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

Microbiological studies of shelf stable noodles – Part 2

Yang Huang¹, Nancy Jensen¹, Ailsa Hocking¹, Di Miskelly²

¹ Food Science Australia
² Allied Mills

Date: January 2003

Value Added Wheat CRC has taken all reasonable care in preparing this publication. Value Added Wheat CRC expressly disclaims all and any liability to any person for any damage, loss or injury (including economic loss) arising from their use of, or reliance on, the contents of this publication.
VALUE ADDED WHEAT CRC PROJECT
FINAL REPORT

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

Part 2:

Microbiological studies of shelf stable noodles

Yang Huang¹, Nancy Jensen¹, Ailsa Hocking¹, Di Miskelly²

¹ Food Science Australia
² Allied Mills

Date: VAWCRC Report No:
Copy No:

CONFIDENTIAL
(Not to be copied)

Valued added wheat CRC has taken all reasonable care in preparing this publication. Valued added wheat CRC expressly disclaims all and any liability to any person for any damage, loss or injury (including economic loss) arising from this use of or reliance on, the contents of this publication.
EXECUTIVE SUMMARY

Microbiological challenge studies using spores of pathogenic and spoilage bacteria were conducted on acidified, shelf stable, commercial and experimental Hokkien and Udon noodles imported from China and Korea. No outgrowth of spores of the pathogen Bacillus cereus was observed in any of the noodles tested.

Spoilage (discolouration) of the experimental noodles (pH 4.3-4.4) by B. coagulans could be induced when moderately high numbers (>10^4 spores/g) of both B. coagulans and B. licheniformis were present in inoculated packs incubated at 37°C. No growth or spoilage occurred in packs at 20°C. The pH of the discoloured area increased above pH 4.5, but this was very localised and did not induce outgrowth of other spores present. Commercially processed noodles are unlikely to have such a high spore load, so the minimum pH for outgrowth of B. coagulans spores in processed noodles is likely to be higher than that observed in this project.

Provided that:

- processed noodle pH is around pH 4.0 and does not exceed pH 4.2
- good quality raw materials are used
- post process contamination is avoided, and
- pack integrity is maintained

there should be no microbiological risks associated with the consumption of these products.
# TABLE OF CONTENTS

Executive summary 2  
Table of contents 3  
1. Introduction 4  
2. Project aim 5  
3. Materials and Methods 5  
   3.1 Culture and spore preparation 6  
   3.2 Preliminary trials 8  
   3.3 Preparation of inoculum 8  
   3.4 Inoculation procedures 9  
   3.5 Microbial counts of noodles samples 9  
   3.6 Measurement of pH 10  
   3.7 Headspace gas analyses 10  
   3.8 Challenge studies 10  
4. Results and discussion 11  
   4.1 Preliminary inoculation trial 11  
   4.2 First challenge study 11  
   4.3 Second challenge study 13  
   4.4 Third challenge study 14  
   4.5 Fourth challenge study 16  
   4.6 Fifth challenge study 17  
5. Conclusions and Recommendations 19  
6. Outcomes 19  
7. Acknowledgements 19  
8. References 20
1. INTRODUCTION

Shelf stable noodles available commercially in Australia are currently imported from various Asian countries and fall into two main categories – acidified or non-acidified. Non-acidified shelf stable noodles generally require a full 12-D botulinum (thermal) process to confer safety and stability and were not included in this project. However, the majority of shelf stable noodles available commercially in Australia are acidified, so their safety and stability involves the combined effects of product characteristics (acidity) and terminal thermal process (in-pack pasteurisation). For the manufacture of these products, the dough is mixed then rolled into sheets, which are then cut into the different noodle shapes. The raw noodles are boiled, cooled, acidified, packaged, thermally processed and cooled. Acidification occurs at two stages of the process: dilute acids are mixed into the dough initially, and after boiling and prior to packaging, the noodles pass through an acid bath. Various organic acids (such as citric, malic, lactic, gluconic and acetic) may be used in the bath, and the final noodle pH will depend largely on the contact time, the type and concentration of acid(s) used. Most manufacturers use medium to high barrier packaging films to minimise moisture loss during storage, which may otherwise result in an increase in noodle fragility and loss of strand elasticity. Products sold in Australia have shelf lives of 6-12 months.

