HONEY CONCENTRATION DELAYS DETERIORATION OF FRESH CUT PUMPKIN FRUITS BY RETARDING MICROBIAL GROWTH

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Abstract

Pumpkin pulp is an important source of β-carotene, a precursor for vitamin A. The increasing awareness of health benefits of pumpkin fruits explains ever rising price of pumpkins fruits at the urban and peri-urban areas beyond reach of most consumers. The pumpkin fruit is normally sliced and wrapped in polyfilm that is sold to consumers albeit rapid deterioration in quality. Currently, there is limited information on the effect of honey syrup concentration on the quality and microbial growth of fresh sliced pumpkin fruits at room temperature. A study was conducted to evaluate the effect of honey concentration on quality and microbial growth of sliced pumpkin fruit at room temperature. The experiment was laid in complete randomized design replicated four times. Four honey concentrations were used: 0 %, 25%, 50% and 100% (w/v). Microbial study was conducted using nutrient agar and Sabround Dextrose agar for the fungi and bacteria tests, respectively. Morphological characteristics and chemical tests were used to identify the bacterial species present. Fungi were identified based on morphology as observed under microscope at ×10 magnification upon methyl blue staining. Pumpkin slices treated with 100% honey concentration exhibited lowest microbial count and species distribution, highest total soluble solids and best sensory rating. The rate of deterioration of sliced pumpkin fruit depends on honey concentration. Pure honey (100% concentration) delays rotting sliced pumpkin fruits by inhibiting growth of spoilage fungi and bacteria. To sustain acceptability and storage quality of sliced pumpkin fruits it is recommend to dip the slices in hundred 100% honey.

Key Words: Cucurbits, β-carotene, total soluble solids

Introduction

Pumpkin is one of the important members of the family cucurbitacea. In Kenya the crop is grown for mature fruits and tender leaves. The fruit flesh is rich in β-carotene a precursor of vitamin A Vitamin A is important for preventing night blindness, improving body immunity (Zhou *et al* ., 2007). Sudheer *et al.* (2017) reported that intake of rich in beta-carotene like pumpkin may reduce the risk of developing certain types of cancer and offer protection against asthma and heart diseases. Promotion of production and consumption of pumpkin is therefore one the sustainable strategies of managing life style diseases such as cancer, hypertension and diabetes (Torkova *et al.*, 2018).

Whole pumpkin fruits devoid of physical bruises, pest and pathogen attack have long keeping quality up to six months under room temperature (Kiharason and Isutsa, 2019). It therefore follows that slicing the whole fruits without employing preservation techniques will jeopardize storability of pumpkins. However, the size of the pumpkin fruit and its affordability forces sellers to slice the whole fruit to suit the requirements of the consumers based. For small households, sliced pumpkin fruits allow them to buy what they can consume in a meal to avoid wastage due to rots. This explains the increasing popularity of sliced pumpkins fruits in Kenya. Previous studies indicated that slicing reduces shelf –life of the pumpkin fruit through microbial decay, weight loss, adverse colour and textural changes (Eman *et al,* 2015). Thus, this study investigated the effect of honey concentration on quality of sliced pumpkins wrapped with plastic films at room temperature.

A study by Kiharason and Isutsa (2019) reported that most consumers prefer ¼ -sliced pumpkins. They further reported that spoilage of such slices occurred by third day especially in market places. Sliced pumpkin fruits are prone to microbial spoilage even under refrigeration due to high moisture content (Tunde-Akintunde and Ogunlakin, 2011; Anju, 2018).

Various preservation methods such as microwave drying (Song *et al.*, 2017), osmotic dehydration (Katsoufi *et al.*, 2017), cooking, canning and chemical use (Anju *et al.*, 2018) have been proposed to reduce the rate of deterioration in quality of cut pumpkin fruits. Although chemical methods of fresh cut vegetables are effective, their continuous use poses serious health concerns such as allergic and hypertensive effects (Kumari *et al.*, 2019). This necessitates development of natural preservatives capable of improving shelf-life of fresh cut fruits without jeopardizing the health of consumers.

