Potential application of spices and pineapple as healthy beverages

.

ABSTRACT

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 spice-infused pineapple beverage and spice beverage (as a control), were chosen based on the optimization of beverage formulation parameters previously conducted. 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. In antioxidant studies, pineapple juices were incorporated at levels of 25% in pineapple spice beverages, with spice beverages without pineapple juice serving as the control. The highest values of DPPH, FRAP value and total phenolic content were observed in samples of pineapple spice beverages. Spice-infused pineapple beverage (25%) exhibited significant levels of vitamin A, C, B3, B5, B7, B12, and zinc. Sensory acceptance was evaluated by 60 adult consumers, who assessed taste, odour, colour, and overall quality. The highest scores in panellist evaluations were attributed to the pineapple spice beverages. 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.

Keywords: baverages, 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 and 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. 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 (El-Sayed et al., 2015). 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. MATERIAL AND METHODS

2.1 Raw materials and samples preparation

Pineapple juice 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.

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

2.2 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*. (2009). The. 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. and Zuwariah et al. 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

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 20o C. Two drops of juice at 20oC then dropped on the lens (sensitive surface) of the refractometer and measured (AOAC, 2004). The pH value was determined using AOAC, (2004) 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.

2.6 Colour determination of spices and pineapple baverages

Colorimetric determination of instant cereals 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.

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.

2.7 Storage study

The samples, packed in 250 ml aluminum cans (open or close condition), 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. 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 (how it is found directly) 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 [Xin-Hua Lu, 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 100g of fresh weight. According to reference [57], 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 [58]. 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 (what is its effect in the product)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.

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±0.53^{b}	146.52±1.12^{b}	243.92± 1.48^{b}
Pineapple-spice beverages (25%)	84.01±0.13^{ab}	239.32±0.51^{a}	262.87±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)

Vitamin and mineral	Spice beverages	Pineapple-spice beverages (25%)

3.1 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 B5and also vitamin B12. Pineapple-spice beverages typically exhibit higher concentrations of vitamin C (effect of pasteurization if any on nutrients as few are heat sensitive) 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. (any other refernece if any regd presence of vitamin C in pineaplle thus improving its nutritiuos value) 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 Nour et al.[6], 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. [USDA Food Data Central]

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 (refrence if any regd incresae in vitamin conents after adding pineapple source). 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 [USDA FoodData Central].

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

Vitamin A (beta carotene) ug/100g	30.7 ± 0.57b	33.85 ± 0.35a
Vitamin C (Ascorbic acid) mg/100g	165.91 ± 0.67b	188.33 ± 0.28a
Vitamin B1 (Thiamine) mg/100g	<0.1	<0.1
Vitamin B2 (Nicotinamide) mg/100g	<0.1	<0.1
Vitamin B3 (Nicotinamide) mg/100g	13.45 ± 0.64a	17.0 ± 1.27a
Vitamin B5 (calcium-D-penthothenate) mg/100g	0.9 ± 0.14b	1.8 ± 0.28a
Vitamin B6 (Pyridoxine) mg/100g	<0.1	<0.1
Vitamin B7 (Biotin) mg/100g	1.75 ± 0.07a	1.25 ± 0.07b
Vitamin B9 (Folic acid) mg/100g	<0.1	<0.1
Vitamin B12 (Cyanocobalamin) ug/100g	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 column are significantly different (p = 0.05)

3.2 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 (Refernece required). This balance can be more pleasing to the panelists. Additionally, the beverage samples in this study were stored properly and well-preserved (it will be better if the authr can rewrite the preservation and staorage process in one line). 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."

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.

