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# Potential Application of Spices and Pineapple as Healthy Beverages

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author SM conducted the antioxidant analysis. Authors NIM, NK and NMFCMN analysed the samples and help in samples preparation. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The purpose of this study was to evaluate pineapple-spice beverages for their potential health benefits and assess their consumer acceptance. Fortifying fruit beverages with spices can enhance their nutritional and health benefits, making them effective carriers for delivering phytochemicals and other nutrients.

**Methodology:** Spices extracts from cinnamon bark, star anise, coriander, cloves, white cumin, and cumin were incorporated into pineapple beverages to enhance their antioxidant, vitamin, and mineral content. Specifically, two selected samples, namely pineapple-spice beverages and spice beverage (as a control), were chosen based on the optimization of beverage formulation parameters previously conducted.

**Results:** The formulation deemed most favourable through sensory evaluation comprised 25% pineapple juice combined with spice extracts and underwent further physicochemical analyses and shelf life studies. The shelf life of both beverages demonstrated 12 months of storage stability at

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ambient temperature, in terms of sensory evaluation, microbiological, and physical analyses. In the 12th month, the sensory evaluation panel for spice beverages and pineapple spice beverages gave scores above 6 for all attributes, which include colour, odour, viscosity, taste, sweetness, sourness, and overall acceptability of the beverages. The highest scores in panellist evaluations were attributed to the pineapple spice beverages. In antioxidant studies, the highest values of DPPH, FRAP value and total phenolic content were observed in samples of pineapple spice beverages. Pineapple-spice beverages (25%) exhibited significant levels of vitamin A, C, B3, B5, B7, B12, and zinc. Colour analysis for the L* value showed a slight increase, while the a* value showed a slight decrease throughout the storage period for both types of beverages. Additionally, pH and brix values were recorded during the storage period of the beverage samples.

**Conclusion:** Thus, spices and pineapple are an excellent potential for creating nutritious and healthy beverages.

*Keywords: Beverages; pineapple; spice; antioxidant; vitamin and physical analysis.*

## 1. INTRODUCTION

Spices come from different parts of the plant are used to impart an aroma and taste to food but several used in beverages. Spices have been used to fortify foods throughout history as preservatives, flavor and therapeutic agents. Spices also have been utilized as food additives all over the world, not only to enhance the organoleptic properties of food, but also to increase the shelf life by decreasing or eliminating the foodborne pathogens (Lai & Roy 2004). Several studies have recommended the use of dietary herbs and spices for their beneficial effects on human health through their antimutagenic, anti-inflammatory, antioxidative, and immune modulatory properties (Conn 1995). Many spices such as coriander, cinnamon, gooseberry, turmeric, clove, fenugreek seed, asafoetida, star anise, garlic, black pepper, bay leaf, and curry leaf, show good antimicrobial and also as excellent sources of antioxidants. Numerous studies show that antioxidants play an essential role in maintaining human health, preventing and treating diseases, due to their ability to reduce oxidative stress (Pizzino et al. 2017, Muscolo et al. 2024). Measuring the antioxidant activity/capacity of foods and biological samples is therefore essential not only in ensuring the quality of functional foods, but more importantly in studying the efficiency of food antioxidants in preventing and treating the diseases related to oxidative stress.

Several antioxidant procedures should be performed in vitro to determine antioxidant activities for the sample of interest. Taking this into account, it is difficult to compare one method completely with another. Therefore, the methods of analysis must be checked before choosing one for the purpose of research. Total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous ion chelating (FIC) and ferric reducing antioxidant power (FRAP) assay have been used to determine antioxidant activity in all samples. Therefore, fortification of fruit drink with spices could help to provide functional beverages with nutritional and health values. Beverages are good carrier that has been successfully used to deliver phytochemicals and other nutrients for health benefits in our nutrition food system (Samah et al. 2019). Furthermore, addition of herbs and spices or its extracts to baverages make these products act as carrier for nutraceuticals. The purpose of this study was to evaluate pineapple-spice beverages for their potential health benefits and assess their consumer acceptance.