The two major types of shelf stable noodles sold in Australia are Udon and Hokkien. Udon are white or creamy-white in appearance, have a soft, elastic texture and are 4-5 mm in diameter, which gives them a plump appearance. Hokkien noodles are generally yellow with thinner strands, 2-3 mm in diameter. Most chilled Hokkien noodles are highly alkaline which promotes the development of the yellow flavone pigment from wheat flour. However shelf stable noodles are acidified to ensure their safety, so the yellow appearance of most shelf stable Hokkien noodles is due to the addition of yellow colouring. Although there may be some variation in acids used and their concentrations, most shelf stable Hokkien and Udon noodles should be around pH 4 at manufacture. Variability in commercial manufacturing processes has sometimes resulted in the production of noodles at higher pH values, but generally not exceeding pH 4.2.

The thermal process is an acid, in-pack pasteurisation, which yields a non-sterile product. Unpublished work conducted in this laboratory had shown the survival of the acid tolerant spore forming bacterium Bacillus coagulans and of the pathogen Bacillus cereus in packaged shelf stable noodles. The presence of B. cereus spores in noodles was also confirmed in the market place survey (Jensen & others 2002) where they were detected in 9% of shelf stable noodle samples and in 30% of refrigerated samples. Low numbers of B. cereus have been detected in flour (Berghofer & others 2003), so its presence in noodles is not unexpected. Although the organism is not notably acid tolerant, its growth has been reported at pH 4.3 at 30-35°C (Jenson & Moir 1997).

Growth of the highly pathogenic bacterium Clostridium botulinum has been reported in foods and laboratory media at pH ≤ 4.6 (Odlaug & Pflug 1978; Tanaka 1982). This occurred where
media were pH adjusted with hydrochloric acid or where significant concentrations of precipitated protein were present. In addition, metabolic effects, where the growth of one organism can alter the environmental conditions sufficiently to support the growth of a second organism, have been reported for *B. coagulans* and *C. botulinum* in model systems (Montville 1982) and for *B. licheniformis* and *B. subtilis* in tomato juice (Rodriguez & others 1993). In the model system, botulinum toxin production was induced following the co-inoculation of *B. coagulans* and *C. botulinum* at low inocula (10 spores each/mL) at pH 4.4.

This project was therefore initiated to closely evaluate the safety and stability of these products. Microbiological challenge studies were conducted using strains of acid tolerant bacterial spore formers (and the pathogen *B. cereus*) inoculated into Udon and Hokkien noodles at pH 3.9-4.0 (commercial noodles, low pH) and pH 4.3-4.4 (experimental noodles, high pH). Samples were incubated at 20°C (to simulate room temperature storage) and 37°C (to provide optimal recovery conditions for most of the spores). The noodle products tested, bacterial strains, incubation times and temperatures are shown in Table 1.

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Noodles</th>
<th>Country of origin</th>
<th>Noodle pH</th>
<th>Bacterial strains</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Temp (°C)</td>
</tr>
<tr>
<td>1</td>
<td>Hokkien &amp; Udon</td>
<td>Korea</td>
<td>Low &amp; high</td>
<td>All <em>Bacillus</em> &amp; <em>Clostridium</em> (Table 2)</td>
<td>20 and 37</td>
</tr>
<tr>
<td>2</td>
<td>Hokkien &amp; Udon</td>
<td>Korea</td>
<td>High</td>
<td>All <em>Bacillus</em> &amp; <em>Clostridium</em> (Table 2)</td>
<td>20 and 37</td>
</tr>
<tr>
<td>3</td>
<td>Hokkien</td>
<td>China</td>
<td>High</td>
<td>All <em>Bacillus</em> &amp; <em>Clostridium</em> (Table 2)</td>
<td>20 and 37</td>
</tr>
<tr>
<td>4</td>
<td>Hokkien</td>
<td>China</td>
<td>High</td>
<td><em>B. coagulans</em> &amp; <em>B. licheniformis</em></td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>Hokkien &amp; Udon</td>
<td>Korea</td>
<td>Low</td>
<td>Lactic acid bacteria</td>
<td>20 and 37</td>
</tr>
</tbody>
</table>

Prior to the commencement of the challenge studies, preliminary tests were conducted to optimise the inoculation and recovery procedures, and to simulate the packaging practices used in the commercial manufacture of the products.