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

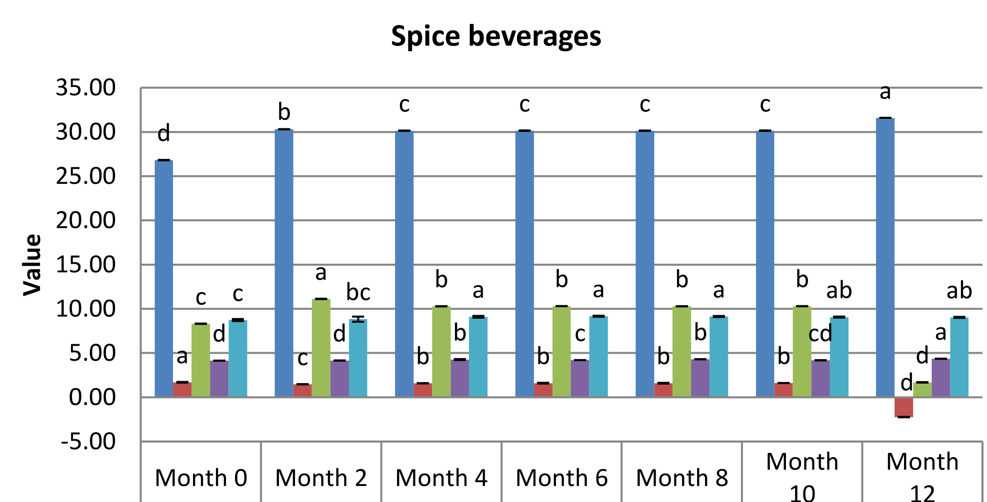
Month	Sample	colour	odour	viscosity	taste	sweetness	sourness	overall acceptability
0	Spice beverages	5.73 ± 1.04^{b}	4.78 ±1.05^{d}	5.33 ± 1.05^{e}	4.70 ±1.11^{c}	4.93 ± 1.14^{e}	4.73 ± 1.04^{d}	4.88 ±1.00^{d}
	Pineapple-spice beverages (25%)	5.75 ± 0.98^{b}	5.18 ± 1.28^{bc}	5.70 ±1.04^{cde}	5.68 ±1.05^{b}	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}

**** Need justification of above data showing incraesing trend in progress with storage days**

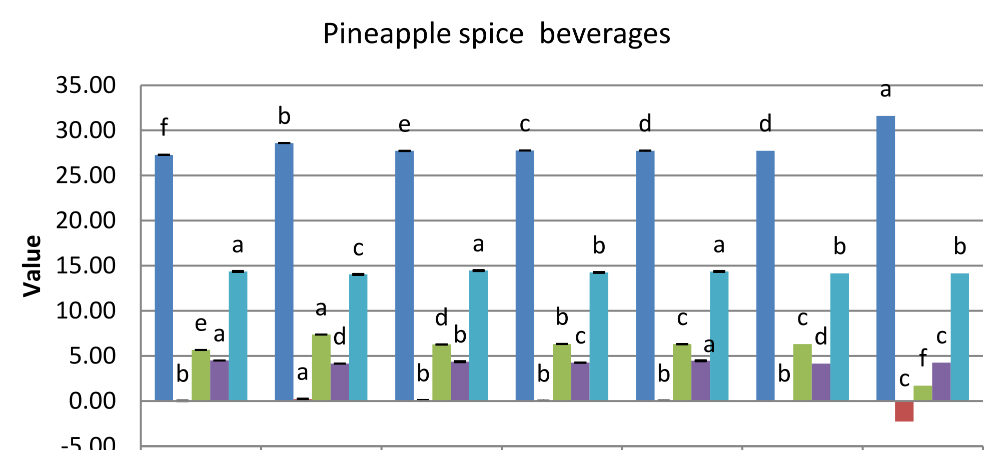
Color occupies an effective role in the appearance of the instant cereal and actively influences the consumer's decision to buy the product. (Ghandehari Yazdi et al., 2020). The color properties in terms of a* (red (+) / green (−), L* (black (0) / white (100)), and b* (yellow (+) / blue (−) values of all beverages are indicated in Figure 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{Hernandez]. . In this studies, both colour of the beverages become lighter after 1 2 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]. 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. [3]). 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, 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, 2017].

A stable pH is important for both beverages and microbial stability. pH can change during storage due to fermentation or chemical reactions within the beverage.Total Soluble Solids (Brix) measures the sugar content, which affects sweetness and taste. Over time, sugar levels might shift due to enzymatic activities or ingredient interactions. (Need more justification about pH changes with refernces as pH increases very slowly after 12 months at ambient temperature which needs clarification, moreover there is no result and discussion about microbiaological storage study which is very important in storage study though it is mentioned in materials and methods portion)



	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



	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

Figure 3. Colour (L*, a*, b*), pH and total soluble solid (°brix) of spice beverages and pineapple spices beverages during storage.

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.

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