FOOD SAFETY AND SECURITY OF SAGO STARCH IN RURAL PAPUA NEW GUINEA

A Thesis submitted by
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I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university of other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

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STATEMENT ON THE CONTRIBUTION OF OTHERS

Financial support for the duration of this project was obtained through a research grant from the Australian Centre for International Agricultural Research (ACIAR), and from a stipend provided by the School of Veterinary and Biomedical Sciences. Project costs were met through the ACIAR funding. The work was completed under the supervision of A/Prof Warren Shipton, Dr Jeffrey Warner, A/Prof Leigh Owens, and Barry Blaney. The ACIAR project was in collaboration with researchers at the Department of Primary Industries and Fisheries Animal Research Institute at Yeerongpilly, The University of Technology in Lae, and the University of Papua New Guinea in Port Moresby.

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DECLARATION OF ETHICS

Relevant research reported in this thesis received approval of the Papua New Guinea Medical Research Advisory Council (MRAC number 05/25) and the James Cook University Ethics Review Committee (Human ethics number H2167).

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ABSTRACT

Sago starch is an important source of dietary carbohydrate in lowland and coastal areas of Papua New Guinea (PNG). There have been sporadic reports of severe haemolytic illness resulting from sago starch consumption, termed sago haemolytic disease (SHD), with most reports coming from the Western Province. Despite the occurrence of SHD, and a high likelihood of less severe foodborne illness resulting from consumption of indigenous foods in general in PNG, there have been no detailed studies of the microbiology of sago starch. The aim of this study was to establish a broad basal knowledge of the microbiology of traditionally prepared sago starch in PNG.

Sago starch samples and sociological information were collected in two of the main sago eating regions of PNG, the East Sepik Province and the Western Province. Sago starch samples were collected predominantly from the houses of sago starch producers in rural areas of the two provinces, and to a lesser degree from markets in some villages in the East Sepik Province. In addition to these samples considered 'fit for consumption', two samples of sago starch that had been associated with outbreaks of SHD were also analysed.

Analysis of the sago starch for common bacterial pathogens was done using accepted methods, and where possible was based on the relevant Australian Standards. The findings suggest that faecal contamination of sago starch is widespread, with over three-quarters of all samples tested for faecal coliforms at the upper limit of detection. The human pathogen *Salmonella* spp. was isolated from approximately 7% of samples tested. The presence of emerging human pathogens such as *Citrobacter freundii* and *Enterobacter sakazakii* was tested, with the former being present in a low percentage of samples tested. Other important bacterial food pathogens such as *Staphylococcus aureus* and *Bacillus cereus* were also detected in sago starch, but none of the 57 samples tested for *Listeria monocytogenes* was positive.

Mycological analysis of sago starch revealed a variety of fungal contaminants. Commonly occurring genera of filamentous fungi included *Penicillium, Scytalidium, Aspergillus,* and *Acremonium*. Mycotoxin analysis of sago starch revealed that the

common mycotoxins such as aflatoxins, ochratoxin A, cyclopiazonic acid, sterigmatocystin, zearalenone and citrinin were not present. Selected fungal isolates were tested for the presence of mycotoxin production in pure culture, with two-thirds found to be capable of citrinin production and one isolate capable of sterigmatocystin synthesis.

In an attempt to determine the aetiological agent of SHD, bacterial and fungal isolates were screened for haemolytic activity on blood agar. A suitable semi-quantitative assay was developed, and extracts from bacterial and fungal cultures were tested. Particular attention was paid to the haemolytic activity of fungal extracts, given the long speculated role of mycotoxins in the aetiology of SHD. The haemolytic activity of numerous fungal species has been demonstrated for the first time, and steps in the optimised extraction and purification of the haemolytic component of some isolates has been completed. Further work was conducted on extracts from *Penicillium steckii*, a common contaminant of sago starch. The chemical properties of the extract suggest that a novel fungal metabolite is responsible for haemolytic activity.

Detailed studies on the microbial ecology of stored sago starch have been conducted, primarily to garner a greater understanding of factors that contribute to the microbial safety of the food. The study has established that sago starch is a naturally fermented product, and this fermentation process contributes greatly to the food safety of the product. Bacterial pathogens such as *B. cereus, L. monocytogenes, S. aureus* and *Salmonella* sp. do not survive well in actively fermenting sago starch, primarily due to the production of weak acids. Furthermore, numbers of filamentous fungi are low in actively fermenting sago starch, presumably due to the reduced oxygen availability.

On the basis of the sociological data and microbial studies, a hazard analysis and critical control point (HACCP) plan was devised that was considered appropriate for application in village based production of sago starch in rural PNG. Through a greater understanding of the microbiology of sago starch, together with the development of an appropriate HACCP plan, this research will lead to increased food safety and food security for sago consumers in rural and remote lowland areas of

PNG. Moreover, studies of the haemolytic metabolites of fungi isolated from sago starch pave the way for further research to determine the aetiology of SHD.

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COMMONLY USED ABBREVIATIONS

ANOVA	Analysis of variance
ATCC	American type culture collection
a_w	Water activity
BHC	Balimo Health Centre
BHIB	Brain heart infusion broth
BPW	Buffered peptone water
BSA	Bismuth sulphite agar
CAST	Council for Agricultural Science and Technology
CCP	Critical control point
cfu	Colony forming unit
CPA	Cyclopiazonic acid
Da	Dalton
DRBC	Dichloran rose Bengal chloramphenicol agar
EHEC	Enterohaemorrhagic *Escherichia coli*
g	gram
g	gravity
HACCP	Hazard Analysis and Critical Control Point
Hb	Haemoglobin
HBA	Human blood agar
HBA-chlor	Human blood agar with chloramphenicol
HPLC	High performance liquid chromatography
HUS	Haemolytic uraemic syndrome
JCU	James Cook University
kDa	kilo Dalton
kg	kilogram
l	litre
LD50	50% lethal dose
M	molar
ml	millilitre
min	minute
MPN	Most probable number
NACMCF	National Advisory Committee on Microbiological Criteria for Foods
nm	nanometre
PBS	Phosphate buffered saline
PLC	Preparatory layer chromatography
PMGH	Port Moresby General Hospital
PNG	Papua New Guinea
ppm	parts per million
ppb	parts per billion (10^9)
Rh	Rhesus factor
rpm	revolutions per minute
RR	Reference range
RTX	Repeat in toxin
SBA	Sheep blood agar
SBA-chlor	Sheep blood agar with chloramphenicol
SBA-gluc	Sheep blood agar with glucose

SHD	Sago haemolytic disease
SMA	Synthetic *Mucor* agar
STEC	Shiga-toxigenic *Escherichia coli*
stx	Shiga toxin
TDS	Toxin diluent solution
TEF	Toluene: ethyl acetate: formic acid
TLC	Thin layer chromatography
UV	Ultraviolet
VFA	Volatile fatty acid
WBC	White blood cell
WHO	World Health Organisation
×	Multiplication
ºC	degrees Celsius
µl	microlitre
µg	microgram