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The Effects of Blanching on the Microbial Load of Processed Cocoyam Leaves (*Xanthosoma sagiitifolium*) During the Storage Period

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Key words: Blanching, *Xanthosoma sagiitifolium*, Microbial quality, coliform count, perishable

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Abstract: Cocoyam leaf (*Xanthosoma sagiitifolium*) (refered to as 'Kontommire' in the Ghanaian setting) is arguably one of the most readily available and cheap indigenous leafy vegetable that is commonly consumed in Ghana. It is noted to be a good source of minerals, vitamins and soluble fibre when consumed in its fresh (not raw though) state. However, this vegetable is highly perishable. Dehydration methods and jute sack storage are usually used for its preservation. However, these lead to discolouration, loss of some nutrients and exposure to microbial contamination thus reducing the general acceptability by consumers. In view of this, this study was conducted to process and preserve the leaves in their fresh state. The preservation method investigated was blanching. The effect of this preservation method on microbial quality (total viable count, total coliforms, yeast and moulds count using serial dilution) was determined. The shelf-life study lasted for three weeks with sampling at weekly intervals. Data obtained were analysed using the Statgraphics Centurion (16th edition). Microbiological quality assessment of the processed leaves also showed that blanching treatment significantly reduced the total viable count of the cocoyam leaf but increased the yeast and mould growth when stored for longer period. Samples stored in hermetically sealed bag package and ambient temperature (300°C) also had increase in the total viable count, yeast and mould count and total coliform count during storage.

INTRODUCTION

Background of the study: Vegetables are an integral food source in the diet of most Ghanaians. Many people obtain their nutrients from food plants which are cheaper, more accessible and of high nutritional value (Kwenin *et al*., 2011). Not only are these vegetables used as food source, some of them are also used for medicinal purposes. Modern science has isolated many natural products with active principles of medicinal importance from many indigenous plants. For example, *Brassica* spp. have been shown to contain glucosinolates which are

highly effective against cancer and heart diseases. Baobab leaves (*Adansonia digitata*) are used for the treatment of kidney and bladder diseases, asthma, diarrhoea, urinary tract diseases and as a blood cleanser, prophylactic and worm expeller, amongst others.

Leafy vegetables such as *Talinium triangulare* ('Bokobokor'), *Amaranthus crentus* ('Aleefu') have also been known to make food more palatable and digestible (FAO, 1990). This is because they contain high amounts of digestible carbohydrates such as starch, sucrose, glucose and fructose. Also, they contain high moisture and are easy to digest. They have also been known to contain antioxidants (lutein and zeaxanthin) that protect the eye tissues against free-radicals. Again, they are believed to minimize ageing related diseases as well as an anti-cancer agent that maintains and fights off infections (Abukutsa-Onyango, 2003; ICRAF., 2004; MacCalla *et al*., 1994). Not only are these vegetables used as food source, some of them are also used for medicinal purposes. Modern science has isolated many natural products with active principles of medicinal importance from many indigenous plants. For example, *Brassica* spp. have been shown to contain glucosinolates which are highly effective against cancer and heart diseases.

In Ghana, some of the popular leafy vegetables consumed include: the leaves of cocoyam *Xanthosoma sagittifolium* locally called 'Kontommire', water leaf (*Talinum triangulare*) locally called 'Bokobokor' *Amaranthus cruentus* locally called 'Aleefu', *Corchorus olitorius* locally called 'Ayoyo', Okro leaves (Hibiscus esculentum) and cassava leaves (Manihot esculanta). For this study, 'kontommire' (*Xanthosoma sagittifolium*) was used because it is one of the most widely consumed local leafy vegetables in Ghana.

Vegetables are highly perishable and contribute as much as 50% of post-harvest loss throughout the developing world as a result of inadequate infrastructure for processing and storage and the reduction of these losses through appropriate preservation methods could reduce the domestic shortfall in food supply by 20-30% (FAO., 1985).