2. PROJECT AIM

To assess the microbiological safety and stability of shelf stable, acidified noodles.

3. MATERIALS AND METHODS

MasterFoods Australia New Zealand provided commercial and experimental Hokkien and Udon noodles. The commercial noodles were pH 3.9-4.0 (low pH) and the experimental noodles were pH 4.3-4.4 (high pH). All the commercial noodles were manufactured in Korea.
The experimental Udon and Hokkien noodles used in Study 2 were also manufactured in Korea; those used in studies 3 and 4 were manufactured in China (Table 1). All noodles were stored at room temperature until required. Sterile, moderate to high barrier pouches (13 x 20 cm) of linear, low density polyethylene with 15 μM nylon (LLDPE+N), similar to those used commercially for shelf stable noodles, were purchased from Cryovac Australia P/L. All microbiological media were purchased from Oxoid Australia Pty Ltd and were prepared as directed.

3.1 Culture and spore preparation

The strains selected for the studies are shown in Table 2. Where possible, strains were sourced from cereal or acidic foods. Freeze-dried and frozen (-80°C) cultures were resuscitated, subcultured and incubated in accordance with the regimen shown in Table 3. General purpose media and incubation conditions commonly used for these groups of organisms were selected for the resuscitation. For spore crop preparation, the selection of media and incubation temperatures was based on data generated previously in this laboratory for optimised spore production by B. coagulans, C. pasteurianum and C. butyricum.

Table 2. Bacterial strains used in the challenge studies and their origin.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Species</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRRB$^a$ 2600, ACM$^b$ 1152</td>
<td><em>Clostridium pasteurianum</em></td>
<td>Unknown</td>
</tr>
<tr>
<td>FRRB 2737, ATCC$^b$ 7041</td>
<td><em>C. pasteurianum</em></td>
<td>Spoiled canned pineapple</td>
</tr>
<tr>
<td>FRRB 2732</td>
<td><em>C. butyricum</em></td>
<td>Blown cheddar cheese</td>
</tr>
<tr>
<td>FRRB 2740, ATCC 19398</td>
<td><em>C. butyricum</em></td>
<td>Pig intestine</td>
</tr>
<tr>
<td>FRRB 2723</td>
<td><em>Bacillus coagulans</em></td>
<td>Satay sauce</td>
</tr>
<tr>
<td>FRRB 2735, DSM$^b$ 2385</td>
<td><em>B. coagulans</em></td>
<td>Unknown</td>
</tr>
<tr>
<td>FRRB 2491</td>
<td><em>B. cereus</em></td>
<td>Fried rice, produces</td>
</tr>
<tr>
<td></td>
<td></td>
<td>diarrheal toxin</td>
</tr>
<tr>
<td>FRRB 2717</td>
<td><em>B. cereus</em></td>
<td>Commercial Udon noodles</td>
</tr>
<tr>
<td>FRRB 2660</td>
<td><em>B. subtilis</em></td>
<td>Ropy bread</td>
</tr>
<tr>
<td>FRRB 2738</td>
<td><em>B. subtilis</em></td>
<td>Thai sauce marinade</td>
</tr>
<tr>
<td>FRRB 2659</td>
<td><em>B. licheniformis</em></td>
<td>Ropy bread</td>
</tr>
<tr>
<td>FRRB 2703</td>
<td><em>B. licheniformis</em></td>
<td>Chocolate sauce</td>
</tr>
<tr>
<td>FRRB 2720</td>
<td><em>Lactobacillus brevis</em></td>
<td>Ryegrass silage</td>
</tr>
<tr>
<td>FRRB 2487</td>
<td><em>L. buchneri</em></td>
<td>Unknown</td>
</tr>
<tr>
<td>L36</td>
<td><em>L. buchneri</em></td>
<td>Probably spoiled acid food</td>
</tr>
<tr>
<td>4</td>
<td><em>L. fructivorans</em></td>
<td>Spoiled tomato sauce</td>
</tr>
<tr>
<td>L21</td>
<td><em>L. plantarum</em></td>
<td>Probably spoiled acid food</td>
</tr>
</tbody>
</table>

