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COMPARATIVE STUDIES ON ANTIOXIDANT PROPERTIES OF SELECTED VARIETIES OF BANANA PEELS AND FORMULATION OF YOGHURT WITH BANANA PEEL EXTRACT TO STUDY THE STORABILITY OF YOGHURT

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Abstract: Banana peel is considered as a natural source of antioxidants and phytochemicals which is rich in free radical scavenging activity. This study aimed to compare the antioxidant properties of banana peels from three different varieties of bananas, '*Seeni'* (ABB), '*Kolikuttu'* (AAB) and '*Ambul'* (AAB). Antioxidant activity was assessed with DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS+ (2-2'-Azino-Bis-3- Ethylbenzothiazoline-6-Sulfonic Acid) assays. The DPPH values of '*Ambul'*, '*Kolikuttu'*, and '*Seeni'* were 55.36 ± 0.89%, 56.55 ± 1.86%, 57.77 ± 8.67% and the ABTS+ values were 7.94 ± 1.45%, 59.74 ± 2.31% and 69.8 ± 0.76% respectively. The highest antioxidant activity was given by the '*Seeni'*. The preliminary consumer survey was confirmed the acceptability for yogurt to incorporate with banana peel extract. *Lactobacillus rhamnosus* was used as the bacteria culture for the preparation of yogurt. The ethanolic banana peel extract of '*Seeni'* was incorporated with yogurt within the range of 0-1000 µL/100 mL milk. Over 90% of added extract was remained in the final product. The results revealed the significantly higher ($p< 0.05$) total phenolic content (TPC), DPPH, ABTS+ compared to control yogurt. The yoghurt which was incorporated the extract up to 800 µL/100g was received the highest rating in the 9 points hedonic scale in terms of appearance, flavor, consistency, and overall acceptability. A higher TPC, DPPH, ABTS+ and lower peroxide value were exhibited compared to control yogurt after 21 days. At the end of the 7th, 14th and 21st day, the DPPH values were varied from 70.67 ± 1.54% to 74.79 ± 2.36%, 71.18 ± 3.21%, 75.30 ± 1.54% and ABTS+ values were varied from 69.31 ± 1.23% to 65.21 ± 0.34%, 62.45 ± 0.63%, and 62.47 ± 1.23% respectively. The color, pH, titrable acidity, the microbial count did not result a significant difference at refrigeration storage. The study was demonstrated that banana peel extract has the potential to be used as a functional food ingredient for promoting the storability of yoghurt.
Keywords: Antioxidant; Banana peel; Ethanolic; Functional; Phenolic

1 INTRODUCTION

The peels of a variety of fruits are considered as a natural source of antioxidants and phytochemical content which are rich in free radical scavenging activity. An antioxidant is any substance that presents at a low concentration compared to that oxidizable substrates. The peel of the fruit contains various antioxidant compounds such as gallocatechin and dopamine [1]. Banana peel is a rich source of antioxidants such as ascorbic acid, tocopherol, beta carotene, phenolic groups, dopamine and gallocatechin. And also anthocyanin, delphinidin, cyaniding and catechins contain in ripen banana peel [2]. They show antioxidant and antimicrobial properties and are potential preservative agents in food. More than 40 individual phenolic compounds. Asia is the largest banana-producing region [3]. The percentage of a banana peel is nearly 35% of the fruit. This accounts for a huge amount of by-products. They discarded rather than consumed. Fruit waste and by-products are formed in great amounts during the industrial processing of fruits. Therefore is important to find effective ways to manage or utilize waste materials and by-products during fruits processing. The utilization of banana peels is an economic concern nowadays. Numerous methods have been proposed for measuring the antioxidants activities of food waste.

The scientific name of banana is Musa, from the Musaceae family of flowering tropical plants. The bananas are clustered at the top of the plant [4]. Banana is 4th on the list of the developing world's food crops, after rice, wheat and maize. Local consumption has accounted for 90% of production, mainly in the poorest countries of Africa, Latin America and Asia. Banana is a good source of vitamin B6, fiber, potassium, magnesium, vitamin C, manganese per one serving, or one medium ripe banana. It presents approximately a hundred and ten calories, zero gram fat, 1 gram protein, 28 grams carbohydrate, 15 grams sugar (clearly occurring), three grams fiber, and 450 mg potassium [4].

Banana peels include plant chemical substances withinside the shape of antioxidants. They can act as an antiseptic and anti- inflammatory to promote wound healing such as for minor burns, and sunburns [4]. Banana is among the most traded fruits not only in Sri Lanka but also in the world [3].

Banana is one of the maximum traded end result in the world. In 2017 alone, 22.7 million tonnes of bananas had been traded, representing nearly 20% of the worldwide manufacturing that year. Asia is the largest banana-producing region [3]. Bananas are produced in 135 countries in the world. A total of 155.2 million tonnes of bananas were produced in 2018. India was the top country in bananas production in the world in 2018 was 30.8 million tonnes [5]. Filipinos have the highest per capita consumption, around 60kg/year, followed by Brazilians (similar number). But in African nations

along with Uganda, Rwanda and Cameroon, in line with capita intake consumption exceeds two hundred kg of banana.
The banana is one of the most widely cultivated and consumed fruits in Sri Lanka. The major districts of cultivating bananas in the country are Kurunegala, Rathnapura, Hambantota, Moneragala, Ampara and Jaffna [6]. It is also an attractive fruit crop for farmers due to its high economic gains throughout the year. In Sri Lanka, 29 banana cultivars consisting of wild species had been reported. Five of these species can be categorized as cooking bananas, the other varieties, except two wild species, are consumed raw. Approximately 54% of the total fruit cultivation lands are used for banana cultivation [6]. Examples of the Mysore group are ever-popular Ambul and Seeni bananas. *Kolikuttu, Suwendel, Puwalu* and *Rath kehel* come with *Kolikuttu* group, and *Anamalu*, *Embon*, *Bin kesel* and *Nethrapalam* come within the Cavendish group [7]. Banana is an attractive fruit crop among farmers due to its higher economic gains throughout the year.

