# EFFECT OF DRY HEAT ON BACTERIAL FLORA OF MACADAMIA NUTS

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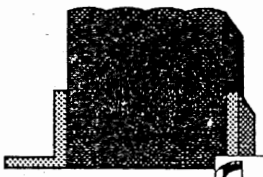

TECHNICAL PAPERS

*This project was commissioned by MacFarms Australia and conducted by IFIQ on a confidential contract basis. MacFarms have kindly agreed to the report being released to the industry.*

## INTRODUCTION

The aim of this project was to assess the feasibility of the use of dry heat treatments to ensure the microbiological safety of raw macadamia kernels.

The decision to confine this initial investigation to dry heat treatments was based on the belief that dry heat treatments were less likely to affect quality and shelf-life of the product than wet (steam) treatments. It was planned to undertake a second project to investigate steam treatments and their effect on shelf-life if dry heat treatments were shown to be not feasible.

## MATERIALS AND METHODS

### Materials and Inoculation

The microbiological effects of dry heating on uninoculated macadamia nuts were studied. The uninoculated material was macadamia kernel style 4 (half kernels) taken from factory lines at MacFarms of Australia at Woombye. For the inoculated studies (challenge tests), dried nut-in-shell (kernel moisture content 1.5%) was used in lieu of kernel in order to avoid the possibility of bacterial contamination of laboratory equipment and because kernels with very low microbial counts were required. This material was also obtained from MacFarms. The challenge tests determined the survival of *Enterobacter cloacae* (isolated from dry-heated nuts) and *Salmonella typhimurium* (isolated from chicken litter used as a fertiliser in macadamia orchards) when seeded into the kernel of macadamia nut-in-shell and subjected to various heat treatments. A small hole was drilled through the shell and into the kernel, the kernel was then seeded with a suspension of the relevant bacterium, the nut-in-shell was air-dried in a biohazard cabinet at ambient temperature to its original weight and the hole was then sealed with a non-bacterial sealer.

### Equipment and Air Flow

Heating was accomplished by passing hot air over the product on wire-mesh trays in a small cabinet dehydrator or a laboratory oven. Air velocity was 4 m/s in the cabinet dehydrator and 0.4 m/s in the laboratory oven. For some treatments, the air flow was unidirectional in regard to product orientation while for other treatments the product trays were turned through 180° after half of the treatment time had elapsed. For most of the treatments, 50g of product was distributed over a 25cm x 25cm area of the tray surface so that the individual nuts were not in contact with each other. However, for some treatments (referred to as "bunched"), the same amount of sample was distributed over an area of 10cm x 10cm.

### Temperatures and Times

For the uninoculated kernels, temperatures studied ranged from 80°C to 155°C and times ranged from 1 minute to 24 hours. Since that study showed that lower temperatures/longer times were more effective than higher temperatures/shorter times with regard to reducing bacterial counts without causing changes in colour and flavour, a temperature range of 70°C to 80°C and times from 7 hours to 96 hours were chosen for the inoculated nut-in-shell study.

### Microbiological Analyses

Total bacterial counts, Gram +ve and Gram -ve counts were conducted in accordance with methods described by Kwee *et al* (1988). The Gram -ve bacterial count is used to estimate the less heat resistant bacteria and thus their relative reduction can be used as an indicator of the effectiveness of the heat treatment in regard to the elimination of certain pathogenic bacteria such as *Salmonella* and *Escherichia coli*. Gram +ve bacterial counts can be used to give an estimate of the number of the more heat-resistant bacteria, such as spore-forming thermoduric bacteria.

Coliform counts were estimated in accordance with AS1766.2.3 (1987) and *E. coli* in accordance with AS1766.2.3 (1987). Coliforms are non-heat-resistant Gram -ve bacteria commonly

found in the environment. *E. coli* are members of the coliform group. They predominate in the alimentary canals of warm-blooded animals and therefore their presence in foods is used as an indication of faecal contamination.

The number of *Salmonellae* in the inoculated samples was estimated by the standard plate count method (AS1766.2.1-1991). The presence or absence of *Salmonellae* was tested by AS1766.2.5-1991.

**Colour and Flavour**
After each heat treatment, the kernels were evaluated by a panel of laboratory staff, in terms of the presence or absence of any flavour change or colour change resulting from the heat treatment. Since inoculated product could not be tasted, uninoculated nut-in-shell was given an identical heat treatment to the inoculated nut-in-shell and that product was evaluated by the panel.

