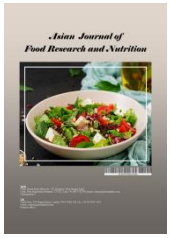

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Development of a Ready-to-serve Beverage Using Coriander *(Coriandrum sativum)*, Ginger *(Zingiber officinale)* and Long Pepper (*Piper longum*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Coriander, ginger and long pepper are well known for the medicinal properties and are commonly used as ingredients in the ayurvedic system of medicine. This study focused on developing a ready-to-serve (RTS) herbal drink using coriander, ginger and long pepper to meet the expectations of health-conscious consumers. Required amounts of roasted coriander seeds, dry ginger and long pepper were mixed with a required amount potable water, boiled at 100℃ until the volume was reduced to the desired level and filtered. The extract was mixed with the three different sweeteners, kithul treacle, coconut treacle and sugar, heated up to 100℃ and filled into heat stable polyethylene

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terephthalate (PET) bottles and capped well. Sensory evaluation was carried out using a screened and trained sensory panel to select the most preferred sweetener. The pH and Brix values of the developed herbal drink were measured. Aerobic plate count, yeasts and molds count and coliforms / *E. coli* count of the herbal drink were determined after 7 days. Freeze-dried herbal drink was used to determine the antioxidant properties using in vitro bioassays. Coconut treacle was selected as the most preferred sweetener. The pH and Brix values of the developed herbal drink were 4.98 and 6.2º, respectively. It complied with the microbiological limits prescribed in the Sri Lanka Standard specification for RTS fruit drinks. The developed herbal drink contains phenolic compounds including flavonoids and possessed abilities to scavenge DPPH (2,2-diphenyl-1-picryl-hydrazyl) radicals, reduce ferric ions and chelate ferrous ions. These findings indicated the antioxidant potential of the developed herbal drink.

Keywords: Coriander; ginger; ready-to-serve drink.

1. INTRODUCTION

Today, due to the emerging trend of non-communicable diseases (NCD) and the risk of viral epidemics, much attention has been paid to the local Ayurvedic plants and their biodiversity and their ability to control the above-mentioned conditions. In this study, a healthy beverage based on three plants in Ayurvedic practice commonly found in Sri Lanka was evaluated. It is important to look at the chemical and ayurvedic value of each plant by conducting laboratory research and to identify the important health and economic values that can be achieved through it more effectively [1].

Ginger (*Zingiber officinale*), coriander (*Coriandrum sativum*), and long pepper (*Piper longum*) are herbs with Ayurvedic values that contribute to enhancing the bioactivity and organoleptic properties of a natural beverage [2]. There are several different species of these three herbs reported in Sri Lanka and this research focused to select the most suitable and effective species for product development.

The main drawbacks were identified as the unavailability of ayurvedic-based therapeutic beverages in ready-to-drink form, and the lack of their use among the young generation. In addition, their complex time-consuming processing and poor sensorial properties further restrict their popularity. To overcome this issue, the objective of this study was to develop a ready-to-serve product that can be used conveniently regardless of age individual age limits, or any other restrictions.

Further potential to enhance a natural product as an immune-boosting beverage and the wide range of opportunities available for making it viable in the market was also important objective [3].

The main objective for this study was:

- Development of coriander (*Coriandrum sativum*), ginger (*Zingiber officinale*) and long pepper (*Pipper longum)* based ready to serve beverage.

The sub objectives were:

- To select the most suitable formula to develop a coriander (*Coriandrum sativum*), ginger (*Zingiber officinale*) and long pepper (*Piper longum*) based ready to serve beverage.
- To carry out the sensory evaluation.
- To determine the antioxidant activity, microbiological quality and physio-chemical properties.

The main advantages of this study can be identified as the successful development ready to serve convenient beverage using locally available ayurvedic plants by following scientifically validated protocols. All activities of this study are in accordance with validated scientific methods.

Further, the natural flavors and aromas of coriander and ginger might be a deterrent for acceptance. Therefore, these products will provide better sensorial properties with extended shelf life.

2. LITERATURE REVIEW

2.1 Introduction

Ayurvedic medicinal plants are widely used for the treatment of different types of diseases. This study was focused on developing a ready-to-

serve herbal beverage using coriander (*Coriandrum sativum*), ginger (*Zingiber officinale*), and long pepper (*Pepper longum*) which are well known for their medicinal properties in the traditional system of medicine.

The raw materials were obtained from pre-identified locations. This enabled the gathering of accurate experimental data for finalizing the best formula. In addition to the product development process, it also aims to perform quality testing, testing of sugar levels, and bioactivity.

2.2 Coriander (*Coriandrum sativum*) as the Raw Material

Coriander (*Coriandrum sativum*) is a valuable plant that belongs to the family *Apiaceae (Umbelliferae)* [4]. It grows well over many years in many temperate climates in the Middle East, Latin America, Africa and Asia [5]. Coriander is popular in ayurvedic medicine as well as in everyday life as a flavoring agent and as an immunomodulatory drink.

The edible part of coriander comprises leaves and seeds. Research suggested that coriander seeds, which are mainly used as a spice, have health benefits and are rich in ayurvedic values in daily life [6]. Coriander seeds are spherical and aromatic. However, its bitter taste restricts its intake [4]. However, coriander which has high medicinal value is a valuable ayurvedic medicine that can be prepared and used in several forms.

Another use of coriander is in the oil extracted from coriander [7]. The oil can be used primarily in the manufacture of perfumes, body care products, and the blending of perfumes [7]. Different parts of this plant can be identified for different uses and properties as different parts of the plant have been reported for different health activities and different biological activities [8].

Coriander has traditionally been used as a treatment for gastrointestinal disorders, anorexia, diarrhea, pain, and vomiting, and the control of fever and body temperature [9].

