*J. Sci. Univ. Kelaniya 16 (1) (2023): 31 - 46* http://doi.org/10.4038/josuk.v16i1.8078

# Evaluation of the changes in physiochemical properties and fatty acid profile of industrially pasteurized coconut (*Cocos nucifera*) milk during storage

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Received: 23.01.2023 Revised: 21.02.2023 Accepted : 27.02.2023 Online: 15.05.2023

**Abstract** The changes in the quality of coconut milk in industrial storage conditions have received scarce attention despite its widespread use. The study was carried out to evaluate the quality of industrially pasteurized coconut milk based on physicochemical parameters; pH, total solid content, acidity, total microbial count, and the changes in the fatty acid profile during storage. The study revealed that pH of the coconut milk samples varied in the range of 5.2-7.2 and acidity drastically increased during storage. The total microbial count increased with an increasing number of days in all samples. The highest total microbial count was observed on day 5 in 3 samples indicating the unfavourable microbial spoilage. Total solids in all samples were higher than 35% throughout the study. The ANOVA confirmed a significant increase ($p>0.05$) in the acidity and total microbial count from day 1 to 5, illustrating the crucial roles which these variables play in determining the quality of industrially pasteurized coconut milk during storage. The presence and changes of fatty acids in industrially pasteurized coconut milk were quantitatively analyzed by GC/MS and a total of 8 fatty acids were identified. The fatty acid contents changed significantly after industrial pasteurization and storage.

**Keywords:** acidity, coconut, coconut milk, fatty acid, pasteurization, pH, physicochemical parameters

## Introduction

Coconut is a significant contributor to foreign exchange and a major component of the rural livelihood and cuisine in coconut growing Asian countries such as Indonesia, India, Philippines and Sri Lanka (Devi & Ghatani, 2022). The coconut tree is frequently referred to as the "Tree of Life" among coconut cultivating communities due to its significant contribution to people's well-being and means of subsistence (Mat et al., 2022). Coconut milk is a white, opaque, protein-oil-water emulsion and is fundamentally free from fiber (Tulashie et al., 2022). It is also specified as a milky fluid produced manually or mechanically from grated coconut kernels with or without the addition of water (Hebbar et al., 2022). Codex Alimentarius defines coconut cream as the emulsion extracted from matured endosperm (kernel) of the coconut fruit with or without any addition of coconut water or water (Rethinam & Krishnakumar, 2022b).

In contrast to traditional approaches, rapid, accurate, and efficient methods have recently been employed in determining the quality of coconut-based products. Nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, mid-infrared (MIR) spectroscopy, near-infrared (NIR) spectroscopy, ultraviolet-visible (UV-VIS) spectroscopy, fluorescence spectroscopy, Fourier-transformm infrared spectroscopy (FTIR), and Raman spectroscopy (RS) are some of the novel techniques used to evaluate the quality of coconut based products (Dzidic et al., 2021; Pandiselvam et al., 2022). Fresh coconut endosperm without testa mainly contains moisture (68.62 ± 0.161 %), 22.82 ± 0.212 % fat, 5.42 ± 0.083 % carbohydrate, 2.39 ± 0.006 % protein, and 0.75 ± 0.015 % ash

(Dzidic et al., 2021). It also contains several minor compounds including gallic acid, catechin acid, chlorogenic acid, p-hydroxybenzoic acid, caffeic acid, syringic acid, ferulic acid, p-coumaric acid and ellagic acid (Beegum et al., 2022). The level of fat in the products, distinguishes coconut milk from cream (Divya et al., 2023). Based on their fat content, coconut milk products can be divided into coconut milk and coconut cream (Divya et al., 2023). Coconut milk should consist of at least 10% fat, 2.7 % non-fat solids, and 12.7-25.3 % total solids, according to the Codex Standards for aqueous coconut (Codex Alimentarius, 2019). Concentrated coconut milk also known as coconut cream should consist of at least 20% fat, 5.4 % non-fat solids, and total solid content of 25.4-37.3 % (Codex Alimentariyus, 2019).

Commercial production of coconut milk and its applications can significantly minimize nut waste and increase the effective use of by-products such as coconut chips, coconut flour and coconut butter (Hebbar et al., 2022). Coconut oil, desiccated coconut, copra, coconut cream, and coconut milk powder are the main kernel products used by consumers (Samarawikrama et al., 2022). The effectiveness of extraction of main products and by-products are much higher when it is done at an industrial level than when it is done at a domestic level (Manikantan et al., 2021a). The effectiveness of extraction largely depends on the scale of the manufacturing facility and the type of technology used. The non-kernel sector products are based on the husk and the shell of the coconut (Aiome et al., 2021). Husk products include bristle fiber, mattress fiber, coir pith and other value-added products for example coir yarn, coir twine, Tawashi brushes, coir brooms, brushes, rubberized coir pads, mattress for bedding, coir mats, rugs, fiber pith, husk chips, geo textiles and molded coir products used in horticulture. Coconut cream and coconut milk play a vital role in the Asian cuisine. Cream and milk extracted from coconut kernel is used in industrial scale for downstream applications such as sprayed coconut milk powder, UHT coconut milk and coconut milk-based beverages. Coconut milk powder and UHT coconut milk products are in high demand in the modern society as a convenient way of preparing meals without having to manually scrape coconut from nut and extracting milk at households (Manikantan et al., 2021b). Therefore, from the perspective of the industry, improving the quality of the milk and cream as well as the extraction efficiency will produce positive economical outcomes. The world's leading coconut producers are Indonesia, the Philippines, India, Sri Lanka, and Brazil, and production is primarily restricted to the Asia and Pacific region (Rethinam & Krishnakumar, 2022a). Over the past years, the demand for coconuts has grown significantly worldwide due to the growing focus on coconut water and Virgin Coconut Oil (VCO), which have lately been shown to offer health benefits (Sulistyo et al., 2018).

The composition of coconut milk is vital for the unique flavour profile (Wang et al., 2020). The type of coconut kernel utilized, can influence the composition of coconut milk (Tulashie et al., 2022). It also depends on the maturity of the crop and the agro-ecological conditions. The physical properties of coconut milk include specific gravity, surface tension, viscosity, refractive index and pH. Specific gravity ranges from 1.0029 to1.0080, surface tension (dyne $cm^{-2}$) ranges from 97.76 to 125.43, viscosity (mPa.s) ranges from 1.61 to 2.02, refractive index ranges from 1.3412 to 1.3446 and pH ranges from 5.95 to 6.30 (Tangsuphoom & Coupland, 2008). Coconut milk is prepared by using a significant amount of separated, whole, disintegrated, macerated or comminute fresh endosperm (kernel) of coconut palm (*Cocos nucifera L*.) and expelled, where most filterable fibers and residues are excluded, with or without coconut water, and/or with additional water (Codex Alimentarius, 2019) . In most of coconut mills the coconut water is segregated and used for different applications such as pasteurized coconut water beverages (Devi & Ghatani, 2022).

