J. Trop. Agric. and Fd. Sc. 52 (2)(2024): 39 – 47

Evaluation on stability and quality characteristics of black tea-roselle mixture during storage

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Abstract

Black tea-roselle mixture is a newly developed tea product that combines black tea from *Camelia sinensis* and *Hibiscus sabdariffa* chunks or known as roselle. The tea leaves undergo natural fermentation followed by drying at 110 °C before being mixed with roselle chunks. A study was conducted to assess the quality of black tea-roselle mixture during 12 months of storage at ambient temperature. Every three months, the individual sachet of the black tea-roselle mixture was analysed for its physical properties, microbiological, sensory qualities, and phytochemical levels. During the storage period, the moisture content of the product remained constant, while water activity varied between 0.30 to 0.49. Microbial analysis showed safe levels of total viable count, yeast and mold and coliform count throughout the storage. The phytochemical content of the tea changed during storage. Catechins, a beneficial compound, decreased significantly by almost 50% by the end of the 12 months storage. Theaflavins content vary unpredictably, peaked at month 6. Overall, the black tea-roselle mixture remained physically stable and safe for consumption for at least 12 months when stored at ambient temperature. However, the degradation of the catechins content over time suggested that the product may experience a decrease in its health-promoting properties.

Keywords: *black tea-roselle, physicochemical changes, phytochemical content, microbial quality, sensory acceptance*

Introduction

Tea is a unique beverage and most widely consumed worldwide, second only to water. Tea is processed from tea plant *Camelia sinensis*. The Camelia plant has two varities, *sinensis* (grown in China, Japan and Taiwan) and *assamica* (grown in south India and South East Asia, including Malaysia). Annual production for green tea, oolong tea and black tea are 20%, 2% and 78%, respectively (Kasim 2019). The most suitable leaf part for tea processing is to harvest one shoot with two parts of the nearest leaves. The appropriate time to harvest tea leaves is between 30 – 45 days after each harvesting period. There are two types of technology to produce black tea; orthodox and Cut, Tear and Curl (CTC) technology. Orthodox technology rolls the leaf to mix the internal constituent whereas CTC technology cut, tear and curl the leaf to achieve the same purpose (Theppakorn 2016). Basically, tea processing involves the following processes: picking, withering, rolling, oxidation, drying, sieving, grading and packing.

There are many benefits of consuming tea that have been reported such as a source of antioxidants (Langley-Evans 2000), helping to reduce cholesterol (Singh et al. 2009; Davies et al. 2003), reducing blood pressure (Grassi et al. 2015; Negishi et al. 2004), reducing body fat (Moitra et al. 2013; Chen et al. 2009), reducing blood sugar (Mackenzie et al. 2007), as antibacterial (Turkmen et al. 2007; Naderi et al. 2011), antivirus (Mhatre et al. 2021; Liu et al. 2005; Cantatore et al. 2013) and anticancer (Lee et al. 2002; Luo and Jiang 2021). Black tea is believed to neutralise germs, reduces risk of stroke (Mineharu et al. 2011; Naveed et al. 2018), strengthens immune system (Sava et al. 2001), and protects lungs

Article history
Received: 26.3.24
Accepted: 20.7.24

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from damage (Yen and Chen 1995). Every fresh tea leaf is rich with antioxidant called catechins which account for about 30% of the dry weight of tea leaves (Theppakorn, 2016). Catechins work to eliminate free radicals which lead to cell aging and death. During fermentation, the catechins undergo chemical reaction that convert them to other types of antioxidants called theaflavins and thearubigins. Tea is known for their unique aroma and flavour as well as health-promoting properties to human body. Those health-promoting properties are contributed by a wide range of phytochemical compounds found in tea such as (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), (+)-catechin (C), (-)-gallocatechin (GC), (-)-catechin gallate (CG), and (-)-gallocatechin gallate (GCG) (Pinto 2013).

Black tea, derived from *Camellia sinensis*, contains a complex mixture of compounds with various health benefits. The main polyphenolic compounds in black tea are theaflavins and thearubigins, which are products of tea fermentation (Khan & Mukhtar 2013; Shevchuk et al. 2018). Other important components include catechins, flavonoids like quercetin and kaempferol, phenolic acids, and purine alkaloids such as caffeine and theobromine (Naveed et al. 2018). These compounds contribute to black tea's antioxidant, antimicrobial, and cholesterol-lowering properties (Naveed et al. 2018). Research has shown that black tea consumption may help prevent chronic diseases like cancer and cardiovascular disorders (Khan & Mukhtar 2013). Advanced analytical techniques like HPLC-MS have been employed to identify and quantify these compounds in tea samples, allowing for differentiation based on origin, processing, and botanical varieties (Shevchuk et al. 2018).