To address the health and environmental concerns of chemical residues in fresh cut pumpkin, a study by Nguyen *et al.* (2019) showed that 10% Vinegar improves storage quality of fresh cut pumpkins by reducing microbial load as well as maintaining colour. This study involved refrigeration and purchase of commercial sanitizers which might not be affordable to resource poor households and fresh produce retailers. Thus simple and affordable techniques like honey dips are considered suitable alternatives in fruit preservation methods. Drying of sliced fruits has been shown to reduce bioavailability of carotenoids (Song *et al.*, 2017). The most effective drying method for fruits is vacuum microwave but relatively expensive for resource poor traders and households in developing economies such as Kenya.

Osmotic dehydration is one of the oldest methods of fruit and vegetable preservation (Yadav and Singh, 2014). It is gaining popularity due to the following advantages increased nutritional and sensory quality attributes such as improved colour and taste (Ahmed *et al* . 2016). Compared to other fruit and vegetable preservation methods, osmotic dehydration is less energy intensive and preferred in minimally prepared foods (Ahmed *et al* ., 2016). Osmotic dehydration slows deterioration of fruits and vegetables by removing moisture from the food and form intermediate water products which substantially reduces the rate of physical, chemical and biological processes responsible for food spoilage (Yadav and Singh, 2014; Suresh and Durvesh, 2015; Ahmed *et al* . 2016).

Honey is one of the oldest preservatives, which has been shown to have antimicrobial, anti-browning of fresh cut fruits, and antioxidants besides other medicinal attributes (Ahmad and Kumaran, 2015; Supapvanich and Boonyaritthongchai, 2016). However, the effect of dipping pumpkin slices in honey preservatives at various concentrations is scanty in literature. Recently, Qiao *et al.* (2020) demonstrated the suppressive effect of low molecular weight filtrates from *Bacillus subtilis* on *Penicllium chrysogenum* in fresh cut pumpkins. Use of such innovative bioagents and honey may be effective for maintaining shelf life fresh cut pumpkins.

The performance of osmotic process that culminates into a final product with desired organoleptic and nutritional attributes is crucial in fresh cut pumpkin fruits. A review by Akbarian *et al.* (2014) mentioned type and concentration of osmotic agent, temperature during dehydration and agitation as crucial factors for successful osmotic dehydration. Phisut (2012) reported increase water loss and solute gain at increasing concentration of osmotic agent. However, another study by Giraldo *et al.* (2003) reported better solid gain of mango at 55˚Brix than at ˚Brix level. The conflicting findings informed the need to investigate the effect of honey concentration on keeping quality of pumpkin slices at room temperature. The current study focused on the effect of honey concentration on the sensory and microbial quality of sliced pumpkin fruits within short storage period at room temperature conditions.

MATERIALS AND METHODS

Pure organic honey was obtained from arid and semi-arid areas of Kitui, Eastern Kenya. Honey was diluted with warm distilled water at 25%, 50% and 100% (v/v) and covered by polyvinylchloride (PVC) film of 11 µm thickness. Mature pumpkin fruits were obtained from one farmer at Chepnyogaa area of Kericho County, Kenya. The fruits were selected based on uniformity in external physical characteristics such colour and size. The fruits were first washed in tap water, rinsed with distilled water and finally wiped with 50 µL/L of sodium hypochlorite. The washed fruits were blotted dry and cut into 12 similar slices lengthwise using a sharp sterile knife. The seeds and placental materials were removed. Three slices from the same fruit was dipped into different levels of honey concentration. The study adopted complete randomized design with four treatments replicated four times. The treatment consisted of four levels of honey concentrations (0, 25, 50 and 100 % w/v). The sliced fruits were weighed and dipped into respective honey concentration for five minutes. Excess honey solution was allowed to drip off before wrapping the slices using permitted food grade polyvinylchloride (PVC) film of 11 µm thickness then placed into trays. The trays were stored at room temperature that averaged 20.6 ˚C.