Traditionally, leafy vegetables have been preserved by sun drying, storing in clay pots and storing in baskets and jute sacks (Nazare *et al*., 2007). For instance, in the Northern Region of Ghana people may preserve leafy vegetables by drying them, keeping them in jute sacks and moistening the sack so as to preserve the freshness of the leaves for a day or two. Also, market women store leafy vegetables in plain polyethylene bags and sprinkle water on them to maintain their freshness. Though these methods may preserve the leaves for some days, there are changes in colour, texture and odour. There also tends to be microbial contamination. Physical contamination and changes may result from contact with dust, soil, insect infestation, exposure to sunlight and contact with other pests. Where most vegetables are not cleaned before drying, the produce is heavily contaminated with bacteria and mould spores. This may result in the formation of mycotoxins and pathogenic microorganism infections (Brennan and Day, 2006). These therefore calls for the use of a more systematic method of preservation of 'kontommire' (*Xanthosoma sagittifolium*) blanching.

Blanching involves the use of mild heat in different heating systems such as steam, hot water and microwave. This causes cell death and physical and metabolic chaos within the cells of the vegetables. The heating effect leads to enzyme destruction as well as damage to the cytoplasmic and other membranes which become permeable to water and solutes. An immediate effect is the loss of turgor pressure. Water and solutes may pass into and out of the cells, a major consequence being nutrient loss from the tissue. Also, cell constituent which had previously been compartmentalized in subcellular organelles become free to move and interact within the cell (Brennan and Day, 2006).

The major purpose of blanching is generally to inactivate enzymes which would otherwise lead to quality reduction in the processed product and also reduce the microbial population on the surface of the fresh produce. It causes the removal of gases from plant tissues, especially, intercellular gas which is useful in avoiding oxidation of the product and corrosion of the cans that may be used for packaging. Removal of gases, along with the removal of surface dust has a further effect in brightening the colour of some products, especially green vegetables (Fellows, 2000). On this note, there is therefore the need to adopt automated modern methods and use of appropriate equipment for preservation (such as blanching) to avoid/reduce possible microbial growth in storage, since, sun drying is unreliable. Thus, this study was carried out to ascertain the effect of blanching as a method of preservation on the microbial load of processed cocoyam leaves during the storage period.

MATERIALS AND METHODS

Sample collection and sample preparation: Fresh fully opened cocoyam leaves which were 2 weeks old were harvested from the Biotechnology and Nuclear Agriculture Research Institute (BNARI) farm and used for the study. After harvesting, the cocoyam leaves were sorted out to remove all debris, bruised leaves and other unwanted particles. They were then washed in 50% brine solution and put into a sterilized bowl. The chopping board used for shredding was first sterilized with 70% alcohol. After shredding, the cocoyam leaves were

Fig. 1: Photograph of shredded cocoyam leaves stored in zip-lock polyethylene bags

packaged into zip-lock and hermetically sealed bags. The shelf-life study was carried out for a period of three weeks and the pH of the samples were monitored weekly.

Blanching: Steam blanching method was used in order to retain most of the nutrients. This was done over boiling water containing 50% NaCl at a temperature of 100°C for 2 min in a steamer for the leafy vegetable. The addition of the salt (NaCl) was to help improve the colour. After blanching, the leaves were allowed to cool to room temperature before packaging into the zip-lock polyethylene bag and hermetically sealed polyethylene bag. Some of the packaged leaves were stored at room temperature of 30±20°C and some in the refrigerator Fig. 1.

Determination of total plate count (Aerobic Mesophiles): The 10 g of the leafy vegetables used was added to 90 mL of 0.1% Peptone water (Pw) to form a stock solution which was agitated and then incubated at 370°C for 15 min. The stock solution was serially diluted using 10 fold serial dilution up to 105 into 10 other sterile McCartney bottles containing 9 mL peptone water. Using the pour plate method, 1 mL of the suspension was picked onto a sterilized petri-dish and nine millilitres (9 mL) of the plate count agar was added. This was mixed by rotating the petri-dishes under the laminar flow at 45-500°C. The media were allowed to cool and set and then incubated at 370°C for 18-24 h in a microbiological incubator (Westach). Plates were selected for count using the colony counter. Based on the dilution factor the count was expressed as A×10 Bcfu/g where A is the colony counted, B is the dilution factor and CFU/g is colony forming unit per gram for microbiological determination. This procedure was carried out before and after treatment by irradiation method.

Determination of Total coliform Count (TCC): Using serial dilution method, one millilitre of the prepared sample was added to the 9 mL of Violent Red Bile Agar (VRBA), mixed well by rotating under the laminar flow and allowed to cool and incubated at 370°C for 18-24 h. Colonies were counted and expressed in CFU/g. This procedure was carried out before and after treatment by blanching method.