The process of cultivation involves a number of technical and physical inputs. The collection and selling of nuts to industry is carried out by dealers or millers who are the intermediaries in the value stream. Then processing occurs on an industrial scale at facilities that can range in size from small to large. After manufacturing, the value stream moves to its final phase, when it is distributed to consumers who use it both commercially and domestically. Both local and international consumers may be supplied through these distribution chains. The distribution channel is chosen based on the customer's characteristics and geography.

Generally, the matured coconut nuts are collected by coconut mills and processing plants (primary or secondary collectors) and pasteurized at their plants to be further processed by manufacturers. The common process flow diagram of a typical coconut mill/ processing plant is illustrated in Figure 1. The coconut milk to be dispatched from coconut mills are used by food manufacturers to produce value added products such as milk powders, ready to drink products and coconut milk-based products.

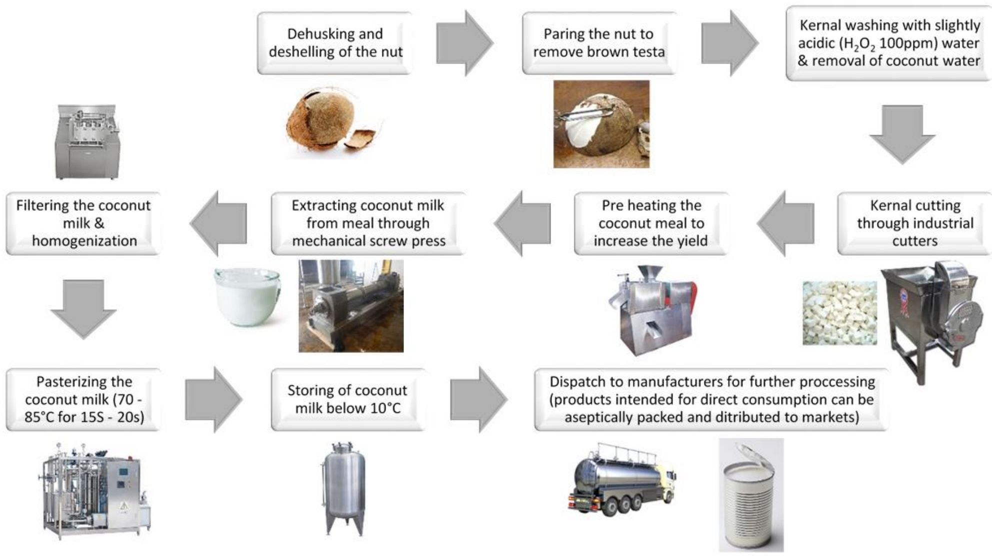


**Figure 1:** Simplified process flow diagram of a coconut processing plant

Untreated coconut milk due to its high nutrient content spoil rapidly even at lower temperatures (Tiravibulsin et al., 2021). In case of treated coconut milk, spoilage microorganisms can find their way into coconut milk through utensils, processing equipment, handlers, operators at coconut mills and environmental contaminants (Kurwijila, 1997). Common genera of spoilage bacteria in coconut milk include *Bacillus*, *Achromobacter, Microbacterium*, *Micrococcus* and *Brevibacterium.* Coliform organisms and fungi such as *Penicillium, Geotricum*, *Mucor*, *Fusarium* and *Saccharomyces* spp. are also commonly found in coconut milk (Onoharigho et al., 2022). The generation time for multiplication of bacterial cells in coconut milk drops from 232 min at 10 °C to 44 min at 30 °C (Vallath & Shanmugam, 2022). Hence, coconut milk is stored at 10 °C – 15 °C temperature to minimize the microbial growth in processing facilities. During storage coconut milk can undergo auto-oxidation and lipid oxidation which will eventually lead to change in composition of coconut milk (Ponnampalam et al., 2022). Formation of organic fatty acids and breakdown of existing organic compounds could be the net result of biological and chemical reactions in coconut milk during storage (Tiravibulsin et al., 2021). Hence, it is important to evaluate the quality of the coconut milk before it is subjected to further processing as it may directly have an impact on the final product quality and production cost (Tiravibulsin et al., 2021).

In spite of the fact that diverse coconut milk preparations are widely utilized in Asian cuisine and other parts of the world as a unique delicacy, very few studies have been conducted on the physicochemical quality parameters and fatty acid profile during storage.

This research features the evaluation of the quality of coconut milk that is processed in a typical coconut processing plant based on physicochemical parameters; pH, total solid content, titratable acidity, total plate count and fatty acid profile change during storage. The study has taken into account the industrial settings and elements that influence the physicochemical properties and fatty acid profile of coconut milk during storage.

## Methodology

### *Sampling*

Coconut milk samples were obtained from five coconut processing plants in Northwestern Province of Sri Lanka. Fifteen samples were collected as test materials. Three samples from different storage tanks belonging to different batches were collected from each coconut processing plant.

### *Preparation of liquid samples of coconut milk at the coconut processing plant*

Matured fresh nuts were de-husked and de-shelled manually at the coconut processing plants. Then the nut was pared off to get rid of the brown skin layer known as 'testa' as the brown skin can bring a bitter taste. The obtained white kernels were washed thoroughly, with 100 ppm $H_2O_2$ solution followed by potable water. The kernels were drained and pre-cut using industrial scale cutters. The pre-cut coconut was steam blanched for 10 minutes and milk was extracted mechanically using screw press without adding water. In some mills the residual kernel from this first press is then mixed with water before it is pressed again to increase extraction yield. Coconut milk was filtered after extraction to remove large contaminants. Then the milk was homogenized at 15 000 rpm for 15 minutes using a homogenizer. Then the milk sample was pasteurized at 80 ± 5 °C for 15 ± 5 seconds. Pasteurized and filtered coconut milk was stored at 4 °C in storages tanks for dispatch.

### *Storage*

The pasteurized milk samples were collected from storages tanks and stored in 500 mL, clean, sterile, airtight PET (Polyethylene terephthalate) bottles, following the aseptic techniques to eliminate the effects of microbiological contaminations throughout the process. The samples were stored between 13 ± 2 °C in the refrigerator for further analysis.

