The Pharma Innovation Journal 2023; 12(6): 1076-1079

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(6): 1076-1079

www.thepharmajournal.com
Received: 02-04-2023
Accepted: 09-05-2023

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Investigating the microbial sporulation of mature coconut water

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Abstract
Mature coconut water (MCW) is the liquid found inside a mature coconut, which can serve as a favorable environment for microbial growth if not properly handled and stored. Some common microbial tests were` conducted on MCW to analyze the extent of microbial spoilage, Fresh MCW was collected, from the hostel mess of Kaunya Institute of Technologies and Science (KITS), and the MCW was sterilized and stored in a freezer for further analyses. The Total plate count (TPC), for yeast and mould enumeration was done by serial dilution, and spread plate method for the 0th, 3rd, 6th hour and the Preservation Efficacy Test (PET) was carried out with control (T_0), and by adding 3% of salt (T_1), 5% of salt (T_2), 3% of sugar (T_3), 5% of sugar (T_4), 3% of salt + 3% of sugar (T_5) and 250 ppm of sodium benzoate (T_6). The results of the plate count for the fresh MCW samples were 1.25 x 10^3 CFU/mL, yeast and mould count were 700000 CFU/ mL and the pH was found to be 5.3. In this study, based on the PET, T_1 (3% of salt) acted as good preserving agent. The TPC in T_1 (3% salt) was found to be 4.5 x10^3 CFU/ ml and yeast and mould count was 1650000 CFU/ml.

Keywords: (MCW) Mature coconut water, microbial analyses, total plate count, yeast and mould count, (PET) Preservation Efficacy Test.

Introduction
MCW refers to the liquid endosperm of coconut at an age of approximately 10 months at which the white meat hardens. It is a pure, sweet, delicious, nutritious, and refreshing natural drink containing rich amounts of sugars, vitamins, minerals, beneficial phytohormones, and electrolytes. It has a caloric value of 17.4 per 100g. MCW is an excellent substitute to tender nut water as a rehydrating drink because it contains more organic ions such as calcium, potassium, magnesium, and phosphorous, compared to tender coconut. These qualities make MCW particularly suitable for the sports drink markets. So, MCW is described as a "sports beverage" and "sports rehydration drink" because of its natural functional properties present in MCW. Apart from functional properties as described, it also possesses typical flavour and aroma (Prades *et al.*, 2012) [5].

Preserving MCW is rather tricky because of external contaminants, microorganisms, enzymatic and non-enzymatic browning all of which lead to fast spoilage. These factors also cause discoloration, off-flavour, degradation, chemical, and structural changes so, preservation methods are to be followed in the processing of MCW, including sterilization, ultraviolet treatment, the addition of chemical preservatives, and filtration, etc., (Naik *et al.*, 2020).

The effect of storage temperature (between 4º-35 ºC) and duration of storage (up to 24 hrs) on physicochemical properties and microbiology of immature and MCW was observed by Tan and Easa (2021) [9].

They reported that when temperature rises above 25 ºC, TSS was slightly reduced and pH also became low with more acidity. Mature water shows more positive changes with an increase in temperature than immature MCW.

Materials and methods
The study was conducted in the Microbiology laboratory, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore.

Chemical and reagents
The chemicals used in media preparation were Nutrient Agar (NA), Potato dextrose agar (PDA), and Sabouraud dextrose agar (SDA).

Sample preparation of MCW

MCW was obtained from mature coconuts which were collected from the hostel mess, of KITS. MCW was filtered through a muslin cloth. The filtered MCW from several nuts were pooled and stored in a 2-liter plastic bottle and brought to the laboratory inside the KITS for further analysis. The MCW samples were collected in batches and sterilized by pasteurizing for 30 minutes @ 63 ºC for each batch of MCW, prior to carrying out the microbiological assessments. (Rukmini *et al*., 2017) [7].

