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Investigation of Microbiological Contamination during Manufacturing Process of Desiccated Coconut

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ABSTRACT

The Desiccated Coconut (DC) industry is one of the major export oriented food processing industries in Sri Lanka and has become a key foreign exchange earner. Market value of DC is greatly associated with its quality. Manufacturers of DC in Sri Lanka exert their maximum effort to maintain higher quality through stringent quality standards and good hygienic practices during the manufacturing process. The recommended aerobic plate count (APC) by Coconut Development Authority is below 10,000 cfu/g which is the only criteria satisfies by the manufacturers currently. However, there is a growing demand for DC with APC of 5,000 cfu/g from many buyers. This indicates that the DC market can be expanded by fulfilling above requirement by further reducing the APC through eliminating possibilities of contaminations. Therefore, this study was carried out to investigate possible ways of microbiological contaminations, to identify possible reasons that cause contaminations and to find out the means of reducing the contaminations. Samples of DC and coconut meat were collected from 12 points of DC manufacturing process. All samples were tested for microbiological tests namely APC, Yeast and Mold Count (YMC). According to the results, wet section and addition of Chiplet fine (CF) to the cooling table had considerable contamination potentials. Addition of CF had a significant effect on YMC of the final product. To avoid contaminations, it was proposed to change the water regularly in washing baths, to follow sterilization methods for CF product before adding directly in to the cooling table, and to inspect the wet section more regularly.

KEYWORDS: Aerobic plate count, Contamination, Desiccated coconut, Yeast and mold count.

INTRODUCTION

Coconut (*Cocos nucifera* L.) is the third most important commercial crop of Sri Lanka. Sri Lanka holds the fourth largest producer of coconut in the world, covering a total area of 395,000 ha. Coconut plantations are concentrated in the south of the Northwestern province and in the north of the Western province, named the coconut triangle, yet it is grown in home gardens and in isolated plots in other parts of the country (Kumar*et al.*, 2003). ,

The average annual coconut production is around 3,000 mn nuts, of which around 65% is directly used for domestic consumption. The rest is mainly used by two industries, namely Desiccated Coconut (DC) (18%) and coconut oil (Kumar *et al.*, 2003).

Sri Lanka bears the third position in world DC exports and in year 2014 total DC export was 50,354 MT. Sri Lanka has exported 42.5 kg mn DC in 2014 with a total value of Rs. 14,377 mn (Anon, 2014).

Desiccated Coconut is the white kernel of fresh mature coconut, shredded and dried down to about 2.5% moisture content under strict hygienic conditions (Anon, 2006). Four standard grades based on particle size are produced namely extra fine, fine (macaroon), medium and coarse. A limited amount of fancy cuts *viz.*, flakes, threads and chips are also produced for special markets. The main uses of DC are for the confectionary industry as a filling for chocolates and candies, the bakery industry for cakes and nut filling products, biscuits cake decorations and ice cream and preparation of various snacks. Unlike many other products DC does not yet have a substitute or alternative. This is because it does not compete with similar products especially from any advanced nation. Also DC is entirely a natural product with no additives (Savanadasa, 2011).

Presently there are 64 DC mills in operation, which are mostly located in the coconut triangle. Of which around 50 are large scale factories, processing over 100,000 coconuts per day (Nandana and Werellagama, 2001). The remaining mills are medium scale; Sri Lanka is the world's second largest DC producer sharing about 25% of the global annual DC production (Kumar *et al.*, 2003).

The quality control and factory hygiene are of paramount importance for maintaining the high quality of DC. Quality control is a set of activities intended to ensure that quality requirements are actually being met (Anon, 2010). Therefore, to assure safety of DC, there should be a well-accepted food

safety system. It is related to the presence of food-borne hazards in food at the point of consumption. Food safety hazards can occur at any stage of the DC processing chain, adequate control throughout the processing chain is essential. Thus, food safety is ensured through the combined effort of all the parties participating in the food processing chain. All the specifications to maintain the quality of DC is recommended by Coconut Development Authority (CDA). The allowable maximum moisture content of DC is 3% and the oil content is about 68%. DC has maximum 0.3% of Free Fatty Acid (FFA) content as Lauric acid. The shelf life of DC is six months (Anon, 2006). As per the CDA recommendation, microbiological limits for desiccated coconut are 10,000 cfu/g of Aerobic plate count and, 100 cfu/g of yeast and mold count.