## RESULTS AND DISCUSSION

**Uninoculated kernels**
The effect of dry heat on bacterial load, colour and flavour of uninoculated macadamia kernels is shown in Table 1. The major findings are as follows:

- None of the heat treatments applied eliminated the Gram -ve or Gram +ve bacterial populations present in the kernels.
- A few of the heat treatments applied reduced coliform counts below detectable levels but, in all cases, some Gram -ve bacteria were still present in the kernels. This indicates that the survival of non-heat-resistant, non-spore-forming bacteria (which could include genus such as *Salmonella*) is still possible.
- For each temperature, the longest heating times investigated resulted in significant changes in colour and/or flavour of the kernels, ie. the kernels were partially roasted. Since this work was aimed at treatment of product to be marketed as raw kernel, there was no point in investigating longer times than those listed in Table 1.
- *E. coli* and *Salmonella* were not detected in any of the samples tested.

**Inoculated Nut-in-shell (Challenge Test)**
The effect of dry heat on bacterial load of inoculated nut-in-shell is shown in Table 2. The effect on colour and flavour is shown in Table 3.

The only heat treatment investigated which eliminated *Enterobacter cloacae* and *Salmonella typhimurium* cultures, seeded in nut-in-shell, was 80ºC for 48 hours with an air flow of 4 m/s. However, the kernels had a semi-roasted flavour and a change in colour after this treatment. Treatment at 80ºC with a 0.5 m/s air flow did not eliminate the cultures after 72 hours. Treatment at 70ºC and 75ºC for 96 hours with air flows of 0.5 m/s and 4 m/s did not eliminate the cultures but resulted in significant flavour changes.

## CONCLUSION

This investigation has shown that the use of dry heat treatments to ensure the microbiological safety of macadamia kernels is not feasible because the treatments required to eliminate organisms of concern would result in significant changes in the flavour and/or colour of the product (partial roasting).

## RECOMMENDATION

It is recommended that the feasibility of using wet heat (steam) treatments, to ensure the microbiological safety of macadamia kernels, be investigated.

## REFERENCES

Kwee, W.S., Dommett, T.W. and Vos, A.C. (1988). "Fluorogenic Medium for Differential Gram-negative, Gram-positive and Total Bacterial Counts in Liquid Milks". *Aust. J. Dairy Technol.*, 43 (2) 50.

Standard Association of Australia (1991). Australian Standard AS1766, Part 2, Section 1.

Standard Association of Australia (1987). Australian Standard AS1766, Part 2, Section 3.

Standard Association of Australia (1991). Australian Standard AS1766, Part 2, Section 5.

Table 3. Effect of dry heat treatments on colour and flavour of inoculated macadamia NIS

| TREATMENT | | | Colour change | Flavour change |
|---|---|---|---|---|
| Temperature (ºC) | Air flow (m/s) | Time (hr) | | |
| 70 | 4 | 0 | -ve | -ve |
| 70 | 4 | 48 | -ve | +ve |
| 70 | 4 | 72 | -ve | +ve |
| 70 | 4 | 96 | -ve | +ve |
| 75 | 4 | 0 | -ve | -ve |
| 75 | 4 | 48 | -ve | +ve |
| 75 | 4 | 72 | -ve | +ve |
| 75 | 4 | 96 | -ve | +ve |
| 80 | 0.5 | 0 | -ve | -ve |
| 80 | 0.5 | 24 | +ve | +ve |
| 80 | 0.5 | 48 | +ve | +ve |
| 80 | 0.5 | 72 | +ve | +ve |
| 80 | 4 | 0 | -ve | -ve |
| 80 | 4 | 7 | -ve | -ve |
| 80 | 4 | 24 | +ve | +ve |
| 80 | 4 | 48 | +ve | +ve |