The effect of honey concentration on the quality on sliced pumpkin slices at room temperature, the following variables were measured within 10 days from the commencement of the study.

Weight loss

Weight loss was measured every two days for 10 days using the formula,

$$\%weight\ loss = \frac{W_1 - W_2}{W_1} x100$$

where, W1 and W2 represent initial weight and final weight, respectively at the i^{th} day. The weights were taken using digital weighing balance.

Total soluble solids and sensory tests

Total soluble solids were estimated at 10 days after slicing the pumpkin fruits. About 2 g of pumpkin flesh for each treatment was cut and homogenized after addition of 2 ml of distilled water. The mixture was vortexed for 5 minutes. About 1 ml of the solution was used to estimate total soluble solids using hand held Refractometer at 20 ±5 ˚ C. the readings were expressed as ˚Brix. A panel of twenty semi-trained panelists was used to conduct sensory evaluation of sliced pumpkin fruits under respective treatments. Variables considered in the sensory evaluation included appearance, decay, colour and overall acceptability based on 5 point hedonic scale: where 1, 2, 3, 4 and 5 denoting extremely dislike, dislike, neither like nor dislike, like, extremely like, respectively.

Microbial morphology and chemical tests

Microbial growth and chemical tests were evaluated at the end of the experiment (10 days post-slicing of pumpkin fruits) to identify the fungal and bacterial isolates. To study the bacterial growth, twelve samples according to the treatments were homogenized. The paste was serially diluted by adding 9 ml of distilled to 1 ml of the paste and adequately centrifuged. The 2^{nd} diluent was constituted by pipetting 1 ml from solution from 1^{st} diluent into clean tube and 9 ml of distilled water and centrifuged. Similarly, 1 ml of 2^{nd} solution was mixed 9 ml of distilled water and centrifuged to form 3^{rd} diluent. The diluent from the 3^{rd} dilution of each sample was cultured in nutrient agar (NA) incubated at 37 $^{\circ}$ C for nutrient for 24 hours to study fungal colony growth. Fungal species were identified using morphology and colony characteristics through microscopy upon staining with methyl blue dye and observed at × 10 magnification. For bacterial study, Sabaround dextrose agar (SDA) and streaking method was used. The plates were then incubated at 25^{o}C for SDA for 72 hours. Using microscopy, bacterial isolates were identified based on morphology, colour and chemical reactions to gram staining, catalase, citrate, indole, glucose, sucrose, mannitol and lactose.

RESULTS

Weight loss

Weight loss in fresh cut vegetables and fruits is an indicator of water loss. In the current study, percent weight loss of the sliced pumpkin fruits increased with time regardless of the treatment (Figure 1). The percent weight loss at 2 and 4 days after slicing pumpkin fruit were not significantly different. Percent weight loss at the 4^{th} and 6^{th} were not significantly different at $p < 0.05$. Weight loss pumpkin slices of at 6^{th} day after slicing was higher than that of 2^{nd} post slicing. Similarly, sliced pumpkin fruits at 6 and 8 days after slicing were not significantly different at $p < 0.05$ with respect to weight loss. The study showed that weight loss at 8^{th} and 10^{th} day was not significantly different at $p < 0.05$. However, weight loss at 10^{th} day was significantly higher ($p < 0.05$) than that of 6^{th} day.

Pure honey (100% concentration) caused the highest weight loss of sliced pumpkin fruits (Figure 2). Though not statistically significant, weight loss of sliced pumpkin fruits increased with increasing concentration of honey.

The rate of weight loss depended on the honey concentration and time after slicing. The weight of sliced pumpkin fruits for 0, 25 and 50% were similar and significantly lower than 100% honey concentration up to sixth day post slicing.