Table 1. Taxonomic Hierarchy of coriander [1]

Kingdom	*Plantae*
	Viridiplantae – green plants
	Streptophyta (land plants)
	Embryophyta
Division	*Tracheophyta*
Subdivision	*Spermatophytina*
Class	*Magnoliopsida*
	Asteranae
Order	*Apiales*
Family	*Apiaceae*
Genus	*Coriandrum L.* – coriander
Species	*Coriandrum sativum L.* – Chinese-parsley, Chinese parsley, coriander

Fig. 1. Coriander seeds and leaves ("coriander | Definition, History, Uses, Seeds, Leaves, & Facts", 2022)

Among the important chemical composition of the coriander plant, the essential oils and fat content of the seeds are crucial. Essential oils and fat contribute to 25% of the seed [7]. Which also contributes to the characteristic aroma of coriander seed. It is mainly composed of petroselinic acid and contains octadeconoic acid, which has an important characteristic chemical composition [8].

In addition, it contains linoleic acid as well as oleic and palmitic acid. It was also found that the neutral lipid composition (NL) ranged from 93.0 to 95.65%. In addition, it contains lipids and triacylglycerol (TAG) and the remaining NLs are glycolipids GL (4.14%), followed by phospholipids (PL) (1.57%). The main markers of sterols (ST) are stigmas sterol, β-sitosterol, Δ5-arenasterol, 24-stigmastadienol, and campsterol. Total ST is estimated to be in the range of 36.93–51.86 mg/g [3].

In addition to fats and oils, coriander seeds contain important nutrients such as sodium, carbohydrates, dietary fiber, sugar, proteins, calcium, iron and potassium [5]. Previous studies revealed that the nutrients contained in it have the potential to protect skin-related health, treat diabetes, and boost immunity and lower cholesterol levels [3,5].

2.3 Ginger (*Zingiber officinale*) as the Raw Material

Ginger (*Zingiber officinale*), is primarily an aromatic spice that belongs to the Zingiberaceae family. Ginger is known as a medicinal plant [10]. It is a perennial plant mainly native to Southeast Asia. Ginger grows well mainly in the tropics and subtropics [11].

Ginger grows best in warm and humid climates, considering the climatic conditions that contribute to its growth [13,14]. Also, growth is best when the average annual rainfall is more than 2000 mm thus plant reach its optimum height of 300-900 m [15].

Table 2. Taxonomic hierarchy of ginger [12]

Kingdom	*Plantae* – plantes, Planta, Vegetal, plants
Subkingdom	*Viridiplantae* – green plants
Infrakingdom	*Streptophyta* (land plants)
Super division	*Embryophyta*
Division	*Tracheophyta*
Subdivision	*Spermatophytina*
Class	*Magnoliopsida*
Superorder	*Lilianae - monocots, monocotyledons*
Order	*Zingiberales*
Family	*Zingiberaceae* – Ginger Family
Genus	*Zingiber Mill.* – ginger
Species	*Zingiber officinale Roscoe* – garden ginger

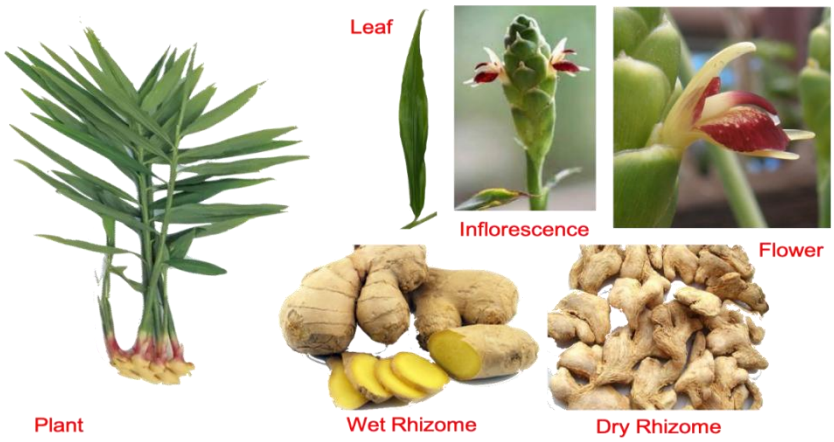

Fig. 2. Parts of the ginger plant [12]

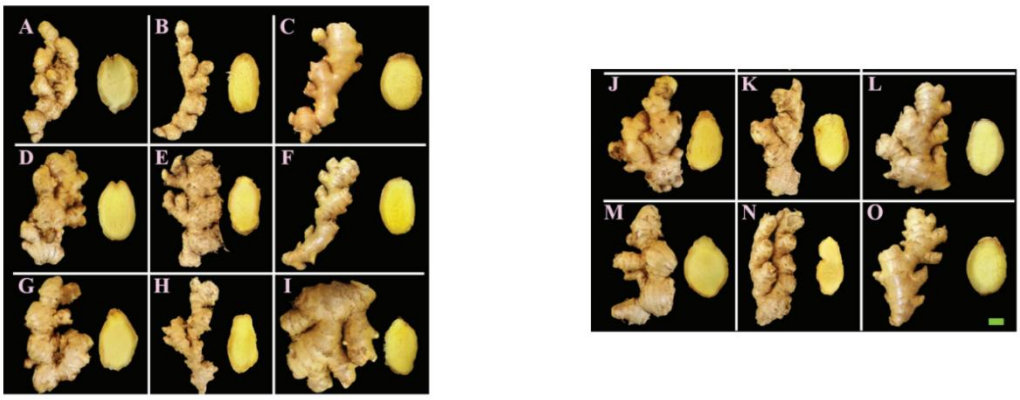

Fig. 3. The external appearance and cross-sectional views of mature ginger rhizomes. Scale bar represents 1 cm. A, D, G, J, and M: Chinese ginger (CG), B, E, H, K and N: Local ginger (LG), C, F, I, L and O: Rangoon ginger (RG) [10]

The cultivation of ginger in Sri Lanka is ubiquitous. However major harvest comes from the wet and intermediate zones of the island. In addition, ginger is popular in Sri Lanka both as a valuable cash crop and export crop [10]. Several major varieties of ginger grown in Sri Lanka can be identified. Local ginger (LG) (*Beheth Inguru* or *Siddha Ginger*), Chinese ginger (CG), and Rangoon ginger (RG) [10]. These ginger varieties are very similar in their morphological features and appearance. There is a slight difference in the size of the ginger rhizomes and leaves. It can also be seen that their growth is vary depend on the growing environmental conditions [11]. Several studies reported on different ginger species of Sri Lanka and also highlighted their genetic features.

