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Pickling as a Preservation Technique for *Solanum aethiopicum;* An Edible Green Leafy Vegetable

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SA, SB and IMM designed the study. Author SA performed the data collection. Authors SA and AN performed the statistical analyses. Author SA wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Solanum aethiopicum is a nutrient rich green leafy vegetable whose utilisation is limited by its short shelf life. Although refrigeration and freezing are effective methods to preserve vegetables, these methods may not be available and affordable in resource-limited settings. A viable alternative is pickling, which is a cost effective and easy to apply method. In this study, *nakati* leaves were fermented in brine with 3% dry salt and 2% sugar at 25°C for 14 days. The fermentation was monitored at intervals by enumerating lactic acid bacteria (LAB), coliforms and fungi as well as measuring the brine pH. Sensory evaluation (n=30) was carried out to assess the acceptability of the vegetables. Shelf stability was evaluated for three weeks. The results showed that lactic acid bacteria increased significantly ($P < .001$) from 2.06 log cfu/ml to 8.2 log cfu/ml during fermentation. Coliforms and fungi reduced from 5.5 log cfu/ml and 1.5 log cfu/ml to undetectable levels within 8 and 6 days of fermentation, respectively. The brine pH decreased from 5.87 at day 0 to 3.35 after 14 days of fermentation ($P < .001$). Pickled *nakati* had lower consumer acceptability scores ($P <$

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.001) than fresh *nakati.* The *nakati* remained stable during the three weeks of storage. Therefore, pickling offers a potentially viable preservation method that can be adopted for green leafy vegetables.

Keywords: Solanum aethiopicum; nakati; pickling; fermentation; vegetables; African eggplant.

1. INTRODUCTION

Africa has a range of indigenous vegetables of nutritional importance due to their rich content of vitamins, minerals, antioxidants, phytochemicals and dietary fibre [1,2]. *Solanum aethiopicum* Shum, known as African eggplant or *nakati* (Luganda language from Uganda), is one such vegetable that holds cultural significance in the Ugandan diet as a commonly consumed side dish [3]. However, *Solanum aethiopicum* has a short shelf life of only 1-2 days at room temperature (25°C), necessitating appropriate preservation techniques to extend its shelf life [4,5].

Although refrigeration, freezing, and canning are effective methods for preserving vegetables, these methods are generally unavailable to, and unaffordable by local traders and farmers, particularly in Africa [6,7]. In Uganda, for instance, fresh consumption of vegetables is predominant. This often leads to wastage of surplus vegetables, especially in rainy seasons [5,7]. In a few instances, preservation techniques such as traditional open-sun drying, solar drying, and oven drying methods are used on a small scale but these result in significant loss of micronutrients, particularly vitamins A and C [5].

An alternative approach that can be used to extend the shelf life of vegetables while preserving their nutritional content is pickling. Pickling has been shown to increase the shelf life of vegetables while retaining their nutritional value [7-9]. Pickling is a cost effective and easily applicable technique, making it a viable alternative [7]. Despite the successful application of acid fermentation for the preservation of various food products, its utilization for indigenous leafy vegetables in Africa remains limited [1,7].

Therefore, this study aimed at investigating the effects of pickling as a preservation technique on the quality, acceptability, and shelf stability of *Solanum aethiopicum* (*nakati*). By evaluating the impact of pickling on the vegetable's sensory attributes, nutritional composition, and microbial stability, the study sought to provide insights into the feasibility of pickling as a means to enhance the shelf life and preserve the nutritional value of this indigenous African vegetable. The findings of this study have the potential to inform the development of sustainable and affordable preservation strategies that benefit both local farmers and consumers of green leafy vegetables.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Freshly harvested *Solanum aethiopicum* (*nakati)* was purchased from Kalerwe market, Kampala district, Uganda. Light brown sugar (Kakira Sugar Ltd, Uganda) and iodized salt (Habari, Krystalline Salt Ltd, Nairobi, Kenya) were purchased from a supermarket in Kampala.

Healthy green leaves of *nakati* were separated from the stems and washed thoroughly with potable water prior to being cut into small pieces (about 5 cm x 5 cm). Based on Wafula [7] and Breidt et al. [10], the following treatments were applied on the vegetables; (i) 5% brine, (ii) 5% dry salt, (iii) brine with 3% salt and 2% sugar and (iv) 3% dry salt and 2% dry sugar. The vegetables were mixed thoroughly with the salt and or sugar and placed in clean sterile glass jars. Weights held in a clean polythene bag were placed on the vegetables to fully submerge the leaves in the brine. The jars were loosely closed and the *nakati* left to ferment for 14 days at room temperature. Brine pH was measured at the start and after 14 days of fermentation. The most effective treatment at lowering pH (3% dry salt and 2% dry sugar) was later used in a separate experiment to determine the effect of fermentation on pH, microbial counts and consumer acceptability. Samples were withdrawn at 0, 2, 4, 6, 8, 10 and 14 days of fermentation for determining microbial counts (lactic acid bacteria (LAB), coliforms, yeasts and molds) and pH.