Note: Colour/Flavour
-ve indicates no change
+ve indicates a change has occurred

# Table 1

## Effect of Dry Heat on Bacterial load, Colour and Flavour of Uninoculated Raw Macadamia Kernels

| Nut Sample | TREATMENT | | | Microbiological Analyses | | | | Colour Change | Flavour Change |
|---|---|---|---|---|---|---|---|---|---|
| | Temperature (°C) | Time* | Air Flow (m/s) | Coliforms/g | Total count/g | Gram +ve/g | Gram -ve/g | | |
| A | Nil treatment | NA | NA | 650 | 7300 | Not Done | Not Done | NA | NA |
| A | Nil treatment | NA | NA | 79 | 6900 | 450 | 6400 | NA | NA |
| A | Nil treatment | NA | NA | 23 | 4600 | 600 | 4000 | NA | NA |
| A | Nil treatment | NA | NA | 130 | 5500 | 500 | 5000 | NA | NA |
| B | Nil treatment | NA | NA | 2 | 800 | 150 | 650 | NA | NA |
| A | 80 | 5+5 min | 4 | 8 | 2800 | 50 | 2750 | - ve | - ve |
| A | 80 | 10+10 min | 4 | 49 | 3450 | 100 | 3350 | - ve | - ve |
| A | 80 | 20+20 min | 4 | 2 | 1800 | <10 | 1800 | - ve | - ve |
| A | 80 | 2h | 4 | <2 | 7050 | 200 | 6850 | - ve | - ve |
| A | 80 | 4h | 4 | 5 | 3250 | 500 | 2750 | - ve | - ve |
| A | 80 | 7h | 4 | <2 | 1400 | 950 | 450 | - ve | - ve |
| A | 80 | 7h | 4 | <2 | 750 | 250 | 500 | - ve | - ve |
| A | 80 | Bunched 7h | 4 | 8 | 600 | 150 | 450 | - ve | - ve |
| A | 80 | Bunched 7h | 4 | <2 | 750 | 100 | 650 | - ve | - ve |
| A | 80 | 24h | 4 | <2 | 950 | 200 | 750 | + ve | + ve |
| A | 80 | Bunched 24h | 4 | <2 | 600 | 50 | 550 | + ve | + ve |
| A | 105 | 1 min | 0.5 | 46 | 4500 | 300 | 4200 | - ve | - ve |
| B | 105 | 1 min | 4 | 13 | 4500 | 100 | 4400 | - ve | - ve |
| A | 105 | 2 min | 0.5 | 110 | 3100 | 300 | 2800 | - ve | - ve |
| B | 105 | 2 min | 4 | 11 | 1300 | 50 | 1250 | - ve | - ve |
| A | 105 | 4 min | 0.5 | 49 | 2400 | 200 | 2200 | - ve | + ve |
| B | 105 | 4 min | 4 | <2 | 1800 | 4100 | 1700 | - ve | + ve |
| B | 115 | 2 min | 4 | 23 | 1350 | 100 | 1250 | - ve | - ve |
| A | 115 | 2 min | 4 | 130 | 6000 | 200 | 5800 | - ve | - ve |
| B | 115 | 4 min | 4 | 23 | 7750 | 150 | 7600 | - ve | + ve |
| A | 115 | 4 min | 4 | 49 | 15300 | <10 | 15300 | - ve | + ve |
| B | 115 | 8 min | 4 | 13 | 1600 | 450 | 1150 | + ve | + ve |
| A | 115 | 8 min | 4 | 31 | 2200 | 600 | 1600 | + ve | + ve |
| A | 125 | 1 min | 0.5 | 23 | 6300 | 300 | 6000 | - ve | - ve |
| A | 125 | 2 min | 0.5 | >1,600 | 13000 | 400 | 12600 | - ve | - ve |
| A | 125 | 2+2 min | 4 | 8 | 2300 | 100 | 2200 | + ve | + ve |
| A | 125 | Bunched 2+2 min | 4 | 109 | 3900 | 350 | 3550 | + ve | + ve |
| A | 125 | 4 min | 0.5 | 2 | 3000 | 400 | 22600 | + ve | + ve |
| A | 125 | 4 min | 4 | 33 | 2700 | <100 | 2700 | + ve | + ve |
| A | 125 | 4+4 min | 4 | <2 | 1550 | 1300 | 250 | + ve | + ve |
| A | 125 | 8 min | 4 | 5 | 7900 | 1100 | 6800 | + ve | + ve |
| A | 135 | 1+1 min | 4 | 31 | 13000 | 400 | 12600 | - ve | + ve |
| A | 135 | Bunched 1+1 min | 4 | 33 | 4800 | 500 | 4300 | - ve | + ve |
| A | 135 | 2+2 min | 4 | <2 | 4150 | 4050 | 100 | + ve | + ve |
| A | 135 | 2+2 min | 4 | 23 | 3600 | 200 | 3400 | + ve | + ve |
| A | 135 | 2+2 min | 4 | 23 | 1250 | 50 | 1200 | + ve | + ve |
| A | 135 | Bunched 2+2 min | 4 | 109 | 3100 | 600 | 2500 | + ve | + ve |
| A | 135 | 4 min | 4 | 5 | 850 | 400 | 450 | + ve | + ve |
| A | 145 | 1+1 min | 4 | 33 | 6600 | 350 | 6250 | + ve | + ve |
| A | 145 | 2+2 min | 4 | 13 | 400 | 100 | 300 | + ve | + ve |
| A | 145 | 2+2 min | 4 | <2 | 400 | 200 | 200 | + ve | + ve |
| A | 152 | 1+1 min | 4 | 8 | 1650 | 100 | 1550 | + ve | + ve |
| A | 154 | 1+1 min | 4 | 2 | 950 | 100 | 850 | + ve | + ve |
| A | 155 | 30s+30s | 4 | 17 | 2150 | 600 | 1550 | + ve | + ve |

