Effect of storage temperature and processing conditions on microbial quality of desiccated coconut

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Abstract

Desiccated coconut (DC) industry is a major export oriented food processing industries in Sri Lanka. Desiccated coconut is a dried, white, particulated or shredded product manufactured from freshly peeled coconut kernel. Highly stringent hygienic conditions are necessary throughout the production line of DC, as it is a direct consumption food product. The current study was undertaken to ascertain the effect of process flow line, storage time and temperature on microbial quality of the final product. Factory conditions and machinery in the production line in Sri Lanka vary from fully automated to semi-automated with manual handling systems. DC samples collected from semi-automated and fully-automated factories were subjected to microbiological analysis after storing at 25°C, 30°C, 37°C and 42°C in calibrated incubators for periods of 24 weeks. Several quality parameters were tested weekly. Moisture content was also measured to ascertain the effect of temperature on available water for microbial growth.

Both storage temperature and storage time significantly affected aerobic plate count (APC) while the moisture level did not ($P<0.05$). The APC showed significant decrease at each storage temperature compared with initial count. Initial APC decreased from 1.4×10^5 to 1.5×10^3 at 25°C and to 1.5×10^2 at 42°C. Both temperature and storage time recorded a negative significant combination effect on APC. A significant decrease in yeast count was observed in the 1st week at all temperatures from 2.1×10^2 to < 10 and DC did not thereafter support the growth of yeast, demarcated by a significant reduction (zero counts). This indicates that the storage time significantly affected yeast count, while storage temperature did not. In general, neither storage time nor storage temperature had a significant effect on mould count ($P=0.208$). Moisture level significantly increased at lower temperature (25°C) from 1.0% to 4.4% and at higher temperature (42°C) to 1.4 %. However, APC decreased with time significantly although moisture level increased. On this basis of principal components analysis, microbial quality of DC did not appear to be dependent on production procedure of DC. This clearly shows that manual handling alone does not account for degraded microbiological quality of DC.

Introduction

Desiccated coconut is a dried product manufactured from freshly peeled coconut kernel, produced in many sizes and textures from extra fine to course grades. About 60-80% of global DC production is used in the bakery and confectionery industries to enhance texture, flavour, aroma, degree of chewiness and eye-appeal. Small quantities are repacked and sold through retail outlets for domestic use, both in its original state and in sweetened forms.

Sri Lanka is one of the pioneers in manufacturing DC, commencing in 1888. Presently several coconut growing countries are competing with Sri Lanka in the global market. However, Sri Lankan DC is very popular due to its inherent flavour and absence of food additives and preservatives.

Highly stringent hygienic conditions are applied throughout the production line of DC, as it is a direct consumption food product. During the manufacture of DC, the product undergoes two heat treatments, firstly when pieces of coconut meat are immersed in boiling water for not less than 90 sec, and secondly when the shredded coconut is dried in hot air for a period of 30-40 minutes at temperatures between 90C - 110C. Although these practices greatly reduce the viable microbial content of the coconut, they do not produce a product that is sterile Yet it is necessary to ensure that the product is free from pathogenic organisms as well as organisms which can cause microbial spoilage.Sri Lankan DC factories include those involving manual handling as well as those with fully automated process lines, which accounts for products of diverse microbial qualities.

Compliance to see standards are met in microbial quality, deterioration during storage is a feasibility. The aims of this study were to investigate the effect of storage temperature and production procedure on microbial quality of DC and to understand the changes of microbial quality throughout the storage period at various temperatures. Particular emphasis was made on determining effects of temperature, time and combined effect on microbial quality.

Materials and methods

Experiments were conducted to determine the effect of storage temperature, storage period and their combined effect on the physical, chemical and microbial quality of DC. The study involved fine grade desiccated coconut, collected directly from the factory at the end of the production line.

Method of sampling

From the end of the production line of desiccated coconut, 3 kg portion of fine grade desiccated coconut samples were collected into pre-sterilized (gamma radiated; dose; 25 kGy, rate; 3.94 kGy/h) polyethylene bags (size-15"x12", gauge-338) in duplicates. Collections were made from two factory types: semi-automated (where manual handling in the dry section is comparatively high) and fully automated (where manual handling is minimized). The bulk sample was used to make fifty grams of DC sub-samples in pre-sterilized polyethylene bags (size-6"x7", guage-338), aseptically. The bag was thermally sealed and packed into two ply Kraft paper bags to simulate factory packing conditions.