$^a$ FRRB = Food Science Australia bacterial culture collection.

$^b$ ACM, ATCC, DSM = Australian, USA and German culture collections, respectively.
Table 3. Resuscitation and incubation conditions for cultures.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Resuscitation medium</th>
<th>Incubation temperature (°C)</th>
<th>Atmosphere</th>
<th>Incubation time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. coagulans</td>
<td>Brain heart infusion agar</td>
<td>45</td>
<td>Aerobic</td>
<td>24</td>
</tr>
<tr>
<td>B. cereus</td>
<td>Brain heart infusion agar</td>
<td>37</td>
<td>Aerobic</td>
<td>24</td>
</tr>
<tr>
<td>Other Bacillus spp.</td>
<td>Brain heart infusion agar</td>
<td>30</td>
<td>Aerobic</td>
<td>24</td>
</tr>
<tr>
<td>Clostridia</td>
<td>Reinforced clostridial agar</td>
<td>30</td>
<td>Anaerobic</td>
<td>48</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>de Man, Rogosa &amp; Sharpe broth and agar</td>
<td>30</td>
<td>Microaerophilic</td>
<td>48</td>
</tr>
</tbody>
</table>

Spore crops were prepared for each strain to provide spore inocula for the challenge studies. Resuscitated cultures were streaked onto approximately 20 plates of the selected medium (Table 4) using the lawn technique of streaking over most of the agar area. This was to induce the development of a large numbers of colonies, to provide high numbers of spores. Plates were incubated under the conditions shown for the specified time. After this time, colonies on at least 2 plates were examined every 2-3 d for the proportion of spores, using the wet preparation technique and phase contrast microscopy. When the proportion of spores reached around 90% or at the end of incubation time (Table 4), crops were harvested.

Spore crops were harvested in a biohazard cabinet to limit the risk of contamination. Approximately 2 mL sterile filtered water was transferred to each agar plate. The growth was scraped from the agar surface using a spreader, and combined with that from other plates from the same strain in a 50 mL centrifuge tube, and then stored on ice. The cell and spore suspensions were centrifuged four times at 4,000 g for 10 min at 4°C. After the first three centrifugations, the supernatant was removed and the cells and spores re-suspended in 30 mL water, to wash them. After the final centrifugation, they were re-suspended in 5 mL water and stored at 1°C for up to 3 months.

Table 4. Media and incubation conditions for spore crops.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sporulation medium</th>
<th>Incubation temperature (°C)</th>
<th>Atmosphere</th>
<th>Incubation time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cereus</td>
<td>Nutrient agar</td>
<td>37</td>
<td>Aerobic</td>
<td>7</td>
</tr>
<tr>
<td>B. coagulans</td>
<td>Campden agar</td>
<td>50</td>
<td>Aerobic</td>
<td>7-14</td>
</tr>
<tr>
<td>B. licheniformis</td>
<td>Nutrient agar</td>
<td>30</td>
<td>Aerobic</td>
<td>7-14</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>Nutrient agar</td>
<td>30</td>
<td>Aerobic</td>
<td>7-14</td>
</tr>
<tr>
<td>C. butyricum</td>
<td>Modified reinforced clostridial agar</td>
<td>30</td>
<td>Anaerobic</td>
<td>16-21</td>
</tr>
<tr>
<td>C. pasteurianum</td>
<td>Modified reinforced clostridial agar</td>
<td>30</td>
<td>Anaerobic</td>
<td>42</td>
</tr>
</tbody>
</table>
3.2 Preliminary trials