Recently, there is also a demand for tea to be mixed with herbs (i.e ginger, lemongrass and tamarind), spices (i.e star anise, clove and cinnamon) as well as other ingredients such as lemon and roselle that provide health benefits, which can be exploited to sell the products. These herbs and spices are generally used to provide an alternate flavour in food and beverages. They rich in flavonoid and phenolic compounds and help to boost immune system. In this study, we developed a black tea from *Camelia sinensis* and mixed it with roselle chunks. Roselle (*Hibiscus sabdariffa* L.) is an edible flower rich in phenolic compounds, particularly in its calyx (Selli et al. 2021). These compounds include anthocyanins, phenolic acids, flavonoids, and their derivatives, which contribute to roselle's potential health benefits and antioxidant properties (Selli et al. 2021; Hapsari et al. 2021). The most abundant phenolic compound in roselle leaves is rutin, with total phenolic content varying among accessions (Lyu et al. 2020). Green roselle extract contains flavonoids, tannins, and triterpenoids, along with 323 compounds identified through LCMS analysis, including 5-hydroxymethyl-2-furaldehyde and curcumin (Fitriaturosidah et al. 2022). The composition and concentration of phenolic compounds in roselle can vary due to growth factors, cultivars, and environmental influences (Hapsari et al. 2021). Proper extraction and separation methods are crucial for reliable identification and quantification of these compounds, which have demonstrated various biological activities and beneficial effects on human health such as antidiabetic properties (Hapsari et al. 2021), antiinflammatory (Mardiah et al. 2015; Nyam et al. 2015), antibacterial (Alaga et al. 2014; Nanthakumar and Dhurgaashini 2020), helps in menstrual pain (Mariod et al. 2021) and aids digestion (Abou-Arab et al. 2011).

The content and stability of these compounds in tea product are depending on many factors during harvesting, processing, and condition of storage. Poor food storage can lead to the growth of harmful bacteria, fungi, and other pathogens that can cause foodborne illness. By studying food storage, it is possible to identify and implement proper hygiene practices, temperature control measures, and packaging methods that help to minimise the risk of contamination and ensure food safety for consumers. The objective of this study was to determine the physicochemical changes, phytochemical content, microbial quality, and sensory acceptance of black tea-roselle mixture when subjected to 12 months of storage at ambient temperature.

Materials and method

Production of black tea sample

Tea leaves were purchased from Bharat Plantation, Cameron Highland, Pahang and was processed according to Kumar et al. (2013) with some modification to produce dried black tea sample. Tea leaves were subjected to withering process for overnight to reduce the moisture content to 50%. Tea leaves then were rolled into a small piece to let it break and release the enzyme that will react to the oxygen. The broken tea leaves were placed on a tray in a room with a controlled temperature of 18 to 22 °C for 60 min to allow natural fermentation to take place. Later, the leaves went through a drying process at a temperature of 110 °C for 4 to 6 hours until the moisture content reached below than 10% of the original weight. The produced dried tea was then kept in a sealed packaging before being stored in a chiller.

Production of roselle chunks

Roselle chunks were prepared using the method outlined by Amoasah et al. (2018). Roselle calyxes were collected from Ekomekar Resources, Terengganu and initially were washed with water to remove impurities such as sand prior to steaming process for 2 min. Steamed roselle calyxes were distributed into oven tray and dried at 60 °C until achieve the moisture content below 5%. The roselle calyxes were then chopped to smaller chunks by food processor and subsequently kept in a sealed packaging before being stored in a chiller.

Packaging and storage study of black tea-roselle mixture

Storage study for black tea-roselle was performed to determine the shelf-life of final product that has been packed with appropriate packaging materials and stored in dedicated storage condition. Two grams of black tea-roselle mixture were packed inside heat sealable cellulose filter paper teabag followed by individual aluminium sachet (OPP/Al/PE, 7 cm x 8 cm). Then, an amount of 20 sachets were packed into a rectangular box (size: length 13 cm x width 9 cm x height 8 cm). The boxes were later kept in a laboratory storage cabinet at ambient temperature (27 ± 3 °C) with a relative humidity of 70 to 80% for 12 months, where sampling intervals for physicochemical changes, phytochemical content, microbial quality, and sensory acceptance analysis were done every three months.

Physicochemical (colour measurement)

The colour changes of black tea-roselle mixture throughout the storage period were determined using a chromameter (Minolta Chroma Meter CR 400, Konica Minolta, Inc, Tokyo, Japan) by measuring the L*, a* and b* parameters. 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. Five measurements on different areas of the sample surface were performed.

Physicochemical (moisture content measurement)

The moisture content of the black tea-roselle mixture was analysed by using infrared moisture analyser (MA 35, Sartorius Lab Instruments GmbH & Co. KG). The sample was placed on an aluminium dish and tested according to the manufacturer's instructions. The sample pan or container of the infrared moisture analyser was opened, and the weighed sample was placed inside. The container was securely closed. Infrared radiation is emitted by the halogen bulb in the instrument, which causes the sample to be heated and the moisture to be evaporated. The weight loss of the sample is continuously measured by the instrument as the moisture evaporated.

Physicochemical (water activity measurement)

Water activity (a_w) was measured with the Aqualab Series 3TE water activity meter (Decagon, USA). Approximately 10 g to 12 g of sample was weighed into a sample cup. The sample cup then was inserted into the water activity device. The meter usually has sensors that touch the sample to measure water activity. The measurement was allowed to stabilise and reach a steady reading, as indicated by the meter. The machine was calibrated with distilled water before commencing measurement of the samples. Triplicate measurements were performed for each sample.

