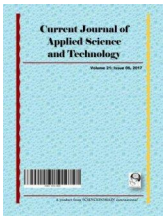

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Effects of Honey on Total Phenolic Content and Antioxidant Activities of Pineapple (*Ananas comosus*) Drink Before and During Storage

T. K. Adebayo[1*], I. A. Abdulraheem[1], A. S. Daramola[1] and T. C. Israel[1]

[1]Department of Food Technology, Federal Polytechnic, Offa, Kwara State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author TKA designed the study. Author IAA carried out the experiment and the statistical analysis, while author ASD wrote the first draft of the manuscript. Authors TKA and TCI corrected the manuscript and confirmed the literatures. All the authors read and approved the final manuscript.

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ABSTRACT

This study determined the effects of honey on total phenolic content and antioxidant activities of pineapple (*Ananas comosus*) drink before and during storage. Packaged honey was added to pineapple drink at ratios, 0, 5, 10, 15 and 20%. Pineapple without honey was used as a control. The pH, total titratable acidity, total soluble solids and vitamin C content of the samples were determined. Total phenolic content and the antioxidant activities of the formulated samples were also determined. Sensory evaluation was carried out with semi-trained panellist. The samples were stored under two different conditions (room temperature (29°C) and refrigeration (4°C)). The antioxidant activities of the stored pineapple drink were determined at an interval of five days for the total of 30 days. The results showed an increase in pH, total titratable acidity and vitamin C content of all the samples as the amount of the honey added increased. The total phenolic content and the antioxidant activity of the samples also increased as the quantity of honey added increases. There

**Corresponding author: E-mail: tesleemkayodeadebayo@gmail.com;*

was a positive correlation (0.98) between the total phenolic content of the samples and their antioxidant activities. Samples containing honey were scored high in all attributes (Colour, Taste, Aroma and overall acceptability) than the control sample. The antioxidant activities of the pineapple drink stored at room temperature increased with the increase in storage time, while the antioxidant activities of pineapple drink stored at refrigeration temperature remain constant. In conclusion, the addition of honey to pineapple drink helped to improve the health benefit of the drink by increase the scavenging power of the drink when compare with pineapple drink without honey.

Keywords: Pineapple drink; honey; antioxidant; storage; total phenolic content.

1. INTRODUCTION

Pineapple (*Ananas comosus var Smooth cayennes*) is one of the common fruits in numerous tropical and subtropical nations. Its natural product can be handled into a few items, for example, canned pineapple cut, pineapple mash, dried pineapple, and sanitised pineapple juice [1]. Pineapple juice is a common juice because of its lovely fragrance, enhanceandvarious practical properties. Pineapple juice contains minerals, particularly manganese, amino acids, sugars, vitamins, and polyphenols [2]. It is considered as a practical juice because of its wellbeing advancing properties, anti-atherosclerotic, and anti-ageing properties [3]. Nutritional composition of pineapple juice differs with geology, culture; harvesting season, and handling time. Just a couple of studies have detailed the general structure of pineapple juice, for example, minerals, sugars, natural acids, and amino acids, and in addition physical properties, for example, pH, Brix, powder, and titratableacidity [4].

The pineapple drink satisfies the “5 A Day” dietary requirement of fruits and vegetables set by many health agencies [5]. Most pineapple drink is sweetened with sugar to enhance the taste since the drink is diluted with water to maximise profit. Consumption of sugar has been attributed to so many diet-related noncommunicable diseases such as diabetes [6]. There are other sweeteners like honey with health benefit that can be used in place of sugar.

Honey has an intricate structure comprising of a high convergence of sugars, water, minerals, proteins, vitamins, natural acids, flavonoids, phenolic acids, and chemicals. These parts characterise both the physical properties and the dietary and nutraceutical attributes of the item itself [7]. Honey phenolic mixes are results of optional plant digestion, and their substance differs as per the species, assortment, organ considered, physiological stage, pedo-climatic attributes [8]. The cell reinforcement limit of the phenolic mixes is ascribed to their concoction structure. They are described by a fragrant ring with at least one hydroxyl substituent and, in numerous cases, serve in plant guard components to balance responsive oxygen species [9].