2.2 Microbiological Analyses

LAB were determined by pour plating appropriate selected serial dilutions of the brine in sterile MRS agar (Laboratorios CONDA, Madrid, Spain)

and incubating at 30℃ for 48 h [11]. Coliforms were determined by pour plating selected serial dilutions of the brine in violet red bile lactose agar (Laboratorios CONDA, Madrid, Spain) and incubating at 37℃ for 24 h [12]. Yeasts and molds counts were determined by spread plating selected serial dilutions of the brine on sterile pre-poured Potato Dextrose Agar (Laboratorios CONDA, Madrid, Spain) acidified with 1% lactic acid followed by the plates at 30℃ for 72 h [13].

2.3 pH

The pH of brine was measured using a pH meter (INE-PHS-3E mrc – Laboratory Instruments, Hagavish st. Israel).

2.4 Consumer Acceptability

An untrained consumer panel (n = 30) comprising of staff and students from Makerere University was used to assess acceptability of fresh and pickled *nakati.* The *nakati* was prepared by steaming for 10 minutes as described by Kinyi et al. [14]. Panelists ranked the acceptability of quality attributes using a nine-point hedonic scale (*9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely*) [15]. A preference test was also carried out between the fresh and pickled *nakati* [16]. Bottled water was provided for rinsing the palate in between sample tasting.

2.5 Shelf Stability

Shelf stability was evaluated by determining pH, coliforms and fungal counts in the *nakati* (with 3% dry salt and 2% dry sugar) after 14 days of pickling. Determinations were done at 1, 2 and 3 weeks of fermentation. Visual observation was also used to check for signs of spoilage.

2.6 Statistical Analysis

The experiments were carried out in duplicate. One-way analysis of variance was used to test for significant differences at α level of 0.05%. The Tukey's HSD test was used to separate the means. An independent samples t-test was carried out for the data obtained from sensory evaluation to determine if there were significant differences between the acceptability scores of the pickled and fresh *nakati.* This was done at 95% level of confidence using SPSS (IBM, USA) software version 20.

3. RESULTS AND DISCUSSION

3.1 pH

Table 1 shows the changes in pH during the pickling process. The treatments with sugar registered the greatest decrease in pH during the pickling process. This is attributed to the availability of fermentable carbohydrates that were utilised by the lactic acid bacteria to produce lactic acid. Oguntoyinbo et al. [1] previously reported that some African indigenous vegetables lack sufficient carbohydrates in a form that can be utilized in lactic acid fermentations and, therefore, required supplementation for the fermentation to proceed. During lactic acid fermentation, LAB break down fermentable carbohydrates to produce various fermentation products depending on the bacterial species involved. Homofermentative bacteria yield lactic acid as the only product of fermentation while heterofermentative bacteria produce lactic acid, ethanol, carbon dioxide, acetic acid, acetaldehyde, diacetyl, and acetoin [8]. Addition of sugar, therefore, provides a fermentable carbohydrate source thus enabling fermentation to proceed with subsequent lowering of pH by the acid produced. Addition of sugar, therefore, promotes lactic acid fermentation and pH reduction.

Table 1. Changes in pH in different *nakati* treatments during pickling

Treatment	pH	
	Day 0	Day 10
5% brine	5.89±0.00	6.09^{a}±0.01
3% salt + 2% sugar brine	5.91±0.01	4.14^{c}±0.02
3% salt + 2% sugar dry	5.87±0.02	3.72^{d}±0.02
5% dry salt	5.94±0.01	5.81^{b}±0.02

Values are means ± standard deviations of duplicate determinations. Means in the same column with different superscripts are significantly different (P < .001)

The treatment with dry sugar and salt was selected for further evaluation for changes in pH and microbial population because it registered the lowest pH after fermentation. Rapid acidification during pickling is desirable because low pH inhibits growth of undesirable microorganisms [8,17]. Salt in pickling inhibits growth of undesirable microorganisms and induces plasmolysis in plant cells which leads to the release of water, minerals and nutrients needed for LAB growth [8,17,18]. Therefore, a combination of sugar and dry salt provides an ideal approach for pickling *nakati*.

3.2 Microbial Counts

Fig. 1 shows the changes in microbial counts during the pickling of *nakati* using the earlier selected treatment containing 3% dry salt and 3% dry sugar.