Treatment structure and Quality tests of DC

Treatment temperatures, 25oC, 30oC, 37oC, 42oC were selected as storage temperatures, representing normal condition to high temperatures. DC is usually sealed in open containers with no temperature control and hence usually subjected to high temperature such as 40-45oC. DC sub-samples were stored in separate calibrated incubators maintained at 25±1oC, 30±1oC, 37±1oC and 42±1oC for up to 24 weeks because shelf life of DC is recommended to be 6 months. Treated sub-samples were tested after 1, 2, 4, 8, 12, 16, 20, 24 weeks for moisture content,5 aerobic plate count (APC),6 yeast and mould count7 according to Sri Lanka Standards. Initial samples and quality control samples were also tested.

Laboratory-based experiments were carried out to ascertain the effect of storage time, storage temperature and their combination effect on DC moisture content, APC count, yeast count and mould count.

Results and discussion

Effect of storage on moisture levels

The initial moisture content of DC samples M001, M002, M003 and M004 were 1.0 (±0.00), 0.9 (±0.00), 1.0 (±0.07) and 1.2 (±0.00) respectively which complied with the specifications of Sri Lanka Standards.5 Significant increases of moisture levels for all types of factories were observed from the 1st week onwards when compared with initial mean moisture content and the increases were highly significant (P-value of 0.000) at all the time points tested. The maximum moisture content achieved for all temperatures were 4.8 (± 0.00) % for 25oC, 4.0 (± 0.00) % for 30oC, 2.2(± 0.00) % for 37oC and 2.0 (± 0.28) % for 42oC. (Fig. 1.)

