VALUE ADDED WHEAT CRC
PROJECT REPORT

Project 2.1.7 – Microbiological safety and stability of noodles, breadcrumbs and steamed breads made from Australian flour

Studies on the microbiological safety of breadcrumb products – Part 3

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EXECUTIVE SUMMARY

Breadcrumbs are often made from bread returns, so there is a potential risk that mouldy bread could be included with the bread used for the crumbing process. There is, therefore a potential risk of mycotoxin contamination of the process. There are no data concerning the presence of moulds or mycotoxins in bread returns in Australia, so this project was initiated to provide such data.

A survey was conducted of four breadcrumb manufacturers in four Australian States. Samples were obtained over four consecutive weeks and then at monthly intervals for three months and were tested for standard plate count, rope spores, yeasts, moulds, pH and water activity (a_w). In addition, the relationship between the moisture content and water activity of breadcrumbs was determined at 22°C.

The results showed that most of the **dried** breadcrumb products had standard plate counts and yeast and mould counts below their detection limits of 10 and 100 colony forming units (cfu)/g respectively. Both categories for the **wet** samples ranged from 10^3–10^5 cfu/g. One potentially toxigenic fungal species, *Penicillium crustosum*, was detected in one batch of wet crumb. This was the only sample containing bread returns beyond the use-by date.

The counts for rope-producing spores for all samples were < 10 cfu/g. The samples ranged between pH 5.2 and 5.7.

The **wet** samples were around a_w 0.96, while the **dry** samples ranged from a_w 0.26-0.64. Most of these samples were $a_w \leq 0.4$, however five samples were $a_w > 0.60$ and three were a_w 0.55-0.60. The (slow) growth of xerophilic moulds can occur at $a_w \geq 0.61$, so it is important that products are dried to well below a_w 0.60 to avoid this risk, and have a large enough safety margin to prevent growth if the moisture content increases during the shelf life of the product.

The moisture sorption isotherm determined during the project showed a_w 0.6 is associated with moisture contents of around 12%. Therefore drying breadcrumbs to $\leq$ 10% should provide an adequate safety margin to prevent the growth of moulds.

The report makes the following recommendations:

- **Only bread returns within their use-by date should be used for breadcrumb manufacture, and**
- **The maximum moisture content for dried breadcrumbs should not exceed 10%.**

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1. INTRODUCTION

Different types of moulds have been isolated from bread in Australia, including *Aspergillus*, *Penicillium* and *Fusarium* spp. If conditions are suitable, these organisms can produce mycotoxins that may be toxic to humans (Appendix 1). Breadcrumbs are often made from bread returns, so there is the potential for mouldy bread to enter the process, as sorting procedures may not always be rigorous. Thus the possibility of mycotoxin contamination exists. The heating process for breadcrumb manufacture is 69-72°C for 7-8 mins, which is unlikely to inactivate mycotoxins since most mycotoxins are heat stable (Appendix 2). There are no data on the presence of moulds or mycotoxins in bread returns in Australia, or on the effects of the breadcrumb processes (crumbing and baking) on pre-formed mycotoxins.

This project commenced in March 2002 with a survey of the microbiological status of bread returns and dried breadcrumbs in four Australian plants. Based on the results of the survey, a literature review was conducted to consolidate published information on the heat stability of the mycotoxins likely to present in these products.

2. PROJECT AIMS

1. To evaluate the microbiological status of breadcrumb manufacture in Australia
2. To evaluate the potential for mycotoxin contamination of breadcrumb
3. To report on the heat-stability of pre-formed mycotoxins.

3. MATERIALS AND METHODS

3.1 Samples

Samples were obtained from four factories in four Australian States (A,B,C and D). Samples of wet and dried breadcrumb products were provided every week for the first month, and then every month for three months. There were two types of breadcrumb products sampled: one was 100% white crumb and the other was a blend of around 60% white and 40% wholemeal breadcrumb.

Samples were transported to Food Science Australia at North Ryde, within 24 h of sampling. Samples were tested immediately after receival, or were sealed and stored at 1°C and tested within 24 h.

3.2 Media

All microbiological media were purchased from Oxoid Australia Pty Ltd.