Determination of moulds and yeasts count: Using the serial dilution technique, 1 mL suspension was dispensed into sterilized petri-dish and 9 mL of OGYEA agar was added. The mixture was agitated and allowed to solidify and incubated at 300°C for 24 h. Counting was done using colony counter from the plate that yielded between 30-300 colonies. This procedure was carried out before and after treatment by blanching method.

Isolation and identification of coliform Bacteria: Using a sterile inoculating loop, stock solution of sample was picked onto the Blood agar and McConkey medium and incubated at 370°C for 18-24 h. Physiological characteristics of the colonies formed were examined separately. Pure colonies were sub cultured onto Nutrient Agar (NA) medium and Eosine Methyl Blue Agar (EMBA) (Merck, Darmstadt-Germany) medium and incubated again at 370°C for 18-24 h. Colonies were Gram stained and observed under light microscope at x100 with oil immersion for physiological characteristics and cellular morphology. Based on these, biochemical tests were performed to identify organisms from coliform group.

Statistical analysis: Data obtained was analyzed using the Statgraphics centurion software (Version 16.0). The factorial experimental design was used for the study. Results were computed in graphs and tables using Microsoft Excel and Microsoft word on the Microsoft Office 2010 package and SPSS version 16.

RESULTS AND DISCUSSION

Effect of blanching and ambient temperature, blanching and refrigeration on total viable count storage had significant effect ($p<0.05$) after blanching and

Table 1: Blanching and refrigeration (Hermetically sealed bag) ANOVA table for total plate count by weeks

Source	Sum of squares	df	Mean square	F-ratio	p-values
Between groups	190.541	3	63.5136	10343.32	0.0000
Within groups	0.0491244	8	0.00614055		
Total (Corr.)	190.59	11			

Table 2: Blanching and refrigeration (Storage in Zip-lock bag) ANOVA table for total plate count by weeks

Source	Sum of squares	df	Mean square	F-ratio	p-values
Between groups	65.9511	3	21.9837	20037.76	0.0000
Within groups	0.0087769	8	0.00109711		
Total (Corr.)	65.9599	11			

Table 3: Blanching and ambient temperature storage (Hermetically sealed bag) ANOVA table for total plate count by weeks

Source	Sum of squares	df	Mean square	F-ratio	p-values
Between groups	139.768	3	46.5894	36477.61	0.0000
Within groups	0.0102176	8	0.0012772		
Total (Corr.)	139.778	11			

Table 4: Blanching and ambient temperature storage (Storage in zip-lock bag) ANOVA table for total plate count by weeks

Source	Sum of squares	df	Mean square	F-ratio	p-values
Between groups	168.074	3	56.0247	35929.69	0.0000
Within groups	0.0124743	8	0.00155929		
Total (Corr.)	168.087	11			

Table 5: Blanching and refrigeration (Hermetically sealed bag) anova table for total coliform by weeks

Source	Sum of Squares	df	Mean square	F-ratio	p-values
Between groups	185.659	3	61.8862	553513.57	0.0000
Within groups	0.000894449	8	0.000111806		
Total (Corr.)	185.66	11			

Table 6: Blanching and refrigeration (Storage in Zip-lock bag) ANOVA table for total coliforms by weeks

Source	Sum of squares	df	Mean square	F-ratio	p-values
Between groups	88.249	3	29.4163	3209.85	0.0000
Within groups	0.0733152	8	0.0091644		
Total (Corr.)	88.3223	11			

Table 7: Blanching and ambient temperature storage (Hermetically sealed bag) ANOVA table for total coliform by weeks

Source	Sum of squares	df	Mean square	F-ratio	p-values
Between groups	155.029	3	51.6763	128440.16	0.0000
Within groups	0.0032187	8	0.000402337		
Total (Corr.)	155.032	11			

Table 8: Blanching and ambient temperature storage (Storage in zip-lock bag) ANOVA table for total coliforms by weeks

Source	Sum of squares	df	Mean square	F-ratio	p-values
Between groups	169.079	3	56.3596	544031.71	0.0000
Within groups	0.00082877	8	0.000103596		
Total (Corr.)	169.08	11			

refrigeration storage (Table 1 and 2). Storage increased the viable counts in both packaging materials and storage temperatures (Fig. 2). During storage in zip-lock polyethylene bag total viable count showed an increased from 0.01-7.98±0.02 log CFU/g during storage (Fig. 1). Samples stored in hermetically sealed bag also recorded an increase from 0.01-8.71±0.02 log CFU/g after the storage period (Fig. 2-4). Also ambient temperature storage had significant effect on viable counts (Table 3 and 4). After the storage period total viable counts in samples stored in hermetically sealed bag increased from 0-8.69 log CFU/g. Total viable counts in samples stored in zip-lock bag was also increased from 0 to at 8.48 log CFU/g. The increase in counts is an indication of spoilage and shorter shelf-life. Comparatively counts in hermetically sealed bag were not significantly different from that recorded higher count that was recorded from zip-lock polyethylene bag at all storage temperatures (Fig. 2).