## 2. MATERIALS AND METHODS

### 2.1 Raw Materials and Samples Preparation

Pineapple juices were bought from juices hawker, for specific variety MD2. Spices extract (Cinnamon bark, star anise, coriander, clove, cumin and fennel) were purchase from MARDI Johor Bahru. Ascorbic acid, sodium benzoate and xanthan gum were bought from Daily Food and Chem Selangor. The formulation of pineapple-spice beverages were based on Table 1. All beverage components were mixed, heated to 80 ◦C, and filled into 250 mL can. Pasteurization was carried out in a batch pasteurizer at 85–87 ◦C for 10 min. The pasteurized samples were stored at room temperature and analysed for their physicochemical and sensory characteristics within one week after production. A shelf life study was conducted over 12 months of storage for both beverage samples. The beverage samples were taken every 2 months to analyze color, pH, total soluble solids, and for sensory evaluation during storage. The preliminary study was conducted to determine the percentage of pineapple juice (15%, 25%, and 35%) to be added to the spice beverages formulation. Based on sensory evaluation, the panelists selected 25% pineapple juice as the final formulation.

## 2.2 Antioxidant Analyses

The free radical-scavenging capacity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical-scavenging assays and *ferric reducing antioxidant potential (FRAP)* of each sample was assessed using the method proposed by Alothman et al. (Alothman et al. 2009). The content of reducing components (expressed as tpc) was estimated using the folin–ciocalteu assay according to a method developed by Velioglu et al. (1998) and Ikram et al. (2009).

## 2.3 Vitamin C, Vitamin A (β-carotene) and Vitamin B Analyses

Methods of vitamin A (β-carotene) and vitamin C were based on Ismail and Fun (2003). The samples were analyzed using High-Performance Liquid Chromatography (HPLC) by Waters Alliance HPLC System. The waters symmetry C18 column (4.6 × 250 mm × 5 µm) was used. The mobile phase consists of 0.1 M potassium acetate, pH 4.9(2) Acetonitrile-water (50:50) for vitamin C while for vitamin A, acetonitrilemethanol- ethyl acetate (88:10:2) was used. The vitamin analyses was evaluated according to Aslam et al. (2008) and Zuwariah et al. (2021) method.

## 2.4 Sensory Evaluation

In the study, pineapple-spice beverages underwent an acceptance test which focused on sensory evaluation, including color, aroma, aftertaste, taste, and overall acceptance. To participate in the evaluation, 60 untrained panelists were invited, who were MARDI staff and practical students from different universities, within the age range of 21 to 58, and met the criteria of good health and non-smoking. The assessment took place at the Food Sensory Laboratory, located within the Food Science and Technology Research Center at MARDI. The evaluation was conducted under normal room temperature and fluorescent lighting conditions. Each panelist was provided with tissue and plain water placed on a tray. Subsequently, the samples were served to them in plastic cups, with each cup labeled with a randomly assigned 3-digit number. After evaluating each sample, the panelists were instructed to rinse their mouths before proceeding to the next one. Following the sensory evaluation, the panelists were required to complete a questionnaire using a 7-point hedonic scale for each sample attribute. The scale ranged from 1 (dislike extremely) to 7 (like extremely), indicating the panelists' preference level. Samples achieving mean scores above 5.00 for overall acceptability were deemed acceptable.

## 2.5 Total Soluble Solid Content and pH

The total soluble solid content (Brix) in the pineapple-spice beverages was determined using a refractometric method, measured with an Abbe refractometer (Atago, Japan). The refractometer was standardised with distilled water at 20°C. Two drops of juice at 20°C then dropped on the lens (sensitive surface) of the refractometer and measured (AOAC 2012). The pH value was determined using AOAC (2012) procedure. Pineapple juice drink was poured into a beaker and the pH probe of the pH meter (Metrohm, Herisau, Switzerland) was inserted into it after the pH meter has been standardised using buffer 4 and 7 solutions at 25°C.