3.3 Methods

3.3.1 Sample preparation

An initial dilution was prepared from each sample using 25 g of sample and approximately 225 mL 0.1% peptone solution. The initial dilution was homogenised by stomaching for 60 s. Serial tenfold dilutions were then prepared using the peptone solution.

3.3.2 Standard plate count

In accordance with Australian Standard 1766.2.1 (Standards Australia 1991), homogenates and dilutions of samples were plated onto plate count agar using the pour plate technique. Plates were incubated under aerobic conditions at 30°C for 72 h and all bacterial and fungal colonies counted. The number of colony forming units per gram of sample was calculated.

3.3.3 Yeast and mould count

Homogenates and sample dilutions were plated onto dichloran rosebengal chloramphenicol (DRBC) agar and dichloran 18% glycerol (DG18) agar using the spread plate technique, as described in the Australian Standard 1766.2.2 (Standards Australia 1997). Plates were incubated under aerobic conditions at 25°C for 5 days (DRBC) and 7 days (DG18). Colonies were counted and moulds identified to genus level, using methods described by Pitt and Hocking (1997). Presumptive yeast colonies were confirmed by microscopic examination. The number of colony forming units of yeasts and moulds per gram of sample was calculated.

3.3.4 Rope spore count

As described by Stevenson and Segner (2001), 10 mL of the initial homogenate of each sample was transferred into a 250 mL flask containing 100 mL of molten (45°C) dextrose tryptone agar. The agar suspension was steamed at 95°C for 15 min, cooled to 45°C in a water bath and poured into 5 sterile plates. Plates were incubated under aerobic conditions at 37°C for 48 h. Subsurface colonies were pulled to the agar surface and incubated for a further 24 h. Colonies positive for 'stringiness' were examined for microscopic morphology. Those displaying macroscopic and microscopic morphology typical of rope-producing spore formers were counted. The number of colony forming units of rope spores per gram of sample was calculated.

3.3.5 Measurement of pH

As described in an approved method of the American Association of Cereal Chemists (2000), 15 g of sample was weighed, and 100 mL of filtered water added. The solution was stirred for 30 min using a magnetic plate stirrer, followed by standing for 10 min. The pH of the supernatant was measured using a Sentron 1001 pH meter (Sentron Europe BV, Roden, The Netherlands).

3.3.6 Measurement of water activity

Water activity measurements were made using an AquaLab CX-3 dew point instrument (Decagon Devices, Washington, USA) according to the manufacturer's instructions.

3.3.7 Measurement of desorption isotherm of breadcrumb

White sliced bread was purchased from a local supermarket. The slices (with crusts) were transferred into a blender for crumbing and were then dried at 30°C for 1–2 days. Crumb samples were collected in triplicate at intervals during the drying process and transferred into preweighed AquaLab cells. The cells and lids were immediately wrapped in aluminum foil to prevent moisture loss and left to equilibrate at 22°C for at least 2 h. After equilibration, the water activity of each sample was measured, and the lid replaced on the container. The entire container was weighed immediately on an AE260 DeltaRange® digital balance (Mettler, Switzerland) and then placed in a vacuum oven (LaBec, Laboratory Equipment, Australia), preheated to 60°C, and dried at 60°C under vacuum for 16 h. The cells were then transferred to a desiccator, and weighed soon after they reached room temperature. The samples were subsequently discarded, and the empty cells re-weighed. The moisture was calculated as the percentage weight loss of the initial weight of crumb.

4. RESULTS

4.1 Weekly samples

The results for the weekly samples are shown in Figures 1 and 2. Detection limits were 10 colony forming units (cfu)/g for standard plate and rope spore counts and 100 cfu/g for fungal counts.

Thirteen of the wet samples showed standard plate counts of around 10^5 cfu/g sample; the remaining three were around 10^4 cfu/g (Figure 1a). Nine samples had yeast and mould counts > 10^5 cfu/g (Figure 1c). The yeast and mould counts were similar to the standard plate counts for most wet samples, indicating that the majority of their microflora were yeasts and moulds. Most of the moulds belonged to non-mycotoxigenic genera, including *Endomyces* and *Cladosporium*. However, the week 2 sample from factory site C was found to contain a potentially toxigenic fungal species, *Penicillium crustosum* at 3.4×10^4 cfu/g. This batch of breadcrumb contained bread returns beyond their use-by date.