There is little research on the addition of honey as a sweetener in fruit drink to the best of our knowledge. With the presence of the phenolic compound in honey, it may serve a good purpose when added to pineapple drink as a sweetener in improving the health benefit. Hence, this study aims to determine the effect of honey on total phenolic content and antioxidant activities of pineapple drink before and during storage.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Pineapple Drink Preparation

Mature, fresh pineapple (*A. comosus var Cayennes*) was bought from the main marketin Ile-Ife, Nigeria. Fresh, mature and ripe pineapple fruits were washed, peeled and cut into smaller bits manually after which they have blended using control blender Breville BBl605XL Hemisphere (Breville, China) and sieved through a muslin cloth to have relatively fine pineapple juice before dilution with 50% water. Pure honey aseptically sealed in a bottle was purchased in a supermarket in Ile-Ife, Nigeria.

2.1.2 Samples Preparation

Different amount of honey (0, 5, 10, 15, & 20 mL) was added to about 250 ml of freshly prepared pineapple drink and homogenised. The 250 mL of pineapple drink used for this study was based on our preliminary study carried out in our

departmental laboratory. The homogenised pineapple drinks with and without honey were filled inside sterile bottles previously washed and dried. The samples were pasteurised using hot water at 90°C for 15 s and cooled under running water.

2.2 Methods

2.2.1 Determination of pH, total soluble solids (^{o}Brix), and total titrateable acidity (TTA)

pH meter (Hanna Instrument, Poroa de Varzim, Portugal) previously calibrated with buffer solutions (4 and 7) was used to determine the pH of the samples. Total soluble solids were determined in ^{o}Brix using a handheld refractometer (Hanna Instruments, Italy) previously adjusted to zero with distilled water. The prism of the refractometer was cleaned with distilled water after each analysis. The titratable acidity of the samples was determined according to the method of AOAC [10]. 5 mL of pineapple drink was diluted in 25 mL of distilled water and titrated by 0.1N sodium hydroxide (NaOH) to pH 8.1. The titrateable acidity of the pineapple drink was expressed as percentage citric acid.

$$TTA = (V \times 0.1 \times 100 \times 0.064)/ m$$

Where V is the volume of NaOH used, 0.064 is the conversion factor for citric acid, 0.1 is the normality of NaOH and m is the amount of drink used.

2.2.2 Ascorbic acid content (AA)

AOAC [10] method was used in determining the ascorbic acid content. About 20 ml of pineapple drink (with and without honey) samples were made up to 50 ml with oxalic acid (0.1 M) (metal chelator) and filtered. The filtrate (5 ml) was dispensed into a beaker with a pipette and titrated with standardised 2,6-dichlorophenol indophenol dye. The coloured solution changed from orange to pink to mark the end point of the titration. The procedure was repeated three times. The titer values were converted to mg of AA/L of fruit drink samples.

2.2.3 Determination of total phenolic content

Total phenolic contents were determined using the Folin-Ciocalteu modified method of Tezcanet al. [11]. 300 ml of diluted pineapple drink samples with and without honey (1 ml of samples: 100 ml of water) with methanol: water (6:4) was mixed with 1.5 ml of 5-fold-diluted Folin–Ciocalteu reagent and 1.2 ml of 7.5% of sodium carbonate. The mixture was allowed to stand for 60 min at room temperature before the absorbance was measured (Hitachi, Model 100-20 spectrophotometer) at 760 nm. Total phenols were expressed as mg Garlic acid equivalents (mg GAE/mL).

