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Identification of the contributing factors that affect the rise of the acidity in milk extracted from Sri Lankan Coconut (*Cocos nucifera*)

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Abstract
Development of acidity in coconut milk leads to deterioration of the qualitative and organoleptic properties. Identifying the factors which majorly contribute to above cause can assist several major food categories that are directly or indirectly based on Sri Lankan coconut milk as a raw material. Study has indicated the baseline acidity level in coconut milk as 0.4011 mg/g ± 0.017. However, the acidity levels have varied according to the locality they were obtained. Maturity level of coconuts indicated a direct relationship with acidity. Enzymatic activity, and the microbiological factors also contribute to the acidity of coconut milk. Keeping time for 8 hours has increased microbial plate count from 4,50E+04 to 7,20E+05 and acidity from 0.41 to 0.52. But there was no significant difference between the increase of acidity in blanched and non-blanched coconut milk over the time.

Keywords: coconut milk, acidity, total plate count, storage time

1. Introduction

Coconut (*Cocos nucifera*) is one of the major crops produced in Sri Lanka and it contributes to 12% of all Sri Lankan agricultural products. Also, Sri Lanka is known as the 5th largest coconut producer globally. Coconut produced in Sri Lanka is majorly used in households and also used as a raw material for value added products. Coconut endosperm is structurally unique with a peculiar mode of development. Generally, endosperm divided into two parts, white kernel and free watery fruit within its cavity. Coconut water is the aqueous part of the coconut endosperm and coconut milk is the liquid made from grated pulp of mature coconut endosperm without the addition of water. It is also considered as the natural oil in water emulsion [1].

Coconut water can be served as a ready to save beverage or a rehydrator while coconut milk is usually used as a food ingredient in various traditional cooking recipes. It is white colour liquid that has no odour. The main components of coconut milk are water, fat, carbohydrates and protein. Additionally, it contains fat soluble and water-soluble vitamins. Coconut milk includes minerals such as Calcium, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium and Zink. Due to above composition it can be considered as a complex biological fluid. Majority of the fat inside coconut milk is saturated and has lauric acid as major fatty acid. Other than food preparation coconut milk has several added health benefits. Aqueous extract of coconut milk exhibited anti-inflammatory and wound healing properties when tested on mice [2].

Coconut milk is prepared by hand or machine pressing fresh grated coconut kernels, when the particle size is lower and the extraction pressure is higher the extraction efficiency also gets higher. Fat percentage is adjusted depending upon local requirement in between 15-40%. Coconut milk is consumed directly or with cooked food as a taste enhancer. Sri Lankan food companies use coconut milk for the production of spray-dried powder and coconut oil. Coconut oil is used a frying medium to deep frying and frying food products [3]. Coconut milk is chilled, enzymatically treated or fermented to separate coconut oil. Coconut milk powder can easily be reconstituted with water either at 30°C or 100°C to obtain a homogeneous coconut milk solution. Coconut milk extraction contains high total solids content thus increasing the output of spray dried coconut milk comparing the output of spray dried fresh milk [4].

Acidity development in coconut milk is a major problem in coconut milk industry as it can lead to rancidity. Therefore, the keeping quality of the coconut milk should be improved meanwhile preserving its organoleptic properties before the secondary production processes. Microbial growth, storage time, geographical variations and heat treatment are assumed as major contributing factors towards the adverse effects on coconut milk and acidity development. They were tested and statically evaluated in following study to quantitatively analyse their effects on acidity.

The acid value of coconut oil major parameter to evaluate the acidity of coconut milk. It is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is considered as a relative measure of rancidity since the formation of free fatty acids normally occurs during the decomposition of oil glycerides. This value then converted to the Soxhlet Henkel degrees. The total plate count is the enumeration of aerobic, mesophilic organisms that grow in aerobic conditions under moderate temperatures of 20-45°C. These microbes utilize metabolites and some of them convert in to acids in the catabolic process increasing the acidity of the media. Enzymatic breakdown of the macromolecules also contributes to the production of acids.