The dried breadcrumb samples all had low (< 10^3 cfu/g) standard plate counts (Figure 1b), with only eight samples having detectable counts. All had yeast and mould counts below the detection limit, except the week 2 sample from factory site C, which registered 3.5×10^2 yeast (only) cfu/g, (Figure 1d). The wet crumb from this sample contained *P. crustosum*.

The rope spore counts of all wet and dried samples were < 10 cfu/g (results not shown). The pH ranged from 5.2 - 5.7 for both wet and dried samples respectively (Figures 1e and 1f).

The water activity results for the wet samples were uniformly high with all around 0.96 (Figure 2a). Those for the dried samples were more varied, ranging from a_w 0.26-0.63 (Figure 2b). Three samples were a_w> 0.60, with three others $\geq$ 0.50. The dried sample from factory site C taken at the same time as the one that contained *P. crustosum* was a_w 0.62.

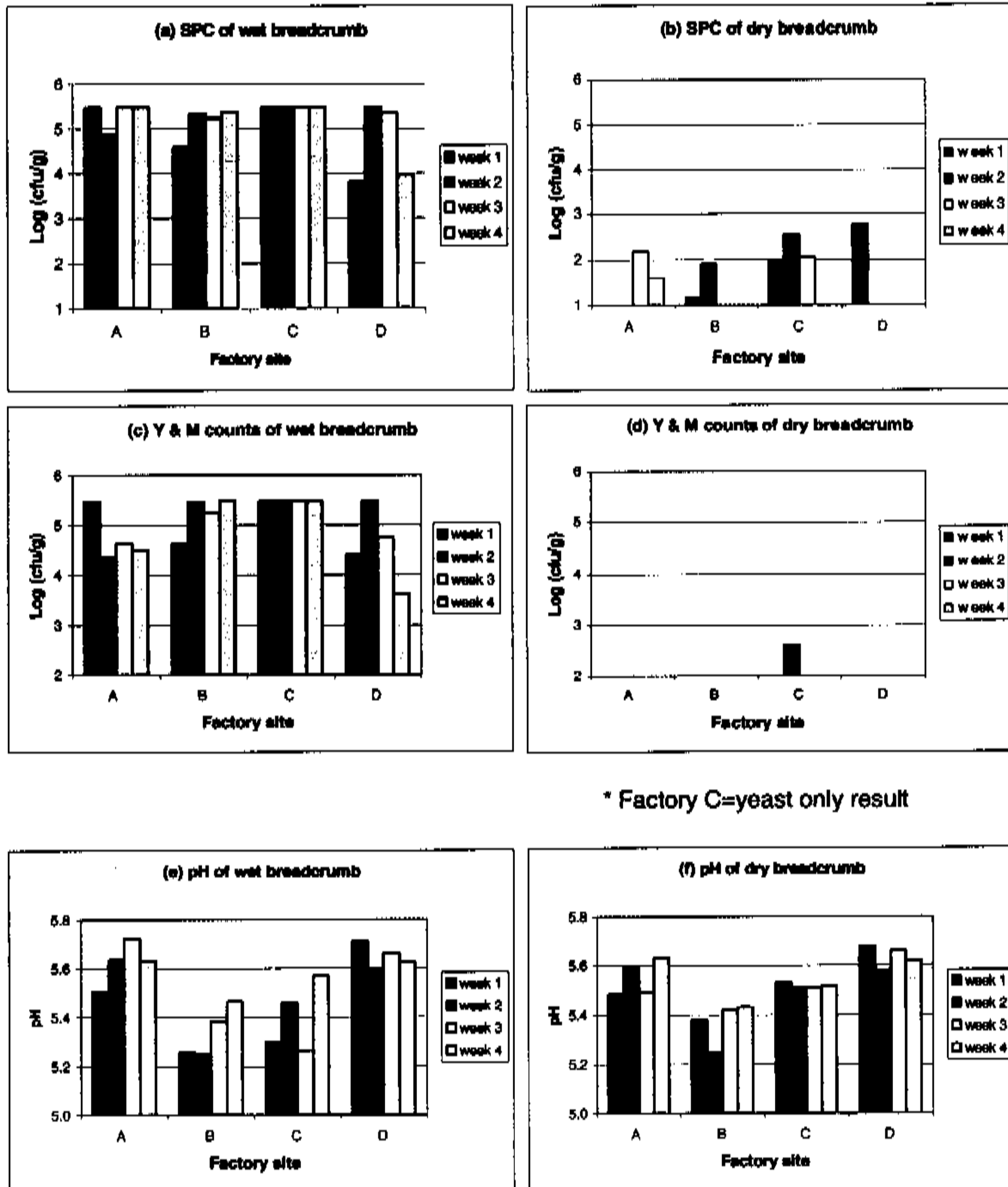

Figure 1. Weekly test results for standard plate counts (SPC), yeast and mould counts (Y & M) and pH of wet and dry breadcrumb.

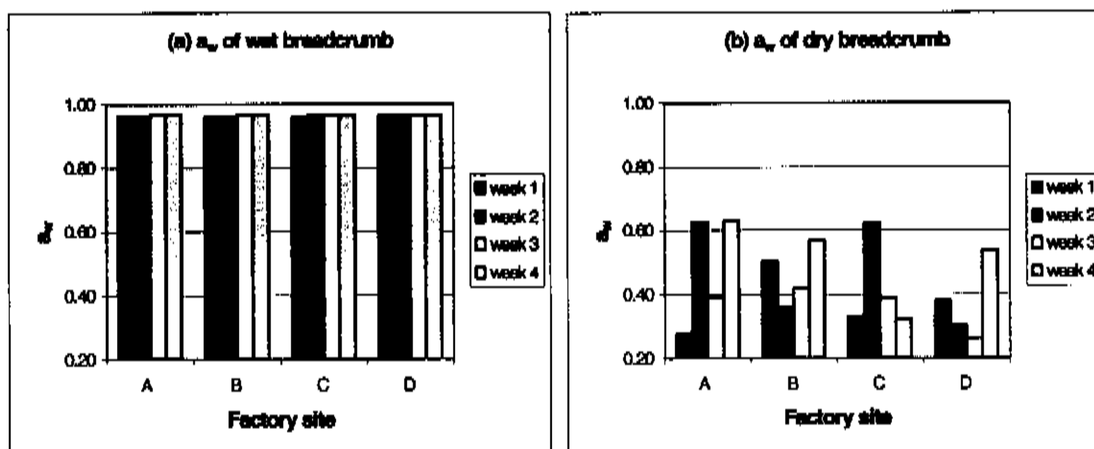

Figure 2. Weekly test results, for water activity (a_w) on wet and dry breadcrumb.

4.2 Monthly samples

The results for the monthly samples are shown in Figures 3 and 4.

Most standard plate count, yeast and mould results were lower for the monthly wet crumb samples compared with the weekly samples (Figures 3a, c). Five samples (only) had standard plate, yeast and mould counts > 10^5 cfu/g. The lower counts may reflect cooler environmental temperatures during the monthly sampling (July to September). For most samples, the counts again indicated that the dominant microflora were yeasts and moulds. No toxigenic fungi were detected amongst the genera.

Few dried samples had detectable total counts (Figure 3b) and none had detectable counts of yeasts and moulds (results not shown). However, one sample from factory site C contained nearly 10^3 yeast cfu/g but these were not recovered on the yeast and mould count (Figure 3b), possibly indicating that they were sublethally damaged. A second sample (from factory site B) contained 5 x 10^2 *Bacillus* spp. cfu/g, which was not observed in any other sample.

The pH ranged from 5.2-5.7 and 5.3-5.6 for wet and dried samples respectively (Figures 3d, e).

The water activity of the wet samples was around a_w 0.96, while that of the dried samples ranged from a_w 0.32-0.64 (Figures 4a, b). Two samples were a_w> 0.60 and one was a_w 0.57.

The rope spore counts for all samples were < 10 cfu/g.