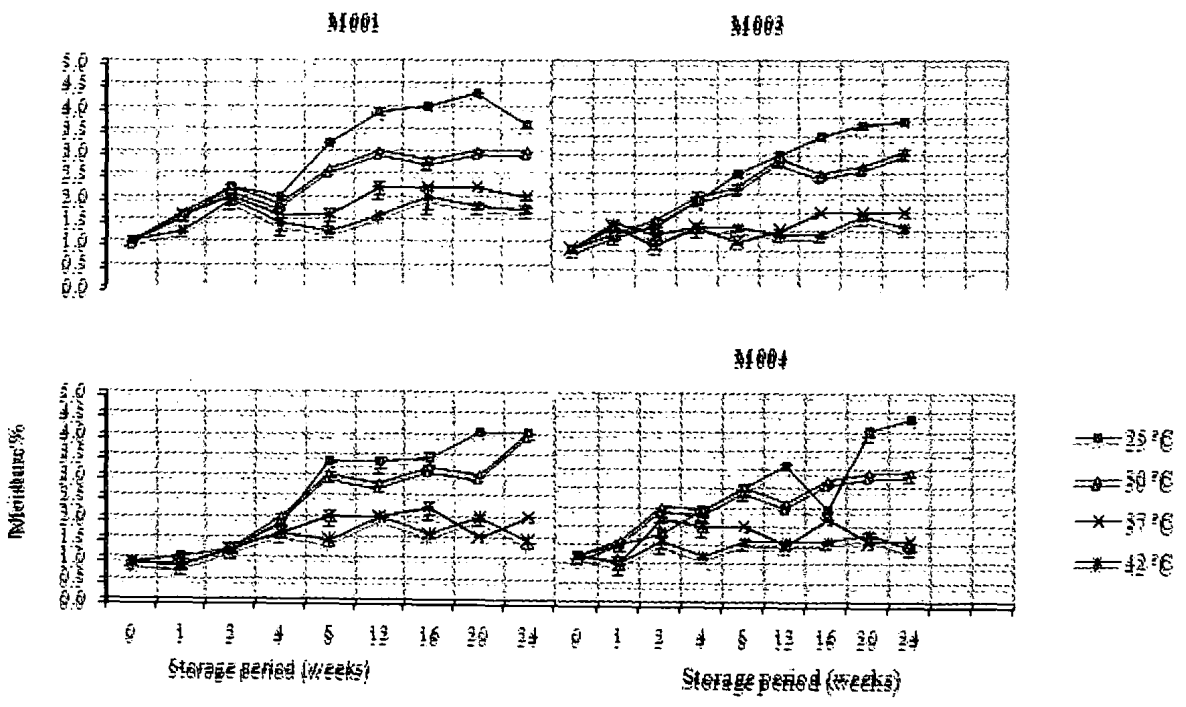

Fig. 1, Variation of DC moisture content over time at different temperatures

In general, both storage temperature and storage period have a complimentary significant effect on moisture levels. At temperatures 25C and 30C, a significant increase in moisture content was observed with time. At 30C and 37C, the effect was not significant. The maximum permissible moisture content of DC is 3.0 %. The packaging material currently used in the industry, low density polyethylene, has been shown to permit absorption of moisture.[8] The samples stored at 25C absorbed moisture up to 3.5 % in 12-16 weeks increasing to 4.8 % in 24 weeks but showed no signs of mold spoilage, neither discolouration nor off-odour. In addition, as a desiccated product, DC can absorb water from the atmosphere and hence relative humidity plays a significant role on DC moisture levels. Relative humidity is affected by temperature as is also shown by the slower increase in moisture content of DC at higher temperatures, i.e.37C and 42C.

Effect of storage on APC count

The fully automated factories M001and M002 maintained initial APC counts at 1.4 x 10^4 and 3.0 x 10^4 cfu/g respectively. The initial APC count of the semi-automated factories, M003 and M004 were ten fold higher at 3.8 x 10^5 and 1.4 x 10^5 cfu/g respectively. Significant increases of APC count was recorded across all the temperatures up to 2^{nd} to 8^{th} weeks with the highest APC count for M003 at 3.1x10^6 (± 14142) cfu/g in the 1^{st} week at 25C. However, the decrease in count was significant from the 8^{th} week onwards when moisture content significantly increased. Lower numbers were recorded at 37 and 42C, 10^2 cfu/g in M001 and M002. But in M003 and M004 which are semi-automated factories, counts were still significantly higher at all temperatures than the M001 and M002. A general trend of APC count reduction was seen for all the temperatures (Table 1) although the reduction was not significant towards the latter time points. Finally, both storage temperatures and storage periods have a complimentary negative significant effect (P 0.000) on APC counts for each sample by end of the 24^{th} week.

Effect of storage on yeast count

There was a recorded high yeast count in the initial samples of DC 205 (± 6.36), 105 (± 57.28), 105 (± 19.09) and 73 (± 12.73) cfu/g for samples M001, M002, M003 and M004 respectively. A significant steady decrease was observed from the 1 week when compared with initial mean yeast counts in all the samples for all storage temperatures. There were no significant differences for other weeks with a few exceptions. This shows that the decrease is constant from the 1 week or almost all the yeast was destroyed in the 1 week. The results suggest that DC is not a favorable medium for growth of yeast and that storage time had no effect on yeast count of DC.

Effect of storage on mould count

The mould counts of original samples were low. After 2-4 weeks of incubation, an increase in mould counts was observed in some samples, although not significant. Significant differences in mould counts did not occur at all storage temperatures for all factories except M003. Factory M003 was observed to have high mould count at the beginning (91±12.73 cfu/g) and significant differences were seen at all tested temperatures. In general, neither storage temperature nor storage period had any significant effect on DC mould count.

Reduction of mould counts to zero cfu/g was observed in all samples collected from both types of factories from the 2 to the 24 week. At end of the experiment, mould was not detected. This means that contaminated moulds in DC were destroyed with time at all the temperatures tested.

Principal component analysis of microbiological characteristics of DC

Component loading of microbiological characteristics of DC at initial stage and after storage at different temperatures for different time periods was carried out for principal component analysis. Variables such as aerobic plate count at different temperatures and mould counts at 25C could be considered as heavily loaded (>0.5) along the first principal component axis (PCA1). On the other hand the second component is heavily loaded with the mould counts in different temperatures (PCA2). The third component is heavily loaded the yeast counts at different temperatures (PCA3).