The major economically important part of ginger is its rhizome. Ginger is one of the most widely used medicinal plants in the world and has been used since ancient times for treating various infections and for synthesizing ayurvedic products. Ginger is currently used in the manufacture of perfumes and related products [16]. In addition, ginger has many medicinal properties that are used to treat various ailments. Ginger is said to be one of the best anti-inflammatory drugs for asthma, cough, nausea, and digestive disorders [17].

Ginger is also known to have anti-microbial properties that are active against many pathogenic microorganisms [17]. It has also been found to have anti-cancer properties. Further, it is active in treating certain NCDs such as diarrhea and skin infections [18]. The chemical composition and nutrient profile of ginger added value to it. Fresh ginger contains a high amount of protein. In addition, it contains lipids, minerals, fiber, sodium, calcium, and vitamins [15,19].

2.4 Long Pepper (*Piper longum*) as the Raw Material

Long pepper is a special aromatic herb that has been with us for centuries owing to its broad curative properties. The conventional herb goes by the botanical name *Piper longum* and comes from the “Piperaceae” family [20]. The all-encompassing science of Ayurveda classifies this herb as a strong herb that has strong anti-bacterial and aphrodisiac effects on the body and is widely utilized for treating diabetes, asthma, indigestion, cough, and cold [20].

Table 3. Taxonomic Hierarchy of long pepper [21]

Kingdom	***Plantae – plantes, Planta, Vegetal, plants***
Phylum	*Spermatophyta*
Subphylum	*Angiospermae*
Class	*Dicotyledonae*
Order	*Piperales*
Family	*Piperaceae*
Genus	*Piper*
Species	*Piper longum*

Clad by the vernacular names ‘Pipli’ in Hindi, ‘Tippili’ in Tamil, ‘Pippalu’ in Telugu, ‘Tippali’ in Malayalam, and ‘Pipul’ in Bengali, the long pepper is indigenous to several parts of India, Malaysia, Indonesia, Singapore, and Sri Lanka [22]. In cultivation, it is mostly found at a height of 1800 meters [23].

Fig. 4. Long pepper [21]

Long pepper contains a lot of health benefits. It is good for diabetics as it helps control the sugar levels in the blood. Long pepper avoids liver ailments from occurring, such as jaundice. It can fight against bacterial infections and is good for weight loss as well [23,20].

2.5 Food Additives

In addition to the main ingredients (coriander, ginger, and long pepper) in the ready-to-serve beverage, additives contribute to the sensory properties of the final products and control the aroma and color. Additionally, various benefits can be achieved through additives. In this study, treacle has been an additive to add sweetener to RTS and control its strong flavors.

The product is primarily intended to be an instant drink that is suitable for a wide range of age groups and should appeal to a wide range of people. Treacle is used as a natural additive and does not contain any artificial chemical substances [24].

Considering natural treacle rich in unique nutritional and medicinal properties. Its contribution to sensory properties [25] and enhance the shelf life of the final product and minimizes the impact of external microbial infections and other environmental influences on the product [25].

Natural treacle is a sticky and viscous solution composed of 80-85% carbohydrates. Glucose and fructose are contained as carbohydrates. In addition, 15-17% water, 0.1-0.4% protein, 0.2% ash, and very small amounts of amino acids, vitamins, enzymes, and phenolic antioxidants [24,25] are present.

2.6 Sensory Evaluation and the Test Comparison

The final product of a newly formulated food/beverage needs to be evaluated for its sensory quality. Primarily in an evaluation survey, different flavors, different colors, and different aromas of the product are evaluated. Researchers will also be able to present an evaluation report on each component of the product and their comparative information. As a result, the product has the potential to be more effective and efficient [26].

Sensory evaluation provides a clear understanding with regards to product quality and consumer acceptance before. It can also lead to a better exploration of the individual ingredients and other food-related ingredients used in the manufacturing process, as well as a broader understanding of the changes that need to be made during production and quality control to suit customer preferences [26].

2.7 Chemical Testing and Microbial Testing for Product

Evaluation of proximate composition as well as microbiological quantity is essential to make an accurate assessment of the quality of the final product. The microbiological evaluation includes total plate count, total coliform count, and yeast and molds count. Further, turbidity, ash content, pH level, acidity level, Brix value, contributes to physio-chemical properties [2]. In addition, a

study of storage temperature and shelf life period is critical [2].

3. MATERIALS AND METHODOLOGY

3.1 Materials

The research study was conducted at the Nutritional Biochemistry and Functional Food Laboratory of Food Technology Section, Industrial Technology Institution, Malabe, Sri Lanka.

Folin-Ciocalteu phenol reagent, gallic acid and sodium carbonate were purchased from Sigma Aldrich, MO, USA.

Coriander (*Coriandrum sativum*), fresh ginger (*Zingiber officinale*), dry ginger (*Zingiber officinale*) and long pepper (*Piper longum*) were purchased from the local market in Galle, Sri Lanka. Kithul treacle, coconut treacle and refined white sugar were purchased from the local market in Malabe, Sri Lanka.