In factory processing of DC, process activities namely dehusking, deshelling, remove pairings, splitting coconut meat, removing of spoiled meat washing them with chlorine water are done manually by workers. After putting these coconut meat into the conveyor, the process is automated up to packing. Chiplet Fine (CF) and chiplet medium are the grades form when producing chips. Chips are exportable products while other two grades are not demanding products. Therefore these two grades are collected for DC processing. Chiplet medium is added to a conveyor just before the dryer. And CF is added directly to the cooling table 2.

During DC manufacturing process, several methods are employed to remove microbes at different locations. Chlorination is the first method used for coconut meat. Second is employed at blanching unit and third one is at the dryer. Most of the microbes and spores are being destroyed during hot water treatment in blanching unit. Generally this unit maintain at 90-100 °C for 90 sec. Further, remaining unkilled microbes are destroyed during drying where drying is practiced at 100 °C temperature for about 45 min.

The recommended aerobic plate count (APC) by CDA is below 10,000 cfu/g and currently manufacturers only satisfies that criteria. However, there is a growing demand for DC with APC of 5,000 cfu/g from many buyers. This indicates that the DC market can be expanded by fulfilling above requirement by further reducing the APC through eliminating possibilities of contaminations.

Therefore, this research was conducted to investigate, whether there are any ways of microbiological contamination, and what are the locations, reasons for cause contamination and means of reducing the contamination during DC manufacturing process.

MATERIALS AND METHODS

Location

This study was conducted at St. Joseph DC and Fibre mills, Katana, Negombo during the period from January to May 2016.

Sample Collection

Samples were collected from both wet and dry sections.

Sample Collection from Wet Section

Samples were collected from four locations (Location 1- 4) in wet section as follows;

Location 1: Washing area of coconut meat,
Location 2: Coconut meat is washed out with chlorinated water for the first time (This method follows in DC processing industry).
Location 3: Coconut meat is washed out of chlorinated water for the second time (Normally this method was followed when producing DC fine to be used for coconut cream production. Because there should not be any single particle of pairings in that product. In order to follow this, the coconut meat is double checked and removed pairings for several times. In this process coconut meat is washed twice with the chlorinated water). Location 4: Grated wet coconut samples from the conveyor.

Ten gram samples from each location was collected separately and APC test was carried out on the same day.

Sample Collection from Dry Section

Samples were collected from five locations (Location 5-9) in dry section as follows;

Location 5: Output from the dryer
Location 6: Cooling table 1
Location 7: Cooling table 2
Location 8: Shifter
Location 9: From the bin

Ten gram samples from each location was collected separately. These samples were kept for seven days before testing.

Microbiological Analysis

Aerobic Plate Count Test-Wet Section and Dry Section

Solutions for APC were prepared using 1 g/L Bacteriological peptone, 8 g/L NaCl and 21.6 g/L Plate count agar. Five 90 mL peptone bottles and twenty two 9 mL peptone bottles

were prepared. Plate count agar was prepared and autoclaved under 121 °C, 1 bar pressure for 15 min.

Ten grams of coconut meat was collected from wet section and weighed into 90 mL peptone bottle. Ten gram samples from dry section were also measured into 90 mL peptone bottles and followed the procedure mentioned below. Serial dilutions were prepared for each and every sample collected at every location up to 10^{-6} and cultured into Petri dishes using pour plate technique. Plates were incubated at 37.5 °C for 48 h. Number of colonies appeared were counted and recorded after incubation (SLS 98:2013).

Yeast and Mold Test

Samples were collected from three locations (location 10-12) of the desiccated coconut process.

Location 10: From the bin without addition of CF

Location 11: From CF adding to the cooling table

Location 12: Samples taken just after adding of CF

Solutions for this test were prepared using 1 g/L Bacteriological peptone, 8 g/L NaCl and 31.6 g/L DG18 Agar. Three 90 mL peptone bottles and three 9 mL peptone bottles were prepared. 250 mL of Agar media was prepared using 66 g of Glycerol and DG18 Agar. All solutions were autoclaved under 121 °C, 1 bar pressure for 15 min. 10 g of location 10 sample was measured and serial dilution was prepared up to 10^{-2}. Then 1 mL from each dilution was cultured into a petri dish using pour plate technique. Same procedure was followed for the point 11 and 12 samples.