High performance liquid chromatography analysis for phytochemical compound

The phenolics content of tea samples were determined by using High Performance Liquid Chromatography (HPLC). HPLC Agilent 1200 was used for analysis completed with a quaternary pump, column oven and photodiode array detector (PDA). The extraction and chromatographic analysis were performed according to Jabit et al. (2022). Chemical standards used in the study were purchased from Sigma-Aldrich. Briefly, 1 g of tea sample was macerated with 7 mL of boiled water for 10 mins followed by 3 mL of methanol and sonicated for 10 mins. Then, the extracts were filtered with nylon syringe filter of 0.45 μm prior to HPLC injection. HPLC analysis was conducted using a Kinetex 5μ C18 column (250 mm length x 4.6 mm ID). The flowrate was 1 mL/min, and the sample injection volume was 10 µL. The mobile phase consisted of a mixture of solvent A (70% distilled water and methanol with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). A solvent gradient system was employed, starting with 100% solvent A and gradually changing to 80% solvent A at the 10th minute. From the 15th minute to the 20th minute, 20% solvent A was used, and then the system returned to 100% solvent A from the 21st to the 26th minute. Detection was performed at 210 nm to identify catechin and theaflavin.

Microbiological analysis

Microbiological analyses were carried out on final products as quality and safety control. The analyses done were total plate count (TPC), yeast and mold (Y&M) and total coliform (TC) according to the AOAC (2012) method; using the pouring plate technique carried out in the biohazard cabinet. For TPC analysis, the media used was Total Plate Agar (Oxoid, United Kingdom), Potato Dextrose Agar (Oxoid, United Kingdom) added with 10% tartaric acid for Y&M, while for TC, Violet Red Bile Agar (VRBA) was used. A total of 10 g of sample was weighed and diluted with 90 mL of Ringer's (Sigma Aldrich, USA) solution. A 10^1 to 10^5 dilution was carried out. A total of 1 mL of each diluent was transferred into a petri dish before the media were poured in (agar temperature set at 50 °C) and the agar was allowed to solidify at room temperature. All petri dishes for TPC and TC were incubated at the temperature of 37 °C for 24 to 48 h while for Y&M at temperature of 32 °C for approximately 72 h. Observation of the microbial colony's growth was done using a colony counting tool.

Sensory evaluation

Tea products were withdrawn every three months and the sensory evaluation of black tea-roselle mixture was carried out using a 7-point Hedonic rating test (7= Like very much; 6 = Like; 5 = Like slightly; 4 = Neither like nor dislike; 3 = Dislike slightly; 2 = Dislike; 1 = Dislike very much). Products were coded by three-digit random number prior to evaluation. The black tea-roselle mixture (2 g) was infused in 250 mL freshly boiled water for 3 min. Then, 30 mL of the tea infusions was transferred to teacups for sensory test where a group of 35 semi-trained panellists evaluated the tea solution samples on colour, odour, bitterness, sourness, intensity of roselle and overall acceptability. The sensory testing was conducted in a designated panel room with controlled temperature. The panel room was completely free of food or chemical odour, unnecessary sound and mixing of daylight.

Statistical analysis

All data were expressed as means ± standard deviations (SD). Results were compared using one-way analysis of variance (ANOVA) using Tukey's HSD post hoc test with 95% confidence using Minitab 19. All experiments and readings were done in triplicates.

Results and discussion

Physicochemical properties

Water activity (a_w), moisture content and colour of black tea-roselle mixture stored at 27 ± 3 °C with a relative humidity between 70 to 80% for 12 months are as shown in *Table 1*.

The a_w showed an inconsistent trend throughout the storage study and ranged between 0.30 to 0.49. This might be due to the storage condition with high humidity between 70-80% which leads to the absorption of moisture from the surroundings, thereby increasing the water activity. Water activity is a measurement of the availability of water in a food product and its ability to support the growth of microorganisms and perform chemical reactions (Gailani et al. 1987). It is defined as the ratio of the vapor pressure of water in a food sample to the vapor pressure of pure water at the same temperature and pressure (Park 2008). The a_w of food is an important parameter that influences its stability, shelf life and safety. It affects the growth of microorganisms in food. Microorganism have varying water activity requirements for growth. The specific range for spoilage depends on the type of microorganism and the food product itself, such as 0.61 to 0.65 for osmophilic yeast (*Sacharomyces rouxii*), 0.65 to 0.75 for Xerophilic molds *(Aspergillus chevalieri, A. candidus, Wallemia sebi),* 0.80 to 0.87 for most molds and *Staphylococcus aureus*, 0.87 to 0.91 for *S. aureus* (aerobic) and many yeasts *(Candida, Torulopsis, Hansenula)* and 0.91 to 0.95 for *Salmonella*, *Clostridium botulinum*, *Listeria monocytogenes* and *Bacillus cereus* (Tapia et al. 2020). The higher the a_w, the more microorganisms can grow on the food. The value of a_w for black tea-roselle products is below the set limit of 0.5 for dry food. In tea products with low water activity, mold growth is less common due to the low moisture content, but certain molds can still thrive under the right conditions. Some molds that might be encountered in tea products, despite the low water activity, include *Aspergillus spp.*, *Penicillium spp*. and *Cladosporium spp*. These molds are widespread and can produce mycotoxins under certain conditions. They can survive in low moisture environments and might be found in dried tea leaves, particularly if the storage conditions are not optimal.