3.1.1 Utensils and equipment

Analytical balance, grinder, chopping boards, knife, spoons, pans, thermometer, measuring cylinders, tea filter and muslin cloths were used for product development. Microplate reader (SpectraMax Plus384, Molecular Devices Inc., USA) and chroma meter (CR-400, Konica Minolta Inc., Japan) were used for analysis.

3.1.2 Research procedure

The research procedure consisted of several key steps. Firstly, raw materials were collected from predetermined locations, taking into consideration factors such as accessibility, availability, and relevance to the research objectives. Subsequently, the collected raw materials underwent washing and cleaning processes to eliminate impurities. Afterward, the raw materials were processed and a suitable formula was determined for the experiment. The next step involved boiling and filtering the materials to extract desired components. Following this, formulas with different flavors were developed to cater to varying preferences. A sensory evaluation was then conducted to assess the organoleptic properties of the formulations. Based on the results of the sensory evaluation, the best formula was selected. In order to further analyze the quality of the selected formula, the pH value, brix value, and total phenolic content were measured. Additionally, microbial analysis was performed to ensure the safety and hygiene of the product. The shelf life of the formulation was also examined to determine its longevity and stability. Finally, the nutritional properties of the product were determined, providing valuable insights into its potential health benefits.

3.2 Methodology

3.2.1 Preparation of the beverage

The first set of ready-to-serve beverage samples was prepared using fresh ginger. Required amounts of coriander seeds, fresh ginger, and long pepper were measured (Table 4). Coriander seeds were roasted at 100℃ for 3 minutes. Ginger and long pepper were cut into small pieces (0.5 – 1.0 cm). Roasted coriander seeds along with ginger and long pepper pieces were mixed with 500 ml of potable water and boiled at 100℃ until the volume was reduced down to 100 ml. The mixture was filtered and mixed with 15 ml of kithul treacle.

According to the pre-decided formula by Industrial Technology Institute several replicates were tested and the most suitable sample was selected for further development.

Table 4. Amounts of coriander, fresh ginger, and long pepper used for 100g mixture for the first set of beverages

Formula	Coriander (g)	Fresh ginger (g)	Long pepper (g)
F1	66.6	25	8.4
F2	53.28	40	6.72
F3	58.11	32.72	9.16
F4	49.92	37.48	12.59

F1: Formula No.1, F2: Formula No.2, F3: Formula No.3, F4: Formula No.4

Table 5. Amounts of coriander, dry ginger, and long pepper used for the second set of beverages

Formula	Coriander (g)	Dry ginger (g)	Long pepper (g)	Initial volume (ml)	The final volume (ml)
FC1	66.6	25	8.33	1725	360
FC2	53.30	39.97	6.71	1500	750
FC3	66.6	25	8.33	1725	150
FC4	66.6	25	8.33	2300	200

FC1: Formula No.1, FC2: Formula No.2, FC3: Formula No.3, FC4: Formula No.4

The second set of ready-to-serve beverage samples was prepared using dry ginger. Required amounts of potable water, coriander seeds, dry ginger, and long pepper were measured (Table 5). Coriander seeds were roasted at 100 ℃ for 3 minutes. Ginger and long pepper were cut into small pieces (0.5 -1.0 cm). Roasted coriander seeds along with ginger and long pepper pieces were mixed with potable water, boiled at 100 ℃ to reduce the volume and the mixture was filtered. From each formula, three 100 ml samples were separated and mixed with kithul treacle (15 ml), coconut treacle (15 ml), and sugar (7 g) separately.

3.2.2 Evaluation of sensory properties

Sensory evaluation was carried out according to the methods described by Bornare and Sumaiya [27] and Boyapati [28] in isolated booths using 10 panelists of the screened and trained sensory panel of ITI with a seven-point hedonic scale where scores 1,2,3,4,5,6 and 7 represented extremely dislike, moderately dislike, slightly dislike, neither like nor dislike, slightly like, moderately like and extremely like, respectively. Each sample was presented in a transparent drinking glass labeled with a random three-digit code. Potable water was provided for mouth–rinsing between each tasting to avoid carryover effects. The panelists were given written instructions and asked to evaluate the drink for appearance, color, odor, texture, taste, after taste, and overall acceptability. The ballet paper is presented in Appendix.

3.2.3 Determination of physico-chemical properties

The pH values of the beverages were measured according to the instructions given in the operating manual of the pH meter (CyberScan pH 510, Eutech Instruments, Singapore). The Brix values of the samples were measured according to the instructions given in the operating manual of the digital refractometer (Atago Co., Ltd, Japan).

After selecting the most suitable sample from the sensory analysis the aerobic plate count, yeasts and molds count and Coliforms / *E. coli* count of the drink were determined according to the methods described in the Sri Lanka Standards. A freeze-dried drink was used to determine antioxidant properties. Total phenolic content (TPC) was determined according to the Folin-Ciocalteu method and total flavonoid content (TFC) was determined according to the aluminum chloride *colorimetric method*. DPPH (2, 2-diphenyl-1-picryl-hydrazyl**)** radical scavenging activity, ferrous ion chelating activity and ferric reducing antioxidant power were determined.

3.2.4 Determination of total phenolic content

Total phenolic content (TPC) was determined according to the Folin-Ciocalteu method as described by Jayawardana *et al.* (2021) using 96-well microplates. A known amount of finger millet extract was initially dissolved in DMSO and diluted with distilled water. Diluted finger millet extract (20 µL) was added to a microplate, mixed with freshly prepared 10 times diluted Folin-Ciocalteu reagent (110 µL) and preplate reading was taken at wavelength of 765 nm using a microplate reader (SpectraMax Plus^{384}, Molecular Devices Inc., USA). Sodium carbonate solution (10% w/v, 70 µL) was added, incubated at the room temperature for 30 min and absorbance was measured at wavelength of 765 nm. Gallic acid was used as the standard phenolic acid to plot the standard curve ($y = 0.0532x + 0.0339$; $r^2 = 0.9992$) and TPC was calculated as mg gallic acid equivalents.