Microbial growth and chemical tests

In all the treatments, microbial contamination on sliced pumpkin fruits was detected albeit at varying counts. 100% honey concentration treated pumpkin slices exhibited significantly ($p < 0.05$) lower microbial counts than other concentrations (Table 1). The microbial count in pumpkin slices treated with honey concentrations less than 100% were not significantly different (Table 1). Microbial analysis showed that bacterial contamination was relatively higher than that of fungi (Table 1). The fungal species identified physical by physical observation under microscopy included *Mucor sp, Fusarium sp, Aspergillus sp., Rhizopus sp., Penicillium sp and Geotrichum sp.* The number of spoilage microbial species decreased with increasing concentration of honey (Table 1). *Fusarium sp* was detected in all treatments except in 50% treated pumpkin slices. *Rhizopus sp* was completely inhibited at 50 and 100% honey concentrations. It is worth noting that *Penicillium sp and Geotrichum sp* were the only fungal species present at 50% honey treated sliced pumpkins. Similarly, only *Fusarium sp* survived the 100% honey concentration.

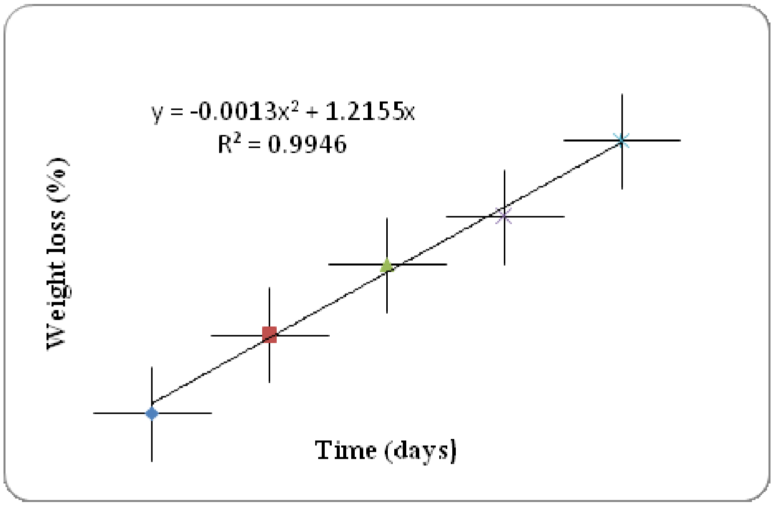

Figure 1: Effect of time on weight loss of sliced pumpkin fruit at room temperature.

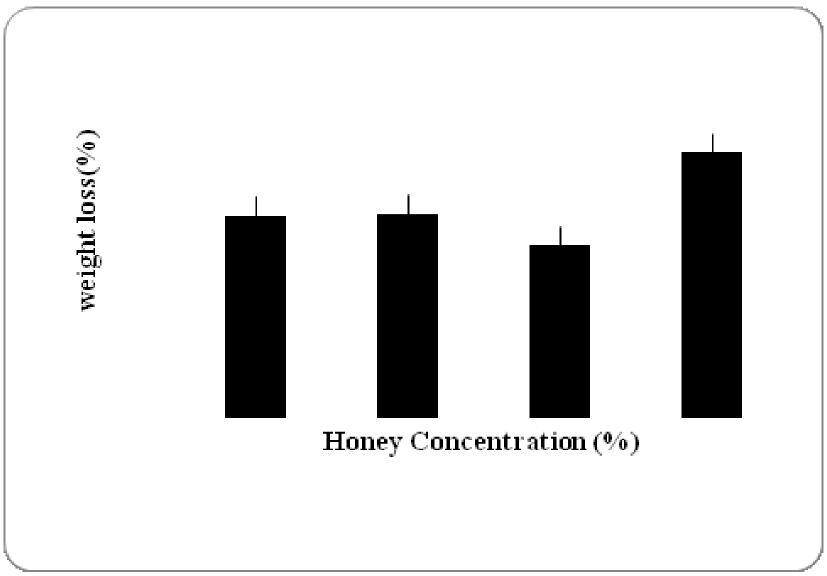

Figure 2: Effect of honey concentration on weight loss of sliced pumpkin fruits at room temperature.