Moisture content of black tea-roselle mixture ranged between 4.54 to 5.99% and no significant changes were observed even though the samples show an inconsistent trend throughout the storage. This indicates the usage of suitable packaging was able to maintain a constant moisture level during storage. Most of the commercial tea samples (70%) have a moisture content of 6.6% or less and another 30% contains more moisture percentage up to 8% and it is being concluded that the moisture content should be controlled between 2.5 to 6.5% to ensure better quality of food product (Yao et al. 1992). Moisture is an important factor in food quality, preservation, and resistance to deterioration. A high amount of moisture content could have a negative effect on product shelf life.

Moisture content and a_w are two important parameters used to characterise the moisture status of a material, especially in food and pharmaceuticals industries context. Even though the two parameters are related, they represent different aspects of moisture in a substance. Moisture content indicates the absolute amount of water present in a substance. In many cases, both parameters are used together to assess and control the quality and stability of products. For example, controlling a_w is important to prevent microbial growth and enzymatic spoilage, while moisture content affects the physical properties and stability of a material, such as texture, shelf life, and chemical reactivity.

Table 1. Physicochemical changes of black tea-roselle mixture during storage

Storage time (month)	Water activity (a_w)	Moisture content (%)	Colour		
			L*	a*	b*
0	0.30 ± 0.01^{d}	4.54 ± 0.75^{a}	24.37 ± 0.56^{a}	2.88 ± 0.27^{a}	4.72 ± 0.70^{a}
3	0.49 ± 0.03^{a}	5.99 ± 0.97^{a}	22.01 ± 0.27^{b}	2.46 ± 0.83^{a}	2.06 ± 0.34^{b}
6	0.38 ± 0.01^{bc}	4.87 ± 0.66^{a}	21.92 ± 0.38^{b}	2.87 ± 0.47^{a}	0.93 ± 0.54^{b}
9	0.37 ± 0.00^{c}	5.68 ± 0.10^{a}	23.81 ± 0.51^{a}	2.18 ± 0.60^{a}	0.97 ± 0.65^{b}
12	0.42 ± 0.00^{b}	5.53 ± 0.13^{a}	24.52 ± 0.46^{a}	2.83 ± 0.15^{a}	4.04 ± 0.31^{a}

*Values are presented as mean±SD. Values with different superscript within the same column are significantly different ($p < 0.05$)

Colour is one of the important parameters when it comes to the food products as it has an impact on customer preference when buying the products. L* values below 50 indicates that black tea-roselle mixture is relatively darker. Black colour of tea products is caused by a natural fermentation process that happened during the processing of black tea. Traditionally, it is known as fermentation process, but it is mainly enzymatic oxidation of polyphenols by polyphenol oxidases (Kosinska & Andlauer 2014). Unlike any other tea which does not undergo a fermentation process such as green tea and white tea or undergo a short time of fermentation process such as oolong tea, which resulted in a brighter colour of final product. The initial green colour of tea leaves turns into light brown, and deep brown during their oxidation, which indicates formation of theaflavins and thearubigins (Pinto et al. 2013). Generally, no significant changes were observed for colour upon 12 months of storage. Inconsistent value for b* during 3 to 9 months of storage is unpredictable as in cases where the samples are derived from natural sources, inherent biological variability among plant raw materials may contribute to differences in colour characteristics and b* values.

Phytochemical properties

In this research work, catechin and theaflavins were analysed over a period of storage, which as summarised in *Table 2*.

Table 2. Phytochemical compound of black tea-roselle mixture during storage

Storage time (month)	Catechin (mg/g)	Theaflavin (mg/g)
0	9.02 ± 0.02^{a}	0.07 ± 0.01^{d}
3	5.55 ± 0.00^{b}	0.14 ± 0.01^{c}
6	5.75 ± 0.05^{b}	0.32 ± 0.03^{a}
9	3.85 ± 0.15^{c}	0.24 ± 0.03^{b}
12	4.02 ± 0.01^{c}	0.06 ± 0.00^{d}

*Values are presented as mean±SD. Values with different superscript within the same column are significantly different ($p < 0.05$)

Initial content of catechin in black tea-roselle mixture was 9.02 ± 0.02. This is in line with the result obtained by another researcher Adnan et al. (2013), who observed the catechin content in 15 samples of commercial black and green tea were in the range of 0.14 to 7.44 mg/g. Catechin showed a decreasing trend ($p < 0.05$) with a value of 4.02 mg/g at final month of storage. There is almost 55% reduction compared to the initial value of catechin at the beginning of the storage study. The results of the HPLC analysis showed that catechin and theaflavin were unstable over storage at ambient temperature. This causes the occurrence of compound degradation and subsequently affects the content of catechin throughout the storage period. The finding of this study suggested that catechin in tea may not be stable during long term storage. Tea may not be susceptible to spoilage even though stored for a long period due to the low moisture content of the tea itself. However, there is a great decrease in the catechin content due to compound degradation during storage at homes, warehouses, restaurants, and stores, and this could affect the tea's health promoting properties (Theppakorn 2016). Tea considered as a dry product and maybe kept for a long-term storage. Even though it is not susceptible to the spoilage, there is a possibility for catechins and other phytochemicals to degrade over storage. A study by Friedman et al. (2009) reported that the catechins in commercial green tea may not be stable for long term storage in the solid state. Factors such as storage time, temperature, humidity, and light may give a significant effect to the phytochemical content in tea (Pou, 2016). The presence of roselle chunks, even in a small percentage (less than 12%), could also potentially influence the degradation of catechin in the tea mixture. Roselle contains organic acids, such as citric acid and tartaric acid, which could potentially react with catechin through chemical interactions. These interactions may lead to the breakdown of catechin molecules into smaller compounds. Roselle also contains various bioactive compounds, including anthocyanins and flavonoids, which may interact with catechin and influence its stability.