The scatter diagrams (Fig. 2) derived from the component scores indicate that there is no particular grouping of the two production types of the factories under consideration. Sample M003 stood separately from the others which clustered together. Of the three clustered together, the sample M004 was collected from a factory using a semi-automated production line, whereas the other two were fully automated. For instance, factory showed a wide range of scatter distribution in the scatter

plots indicating its wide variability. On this basis, it could be suggested that above mentioned microbiological characteristics were not dependent on the type of production procedure of factories. i.e. fully automated and semi-automated. It clearly shows that manual handling along does not account for the degraded microbiological quality of DC and that with good manufacturing practices, quality can be maintained irrespective of the production method used.

Table 1: The effect of a range of storage periods and storage temperatures on APC count of DC

Storage period	**Mean APC counts at different temperatures (microorganisms / g) ± SEM for M001**				**Storage period**	**Mean APC counts at different temperatures (microorganisms / g) ± SEM for M002**			
(weeks)	**25°C**	**30°C**	**37°C**	**42°C**	**(weeks)**	**25°C**	**30°C**	**37 °C**	**42°C**
0	1.4x10 ± 707	1.4x10 ± 707	1.4x10 ± 707	1.4x10 ± 707	0	3.0 x10 ± 707	3.0x10 ± 707	3.0 x10 ± 707	3.0x10 ± 707
1	1.4x10 ± 707	1.1x10 ± 2192	1.3x10 ± 0	1.6x10 ± 707	1	2.6 x10 ± 707	2.9x10 ± 7071	2.1 x10 ±4243	1.3x10 ± 0
2	1.4x10 ± 1414	4.4 x10 ± 4879	1.0x10 ± 2475	5.6 x10 ± 566	2	2.4 x10 ± 1414	1.6x10 ± 707	1.4 x10 ± 5657	1.4x10± 707
4	1.7x10 ± 778	1.0x10± 3536	7.8x10 ± 1980	3.2 x10 ± 495	4	3.3 x10 ± 1414	1.0x10 ± 0	2.1 x10 ± 15627	7.1x10 ± 1273
8	1.0x10 ± 778	7.1 x10 ± 1909	3.6 10 ± 778	2.4x10 ± 778	8	1.1 x10 ± 707	4.5x10 ± 990	3.4x10± 849	1.8x10 ± 566
12	1.1x10 ± 283	3.6 x10 ± 71	1.8x10 ± 71	1.9 x10 ± 141	12	4.0x10± 141	2.0x10 ± 212	4.2 x10 ± 106	1.3x10 ± 424
16	3.2x10 ± 0	1.8 x10 ± 212	5.0x10± 226	5.6 x10 ± 1124	16	2.9x10± 141	1.0x10 ± 85	5.4 x10 ± 149	1.4x10 ± 495
20	2.2x10 ± 0	1.3 x10 ± 0	2.6x10± 21	4.4 x10 ± 14	20	1.8 x10 ± 1061	7.7x10 ± 240	5.5 x10 ± 99	3.8x10 ± 64
24	1.8x10 ± 71	1.2 x10 ± 0	2.2x10 ± 50	4.9 x10 ± 429	24	1.5 x10 ± 424	9.9x10 ± 7	6.2 x10 ± 35	7.1x10 ± 184