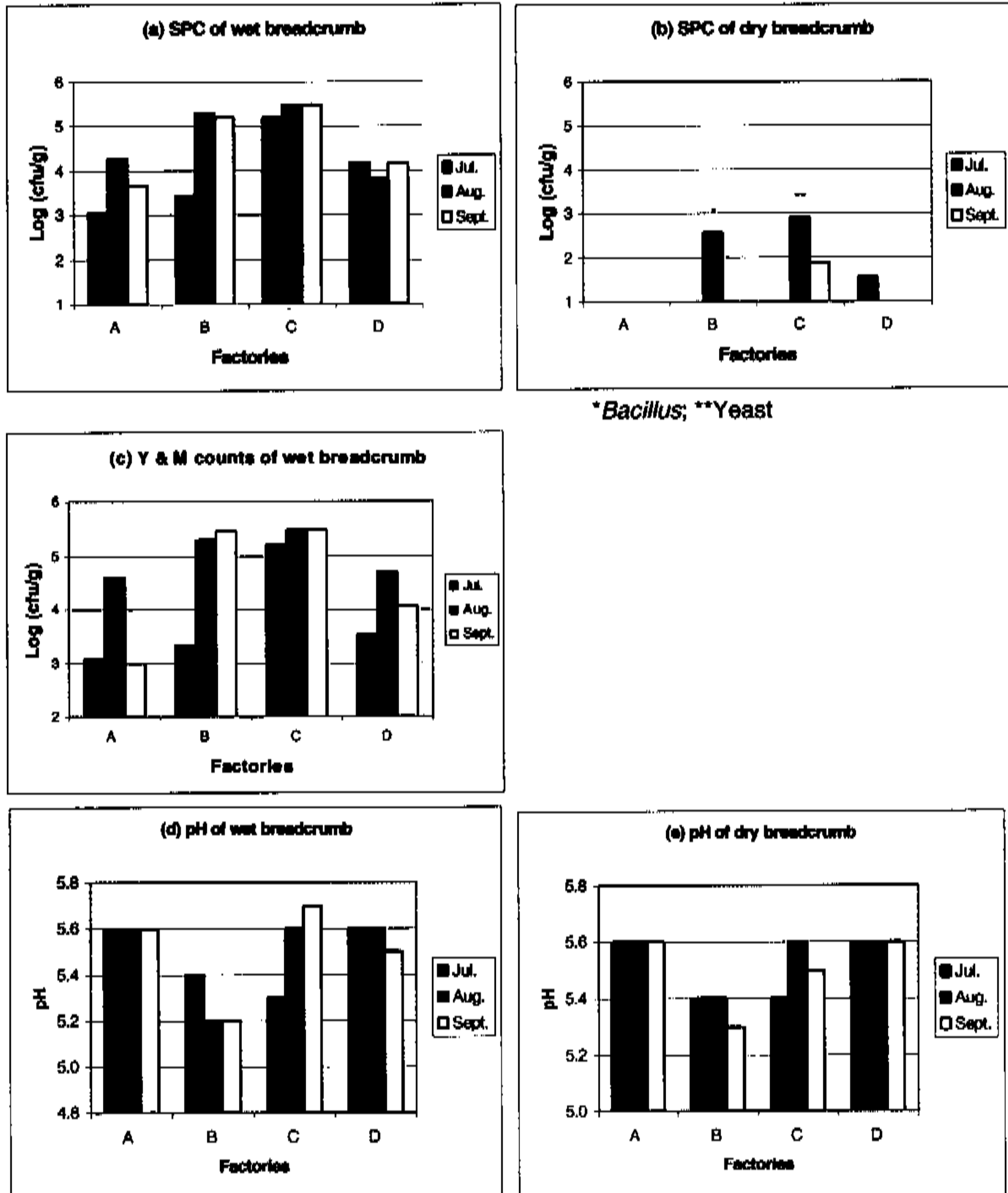

Figure 3. Monthly test results for standard plate counts (SPC), yeast and mould counts (Y & M) and pH of wet and dry breadcrumb.

