RESEARCH ARTICLE

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Substituting Natural Honey for Cane Sugar (Sucrose) Retards Microbial Growth Independent of Water Activity During Storage of Tomato Jam

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ABSTRACT

Today, there are several attempts to make nutritious food products low in glycemic carbohydrates as this can partly help in solving the ever-growing numbers of type 2 diabetes and obesity. We thus made two types of jam, one using conventional sucrose with a high glycemic index and the other using honey which is regarded as a low glycemic index carbohydrate. Honey jam had comparable physical properties with sucrose jam. The total soluble solid (Brix) was significantly lower in honey jam than in sucrose jam (p=0.04) indicating less sugar in honey jam. Both jams had comparatively higher percent moisture content than other jams reported in literature potentially suggesting reduced storage stability. Despite having higher water activity, honey jam had progressive decrease in microbial colony counts suggesting honey was inhibitory to growth of microorganism compared to sucrose jam. This effect was independent of water activity as sucrose jam which had lower water activity promoted growth of microorganism during storage. Therefore, substituting natural honey for sucrose during formulation of tomato jam retards microbial growth thereby prolonging shelf life.

Keywords: honey jam, sucrose, titratable acidity, total soluble solids.

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I. Introduction

Fruits and vegetables are rich sources of vitamins, minerals and phytochemicals and are promoted as one of the dietary approaches to fighting chronic diseases [1], [2]. The low consumption of fruits and vegetables below the recommended 400/g is partly due to their seasonality, high cost, uneven distribution, and diminished production [3]. In regard to seasonality, low shelf life has been attributed to high water content which facilitates rapid growth of micro-organisms, is a common reason for rapid deterioration of fruits and vegetables. Possible ways to overcome this obstacle are through making jams, modified atmosphere packaging, drying and fermentation which can extend their shelf life [1], [4], [5].

One crop commonly used to make jams is the tomato fruit. Tomato plants belong to the *Solanaceae* family and is a rich in lycopene, anthocyanin, carotenoids, vitamins such as vitamin C and E [6], [7]. The several bioactive compounds present in tomatoes are lycopene, ascorbic acid, β-carotene and other phytochemicals have been linked to play a role in the protective effects of tomatoes against chronic diseases [7]. In a placebo-controlled study, Engelhard *et al.* [8] demonstrated that tomato extracts with antioxidant potential reduced blood pressure in hypertensive patients. They also reported a reduction in lipid peroxidation products, especially thio barbituric acid. Further in a meta-analysis and systematic review, Cheng *et al.* [9] demonstrated that lycopene and tomato supplementation lead to a reduction in low density lipoprotein cholesterol, interleukin-6 (IL-6) while lycopene in particular significantly lowered systolic blood pressure. In mice exhibiting type 2 diabetes, a 10-week lycopene intake led to decrease in fasting blood glucose as well as markers oxidative stress such as tumor necrosis factor-α (TNF-α), malondialdehyde, oxidized low density lipoprotein and glycosylated hemoglobin [10]. In a double-blind study, the effect of breakfast diets containing approximately 450 Kcal of honey or sucrose on appetite stimulating hormones was investigated [11]. They found out that consumption of meals containing honey compared to sucrose ones slowed ghrelin response, reduced glucose sparks, and improved overall amounts of peptide YY (PYY). Taken together, these data suggest that intake of tomatoes or products low in calories can positively impact health.

Sucrose has widely been used to make jams and there have been attempts to replace sucrose with other sugars or sweeteners such sativoside to reduce sugar content and make the jam suitable for special groups for people with diabetics [12]. These authors observed a reduction in jam firmness, brix, yield stress, consistency index but with an increase in flow behavior and water activity. They also observed that at a lower sugar content syneresis was paramount due to less water being unbound. In another study, replacement of sucrose with sativoside and sucralose during manufacture of mango jam reduced yield stress and consistency index because of reduced amounts of total soluble solids but with an increase in flow patterns of the jams [13]. These studies show that substitution of sucrose, the commonly used sugar in jam manufacture with alternatives can have an impact on the characteristics of jam. Basu *et al*. [14] also reported that replacement of sucrose with sorbitol decreased jam gel strength due to weaker junction points within the pectin gel. We thus investigated the effects of substituting sucrose with natural honey of the quality attributes of the jam with an aim of promoting honey in jam processing.