Storage period	**Mean APC counts at different temperatures (microorganisms / g) ± SEM for M003**				**Storage period**	**Mean APC counts at different temperatures (microorganisms / g) ± SEM for M004**			
(weeks)	**25°C**	**30°C**	**37°C**	**42°C**	**(weeks)**	**25°C**	**30°C**	**37°C**	
0	3.8 x10 ± 35355	3.8 x10 ± 35355	3.8 x10 ± 35355	3.8 x10 ± 35355	0	1.4 x10 ± 7071	1.4 x10 ± 7071	1.4 x10 ± 7071	1.4x10 ± 7071
1	3.1 x10 ± 14142	4.2 x10 ± 35355	4.8 x10 ± 84853	2.8 x10 ± 21213	1	1.3 x10 ± 14142	1.7 x10 ±14142	3.6 x10 ± 42426	6.8x10 ± 5657
2	4.7x10± 28284	3.4 x10 ± 49497	6.0x10± 76777	5.0 x10 ± 84853	2	1.6x10 ± 14142	1.5 x10 ±28284	9.6 x10 ±5657	4.4x10 ± 2828
4	6.9 x10 ±14142	5.3 x10 ± 0	6.4 x10 ± 63640	5.0 x10 ± 7071	4	1.1 x10 ± 19799	7.4 x10 ±1828	1.6x10± 707	1.0x10 ± 707
8	6.2 x10 ± 14142	6.3 x10 ± 12728	8.4 x10 ± 22627	4.5 x10 ± 7071	8	7.7 x10 ± 21213	2.2 x10 ±4950	6.6 x10 ±354	8.0x10 ± 71
12	2.9 x10 ± 42426	1.3 x10 ± 14142	5.2 x10 ± 707	4.2 x10 ± 10607	12	3.010± 707	1.2 x10 ±707	4.8 x10 ±1061	5.9x10 ± 424
16	2.4 x10 ± 21213	2.6 x10 ± 35355	3.6 x10 ± 2828	3.1 x10 ± 15556	16	4.2 x10 ± 283	2.0x10± 212	2.3 x10 ±141	1.0x10± 71
20	5.0 x10 ± 9192	2.2 x10 ± 35355	4.4 x10 ± 71	2.2 x10 ± 4243	20	3.0 x10 ± 1344	2.0x10± 283	1.1 x10 ±184	1.5x10 ± 283
24	4.5 x10 ± 21213	6.4 x10 ± 3111	4.5 x10 ± 0	6.4 x10 ± 636	24	5.6 x10 ± 71	8.3 x10 ±106	7.0 x10 ±49	1.6x10 ± 21

$\alpha = 0.05$, n = 2, NS- Not significant

Note: Values are rounded off to the nearest integer

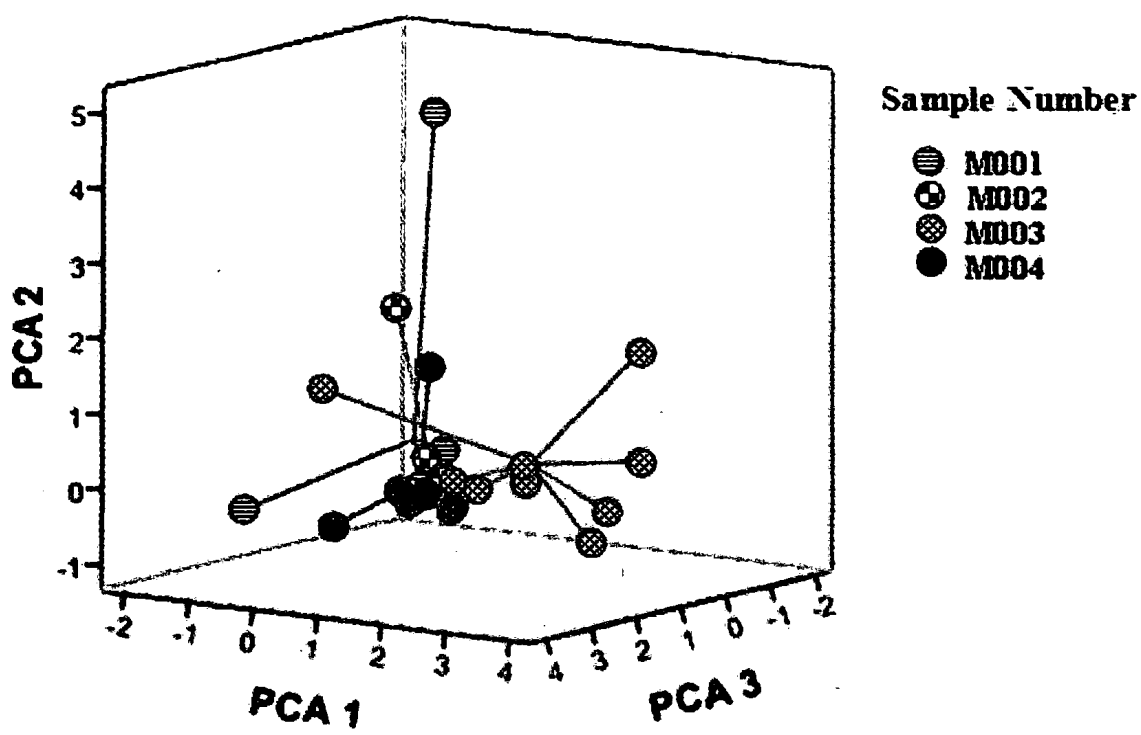

Fig. 2. Biplot produced by plotting principal component 1 (PCA 1), principal component 2 (PCA 2) with principal component 3 (PCA3) for microbiological characteristics of DC collected from different factories stored at different temperatures and different time periods. Percentage of trace along the first PC = 34.178%, along the second PC = 29.556% and along third PC = 22.186%, Total = 85.920%.