2.2.4 Determination of total antioxidant activity

The $ABTS^{+}$ radical scavenging activity of the pineapple drink (with and without honey) samples were measured using the procedure described by Awika et al. [12]. ABTS stock solution (2 ml of ABST stock solution (0.01 M) was added to 58 ml of phosphate buffer saline pH 6.9) was incubated for 12 h and the reaction mixture was incubated for 30 min. The absorbance was read at 734 nm. Trolox served as the standard and results were expressed as micromole Trolox equivalents per gram sample (µmol TE/100 mL)

2.2.5 Sensory evaluation

A 15-member sensory panel randomly chosen among students based on consumption of various commercially sold drinks. They were trained with different blank labelled fruit drinks with a mixture of various commercially sold drinks to hedonically evaluate the sensory characteristics of the drinks before introducing pineapple drink with and without honey to them. All samples were randomlylabeledalphabetically, and the panellist evaluated each sample for taste, colour, aroma, and overall acceptability on a 9-point hedonic scale from one for dislike extremely to nine for like extremely. The sensory parameters were rated to the popular Five Alive Just Drink.

2.2.6 Storage study pineapple drinks with and without honey

The bottled pineapple drink (with and without honey) were immediately stored under two conditions: refrigerated (4°C) and room temperature (29°C). The samples were evaluated for antioxidant properties (using ABTS radical scavenging) for a 30-day period five day's interval.

2.2.7 Statistical analysis

All analyses were performed in triplicates. Data were analyzed using IBM SPSS 20.0. ANOVA

(One-way) was performed to determine significant differences between the means, and the means were separated using the least significant difference (LSD).

3. RESULTS AND DISCUSSIONS

3.1 Chemical Characteristic of Pineapple Drink Samples (with and without Honey)

The chemical characteristics of the pineapple drink (with and without honey) are shown in Table 1. The pH of the pineapple drink sweetened with honey was the same (Samples C, D, & E) at $p > 0.05$, but different from the 100% pineapple drink and sample that contained the least amount of honey (Sample B). The pH of all the pineapple drinks ranged between 7.02 and 8.60. The similarity in the pH value for the samples that contained a higher amount of honey (10, 15, and 20 mL) may be attributed to the general effects of honey on the pineapple drink. The effect of honey added to pineapple drink (sample B (5 mL)) also showed in the pH, and it was significantly different from the remaining sweetened samples at $p < 0.05$. The pH of the pineapple drink sweetened with honey was above the range of drink blends reported by Bamidele and Fasogbon [13] who reported a decrease in pH for pineapple juice blend with snake tomato drink and JGB et al. [14] who reported that there was no significant difference in pH for in Kefir ice cream sweetened with honey during storage.

The total titratable acidity (TTA) values of all the samples ranged between 0.47 and 0.96, with pineapple drink(without honey) having the highest(0.96) and pineapple drink sweetened with honey (20 ml) the least (0.37) value (Table 1). The increase in amount of honey in the pineapple drink had an effect on TTA. The decrease in TTA observed in pineapple drink sweetened with honey may be due to a decrease in the acid content of the pineapple drink brought by the addition of honey. This result was different from the report of Bamidele and Fasogbon [13] who reported an increase in TTA of snake tomato and pineapple juice blend.

Total soluble solids (TSS) of the drink blend were between 10.40 for pineapple drink without honey and 53.60 for pineapple drink with 20 ml honey. The high amount of sucrose present in pineapple drink may be responsible for the high value of TSS. The TSS of all the pineapple drink increased with increase in the amount of honey added. This may be attributed to the higher amount of TSS in pineapple drink which the TSS in honey was complementing. The addition of honey with pineapple drink helps in improving the organoleptic properties of the pineapple drink. Pineapple drink and honey is associated with having high soluble solid and this was reflected in the mixture of pineapple drink and honey as the sample with the highest amount of honey had the highest soluble solids. Similar results were reported for clarified juice by Bamidele and Fasogbon [13], Laorko et al. [15] and Oludemi and Akanbi [16].