II. Materials And Methods

A. Raw Materials

Mature, well ripened tomatoes free from molds and bruises were bought, sorted and used for jam making. First, the tomatoes were cleaned using clean running water to remove dirt and then peeled to remove the skins by immersing the tomatoes in warm water in order to soften it, and then hand peeling was eventually done [15], [16]. Finally, the tomatoes were cut into small pieces using a sharp knife and then blended into a pulp before boiling.

B. Preparation of Jam and Storage

Sucrose jam was prepared by weighing 1 kg of the pulp, which was boiled while adding 1250 g of sugar and stirring the mixture as previously reported [17]. Lemon juice was slowly added till optimum pH of 3.0–3.3 was reached to enhance gelation. The heating continued until the pulp formed a gel. The set point of the jam was checked using a drop test where jam was scooped with spoon and dropped into clear cold water. When the jam did not disperse, within a minute it was assumed the jam was ready. This process occurred concurrently with heating. Then the jam was removed from the heating pan and cooled. The same procedure was used for honey jam where 625 grams of honey was used instead of sugar, as per the criteria for substituting sucrose with honey [18]. Finally, the jam was packed in 250 ml bottles for further analysis and storage was done at ambient temperature (25–27°C) for 30 days. There were 12 bottles per treatment and three bottles were opened at each testing interval. The other jams were used to determine total solids, moisture, ash, titratable acidity, pH, water activity, yeast and molds as explained in subsequent sections. These determinations were done in triplicates every week for three weeks.

C. Determination of Total Soluble Solids (Brix)

Total soluble solids were determined using a digital refractometer (Bellingham Stanley RFM340M, UK) as described by Bekele *et al*. [19]. 1 ml of each tomato jam was placed on the prism of the refractometer after calibration and wiping the prism with soft tissue to clean it. Readings were displayed on the brix meter and the readings were determined in triplicates.

D. Determination of Dry Matter and Ash Content

The dry matter content of the tomato jam samples was determined in triplicate by drying in an oven at 105°C for 24 h Sulejmani *et al*. [20]. The samples were cooled in desiccator while properly covered and the samples were weighed as soon as they reached the room temperature. The percentage of dry matter was calculated using the following formula:

$$Percentage\ dry\ matter = \frac{(A-B)*100\%}{A} \tag{1}$$

Where:
A – mass of the sample,
B – loss of mass after drying.

Ash content was determined by weighing 10 g of the sample into crucibles in triplicate as described by Sulejmani *et al*. [20]. Then the crucible was placed in a muffle furnace set between 550ºC to 600ºC for 5–6 hours. The ash content was calculated using the following formula:

$$Percentage\ ash = \frac{\text{weight after ashing} - \text{tare weight of crucible original}}{\text{sample weight} \times \text{dry matter coefficient}} \tag{2}$$

where:
dry matter coefficient – % solids/100.

E. Determination of Titratable Acidity and Ph

Titratable acidity is the total amount of acid in the solution determined by titration using a standard solution of sodium hydroxide as titrant as described by Sulejmani *et al*. [20] with modifications where citric acid milliequivalent factor of 0.064, the dominant acid in tomatoes was used. Six grams (6 g) of each sample was placed in 100 ml beaker and to each sample 50 ml of water was added. Titration was done using 0.1N NaOH to an end point of pH 8.2 which was determined by a pH meter and millilitres of NaOH used were recorded. The percentage of titratable acidity was calculated using the following formula:

$$Percentage\ acidity = \frac{(\text{mls of NaOH used}) \times (0.1\text{N NaOH}) \times (\text{milliequivalent factor}) \times (100)}{\text{grams of sample}} \tag{3}$$

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where:
milliequivalent factor – 0.064 for citric acid (a dominant acid in tomatoes)

The pH was determined by diluting 6 g of the jam with of water and measurements taken using a pH meter.

F. Determination of Yeast and Molds

Nutrient Agar (NA) and Yeast Extract Algae (YEA) media were used to determine CFU/ml of yeasts and molds using total plate count. The dilutions for NA were 14 g of NA was diluted in 500 ml of water while 12 g of YEA was diluted in 500 ml of water. Nine milliliters (9 ml) of water were measured into 20 different test tubes (10 test tubes for each sample). One milliliter (1 ml) of each sample was pipetted into a test tube labeled 1 and 2 representing the sucrose jam and honey jam, respectively. The mixtures were shaken using a spin mix. Another 1 ml was removed from the test tube containing the mixture and added to the following test tubes until the 10th test tube was reached. Then 40 microliters of the mixture in the final test tube were pipetted into different petri-dishes containing either NA or YEA and then incubated for 2 days. The colony count was done using a colony counter.