### *Physicochemical evaluation of coconut milk*

Samples were taken in triplicates and used for further analysis for pH, total solid content, titratable acidity and total plate count. The coconut milk samples were periodically (at 24 hour's intervals from processing day up to day 05) observed during storage.

### *Determination of pH of coconut milk*

The pH value was measured using a pH meter (Eutech pH 2700). The pH meter was calibrated with buffer solutions with pH values of 4.0, 9.0 and 10.0 before use. The samples in airtight PET (Polyethylene terephthalate) bottles were transferred into a plastic 50 mL cup. Subsequently, the pH meter's probe was submerged into the coconut milk sample, and the values were recorded. The pH was determined in triplicate for each sample.

### *Determination of total solids of coconut milk*

Hot air oven drying method was used to determine the total solids of coconut milk. Three grams of the sample were accurately weighed and placed in a flat-bottomed metal dish with a lid. The open dish was heated in a boiling water bath for 30 minutes to remove most of the moisture. Afterwards, the dish's bottom was washed and dried. A ventilated oven set to 102 ± 2 °C was used for further drying. The dish was placed in the oven with the lid, uncovered. After drying for 2 hours in the oven, the dish was covered with the lid, allowed to cool in a desiccator for 30 minutes, and weighed. The dish with the cover was heated in the oven again for 30 minutes, subsequently cooled and weighed until the difference between the two weightings was less than 1 mg. The total solids were determined in triplicate for each sample (Lakshanasomya et al., 2011).

### *Determination of titratable acidity of coconut milk*

Ten grams of coconut milk was accurately measured and placed in a 250 mL Erlenmeyer flask. 20 mL of distilled water and 2 mL of phenolphthalein indicator solution were added to the Erlenmeyer flask. After mixing thoroughly the solution was neutralized with 0.1 mol/L NaOH standard solution. Volume of NaOH used (V) was recorded at the endpoint of titration. The titratable acidity was calculated as V X 10. The titratable acidity was determined in triplicate for each sample (Yan et al., 2017).

*Microbiological analysis of coconut milk*

The coconut milk was homogenized and diluted to obtain $10^{-3}$ dilution. A total of 1 mL of suspension of the dilution introduced into the petri dish. Plate Count Agar (PCA) that had been cooled to 45 °C ± 1 °C was added on to each petri dish containing the diluted suspension. After agar solidification, the culture plates were overturned and incubated at 36 °C for 48 h. Finally, the microbial colonies were counted and expressed as colony forming units (CFU) per gram. The total microbial count was determined in triplicate for each sample (Yan et al., 2017).

*Fatty acid analysis of coconut milk during storage*

The analysis of the samples was performed on an Agilent 7890B gas chromatography instrument. An Agilent CP-SIL-88 (FAME, 100 m x 250 µm x 0.2 µm) capillary column was used to separate volatile compounds. The temperature of the injector port was 250 ℃. The oven temperature program was as follows: the column temperature started at 50 ℃ and was held for 5 min, then raised at 8 ℃/min to 180 ℃; it was finally increased at 15 ℃/min to 200 ℃ and maintained for 7 minutes. The carrier gas was helium (He) set at a flow rate of 1.7 ml/min and was held at a constant pressure of 45 psi. The mass spectrometry conditions were as follows: The MS Quad temperature is set at 150 ℃ and MS source is set at 250 ℃, respectively. The ionization energy was 70 eV.

*Statistical analysis*

Each experiment was repeated in triplicate. The data obtained were analyzed by SPSS (version 20.0). The results were expressed as mean ± standard error mean (SEM). Significant differences between the samples were assessed by one-way analysis of variance (ANOVA) test at a 95 % confidence level ($p < 0.05$).

## Results and Discussion

*pH and the* titratable a*cidity*

Results show that pH of the coconut milk samples varied in the range of 5.2 - 7.2 during the storage period (Table 1). The highest pH was recorded in sample 3 on the 5$^{th}$ day of storage that is 13.44 ± 0.97. The least pH value at day 5 was recorded in sample 5 which was 7.88 ± 0.87. The ANOVA showed there is no significant difference ($p>0.05$) in pH value between day 1 and day 5 coconut milk samples tested ($p= 0.08$).

Titratable acidity showed an increasing trend with increasing number of days in storage. Titratable acidity varied in the range of 7.02 to 13.44 in all samples in the duration of 5 days (Table 2). Contrary to pH variation, the increase in titratable acidity is more prominent since there was a substantial difference between the titratable acidity on day 1 and 5 while it increased gradually from day 1 to day 5. Further, a more drastic increase in the titratable acidity was observed moving from day 3 to day 5 in all samples except sample 5. A clear change in the titratable acidity was observed in sample 3 followed by other samples in the order of sample 4, 1, 2 and 5. The ANOVA showed that there is significant difference ($p>0.05$) in titratable acidity between day 1 and day 5 coconut milk samples tested ($p= 0.02$).

**Table 1:** pH variation of coconut milk samples (mean) during storage

| pH | Day 01 | Day 02 | Day 03 | Day 04 | Day 05 |
|---|---|---|---|---|---|
| **Sample 1** | 6.01 ± 0.14 | 6.1 ± 0.16 | 6.19 ± 0.13 | 6.1 ± 0.17 | 6.08 ± 0.16 |
| **Sample 2** | 6.06 ± 0.7 | 6.1 ± 0.12 | 6.09 ± 0.13 | 6.11 ± 0.11 | 6.02 ± 0.91 |
| **Sample 3** | 7.2 ± 0.16 | 6.04 ± 0.12 | 6.14 ± 0.13 | 6.03 ± 0.90 | 5.23 ± 1.10 |
| **Sample 4** | 6.12 ± 0.34 | 6.11 ± 0.82 | 6.06 ± 1.01 | 5.68 ± 0.72 | 5.55 ± 0.77 |
| **Sample 5** | 6.17 ± 0.7 | 6.15 ± 0.9 | 6.14 ± 1.03 | 6.1 ± 0.90 | 5.98 ± 1.10 |

Note: No significant difference was observed ($p=0.08$) from Day 01 to Day 05 at 0.05% level of significance. Each data point represents the mean of three replicates ± standard deviation.