**Table 1. Formulation of pineapple-spice beverages**

| Ingredients | Spice baverages (Control)(%) | Spices-infused pineapple beverages (%) |
|---|---|---|
| Filtered water | 76.86 | 51.86 |
| Pineapple juice extract | - | 25 |
| Xanthan gum | 0.04 | 0.04 |
| Sugar | 8.0 | 8.0 |
| Ascorbic acid | 0.1 | 0.1 |
| Spices extract | 15.0 | 15.0 |
| Sodium Benzoate | 0.025 | 0.025 |

### 2.6 Colour Determination of Spices and Pineapple Baverages

Colorimetric determination of beverages were performed by using Minolta CR-400 colorimeter (Konica Minolta Sensing Inc., Japan), according to L*, a*, b* system. The L* (lightness), a* (red intensity) and b* (yellow color intensity) values were measured at six different points for each sample. The L value states the positions on the white/black axis, the a value the position on the red/green axis and the b value the position on the yellow/blue axis.

### 2.7 Storage Study

The samples, packed and sealed in 250 ml aluminum cans, were stored at ambient room temperature. Sampling was conducted every two months, from month 0 to month 12, and the physicochemical properties data were plotted over time. Throughout the 12-month storage period, the microbiological quality, sensory evaluation, pH, and total soluble solids (Brix) of the spice beverages and pineapple-spice beverages were assessed every two months.

## 3. RESULTS AND DISCUSSION

### 3.1 Antioxidant Capacity of Pineapple-spice Beverages

The presence of different groups of antioxidants was reflected in the antioxidant capacity (AC) of the products, as measured by three different assays (Table 2). The addition of pineapple extract contributed to the vitamin content and increased the antioxidant capacity of the pineapple-spice beverages. Samples containing 25% pineapple extract showed the highest absorption in the DPPH assay, as well as the highest FRAP and total phenolic content, compared to spice beverages without pineapple extract. Pineapple-spice beverages exhibited the highest antioxidant capacity values across all three methods, likely due to the combined action of vitamin C, polyphenols, and carotenoids. The antioxidant capacity of pineapple was correlated with its phenolic, flavonoid, and ascorbic acid content (Lu et al. 2014).

A significant variation in total phenolic content (TPC) was observed between the beverages. The pineapple-spice beverages showed 262 mg of gallic acid equivalents (GAE) per 100g, compared to the spice beverages, which had 243 mg GAE per 100g. This result is higher than earlier reports from other researchers, where pineapple extract showed a total phenolic content (TPC) ranging from 31.48 to 77.55 mg GAE per 100 g of fresh weight. Pineapple is known for its high phenolic content, with values varying depending on the extraction method and the part of the fruit used (such as peel, stem, or juice). Almeida, et al. (2011) reported 298.6 mg Gallic acid equivalents (GAE)/100 g dry basis for pineapple by-products. According to reference (Cano-Lamadrid et al. 2018), the addition of a sugar solution does not significantly affect the antioxidant capacity (AC). In contrast, the addition of stevia in fruit juice can enhance its antioxidant capacity (Bender et al. 2018). This effect might be due to the stabilization of antioxidants in the presence of sugar. A significant increase in antioxidant capacity was observed in pineapple spice beverages, as determined by the DPPH, FRAP, and total phenolic content methods. The addition of pineapple extract can further increase the antioxidant capacity of the beverage, thereby enhancing its overall value. Although pasteurization is applied in the beverage-making process, some antioxidants in the pineapple spice beverages transform into more active forms, such as those with a smaller molecular weight.

### 3.2 Vitamin Content of Spice Beverages and Pineapple-spice Beverages

Table 3 shows the vitamin and zinc content of the spices studied. Both samples had vitamin A, vitamin C, vitamin B3, vitamin B5, vitamin B7, vitamin B12 and zinc. Pineapple spice beverages had significantly ($p<0.05$) higher vitamin A, vitamin C, Vitamin B3, vitamin B5 and also vitamin B12. Pineapple-spice beverages typically exhibit higher concentrations of vitamin C compared to spice beverages. This is attributable to the natural abundance of ascorbic acid (vitamin C) in pineapples, which serve as a rich source of this essential nutrient (Lobo & Siddiq 2017). The incorporation of pineapple into spice beverages thus elevates the overall vitamin C content. Spices are known for their bioactive compounds, such as polyphenols and flavonoids, but their contribution to vitamin C intake is less prominent. However, some spices do contain measurable amounts of vitamin C, though the concentrations are generally lower compared to fruits and vegetables. According to U.S. Department of Agriculture, nutrient database (U. S. Department of Agriculture, Nutrient database 2008), fresh cumin seeds may have around 7.7