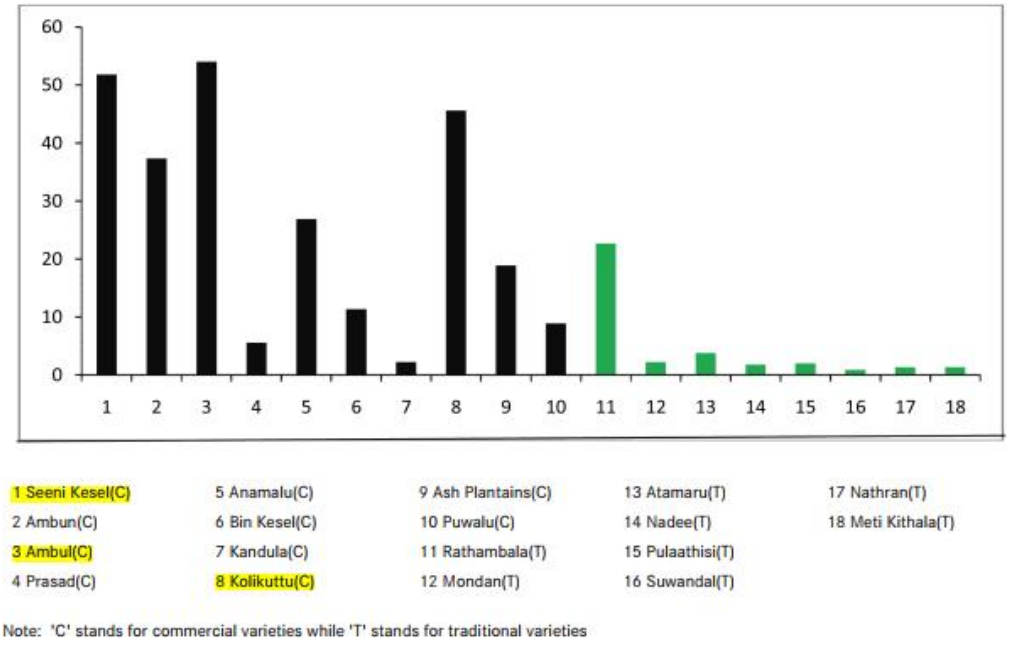

Figure 1 Percentage of Farmers Who Cultivate Each Banana Variety in Sri Lanka. Adopted From [8]

Banana peels of *Ambul*, *Seeni*, and *Kolikuttu* were used in this study as three major varieties of bananas grown in Sri Lanka. The highest percentage of farmers who cultivate *Ambul* banana. Then *Seeni* banana and the next common variety of banana is *Kolikuttu* compare to other banana varieties grown in Sri Lanka [8].
Banana can be considered an important part of a healthy and balanced diet. A significant amount of organic waste is generated due to the removal of banana peel in the food industry. These have usually been discarded creating a serious problem for the environment. Therefore it is important to find an effective way to manage or utilize the waste materials and by-products formed during the food industry that use bananas as the raw materials.
The thick, fibrous outermost cover of the banana is the banana peel. The fruit peel contributes 35% of the whole fruit weight [9]. Banana peel is the less popular source of nutrition even in Western countries. Many people believe banana peels are not safe to eat. But they offer several health benefits. In addition to the nutritional benefits of eating the banana peel, there are environmental benefits as well. Bananas are the most widely eaten fresh fruit in most of the countries in the world. By eating banana peels, it can help to minimize the amount of food that goes to landfills [10]. Many bioactive compounds have been detected in dried banana peel powder, including epicatechin, and gallocatechin. Gallocatechin was 5 times higher in the banana peel than in its pulp, indicating the peel is a high source of antioxidant compounds [11].
Both banana and banana peels offer different health benefits depending on their level of ripeness. Green banana may be more effective in treating digestive issues, while riper, blackened bananas as the previous study findings. Banana peels have high levels of tryptophan that combine with B6 in the banana peel. It helps to relieve some symptoms of depression, mood disorders. They fight cancer-causing free radicals in the body. Eating banana peels, especially green, unripe peels can increase the antioxidant levels and reduce the risk of cancer [10].
Ambul bananas are famous for being slightly sour but sweet at the same time. They are petite, slender, sharp ended and easy to spot in a crowd. Skin is relatively thin and covers pale yellow smoothly soft deliciousness. *Seeni* bananas are similar to *Ambul* bananas in that they are too small in size. They are rounder in shape. And also has a distinctly sweet taste. Its skin is thin, rubbery, apple green in color when unripe and buff- yellow when it ripens. *Kolikuttu* bananas are stout, plump and blunt-ended. Thinner skin, which splits during early ripening, and their creamy white fleshgives out an appetizing aroma [7].

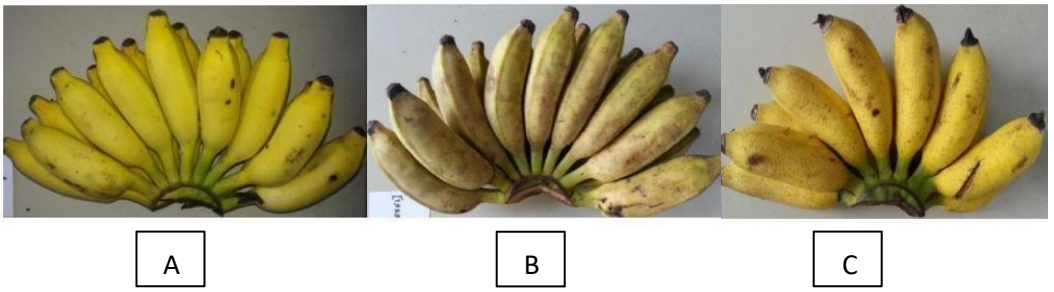

Figure 2 A - Ambul Banana, B - Seeni Banana and C – Kolikuttu Banana.
Adopted from [12]