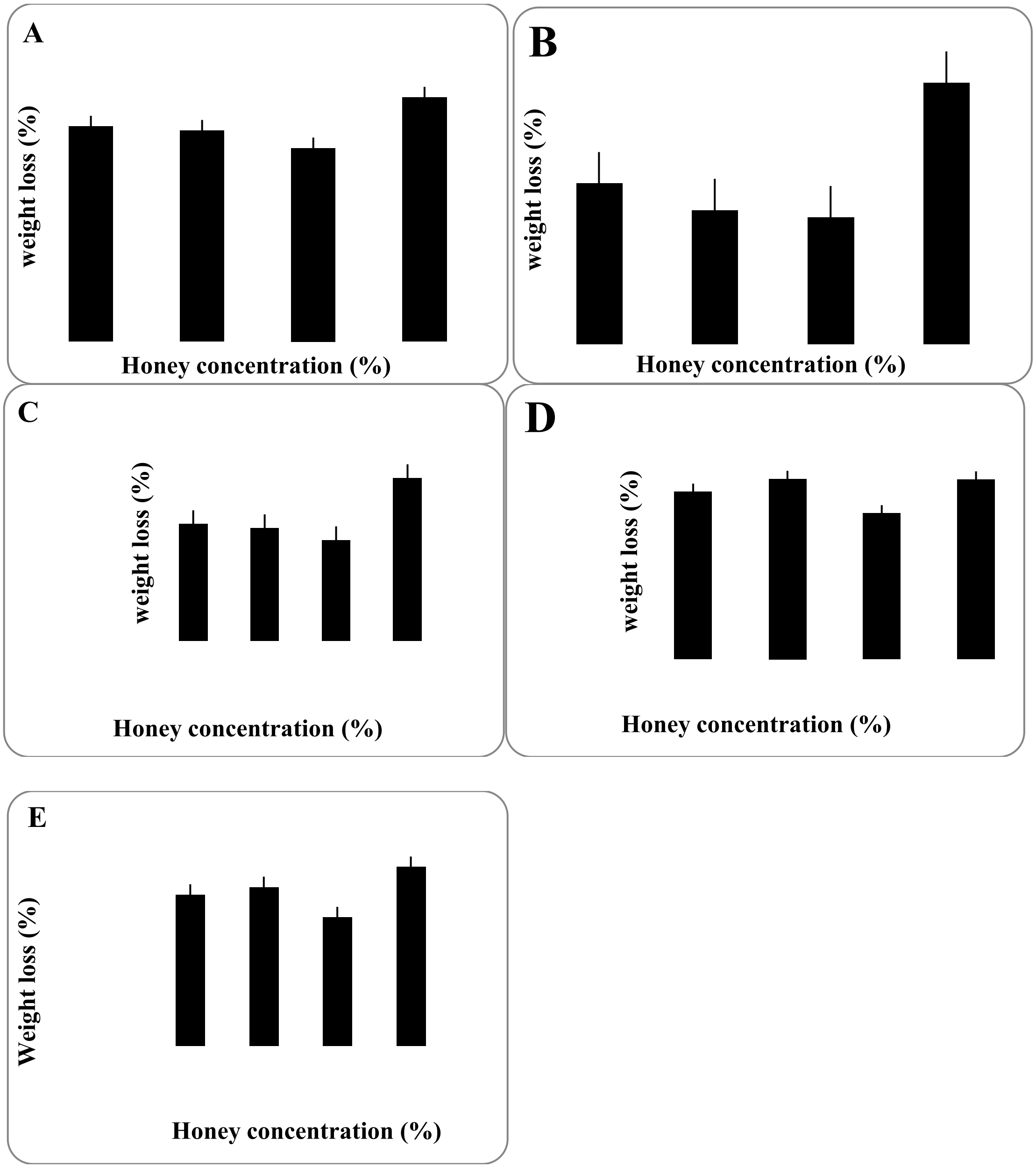

Figure 3: Effect of Honey concentration on weight loss of sliced pumpkin fruits after 2 **(A),** 4 **(B),** 6 **(C),** 8 **(D)** and 10 **(E)** days postslicing.