mg of vitamin C per 100 grams. However, cumin's primary health benefits are derived from its phytochemical composition rather than its vitamin C content. Cinnamon and star anise contain very low amounts of vitamin C compared to other spices like chili peppers or paprika. Cinnamon bark contains negligible amounts of vitamin C. It is not considered a significant source of ascorbic acid, with estimates of vitamin C content being less than 1 mg per 100 grams in dried cinnamon. The vitamin C content in star anise is reported to be approximately 0 mg per 100 grams, indicating that it is not a notable source of this nutrient (U.S. Department of Agriculture, Agricultural Research Service 2014).

Pineapple, when added to spice beverages, enhances the overall nutritional profile, especially by increasing the content of certain vitamins, including B vitamins. When pineapple is incorporated into a spice beverage, it can increase the levels of thiamine, niacin, and vitamin B6, though the overall contribution still depends on the concentration of pineapple juice in the drink and also processing method. Siddiq et al. (2011) and Hounhouigan et al. (2014) reported that adding pineapple juice increases the vitamin C content and other nutrients in beverages, improving their health benefits. Cooking and prolonged exposure to heat and light can degrade vitamin B and C due to its sensitivity to environmental factors. Cinnamon and cloves contain trace amounts of vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), and vitamin B6, but the quantities are very small (U.S. Department of Agriculture, Agricultural Research Service 2014).

According to Campos et al. (2020) pineapple crude juice contains 212.9 mg of vitamin C per 100 g. However, vitamin C is highly sensitive to heat and can degrade during the pasteurization process. This degradation occurs because vitamin C (ascorbic acid) is unstable at elevated temperatures, especially above 70°C. During pasteurization, which typically involves heating the beverage to temperatures around 70-85°C, a significant portion of the vitamin C can be lost due to both heat exposure and oxidation (Campos et al. 2020, Polanía et al. 2022). B vitamins may lose around 10-30% of their content depending on the conditions used during pasteurization. Vitamins A tend to be more stable under heat compared to water-soluble vitamins. However, some degradation can still occur, especially when exposed to high heat for long periods (Ottaway 2010).

**Table 2. Antioxidant capacity of spice beverages and pineapple-spice beverages**

| Sample/Analysis | DPPH (% absorption) | FRAP (mg $FESO_4$ Eq/100 g) | Total phenolic content TPC (mg GA Eq/100 g) |
|---|---|---|---|
| **Spice beverages** | $83.38 \pm 0.53^{b}$ | $146.52 \pm 1.12^{b}$ | $243.92 \pm 1.48^{b}$ |
| **Pineapple-spice beverages (**25%) | $84.01 \pm 0.13^{ab}$ | $239.32 \pm 0.51^{a}$ | $262.87 \pm 1.07^{a}$ |

*Data was expressed as mean±SD, each value is a mean of triplicate reading (n=3), means with different lower case letters in the same column are significantly different ($p < 0.05$)*

**Table 3. Vitamin content in spice and pineapple-spice beverages**

| Vitamin and mineral | Spice beverages | Pineapple-spice beverages (25%) |
|---|---|---|
| Vitamin A (beta carotene) ug/100 g | 30.7 ± 0.57b | 33.85 ± 0.35a |
| Vitamin C (Ascorbic acid) mg/100 g | 165.91 ± 0.67b | 188.33 ± 0.28a |
| Vitamin B1 (Thiamine) mg/100 g | <0.1 | <0.1 |
| Vitamin B2 (Nicotinamide) mg/100 g | <0.1 | <0.1 |
| Vitamin B3 (Nicotinamide) mg/100 g | 13.45 ± 0.64a | 17.0 ± 1.27a |
| Vitamin B5 (calcium-D-penthothenate) mg/100 g | 0.9 ± 0.14b | 1.8 ± 0.28a |
| Vitamin B6 (Pyridoxine) mg/100 g | <0.1 | <0.1 |
| Vitamin B7 (Biotin) mg/100 g | 1.75 ± 0.07a | 1.25 ± 0.07b |
| Vitamin B9 (Folic acid) mg/100 g | <0.1 | <0.1 |
| Vitamin B12 (Cyanocobalamin) ug/100 g | 0.3 ± 0.01b | 0.4 ± 0.01a |