Banana has protective factors against certain chronic diseases such as cardiovascular diseases, cancer, diabetes, Alzheimer's disease, cataracts, and age-related functionaldecline [12].
The peels of a variety of fruits can act as the natural source of antioxidants and phytochemical content which are rich in compounds with free radical scavenging activity [13].
Banana peel is a by-product in the food industry that is rich in dietary fiber and phenolic compounds [9]. They are not only used in the food industries but also used in the nutraceutical and pharmaceutical industries. The banana peels are also more nutritious same as the banana flesh. The nutritious valueof banana peels depends on the stage of maturity, soil conditions and climatic conditions they have grown. The discarded banana peels contain diverse nutrients and antimicrobial compounds. Banana peel extracts are known to possess the antioxidative activity and can protect fish oil and meat from oxidation [9].
And also anthocyanin, delphinidin, cyaniding and catechins contain in ripen banana peel [2]. They show antioxidant and antimicrobial properties and are potential preservative agents in food. Banana peels are a rich source of phenolic compounds, flavonoids such as quercetin, myricetin, kaempferol, and cyaniding [2].
In bananas, the peel can be the major source of obtaining natural antioxidants. Ascorbic acid, tocopherol, beta carotene, phenolic groups, dopamine and gallocatechin are antioxidant compounds also identified in the banana peel. Anthocyanin, delphinidin, cyaniding and catecholamines contain in the ripe banana peel [2].
Banana peel is one of the underutilized sources of phenolic compounds. It is considered as a good source of antioxidants for foods and functional foods against cancer and heart disease. The peel of the banana incorporates diverse antioxidant compounds together with gallocatechin and dopamine. But the recent trends focus on the isolation, characterization and utilization of natural antioxidants. These compounds are found in most of the fruit tissues such as polyphenols that are present in the banana peel. It is a potential source of antioxidant and antimicrobial activities.
Free radicals can attack various substrates of interest in the body. It contributes to chronic disease development such as oxidatively modified LDL. Oxidatively changed LDL has been hypothesized to be a causative agent in the improvement of cardiovascular disease.

2 MATERIALS AND METHODOLOGY

2.1 Reagents

Ethanol (70%), Gallic acid (dry powder), Anhydrous sodium carbonate, Rutin (dry powder), 5% Sodium nitrite, 10% (w/v) Aluminum chloride, 1M Sodium hydroxide, Follin-Ciocalteu reagent, 0.2M Phosphate buffer, 1,1-diphenyl-2-picrylhydrazyl (DPPH), MRS agar, Acidified potato dextrose agar (PDA).

2.2 Sample Collection

Healthy plants of three varieties of fully ripe banana *Ambul* banana, *Kolikuttu* banana and *Seeni* banana were purchased from retail vendors and brought into the laboratoryunder humid conditions.

2.3 Feedstock Preparation Pre-Treatment and Surface Sterilization of Samples

Banana fruits were washed with chlorinated water and then it was washed with distilled water. Fruits peels were removed and sliced into small pieces. They were shade dried at room temperature (30-32 ^{O}C) for three days. The dried peels were grounded using a lab grinder and passed through a sieve to obtain the powder. It waskept in an airtight jar at 4 ^{O}C until further use [2].The moisture content of the dry powder was determined by the oven drying method.

2.4 Ethanolic Banana Peel Extraction

To extract bioactive compounds, 2.5g of banana peel powders were dissolved in 50 mL of ethanol at a solid-liquid ratio

of 1:20 (g/mL) and extracted at room temperature for 1 hour in a shaking water bath at 100 rpm, followed by centrifugation at 4000 rpm for 10 minutes and filtration over Whatman filter paper. The filtrate was concentrated by a rotary evaporator at 35 ^{O}C to remove ethanol and obtained highly concentrated liquid banana peel extract [14]. It was dissolved in water to make a total volume of 10 mL.

2.5 Preparation of Gallic Acid Standard

Gallic acid stock was prepared by dissolving 0.2 g of dry gallic acid in 10 ml of ethanol. It was diluted to a volume of 100 ml with distilled water. For the sodium carbonate solution, 20 g of anhydrous sodium carbonate was dissolved in 80 ml of distilled water. It was brought to a boil. To prepare the calibration curve 0, 1, 2, 3, 4and 5 ml of prepared stock the solution in a 100 ml volumetric flask was diluted to volume with distilled water. These solutions were obtained with the concentration of 0, 20, 40, 60, 80 and 100 mg/L gallic acid. For each calibration solution, pipette 0.5 ml to each solution, 2 ml of distilled water and 0.5 ml of sodium carbonate solution for 30 minutes at 40 ^{O}C. The absorbance of each solution was measured using a spectrometer at 765 nm. The standard curve was obtained by plotting the absorbance against the gallic acid concentration. The total phenolic content of the banana peel extracts was obtained by the curve [15].

2.6 Preparation of Rutin Standard

Accurately 0.2 g of dry rutin was dissolved in 10 ml of ethanol and diluted to a volume of 100 ml with distilled water. To obtain the calibration curve, 0, 1, 2, 3, 4 and 5 ml of the above the stock solution in 100 ml volumetric flasks was diluted to volume with distilled water. They had the solutions with concentrations of 0, 20, 40,60, 80 and 100 mg/L rutin. For each calibration, pipette different amount of samplein 3.0 ml of distilled water to which 0.3 ml of 5% sodium nitrite was added and properly mixed. After 5 minutes at room temperature, 0.6 ml of 10% Aluminum chloride was added. Then 2 ml of 1M sodium hydroxide was added after 6 minutes and absorbance was read at 510 nm. The total flavonoid content of banana peel extracts was determined by the standard curve obtained [15].