Vitamin C content ranged between 15.20 and 36.25 mg for all the samples with pineapple drink sweetened with 20 ml honey had the highest (36.25 mg) while pineapple drink without honey had the least (15.20 mg) content (Table 1). The vitamin C in the pineapple increased with increase in the amount of honey added. This result was similar to the report of Bamidele and Fasogbon [13]. They reported an increase in the vitamin C content of Snake tomato and pineapple juice blend. The influence of the different amount of honey added was reflected in the entire chemical characteristics tested for in the pineapple drink. The increase in pH, TTA, TSS

Table 1. Chemical characteristic of Pineapple (*Ananas comosus*) drink with and without honey

Samples	P^H	TSS (°Brix)	TTA (% citric acid)	Vit C (mg/100 mL)
A	7.02^{a}±0.1	10.40^{a}±0.2	0.96^{c}±0.1	15.20^{a}±0.6
B	7.78^{b}±0.2	25.46^{b}±0.3	0.74^{b}±0.1	22.26^{b}±0.5
C	8.20^{c}±0.3	35.44^{c}±0.3	0.51^{a}±0.2	28.33^{c}±0.3
D	8.40^{c}±0.2	42.61^{d}±0.4	0.49^{a}±0.1	32.42^{d}±0.5
E	8.60^{c}±0.4	53.60^{e}±0.5	0.47^{a}±0.2	36.25^{e}±0.6

Key: A = 100% pineapple drink without honey, B = Pineapple drink + 5 mL of honey, C = 3 Pineapple drink + 10 mL of honey, D = Pineapple drink + 15mL of honey, and E = Pineapple drink + 20 mL of honey. Values are means of duplicates ± standard deviations. Means in a row with different superscripts are not significantly different ($p > 0.05$) from each other using the least significant difference (LSD)

and vitamin C content of pineapple drink that contained honey has sweetener were noticeable with all the chemical properties of the drink higher than pineapple drink without sweetener (honey). With the high content of vitamin C in the samples with honey, the pineapple drinks will have higher scavenging power when compared with pineapple drink without honey.

3.2 Total Phenolic Content and the Antioxidant Capacities of Drink Blends

The total phenolic content (TPC) and antioxidant activities (AA) of the pineapple drink with and without honey as a sweetener are shown in Table 2. The TPC values ranged between 10.56 and 50.14 mg GAE/mL for all the pineapple drink samples. The TPC was highest (50.14 mg GAE/mL) in pineapple drink that contained 20 ml of honey and was least in pineapple drink without honey (19.56 mg GAE/mL). The increase in TPC of the samples increased with an increase in the amount of honey added to the pineapple drink with significantly different at $p < 0.05$ when compared with pineapple drink without honey. The increase in the value of TPC recorded for the samples with honey may be due to the interaction between the phenolic compounds present in both pineapple drink and honey. Mullen et al. [17] reported the phenolic compounds in commercial juice and pineapple juice was ranked one of the best fruit drink with phenolic compounds. The report of Perna et al. [8] showed the phenolic profile of three types of honey and all the three has high phenolic compounds.

The antioxidant activities (ABTS) were higher in pineapple drinks that contained honey as sweeteners than in the pineapple drink without honey (Table 2). The pineapple drink without honey had the least antioxidant activities (36.14 µmol TE/100 mL) followed by pineapple drink that contained 5 ml of honey (59.78 µmol TE/100 mL). Antioxidant activities of the pineapple drink with honey increased with the increase in the amount of honey added. There was a positive correlation (0.98) between the total phenolic content of the samples and their antioxidant activities (Supplementary Table 1). The increase in the antioxidant activities of the samples may be attributed to the honey added to the pineapple drink. Blasa et al. [18] reported that honey contained biologically active compounds such as polyphenols, vitamin C and organic acids that can scavenge free radical such as reactive oxygen species in the body. With the report of Blasa et al. [18], it can be stated that the increase in antioxidant activities observed in this studies could be attributed to the presence of honey which helps in boosting the antioxidant activities of pineapple drink.

