

**AN ALTERNATIVE (MOLECULAR) METHOD  
FOR THE RAPID DETECTION OF SALMONELLA  
IN DESICCATED COCONUT**

**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE  
MASTERS DEGREE IN APPLIED MICROBIOLOGY  
TO THE DEPARTMENT OF MICROBIOLOGY  
UNIVERSITY OF KELANIYA  
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## ABSTRACT

A survey conducted by the Sri Lanka Coconut Board show that the overall incidence of the *Salmonella* contamination is 0.9% of the total samples tested. Conventional methods used by the Coconut Development Board (CDA) to detect *Salmonella* in desiccated coconut (DC) takes an average of 6-7 days. Therefore, some urgent shipments are done prior to obtaining the test results to avoid delays. This results in occasional rejection of shipments by importing countries. For instance, the amounts of DC rejected were 84, 16 and 115 metric tons in 1977, 1978 and 1979 respectively. The objective of this work is to identify a quick method of detecting *Salmonella* in DC samples and to enhance export earnings by minimizing rejections.

Conventional methods consist of, pre-enrichment of DC samples in buffered peptone water (BPW), selective enrichment in Rappaport Vassiliadis Soya peptone (RVS) broth and Tetrathionate broth, plating on selective media – Xylose Lysine Decarboxylase (XLD) and Brilliant Green Bile Agar (BGA), and suspected colony identification, confirmation with Triple Sugar Iron (TSI) agar and biochemical confirmation by using “api 20E ” system and serological confirmation by slide agglutination test with polyvalent ‘O’ and ‘H’ *Salmonella* antigen. About 4000 DC samples were tested with the conventional method, and only four samples were found positive and confirmed for contamination, for a period of four months.

For the Polymerase Chain Reaction (PCR) based molecular method, Genomic Deoxyribo Nucleic Acid (DNA) was extracted with the boiling method from DC samples artificially contaminated with known *Salmonella* cultures, after pre-enrichment in BPW. PCR was carried out with *Salmonella* genus-specific primers. Gel electrophoresis was done, and visualized. The anticipated size Amplified DNA product (457 bp) was observed.

It was essential to pre-enrich and to use Triton X 100 in order to obtain genomic DNA of *Salmonellae* from contaminated DC samples.

Confirmation of contamination obtained as a result of generation of 457 bp size products with four DC samples subjected to PCR based assay gave more validation to the method as they had already been found to be positive and confirmed with the conventional method.

To check the sensitivity of the developed method, experiments were carried out with extracted DNA from DC samples contaminated with various strengths of inoculum of known *Salmonella* species (*Salmonella* M 1 type). It was revealed that the minimum of 3-4 cells if present in 1  $\mu$ l of the pre-enriched solution could be detected precisely with this method.