2. Materials and methods

2.1 Basic tests

2.1.1 Acidity in Soxhlet Henkel Degrees

Acid value (the amount of fatty acids which have been liberated from the glycerides) is determined by directly titrating the fat/oil sample in a solvent medium against a standard potassium hydroxide solution. Micro burette, 10ml pipette, pipette bulb, 50ml beaker, magnetic stirrer and burette holder is used as for the experiment along with 0.1N Potassium Hydroxide and Phenolphthalein as chemical reagents. First, 10ml of Coconut milk sample was taken into clean dry beaker. Then 1ml of Phenolphthalein was added to the sample. After that sample was kept on the Magnetic stirrer and titrate with 0.1N KOH solution until the Coconut milk colour changed to Pale Pink colour. As the final step, titrated KOH volume was measured. Coconut milk acidity calculating equation is as follows:

Acid value (milligrams of KOH per gram sample)

$$= (\mathrm{A} - \mathrm{B}) \times \mathrm{N} \times \frac{56.1}{W}$$

(10ml of sample weight is considered approximately equal to the 10g of sample. "A" stands for millilitres of standard alkali used in the titration, "B" represents millilitres of standard alkali used in titrating the blank, **"**N**"** equals the normality of standard alkali, Meanwhile,**"**W**"** stands for grams of sample***)***

Then converted the volume obtained via 0.1 KOH to volume of 0.25N NaOH.

$$Acidity\ (o_{SH}) = \frac{Volume\ of\ 0.25N\ NaOH}{Weight\ of\ Coconut\ Milk\ Sample} \times 100g$$

$$Acidity\ (o_{SH}) = \frac{Volume\ of\ 0.25N\ NaOH}{10g} \times 100g$$

$$Acidity\ (o_{SH}) = Volume\ of\ 0.25N\ NaOH \times 10$$

2.1.2 Total solids measurement

Total Solids (TS %) amount was measured in the coconut milk samples. TS is the net weight of coconut milk after the evaporation of moisture. TS vary with the extraction procedure and the force given to the extraction. For this experiment, MB45 moisture analyser along with fibre pads was used. First the preheating was conducted. Next the samples were analysed for 6 minutes for a direct reading from analyser.

2.1.3 TPC (Total Plate Count) analysis

TPC of the coconut milk was conducted after the extraction to identify the contamination and its relationship to the acidity. Preparation of dilution series was done in accordance with the ISO 6887-6:2013. Petri dishes, micro pipets, para films, Bunsen burners and incubators were used as instruments. Initially, the PCA (Plate Count Agar) media was prepared using casein enzymic hydrolysate, yeast extract, dextrose and agar. Next, 23.5g of media was dissolved in 1000 ml of distilled water. Then heated till boiling to dissolve the medium completely and sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. At inoculation phase, 1ml of dilution was measure by a micro pipette and then added to a sterilized petri dish (Oven sterilized at 160ºC for 1 hour). Temperature of the media was checked until it cooled to temperature around 45ºC. After the cooling process, about 20ml of PCA media was added petri dish. The lid was closed and the mixture was mixed inside lamina flow rotating on table for 5 times clockwise and 5 times anticlockwise. Above process was repeated on all petri dishes and then the petri dishes were allowed to settle at ambient temperature. After that the petri dishes were covered with para films and inverted and then incubated at 30ºC for 48 hours. After incubation, the colonies (including pinpoint colonies) were counted. If less than one-quarter of the dish was overgrown by spreading colonies, the colonies on the unaffected part of the dish were counted and the corresponding number of the entire dish was calculated. If more than one-quarter was overgrown by spreading colonies, the count was discarded. Plates with more than 15 and less than 300 colonies were retained. After counting number of colonies, CFU/g (Colony Forming Units per gram) was calculated. Following equation was used for the calculation.

$$N = \frac{\Sigma C}{(n1 + 0.1n2)D}$$

(N – CFU/g, ΣC – Sum of colonies counted on all petri plates, n1 – Number petri plates retained in the first dilution, n2 – Number petri plates retained in the second dilution, D – Dilution factor corresponding to first dilution. After calculating the result, the result was rounded off to two significant figures. The results were reported as a number between 1.0 and 9.9 x 10^x where x is the appropriate power of 10.)

2.2 Tests to determine relationships between different variables.

2.2.1 Determination of the acidity baseline

Coconuts were collected from coconut cultivations in Gampaha, Puttalama and Kurunegala areas. Amount of 22 samples at same level of maturity were selected with minimum to no abnormalities. Collected samples were subjected to coconut milk extraction process. All the equipment and utensils were disinfected using 70% alcohols. All coconuts were washed properly using water and dried in natural air to remove the moisture on the shell. Then coconut was separated into two halves and grated using a clean stainless-steel blender with distil water at pH 7 for about 1 minute. Volume of 8% of water added as a proportion to the weight of grated coconut milk. From this blended coconut, coconut milk was extracted using a cheese cloth. Then milk was filtered using 0.5mm sieves. All the 22 coconut milk samples were analysed for acidity and TS respectively as mentioned above.