Plates were kept for incubation at 23 °C for 3 to 5 days. Colony counts were recorded after incubation (SLS 98:2013).

Statistical Analysis

Collected data generated from *in vitro* experiment were statistically analyzed by ANOVA General Linear Model using SAS statistical package (SAS version 9.40).

RESULTS AND DISCUSSION

Aerobic Plate Count Test

Wet Section

There was a significant difference between APC of raw coconut meat and coconut meat washed after the second time (Table 1). Also a significant difference between washing with chlorinated water and after blanching of coconut meat was also found (Figure 1). Microbial population was minimized by sterilization with boiling water Therefore, it was the main technique of sterilization in this process.

Table 1. Differences between aerobic plate counts of points in wet section

Location	N	APC×10^3 (cfu/g)	
		Mean	SD
1	5	218.0^{a}	±21.68
2	5	156.40^{b}	±11.61
3	5	131.80^{b}	±10.21
4	5	26.23^{c}	±7.6
9	5	5.84^{d}	±0.74

Cfu- Colony forming unit per gram, N-Number of samples, APC– Aerobic plate count, SD– Standard deviation. Mean values with different superscript letters are significantly different at 0.05 level

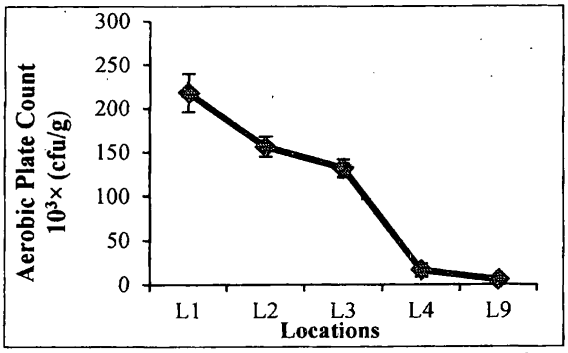

Figure 1. Aerobic plate count of the points selected in wet section

In a previous investigation *Acinetobacter, Flavobacterium, Microbacterium, Micrococcus* and various members of the *Enterbacteriaceae* were found on coconut shells (Kajs *et al.*, 1976). It is likely that the coconut meat would be contaminated with these organisms.

Microbial contamination is a risk to food product quality and safety. Therefore, Chlorine exposure might destroy the cell wall by altering it physically, chemically and biochemically and terminate the cell's vital functions, killing the microorganisms. This disinfectant was able to inactivate extracts of various enzymes, attacking a variety of bacterial molecules or targets, including enzymes, nucleic acids and membrane lipids (Calomiris and Christman, 1998). According to the present study, washing out of chlorinated water for several times could help to reduce surface contaminations than washing them once. However chlorine concentration must not exceed 3 ppm as recommended by CDA, as desirable colour can be deteriorated by Chlorine.

APC of spoiled coconut meat was ranged 410,000 to 310,000. Some of these type of spoiled coconut pieces were found from washing baths. This is due to less concern by workers and supervisors. Frequent inspection by quality controllers should be carried out to minimize those actions.

Storage of wet coconut meat for a long time is a favorable condition for multiplication of microbes on coconut meat. Remedies can be applied by fixing of a conveyor system from splitting of coconuts through washing area to the chlorine washing baths and to the blanching unit. Then it quickly moves to the process without delaying. Any delay in moving the exposed coconut to the drying process obviously increased the microbial population, the time between deshelling, paring, and desiccating should be carried out less than one hour. This ensures the regular changing of water in washing baths.

There were no significant difference among two level of methods. But there was a small reduction of APC when coconut meat washed out with chlorinated water for the second time in location 4 and 5. Although there was no significant difference between two washing methods, second washing with chlorinated water had minimized APC compared to the first washing (Table 2).