Effect of blanching and ambient temperature, blanching and refrigeration on total coliforms: Storage had significant effect on the total coliform count after blanching and refrigeration (Table 5 and 6). Storage

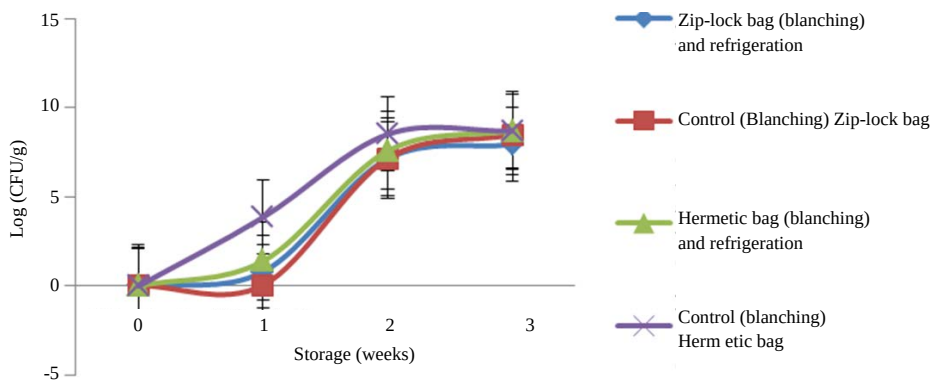

Fig. 2: Total viable count after blanching and ambient temperature storage and blanching and refrigeration storage