Table 2. Total phenolic content and the antioxidant capacities of Pineapple (*Ananas comosus*) drink sweetened with honey

Samples	TPC (mg GAE/mL)	ABTS (µmol TE/100 mL)
A	10.56^{a}±1.2	36.14^{a}±2.5
B	22.22^{b}±0.9	59.78^{b}±3.5
C	32.44^{c}±0.8	79.65^{c}±4.1
D	41.69^{d}±0.7	85.14^{d}±5.2
E	50.14^{e}±0.9	96.25^{e}±6.5

Key: A = 100% pineapple drink without honey, B = Pineapple drink + 5 mL of honey, C = 3 Pineapple drink + 10 mL of honey, D = Pineapple drink + 15 mL of honey, and E = Pineapple drink + 20 mL of honey. Values are means of duplicates ± standard deviations. Means in a row with different superscripts are not significantly different (p > 0.05) from each other using the least significant difference (LSD)

3.3 Sensory Attributes of the Drink Blends

Sensory attributes of the pineapple drink with and without honey as a sweetener are shown in Table 3. The pineapple drink without honey was rated least (7.02) in term of colour, with significant ($p > 0.05$) difference when compared with the rest of the samples that contained honey as a sweetener. There was no significant difference ($p > 0.05$) among the samples containing honey (sample B to E) for colour. The similarity in the scores given to pineapple drink containing honey may be due to the familiarity of the panellist to the golden colour of honey used in sweetening the pineapple drink. Although, the colour score for all the samples were similar. Sample A (pineapple drink without honey) scored the least for taste (6.05) and sample E (pineapple drink with 20 mL of honey) was scored the highest (7.99). There was no significant difference ($p > 0.05$) between samples C (pineapple drink with 10 mL honey) and sample D (pineapple drink with 15 mL) in term of tatse. It was expected for the panelist to score samples (pineapple drink) with honey higher than sample without honey (sample A). This is due to the increase in the sweetness of the sample brought about by the addition of honey.

The aroma of sample E was scored the highest (7.54) followed by sample D (6.96), followed by Sample C (6.64), followed by sample B (6.44) and sample A (6.12) having the least flavour. There was a significant ($p < 0.05$) difference between sample A (pineapple drink without honey) and the rest of the samples. The increase in the aroma score may be attributed to masking of the pineapple drinkaroma commonly known to the panelist to another sensational flavour produced by the addition of honey to pineapple drink.

Sample D and E were rated highest in overall acceptability (7.45 & 7.62) without a significant difference at $p > 0.05$. Sample A was scored the least (6.30), followed by sample B (6.61) and sample C (6.90). There was no significant difference among samples A, B & C at $p > 0.05$. All the various attributes of the pineapple drink (with and without honey) influenced their overall acceptability by sensory panellists. The pineapple drink (without honey) had the lowest value for taste, colour, flavour, and overall acceptability; this may be due to the absence of sweetener which the sensory panellists were not familiar with. The addition of honey to pineapple drink improves the colour, taste and the flavour of the drink and this gave better acceptability of the sweetened pineapple drink by the panellist.

3.4 Changes in Antioxidant Activities of the Pineapple Drink with and without Honey

Effects of storage changes in the antioxidant capacity of the pineapple drink with and without in Figs. 1 and 2 showed that the antioxidant activities of the samples stored at the room temperature (29 °C) began to increase from day 20 to 30 days of storage. All the samples (samples A-E) showed a slight increase in the antioxidant activities and sample E have the highest antioxidant activities among them. The antioxidant activity provides information on chemical substance in the food sample to inhibit oxidation. This was determined by analysing the scavenge power of the drink on artificial free radicals after various storage time intervals and conditions. Antioxidant is an important barrier against spoilage; it, therefore, helps to extend the shelf life of drink. It has been described to neutralise the physical, chemical and sensory deterioration of the food product [19].