Total soluble solids and sensory tests

A 100% honey concentration significantly had the highest total soluble solids of sliced pumpkin fruit at room temperature (Table 1). A similar trend was recorded for overall consumer acceptability (Table 1). Based on sensory attributes, acceptability of sliced pumpkins at day 10 reduced for all the treatments. However, 100% treated honey pumpkin slices could be accepted by 80% of the panelists (Table 1). For 0, 25 and 50% honey concentration, at day 10 the panelist generally agreed that they were unfit for human consumption.

Table 2 summarizes chemical and physical features of the bacterial species present in sliced pumpkin fruits. Three gram positive bacteria species were detected. The chemical reactions and physical features indicate that the probable bacterial species could be

Bacillus sp (white colony), *Micrococcus sp* (yellow colony) and *Staphylococcus sp* (white opaque colony).

Table 1. Effect of honey concentration microbial growth, total soluble solids and consumer acceptability of fresh cut pumpkin fruits at 10 days post cutting under room temperature

Honey concentration	Bacterial colony forming units count (cfu/ml)	Fungal colony forming units (cfu/ml)	Fungal species detected	Total soluble solids (°Brix)	Acceptability (%)
0	38.45 a	2.67a	*Mucor sp* *Fusarium* sp. *Aspergillus sp* *Rhizopus*	6.51b	17.50b
25	51.33a	3.00a	*Fusarium* sp. *Aspergillus sp* *Rhizopus sp*	5.77b	0.00b
50	41.67a	2.00a	*Penicillium sp.* *Geotrichum sp.*	7.32b	2.50b
100	28.12b	0.67b	*Fusarium* sp.	8.80a	80.00a

[a]Means followed by the same letter within a column are not statistically significant at $\alpha = 0.05$

Table 2. Chemical and physical of characterization of bacterial species in sliced pumpkin at room temperature at 10 days after cutting

	Chemical characteristics								Physical characteristics
Colony colour	Gram Staining	Catalase	Citrate	Indole	Glucose	Sucrose	Mannitol	Lactose	
White	+ Rods	+	+	-	+	+	+	+	Elevation-Flat Margin-Entire Form-Circular
Yellow	+ Cocci	+	-	-	+	+	-	+	Elevation-Raised Margin-Irregular Form-Undulate
White Opaque	+ Cocci	+	+	-	+	-	+	+	Elevation-Flat Margin-Entire Form-Circular

DISCUSSION

Weight loss

Weight loss is an indication of water loss from tissue of the sliced pumpkins. In this study, the weight of water loss linearly increased with increase in storage time. The extended storage period enhanced water evaporation form the cut fruit surfaces hence the reduced weights. The higher honey concentration (100%) exerted higher osmotic pressure in pumpkin slices hence highest weight loss. Our findings are supported by a study by Pedapati and Tiwari (2014) who reported increased weight loss and better preservation of guava fruits at higher concentration of sucrose.

Microbial growth and chemical tests

In the current study, five fungal species were detected at honey concentrations.. This shows that the species could be present in the flesh, skin or seed tissues of the pumpkin fruits (Patterson and Lima, 2017). The bacteria detected were Gram positive of cocci and rod types. This is similar to the findings of Kiharason and Isutsa (2019) who observed that gram positive bacteria were dominant in sliced pumpkins. The finding could be explained by the experimental conditions (room temperature that ranged from 18-26 °C) which are known to increase the competitive ability of gram positive bacteria over gram negatives groups (Manell and Ackland, 1986). The antimicrobial activities of honey in this study agree with findings by Goślińsk *et al* . (2020) that demonstrated that manuka honey was effective against both gram positive and gram negative bacteria.