Preliminary trials of the inoculation and recovery procedures were conducted using spores of *B. cereus* and 2 x 220 g packs of each of (commercial) Udon and Hokkien noodles. The spore suspension was heated for 10 min at 80°C as described in Section 3.1. The noodles were loosened (aseptically) and transferred into a stomacher bag. They were inoculated with 0.2 mL of spore suspension, which was added dropwise at four points on each of the upper and lower noodle surfaces. The noodles were massaged gently for 1 min and stomached for 1 min. This procedure was then repeated 5 times, after which triplicate samples of 25 g were removed from each pack for spore counts. They were tested as described in Section 3.5.

Trials were also conducted to simulate the commercial packaging of noodles. For the challenge studies, noodles were to be removed from their outer package, inoculated and re-packed in smaller volumes. Shelf stable noodles are not packed under vacuum commercially, but some air is removed from the packs. Trials using a Multivac A300 vacuum packaging machine (Multivac Inc, Germany) showed a vacuum setting of 700 mbar and 1.8 s sealing time produced packs that appeared similar to those packed commercially.

3.3 Preparation of inoculum

3.3.1 Spore inocula

The inoculum cocktail was prepared on the day of inoculation of each study. The spore counts were used to calculate the volume of spore suspension and diluent required to result in a cocktail of approximately equal numbers of each strain, i.e. approximately 10^8 spores of each strain/mL cocktail. Spore suspensions (stored on ice), were mixed, diluted with peptone diluent and combined. The cocktail was heated at 80°C for 10 min as described, to inactivate any vegetative cells. This was then cooled in ambient temperature water and stored at room temperature before the inoculation.

3.3.2 Lactobacilli

For challenge study 5, where lactic acid bacteria were used, the resuscitated cultures were grown in MRS (de Man, Rogosa & Sharpe) broth in 10 mL amounts at 30°C for 2 d, microaerophilically. The broths were centrifuged at 4,000 g for 10 mins at 4°C to concentrate
the cells, which were then re-suspended in 2 mL 0.85% saline. The cell suspensions were
counted (without heating) on MRS agar using the spread plate technique and peptone
solution as diluent. Plates were incubated microaerophilically at 30°C for 3 d, and the
colonies counted. The counts were used to calculate the volume of cell suspension and
diluent required to result in a cocktail of approximately equal numbers of each strain, i.e.
approximately 10^6 colony forming units (cfu) of each strain/mL cocktail. The cocktail was
prepared on the day of inoculation and added to the noodles without heating.

3.4 Inoculation procedures
The challenge studies were inoculated in a biohazard cabinet to minimize contamination.
The outer surfaces of the noodle packs were sanitised and then opened aseptically. The
noodles were transferred into a stomacher bag in 220 g amounts and the noodle strands
loosened aseptically. The heated inoculum (0.2 mL) was added dropwise at four points on
each of the upper and lower noodle surfaces. Each stomacher bag was massaged for 1 min
and then removed from the biohazard cabinet. The bag was placed inside a second
stomacher bag and sealed. The double bag was stomached for 1 min, then loosened and
shaken. This procedure was repeated 5 times. The bags were opened aseptically (in the
biohazard cabinet) and the contents transferred into sterile LLDPE+N pouches in
approximately 100 g amounts. The pouches were reduced in size to allow for the smaller
noodle volume (100 g versus 220 g), and sealed using the packaging machine set at 700
mbar vacuum and 1.8 s sealing time. Pouches were incubated according to the schedule
shown in Table 1.

3.5 Microbial counts of noodles samples
Duplicate samples of each condition were tested at each sampling. Packs were examined
visually for spoilage and then opened aseptically. Approximately 25 g sample was combined
with approximately 225 mL peptone solution and homogenised by stomaching for 60 s.
Additional tenfold dilutions were prepared using peptone solution.