There is limited research done on the stability of catechin during storage especially in sachet-packed dried tea. Chen et al. (2001) conducted a six-month stability study on canned and bottle tea drinks during storage. The study observed the degradation of green tea catechins (GTC) under different pH condition. After 6 months of storage, 23% of GTC dissolved in distilled water was degraded, compared to 55% degradation when dissolved in a pH 4 buffer. The highest degradation occurred when the pH was raised to 5, with over 90% of GTC being lost. These findings highlight the pH sensitivity of GTC, indicating that its stability is significantly affected by the acidity of the solution. Kim et al. (2020) reported that the quality of matcha tea gradually deteriorated upon storage. The moisture content, brightness (L*), green value (G*), total phenolic content, total flavonoid content, antioxidant activity and catechins level of matcha tea gradually decreased at higher temperature and longer storage period.

The inconsistency trend in theaflavins during storage also has been observed. The amount of theaflavins was found to be peak at 6 months of storage, followed by a significant decreased to a final value of 0.06 mg/g sample. Even though moisture content did not show any significant decreased throughout the storage, this inconsistency might be due to any other volatile ingredients-that are slowly lost during long-term storage, as reported by Friedman et al. (2009). Theaflavins is known to be responsible for astringent taste to the black tea and thearubigins causes the bright-orange-red colour in black and oolong tea solution (Wong et al. 2022). The amount of theaflavins for black tea-roselle mixture is considered low compared to previously reported research work by others. Yashin et al. (2015) suggested that the low amount (0.29% to 1.25% of tea dry basis) of theaflavins is due to incomplete fermentation and/or long storage period. Although the complete fermentation process can oxidize more than 75% of the catechins present in tea leaves, resulting in the formation of theaflavins, the concentration of theaflavins in black tea was not high. This is most likely due to the theaflavins has undergo further oxidation, leading to the formation of thearubigins (Wong et al. 2022). Storage of black tea for up to 12 months can affect theaflavins and thearubigins content. The changes in these compounds might happened gradually and continuously throughout the storage period. Light, oxygen, and temperature are the primary factors that can impact tea quality and the stability of catechins during storage (Thomas et al. 2008).

Microbiological properties

Microbiological properties of black tea-roselle mixture during storage were as shown in *Table 3*. Result shows that all products are within the acceptable limits for dried or powdered botanicals according to the Recommended Microbial Limits for Botanical Ingredients, United States Pharmacopoeia Convention, USP-NF 35-30 (2012) indicating that maximum limit for Total Viable Count should not exceed 1.0×10^5 CFU/g and total coliform must be lower than 1.0×10^3 CFU/g. Microbiological analysis shows that total viable count (PCA), yeast and mold (PDA) and coliform count (VRBA) readings are at safe levels throughout the storage period as shown in *Table 3*. Even though microbial monitoring revealed that no harmful bacteria was detected during the storage, attention should be given to practice the hygienic processing procedure and proper storage throughout the production chain, especially for retailers and consumers. Avoid storing the tea in a humid condition as this can lead to the growth of potentially pathogenic bacteria in the tea samples. Carraturo et al. (2018) examined 32 samples of black and green teas available in southern Italy markets and online shops. Microbial levels in over 80% of the samples ranged from 1.0×10^2 to 2.8×10^5 CFU/g of tea, with most microorganisms identified as *Bacillaceae*. Fungi were found in 70% of the samples, with *Aspergillus niger* strains being the most common, followed by *Aspergillus tubingensis*.

Sensory properties

Table 4 presents the sensory characteristics of black tea-roselle mixture upon storage for 12 months at ambient temperature.

The colour, odour, bitterness, sourness, intensity of roselle and overall acceptability of black tea-roselle mixture were evaluated by the panelists. Generally, all attributes possessed the highest score at 12-months of storage, except no significant changes for colour. The respondents assigned the highest scores to the aged tea mixture (stored for 12 months) for specific attributes, including bitterness, sourness, intensity of roselle, and overall acceptability. Aged tea mixtures often undergo complex chemical changes during storage, leading to the development of unique and desirable flavour profiles.