G. Determination of Water Activity During Storage

Water activity for each sample was determined using data loggers (Lascar Electronics, Inc, PA, US) which were placed inside the packaging bottle during storage at an ambient temperature. These bottles were relatively bigger in size than those used for packing samples for storage. This was done to accommodate data loggers inside the bottles. The data logger was left inside the packaging bottle for three days in order to allow the humidity to equilibrate. During that period the data loggers were recording relative humidity every 6 hours for 3 days. The data was downloaded using Easylog USB software (Lascar Electronics, Inc, PA, US) for analysis.

H. Data Analysis

The data was analysed using a t-test in SPSS 25 for windows and separation of the mean values was carried out at (α=0.05) to determine whether significant differences in pH, water activity and preference scores in sensory attributes.

III. Results and Discussions

A. Physicochemical Properties

The physicochemical properties of the two jams are presented in Table I. The pH, ash content and moisture were significantly different between sucrose jam and honey jam. Titratable acidity was higher in honey jam possibly due to organic acids inherent in honey. The acids of honey account for approximately 0.57 % of the solids and this level contributes not only to the flavor, but also to stability of honey against microorganisms [21], [22]. The major acid found in honey is gluconic acid which arises from glucose oxidation by glucose oxidase [21]. Other acids in honey are formic, acetic, butyric, lactic, oxalic, succinic, tartaric, maleic, pyruvic, pyroglutamic, a-ketoglutaric, glycolic, citric, malic [22]. The Brix value was higher in sucrose jam than in honey jam signifying less sugar in the honey jam. Sugars are the main constituents of honey, comprising about 95 % of honey's dry weight. Natural honey contains approximately 38.2% fructose, 31% glucose, 9% disaccharides (sucrose, maltose, isomaltose, maltose, turanose and kojibiose) and 4.2% of oligosaccharides [23].

TABLE I: Physicochemical properties of honey and sucrose jam

Physicochemical properties	Mean±SD (Honey)	Mean±SD (Sucrose)	P-value
TA (in citric acid)	1.79±0.41	1.01±0.22	0.000
Total Soluble Solids (Brix)	60.03±4.37	66.72±1.28	0.004
pH	3.75±0.41	3.47±0.11	0.147
Moisture content (%)	40.16±2.24	42.95±1.87	0.237
Water activity	0.88±0.01	0.83±0.01	0.000
Ash content (%)	0.27±0.01	0.28±0.01	0.066
Total solids (%)	59.84±0.71	57.05±0.14	0.000

Tomato is a rich source of natural bioactive compounds. Consumption of tomato and tomato products has been linked with decreased risk of various chronic diseases, such as cardiovascular and cancer diseases as tomatoes contain lycopene which may be responsible for such effects [24]. Similarly, in another study [25] the authors showed that tomatoes contain polyphenols, vitamins and minerals that contribute to beneficial effects of tomato consumption. It has been reported that the nutritional value of tomatoes varies with the stage of maturity, with the highest concentration of bioactive compounds present in fully ripe tomatoes [26], [27].

B. Water Activity

The relative humidity (% rh) of the storage room was 57.1%. The results showed that there was a significant difference in terms of water activity (a_w) between the two jams. The honey jam had a water activity of 0.885 whilst the sucrose jam had a water activity of 0.834 (p=0.000003). This difference in water activity might be due to the difference in sugar content and composition as honey jam had lower brix value than sucrose jam (Table I). As honey contains many sugars as mentioned earlier, these sugars have a profound effect on the water activity of honey. Generally, honey has a water activity (aw) of 0.5–0.65 and glucose crystallization can lower soluble solids leading amorphous solution dilution and hence an increase in water activity [22]. Our data support this notion as change in moisture content (moisture lost as a percentage of initial moisture) was higher in honey jam than sucrose jam for 3 weeks storage (Fig. 1) indicating honey absorbed more water. It has been reported that honey has hygroscopic properties perhaps because 95–99% of solids in honey are sugars [28]. The water activities of most jam reported in literature are not reported (Table II). Other studies have reported water activity of most honey ranges between 0.56 and 0.62, pH 3-4, total soluble solid of 65-68% and 40-45% fruit pulp [29], [15]; Table II. These differences are attributed to differences in formulation as well as differences in evaporation of water as some jams retain more water to retain the spread ability (Table II). Similarly, in this study we observed that further evaporation of water resulted in honey becoming more viscous and eventually solidified. It also made honey darker possibly due to non-enzymatic browning and caramelization occurring in the jam during heating. Despite these differences both jams had better properties than other jams reported in literature (Table II). However, higher moisture content of jams in this study might reduce shelf stability.