2.7 Banana Peel Extracts

2.7.1 Analysis of the total phenolic content (TPC)

Samples were analyzed for total polyphenol content according to the Folin-Ciocalteu method. To 0.5 ml aliquot of the extract solution, 0.2 ml of Folin-Ciocalteau reagent and a saturated solution of Na2CO3 0.5 ml was added. This was increased to 10 ml with distilled water and incubated at 27 ^{O}C for 30 minutes. Optical density was measured at 765 nm using a UV visible spectrophotometer. The concentration was calculated using gallic acid as a standard. The results were expressed as gallic acid equivalents/100 g of sample [15].

2.7.2 Analysis of the total flavonoid contents

The total flavonoid content of the extracts was determined according to the colorimetric assay following the procedure of with slightmodifications[15]. Accurately 1 ml of aqueous extract containing 0.01 g/ml of dry matterwas placed in 10 ml volumetric flasks. Then 5 ml of distilled water was added. At zero time, 0.3 ml of (5%) NaNO2 was added.

After 5 minutes 0.6 ml of (10% w/v) AlCl3 was added. After another minute 2 ml of 1M solution of NaOH was added. Then the volume was made up to 10 ml with distilled water. The mixture was shaken vigorously and the absorbance of the pink color of the mixture was read at 510 nm using a UV-visible spectrophotometer. A calibration curve was prepared using a standard solution of rutin and the results wereexpressed as mg rutin equivalents/100 g of dry matter. All samples were analyzed in triplicates and results were averaged.

2.7.3 Free radical scavenging ability using DPPH assay

Free radicals scavenging activity of banana peel extracts were determined following the procedure of with slight modifications using DPPH[15]. The antioxidant capacity of the banana peel extracts was evaluated through the free radicals scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. Accurately 5.0 ml of freshly prepared solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) ethanolic solution at concentration 0.025 g/L was added to 1.0 ml of extractcontaining and 25 µg/ml of dry matter in ethanol. The mixture was shaken and keptin the dark. It was left to stand at room temperature for 30 minutes. The absorbanceof the solution was measured at 515 nm, against a blank of ethanol without DPPH, using a UV visible spectrophotometer. Results were expressed as a percentage of inhibition of the DPPH radical which was calculated according to the followingequation.

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of the sample}) \times 100}{\text{The absorbance of control}}$$

Equation 1: Radical scavenging activity %[15].

2.7.4 Free radical scavenging ability using ABTS+ assay

ABTS+ radical scavenging activity was determined according to the method Rajurkan and Hande, ABTS+ radical was generated by reacting 7mM ABTS in water and 2.46 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 hours before use. ABTS+ solution was diluted to obtain an absorbance of 0.70 at 734 nm. After the addition of 5 µl of diluted 25, 50 and 100% to 3.99 ml diluted ABTS+ solution, the absorbance was measured 30 minutes after initial mixing at 517 nm. A percentage of inhibition of the ABTS+ radical was calculated according to equation 1.

2.7.5 Consumer preference survey

A consumer preference survey was conducted to determine the consumer preference for fortified yogurt with banana peel extract. It was included 21 questions as three sections. A random sample was selected from the population who are of different gender, ages, income levels and educational levels.

2.7.6 Formulation of yogurt with banana peel extract

The liquid banana peel extract containing the highest free radical scavenging activity was selected from three different varieties. It was used to formulate the yogurt. The filtrate was concentrated by a rotary evaporator at 35 ^{O}C temperature. The extract was ready for fortification in yogurt.

The whole milk and 4% (w/v) sugar, the gelatin were added to manufacture the yogurt. Sugar was dissolved in milk and heated to 95 ^{O}C (pasteurization) with continuous stirring for 30 minutes. It was allowed to cool down to 45 ^{O}C. The *Lactobacillus rhamnosus* starter culture (1%) and different volumes of the banana peel extracts such as 0, 200, 400, 600, 800 and 1000 ml were added into 100 ml of milk. The sample was poured into the 85 ml standard yogurt cups separately. The mixtures was be incubated at 45 ^{O}C for 12 hours. The yogurts were stored in a refrigerator at 4 ^{O}C for quality analysis. They were tested every 3 days. The formulated yogurt was subjected to analysis until the failure rate reached 50% [14].

2.8 Characterization of the Formulated Yogurts on 1st, 7th, 14th and 21st Day during the Storage Period

2.8.1 Extraction of bioactive compounds from yogurt for analysis

Accurately 20 g of yogurt was mixed with 40 ml ethanol in a glass conical flux. The mixture was left at 4 ^{O}C for 6 hours incubation ad stirred for 30 minutes to perform extraction at room temperature. The resulted slurry was filtered through Whatman filter paper [14].

2.8.2 Total phenolic content (TPC)

The TPC in extracts and yogurts were determined by the Folin-Ciocalteu assay with gallic acid equivalent as standard and expressed as µg gallic acid equivalent per g yogurt [14].

2.8.3 Determination of remaining antioxidant activity with DPPH assay

Accurately 20 µL of yogurt extract was treated with 0.08 mmol/L DPPH and the decrease in absorbance was estimated at 517 nm. The analysis was repeated for 3 weeks with 2 days' time intervals. The activity was expressed as % of DPPH radicalscavenging [16].

2.8.4 Determination of remaining antioxidant activity with ABTS+ assay

The absorbance was recorded at 734 nm after 6 minutes of incubation of the mixture of 20 µL extracts 1.950 mL ABTS and potassium persulfate solution at room temperature. The percentage DPPH and ABTS+ scavenging activity of extracts werecalculated according to equation (1).

2.8.5 Peroxide value

The peroxide value of each yogurt was measured by the acetic acid chloroform AOAC method described by Tseng and Zhao. All the experiments were done in triplicate. The peroxide value of both control and fortified yogurts were expressed as Milliequivalent peroxide per kg yogurt.