*Data was expressed as mean±SD, each value is a mean of triplicate reading (n=3), means with different lower case letters in the same raw are significantly different ($p < 0.05$)*

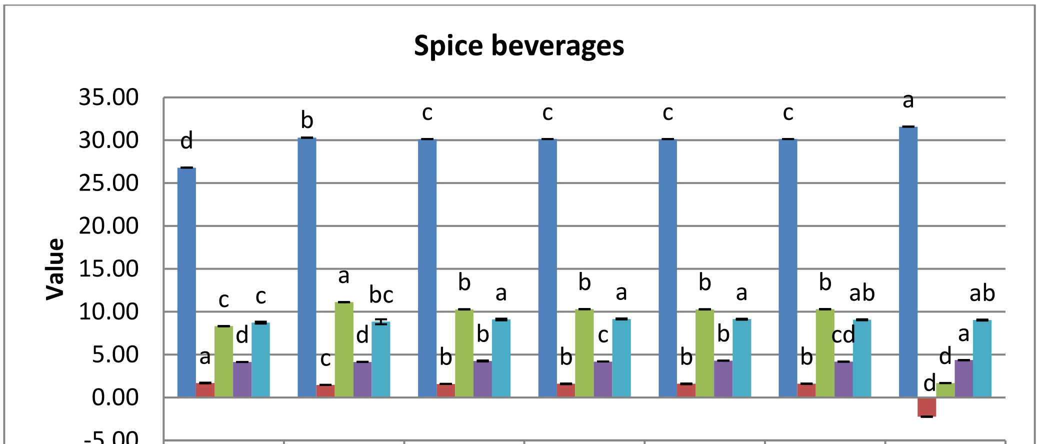
### 3.3 The Stability of Spice Beverages and Pineapple-spice Beverages during Storage

In overall, the sensory evaluation scores over the 12-month storage period showed an increasing trend for all evaluation attributes. In the 12th month, the sensory evaluation panel for spice beverages and pineapple spice beverages gave scores above 6 for all attributes, which include colour, odour, viscosity, taste, sweetness, sourness, and overall acceptability of the beverages. Some studies showed that, natural sugars in the pineapple may become more pronounced or balanced with the acidity, improving the sweetness-sourness profile (Gao et al. 2022). This balance can be more pleasing to the panelists. Additionally, the beverage samples in this study were stored properly and well-preserved. This condition may have maintain their quality or even improve certain sensory attributes, such as color, aroma, and taste, especially in beverage recipe includes spices or natural preservatives. According to Polania et al. (2022) and Campos et al. (2020), certain flavors in beverages can develop or blend more harmoniously over time. The spices in spice-based beverages might undergo slow extraction, enhancing their aromatic and flavor profiles. Similarly, pineapple juice can mellow out, reducing sharp acidic notes and creating a balanced sweetness, which enhances the overall taste appeal. From a previous literature review, it appeared that the quality of pineapple juice is largely influenced by the technology used during processing (Nauman et al. 2016). Little studies have been performed on the effect of storage time and pasteurization on the quality of pineapple juice parameters such as colour, sugar content, acidity, vitamin and bromelain content.