3.2.5 Determination of total flavonoid content

Total flavonoid content (TFC) was determined according to the aluminium chloride colorimetric method as described by Jayawardana et al. (2021) using 96-well microplates. A known amount of finger millet extract was dissolved in methanol. Dissolved finger millet extract (100 µL)

was added to a microplate and preplate reading was taken at wavelength of 415 nm using a microplate reader (SpectraMax Plus^{384}, Molecular Devices Inc., USA). Aluminium chloride solution (2% w/v, 100 µL) was added, incubated at the room temperature for 10 min and absorbance was measured at wavelength of 415 nm. Quercetin was used as the standard flavonoid to plot the standard curve ($y = 0.0349x - 0.2091$; $r^2 = 0.9974$) and TFC was calculated as mg quercetin equivalents.

3.2.6 Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity was determined according to the method described by Jayawardana et al. (2021) using 96-well microplates. A known amount of finger millet extract was dissolved in methanol. Dissolved finger millet extract (50 µL) was added to a microplate, mixed with methanol (90 µL) and preplate reading was taken at wavelength of 517 nm using a microplate reader (SpectraMax Plus^{384}, Molecular Devices Inc., USA). DPPH solution (0.02% w/v, 60 µL) was added, incubated at the room temperature in dark for 10 min and absorbance was measured at wavelength of 517 nm. Reaction mixture without the sample was used as the control. Trolox, a water-soluble derivative of vitamin E, was used as the standard antioxidant. DPPH radical scavenging activity as percentage inhibition was calculated using the following equation.

$$\text{Inhibition \%} = [(A_C - A_S) / A_C] \times 100$$

Where,
A_C = Absorbance of control
A_S = Absorbance of sample

3.2.7 Determination of ferrous ion chelating activity

The FIC activity was determined according to the method described by Jayawardana et al. (2021) using 96-well microplates. A known amount of finger millet extract was initially dissolved in DMSO and diluted with distilled water. Diluted finger millet extract (100 µL) was added to a microplate, mixed with 1 mM ferrous sulphate solution (20 µL) and distilled water (40 µL) and preplate reading was taken at wavelength of 562 nm using a microplate reader (SpectraMax Plus^{384}, Molecular Devices Inc., USA). Then, 40 µL of 1 mM ferrozine solution was added, incubated at the room temperature for 10 min and absorbance was measured at wavelength of 562 nm. Reaction mixture without the sample was used as the control. EDTA, a frequently used metal chelating agent, was used as the standard. FIC activity as percentage chelation was calculated using the following equation.

$$\text{Chelation \%} = [(A_C - A_S) / A_C] \times 100$$

Where,
A_C = Absorbance of control
A_S = Absorbance of sample

3.2.8 Determination of ferric reducing antioxidant power

The FRAP value was determined according to the method described by Jayawardana et al. (2021) using 96-well microplates. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride and 10 mM TPTZ in a ratio of 10:1:1 and incubating at 37 ℃ for 10 min. A known amount of finger millet extract was initially dissolved in DMSO and diluted with acetate buffer. Diluted finger millet extract (20 µL) was added to a microplate, mixed with acetate buffer (30 µL) and preplate reading was taken at wavelength of 600 nm using a microplate reader (SpectraMax Plus^{384}, Molecular Devices Inc., USA). Then, freshly prepared FRAP reagent (150 µL) was added, incubated at the room temperature for 8 min and absorbance was recorded at wavelength of 600 nm. Trolox was used to plot the standard curve ($y = 0.17x + 0.15$; $r^2 = 1.00$) and FRAP value was calculated as mg Trolox equivalents.

3.2.9 Determination of aerobic plate count

Aerobic plate count was determined according to the method described in the part 1 of the SLS specification for microbiological test methods namely, General guidance for enumeration of microorganisms - colony count technique (Sri Lanka Standards Institution, 1991a). Plate count medium was prepared by dissolving dehydrated yeast extract (2.5 g), anhydrous D-glucose (1 g), tryptone (5 g) and agar (15 g) in distilled water (1 L). Then, it was sterilized in an autoclave at 121 ℃ for 20 min. Peptone diluent was prepared by dissolving peptone (1 g) and sodium chloride (8.5 g) in distilled water (1 L) and sterilizing in an autoclave at 121 ℃ for 20 min. Sample (10 g) was added into a Stomacher bag which containing 90 mL of peptone diluent and mixed well using a Stomacher blender for 1 min. It was considered as the first dilution (10^{-1}). Another two

serial dilutions (10^{-2} and 10^{-3}) were prepared. Then, 1 mL portions of each dilution were transferred into 3 sterile petri dishes and 15 mL of the medium was added to each dish. Dishes were covered with lids, the inoculum and the medium were mixed well by rotating the dishes and allowed to solidify. Then, the dishes were inverted, incubated at 30 ℃ for 72 hrs. and colonies were counted using a colony counter.

3.2.10 Determination of yeasts and molds count

Yeasts and molds counts were determined according to the method described in the part 2 of the SLS specification for microbiological test methods namely, Enumeration of yeasts and molds (Sri Lanka Standards Institution, 1991b). Yeast extract-dextrose-chloramphenicol agar medium was prepared by dissolving yeast extract (5 g), dextrose (20 g), chloramphenicol (0.1 g) and agar (15 g) in distilled water (1 L). Then, it was sterilized in an autoclave at 121 ℃ for 20 min. Three serial dilutions of the sample were prepared using the peptone diluent. Then, 1 mL portions of each dilution were transferred into 3 sterile petri dishes and 15 mL of the medium was added to each dish. Dishes were covered with lids, the inoculum and the medium were mixed well by rotating the dishes and allowed to solidify. Then, the dishes were inverted and incubated at 25 ℃ for 72 hrs. Yeast and mold colonies were counted using a colony counter.