Microbial Enumeration

The total microbial count of the fresh MCW sample was determined using an agar plating technique. One ml in 9 ml of sterile distilled water solution was serial diluted up to 10^{-3}. From the dilutions, 0.1 ml was inoculated into 20 ml of molten media (Nutrient agar and potato dextrose agar) (Adubofuor *et al*., 2016) [1].

Plating was done at time intervals of 0, 3, and 6 hours and was analyzed for the microbial load. The different MCW dilutions were plated on TPC and yeast and mould plate counts in petri plates. The TPC and yeast and mould plate count petri plates were incubated at 37 °C for 24 h [Tan *et al*., 2021] [9].

Preservative efficacy testing

The common preservatives, sugar and salt were used to test the efficacy, with concentration levels of T0- control, T_1 - salt 3%, T_2 - salt 5%, T_3 - sugar 3%, T_4 - sugar 5 %, T_0 - 3% salt +3% sugar, and T_6 - 250 ppm of sodium benzoate were added to 100ml of MCW for each test to ensure that the chosen preservatives were effective in inhibiting microbial growth and maintaining product safety. Wijnker *et al*. (2006) [10] recognized the antibacterial characteristics of salt employed in conventional procedures for preservation.

Results and Discussion

Viable Count Measurements

Viable count measurements (Total plate count and yeast & mould count) were carried out. The nutrient agar was used to find bacterial colonies and sabouraud dextrose agar was used to find the number of fungal colonies in the sample.

Total Plate Count (TPC)

TPC test was done according to Beuchat *et al*. (1998) [2]. The total number of viable microorganisms present in a given sample of MCW was counted to provide a general assessment of the microbial load and is an indicator of overall hygiene and quality of the produce. The total plate count is 1.25 x 10^6 CFU/ml.

Yeast and Mould Count

Yeasts and moulds count were enumerated according to Tournas *et al*. (2001) [8]. These are common spoilage microorganisms that can grow in MCW under favourable conditions. Testing for yeast and mould counts help to assess the shelf life of the product and determine the effectiveness of preservation methods. The yeast and mould count was 700000 CFU/ml.

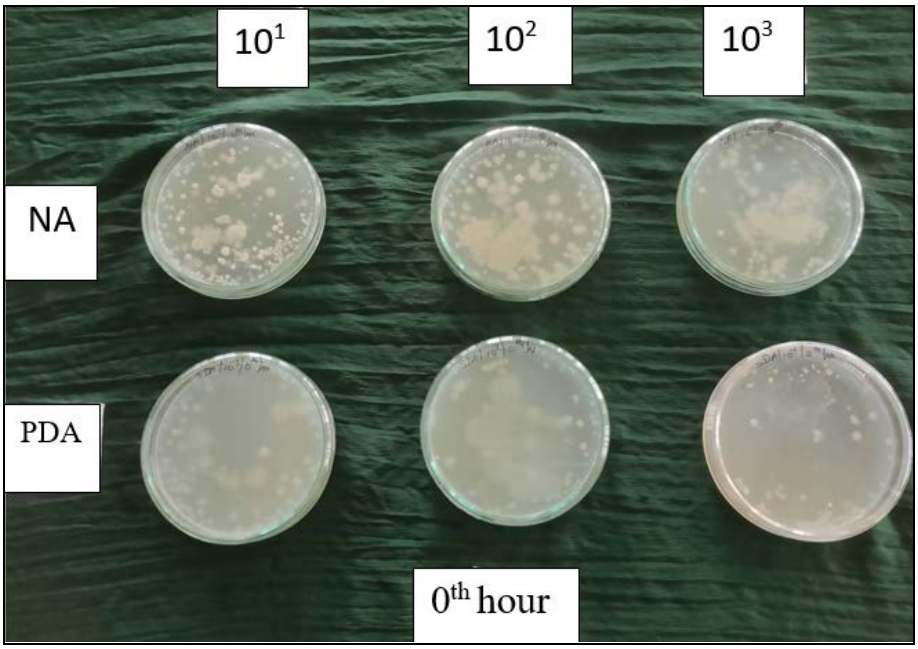

Fig 1: Growth of bacteria [NA] and fungi [PDA] at 0^{th} hour with dilutions of 10^1, 10^2, 10^3