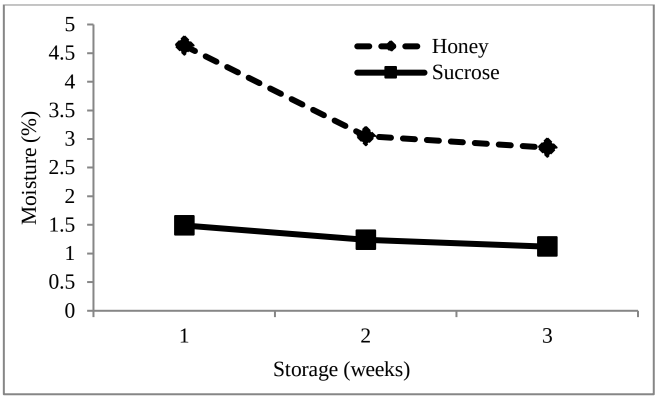

Fig. 1. Difference in loss of moisture relative to initial moisture of honey and sucrose jams stored at ambient temperature (26-27ºC) for 3 weeks.

C. *Ash Content and Total Soluble Solids*

The ash content of different jams is presented in Table II. There was no significant difference in ash content between honey jam (0.27%) and sucrose jam (0.28%) (p=0.066). The total ash content is higher than those reported by Naeem *et al.* [30] on grape (0.18%) and blueberry (0.12%) jams. Ash content represents the quantity of minerals such as calcium, phosphorus, and iron in the jam. This data suggests that honey jam may provide a similar total mineral content as the jam made using sucrose.

The total soluble solid is a measure of the amount of material that is soluble in water, and it is expressed as a percentage. This simply means a product with 100% soluble solids has no water and the one with 0% soluble solids is all water. The total soluble solids of honey jam were significantly lower (60.03%) compared to sucrose honey (66.72%) (p=0.004) as shown in Table I. The total solids in jam are largely accounted for by sugar content. Therefore, the difference in total solids between honey and sucrose jams is mainly due to the difference in moisture content and sugar concentration.

During jam preparation the mix is heated to reduce the water content of the mixture and concentrate the fruit and sugar so that final total soluble solids content of a jam should be between 65 to 68% which is considered end point of jam [31]. However, because of difficulties in heating a more viscous jam, we were unable to achieve 65-68% total solids for the honey jam. Obviously, this might have affected the shelf stability of our product as jams with lower than 65–68% total soluble solids tend to have shorter shelf life because of high water activity which can tolerate the growth of bacteria and molds [31]. However, at 60% and 66% for honey and sucrose jam, respectively, we were able to get jam with good viscosity and consistency therefore further evaporation of water through heating was abandoned.

TABLE II: COMPARISON OF QUALITY PARAMETERS OF DIFFERENT FRUITS JAM WITH JAM MADE FOR THIS STUDY

Sample name	Sweetener	TSS (Brix)	TA	Moisture	Dry matter (%)	pH	Water Activity	Ash	Reference
Our sample	Sucrose	66.72	1.01	40.16	57.05	3.47	0.83	0.28	
	Honey	60.03	1.79	42.95	59.84	3.75	0.88	0.27	
Tomato jam		68.9–73			51–57	3.1–3.3	0.7–0.8	ND	(32)
Aronia jam	Sucrose	ND	0.94	28.4	ND	5.2	ND	ND	(33)
Pineapple peel jam	Sucrose	77	0.25	62.6	37.4	4.91	ND	ND	(34)
Musk melon jam	Sucrose	68	0.33	30.55	69.45	5.45	ND	ND	(35)
Black-pum jam	Sucrose	68	0.34	21.65	78.36	3.42	ND	4.2	(36)
Mandarin	Sucrose	70	0.98	29.62	70.38	2.87	ND	0.07	(37)
Pineapple jam	Sucrose	64	1.38	21.64	78.36	3.95	ND		(38)
Different fruits jams	Sucrose	ND	ND	31.32–33.36	66.7–68.68		ND		(30)
Common standard	Sucrose	65–68				2.5-3.45			(31)

ND not determined, TSS, total soluble solids.