3.2.11 Determination of Coliforms / *E. coli* count

The Coliforms / *E. coli* counts were determined according to the method described in the part 3 of the SLS specification for microbiological test methods namely, Detection and enumeration of Coliforms, fecal Colifroms and *E. coli* [29]. MacConkey broth was prepared by dissolving MacConkey broth powder (34.51 g) in distilled water (1 L). Then, 9 mL portions of the broth were transferred to tubes containing an inverted Durham tube and sterilized by autoclaving at 121 ℃ for 20 min. Three serial dilutions of the sample were prepared and 1 mL portions of the each dilution were transferred in to three MacConkey tubes. Tubes were covered with cotton plugs, incubated at 30 ℃ for 48 hrs and color changes of the tubes and gas production were recorded.

3.3 Data Analysis

Data of three different sensory analysis samples were statistically analyzed using the IBM SPSS Statistics (Version 28.0.1.1) software and the Minitab 21 statistical software. Statistical significance was set at 95% confidence level. Data of sensory evaluations were statistically analyzed using the Friedman test.

4. RESULTS AND DISCUSSION

4.1 Physico-chemical Properties of the Beverages

The pH values of this RTS drink product were recorded as the different samples and these pH values were detected by a calibrated pH meter (EUTECH instruments pH700) in triplication and shown in Tables 6 and 7. The 4.5 to 6.0 range of pH in a product is ideal for human health. The pH value means the presence of free hydrogen ions and buffering capacity of the juices (Beverage and Saranyah, 2015). The pH values show the acidity levels of each sample. Every sample has pH values between 4.0 -6.0 Ph. Results show the samples are in the litter lower than the neutral pH values. Because of this can be observed that all the tested samples are at the acidity levels. And also according to the results, the fresh ginger and dry ginger have not specifically affected the pH. However, it does not comply with the requirement specified in Sri Lankan standards for ready-to-serve drinks (SLS 729: 2010) the pH range of RTS must be below 3.8. Addition of the citric acid to the formulation can help to overcome that issue.

Table 6. The pH values of the first set of beverages prepared using fresh ginger

Formula	pH value
F1	5.25 ± 0.25
F2	5.40 ± 0.25
F3	4.98 ± 0.25
F4	4.80 ± 0.25

F1: Formula No.1, F2: Formula No.2, F3: Formula No.3, F4: Formula No.4

The Brix value of both the product development trials was recorded in Table 9. Brix was recorded at room temperature using a digital refractometer. One degree of Brix means a hundred grams of liquid solution contains one gram of sugar. After the results, it was observed that the higher the Brix value sweeter the formula.

According to the results, the formula containing coconut treacle has the highest Brix values compared to the formula containing kithul treacle and formula containing sugar.

Table 7. The pH values of the second set of beverages prepared using dry ginger

Formula	Sweetener	pH Value
FC1	Sugar	5.38 ± 0.25
	Kithul treacle	5.42 ± 0.25
	Coconut treacle	5.17 ± 0.25
	None	5.40 ± 0.25
FC2	Sugar	5.03 ± 0.25
	Kithul treacle	5.15 ± 0.25
	Coconut treacle	5.05 ± 0.25
	None	5.17 ± 0.25
FC3	Sugar	5.33 ± 0.25
	Kithul treacle	5.56 ± 0.25
	Coconut treacle	5.17 ± 0.25
	None	5.42 ± 0.25
FC4	Sugar	5.01 ± 0.25
	Kithul treacle	5.05 ± 0.25
	Coconut treacle	4.98 ± 0.25
	None	5.15 ± 0.25

FC1: Formula No.1, FC2: Formula No.2, FC3: Formula No.3, FC4: Formula No.4

Table 8. The Brix values of the first set of beverages prepared using fresh ginger

Formula	Brix value
F1	4.2
F2	5.2
F3	4.9
F4	5.0

F1: Formula No.1, F2: Formula No.2, F3: Formula No.3, F4: Formula No.4

Table 9. The Brix values of the second set of beverages prepared using dry ginger

Formula	Sweetener	Brix value
FC1	Sugar	4.0
	Kithul treacle	5.0
	Coconut treacle	6.1
	None	0.3
FC2	Sugar	5.0
	Kithul treacle	5.8
	Coconut treacle	6.1
	None	1.0
FC3	Sugar	4.5
	Kithul treacle	5.4
	Coconut treacle	6.5
	None	1.7
FC4	Sugar	4.8
	Kithul treacle	6.0
	Coconut treacle	6.2
	None	1.2

FC1: Formula No.1, FC2: Formula No.2, FC3: Formula No.3, FC4: Formula No.4

For the sensory analysis, the different sweeteners added samples were named as different codes. The coconut treacle added sample was named C 851, the kithul treacle added sample was named K185, and the sugar added sample was named an S 581.

According to sensory statistically analyzed data, the most acceptable and overall successful sample is sample number C 851 (Coconut treacle added formula). The tested criteria for the sensory evaluation were appearance, color, odour, taste, after taste, and overall acceptability. According to the statistical data sample, C 851 has the highest mean value (23.4) than K 185 and S 581 for the overall acceptability. For this also the highest Brix values were recorded for the coconut treacle (C 851) added sample.