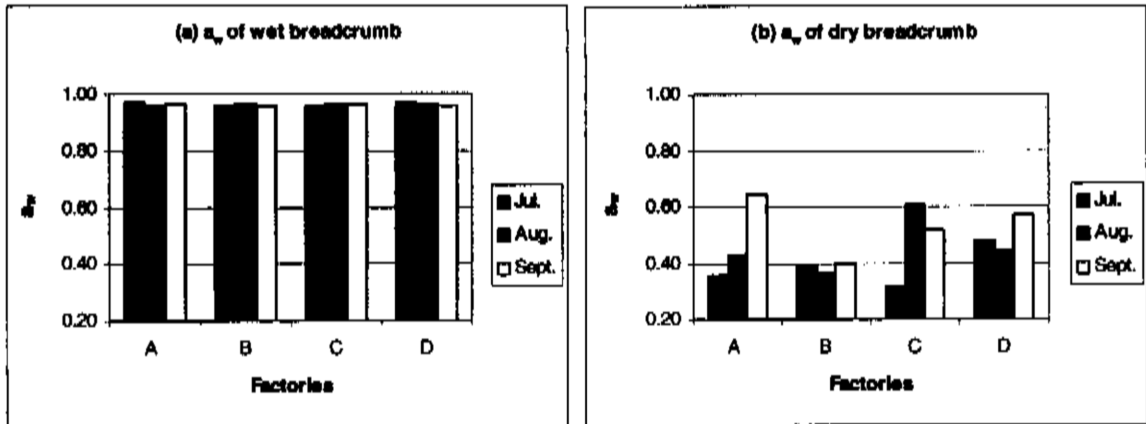

Figure 4. Monthly test results for water activity (a_w) on wet and dry breadcrumb.

4.3 Desorption isotherm of breadcrumbs

The desorption isotherm of breadcrumb at 22°C is presented in Figure 5. The isotherm shows that at a moisture content of 10%, the expected a_w would be 0.45, a safe level for preventing mould growth. However, if the moisture content is 12%, the resultant a_w is 0.6, which is marginal for microbiological stability, due to the potential for growth of xerophilic moulds.