**Table 2:** Titratable acidity variation of coconut milk samples (mean) during storage

| Titratable Acidity | Day 01 | Day 02 | Day 03 | Day 04 | Day 05 |
|---|---|---|---|---|---|
| **Sample 1** | 7.34 ± 0.51 | 7.26 ± 0.61 | 6.72 ± 0.13 | 7.34 ± 0.91 | 8.42 ± 0.92 |
| **Sample 2** | 7.02 ± 0.73 | 6.52 ± 0.11 | 7.34 ± 0.13 | 7.8 ± 0.16 | 9.34 ± 0.90 |
| **Sample 3** | 7.2 ± 0.24 | 7.14 ± 0.37 | 6.86 ± 0.71 | 10.15 ± 0.91 | 13.44 ± 0.97 |
| **Sample 4** | 7.22 ± 0.97 | 5.94 ± 0.73 | 6.62 ± 0.83 | 8.28 ± 1.10 | 10.28 ± 0.74 |
| **Sample 5** | 7.08 ± 0.82 | 7.1 ± 0.71 | 6.78 ± 0.82 | 7.88 ± 0.58 | 7.88 ± 0.87 |

Note: Significant difference was observed (p=0.02) from Day 01 to Day 05 at 0.05% level of significance. Each data point represents the mean of three replicates ± standard deviation.

The increase in acidity may be due to the production of microbial metabolic products such as acetic acid and lactic acid in coconut milk (Kurwijila, 1997). Lactic, acetic and succinic acids at concentration below 1.2 g/L, are being produced during the fermentation of coconut milk by *Lactobacillus reuteri* LR 92 (Onoharigho et al., 2022). However, coconut milk fermented by *Lactobacillus reuteri* DSM 17938, showed higher production of lactic acid ($5.45 \pm 0.33$ g/L) and acetic acid in a lower proportion ($0.56 \pm 0.08$ g/L) (Mauro & Garcia, 2019). Further, malic and citric acids naturally occur in the mature coconut pulp which also contribute to the acidity of the coconut milk (Mauro & Garcia, 2019). Acetic acid is also formed during heat treatment of milk (Ng et al., 2021). Acetic acid formed can be a minor product of carbohydrate degradation in the process (Harper et al., 2022). The heat treatment can form significant transitional compounds in coconut milk and reduce them to acetic acid during storage (Vallath & Shanmugam, 2022). The anaerobic microbial disintegration of carbohydrates in coconut milk into lactic and acetic acids show an increase in acidity and reduction in the pH value (Vallath & Shanmugam, 2022). In addition, breakdown of fat and protein were more pronounced in the raw samples resulting in the release of free fatty acids and amino acids (Garavand et al., 2023), respectively, which also contributed to the above increase observed with respect to the titratable acidity.

*Microbiological analysis*

The sample's total microbial count provides a reliable indication of the coconut milk's quality (Table 3). Total microbial count is expressed in terms of colony forming units. Total microbial count increased with increasing number of days in all 5 types of samples in the range of 2600 CFU/g to >30000000 CFU/g. The highest Total microbial count was monitored on day 5 of sample 3, 4 and 5 indicating the prominent growth of microorganisms in coconut milk. The ANOVA showed there is a significant difference (p>0.05) in average total microbial count (CFU/g) between day 1 and day 5 coconut milk samples tested (p= 0.01).

**Table 3:** Total microbial count variation of coconut milk samples during storage

| Average Total Microbial Count (CFU/g) | Day 01 | Day 02 | Day 03 | Day 04 | Day 05 |
|---|---|---|---|---|---|
| **Sample 1** | $3.40 \times 10^4$ | $5.60 \times 10^5$ | $2.53 \times 10^6$ | $4.66 \times 10^6$ | $1.40 \times 10^7$ |
| **Sample 2** | $2.60 \times 10^3$ | $3.00 \times 10^3$ | $1.56 \times 10^4$ | $1.63 \times 10^5$ | $2.27 \times 10^5$ |
| **Sample 3** | $2.10 \times 10^6$ | $2.30 \times 10^6$ | $1.50 \times 10^7$ | $1.53 \times 10^7$ | $>3.00 \times 10^7$ |
| **Sample 4** | $1.93 \times 10^5$ | $4.66 \times 10^6$ | $8.13 \times 10^6$ | $2.97 \times 10^7$ | $>3.00 \times 10^7$ |
| **Sample 5** | $1.12 \times 10^4$ | $5.06 \times 10^5$ | $1.50 \times 10^7$ | $>3.00 \times 10^7$ | $>3.00 \times 10^7$ |

Note: Significant difference was observed (p=0.01) from Day 01 to Day 05 at 0.05% level of significance. Each data point represents the mean of three replicates ± standard deviation.

Total microbial count drastically increased day by day due to the rapid growth of bacteria and/or fungi. The common microbes detected in coconut milk are *Bacillus, Achromobacter, Microbacterium, Micrococcus, Brevibacterium* and some coliform bacteria, while *Penicillium, Geotricum, Mucor, Fusarium* and *Saccharomyces* species are considered to be the dominant fungi screened from coconut milk (Codex Alimentarius, 2019). The possible sources of the microorganisms found in coconut could be transportation, contaminated shells, uncleansed machinery, collectors, operators at mills, etc. Coconut kernel is comprised with carbohydrates and oils mainly with medium chain triglycerides and aromatic compounds which supply the required nutrient medium for the microorganisms to grow. Depending on the type of bacteria present, spoiling of fresh coconut milk may also occur while fast-chilling in the refrigerator (Tarek et al., 2020). Spoilage of milk resulting from the contamination of dairy products with psychrotrophic microorganisms can result in significant quality loss of milk according to a study conducted by Dogan and Boor in 2003 (Dogan & Boor, 2003). During storage, the micro-biota shifts toward psychrotrophic microorganisms, which can easily reduce the quality of raw milk in low temperatures (Celano et al., 2022). *Pseudomonas* has been identified as predominant milk-associated psychrotrophic bacteria (Lampugnani et al., 2019). The most commonly identified *Pseudomonas* species in milk are *P. fluorescens, P. gessardii, P. fragi*, and *P. lundensis* (Maier et al., 2021). *Pseudomonas* spp. can grow in a temperature range of 4 – 42 °C, with an optimal growth temperature above 20 °C (Maier et al., 2021). They exist in different environmental conditions and are often associated with raw milk spoilage (Azad et al., 2019). Bacteria grow in neutral or slightly alkaline medium while molds and yeasts tend to develop in acidic medium (Azad et al., 2019). According to the present study pH of coconut milk of all samples in all 5 days arrayed in the range of 5.2 - 7.2 proving a desired environment for the growth of microorganisms.