The highest Brix value means the sweetness of the sample is high. Because these results can be concluded, the most acceptable sample is based on its sweetness and taste. The concentration value of long pepper is highly affected by the final taste of the samples. The dry ginger can give some more taste than the fresh ginger. Sensory evaluation results show the difference between fresh ginger and dry ginger.

According to the results of the sensory evaluation, coconut treacle was selected as the most preferred sweetener. Aerobic plate count, yeast and mould count, and coliforms count of the RTS herbal drink are presented in Table 10.

According to the SLS specification for RTS fruit drinks Sri Lanka Standards Institution [29], the aerobic plate count should be less than 50 per 1 ml, and no growth of yeasts, moulds, and coliforms should be detected. Therefore, the developed RTS herbal drink was complying with the microbiological limits mentioned in the SLS specification for RTS fruit drinks Sri Lanka Standards Institution [29]. Although sulphites, benzoates, and sorbates can be added to RTS drinks as preservatives, this RTS herbal drink was developed without adding preservatives. Even after a storage period of 1 month, no growth of yeasts, moulds and coliforms was detected and it can be attributed to the inherent antimicrobial properties of coriander, dry ginger and long pepper. Phenolic compounds including flavonoids are known to be strong antioxidants and therefore, any herbal drink having these compounds can be potentially used as an effective source of natural antioxidants. Total phenolic and total flavonoid contents of the RTS drink are given in Table 11.

Table 10. Results of the microbiological tests of the RTS herbal drink

Sample	Aerobic plate count (CFU per ml)	Yeast and mould count (CFU per ml)	Coliforms count (MPN per ml)
RTS herbal drink	2.5×10^1	Not detected	Not detected

CFU: Colony forming unit, MPN: Most probable number

Table 11. Total phenolic and total flavonoid contents of the RTS herbal drink

Sample	Total phenolic content (mg gallic acid equivalents per 200 ml of the drink)	Total flavonoid content (mg quercetin equivalents per 200 ml of the drink)
RTS herbal drink	241.34 ± 0.20	25.55 ± 0.72

Results are presented as mean ± SD (n = 3)

Table 12. Antioxidant properties of the RTS herbal drink

Sample	DPPH radical scavenging activity (Inhibition % at 1 mg/mL assay concentration)	Ferrous ion chelating activity (Chelation % at 1 mg/mL assay concentration)	Ferric reducing antioxidant power (mg Trolox equivalents per 200 ml of the drink)
RTS herbal drink	24.08 ± 0.36	85.54 ± 1.56	28.81 ± 1.12

Results are presented as mean ± SD (n = 3)

The abilities of the RTS drink to reduce DPPH radicals, chelate ferrous ions, and reduce ferric ions were evaluated and the results are presented in Table 12.

Owing to the phenolic content including flavonoids, the developed herbal drink possesses abilities to scavenge DPPH radicals, and reduce ferric ions and chelate ferrous ions. Since a good antioxidant should be capable of quenching free radicals and chelating redox metals, these findings revealed the antioxidant potential of the developed herbal drink.

The color of the drink is mainly based on the coriander concentration of the drink and a lower coriander concentration gives the lighter color than the others. The more concentrated coriander amount results in the dark color of the final sample. The best color and the best taste of the coriander can be determined as the 66.6% concentration from the final formula.

The selected sample (C 851) has the specific raw material formula for coriander, dry ginger, and long pepper. Coriander 66.6%, dry ginger 25%, and long pepper 8.33% is the most acceptable sample and the coconut treacle is the most acceptable sweetening agent for further product development [30-34].

5. CONCLUSION AND RECCOMENDA-TIONS

In the production of the final product, attention is paid to maintaining the temperature at 100℃ levels and ensuring optimal performance throughout the production process. Furthermore, the selection of raw materials takes into consideration their quality, keeping in mind the desired qualities of the end product. Additionally, thorough research and verification of relevant certificates are conducted to improve the relevant samples and ensure adherence to quality standards. All samples' pH was between 4.0 – 6.0 pH. The highest Brix value was detected from coconut treacle-added samples and the lowest Brix value was detected from sugar-added samples. The most acceptable sample was the sample, C 851 coconut treacle added coriander 66.6%, dry ginger 25%, and long pepper 8.33% sample. The pH value and the further developments of the selected sample must be done with the controlled temperature and required standards.

It is recommended to use an approved suitable acid to obtain the required pH value as per the relevant RTS standard quality certificates. It is also expected to identify the impact on the final product using different variants of the raw materials used. It is also recommended that the

product be inspected for processing, packaging, and storage and that the most appropriate packaging method be identified.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Appendix A. Minitab results for sensory evaluations trail no.01

Kruskal-Wallis Test: Appearance versus Sample

1. Descriptive Statistics

Sample	N	Median	Mean rank	Z-Value
185	6	6.0	11.7	1.22
581	6	5.5	10.8	0.70
851	6	4.5	6.1	-1.92
Overall	18		9.5	

2. Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	3.77	0.151
Adjusted for ties	2	4.14	0.126

Kruskal-Wallis Test: Colour versus Sample

1. Descriptive Statistics

Sample	N	Median	Mean Rank	Z-Value
185	6	5	10.5	0.56
581	6	4	7.9	-0.89
851	6	5	10.1	0.33
Overall	18		9.5	

2. Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	0.81	0.667
Adjusted for ties	2	0.94	0.626

Kruskal-Wallis Test: Odour versus Sample

1. Descriptive Statistics

Sample	N	Median	Mean Rank	Z-Value
185	6	3.5	8.3	-0.70
581	6	3.0	6.3	-1.78
851	6	5.5	13.9	2.48
Overall	18		9.5	

2. Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	6.55	0.038
Adjusted for ties	2	6.93	0.031