Table 3. Microbiological changes of black tea-roselle mixture during storage

Storage time (month)	Microbiological parameters		
Black tea-roselle mixtures	Total viable count (CFU/g)	Yeast and mold (CFU/g)	Total coliform count (CFU/g)
0	4.0×10	$<1.0 \times 10$	$<1.0 \times 10$
3	4.0×10^2	$<1.0 \times 10$	$<1.0 \times 10$
6	3.8×10^3	$<1.0 \times 10$	$<1.0 \times 10$
9	1.5×10	$<1.0 \times 10$	$<1.0 \times 10$
12	3.8×10	$<1.0 \times 10$	$<1.0 \times 10$

Table 4. Sensory attributes evaluation of black tea-roselle mixture during storage

Storage time (month)	Colour	Odour	Bitterness	Sourness	Intensity of roselle	Overall acceptability
0	6.03 ± 0.80^a	5.47 ± 1.15^c	5.13 ± 1.23^b	5.20 ± 1.25^b	5.07 ± 1.15^b	5.07 ± 1.21^c
3	6.17 ± 0.82^a	6.00 ± 0.82^a	4.97 ± 1.47^c	4.83 ± 1.37^c	4.77 ± 1.36^c	5.10 ± 1.37^c
6	6.13 ± 0.88^a	5.60 ± 1.31^b	5.20 ± 1.17^b	5.07 ± 1.18^b	4.87 ± 1.09^c	5.17 ± 1.13^c
9	6.17 ± 0.69^a	5.63 ± 0.98^b	5.20 ± 1.05^b	5.17 ± 1.27^b	5.10 ± 1.25^b	5.30 ± 1.04^b
12	6.30 ± 0.64^a	5.90 ± 0.91^a	5.43 ± 1.02^a	5.40 ± 0.80^a	5.33 ± 1.01^a	5.57 ± 0.84^a

*Values are presented as mean±SD. Values with different superscript within the same column are significantly different ($p < 0.05$)

Despite a significant decrease in the quantity of chemical compounds upon storage, this result suggests that the panelists prefer tea samples that have been aged for a longer period. Decreased catechins content contributed to the less astringent and bitter taste of the black tea. This might also be contributed possibly by the presence of roselle calyx which could make it sweeter rather than bitter. During storage, tea undergoes chemical changes that can significantly impact its flavour, aroma and overall characteristic (Friedman et al. 2009). Catechins and caffeine, which are major bioactive compounds in tea, can undergo chemical transformation during storage. For example, catechins may undergo polymerisation reactions, leading to the formation of complex compounds that can impact the taste and health benefits of the tea.

Conclusion

The tea product packed in aluminium individual sachet made of OPP/Al/PE with 0.10 mm thickness were stable in physical and microbiological qualities up to 12 months of storage at 27 ± 3 °C (70 – 80% RH). The product did not show any sign of microbial growth and any significant changes in the value of moisture content and colour parameter, but some slight changes in a_w were detected throughout the storage periods. The microbial results of black tea-roselle mixture were within the range limits for the dried or powdered botanicals foods category according to the United States Pharmacopeial Convention, USP-NF 35–30 (2012). However, the phenolics content in the black tea-roselle mixture sample was relatively unstable for a period of 12 months of storage. Catechins decreased significantly resulted with final value of 4.02 mg/g sample at 12 months. Theaflavins showed inconsistent trends throughout the storage periods. Further investigation on the potential health effects of consuming the black tea-roselle mixture over an extended period could be explored. Long-term studies could focus on parameters such as antioxidant activity, cardiovascular health markers, or immune function to determine if the decrease in catechin content affects the product's health-promoting properties.

Conflict of interest

The authors declare no conflict of interest.

References

Adnan, M., Ahmad, A., Ahmed, A., Khalid, N., Hayat, I. & Ahmed, I. (2013). Chemical composition and sensory evaluation of tea (*Camellia sinensis*) commercialized in Pakistan. *Pakistan Journal of Botany, 45*, 901–907.

AOAC. (2012). Association of Official Analytical Chemists, Official methods of analysis. 19th ed., Washington, DC: Association of Analytical Chemists.

Abou-Arab, A. A., Abu-Salem, F. M. & Abou-Arab, E. A. (2011). Physico- chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (*Hibiscus subdariffa*). *Journal of American Science*; 7(7): 445–456.

Alaga, T. O., Edema, M. O., Atayese, A. O. & Bankole, M. (2014). Phytochemical and in vitro anti-bacterial properties of *Hibiscus sabdariffa L* (Roselle) juice. *Journal of Medicinal Plants Research, 8(7),* 339–344.

Amoasah, B., Appiah, F. & Kumah, P. (2018). Effects of Different Drying Methods on Proximate Composition of Three Accessions of Roselle (*Hibiscus sabdariffa*) Calyces. *International Journal of Plant and Soil Science, 21*, 1–8.

Cantatore, A., Randall, S. D. & Traum, D. (2013). Effect of black tea extract on herpes simplex virus-1 infection of cultured cells. *BMC Complementary and Altern Medicine* 13, 139.

Carraturo, F., De Castro, O. & Troisi, J. (2018). Comparative assessment of the quality of commercial black and green tea using microbiology analyses. *BMC Microbiol.* 18, 4 (2018).

Chen, Z.-Y., Zhu, Q. Y., Tsang, D. & Huang, Y. (2001). Degradation of Green Tea Catechins in Tea Drinks. *Journal of Agricultural and Food Chemistry,* 49(1), 477–482.

Chen, N., Bezzina, R., Hinch, E., Lewandowski, P. A., Cameron-Smith, D., Mathai, M. L., Jois, M., Sinclair, A. J., Begg, D. P., Wark, J. D., Weisinger, H. S. & Weisinger, R. S. (2009). Green tea, black tea, and epigallocatechin modify body composition, improve glucose tolerance, and differentially alter metabolic gene expression in rats fed a high-fat diet. *Nutrition Research.* 29(11):784–93.