Color occupies an effective role in the appearance of the spice beverages and actively influences the consumer's decision to buy the product (Ghandehari Yazdi et al. 2020). The color properties in terms of a* (red (+) / green (−), L* (black (0) / white (100)), and b* (yellow (+) / blue (−) values of all beverages are indicated in Figs. 1 and 2. L, a, b color analysis is used in the evaluation of spice beverages and pineapple spice beverages to quantify and track color changes during processing, storage, and over time. There were significant differences during 12 month of storage in L*, a* and b* values. Both beverages had lighter colour (L*) after 12 month of storages as compared to initial storages. **L*** is particularly relevant in spice beverages and pineapple spice beverages where color changes due to oxidation or degradation can cause darkening or lightness over time. For example, a cinnamon beverage might become darker during storage due to the breakdown of pigments (Ríos-Pérez et al. 2023). In this study, both colour of the beverages become lighter after 12 month of storage. Over time, certain pigments in the beverage may oxidize, leading to a reduction in their intensity. For example, anthocyanins in fruit-based beverages can degrade, causing the beverage to appear lighter (Rojas & Paredes-López 2016). Ingredients in the beverage, such as spices or preservatives, may interact during storage, leading to changes in color. Some interactions may cause the breakdown of darker pigments, resulting in a lighter beverage.

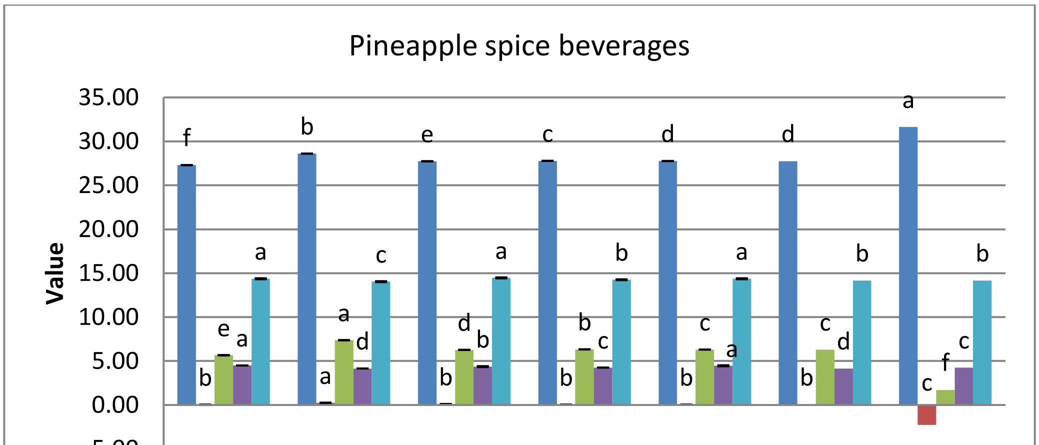
The results showed that the pineapple-spice beverages had a yellow color *(*b* = 5.66) at 0 months of storage, and the samples became darker after 12 months of storage. The trend of darkening also occurred in the spice beverage samples throughout the storage period. The browning effect on the beverages' color was due to the high saccharide content and their reactions during storage, which were influenced by temperature (Sacchetti et al. 2005). On the other hand, both beverages yielded decreasing yellow colour intensity by having the lower b* value (1.69 and 1.72) after 12 month of storages. While the same trend happened for both samples after 12 month of storage yielded the decreasing a* colour intensity by having lowest a* value (-2.24 and -2.27) at the end of storage. According to Hee et al. (2011), acidic pH of less than 3.5 is essential to attain the desired red colour and stability of anthocyanins. Over prolonged storage, light exposure or oxidation can lead to the degradation of these pigments, causing a reduction in **b*** values and resulting in a loss of yellow intensity (Benedetti & de Oliveira 2020). Conversely, if red pigments degrade or if there's a loss of color intensity, the **a*** value may decrease, leading to a more muted appearance (Wang & Weller 2017).

A stable pH is important for both beverages and microbial stability. The pH of beverages can change during shelf-life storage due to various factors such as microbial activity, chemical reactions, and the breakdown of certain ingredients. A pH range of 4.1–4.5 in the both baverages is generally acceptable during 12 months of storage, because the preservation methods such as pasteurization and proper packaging are in place to prevent microbial growth. Beverages with a pH below 4.6 are typically considered acidic enough to inhibit the growth of harmful pathogens like *Clostridium botulinum*, which reduces the risk of foodborne illness (Campos et al. 2020). Total Soluble Solids (Brix) measures the sugar content,