Increase in the antioxidant activities observed during storage of the pineapple drink (with and without honey) may be attributed to redox reactions between the photochemical, the pineapple drink components atmospheric oxygen and/or Maillard reaction products [20]. At room temperature, an increase in the antioxidant activity was not significant until day 20 when it significantly increased until the end of the storage period. Sample E had the highest antioxidant activities (110 µmol TE/100 mL) while sample A had the least (65 µmol TE/100 mL).

Pineapple drink (with and without honey) stored inside refrigeration showed a slight increase in antioxidant activities during storage (Fig. 2). There was a slight increase in the antioxidant activities of the sample at days 25 and 30. The slight increase in the antioxidant activities observed in the samples inside the refrigerator may be attributed to control temperature which may reduce the rate at which the redox reaction may take place. Storage of fruit drinks is encouraged in refrigeration temperature to prevent deterioration and reactions which may reduce the shelf life of the fruits drinks [21].

Table 3. Sensory attributes of Pineapple (*Ananas comosus*) drink sweetened with honey

Samples	Colour	Taste	Aroma	Overall acceptability
A	7.02^{a}±0.2	6.05^{a}±0.2	6.12^{a}±0.5	6.30^{a}±0.5
B	7.56^{b}±0.1	7.05^{b}±0.3	6.44^{b}±0.2	6.61^{a}±0.3
C	7.78^{b}±0.3	7.56^{c}±0.5	6.64^{b}±0.3	6.90^{a}±0.4
D	7.96^{b}±0.4	7.86^{c}±0.3	6.96^{b}±0.4	7.45^{b}±0.4
E	7.98^{b}±0.2	7.99^{cd}±0.3	7.54^{b}±0.3	7.62^{b}±0.5

Key: A = 100% pineapple drink without honey, B = Pineapple drink + 5 mL of honey, C = 3 Pineapple drink + 10 mL of honey, D = Pineapple drink + 15 mL of honey, and E = Pineapple drink + 20 mL of honey. Values are means of duplicates ± standard deviations. Means in a row with different superscripts are not significantly different (p > 0.05) from each other using the least significant difference (LSD)

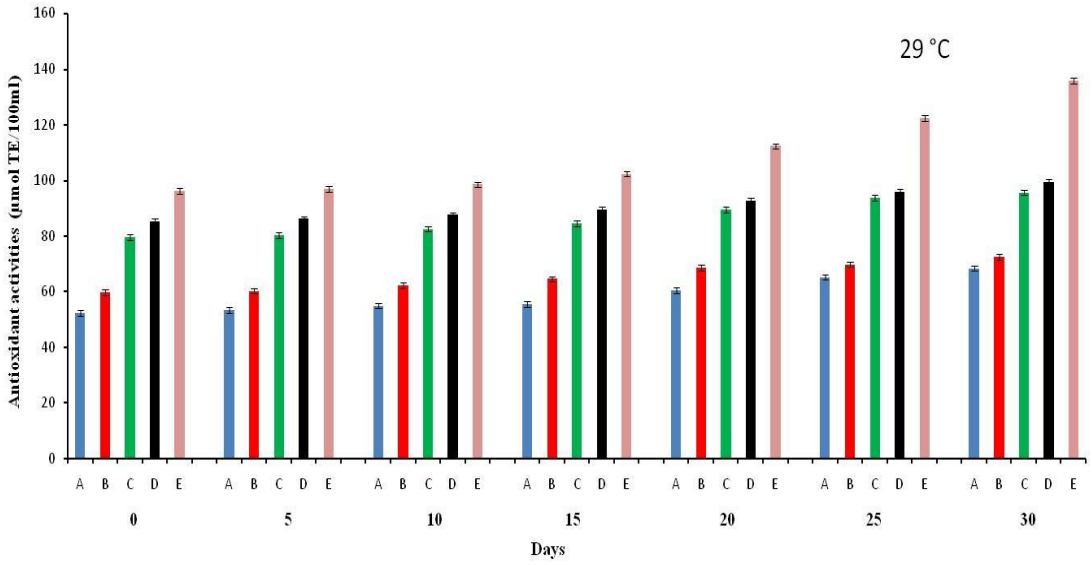

Fig. 1. Effects of storage condition (room temperature (29°C)) on antioxidant activities of pineapple (*Ananas comosus*) drink sweetened with honey