2.2.2 Determine the acidity variation with the maturity of coconut

Coconut samples of different maturity levels were collected to check the acidity variation with maturity. Samples with a

maturity level of 12 months, 11 months and 10 months were selected as 5 samples from each category from the same tree. These 15 nut samples were checked for any abnormality before further analysis These coconut samples were used to check the acidity changes with the maturity. The same procedure as explained above was followed to extract coconut milk from nuts with the maturity at 10, 11 and 12 months. PH meter was used to check the pH levels of each sample at different maturity stages.

2.2.3 TPC and acidity variation with the time at room temperature

Coconut milk is a rich source of nutrient and water which creates an ideal source to grow microorganisms quickly. In order to perform the analyses, coconut milk was extracted from the coconut and then blanched in water bath at 72^0C for 15 sec. Then coconut milk was kept at 25^0C for 8 hours. In 2-hour intervals samples were checked for TPC. Acidity and pH from these samples were checked hourly. In order to measure TPC, 1 ml of coconut milk sample was added to 9ml of sterilized peptone water containing McCartney bottle and mixed thoroughly by using the Vortex mixer to prepare 10^{-1} dilution. Then 1ml of 10^{-1} solution was added to 9ml of sterilized peptone water in McCartney bottle and mixed thoroughly from vortex mixture to prepare 10^{-2} dilution. Same method was repeated until the preparation of 10^{-1} to 10^{-5}. Preparation is followed by inoculation and incubation of the samples from dilution series. Eventually, TPC was calculated.

2.2.4 Determination of the relationship between TS and variation of average acidity

Here the coherence of acidity and total solids of coconut milk was analysed. Coconut milk was extracted by applying different forces and then evaluated for total solids and acidity (All the statistical analysis related to this test was conducted with SPSS 22 software.)

3. Results and discussion

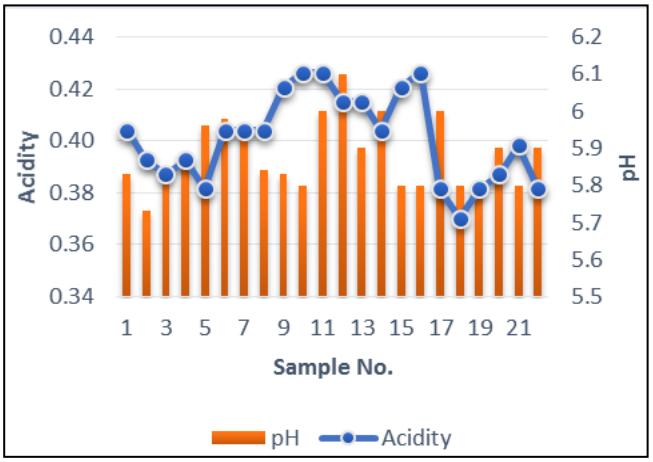

Fig 1: Acidity and pH value of coconut samples

Mean acidity value of the coconut milk samples is 0.4011 with a ±0.01712 standard deviation. According to figure one the acid value can range from 0.418 to 0.383 in Sri Lankan coconut. Also, Pearson correlation test between acidity and pH has indicated no correlation between two variables as the correlation coefficient between them is 0.048. pH value of the coconut milk varied from 5.7 to 6.2 and the optimum pH level with minimum contamination for coconut milk is known as 6.2 [5].