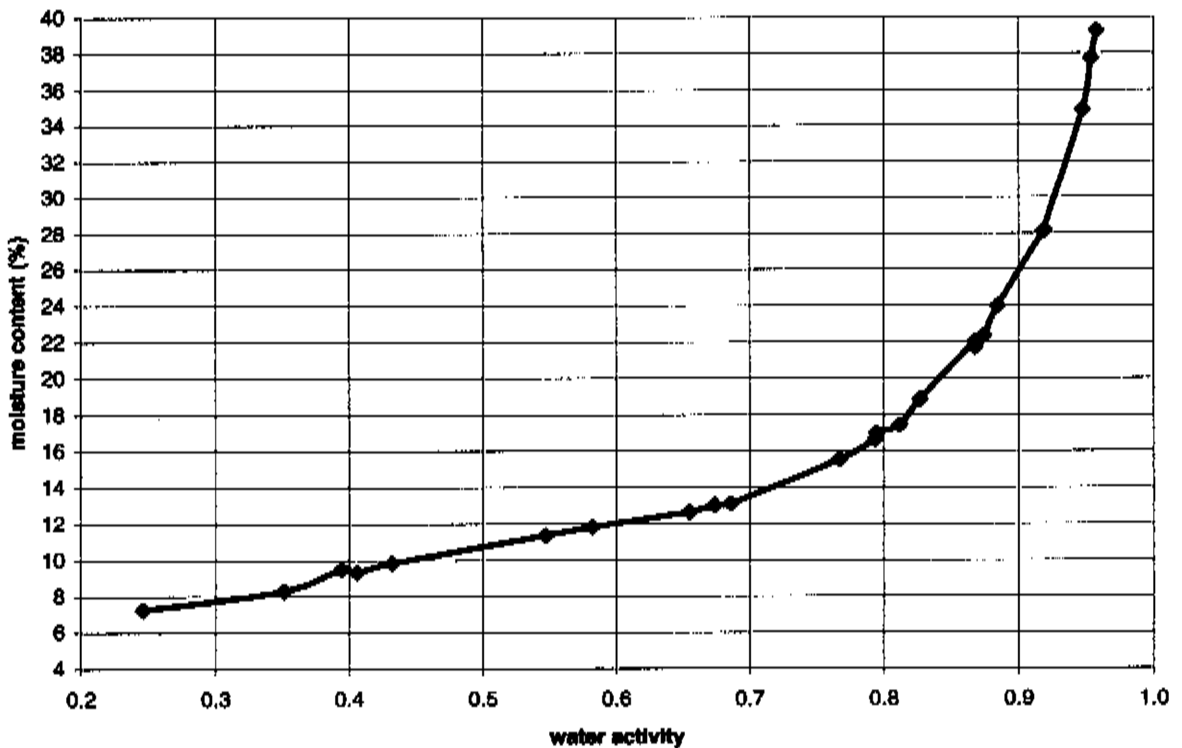

Figure 5. Desorption isotherm for white breadcrumb at 22°C.

5. DISCUSSION AND RECOMMENDATIONS

A variety of moulds have been isolated from bread (Appendix 1), including the potentially mycotoxigenic species *Aspergillus*, *Penicillium* and *Fusarium*. Most mycotoxins are heat stable and are unlikely to be inactivated by the heat process during breadcrumb manufacture (69-72°C for 7-8 min). There are, therefore, potential risks that mycotoxins could be present in dried breadcrumb products.

In this study, most moulds detected belonged to the genera *Endomyces* and *Cladosporium*. Only one potentially mycotoxigenic species, *Penicillium crustosum*, was detected in one batch of wet breadcrumbs from factory site C at 3.4×10^4 cfu/g. The other results for this sample were $> 10^5$ cfu/g for standard plate, yeast and mould counts, and a_w 0.96. Although moulds were not detected in the dried crumb sampled at this time, yeasts were detected at 3.5×10^2 cfu/g. This was the only sample of dried crumb to show a positive fungal count. These results indicate that the high microbial load in the original wet crumb may have reduced the effectiveness of the drying process, and that it may not have been dried sufficiently (a_w 0.62).

P. crustosum has been isolated from a variety of cereal products (Pitt and Hocking, 1997) and can produce a powerful neurotoxin, penitrem A. The toxicity of penitrem A to humans is unknown, however it is known to be toxic to large domestic animals, such as dogs. It is therefore likely that it is also toxic to humans. There are no published data of the heat stability of penitrem A. The batch in which *P. crustosum* was detected was the one batch that included bread returns past their use-by date. This result therefore shows the potential contamination of breadcrumbs with mycotoxins such as penitrem A.

- **It is recommended that only bread returns within their use-by date be used for breadcrumb manufacture.**

The water activity of a substrate is most conveniently defined as the ratio of the partial pressure of water in the atmosphere in equilibrium with the substrate, P, compared with the partial pressure of the atmosphere in equilibrium with pure water at the same temperature (P_0). Effectively, it is a measure of the amount of water available for microbial growth.

Fungi are able to grow on dry foods with a_w as low as 0.61, although mycotoxin production at such low water activities is unlikely (Adams and Moss, 2000). To avoid fungal growth after processing, we recommend that the water activity of breadcrumbs should be controlled under 0.6, preferably 0.55 or lower, to ensure an adequate safety margin. Breadcrumbs are generally dried to moisture contents lower than 10%, corresponding to $a_w \sim 0.45$. During this survey, some breadcrumb samples were $a_w > 0.6$, which could present a risk for fungal growth during storage. A higher moisture content also increases the probability of moisture migration, should the product be subjected to extreme temperature variation. Good control of the moisture content of dried breadcrumb products is critical to prevent the risk of mould growth during transport and storage.

- **It is recommended that the maximum moisture content for dried breadcrumbs not exceed 10%.**

6. OUTCOMES

In investigating the microbiological status of breadcrumb samples from an Australian processor, the following outcomes have been achieved:

- Wet and dried breadcrumb samples were tested and were generally observed to be of good microbiological quality, although some variation in water activity and hence moisture content were observed in the dried crumb

- There is a small but finite risk of the presence of mycotoxins in breadcrumb

- Using bread returns that are within the use-by date of the bread and controlling the moisture content of dried breadcrumb to less than 10% can minimize the risk of mould growth and associated mycotoxin contamination.