The microbial load at the start of the fermentation was in the order coliforms > LAB > fungi. LAB then increased from 2.06 log cfu/ml to a maximum of 8.2 log cfu/ml within 6 days and then reduced gradually to about 7 log cfu/ml until day 14. Coliforms and fungi reduced from about 5.5 log cfu/ml and 1.5 log cfu/ml to undetectable levels within 8 and 6 days of fermentation, respectively.

Fig. 2 shows the changes in the pH of the brine during pickling. The pH of the brine decreased significantly (P <0.001) throughout the pickling process from 5.9 to 3.4.

The initial microbial population can be attributed to various sources including the environment, handling equipment, soil etc. and is similar to that described by Montet et al. [19] who noted that after harvest, the microbial population on the surface of vegetables can be as high as 8 log cfu/ml with LAB contributing about only 0.1% of the population (close to 5 log cfu/ml).

LAB rapidly increased because they were able to utilize the nutrients present in the vegetables together with the added sugar to rapidly multiply and out-compete other microorganisms initially present [17]. This result was consistent with that obtained by Wafula [7] in a study on fermentation of African nightshade. Coliforms were highest at the start of the fermentation probably due to contamination from external sources, such as soil, and presence of favourable conditions for their survival in the environment. The reduction of coliforms and fungi could be attributed to reduction in pH below values favourable for their growth. The lactic acid produced during fermentation is responsible for reduction in pH [7]. The results are in agreement with Montet et al. [19] who found that during fermentation of cabbage, pH drops from near neutral to 3.8-3.2 at the end of the fermentation. Wafula [7] reported similar observations during the fermentation of African nightshade. In this study, initial LAB counts of 1 log cfu/ml increased rapidly in 24 hours to counts of 3 log cfu/ml then peaked at 5 log cfu/ml. Yeasts and molds remained at a concentration below 1 log cfu/ml.

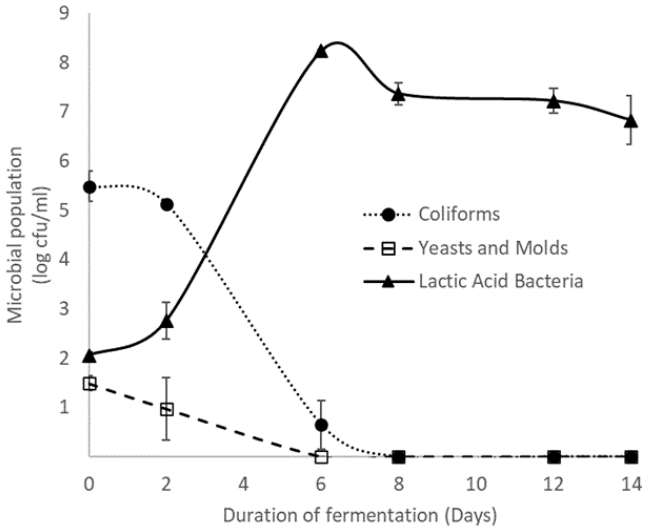

Fig. 1. Changes in microbial counts during the pickling of *nakati* containing 3% dry salt and 2% dry sugar

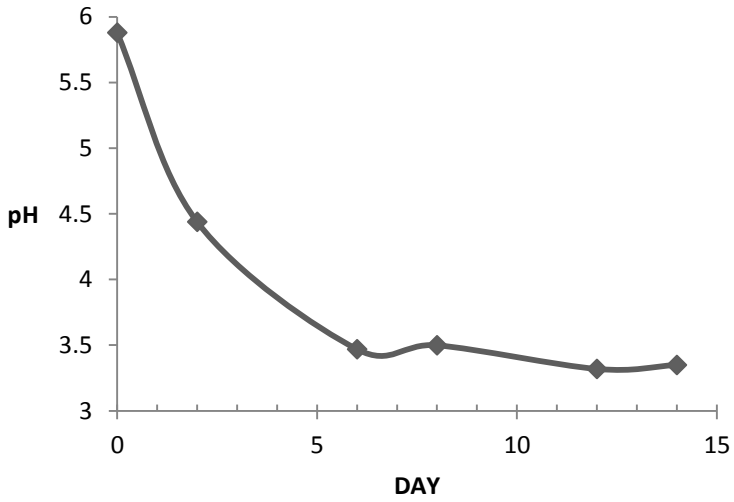

Fig. 2. Change in brine pH during fermentation of *nakati* containing 3% dry salt and 2% dry sugar

Some LAB strains also produce bacteriocins with antibacterial and antifungal properties contributing to the elimination of pathogenic bacteria, coliforms, yeasts and molds [20]. This was illustrated in another study [7] in which inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* was observed during controlled fermentation where African nightshade was inoculated with the microbes at concentrations of 3 log cfu/ml each and LAB at concentration of 7 log cfu/ml. The concentration of *S. enteritidis* increased significantly to log 7 cfu/ml within 24 hours in controls (no LAB added) before reducing to undetectable levels within 144 hours. *L. monocytogenes* remained at the same concentration and reduced to undetectable levels in 48 hours.

