.V. absorbance peaks in a similar way to reaction with oxygen. It would appear that the rates of two reactions, photodimerization and oxidation of DMA, are similar if the film is wet.

This hypothesis was tested. First, under dry conditions the detector film gave comparable results to other methods of measuring rates of oxygen transfer. Secondly, by reducing the light intensity or exposure time to a level just sufficient to remove the incoming oxygen, correct permeability values were also obtained. This demonstrates that the photodimerization reaction occurred only when the oxygen in the film had been consumed; normally, in the dry film, excess illumination does no damage, and ensures all the oxygen in the headspace is scavenged. Thirdly, the concentration of DMA in the detector film was reduced from the usual 0.3 M to <0.15 M, when neither blotching or high permeabilities were obtained. This latter modification would probably be the simplest way to obtain a detector film that gives satisfactory results at high R.H. but the use of this film requires care when scavenging oxygen initially trapped in the system. Fourthly, detector films were made containing 1% glycerol; such films had high rates of oxygen transmission at 97% R.H.

with all types of laminates for wrapping cheese. Finally, a number of other detector materials were tried. Most proved unsatisfactory for similar reasons as for DMA, but one, diphenylanthracene, worked well under all humidity conditions, possibly because the concentration obtainable in the film matrix was somewhat lower than for DMA, or perhaps because the bulky phenyl groups hinder dimerization.

Discussion

The detector film used under normal conditions (i.e. dry) in the spectrophotometric permeability method needs modification when the film is wet. This may occur around 100% R.H. especially in the presence of cellulosic test films. Lower concentrations of DMA or diphenylanthracene (<0.15M) in the detector film are satisfactory. In many cases the problems could be circumvented by measuring test films under more appropriate humidity conditions. Even when other permeability methods are used, the possibility of leaching out components of the test film should be avoided. It is recommended that unless the test films are to be used in an environment that is wet, a R.H. lower than 100% be used for oxygen permeability measurements; standard R.H. of 75% or 94% should be suitable for most purposes.

References

Holland, R.V., Rooney, M.L., and Santangelo, R.A. (1980). Measuring oxygen permeabilities of polymer films by a new singlet oxygen technique. *Angewandte Makromolekulare Chemie* **88,** 209-21.

Holland, R.V., Rooney, M.L., and Santangelo, R.A. (1981). New methods of measuring permeabilities of packaging films. *CSIRO Food Research Quarterly* **41,** 47-50.

News from the Division

Review of the Division

The Division of Food Research underwent in March 1985 its first external review for 17 years. The report of the Committee of Review, which was considered by the CSIRO Executive in December, stated that the Division had been experiencing a period of 'unprecedented difficulty' with declines in secure funding only partly compensated for by short-term contributory funds.

The Committee concluded that, in view of the present and prospective pre-eminence of the processed food industry within the manufacturing sector, it would be in the national interest for strategic research for the food industry to be accorded a higher priority by CSIRO.

Following Executive consideration of the report, the Division is now preparing a proposal for making strategic research for the food industry a possible Executive growth area for 1987-88.

The Review Committee praised the Division's high standard of research and input into the food processing industry, which constitutes more than 20 per cent of all Australian manufacturing.

Only minor variations in structure and composition of the Division were recommended. However, a recommendation to shift responsibility for the seafood research laboratory in Tasmania to the Division of Fisheries Research was accepted by the Executive.

The report recommended the termination of only seven of the Division's 127 current projects.

Whether or not food research does become a priority research area within CSIRO, it is clear that the Division must expand its non-Treasury income if it is to adequately serve the food industry's needs. In response to review committee recommendations, customers will be charged for all but minor services and consultations. Industry will be encouraged to contract research to the Division, taking advantage of the new tax concessions which apply to such arrangements. In addition, advisory committees will be set up to increase the input from the Divison's customers into the research planning process.

Appointments

Mr Colin Chandler

Mr Colin Chandler has been appointed Communications Officer at the Meat Research Laboratory, Cannon Hill, Queensland, taking up duties on 3 March. The appointment of a "science writer" for MRL was one of the recommendations of the Review Committee (see article above). Mr Chandler gained a B.Sc. degree at the University of Queensland in 1981 with a Major in Zoology and completed his B.Sc.

(Honours) in 1982 majoring in Botany and Zoology. His background includes work as a consultant and lecturer, and as a communications officer in several Queensland Government departments.

Ms Sheila Spraggon

The New South Wales Department of Agriculture has appointed Ms Sheila Spraggon as Horticulturist (Postharvest) to be located at FRL and work as extension officer in the area of postharvest handling of fresh fruit and vegetables. Ms Spraggon holds a B.Sc. degree from the University of New South Wales. She was previously attached to the Division as part-time Technical Officer, working on Kiwifruit with

Mr K.J. Scott (New South Wales Department of Agriculture) on a Rural Credits Development Scheme grant.

Transfer

As mentioned in the first item, the Tasmanian Food Research Unit (TFRU) has been formally transferred from the Division of Food Research to the Division of Fisheries Research (Castray Esplanade, Hobart) with effect from 1 March 1986.

The group of ten people, led by Dr June Olley, will continue to work on fish as food and will maintain strong links with the Division of Food Research.

Retirement

At the end of December 1985, FRL farewelled Mrs Halina Podhaiski, on her retirement from CSIRO after 29 years of service. Mrs Podhaiski had been an invaluable member of FRL's team of microbiologists.

General

ENSBANA visitors

As reported in previous issues of the Quarterly the attachment (in 1981-82) of Dr D.J. Casimir (FRL) to the Ecole Nationale Superieure de Biologie Appliquee a la Nutrition et a l'Alimentation (ENSBANA), of the University of Dijon in France, has resulted in several ENSBANA students visiting Australia.

At present, two more students are spending around seven months at FRL: Miss Laurence Noel is working with Dr R.J. Steele and Mr Jacques Beaujeu with Dr Casimir, receiving research training in food technology and food engineering.

Consumer leaflet — multi-lingual

The most widely used of the Division's consumer leaflets, 'Handling Food in the Home' is now available in 14 languages in addition to English. It was translated into Greek, Italian and Vietnamese some years ago and used in courses for food handlers run by Local Government authorities in conjunction with the AIFST Food Microbiology Group.

With funds generously provided through the Multi-lingual Information Project of the Department of Immigration and Ethnic Affairs, the leaflet has now also been printed in Arabic, Croatian, Dutch, French, German, Lao, Polish, Portuguese, Serbian, Spanish and Turkish.

The leaflets are available free on request from the Division.