Table 1: TPC and bacterial growth in fresh MCW sample (TNTC- Too numerous to count)

Hours (Time gap)	TPC (CFU/ml)	Yeast and mould count (CFU/ml)
0^{th}	1.25 x 10^3CFU/ml	700000 CFU/ml
3^{rd}	TNTC	TNTC
6^{th}	TNTC	TNTC

The results obtained were expressed as colony-forming units per milliliter (CFU/mL) using the following equation: Where N is the number of CFU/mL, EC is the total colonies on all the plates counted, n1 is the number of plates counted at the first dilution, n2 is the number of plates counted at the second dilution, and d is the dilution factor of the first dilution.

PH Analysis

The pH of the MCW sample was measured as 5.3 using a digital pH meter. A pH of 7 is neutral, less than seven are acidic, and greater than 7 is alkaline, (Rolle *et al*., 2007) [6].

Preservative Efficacy Testing (PET)

Salt acts as a good preserving agent for MCW- 3% of salt (T1) and 5% of salt (T2) produced differences in appearance and odour. The appearance of MCW was found to be turbid (gas was produced during fermentation), as seen in Fig 2, and no browning occurred till the 14th day. The breakdown of small quantities of protein in MCW produced sulphur smell, while other tests (T_0, T_3, T_4, T_5, T_6) showed browning appearance. The treatments, T_3, T_4, T_6 produced rancid smell and sulphur smell was observed in T0 and T5. The evaluations were performed according to Rolle *et al*. (2007).

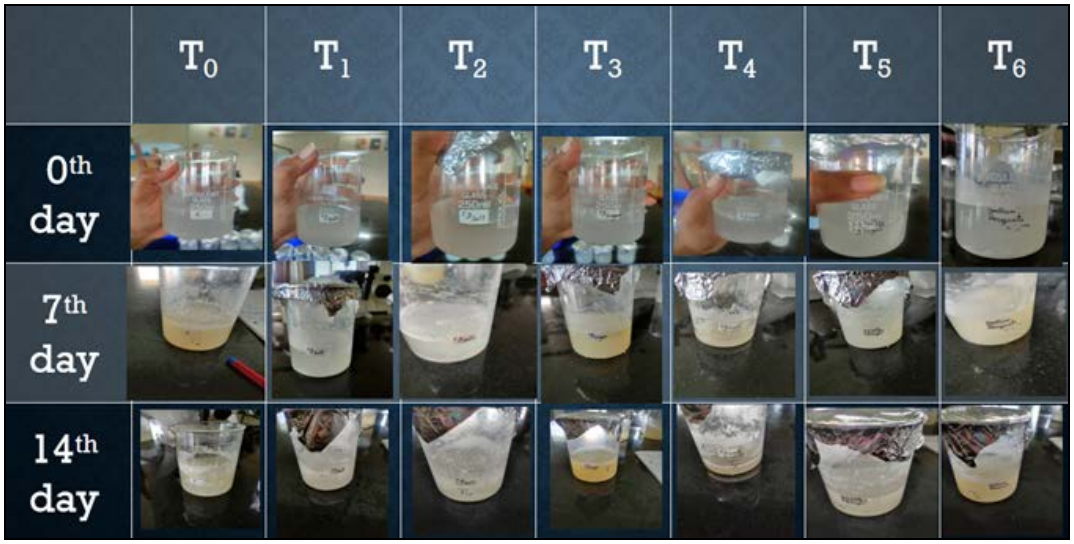

Fig 2: Appearance of samples (colour change) as observed on the 0th, 7th, and 14th days)

The microbial enumeration test was also taken for the PET, the T_1 (3% salt) and T_2 (5% salt) with the dilution of 10^{-2}, 10^{-3} and 10^{-4}, the TPC was TLTC (too less to count), the yeast and mould count for T_1 is 17500000 CFU/mL and the growth of yeast and mould in T_2 is TMTC (Too many to count).