Coconut milk pasteurized at 72 °C for 10 minutes can contain about 2.23 x $10^5$ colonies per mL and can be increased up to 1.02 x $10^6$ colonies per mL by during storage at $7^{th}$ day from day of manufacturing (Reyes-Jurado et al., 2021). The total microbial count values enumerated in sample 1, 2 and 5 exceed 50 000 CFU/g of total plate count in day 2, 4 and 2. Total microbial count values enumerated in sample 3 and 4 are significantly high starting from day 1 indicating higher level of contamination after processing or ineffective heat treatment at coconut processing plant. Another study carried on a physicochemical evaluation in a laboratory setup of coconut milk, suggest that coconut milk pasteurized at 72 °C for 20-30 minutes can be preserved for 4 weeks at 4 °C without affecting its overall acceptability in laboratory setup (Patil & Benjakul, 2018). Tarek, et al. (2020) suggested that coconut milk pasteurized at 72 ± 1 °C for 15 minutes can be stored up to 2 weeks at 4 °C without affecting its overall acceptability in laboratory setup. In contrast to the laboratory setup used in previous studies, in the current study industrial setting and environment that influence the physicochemical properties and fatty acid profile of coconut milk have been taken into account, such as processing in a typical coconut mill/ processing plant with laborers, manual de-husking, manual de-shelling and paring by laborers, manual handling by operators, contamination in transportation, commercially feasible storage conditions such as 10 °C – 15 °C (Maier et al., 2021). The results of standard plate count of coconut milk sample showing the growth of microorganisms with increasing days during storage, are illustrated in Figure 2.

The microbial activity may also impact the fat percentage of coconut milk. According to the Codex definition (Codex Alimentarius, 2019), coconut milk and cream must contain at least 10% oil content respectively. With such fat content that may act as a nutrient source, it is vital to minimize microbial damage caused by lipolysis (Azad et al., 2019), also known as oil breakdown which eventually results in rancidity, giving rise to off-flavour development in coconut milk (Ariyaprakai & Tananuwong, 2015). Even though pure oil cannot be broken down by microorganisms, oil in water emulsions or oil in contact with water can be easily broken down by microorganisms since water is essential for microbial enzymatic split (Yalegama et al., 2016). The oil-in-water emulsion present in coconut milk, stabilized by proteins in the aqueous phase is illustrated in Figure 3a. The fat globules are disrupted by the homogenization during processing (Figure 3b).

