Putting the pressure on spoilage fungi

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Heat processing has been a mainstay of the food industry for many years and is used to destroy microorganisms in foods to render the foods safe and extend the shelf life. However, heat processing is detrimental to the flavour and texture of many foods, and canned foods are regarded as 'old-fashioned' by some consumers. Consequently, some manufacturers of canned fruits have moved to flexible packaging to make their product more appealing to consumers, but this does not really change the organoleptic profiles of the heat processed product.

Non-thermal food processing techniques such as high pressure processing (HPP) can be used to produce foods that are closer in flavour and texture to fresh foods, but have enhanced shelf life compared with minimally processed foods[1]. Because foods can be processed in their final packaging, the addition of preservatives is unnecessary. HPP provides a process that is comparable with pasteurisation rather than the full heat process applied in canning. Fruit products are particularly suited to HPP as they are low pH and thus not susceptible to spoilage by spore-forming bacteria which are not inactivated by pressure treatment alone.

Pears

Because HPP is a relatively new process, there is little information about the response of common spoilage yeasts and moulds to pressure treatment. As part of a project to develop a shelf-stable pear product, we undertook a study of the effects of HPP on some yeasts and moulds that commonly cause spoilage of these products.

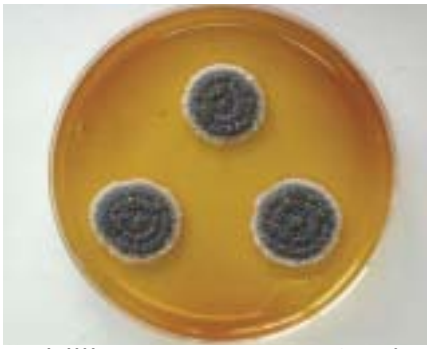
A single strain of each of two yeast and four filamentous fungi was chosen for inclusion in this study. The species examined were *Saccharomyces cerevisiae* isolated from beer; *Pichia anomala* from fermenting fruited yoghurt; *Penicillium expansum* from mouldy pears; *Fusarium oxysporum* from spoiled UHT treated fruit juice; *Byssochlamys fulva* from heat treated strawberry puree; and *Neosartorya fischeri* from heat-treated strawberries.

The two yeast species were selected because of their propensity for spoilage of fruit products and their ability to form ascospores, which may be more resistant to high pressure treatment. *P. expansum* was included because of its significance in post harvest spoilage of apple and pears and the consequent possibility of high numbers of spores on pears before processing. *F. oxysporum* has recently been causing spoilage problems in UHT processed juice products, indicating that it may be heat resistant, and therefore, possibly pressure resistant also. The two heat resistant moulds were included because it was considered important to be able to control these species if a shelf-stable fruit product were to be developed.

Cell suspensions (around 10^7 cfu/ml) were made for each species in a solution similar to that used in fruit processing: 20°Brix sucrose solution adjusted to pH 4.2 with citric acid. Yeast suspensions contained a mixture of vegetative cells and ascospores, and the heat resistant mould suspensions contained ascospores that were 14-21 days old.

The high pressure treatments were applied using apparatus available at Food Science Australia in North Ryde. The U-111 High Pressure Multi-vessel Apparatus [High Pressure Research Centre, Polish Academy of Sciences, Warsaw, Poland], comprises five separate vessels that can be pressurised simultaneously, but depressurised individually. The equipment also has the capacity for temperature control, but these

Typical budding yeast cells (not subjected to HPP!)



Penicillium expansum on Creatine Sucrose Nitrate agar

experiments were performed at ambient temperature (25°C).

For the two yeasts and *P. expansum* and *F. oxysporum*, the unit was pressurised to 400 MPa, with pressure applied for 15, 30, 45, 60 and 120s. The time to reach maximum pressure was 90s and decompression took 30s. This pressure treatment regimen was selected to allow the acquisition of data that would provide an inactivation response curve. Treatment at the pressure intended for the pear product (600 MPa) would have resulted in inactivation times that were too short to measure with the equipment available.

For the two heat resistant moulds, the pressure treatment was 600 MPa applied at the same time intervals as used for the yeasts and heat sensitive moulds, using a 2L high pressure processing unit [Flow International Corporation, USA]. The time to reach maximum pressure was 30s and decompression took <10s.