2.8.6 pH variation in the refrigerated storage period

The pH value of the yogurt sample was measured three times using a pH meter at 7 days intervals. After calibrating with the buffer solution pH 7.0 [17].

2.8.7 Titrable acidity

Titrable acidity values of each yogurt sample were determined by measuring the amount of 0.1N NaOH determined to adjust to pH 8.3 [18].

2.8.8 Microbiological count

2.8.8.1 Lactic acid bacteria count,

Yogurt samples were diluted until appropriate concentration. A series of test tubes (10), each containing 9 ml diluents Then 1g yogurt sample was homogenized in 10 mL diluents and made suspension. From the ordinal sample, 1 ml was transferred into test tube no 1 and mixed thoroughly. Then 1 ml of the sample was transferred from 1st test tube to 2nd test tube. It will be continued up to the last test tube and 1 ml was discarded from the last test tube [19]. And 1 mL of the final diluent was spread on the surface of the MRS medium and incubated at 37 ^{O}C for 72 hours under anaerobic conditions [18].

2.8.8.2 Yeast and mold count

This test was done using the acidified potato dextrose agar (PDA).

2.9 Sensory Evaluation

Yogurts with and without banana peel extract were evaluated for consumer acceptance by 30 numbers of panelists. Panelists were asked to rate the likeness on appearance, flavor, consistency, taste and overall acceptability of the yogurt by using 9 points hedonic scale (9=like extremely and 1=dislike extremely).

2.10 Statistical Analysis

All statistical analyses were conducted using Minitab 16 software. The significance among different data was evaluated by analysis of variance (One Way ANOVA) using a general linear model with a 95% confidence level.

3 RESULT

3.1 Dried Powder of Banana Peels

Table 1 represents the moisture contents of three different varieties of dried powder of banana peels. The mean moisture contents of the three varieties were ranged from 15.67% to 19.53%. The experiment results indicated that the moisture content of dried powder of *Kolikuttu* was maximum and minimum was *Seeni* followed by *Ambul*. The mean values of the moisture content of *Kolikuttu* and *Ambul* were not significantly different. The moisture content of the dried powder of *Seeni* was significantly different from the two other varieties.

Table 1 Mean (±SD) Moisture Contents of Three Different Varieties of Dried Powder of Banana Peels

Banana variety	Moisture content ±SD %
Seeni	15.67 ± 1.15^{b}
Kolikuttu	19.53 ± 1.47^{a}
Ambul	19.20 ± 0.40^{a}

Values are expressed as mean ± SD values followed by different letters for each assay in the same column are significantly different ($P<0.05$)

3.2 Ethanolic Banana Peel Extracts

3.2.1 Total phenolic content

Table 2 represents the total phenolic content of ethanolic extracts of three different banana varieties. The mean total phenolic contents of the three varieties were ranged from 3.07µg GAE/ mL extract to 114.18µg GAE/ mL extract. The experiment results indicated that the amount of phenolic content of *Ambul* extract was maximum and minimum was *Seeni* extract followed by *Kolikuttu* extract. The mean values of the total phenolic content of extracts were significantly different from each other.

3.2.2 Total flavonoids content

Table 2 represents the total flavonoid content of ethanolic extracts of three different banana varieties. The mean total flavonoid contents of the three varieties were ranged from 1.65 µg Rutin/ mL extract to 75.40 µg Rutin/ mL extract. The experimental results showed that the total flavonoids content in three different varieties of banana peels, the maximum amount was obtained in *Ambul* and minimum was *Seeni* followed by *Kolikuttu*. The mean values of the total flavonoid content of extracts were significantly different from each other.

3.2.3 DPPH assay

Table 2 represents the antioxidant potential of ethanolic banana peel extracts determined by the well-established DPPH method. The mean % inhibitions were ranged from 55.36% to 57.77%. The inhibition as a percentage of each banana extract of three samples were determined by using established formulas. These results indicated that the scavenging potential for ethanolic *Seeni* banana peels extract was the maximum and the *Ambul* banana peel extract was the minimum followed by the *Kolikuttu*. The mean values of DPPH% inhibition of extracts were not significantly different from each other.

3.2.4 ABTS+ assay

Table 2 represents the antioxidant potential of ethanolic banana peel extracts determined by the well-established ABTS+ assays. The mean % inhibition was ranged from 7.94% to 69.8%. The inhibition as a percentage of each banana extract of three samples were determined by using established formulas. These results indicated that the scavenging potential for ethanolic *Seeni* banana peel extract was the maximum and the *Ambul* banana peel extract was the minimum followed by the *Kolikuttu*. The mean values of ABTS+ % inhibition of extracts were significantly different from each other.

Table 2 Mean (±SD) Total Phenolic Contents, Total Flavonoid Content, DPPH and ABTS+ Assays Values of Three Different Varieties of Ethanolic Banana Peel Extracts

Banana variety	Total phenoliccontent (µg GAE / mL extract)

Total flavonoid content (µg Rutin /mL extract)	**DPPH radical scavenging activity (% inhibition)**	**ABTS·+ radical scavengingactivity**	**(% i**	**nhibitio n)**
Seeni	3.07 ± 1.32[c]	1.65 ± 0.48[c]	57.77 ± 8.67[a]	69.8 ± 0.76[a]
Kolikuttu	52.87 ± 2.29[b]	35.40 ± 1.46[b]	56.55 ± 1.86[a]	59.74 ± 2.31[b]
Ambul	114.18 ± 1.33[a]	75.40 ± 0.64[a]	55.36 ± 0.89[a]	7.94 ± 1.45[c]

Values are expressed as mean ± SD values followed by different letters for each assay in the same column are significantly different (P<0.05)

3.2.5 Survey

Figure 3 shows the results of the consumer preference survey conducted to identifythe food for the formulation with banana peel extract. As the result, 81%, 17% and 2% of the selected population prefer to consume yogurt, bakery products and milkshakes respectively. It included 21 questions as three sections and 225 people who participated in the survey.