7. ACKNOWLEDGEMENTS

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Appendix 1. Literature review of the presence of fungi and heat stable mycotoxins in bread

Species	Type of bread	Source	Toxin potential
Aspergillus fumigatus, A. niger, A. flavus, A. glaucus, A. carneus, A. terreus, A. ochraceus, Rhizopus stolonifer, Trichoderma harzianum, Trichothecium roseum		(Rai et al. 1990)	fumitremorgens; aflatoxins; ochratoxin A; territrems
Aspergillus terreus	Balady	(El Sayed Amabdalla et al. 1998)	territrem A, territrem B
Penicillium, Aspergillus	White and Raisin	(Bullerman and Hartung, 1973)	luteoskyrin, ochratoxin A, penicillic acid
Penicillium, Aspergillus		(Viljoen and von Holy, 1997)	unspecified *Penicillium* and *Aspergillus* toxins
Moniliella suaveolens	White	(Naito et al. 2000)	
Penicillium, Rhizopus, Aspergillus niger, Alternaria, Fusarium, Mucor	Khamir	(Gassem, 1999)	unspecified *Penicillium* and *Aspergillus* toxins
Aspergillus, Penicillium, Rhizopus, Paecilomyces	Turkey	(Gocmen and Sahin, 1997)	unspecified *Penicillium* and *Aspergillus* toxins
Penicillium roqueforti, P. corylophilum, Eurotium, P. decumbens, Paecilomyces variotii, Aspergillus flavus, P. commune, P. solitum, A. niger, Mucor	Rye	(Lund et al. 1996)	roquefortine, PR toxin, aflatoxins, cyclopiazonic acid
Cladosporium herbarum, Eurotium repens, Penicillium expansum, Rhizopus stolonifer	Wheat	(Kunz et al. 1995)	patulin, citrinin
Aspergillus, Penicillium	NS[a]	(Spicher, 1986)	unspecified *Penicillium* and *Aspergillus* toxins
Mucor, A. amstelodami, A. niger	NS	(Paramasivan and Kalyanasumdarm, 1986)	
A. flavus	NS	(Augustine et al. 1984)	aflatoxins
Penicillium verrucosum var. cyclopium, Penicillium expansum, Aspergillus niger, A. flavus. Neurospora, Paecilomyces, Penicillium brevicompactum	White, Brown	(Andersson et al. 1978)	patulin, citrinin, aflatoxins
NS	NS	(Osborne, 1980)	ochratoxin A, patulin, citrinin
Aspergillus niger, Penicillium cyclopium	Rye	(Tyllinen et al. 1977)	
A. niger, Penicillium cyclopium, Pencillium steckii, Paecilomyces	White	(Tyllinen et al. 1977)	citrinin

Species	**Type of bread**	**Source**	**Toxin potential**
A. niger, Penicillium cyclopium, Pencillium steckii, Paecilomyces, A. glaucus, A. terreus, A. flavus, Penicillium spp. citrinum, viridicatum, rugulosum	Sweet white	(Tyllinen et al. 1977)	citrinin
Aspergillus niger, Penicillium corylophilum		(Davis et al. 1975)	
A. fumigatus, A. niger	Baladi	(Megalla et al. 1985)	fumitremorgens

[a] Not stated.

Appendix 2. Effect of temperature on stability and inactivation of some mycotoxins

Mycotoxin	Substrate	Heating method	Temperature (°C)	Time (min)	Reduction (%)	Reference
Aflatoxin	Cottonseed meal	Jacketed kettle	100	120	70	(ICMSF, 1996)
	Corn grits	Boiling	100	30	30	
	Corn grits	Baking	220[a]	25	16	
	Corn grits	Frying	175	20	35-50	
	Peanuts	Roasting	235	NS[b]	20-50	
	Peanuts	Boiling with 5% salt	116	30	80-100	
	Peanuts	Boiling without salt	116	30	32-37	
	Peanuts	Microwave (1.6 kW)	137	15	69	
	Peanuts	Microwave (3.23 kW)	150	5	95	
	Coconut oil	Sunlight	Ambient	NS	75	
Fusaproliferin	Wet flour	Heating	240	20	78	(Ritiene et al. 1999)
Fumonisin B2	Citrate-phosphate-borate buffer	Heating	125	60	No significant loss	(Jackson et al. 1996)

[a] Temperature of oven, not product.

[b] Not stated.