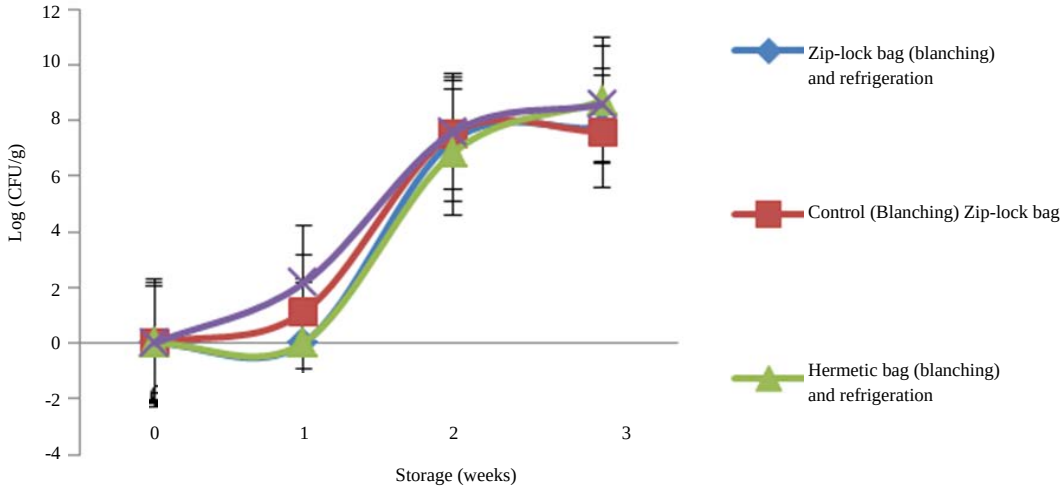

Fig. 3: Total coliforms after blanching, blanching and refrigeration storage

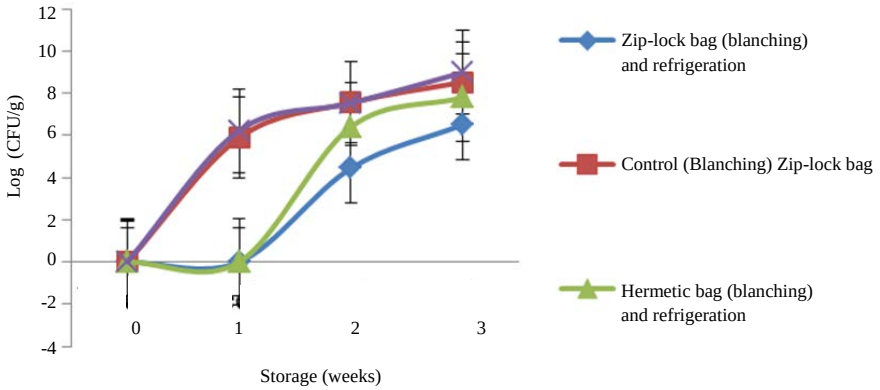

Fig. 4: Yeast and moulds counts after blanching, blanching and refrigeration storage

increased the coliform counts at all storage conditions (Fig. 3). Total coliform count after blanching and refrigeration and storage in hermetically sealed bag increased from 0.01-8.71±0.01 log CFU/g after the storage period. Samples stored in zip-lock polyethylene bag also recorded an increase from 0-7.73±0.01 log

Table 9: Blanching and refrigeration (Hermetically sealed bag) ANOVA table for yeast and moulds by weeks

Source	Sum of squares	df	Mean square	F-ratio	p-values
Between groups	154.386	3	51.462	63163.68	0.0000
Within groups	0.00651793	8	0.000814741		
Total (Corr.)	154.393	11			

Table 10: Blanching and refrigeration (Storage in Zip-lock bag) ANOVA table for yeast and moulds by weeks

Source	Sum of squares	df	Mean square	F-ratio	p-values
Between groups	24.192	3	8.064	84841.29	0.0000
Within groups	0.000760384	8	0.0000950481		
Total (Corr.)	24.1928	11			

Table 11: Blanching and ambient temperature storage (Hermetically sealed bag) ANOVA table for yeast and moulds by weeks

Source	Sum of squares	Df	Mean square	F-ratio	p-values
Between groups	140.838	3	46.9461	1132963.06	0.0000
Within groups	0.000331492	8	0.0000414366		
Total (Corr.)	140.839	11			

Table 12: Blanching and ambient temperature storage (Storage in zip-lock bag) ANOVA table for yeast and moulds by weeks

Source	Sum of squares	Df	Mean square	F-ratio	p-values
Between groups	96.5807	3	32.1936	7361.93	0.0000
Within groups	0.0349838	8	0.00437298		
Total (Corr.)	96.6157	11			

CFU/g. Ambient temperature storage also had significant effect on counts (Table 7 and 8) as it increased counts (Fig. 3). Total coliform counts in samples stored in hermetically sealed bag increased from 0-8.57±0.01 log CFU/g and that of zip-lock polyethylene bag stored samples increased from 0.03-8.48. Comparatively, there were no significant differences ($p<0.05$) in counts recorded of the mean values recorded at all storage conditions (Fig. 3).

Effect of blanching and ambient temperature storage, blanching and refrigeration on yeast and moulds count: Storage had significant effect on the yeast and mould counts in blanched and refrigerated samples (Table 9 and 10). Storage increased counts in both packaging materials (Fig. 4). Counts in samples stored in hermetically sealed bag increased from 0-7.80±0.02 log CFU/g after the storage period whiles counts in samples stored in zip-lock bag also increased from 0-6.51±0.04 log CFU/g. Ambient temperature storage also had significant effect on the yeast and mould growth (Table 11 and 12). There was an increase in counts during storage (Fig. 4). Samples stored in zip-lock bag recorded an increase from 0-8.53±0.02 log CFU/g and hermetically sealed bag counts was also increased from 0-8.99±0.02 log CFU/g. Comparatively yeast and moulds count was significantly different from storage temperatures during the first week of storage (Fig. 4).