Key: A = 100% pineapple drink without honey, B = Pineapple drink + 5 mL of honey, C = 3 Pineapple drink + 10 mL of honey, D = Pineapple drink + 15 mL of honey, and E = Pineapple drink + 20 mL of honey

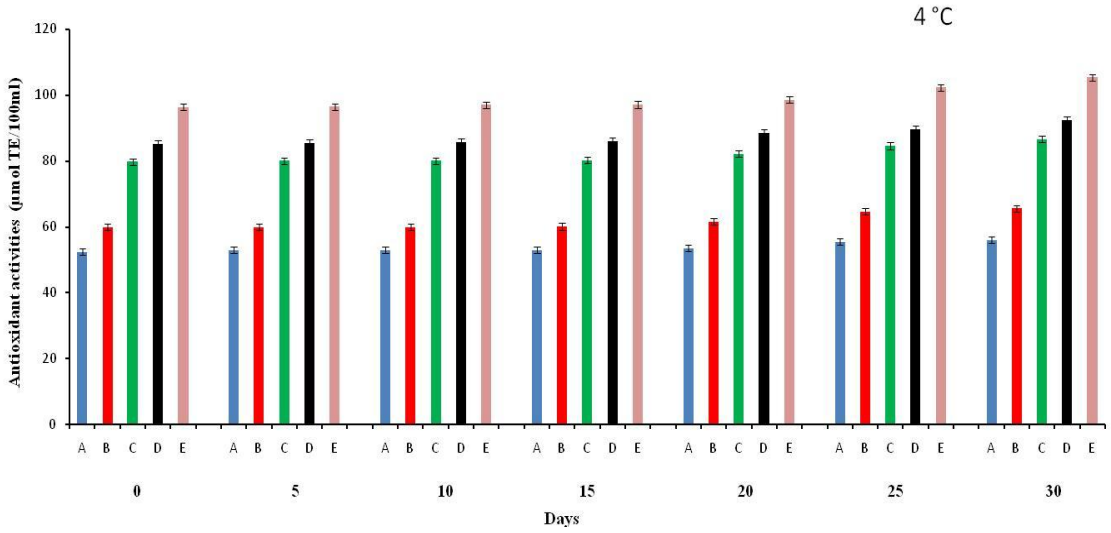

Fig. 2. Effects of storage condition (refrigeration temperature (4°C)) on antioxidant activities of pineapple (*Ananascomosus*) drink sweetened with honey

Key: A = 100% pineapple drink without honey, B = Pineapple drink + 5 mL of honey, C = 3 Pineapple drink + 10 mL of honey, D = Pineapple drink + 15 mL of honey, and E = Pineapple drink + 20 mL of honey

4. CONCLUSION

The addition of honey to pineapple drink contributes to the health benefit of the drink when compared with pineapple drink without honey. Also, the addition of honey to pineapple drink contributes to the acceptability of drink in terms of colour, taste, andaroma by the sensory panellists. Sensory scores show that samples with a high amount of honey are more acceptable by the panellists. The study reveals that storage time and the temperature has a significant effect on the antioxidant activities of pineapple drink with and without honey.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Supplementary Table 1. Showing correlations between total phenolic content and antioxidant of Pineapple drink

Experiments	TPC (mg GAE/mL	ABTS (µmol TE/100 mL
TPC (mg GAE/mL	1	0.98
ABTS (µmol TE/100 mL	0.98	1

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