* Where a single figure is entered in the time column, air flow was unidirectional in regard to product orientation. Where two figures are entered e.g. 10+10 min, the product trays were turned through 180° after half of the total treatment time had elapsed

## Table 2

## CHALLENGE TESTS

### Effect of Dry Heat Treatments on Bacterial load of Inoculated Macadamia NIS

| | TREATMENT | | Microbiological Analyses (cfu/g of kernel) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Inoculum | Temperature (°C) | Air Flow | 0 hr | 7 hr | 24 hr | 48 hr | 72 hr | 96 hr |
| Nil | 70 | 0.5 m/s | <10 | Not Done | Not Done | <10 | <10 | <10 |
| *Ent.cloacae* | 70 | 0.5 m/s | 11000 +ve /10g | ND ND | ND ND | 30 +ve /10g | 10 +ve /10g | <10 +ve /10g |
| *S.typhimurium* | 70 | 0.5 m/s | 9500 +ve /25g | ND ND | ND ND | 240 +ve /25g | 100 +ve /25g | 90 +ve /25g |
| Nil | 70 | 4 m/s | 150 | ND | ND | <10 | <10 | 10 |
| *Ent.cloacae* | 70 | 4 m/s | 9300 +ve /10g | ND ND | ND ND | 410 +ve /10g | 270 +ve /10g | 50 +ve /10g |
| *S.typhimurium* | 70 | 4 m/s | 6400 +ve /25g | ND ND | ND ND | 1400 +ve /25g | 400 +ve /25g | 430 +ve /25g |
| Nil | 75 | 0.5 m/s | 420 | ND | ND | 30 | 10 | 40 |
| *Ent.cloacae* | 75 | 0.5 m/s | 5000 +ve /10g | ND ND | ND ND | 70 +ve /10g | 100 +ve /10g | 20 +ve /10g |
| *S.typhimurium* | 75 | 0.5 m/s | 2600 +ve /25g | ND ND | ND ND | 10 +ve /25g | 10 +ve /25g | 10 +ve /25g |
| Nil | 75 | 4 m/s | 20 | ND | ND | <10 | <10 | 10 |
| *Ent.cloacae* | 75 | 4 m/s | 4200 +ve /10g | ND ND | ND ND | 430 +ve /10g | 150 +ve /10g | 120 +ve /10g |
| *S.typhimurium* | 75 | 4 m/s | 2000 +ve /25g | ND ND | ND ND | 160 +ve /25g | 50 +ve /25g | 80 +ve /25g |
| Nil | 80 | 0.5 m/s | 10 | ND | <10 | <10 | <10 | ND |
| *Ent.cloacae* | 80 | 0.5 m/s | 4800 +ve /10g | ND ND | 20 +ve /10g | 10 +ve /10g | 30 +ve /10g | ND ND |
| *S.typhimurium* | 80 | 0.5 m/s | 9400 +ve /25g | ND ND | 85 +ve /25g | 25 +ve /25g | 30 +ve /25g | ND ND |
| Nil | 80 | 4 m/s | <10 | <10 | <10 | ND | ND | ND |
| *Ent.cloacae* | 80 | 4 m/s | 3400 +ve /10g | 100 +ve /10g | <10 ND | <10 -ve /10g | ND ND | ND ND |
| *S.typhimurium* | 80 | 4 m/s | 10000 +ve /25g | 120 +ve /25g | <10 +ve /25g | <10 -ve /25g | ND ND | ND ND |

ND Not done