Dilutions and homogenates were plated onto RCA using the spread plate technique. Sets of
plates were incubated aerobically and anaerobically at 37°C for 3 d, and were examined
daily for growth. Colonies were examined microscopically for spores and all spore-forming
colonies were counted. During the third and fourth studies, colonies of the different groups of
organisms were counted separately to obtain an estimate of the individual populations.
Generally B. cereus, B. coagulans, B. licheniformis and B. subtilis were detected on the
aerobic plates, and could be differentiated by their colony morphologies. B. coagulans, B.
cereus, B. licheniformis and the clostridia were detected on the anaerobic plates and
differentiated by their colony morphologies and catalase reactions: clostridia (catalase
negative) and Bacillus spp. (catalase positive). The number of colony forming units (cfu) of
bacteria detected aerobically and anaerobically per gram of sample was calculated, and
estimates made of the individual species’ populations where possible.
For challenge study 5, where noodles were inoculated with lactic acid bacteria, MRS agar was used as the plating medium. Plates were incubated microaerophilically at 30°C for 6 d and the colonies counted. Colonies were examined microscopically to confirm the absence of spores and tested for (negative) catalase reaction.

3.6 Measurement of pH

3.6.1 Supernatant of diluted noodles

Approximately 20 g of sample was combined with 180 mL of filtered water and stomached for 60 s. This mixture was allowed to stand for 5 min and the pH of the supernatant measured using a Sentron 1001 pH meter (Sentron Europe BV, Roden, The Netherlands). This method was provided by one of the commercial manufacturers of shelf stable noodles.

3.6.2 Direct measurement of noodle strands

The surface pH of noodle strands was measured by pressing a strand directly against the surface probe attached to the Sentron pH meter.

3.7 Headspace gas analyses

Headspace gas concentrations were measured on selected packs (in Challenge study 3 only) using a Gaspace2 analyser (Systech Instruments, UK).

3.8 Challenge studies

Five challenge studies were conducted (Table 1). The first study was conducted over a short period only. It was anticipated that growth would occur in the 'high pH' noodles, so this study was conducted as a preliminary guide for subsequent studies. Study 2 was conducted over a longer period to confirm the results from the first study. Spoilage was observed in one pack of one type only, so studies 3 and 4 were conducted to examine this more closely. Study 5, using lactic acid bacteria, was conducted (before study 2 was complete) to confirm the ability of the products to support bacterial growth. Their ability to support mould growth had been inadvertently confirmed by sporadic pack spoilage. In studies 1-3 and 5, mixed strain inocula were used in each inoculated pack. In study 4, some packs were inoculated with strains of *B. coagulans* or *B. licheniformis* only; others were inoculated with strains of both species, to ascertain if a synergistic effect could be observed.
4. RESULTS AND DISCUSSION

4.1 Preliminary inoculation trial
The results for the preliminary study of commercial Hokkien and Udon noodles inoculated with *B. cereus* spores are shown in Table 5. Each count is the average of three subsamples, plated in duplicate. The results showed excellent recovery of the inoculated spores with limited variability in numbers, and indicate that the spores were evenly distributed throughout the noodles. This confirms that the inoculation and recovery procedures were acceptable.

Table 5. Microbial counts of noodles inoculated with spores of *B. cereus*.

<table>
<thead>
<tr>
<th>Noodle samples</th>
<th>Counts (log cfu/mL)^b^</th>
<th>Inoculum recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Udon pack 1</td>
<td>5.86 ± 0.02</td>
<td>102.4</td>
</tr>
<tr>
<td>Udon pack 2</td>
<td>5.84 ± 0.01</td>
<td>101.9</td>
</tr>
<tr>
<td>Hokkien pack 1</td>
<td>5.84 ± 0.15</td>
<td>102.1</td>
</tr>
<tr>
<td>Hokkien pack 2</td>
<td>5.94 ± 0.06</td>
<td>103.7</td>
</tr>
</tbody>
</table>

^a^ Colony forming units.