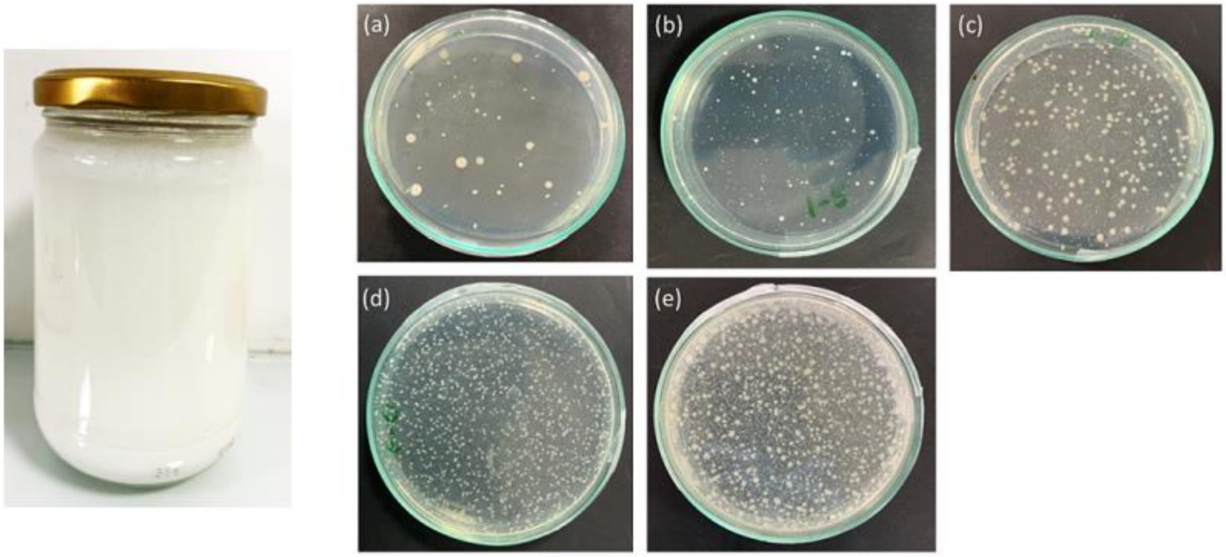


**Figure 2:** Standard plate count of coconut milk sample on (a) Day 1 (b) Day 2 (c) Day 3 (d) Day 4 and (e) Day 5

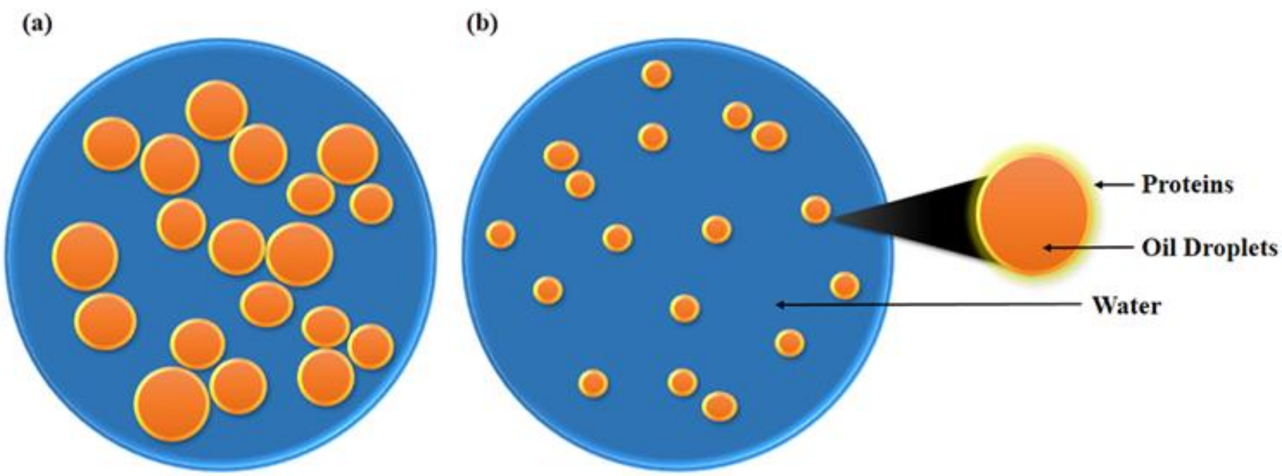


**Figure 3:** (a) Fat globules present in coconut milk (b) Disruption of fat globules after homogenization

Coconut milk is susceptible to various biochemical reactions and microbial activity (Tarek et al., 2020). Therefore, during the storage period, the short and medium–chain fatty acids and aldehydes of thermally treated coconut milk could be increased due to lipid oxidation. The increase of storage period can reduce the nutritive significance of coconut milk (Chuah et al., 2022). During the storage period, the PET bottles were opened for analysis purpose and $O_2$ may have dissolved from the refrigerator environment to sample bottles. A slight volume of oxygen can lead to a self–speed up oxidation chain reaction increasing rate of oxidation (Ruengdech & Siripatrawan, 2021). Water vapor transmission rate WVTR (g $m^{-2}$ per day) at 37.8–40 °C is 3.9–17 and oxygen transmission rate OTR ($cm^3$(STP) $m^{-2}$ per day) at 20 – 23 °C is 1.8 – 7.7 in PET (Poly ethylene terephthalate) bottles (Setiawan et al., 2022). The manual handling and packing procedure were unable to completely eliminate air entrapment in sample bottles. The ANOVA showed that there is significant difference ($p>0.05$) in total microbial count between day 1 and day 5 coconut milk samples tested ($p= 0.009$).

*Total Solids*

**Table 4:** Total solids of coconut milk samples during storage

| Total Solids (% m/m) | Day 01 | Day 02 | Day 03 | Day 04 | Day 05 |
|---|---|---|---|---|---|
| **Sample 1** | 39.15 ± 0.73 | 39.05 ± 0.34 | 38.51 ± 1.31 | 36.28 ± 1.71 | 38.54 ± 0.92 |
| **Sample 2** | 38.64 ± 0.24 | 78.1 ± 1.31 | 38.03 ± 1.22 | 38.1 ± 1.07 | 38.08 ± 0.92 |
| **Sample 3** | 38.42 ± 0.76 | 41.25 ± 0.34 | 34.38 ± 0.61 | 32.15 ± 0.82 | 34.06 ± 0.45 |
| **Sample 4** | 38.46 ± 0.78 | 38.41 ± 1.01 | 38.32 ± 0.82 | 38.38 ± 0.64 | 37.87 ± 0.83 |
| **Sample 5** | 38.32 ± 1.3 | 38.22 ± 0.95 | 39.11 ± 0.87 | 38.16 ± 1.02 | 38.15 ± 0.91 |

Note: No significant difference was observed ($p=0.17$) from Day 01 to Day 05 at 0.05% level of significance. Each data point represents the mean of three replicates ± standard deviation.

Except in one sample, total solids in other four samples were higher than 35% throughout the study and the values did not change with increasing number of days of storage (Table 4). The "Total solids" indicate the level of macro and micronutrients present in coconut milk (Zhang et al., 2018). Coconut milk contains carbohydrates, proteins, fats and micronutrients. The predominant carbohydrates present in coconut milk are sugars; mainly sucrose, and starch (Han et al., 2022). Predominant proteins in coconut milk are albumins and globulins (Patil & Benjakul, 2018). These proteins contains high amounts of glutamic acid, arginine and aspartic acid but are deficient in methionine (Patil & Benjakul, 2018). The major minerals found in raw coconut milk appear to be phosphorus, calcium, and potassium (Rincon et al., 2020). During storage, oxidation of lipids could have occurred resulting in formation of new organic compounds such as methyl ketones, aldehydes, alcohols, lactones, and hydrocarbons as other secondary products (Garavand et al., 2023) and this may have contributed to the increase in total solids. Eventually, these organic substances might have been metabolized by microorganisms in samples further producing compounds with lower molecular weight further adding to the net total solid values. Total solids present in a food sample could be an index of the dry matter content of the samples (Tarek et al., 2020) and it is considered as substrates for many biochemical reactions. Further, microorganisms use the total solids for their metabolic activity (Mat et al., 2022). The ANOVA showed that there is no significant difference ($p>0.05$) in total solids between day 1 and day 5 coconut milk samples tested ($p= 0.17$).

*Analysis of fatty acid profile in industrially processed coconut milk during storage*

In natural coconut milk, four groups of volatile compounds; alcohols, ketones, ester and lactones have been identified and the most abundant group of compounds was alcohols followed by ketones and esters (Bottiroli et al., 2021). Coconut milk contains about 24% of the total fat in a coconut while the cream and meat together accounts for about 34% of the total fat (Guimarães et al., 2021). Fatty acids, reported to be present in coconut milk are, caprylic acid C-8:0 (8%), capric acid, C-10:0 (7%), lauric acid C-12:0 (49%), myristic acid C-14:0 (8%), palmitic acid C-16:0 (8%), stearic acid C-18:0 (2%), oleic acid C-18:1 (6%) and 2% of C-18:2 linoleic acid (Nasution et al., 2019).

Presence of fatty acids in industrially processed coconut milk was quantitatively analyzed by GC MS and a total of 8 fatty acids were identified including pentanoic acid (valeric acid- C15), decanoid acid (capric acid- C10), dodecanoic acid (lauric acid- C12), tetradecanoic acid (myristric acid- C14), hexadecanoic acid (palmitic acid- C16), octadecanoic acid (stearic acid- C18), 9-Octadecenoic acid (oleic acid- C18) and 9, 12-Octadecadienoic acid (linolelaidic acid- C18).

Variation of the area under the peak and hence, the concentration of the fatty acids from day 1 to day 5, are exhibited in Figure 4. Dodecanoic acid was found to be the most abundant fatty acid during the storage period of all 5 days from day 1 to day 5 from day of manufacturing. Their concentrations increased from day 1 to 4 and decreased at day 5 and a dramatic increase in the fatty acid concentration was observed moving from day 3 to 4. The concentrations of decanoid acid (capric acid), dodecanoic acid (lauric acid), tetradecanoic acid (myristric acid), hexadecanoic acid (palmitic acid), octadecanoic acid (stearic acid), 9-Octadecenoic acid (oleic acid) and 9, 12-Octadecadienoic acid (linolelaidic acid) were increased by 91.74%, 82.03%, 88.80%, 89.00%, 83.75%, 84.62%, and 81.23%, respectively by day 4, while the concentration dropped from 32.08%, 16.35%, 26.36%, 32.92%, 30.94%, 34.92% and 35.76% respectively at day 5. Those results of ANOVA showed that the fatty acids in coconut milk changed significantly after sterilization and storage.