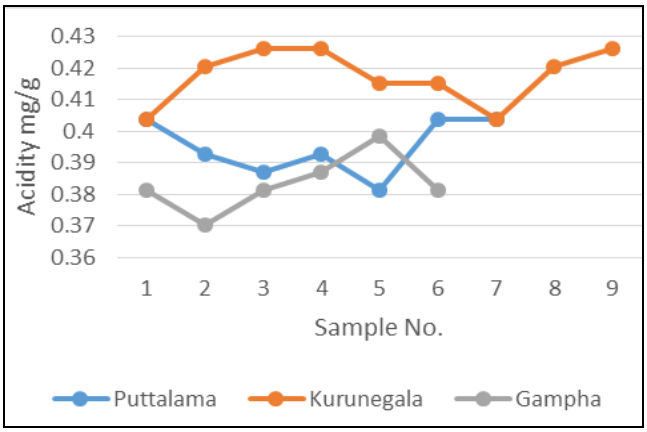

Fig 2: Acidity variation of coconut milk in selected areas

According to the results in the figure 2, milk obtained from coconuts in Gampaha area has the lowest acidity meanwhile milk from coconuts in Kurunegala has the highest acidity. Even though it cannot be concluded that coconuts in interior part of the country have higher acidity due to lack of samples, these results provide qualitative information which supports the above assumption. Other than demographics, acidity in coconut milk depends on many factors. Acidity and the pH of the nuts would show wide variation with variety, geographical location, culture practices, maturity of the nuts, method of extraction and the degree if dilution [6, 7]. Analysis of variance in between these regional coconut types have indicated a significant difference between the mean values of the group at 95% confident level. Results from the multiple comparison test have indicated there is no significant difference between the mean values of Puttalam and Gampaha coconut milk samples. But there is a significant difference between acidity of coconut milk in Kurunegala area with other two areas.

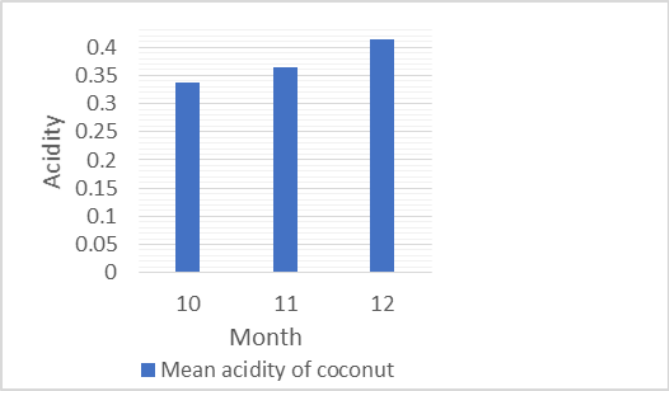

Fig 3: Acidity of coconut milk against the maturity

Figure 3 indicates the mean acidity of the coconut samples at different maturity levels they are being plucked. According to the multiple comparisons method there is a significant difference between the values of 10 months matured coconut

and 11 months matured coconut (p=0.000). There is also a significant difference in the coconut of the maturity level of 11 months and 12 months (p=0.000). It was clear that the analysis acidity of coconut milk changes with the maturity of coconut and with the maturity, acidity was increased.

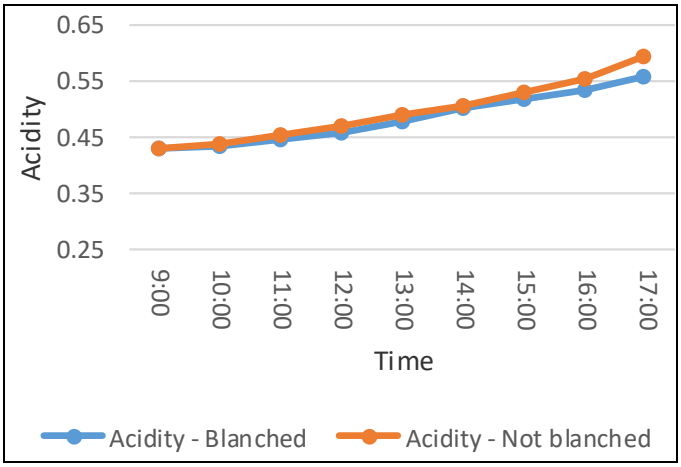

Fig 4: Acidity comparison over time of blanched and non-blanched coconut milk

Over the time, production of acid has increased due to many factors such as enzymatic activity and microbial activity. According to the Seow and Gwee, 1997, the hydrolysis of acylglyccerols can be particularly rapid when catalyzed by the enzyme lipase. The release of short-chain fatty acid such as butyric, caproic caprylic and capric. As proven by the statistical analysis and figure 4, there is no significance difference (p=0.640) between the blanched and non-blanched coconut water samples for the increase of acidity with time. Coconut milk samples were blanched to deactivate the lipase enzyme in order to reduce free fatty acid generation of the milk sample. But the results indicated, increase of the acidity in ambient and open environment could be mainly from the growth of the bacteria and fungi of the coconut milk.