The microbial load of the fungi and bacteria decreased with increasing concentration of honey. Studies by Mancuso *et al.* (2019), showed the antimicrobial activity Manuka honey increased with increasing concentration. Antimicrobial capacity of honey is influenced by osmolarity, acidity and hydrogen peroxide content which are decreased by reduction in concentration (Jenkins, Roberts and Brown, 2015). Our study shows that the source of honey and time of harvesting may be critical while using honey as a preservative in cut pumpkin fruits. Honey harvested in wet areas or during rainy season have relatively higher water activity. Our finding is supported by a comprehensive review by Miguel *et al*. (2017) and references therein, reported that the antimicrobial property of honey is determined by season, source of honey and handling of honey during storage. This might jeopardize the antimicrobial activity of the honey. Further, diluting honey at room temperature especially at retail outlets without temperature controls poses health risk due to microbial growth. Our findings are supported by Santagata *et al*. (2018) who demonstrated the bioactivity of pectin-honey coating against microorganisms. A study by Ankley *et al*. (2020) demonstrated that iron chelating by honey induces iron-limiting environment that differentially influence distribution of bacteria. The iron chelating capacity of honey is expected to increase at high concentrations (Ankley *et al*., 2020). The low bacterial and fungal count at highest honey concentration confirms that antimicrobial property of honey depends on water activity and other mechanisms that require further investigation. Whereas our study showed increasing microbial counts with time (data not presented), Nguyen *et al*. (2019) reported decreasing microbial counts especially fungi in vinegar treated fresh cut pumpkin fruits. The variation in results may be attributed to higher potency of vinegar on the microorganisms coupled with freezing storage conditions used in their study.

This study failed to show statistically different TSS values at honey concentrations up to 50%. This agrees to findings by (Ergun and Ergun, 2009) who reported no difference in total soluble solids pomegranate fruits treated with 50% honey. This is contrary to study by

Ahmad and Kumaran (2015) who reported higher TSS at 0% than 15% honey concentration in aonla preserve. In the current study, sliced pumpkin fruits were dipped in honey while a study by Ahmad and Kumaran (2015) the aonla preserve honey was incorporated hence the differences in the findings. Wen *et al.* (2018) reported that honey dips maintained TSS of fresh cut nectarine. The decreasing TSS in our study could be attributed to breakdown of sugars by microorganisms, which were relatively minimal in 100 % treated pumpkin slices hence sustained soluble sugar contents.

Overall, acceptability of the sliced pumpkins fruit depended on the concentration of honey. The panelist employed in the current study considered appearance, aroma, visual colour to rate the quality of pumpkin slices. The study revealed that the addition of water reduced sensory attributes of honey slices by increasing activity of spoilage microorganisms. Previously, Lee and Lim (2011) reported higher acceptability of pumpkin slices dipped in higher sucrose concentrations. It is interesting to note that distilled water performed better in terms of sensory attributes of sliced pumpkin fruits. The effect of low sugars in 25 and 50% honey concentrations provided a suitable substrate fro the decaying bacteria and fungi. According to Dutocosky (2011), acceptability index of ≥ 70% is the minimum for consumer acceptance. Thus, in this study, only 100% honey met the criterion. However, the length of time in a given solution, storage temperatures and packaging are critical in sensory evaluation (Lee and Lim, 2011).

Conclusion

High microbial count coupled with low sensory acceptability was the main factors reducing quality and shelf life of sliced pumpkin fruits. Pure honey prolongs shelf life of fresh cut pumpkin fruits wrapped in polyfilm at room temperature by lowering the growth of spoilage microorganisms. The reduced microbial load to the pumpkin slices is sustained by high total soluble solids. Addition of water reduces antimicrobial activity of honey. This study provides a pointer that honey for preservation of fresh cut fruits and vegetables should be sourced from dry areas or harvested during dry periods.

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