Table 2. Difference between aerobic plate count of points in wet section when coconut meat wash for the second time and washed only once a time

Method	Location	APC×10³ (cfu/g) Mean±SD
1	4	32.4^{a}±12.76
2	4	26.23^{a}±10.60
1	5	8.42^{b}±0.81
2	5	5.84^{b}±0.74

Level of method 1- Coconut meat washed with chlorinated water once. Method 2- Coconut meat washed with chlorinated water for first time

There was no significant difference among location 5 and 6 or between location 7 and 8. But there were a significant difference between locations 5 and 8, 5 and 7, 5 and 9, 8 and 9 (Table 3). The least APC observed in the final output while highest APC recorded in location 6 and 5 (Figure 2).

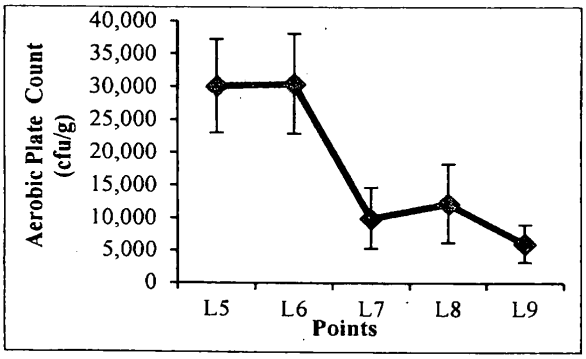

Figure 2. Aerobic plate count of the points selected in drying section

Table 3. Aerobic plate count of points in dry section

Location	N	APC (cfu/g) Mean±SD
5	6	$30,104.17^{b}$±7,103.88
6	5	$30,450.0^{b}$±7,579.60
7	9	$10,032.4078^{c}$±4,663.63
8	7	$12,259.52^{c}$±5,998.51
9	7	$6,105.36^{d}$±2,901.42

This reveals contamination throughout the dry section was negligible. Time taken for cooling process can be reduced by fixing of blowers. After desiccation, a process is not conductive to further growth since desiccated coconut has a moisture content of only 3%, and reinfection is minimal owing to thorough sanitary precautions (Kinderlerer and Clark, 1985).There was a consecutive relationship among APC in drying section and moisture content in each location. So high moisture content in early locations could be a reason for increase in APC during first few points (Figure 3).

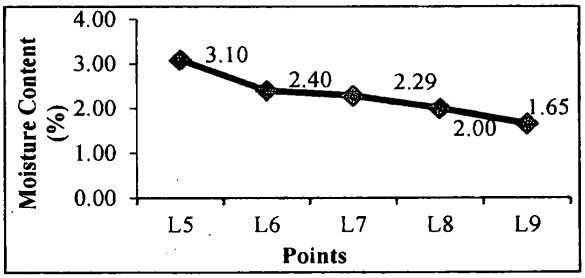

Figure 3. Average moisture content flows in the dry section

Yeast and Mold Count Test

There was a significant difference of YMC among locations 10, 11 and 12 (Table 4).

Table 4.Yeast and Mold Count

Location	N	YMC (cfu/g) Mean±SD
L10	5	152.0^{e} ± 36.50
L11	7	71.43^{f} ± 8.99
L12	7	36.0^{g} ± 12.30

YMC-Yeast and mold count

Therefore, addition of CF has caused an increase in YMC in the final product. According to the results, addition of CF directly to the cooling table has increased the yeast and mold count in the final output. Chiplet fine is the byproduct of chips and it is not an exportable product as chips. Therefore it is packed and stored for several days prior to use in the DC process. Chiplet fine contained some pieces of parings. It is a waste product for DC and pairings contains high amount of Free Fatty Acids. So it could be a cause for the growth of the yeast and molds. Pieces of pairings were

removed when adding it into the cooling table. However, under storage condition of CF yeast and mold can grow and multiply. As a solution, before adding CF to the cooling table, sterilization method namely steam sterilization under 80 °C for 30 min or peroxide sterilization can be applied.

CONCLUSIONS

More contaminations were experienced in the wet section while minimum contaminations were observed in dry section. Addition of CF is another possible means of contaminations during the DC manufacturing process. As per the results, remedial measure to minimize the APC further is proposed. Following sterilization techniques, frequent inspection of washing area, frequent changing of washing baths, maintaining the temperature of dryer and blanching unit could ensure the minimum APC up to 5,000 cfu/g. CF can be treated and applied to the cooling table. This will help to reduce growth of yeast and molds and also improve the quality of DC.

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