3.3 Consumer Acceptability of Pickled *nakati*

Table 2 summarizes the consumer acceptability scores of the *nakati*. The scores of pickled *nakati* ranged from 5 (neither like nor dislike) to 6 (like slightly) while those of fresh *nakati* ranged from 6 (like slightly) to 7 (like moderately). Pickling significantly reduced the consumer acceptability of *nakati*. Fresh *nakati* was preferred by 70% of the panelists. However, these observation were not in agreement with Wafula [7] and Montet et al. [19] who found that fermentation improved the sensory characteristics of vegetables.

Generally in Uganda, most vegetables are consumed after cooking but without any other pre-treatments or processing done to them. This may explain why the fresh *nakati* was more acceptable (Table 2) than pickled *nakati.* Apolot et al. [4] also reported that processed vegetables were generally less acceptable than fresh ones. Pickled vegetables are not commonly consumed in Uganda which makes their taste unfamiliar and could have contributed to less acceptability than that observed by Wafula [7] and Montet et al. [19].

Table 2. Consumer acceptability scores of fresh and pickled *nakati*

Sensory attribute	*Nakati*		P values
	Fresh	Pickled	
Colour	$7.9^{a} \pm 1.2$	$6.1^{b} \pm 1.8$	<0.001
Aroma	$6.9^{a} \pm 1.5$	$4.9^{b} \pm 1.9$	<0.001
Taste	$6.5^{a} \pm 1.7$	$4.8^{b} \pm 2.5$	0.002
Aftertaste	$6.5^{a} \pm 1.9$	$5.1^{b} \pm 2.5$	0.008
Overall acceptability	$7.5^{a} \pm 1.2$	$5.5^{b} \pm 2.5$	<0.001

N=30. Values are means ± standard deviation. Means in the same row with different superscripts are significantly different

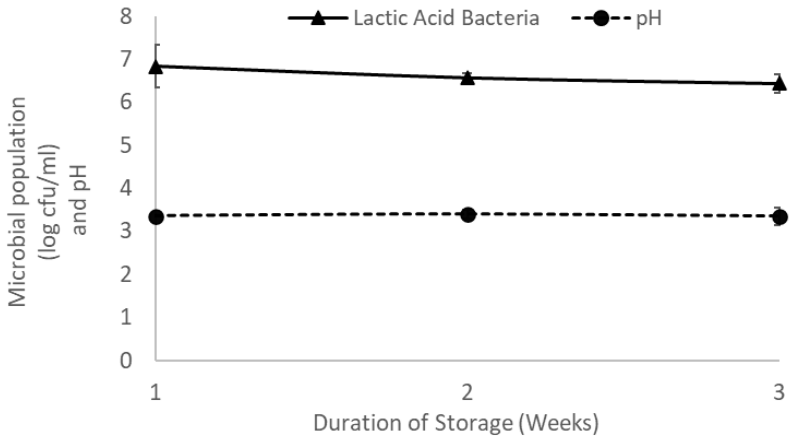

Fig. 3. Changes in LAB and pH during storage of pickled *nakati*

3.4 Shelf Stability of Pickled *nakati*

Fig. 3 shows the changes in pH and LAB counts during storage of pickled *nakati* at room temperature. LAB counts (6.4 – 6.8 log cfu/ml) and pH (3.35 -3.4) did not vary significantly during the three weeks of storage. Coliforms, yeasts and molds were not detected throughout storage. There were also no visible signs of spoilage.

These results indicated that the product was stable throughout the storage period. This can be attributed to depletion of nutrients in the fermentation medium [17] as well as accumulation of organic acids which inhibited further metabolic activity of the LAB [7,19]. The acids create an unfavourable environment for growth of spoilage microorganisms thus creating a relatively stable product [19,20]. According to Erten et al. [8], pickles and other fermented vegetables have a shelf life that is greater than one year. This suggests that pickled *nakati* could as well have a shelf life that is longer than the three months evaluated in this study.

4. CONCLUSION

Pickling offers a potentially viable alternative for extending the shelf life of *nakati (Solanum aethiopicum)* and possibly other indigenous green leafy vegetables. This can be achieved by addition of sugar and salt for rapid and successful pickling to occur. Pickling can increase the shelf stability of *nakati* from 1-2 days to at least three weeks at room temperature thus reducing wastage during the surplus periods. Further studies should evaluate the shelf stability beyond three weeks at room temperature, as well as at refrigeration temperatures.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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