| | Month 0 | Month 2 | Month 4 | Month 6 | Month 8 | Month 10 | Month 12 |
|---|---|---|---|---|---|---|---|
| L* | 26.81 | 30.30 | 30.14 | 30.14 | 30.14 | 30.14 | 31.59 |
| a* | 1.70 | 1.47 | 1.59 | 1.60 | 1.59 | 1.61 | -2.24 |
| b* | 8.32 | 11.12 | 10.29 | 10.31 | 10.29 | 10.30 | 1.69 |
| pH | 4.14 | 4.16 | 4.27 | 4.20 | 4.30 | 4.18 | 4.36 |
| °brix | 8.73 | 8.83 | 9.10 | 9.17 | 9.13 | 9.07 | 9.03 |

**Fig. 1. Colour (L*, a*, b*), pH and total soluble solid (°brix) of spice beverages during storage**




| | Month 0 | Month 2 | Month 4 | Month 6 | Month 8 | Month 10 | Month 12 |
|---|---|---|---|---|---|---|---|
| L* | 27.30 | 28.60 | 27.73 | 27.78 | 27.76 | 27.76 | 31.63 |
| a* | 0.02 | 0.23 | 0.07 | 0.03 | 0.03 | 0.04 | -2.27 |
| b* | 5.66 | 7.37 | 6.27 | 6.33 | 6.31 | 6.30 | 1.72 |
| pH | 4.50 | 4.15 | 4.37 | 4.26 | 4.47 | 4.16 | 4.27 |
| °brix | 14.37 | 14.03 | 14.47 | 14.25 | 14.37 | 14.17 | 14.17 |

**Fig. 2. Colour (L*, a*, b*), pH and total soluble solid (°brix) of pineapple spices beverages during storage**

**Table 4. Sensory evaluation of spice beverages and pineapple-spice beverages during storage**

| Month | Sample | Colour | Odour | Viscosity | Taste | Sweetness | Sourness | Overall acceptability |
|---|---|---|---|---|---|---|---|---|
| 0 | Spice beverages | 5.73 ± 1.04$^{b}$ | 4.78 ±1.05$^{d}$ | 5.33 ± 1.05$^{e}$ | 4.70 ±1.11$^{c}$ | 4.93 ± 1.14$^{e}$ | 4.73 ± 1.04$^{d}$ | 4.88 ±1.00$^{d}$ |
| | Pineapple-spice beverages (25%) | 5.75 ± 0.98$^{b}$ | 5.18 ± 1.28$^{bc}$ | 5.70 ±1.04$^{cde}$ | 5.68 ±1.05$^{b}$ | 5.7 ± 1.11$^{bcd}$ | 5.30 ± 1.14$^{c}$ | 5.75 ± 0.95$^{b}$ |
| 4 | Spice beverages | 5.72 ± 1.02$^{b}$ | 4.88 ± 1.88$^{c}$ | 5.58 ± 0.94$^{de}$ | 5.55 ± 0.91$^{b}$ | 5.38 ± 1.14$^{d}$ | 5.12 ±1.22$^{d}$ | 5.2 ±1.05$^{cd}$ |
| | Pineapple-spice beverages (25%) | 6.08 ±0.70$^{ab}$ | 5.87 ±1.21$^{a}$ | 6.13 ±0.83$^{ab}$ | 6.18 ±0.89$^{a}$ | 6.08 ±0.89$^{ab}$ | 5.97 ±1.00$^{ab}$ | 6.15 ±0.90$^{a}$ |
| 8 | Spice beverages | 6.02 ±0.70$^{ab}$ | 5.25 ±1.16$^{bc}$ | 5.88 ±0.85$^{bcd}$ | 5.37 ±1.19$^{b}$ | 5.6 ±1.11$^{cd}$ | 5.37 ±1.33$^{c}$ | 5.45 ± 1.00$^{bc}$ |
| | Pineapple-spice beverages (25%) | 6.3± 0.83$^{a}$ | 6.22 ±0.92$^{a}$ | 6.28 ±0.90$^{a}$ | 6.32 ± 0.89$^{a}$ | 6.22 ±0.92$^{a}$ | 6.23 ± 0.93$^{a}$ | 6.32 ± 0.85$^{a}$ |
| 12 | Spice beverages | 5.83 ± 0.99$^{b}$ | 5.35 ±1.12$^{b}$ | 5.78 ±0.99 $^{bcd}$ | 5.53 ±1.20$^{b}$ | 5.7 ±1.05$^{bcd}$ | 5.55 ±1.05$^{bc}$ | 5.65 ±1.01$^{b}$ |
| | Pineapple-spice beverages (25%) | 6.03 ±0.96$^{ab}$ | 6.18 ±1.00$^{a}$ | 6.07 ±1.04$^{abc}$ | 6.22 ±0.98$^{a}$ | 6.03 ±1.07$^{abc}$ | 6.07 ±1.07$^{a}$ | 6.2 ±0.94$^{a}$ |