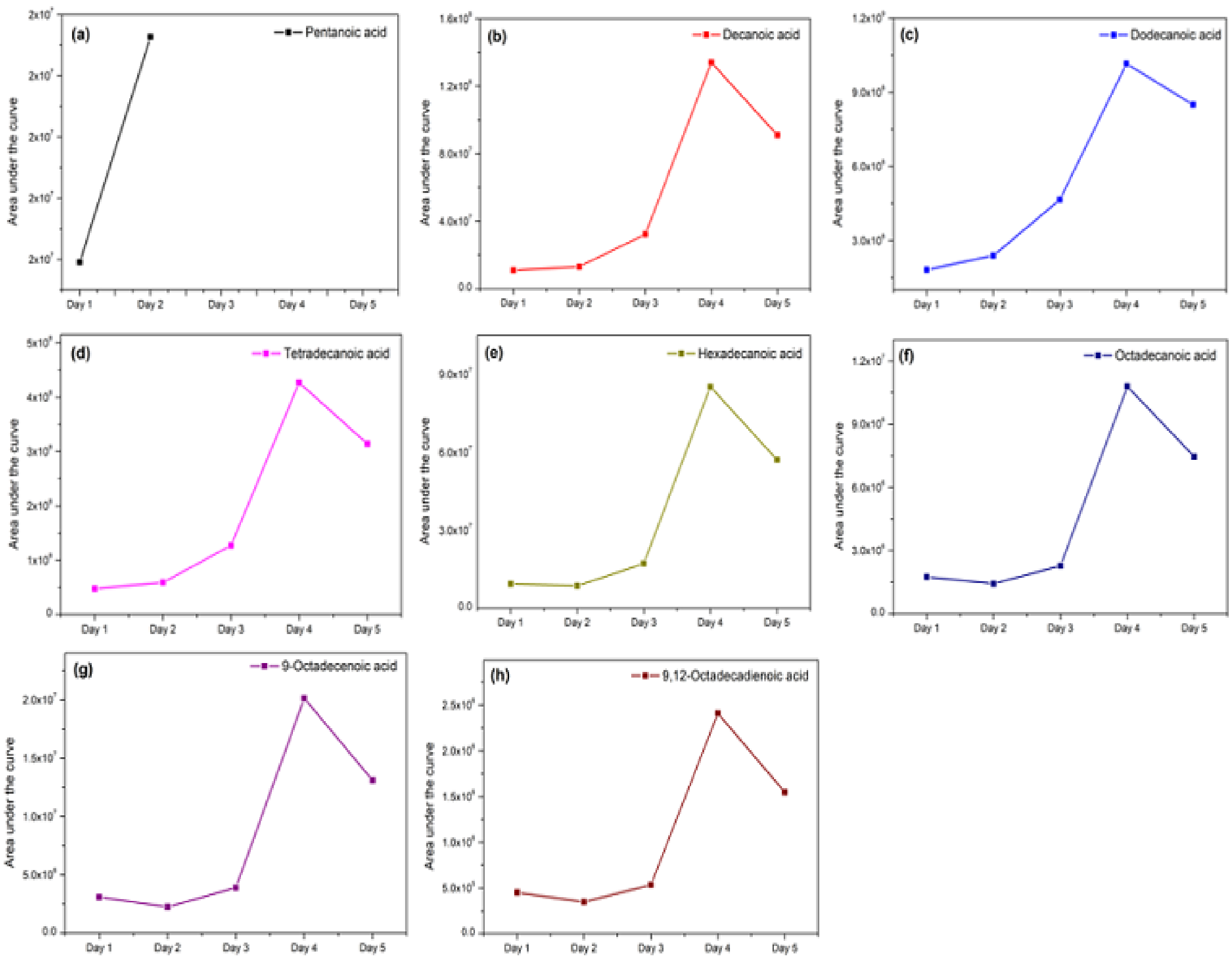


**Figure 4:** The presence and changes of fatty acids in industrially pasteurized coconut milk during storage (a) Pentanoic acid (b) Decanoic acid (c) Dodecanoic acid (d) Tetradecanoic acid (e) Hexadecanoic acid (f) Octadecanoic acid (g) 9-Octadecenoic acid (h) 9,12-Octadecadienoic acid

Increase in the fatty acid concentration could be attributed to several factors. Increase in the fatty acid concentration is consistent with the increase in the total microbial count. The total microbial count increases from day 1 to 5 and fatty acids are generated due to their biochemical action on the substrates present in coconut milk such as carbohydrates (primarily sucrose and some starch), lipids and minerals such as phosphorous, calcium, potassium (Zhang et al., 2020), and coconut proteins consisted of lysine, methionine and tryptophan as amino acids (Zandona et al., 2020). Further, the increase in the fatty acid concentration could be assigned to the lipid oxidation and autoxidation of heated coconut milk during storage. Auto-oxidation could occur during the preheating and exhausting processes, in which the raised temperature during preheating and processing could have a significant influence on autoxidation (Boeck et al., 2021). Moreover, lipid oxidation in food processing is auto-catalytic and the reaction is self-propagating and self-accelerating (Cao, et al., 2021). Some of bacterial strains such as *Pseudomonas fluorescens, Pseudomonas gessardii* present in milk also have the ability to produce heat-stable extracellular lipases which can break down lipids into free fatty acids contributing to the increase of fatty acids and subsequent milk (Singh et al., 2022). The breakdown of lipids into fatty acids and glycerols by lipase is shown in Figure 5. Lipid oxidation, following Maillard reactions, and lipid hydrolysis may indeed contribute to the fluctuation of the volatile compound profile during storage (Narvhus et al., 2021). Most of the fatty acids identified had a drastic decrease in concentration after day 4. It is possible that the oxidation reaction started to slow down, due to a lowering availability of oxygen in the headspace of the sample containers (Liu et al., 2019) and the fatty acids were metabolized into different organic compounds due to metabolic actions of microorganisms. The released fatty acids might be vital contributors to the flavor of processed coconut milk (Ng et al., 2021). Fatty acids with fewer than 8 or 10 carbon atoms are known to impart acidic, pungent, and rancid notes in food products, while fatty acids with more than 12 carbon atom contribute for a soapy taste(Nimbkar et al., 2022).