D. *Comparative changes of physicochemical parameters during storage of honey*

1) *Brix value*

After 14 days storage, Brix dropped significantly (p-value<0.05) in honey jam compared to sucrose jam (Fig. 2) suggesting that sucrose degraded or fermented by microorganisms. The rapid drop in Brix value in honey could suggest that either honey jam was more favourable to the growth of fermenting microorganisms or concentration effect as honey absorbed more water from the environment thereby diluting the concentration of sugar (Fig. 1) than sucrose jam. However, the former is not supported by data as microbial growth was significantly inhibited by honey (Fig. 4). Since jam is high acidic-low water activity food it's more likely that the fermenting organisms were yeasts and moulds.

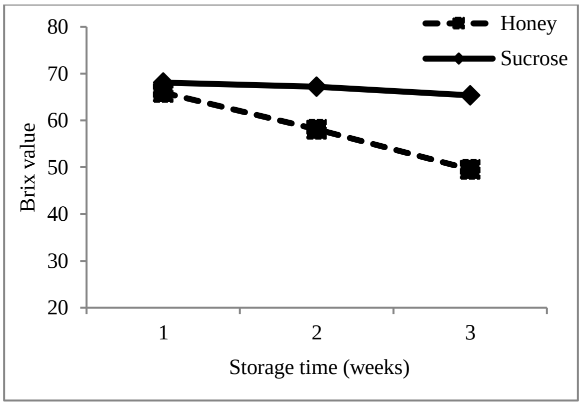

Fig. 2. Changes in Brix values in sucrose and honey jam stored at ambient temperature (26–27ºC) for 14 days.

2) *Titratable acidity*

The change in titratable acidity for sucrose jam was significantly lower (p=0.001) than honey jam (Fig. 3). The titratable acidity of honey jam was 1.47%, 1.57% and 2.05% at 1st, 2nd, and 3rd week while that of sucrose jam was 0.83%, 0.89% and 1.24% at 1st, 2nd and 3rd week (Fig. 3). Increase in titratable acidity during storage of jam has been previously reported and was attributed to degradation of ascorbic acid, hydrolysis of pectin, degradation of polysaccharides and oxidation of reducing sugar resulting in rise in concentration of weakly ionized acids and their salts [39]. The rise in titratable acidity corresponds with an increase in pH. These findings were higher than those found by [40], who reported a total titratable acidity of 0.82% in apricot jam and higher than those reported by [41] on apple, pineapple, peach and a mixed fruit jam. These differences might be due to differences in contents of acids in these fruits.

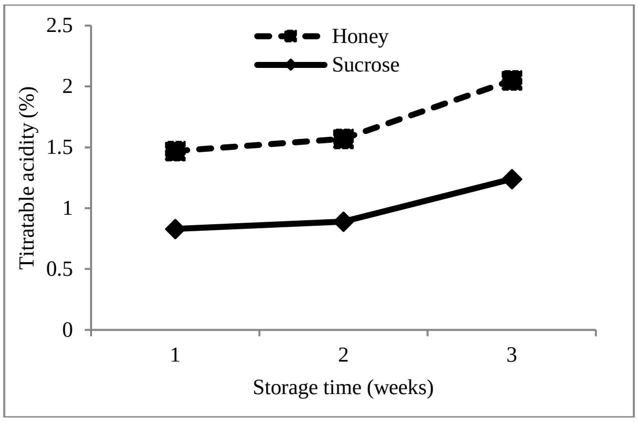

Fig. 3. Change in titratable acidity in sucrose and honey jams stored at ambient temperature (26-27ºC) for 14 days.

3) *Loss of moisture during storage*

The loss in moisture was higher in honey jam than sucrose jam throughout storage period consistent with the fact that honey is more hygroscopic and therefore adsorbed more water (Fig. 1). The loss in moisture for honey and sucrose on 1st, 2nd and 3rd week was 4.63%, 3.05%, 2.35% and 1.49%, 1.24%, 1.12%, respectively. The decrease in moisture content resulting from loss of moisture might be due to differences in humidity between the environment and the packaging. The relative humidity in storage room (57.1%) was lower than in the packaging bottles (88% for honey jam and 83% for sucrose jam). This created a steep differential gradient and moisture might have diffused out of the bottle into the environment.