^b^ Values are mean ± SD (n=3).

4.2 First challenge study
The results for the first challenge study in commercial and experimental Udon and Hokkien noodles manufactured in Korea are shown in Figure 1. No growth was observed and the spore numbers remained relatively unchanged over the 4 week period. The pH of the noodles did not change significantly during the study (results not shown).
Figure 1. Counts of experimental and commercial Korean Udon and Hokkien noodles inoculated with a *Bacillus* and *Clostridium* spp. spore cocktail, incubated for 4 weeks at 20°C and 37°C. Bars on the points show the standard error of mean (n=2).
4.3 Second challenge study

The results for the second challenge study of experimental noodles sourced from Korea and incubated for 6 months are shown in Figure 2. Udon noodles incubated at 37°C were not tested after 12 weeks as there was an insufficient number of samples, however by 12 weeks, one pack had spoiled, evidenced by discolouration of a small area and the presence of a small amount of turbid liquid at the edge of the pouch. The dominant colonies were *Bacillus* spp., but were not *Bacillus cereus*.

No spoilage or significant growth was observed in any other pack over the 6 month period. Generally, spore numbers declined slowly across both products at both temperatures. The pH of the inoculated noodles showed no significant change at either 37°C or 20°C (results not shown).

![Experimental Udon noodles, pH 4.3-4.4](image1)

![Experimental Hokkien noodles, pH 4.3-4.4](image2)

Figure 2. Counts of experimental Korean Udon and Hokkien noodles inoculated with a *Bacillus* and *Clostridium* spp. spore cocktail, incubated for 6 months at 20°C and 37°C. Bars on the points show the standard error of mean (n=2). The red square shows the aerobic count in the one spoiled Udon noodle pack, the green square shows the anaerobic count in that pack.
The results for the third challenge study of experimental Hokkien noodles sourced from China and incubated at 20 and 37°C are shown in Figure 4. Results are shown for the total aerobic and anaerobic counts, and also estimates of the individual populations. Spoilage of a number of packs incubated at 37°C was observed, evidenced by discoloration (Figure 3). Discoloured spots were generally included in the product sampled, but these areas were small and the degree of discoloration varied between packs, evidenced by variability in the counts recovered, e.g. those for month 4. The dominant colonies in the spoiled packs were *B. coagulans*, although *B. licheniformis* was also recovered in significant numbers, so further evaluation of the role of each organism appeared warranted.

The headspace oxygen concentration decreased in spoiled packs to 40 ppm, compared with 20% at packaging, and 15-18% in the unspoiled packs.

Figure 3. Spoiled Hokkien noodles showing discoloration.
Figure 4. Counts of experimental Chinese Hokkien noodles (pH 4.3–4.4) inoculated with a Bacillus and Clostridium spp. spore cocktail, incubated for 6 months at 20°C and 37°C.
The pH values of noodles during this study are shown in Figure 5. The unspoiled noodles in spoiled packs showed similar values to those in the unspoiled packs, however, the discoloured spots showed higher pH values. This increase in pH was presumably due to metabolic activities associated with growth of the spoilage organisms.

![Experimental noodle pH](image)

**Figure 5.** pH values of experimental Chinese Hokkien noodles (pH 4.3-4.4) inoculated with a *Bacillus* and *Clostridium* spp. spore cocktail, incubated for 6 months at 20°C and 37°C. Yellow spots denote pH of undiscoloured noodles from spoiled packs. Red spots denote pH of discoloured noodles from spoiled packs.

### 4.5 Fourth challenge study

The results are shown in Figure 6 for the fourth challenge study on high pH, experimental Chinese noodles inoculated with *B. coagulans* and/or *B. licheniformis* incubated at 37°C for 7 weeks. No growth or discoloration was observed in packs inoculated with either *B. coagulans* or *B. licheniformis* only (counts not shown), but was observed in two packs inoculated with the mixture of species. In these packs, the discoloured areas showed increased *B. coagulans* counts but decreased *B. licheniformis* counts. This indicates that the spoilage required a synergistic interaction between the two *Bacillus* spp., and confirmed the spoilage observed sporadically in the second and third studies.