Davies, M. J., Judd, J. T., Baer, D. J., Clevidence, B. A., Paul, D. R., Edwards, A. J., Wiseman, S. A., Muesing, R. A. & Chen, S. C. (2003). Black tea consumption reduces total and LDL cholesterol in mildly hypercholesterolemic adults. *The Journal of Nutrition*, *133*(10), 3298S–3302S.

Fitriaturosidah, I., Kusnadi, J., Nurnasari, E., Nurindah, & Hariyono, B. (2022). Phytochemical screening and chemical compound of green roselle(Hibiscus sabdariffa L.) and potential antibacterial activities. *IOP Conference Series: Earth and Environmental Science, 974*.

Friedman, M., Levin, C. E., Lee, S. U. & Kozukue, N. (2009). Stability of green tea catechins in commercial tea leaves during storage for 6 months. *Journal of Food Science*, *74*(2), H47–H51.

Gailani, M. B., Fung, D. Y. C. & Kraft A. A. (1987) Critical review of water activities and microbiology of drying of meats, CRC *Critical Reviews in Food Science and Nutrition*, 25:2, 159-183.

Grassi, D., Draijer, R., Desideri, G., Mulder, T. & Ferri, C. (2015). Black tea lowers blood pressure and wave reflections in fasted and postprandial conditions in hypertensive patients: a randomised study. *Nutrients*, *7*(2), 1037–1051.

Hapsari, B. W., Manikharda & Setyaningsih, W. (2021). Methodologies in the analysis of phenolic compounds in Roselle (*Hibiscus sabdariffa* L.): Composition, biological activity, and beneficial effects on human health. *Horticulturae*, 7: 35, 1–41.

Jabit, M. L., Daud, M. N., Abidin, N. Z. & Hussin, F. (2022). Quality assurance of tea leaf and its products using selected major chemical markers. *Food Research* 6 (Suppl. 2): 64–68.

Kasim, M. (2019). Tea production in Malaysia: Culture versus challenges and prospect to Malaysian economics. *International Journal of Research and Innovation in Social Science*, Vol. III, Issue VI.

Khan, N. & Mukhtar, H. (2013). Tea and health: studies in humans. *Current Pharmaceutical Design, 19 34*, 6141-7.

Kim, J. M., Kang, J. Y., Park, S. K., Han, H. J., Lee, K. Y., Kim, A. N., Kim, J. C., Choi, S. G. & Heo, H. J. (2020). Effect of storage temperature on the antioxidant activity and catechins stability of Matcha (*Camellia sinensis*). *Food Science and Biotechnology*, *29*(9), 1261–1271.

Kosinska, A. & Andlauer, W. (2014). Chapter 12-Antioxidant capacity of tea: effect of processing and storage. In: Preedy, V. (Ed.), Processing and impact on antioxidants in beverages (pp. 109-120). Academic Press, San Diego.

Kumar, R. S. S., Murugesan, S., Kottur, G. & Gyamfi, D. (2013). Black tea: The plants, processing/ manufacturing and production. *Tea in health and disease prevention*, *5*, 41-57.

Langley-Evans S. C. (2000). Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. *International Journal of Food Sciences and Nutrition*, *51*(3), 181–188.

Lee, K. W., Lee, H. J. & Lee, C. Y. (2002). Antioxidant activity of black tea vs. green tea. *The Journal of Nutrition*, *132*(4), 785–786.

Liu, S., Lu, H., Zhao, Q., He, Y., Niu, J., Debnath, A. K., Wu, S. & Jiang, S. (2005). Theaflavin derivatives in black tea and catechin derivatives in green tea inhibit HIV-1 entry by targeting gp41. *Biochimica et biophysica acta*, *1723*(1-3), 270–281.

Luo, T. & Jiang, J. G. (2021). Anticancer effects and molecular target of theaflavins from black tea fermentation in vitro and in vivo. *Journal of Agricultural and Food Chemistry*, 69(50), 15052-15065.

Lyu, J. I., Kim, J. M., Kim, D., Kim, J., Kim, S. H., Ahn, J., Kang, S., Ryu, J. & Kwon, S. (2020). Phenolic Compound Content of Leaf Extracts from Different Roselle (Hibiscus sabdariffa) Accessions. *Plant Breeding and Biotechnology*. 8: 1–10.

Mackenzie, T., Leary, L. & Brooks, W. B. (2007). The effect of an extract of green and black tea on glucose control in adults with type 2 diabetes mellitus: double-blind randomized study. *Metabolism: Clinical and Experimental*, *56*(10), 1340–1344.

Mardiah, Zakaria, F. R., Prangdimurti, E. & Damanik, R. (2015). Anti-inflammatory of Purple Roselle Extract in Diabetic Rats Induced by Streptozotocin. *Procedia Food Science, 3*, 182–189.

Mariod, A. A., Tahir, H. E. & Mahunu, G. K. (2021). Composition of *Hibicus sabdariffa* calyx, pigments, vitamins. In: C Newman (Ed.). Roselle (*Hibicus sabdariffa*): Chemistry, production, products, and utilization (69-75). Academic Press, London.

Mhatre, S., Srivastava, T., Naik, S. & Patravale, V. (2021). Antiviral activity of green tea and black tea polyphenols in prophylaxis and treatment of COVID-19: A review. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *85*, 153296-153286.