Effect on microbial quality: A study in 2003 reported that microbial load of log 104-7/g in vegetables is acceptable by the Foods and drugs quality assurance department (Jacxsens *et al*., 2003). After refrigeration storage, samples stored in hermetically sealed bag recorded total viable counts of 4.72 log CFU/g, total coliform counts of 4.98 log CFU/g and yeast and mould counts of 4.82 log CFU/g. All microbial counts recorded were within the acceptance range of log 104-7/g. Whiles samples stored in zip-lock polyethylene bag recorded viable count of 7.37 log CFU/g, coliform counts of 6.97 log CFU/g and yeast and mould counts of 3.46 log CFU/g after the storage period. With the exception of the yeast and mould counts all other microbial counts were above the acceptable level. Also, microbial counts recorded from cocoyam leaves stored at ambient temperature were above the acceptance level by the quality assurance department of the Food and Drugs Board of the USA. Samples stored in hermetically sealed bag recorded viable counts of 8.8 log CFU/g, coliform counts of 6.67 log CFU/g and yeast and moulds counts of 7.24 log CFU/g whiles samples stored in zip-lock bag during storage recorded viable counts of 6.93 log CFU/g, coliform counts of 6.93 log CFU/g and yeast and mould counts of 4.82 log CFU/g. After blanching and storage, microbial counts were higher in samples stored at ambient temperature than in refrigerated samples in both the zip-lock bag and hermetically sealed bag. However, there were no growth of yeast and moulds, less growth of total viable counts and total coliforms in all blanched samples at the initial week. Thus blanching was effective in controlling microbial growth during the first week of storage. A similar study in 1987 reported that blanching is effective in removing pesticide residues or radionuclides from the surface of vegetables as well as reducing micro-organisms

and toxic constituents that are naturally present (nitrites, nitrates and oxalate). Moreover, after the storage period, microbial growth was very high because moisture content was higher at the end of the storage period. This is an indication that moisture supports the growth of microbes and research have shown that higher moisture content is an indication that a product will not store for long period without spoilage by micro-organism during storage (Ejoh *et al*., 2007).

From the study the pH of the preserved cocoyam leaf ranged was at 6.54 in most of the stored samples at the initial week but increased after every storage week to a figure nearer to the neutral pH and this may have accounted for the increase in yeasts and mould growth and the softening of the leaves. Previous study reported that softening of vegetables result from yeasts consuming lactic acid, resulting in an increase of pH which produces degradable enzymes (Fleming *et al*., 1989). Similarly, another study reported that the optimum pH for growth of most microorganisms is near neutrality (pH 7.0) (Toumas, 2005). However, yeasts and moulds are acid tolerant and can even grow in acidic foods. Yeasts can grow in a pH range of 3-10 whiles moulds can grow from pH 2-11. In terms of water requirements for growth, yeasts are intermediate between bacteria and moulds.

Total coliform count increased under all the treatments of preservation. However, no faecal contaminating microbes (*Escherichia coli and Samonella* spp.) were present. Thus, the presence of coliforms or faecal coliforms on the vegetables did not necessarily provide an index of faecal contamination but rather other microorganisms capable of causing human disease were present. The absence of these common faecal coliforms (*E.coli and Salmonella* spp.) may be due to the fact that cocoyam leaf cultivation is mostly rain-fed and also was grown under a confined environment (green-house). Contamination of raw vegetables with enterhaemorrhagic *E. coli* O157:H7 and salmonella spp usually occurs when herbivorous accidentally enter fields or when improperly composted faecal manure is applied as a fertilizer to enrich the soil and this contaminates the airborne dust particles that cause possible contamination in the vines and on the surface of the leafy vegetable (Abdul-Raouf *et al*., 1993), however because cocoyam leaves contain an irritant, it deters herbivorous from grazing on them and also because they can grow under harsh conditions without any enrichment of the soil, no faecal coliform was found (Ramanatha Rao *et al*., 2010).

On the contrary, *Pseudomonas* fluorescens and Pseudomonas viridiflava were present and might have been responsible for the decay of the leaves. In a similarly study, *Pseudomonas* spp was reported to be capable of decaying plant tissue at temperatures at or below 4°C.

CONCLUSION

Blanching was an effective method in reducing microbial load of fresh cut cocoyam leaf. The colour of the leaf was appealing and the microbial load recorded was least after the storage period especially in those that were refrigerated. Yeast and moulds do not grow rapidly on blanched cocoyam leaf when refrigerated. Nevertheless, blanching and ambient temperature storage is not an effective method for leafy vegetable storage since it renders the leaves marshy, smelly and unappealing. In order to reduce the microbial counts in cut leafy vegetables, it is recommended to sanitize the equipment used for processing and also to wash the leafy vegetables in either brine solution or vinegar. It is also recommended to store blanched leafy cocoyam leaf in hermetically sealed polyethylene bag. Finally, cut-blanched cocoyam leaf should be stored in the refrigerator at a temperature of -4°C for a longer period of storage and a temperature range of -8-10°C for shorter periods.

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