In all these studies, relatively high inocula were used so that trends could be readily observed. These inocula are far higher than would be expected to be present in raw or processed noodle doughs. This may have influenced the likelihood of spore germination, as the higher the inocula, the lower the minimum pH at which germination can occur (Rice & Pederson 1954; Rodrigo & others 1990). Conversely, higher pH environments are required for the outgrowth of a lower numbers of spores.
Figure 6. Counts of experimental Chinese Hokkien noodles (pH 4.3-4.4) inoculated with both *B. coagulans* and *B. licheniformis* spores, incubated at 37°C for 7 weeks. Counts at 7 weeks are from two spoiled packs.

4.6 Fifth challenge study

The results for the fifth study on low pH Korean Hokkien and Udon noodles, inoculated with lactobacilli, incubated at both 20 and 37°C, are shown in Figures 7 and 8.

At 37°C, growth to high numbers occurred rapidly in both noodle types and was accompanied by a relatively rapid pH decrease (Figure 8) and reduction in cell viability. At 20°C, the counts increased more slowly in both types and stayed at the same level over the remaining incubation period. The *Lactobacillus* counts in all uninoculated packs were all < 100 cfu/g, the detection limit of the test (results not shown).
Figure 7. *Lactobacillus* counts in inoculated, commercial, Korean Hokkien and Udon noodles (pH 3.9-4.0) incubated at 20°C and 37°C for 4 weeks. Bars on the points show the standard error of mean (n=2).

Figure 8. pH values of commercial Korean Hokkien and Udon noodles (pH 3.9-4.0) incubated at 20°C and 37°C for 4 weeks. The (uninoculated) controls were incubated at both temperatures. Bars on the points show the standard error of mean (n=2).
5. CONCLUSIONS AND RECOMMENDATIONS

Five challenge studies were conducted to assess the microbiological safety and stability of shelf stable noodles. No growth of spores of the pathogen B. cereus was observed in any noodles tested. No outgrowth of spores of Bacillus and Clostridium was observed in current commercial Udon and Hokkien noodles pH 3.9-4.0 sourced from Korea or China. However, not unexpectedly, the noodles were able to support the growth of lactobacilli and moulds. The growth of these organisms should be prevented by the in-pack, thermal process and avoidance of post process contamination.

Outgrowth of B. coagulans spores and subsequent product discolouration was observed in the experimental noodles (pH 4.3-4.4), in the presence of B. licheniformis. The likelihood of spore germination was probably increased by the inoculum size, which was considerably greater than would be expected in processed noodles.

The results of the studies therefore indicated that provided:

- the processed noodle pH is around pH 4.0, and not exceeding pH 4.2
- good quality raw materials are used
- post process contamination is avoided, and
- pack integrity is maintained

there should be no risks to the microbiological safety or stability of these products.

Although the minimum inoculum size and pH for outgrowth of B. coagulans spores were not determined in the products, it is recommended that commercial acidified, shelf stable noodles be manufactured to around pH 4.0 or below.

6. OUTCOMES

In investigating the safety and stability of shelf stable noodles, knowledge has been gained of the microbiology of these products and the factors that need to be controlled to maintain their safety and stability. A paper will be prepared detailing this work and its outcomes, and will be submitted to Food Australia for publication. It is anticipated that the results will be of considerable interest to noodle manufacturers, public health authorities and consumers.

7. ACKNOWLEDGEMENTS

This work was made possible by the financial contributions of Value Added Wheat CRC and MasterFoods Australia New Zealand, and the in-kind contribution of Food Science Australia. The authors thank MasterFoods and Goodman Fielder Milling and Baking for their valuable contributions to the project.
8. REFERENCES


Rice, AC & Pederson, CS. 1954. Factors influencing growth of Bacillus coagulans in canned tomato juice. I. Size of inoculum and oxygen concentrations. Food Res. 19:115-123.