*Data was expressed as mean±SD, each value is a mean of triplicate reading (n=3), means with different lower case letters in the same column are significantly different ($p < 0.05$)*

**Table 5. Microbiological analysis of spice beverages and pineapple-spice beverages during storage**

| Month | Sample | Microbiological analysis | | | | |
|---|---|---|---|---|---|---|
| | | Total plate count | Total anaerobic count | Total yeast & mould count | Total coliform | *Escherichia coli* |
| 0 | Spice beverages | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 |
| | Pineapple-spice beverages (25%) | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 |
| 4 | Spice beverages | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 |
| | Pineapple-spice beverages (25%) | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 |
| 8 | Spice beverages | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 |
| | Pineapple-spice beverages (25%) | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 |
| 12 | Spice beverages | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 |
| | Pineapple-spice beverages (25%) | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 | <1.0 x 10 |

*Notes: <1.0 x 10 indicates the microorganisms tested were not detected in the analysed samples* which affects sweetness and taste. Over time, sugar levels might shift due to enzymatic activities or ingredient interactions. Higher Brix levels can slightly improve shelf stability by reducing the available water content for microbial growth, but this alone is not sufficient for preservation. It must be combined with methods like pasteurization and acidic pH control. The Brix value of the pineapple spice beverage is higher than that of the spice beverage due to the addition of pineapple juice. This is because pineapple juice contributes natural sugars and other soluble solids, which increase the Brix level, adding sweetness and richness to the flavor profile.

Table 5 shows that the total plate count, total yeast and mold count, total coliform, and *Escherichia coli* count are all <1.0 × 10 CFU/g, which indicates no growth detected. According to Australian Standards, the presence of coliform microorganisms in ready-to-eat food over 1.0×10 CFU/g is considered exceeding the acceptable limits. Coliform contamination in food products can relate to the mishandling of raw material, cross-contamination that might occur after heat treatment, failure of heat treatment, or inadequate sanitation process. In conclusion, there was no microbial growth throughout the 12-month storage period for both beverages, indicating that the product is stable and safe for consumption. The pasteurization technique used in this beverage process kills harmful microorganisms and extends the shelf life of the drink by eliminating pathogens, such as bacteria, yeast, and molds, that could cause spoilage or pose health risks. Pasteurization is a heat treatment process used in the beverage industry to kill harmful microorganisms and extend the shelf life of drinks without significantly affecting their taste or nutritional value. During pasteurization, beverages are heated to a specific temperature for a set period and then rapidly cooled. Pasteurization helps to improve food safety, prevent spoilage, and preserve the quality of the product for longer periods without the need for refrigeration (Ramesh 1995).

## 4. CONCLUSION

The physico-chemical properties and sensory acceptance of spice beverages and pineapple spice beverages were assessed. The results showed that the antioxidants, vitamins, sensory evaluation, and storage studies in terms of colour, pH, and total soluble solids of the formulated samples were influenced by the inclusion of spices and pineapple juice in the mixed drinks. The addition of pineapple juice to the spice beverages significantly increased antioxidant capacity, sensory acceptance scores, vitamin content, and shelf life stability compared to the spice beverages. Therefore, it can be concluded that the pineapple spice beverage formulation was the best, and it is recommended to conduct animal studies to investigate the effectiveness of this beverage in enhancing immunity.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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