Kruskal-Wallis Test: Taste versus Sample

1. Descriptive Statistics

Sample	N	Median	Mean Rank	Z-Value
185	6	3.5	8.3	-0.66
581	6	3.0	6.8	-1.50
851	6	5.0	13.3	2.15
Overall	18		9.5	

2. Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	4.88	0.087
Adjusted for ties	2	5.57	0.062

Kruskal-Wallis Test: After taste versus Sample

1. Descriptive Statistics

Sample	N	Median	Mean Rank	Z-Value
185	6	4.0	8.7	-0.47
581	6	3.5	7.5	-1.12
851	6	5.0	12.3	1.59
Overall	18		9.5	

2. Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	2.68	0.262
Adjusted for ties	2	3.06	0.216

Kruskal-Wallis Test: Overall Acceptability versus Sample

1. Descriptive Statistics

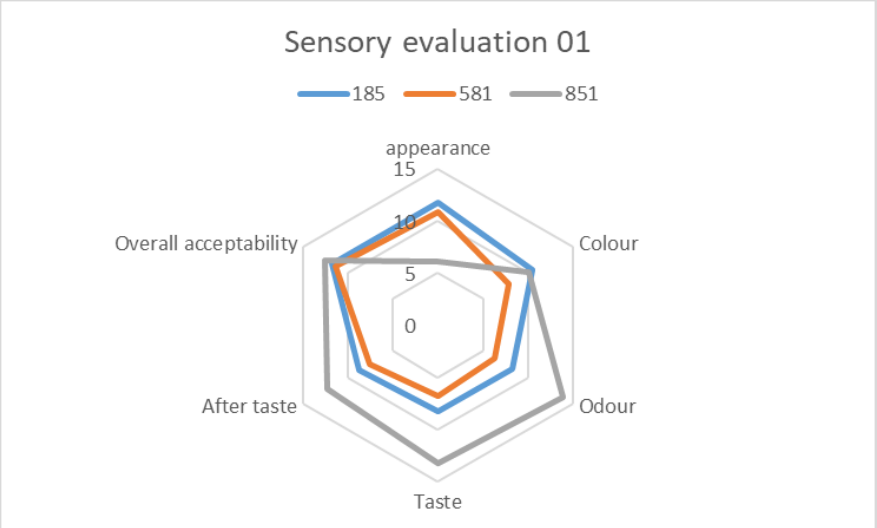
Sample	N	Median	Mean Rank	Z-Value
185	6	6	11.7	1.22
581	6	6	11.3	0.98
851	6	4	5.6	-2.20
Overall	18		9.5	

2. Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	4.86	0.088
Adjusted for ties	2	5.38	0.068

Appendix B. The average distribution of characteristics in trial no.01



Appendix C. Minitab results for sensory evaluations trail no.02

Kruskal-Wallis Test: Appeance versus Sample

1. Descriptive Statistics

Sample	N	Median	Mean Rank	Z-Value
C 851	11	6	19.6	1.09
K 185	11	6	17.1	0.06
S 581	11	6	14.3	-1.15
Overall	33		17.0	

2. Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	1.67	0.435
Adjusted for ties	2	2.02	0.364

Kruskal-Wallis Test: Colour versus Sample

1. Descriptive Statistics

Sample	N	Median	Mean Rank	Z-Value
C 851	11	6	19.3	0.97
K 185	11	6	16.2	-0.32
S 581	11	5	15.5	-0.65
Overall	33		17.0	

2. Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	0.98	0.612
Adjusted for ties	2	1.07	0.587

Kruskal-Wallis Test: After taste versus Sample

1. Descriptive Statistics

Sample	N	Median	Mean Rank	Z-Value
C 851	11	6	20.7	1.57
K 185	11	5	16.5	-0.19
S 581	11	5	13.7	-1.37
Overall	33		17.0	

2. Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	2.92	0.232
Adjusted for ties	2	3.21	0.201

Kruskal-Wallis Test: Overall Acceptability versus Sample

Descriptive Statistics

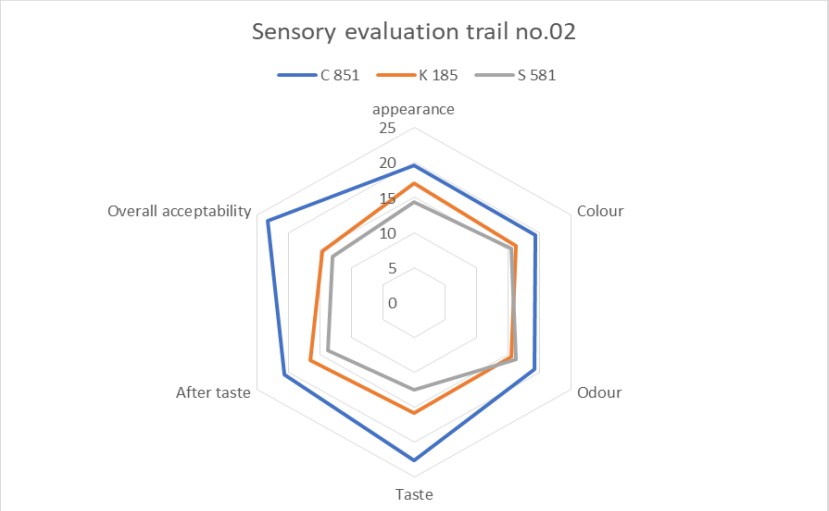
Sample	N	Median	Mean Rank	Z-Value
C 851	11	6	23.4	2.69
K 185	11	5	14.6	-1.01
S 581	11	5	13.0	-1.68
Overall	33		17.0	

Test

Null hypothesis	H_0: All medians are equal
Alternative hypothesis	H_1: At least one median is different

Method	DF	H-Value	P-Value
Not adjusted for ties	2	7.40	0.025
Adjusted for ties	2	8.19	0.017

Appendix D. The average distribution of characteristics in trial no.01



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