Identification of Potential Spoilage and Pathogenic Microorganisms Associated with Production of Thermally Processed King Coconut Water (*Cocos nucifera* var. aurantiaca) in Sri Lanka

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Thermally processed king coconut (*Cocos nucifera* var. aurantiaca) water is one of the fastest growing export commodities in the food and beverage sector in Sri Lanka. Microbial safety of thermally processed king coconut water is demanded by Food and Drug Administration to ensure safe consumption. This study aimed to identify potential spoilage and pathogenic microorganisms associated with king coconut water processed in Sri Lanka. King coconut water samples were collected at pre-identified sampling points; P_1-Nut water extraction, P_2-Bulk collection, P_3-Standardization/Acidification, P_4-Pre-heating prior to hot filling and P_5; Sterilization/Pasteurization in three processing facilities; F_1 (semi-automated), F_2 (automated) and F_3 (manual), where Pasteurization (100 °C/12.5 min), UHT Sterilization (140 °C/3s) and Pasteurization (100 °C/20 min) were practiced, respectively. Serially diluted king coconut water were plated on Nutrient Agar, Potato Dextrose Agar, Eosin Methylene Blue Agar and Reasoner's 2A Agar, incubated at 30 ± 1 °C for 48 h, 25 ± 1 °C for 2 - 5 days, 37 ± 1 °C for 48 h and 37 ± 1° C for 48 h, respectively. Purified bacterial and fungal colonies were morphologically characterized. A total of 29 bacterial isolates and 24 fungal isolates were identified by 16S rRNA and 26S/5.8S rRNA/ITS gene amplification, respectively followed by sequencing using 27F/1492R and ITS-1/ITS-4 primers, respectively. Evolutionary relationships of identified species were predicted using MEGA 7. The study revealed that thermal resistant, facultative-anaerobic, spoilage and pathogenic bacteria (*Pantoea dispersa*, *Bacillus siamensis*, *Pseudomonas stutzeri*, *Acinetobacter lactucae*) and fungi (*Candida carpophila*, *Pichia kudriavzevii*, *Debaryomyces nepalensis*, *Microdochium fisheri*, *Penicillium citrinum*) were survived in the thermally processed finished product. Further, potential risk of *Klebsiella pneumoniae*, *Enterobacter roggenkampii*, *Enterobacter kobei*, *Escherichia fergusonii*, *Bacillus nealsonii*, *Serratia rubidaea*, *Trichosporon asahii*, *Wickerhamomyces anomalus*, *Saccharomycetales* species and *Fusarium* species were identified at initial processing steps (P_1-P_3) in studied processing facilities. In conclusion, the study revealed that the existing thermal treatments are not sufficient for the destruction of identified potential spoilage and pathogenic microorganisms associated with studied processes. Thereby, suggesting thermal process validation, while targeting identified potential harmful microorganisms with optimum time-temperature combinations to ensure product safety.

Keywords: King coconut water; Spoilage and pathogenic microorganisms; Molecular identification; Thermal processing