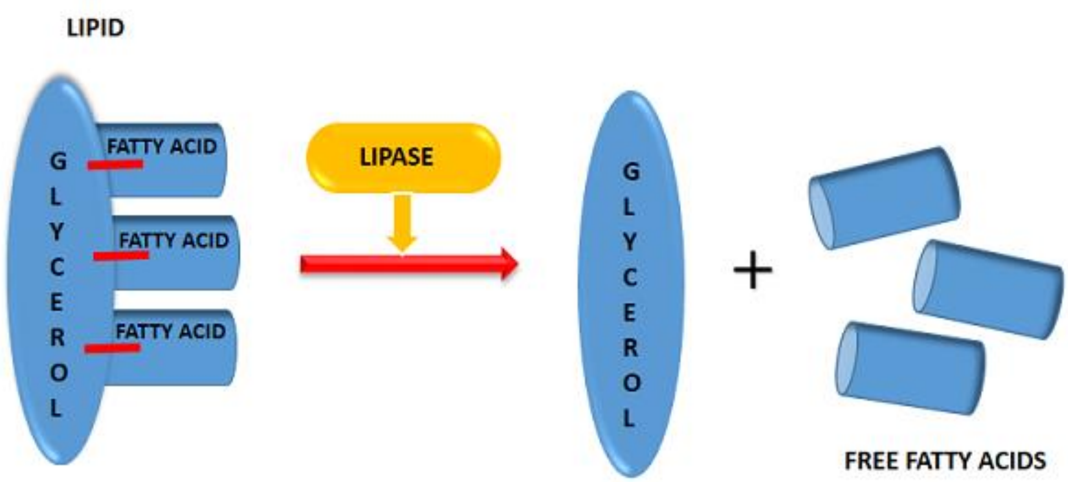


**Figure 5:** Lipid is broken down to free fatty acids and glycerol by the lipase enzyme

The criteria established by Codex Alimentarius guideline for concentrated coconut milk (when there is no addition of water in the processing) are total solids 25.4 – 37.3 (% m/m), pH 5.9 (minimum), moisture (%m/m) 74.6 (maximum) (Codex Allimentarius, 2019). When compared with guidelines recommended by Codex Alimentarius the criteria have remained within norms up to 3 days from processing at mill, suggesting it has met the Codex requirements for further processing or repacking if required.

In contrast to the results of previous studies, it is evident that the physicochemical parameters, including titratable acidity and total microbial count change drastically during storage in industrially pasteurized coconut milk as opposed to coconut milk pasteurized and stored in a laboratory setting. As a result, this work paves the way for future studies to explore and standardize effective techniques for preserving coconut milk employing improved technology as well as pretreatment techniques in industrial scales. More studies concerning sensory evaluations with expert panellists and with higher numbers of coconut milk samples processed and stored in the industrial environment can be conducted to further improve the quality of commercially sterile coconut milk. Basic good manufacturing practices, good agricultural practices, reliable food safety management system including HACCP, comprehensive training workshops to labourers and frequent quality monitoring systems are some of the proposed key strategies to improve the quality of coconut milk by commercial producers.

## Conclusions

The results revealed that pH, titratable acidity, total solids and total microbial count of coconut milk pasteurized in an industrial plant/ coconut mill fall in the range of 5.2 - 7.2, 7.02 - 13.44, higher than 35% and 2600 CFU/g - >30000000 CFU/g respectively from day 01 to day 05 during storage. A total of 8 fatty acids were identified and quantified including pentanoic acid (valeric acid-C15), decanoid acid (capric acid- C10), dodecanoic acid (lauric acid- C12), tetradecanoic acid (myristric acid- C14), hexadecanoic acid (palmitic acid- C16), octadecanoic acid (stearic acid- C18), 9-Octadecenoic acid (oleic acid- C18) and 9, 12-Octadecadienoic acid (linolelaidic acid- C18). The ANOVA showed that there is significant difference ($p>0.05$) in titratable acidity and total microbial count between day 1 and day 5 in the coconut milk samples tested, demonstrating the substantial role that titratable acidity and total microbial count play in influencing the quality of industrially pasteurized coconut milk over the course of storage. Hence, the key variables contributing to the deterioration of coconut milk during storage would be increased acidity and total microbial count. The drastic increase in fatty acids including decanoid acid (capric acid- C10), dodecanoic acid (lauric acid-C12), tetradecanoic acid (myristric acid- C14), hexadecanoic acid (palmitic acid-C16), octadecanoic acid (stearic acid- C18), 9-Octadecenoic acid (oleic acid-C18) and 9, 12-Octadecadienoic acid (linolelaidic acid-C18) determined by the gas chromatography-mass spectrometry must have contributed to the observed increase in acidity of coconut milk over the course of storage.

Therefore, it is evident that pH, total solids %, in the coconut milk samples collected from the coconut mills from the day of processing fall in the range of norms defined by Codex Alimentarius guidelines up to 3 days from processing at mill. However, the total plate count (CFU/g) of the coconut milk in the final product/ finished good such as coconut milk powder or coconut ready to drink product should align with the Codex Alimentarius guidelines, standards of Asian and Pacific Coconut Community and the applicable national quality guideline before releasing to the market. Quality criteria of Codex Alimentarius guideline states that the Coconut milk and coconut cream shall have normal colour, flavour and odour characteristic of the products. Hence, coconut milk pasteurized at a typical mill under the aforementioned circumstances and stored below 15 °C can be given a shelf life of 72 hours from the start of processing at the coconut mill to be used at a manufacturing facility, provided it meets the microbiological quality necessary to meet the national standard. Such coconut milk can be used as a raw material for additional value addition and repackaging for 3 days below 15 °C.

## Conflicts of interest

Authors declare no conflicts of interest.

## Acknowledgement

Authors acknowledge Charindu Makawita for the support given in intellectual discussions.

## Funding

This research was funded by the research grant provided by Sri Lanka Institute of Information Technology, Sri Lanka (SLIIT, FGSR/ RG/FHS/2022/13, SLIIT Research Grant).

## Author contribution

Conceptualization, MJ, CT; methodology, MJ, SJ; formal analysis, MJ, RR, CT; investigation, MJ, SJ;

resources, SJ, CT; data curation, MJ, CT; writing-original draft preparation, MJ; writing—review and editing, CT; supervision, CT; project administration, CT; funding acquisition, CT. All authors read and approved the final manuscript.

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