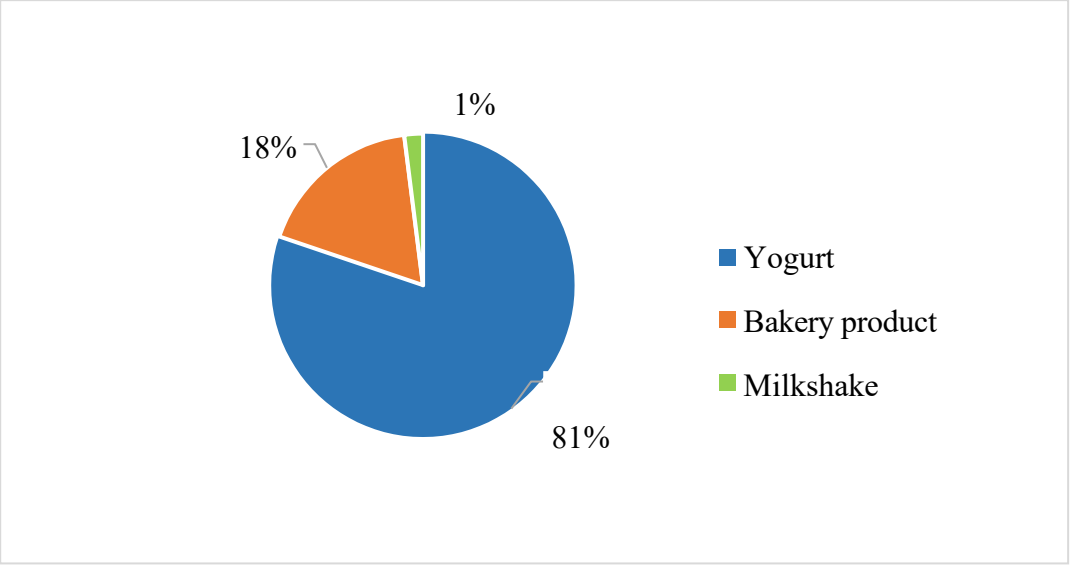

Figure 3 The Consumer Preference Survey to Select the Food Product for the Formulation with Banana Peel Extract

3.3 Formulated Yogurts with Different Concentrations of Seeni Banana Peel Extracts

3.3.1 Total phenolic content (TPC)

Figure 4 shows the total phenolic content of six yogurts formulated with different concentrations of banana peel extracts. The mean total phenolic contents of yogurtswere ranged from 225.75 mg GAE/ g yogurt to 525.75 mg GAE/ g yogurt. The TPC of fortified yogurts were increased while increasing the concentrations of extracts. The fortified yogurts were shown significantly ($p < 0.05$) higher amount of TPC thanthe control yogurt sample.

3.3.2 DPPH assay

Figure 4 shows the antioxidant capacity of six yogurts formulated with different concentrations of banana peel extracts using DPPH assay. The mean % inhibitions were ranged from 24.82% to 67.90%. The inhibition as a percentage of each yogurt was determined by using established formulas. The DPPH % inhibition of fortified yogurts were increased while increasing the concentrations of the extracts as increased the radical scavenging ability. The fortified yogurts were shown significantly ($p < 0.05$) higher amount of DPPH % inhibition than the control yogurtsample.

3.3.3 ABTS+ assay

Figure 4 shows the antioxidant capacity of six yogurts formulated with different concentrations of banana peel extracts using ABTS+ assay. The mean % inhibitionswere ranged from 42.24% to 71.12%. The inhibition as a percentage of each yogurt was determined by using established formulas. The ABTS+ % inhibition of fortifiedyogurts were increased with increasing the concentrations of the extracts as increased the radical scavenging ability. The fortified yogurts were shown significantly ($p < 0.05$) higher amount of ABTS+ % inhibition than the control yogurt sample.

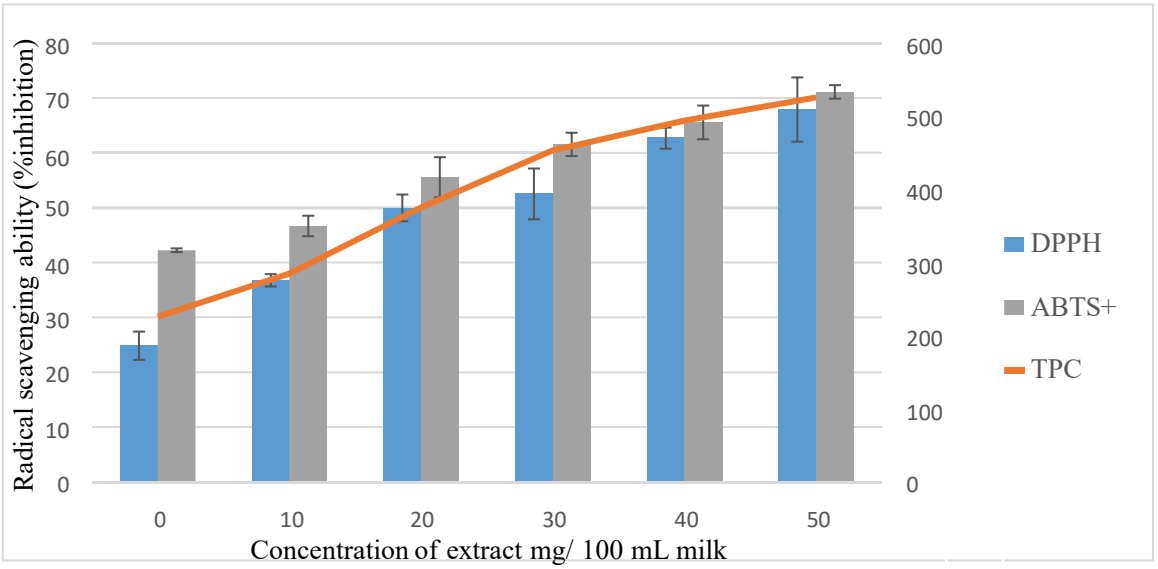

Figure 4 TPC, the Antioxidant Capacity of Yogurts by DPPH and ABTS+ as a Function of Extract Concentration