Inactivation of yeasts and heat sensitive moulds

Yeasts have been shown to be relatively sensitive to high pressure treatment [2, 3], although there has been less attention given to the spores of common food spoilage moulds. In our study, we found that yeasts and mould conidia were successfully inactivated by the chosen high pressure treatments.

For both yeast species, a 3-4 $\log_{10}$ reduction was achieved after 60s at 400 MPa (Figure 1), with a 4-5 $\log_{10}$ reduction after 120s at 400 MPa. For *P. expansum*, a 3-4 $\log_{10}$ reduction was achieved after 15-30s (Figure 2). After 60s, the reduction was >5 $\log_{10}$ and, by 120s, no survivors were detected (limit of detection 10 cfu/ml). *F. oxysporum* conidia and chlamydoconidia were even more sensitive to pressure treatment. A suspension of $2.5x10^7$ mixed microconidia and chlamydoconidia was reduced to $1.4x10^2$ cfu/ml after 15s at 400 MPa, and, after 30s, no survivors were detected.

Inactivation of heat resistant moulds

Both *B. fulva* and *N. fischeri* spore suspensions were pressure treated at 600 MPa, as heat resistant moulds are known also to be pressure resistant [4, 5]. Despite the higher pressure treatment, little inactivation of the ascospores was achieved.

B. fulva ascospores were more pressure sensitive than those of *N. fischeri* (Figure 3), with a 1.5 $\log_{10}$ reduction after 15s at 600 MPa (Figure 3a). The decrease with longer pressure treatments was more gradual, with only 2 $\log_{10}$ reduction after 60s and 2.5 $\log_{10}$ reduction after 120s. Ascospores of *N. fischeri* showed a slight reduction (less than 1 $\log_{10}$) after 15s at 600 MPa (Figure 3b). Longer treatments (up to 120s) appeared to have no further effect on ascospore viability, with barely 1 $\log_{10}$ reduction after 120s treatment.

Conclusions

High pressure processing of fruit

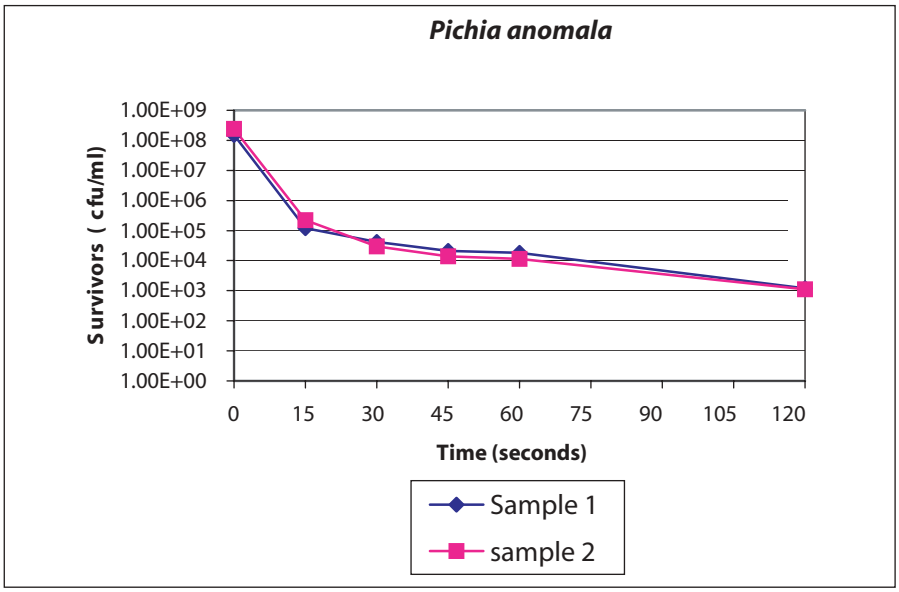


Figure 1. Effect of treatment at 400 MPa pressure at 25°C on a suspension of mixed vegetative cells and ascospores of *Pichia anomala.* Cells were suspended in 20°Brix sucrose solution, pH 4.2 (acidified with citric acid).