Mineharu, Y., Koizumi, A., Wada, Y., Iso, H., Watanabe, Y., Date, C., Yamamoto, A., Kikuchi, S., Inaba, Y., Toyoshima, H., Kondo, T. & Tamakoshi, A. (2011). Coffee, green tea, black tea and oolong tea consumption and risk of mortality from cardiovascular disease in Japanese men and women. *Journal of Epidemiology and Community Health*, *65*(3), 230–240.

Moitra, S. & Sen, G. (2013). Black tea as a part of daily diet: a boon for healthy living. *Journal of Tea Science, 9*, 51–55.

Naderi, N. J., Niakan, M., Kharazi Fard, M. J. & Zardi, S. (2011). Antibacterial activity of Iranian green and black tea on *Streptococcus mutans*: an in vitro study. *Journal of Dentistry (Tehran, Iran)*, *8*(2), 55–59.

Nanthakumar, T. & Dhurgaashini, V. (2020). Preliminary phytochemical screening and antibacterial evaluation of *Hibiscus sabdariffa L.* (Roselle leaf) against *Staphylococcus aureus* (food borne bacteria). *City University eJournal of Academic Research (CUeJAR)*, 2(2), 124–132.

Naveed, M., BiBi, J., Kamboh, A. A., Suheryani, I., Kakar, I., Fazlani, S. A., FangFang, X., Kalhoro, S. A., Yunjuan, L., Kakar, M. U., Abd El-Hack, M. E., Noreldin, A. E., Zhixiang, S., LiXia, C. & XiaoHui, Z. (2018). Pharmacological values and therapeutic properties of black tea (*Camellia sinensis*): A comprehensive overview. *Biomedicine & Pharmacotherapy*, *100*, 521–531.

Negishi, H., Xu, JW., Ikeda, K., Njelekela, M., Nara, Y. & Yamori, Y. (2004). Black and green tea polyphenols attenuate blood pressure increase in stroke-prone spontaneously hypertensive rats, *The Journal of Nutrition*, Volume 134, Issue 1, January 2004, Pages 38–42.

Nyam, K. L., Sin, L. N. & Kamariah, L. (2015). Phytochemical analysis and anti-inflammatory effect of kenaf and roselle seeds. *Malaysian Journal of Nutrition, 21*, 245–254.

Park, Y. W. (2008). Moisture and water activity. In Handbook of Processed Meat and Poultry Analysis, 1st Ed.; Nollet, L.M.L., Toldra, F., Eds.; pp. 35–40. CRC Press: Boca Raton, FL, USA; London, UK; New York, NY, USA.

Pinto, M. D. (2013). Tea: A new perspective on health benefits. *Food Research International, 53*, 558–567.

Pou, K. R. (2016). Fermentation: The key step in the processing of black tea. *Journal of Biosystems Engineering*. *41*(2), 85–92.

Sava, V. M., Galkin, B., Hong, M., Yang, P. & Huang, G. S. (2001). A novel melanin-like pigment derived from black tea leaves with immuno-stimulating activity. *Food Research International, 34*, 337–343.

Selli, S., Guclu, G., Sevindik, O. & Kelebek, H. (2021). Chapter 10- Aroma, Aroma-Active, and Phenolic Compounds of Roselle. Production, Products and Biocomposites. *Roselle*. 143–164.

Shevchuk, A., Jayasinghe, L. & Kuhnert, N. (2018). Differentiation of black tea infusions according to origin, processing and botanical varieties using multivariate statistical analysis of LC-MS data. *Food Research International, 109*, 387–402.

Singh, D. K., Banerjee, S. & Porter, T. (2009). Green and black tea extracts inhibit HMG-CoA reductase and activate AMP kinase to decrease cholesterol synthesis in hepatoma cells. *The Journal of Nutritional Biochemistry, 20(10)*, 816–22.

Tapia, M. S., Alzamora, S. M. & Chirife, J. (2020). Effects of water activity (a_w) on microbial stability as a hurdle in food preservation in Water activity in foods: Fundamental and Application, 2^{nd} Edition, 323–325.

Theppakorn, T. (2016). Stability and chemical changes of phenolic compounds during Oolong tea processing. *International Food Research Journal, 23*, 564–574.

Thomas, J. P., Senthilkumar, R., Kumar, R. R., Mandal, A. & Muraleedharan, N. (2008). Induction of γ irradiation for decontamination and to increase the storage stability of black teas. *Food Chemistry, 106*, 180–184.

Turkmen, N., Velioglu, Y. S., Sari, F. & Polat, G. (2007). Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. *Molecules, 12(3)*, 484–496.

United States Pharmacopoeia Convention, USP-NF 35-30 (2012)

Wong, M., Sirisena, S. & Ng, K. (2022). Phytochemical profile of differently processed tea: A review. *Journal of Food Science*, *87(5)*, 1925–1942.

Yao, L. H., Cheng, C., Chen, Y. & Liu, Y. (1992). The kinetics of green tea infusion. *Journal Food Sci*ence, *13(1)*: 3–6

Yashin, A. Y., Nemzer, B., Combet, E. & Yashin, Y. I. (2015). *Determination of the Chemical Composition of Tea by Chromatographic Methods: A Review*. *Journal of Food Research*, *4(3),* 56–87

Yen, G. & Chen, H. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry, 43*, 27–32