3.3.4 Sensory evaluation

The addition of banana peel extract up to 800 µL in yogurt (40mg/ 100 g yogurt) was received "like very much" liking score on appearance, flavor, consistency and overall acceptability with control yogurts by the panelist (figure 5). This yogurt was selected for further analysis. But 1000 µL in yogurt (50mg/ 100g yogurt) extract was received a lower liking score on appearance, flavor, consistency and overall acceptability. The sensory evaluation scores were not significantly ($p < 0.05$) different among the fortified yogurt compared to control yogurt.

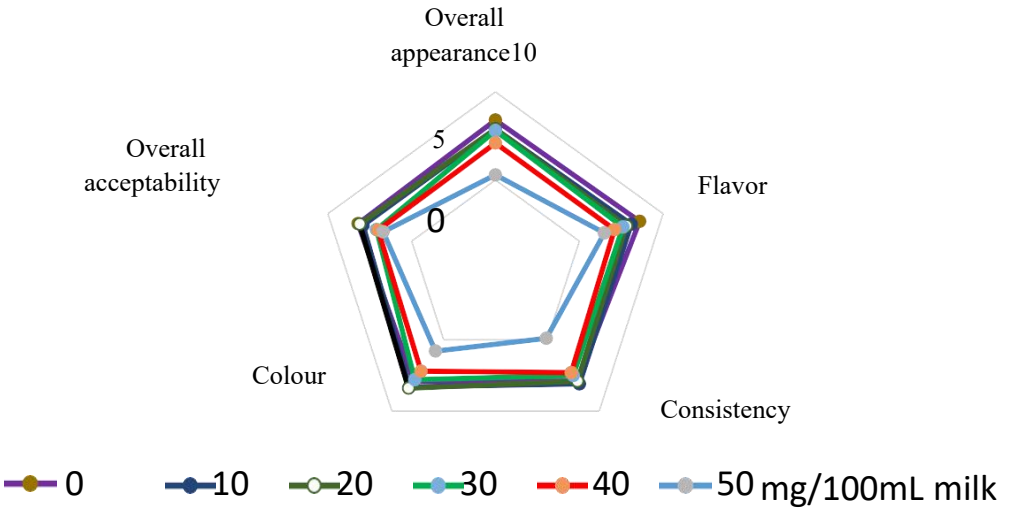

Figure 5 Influence of Different Concentration Banana Peel Extracts on the Sensory Properties of Yogurts (Panelist n = 30)

A 9 points hedonic scale: 9 =like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 =neither like or dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislikevery much and 1 = dislike extremely

3.4 The Selected Yogurt (40 Mg/100ml Milk) Was Formulated with Seeni Banana Peel Extract during the Storage Period

3.4.1 Total phenolic content

Table 3 represents the total phenolic content of formulated yogurt with *Seeni* bananapeel extract on the 1st, 7th, 14th and 21st days. The mean total phenolic contents of control and fortified yogurts were ranged from 198.62 µg GAE/ g yogurt to 185.98 µg GAE/ g yogurt and 498.39 µg GAE/ g yogurt to 483.68 µg GAE/ g yogurt respectively. The TPC of both control and fortified yogurts were reduced during the storage period. The fortified yogurts were shown a significant difference in TPC of yogurt after the 21st day compared to the initial.

3.4.2 DPPH assay

Table 3 represents the DPPH% inhibition of formulated yogurt with *Seeni* banana peel extract on 1st, 7th, 14th and 21st days. The mean DPPH% inhibition of control and fortified yogurts were ranged from 19.73% to 38.25% and 70.67% to 75.30% respectively. The DPPH% inhibition of both control and fortified yogurts were increased during the storage period. The fortified yogurts were shown a significant difference in DPPH% inhibition of yogurt after the 21st day compared to the initial.

3.4.3 ABTS+ assay

Table 3 represents the ABTS+ % inhibition of formulated yogurt with *Seeni* banana peel extract on 1st, 7th, 14th and 21st days. The mean ABTS+ % inhibition of control and fortified yogurts were ranged from 38.31% to 42.91% and 69.31% to 62.47% respectively. The ABTS+ % inhibition of control yogurts were increased during the storage period. The ABTS+ % inhibition of fortified yogurts were decreased during the storage period. The fortified yogurts were shown a significant difference in DPPH% inhibition of yogurt after the 21st day compared to the initial.

3.4.4 Lipid peroxidation

Table 3 represents the data analyzed to evaluate the lipid peroxidation of formulated yogurt with *Seeni* banana peel extract on 1st, 7th, 14th and 21st day. The mean peroxidation value of control and fortified yogurts were ranged from 0.15 Milliequivalent/ kg yogurt to 1.92 Milliequivalent/ kg yogurt and 0.23 Milliequivalent/ kg yogurt to 1.02 Milliequivalent/ kg yogurt respectively. The peroxide value of both control and fortified yogurts were increased during the storage period. The fortified yogurts were shown a significant difference in DPPH% inhibition of yogurt after the 21st day compared to the initial.

3.4.5 pH

Table 3 represents the pH values of formulated yogurt with *Seeni* banana peelextracton the 1st, 7th, 14th and 21st days. The

mean pH values of control and fortified yogurts were ranged from 4.52 to 4.30 and 4.61 to 4.66 respectively. The pH values of control yogurts were decreased during the storage period. The pH values of fortified yogurts were increased during the storage period. The fortified yogurts were shown a significant difference in pH values of yogurt after the 21st day compared to the initial.