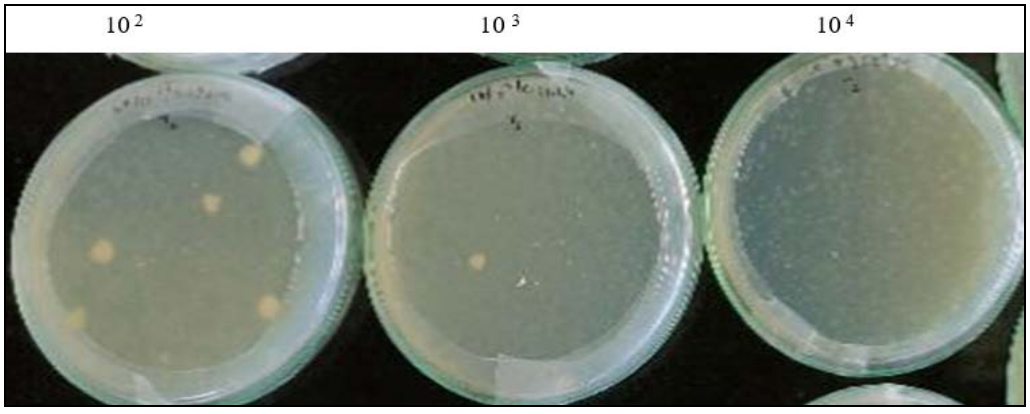

Fig 3: (T_1 - 3% salt) Bacterial growth using NA

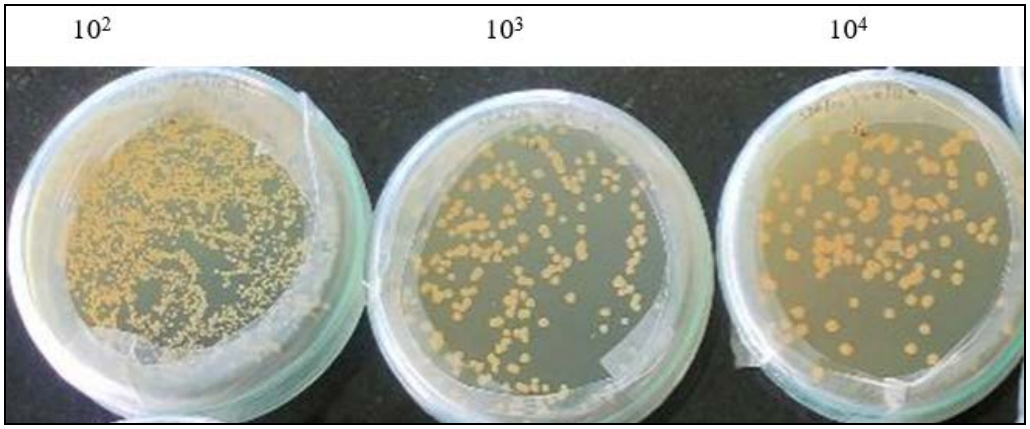

Fig 4: (T_1 – 3% salt) yeast and mould growth using SDA

Table 2: Total plate count and Yeast and mould count in PET for T_1-3% salt

Time	TPC CFU/ml	Yeast and mould (CFU/ml)
7th day	4.5x10 ^{3} CFU/ml	17500000 CFU/ml
14th day	TNTC	TNTC

Conclusion

To minimize the microbial contamination in MCW, a microbial enumeration test was done with a fresh MCW sample, and plating was done within a time gap of 3 hours (0th, 3rd, and 6th hour) as MCW gets spoiled very easily. The microbial enumeration count as observed in the 3rd and 6th hour was found to be more (too numerous to count). In the PET, out of the seven treatments, the result shows that T1 (3% salt) acts as a good preserving agent with minimal microbial growth, while other treatments showed a change in appearance (browning), odor (sulfur and rancid).

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