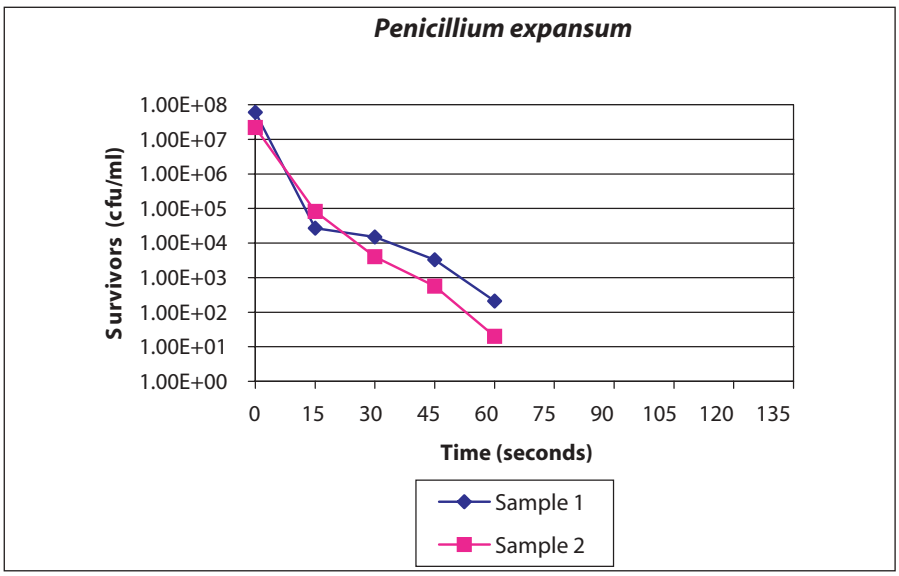


Figure 2. Effect of treatment at 400 MPa pressure at 25°C on *Penicillium expansum* conidia. Cells were suspended in 20°Brix sucrose solution, pH 4.2 (acidified with citric acid).

products can effectively inactivate yeasts (including yeast ascospores), and the conidia of common spoilage moulds such as *Penicillium* species, producing high quality fruit products with extended refrigerated shelf life. However, ascospores of heat resistant moulds are not inactivated by pressure treatment, and these microorganisms regularly cause spoilage of heat processed fruit products such as fruit purées used in dairy manufacturing. Combined treatments such as heat and high pressure may be able to control these fungi. Work is continuing at Food Science Australia to investigate the resistance of these moulds to HPP.

References

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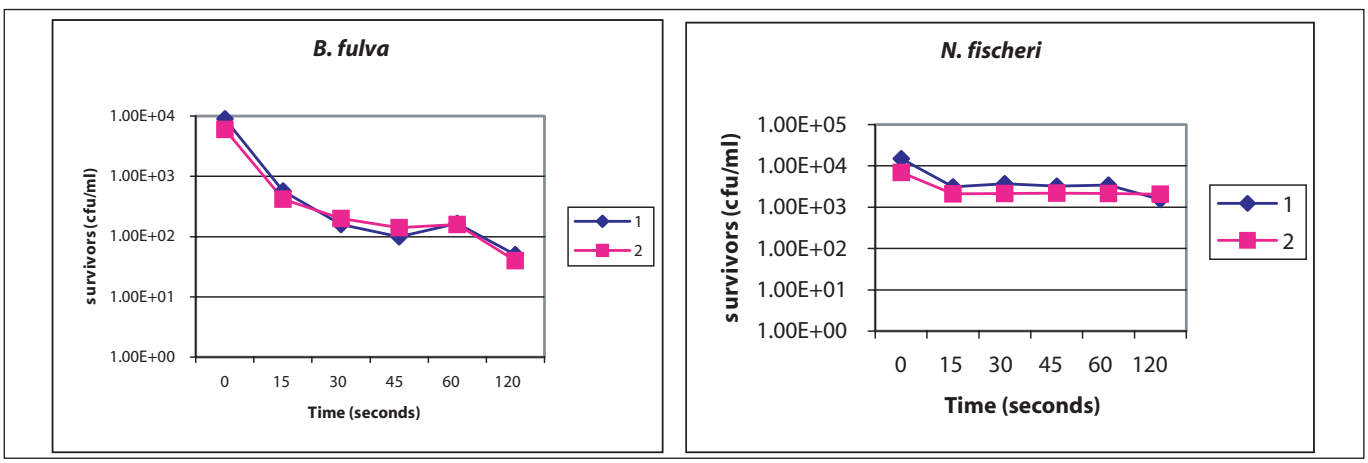

Figure 3. Effect of treatment at 600 MPa pressure at 25°C on *Byssochlamys fulva* and *Neosartorya fischeri* ascospores. Cells were suspended in 20°Brix sucrose solution, pH 4.2 (acidified with citric acid).