Discussion

The Sri Lanka DC industry does not use any preservative and/or microwave irradiations as done in other countries. The only approach for preservation is drying which reduces water activity and storing at an ambient temperature.

Monoglyceride of lauric acid and other medium chain fatty acids have proved to have adverse effects on bacteria, yeast, fungi and even some viruses. The oil content of DC is 68% minimum, while coconut oil contains 48% of lauric acid derivatives. It has been claimed that DC will not to be spoiled either by bacteria or moulds. A similar situation was observed in the present study bacteria, moulds or yeast did not grow in any of the samples and microbial spoilage was not detected with no clumps or visible fungal mycelia observed even with moisture leves at 4.8%. The antibacterial effect may have caused a reduction in APC counts.

The medium chain fatty acids (C_6- C_{12}) in coconut are readily degraded by filamentous fungi belonging to the genera, *Penicilium, Aspergillus, Cladosporium, Fusarium, Trichoderma* and *Monascus* to give methyl ketone one carbon atom less through ketonic rancidity.Short chain fatty acids, methyl ketones and secondary alcohols accumulate in coconuts returned as spoiled. The presence of such chemicals have been shown to cause death of microorganisms. They also have a fungicidal effect inhibiting growth of much of the normal fungal microflora.The degree of inhibition depends on the concentration of the un-ionised acids. A low microbial count may suggest that the commodity has undergone considerable microbiological spoilage and as such, is unfit for human consumption.

The same observations were made in the present study. Although, significant increases of APC counts were observed in first few weeks, they significantly decreased towards the end of the 24 week, even though moisture levels had increased significantly. Even in initial samples with high APC counts of 3.8x10(± 35355) cfu/g, no signs of spoilage were detected. The number of bacteria and mould in spoiled coconut has been found to be significantly lower than that in coconuts obtained from a processor or purchased from retail outlets. Other than that, a significant decrease of APC counts was recorded with increase in storage temperatures. It has also been shown that moisture content decreases with increased temperature. Water content is an important factors in microbial growth. An unfavorable a_w will result both in reduction in rate of the growth and lowered maximal yield of cells. Temperature is also an important environmental factor affecting the growth and viability of microorganisms.

Mesophiles prefer moderate temperatures with an optimum generally between 30C and 45C. In the present study high storage temperature were seen to reduce the growth of bacteria.

Yeasts were detected in high numbers in original samples of DC but not detected after the 1 week of storage at all tested temperatures. At that time taste and smell of DC was sweet and ketonic rancidity was not detected. Yeast and moulds can be suppressed by antimycotics, which are agents that destroy or prevent growth of fungi. Coconut has adequate amounts of lauric, capric and caprylic acid which are medium chain fatty acids that are considered as antimycotic agents. Low water activity, low pH, (pH of DC is 6.1-6.7) and low sugar content (Natural sugar 5.92 g/100 g) may be unfavorable for the growth of yeast.

The mould counts of original samples were low. After 2-4 weeks incubation an increase, although not significant, of mould counts in some samples was observed. It is claimed that the organisms had suffered from metabolic injury after the sequence of production steps and that they required time to recover prior to germination. Since moulds were not detected at the end of the experiment, suggesting that the contaminated moulds in DC were destroyed with time at all the temperatures tested. However it has been reported that moisture levels as high as 4 - 7 % can be observed due to absorption through packing materials, supporting mould growth and causing off-odours due to growth of xerophilic fungi in DC. Visible growth of moulds were also observed after 90 days of experiment.

Production procedure were seen to not affect microbiological characteristics of DC. Results from three factories were clustered together and of them two had fully automated production lines (M001 and M 002), and the other a semi-automated production line (M004). This suggests that good production was not dependent on factory type. The factory that completely deviated from the others had loose adherence to good manufacturing practices and also poor standards of housekeeping

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