Figure 5 indicates the increase of the microbial count within 8-hour time period of blanched coconut water. A significant higher rate of microbial growth was observed at 4-7 hours and the rate of growth acceleration dropped within 7-8 hours

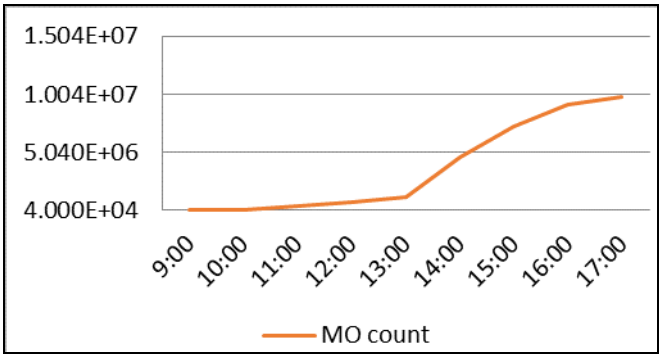

Fig 5. Microbial growth on coconut milk over time

The relationship between the microbial count and the acidity was checked to identify the impact of acidity towards microbial growth.

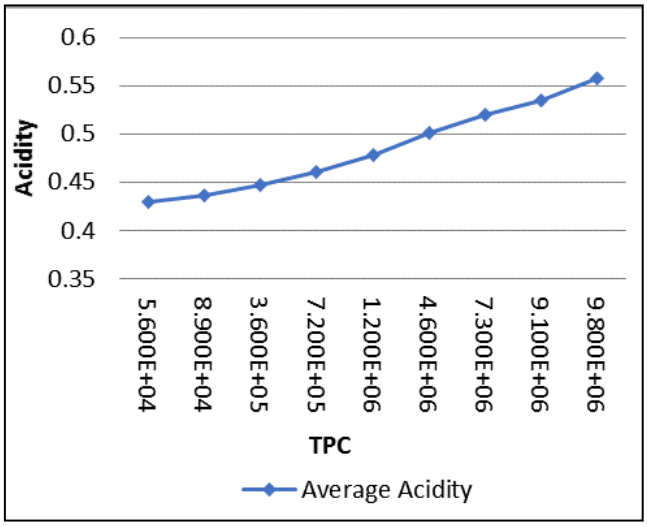

Fig 6: Acidity increment over microbial growth

Within 8-hour time period, acidity of coconut milk varied from 0.43 to 0.555 mg/g of 0.1M KOH. Figure 6 indicates a direct relationship between microbial level and acidity in the coconut milk. Further statistical analysis indicated a stronger relationship between both variables with a Pearson correlation value of 0.965 and P value of 0.000.

Acidity value of the coconut milk changed with the TS of the coconut milk. TS of the coconut milk could be changed with the force used for the extraction, and with this force amount of fatty acids in the milk or the amount of extraction from the kernels could be increased and this could lead to increase the acidity of coconut milk.

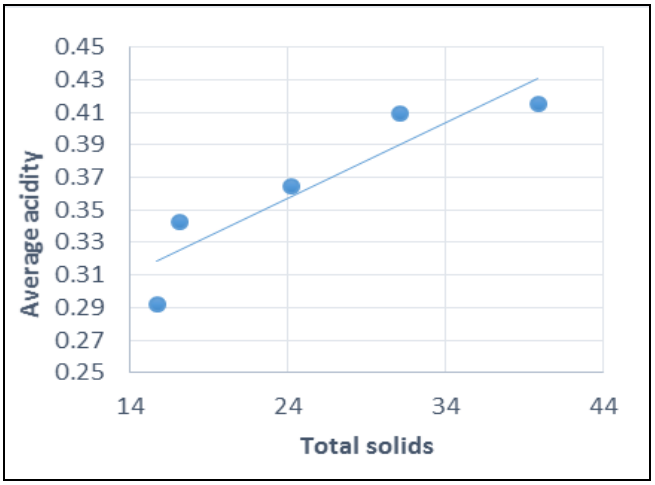

Fig 7: Average acidity variation with selected TS range

With the use of different forces in the extraction, coconut milk average TS values was checked against the average acidity. Then it was statistically analyzed to check the relationship between the acidity and the TS. Results indicated a strong relationship between the two variables. The relationship correlation was 0.916 with p value of 0.29.

4. Conclusion

Study has indicated mean acidity value of coconut milk is 0.4011 but it varies with in a range. Also, it can be concluded

that variations exist between acidity among the coconut obtained from different localities even with in the same country. Therefore, the coconut milk arriving for secondary production process should be standardized. Acidity has an impact from maturity level of the coconut. Therefore, optimum level of plucking should vary according to the required acidity for end product coconut milk is used. Nevertheless, coconut milk was blanched to deactivate the enzymes, acidity increased at room temperature indicating, enzyme's contribution to increase acidity is quite low. It was further confirmed by the acidity increment with total plate count over the time. Total solids tend to increase with increasing acidity in coconut milk as well.

5. References

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