4) *Microbial growth*

Since jam is a high acidic food, we tested how substituting sucrose for honey would affect growth of yeasts and molds and some acid tolerant bacteria [42]. Yeast extract agar is suitable for the enumeration of a wide spectrum of bacteria, yeasts, and molds while nutrient agar, while it supports other microorganisms, is most suited for bacterial and mold growth. Within the first week of storage honey jam had higher microbial load than sucrose jam (Fig. 4). After one week of storage, sucrose honey supported the growth of more microorganisms unlike honey jam which was inhibitory to microbial growth in both media (Fig.5). Since the acidity between honey and sucrose jams was not significantly different (p=0.147) other factors might explain the observed difference. Moreover, honey jam had higher water activity (0.88 vs. 0.83) than sucrose jam; we therefore expected higher microbial growth in honey jam based on the parameter. Nevertheless, honey contains a number of phytochemicals depending on the botanical source with polyphenols being the most dominant phytochemicals [43], [44]. These phytochemicals are known to inhibit microbial growth [34], [44], [45] and may, in part, explain low bacterial count in honey jam during storage. Most importantly, these phytochemicals are responsible for various health benefits of honey [23], [43]. Though honey contains yeast, molds and bacteria, most species do not grow because of concentrated sugar, acidity that lowers water activity and pH, respectively, thereby allowing only a few that can survive these conditions [42].

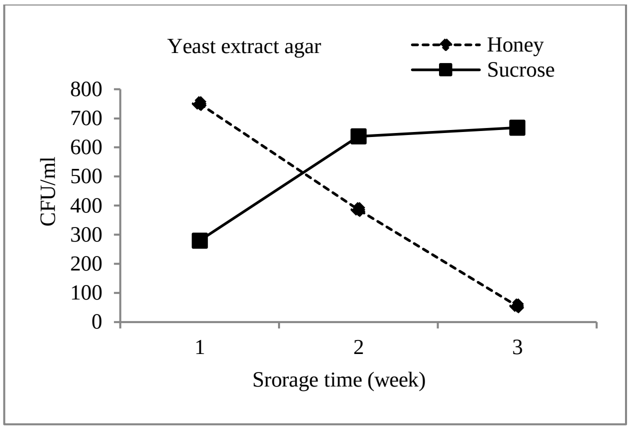

Fig. 4. Microbial growth in honey and sucrose jam on yeast extract agar after storage at ambient temperature for 3 weeks.

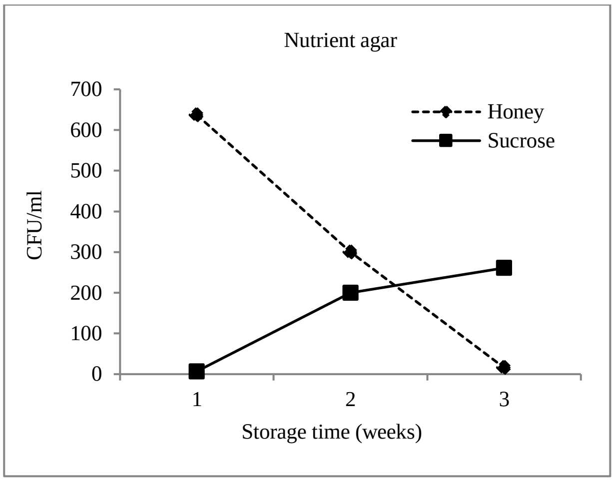

Fig. 5. Microbial growth in honey and sucrose jam on nutrient agar after storage at ambient temperature for 3 weeks.

IV. Conclusion

Replacing sugar with honey produced tomato jam with comparable physicochemical properties. Honey inhibited microbial growth independent of water activity and moisture. However, both sucrose and honey jam had higher moisture content than the general moisture content range reported in literature which might reduce the shelf stability of the jam. Unfortunately, further heating hardened and browned both jams leading to abandonment of heating and consequently producing jams with higher moisture content. Therefore, it is imperative to maintain acceptable standards of honey jam in terms of moisture level, titratable acidity, brix and pH for improved acceptability and shelf stability.

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CONFLICT OF INTEREST

The authors do not declare any conflict of interest.

ETHICAL REVIEW

This study does not involve any human or animal testing.

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