3.4.6 Titrable acidity

Table 3 represents the titrable acidity of formulated yogurt with *Seeni* banana peel extract on the 1st, 7th, 14th and 21st days. The mean titrable acidity of control and fortified yogurts were ranged from 0.48 to 0.52 and 0.50 to 0.54 respectively. The titrable acidity of both control and fortified yogurts were increased during the storage period. The fortified yogurts were shown a significant difference in titrable acidity of yogurt after the 21st day compared to the initial.

3.4.7 Microbial tests

3.4.7.1 Lactic acid bacteria (Lactobacillus rhamnosus) count

Table 3 represents the Lactic acid bacteria count of formulated yogurt with *Seeni* banana peel extract on the 1st, 7th, 14th and 21st days. The mean Lactic acid bacteria count of control and fortified yogurts were ranged from 8.68 log10 CFU/ g yogurt to 6.53 log10 CFU/ g yogurt and 8.61 log10 CFU/ g yogurt to 6.12 log10 CFU/ g yogurt respectively. The Lactic acid bacteria count of both control and fortified yogurts were decreased during the storage period. The fortified yogurts were shown a significant difference in the Lactic acid bacteria count of yogurt after the 21 st day compared to the initial.

3.4.7.2 Yeast and mold count

Table 3 represents the yeast and mold count of formulated yogurt with *Seeni* banana peel extract on the 1st, 7th, 14th and 21st day. The mean yeast and mold count of control and fortified yogurts were ranged from 3.45 log10 CFU/ g yogurt to 5.86 log10 CFU/ g yogurt and 2.21 log10 CFU/ g yogurt to 3.45 log10 CFU/ g yogurt respectively. The yeast and mold count of both control and fortified yogurts were increased during the storage period. The fortified yogurts were shown a significant difference in yeast and mold count of yogurt after the 21st day compared to the initial.

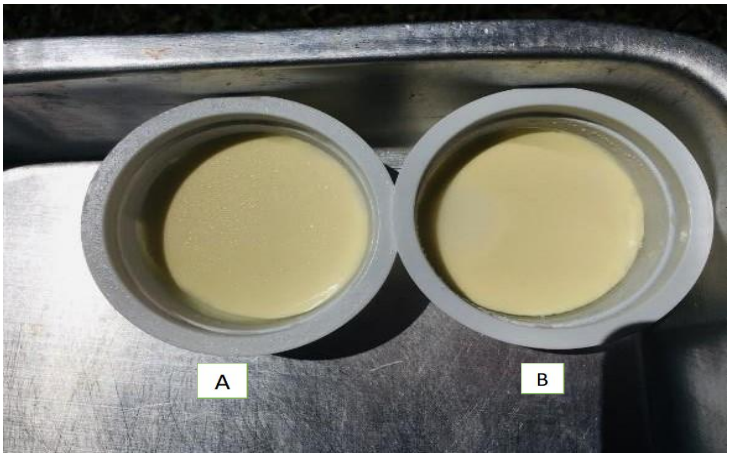

Figure 6 A – Control Yogurt, B – Fortified Yogurt

Table 3 Parameters of the Fortified Yogurt with Seeni Banana Peel Extract during the Storage Period

parameter	Yogurt	Storage time (in day)			
		1	7	14	21
TPC - µg / g yogurt	Control	198.62 ± 0.68[a]	196.78 ± 0.39[b]	188.05 ± 1.73[c]	185.98 ± 1.46[cd]
	Fortified	498.39 ± 1.05[a]	463.68 ± 2.42[b]	459.31 ± 10.41[bc]	483.68 ± 4.51[d]
DPPH % Inhibition	Control	19.73 ± 1.54[d]	24.45 ± 1.54[bc]	34.14 ± 11.99[b]	38.25 ± 1.54[a]
	Fortified	70.67 ± 1.54[c]	74.79 ± 2.36[b]	71.18 ± 3.21[c]	75.30 ± 1.54[a]
$ABTS^+$% Inhibition	Control	38.31 ± 0.23[b]	42.51 ± 1.24[a]	43.01 ± 3.21[a]	42.91 ± 0.01[a]
	Fortified	69.31 ± 1.23[a]	65.21 ± 0.34[b]	63.41 ± 1.09[b]	62.47 ± 1.23[cd]
Peroxide value (Milliequivalent peroxide/kg yogurt)	Control	0.15 ± 1.23[a]	0.51 ± 2.13[b]	1.12 ± 1.43[ab]	1.92 ± 2.31[c]
	Fortified	0.23 ± 2.33[a]	0.35 ± 3.12[c]	62.45 ± 0.63[c]	1.02 ± 0.01[b]
pH	Control	4.52 ± 0.11[a]	4.45 ± 0.03[ab]	43.01 ± 3.21[a]	4.30 ± 0.02[c]
	Fortified	4.61 ± 0.04[bd]	4.53 ± 0.01[c]	62.45 ± 0.63[c]	4.66 ± 0.02[a]
Titrable acidity	Control	0.48 ± 0.005[d]	0.49 ± 0.006[c]	0.50 ± 0.006[b]	0.52 ± 0.006[a]
	Fortified	0.50 ± 0.006[d]	0.51 ± 0.005[c]	0.52 ± 0.01[b]	0.54 ± 0.03[a]
Lactic acid bacteria count (log10 CFU/ g yogurt)	Control	8.68 ± 0.25[a]	8.47 ± 0.32[ab]	7.18 ± 0.01[c]	6.53 ± 0.41[d]
	Fortified	8.61 ± 0.41[a]	8.34 ± 0.21[ab]	7.24 ±0.42[c]	6.12 ± 0.21[d]
Yeast/ mold (log10 CFU/ gyogurt)	Control	3.45 ± 0.07[ab]	2.96 ± 0.31[c]	3.63 ± 0.23[a]	5.86 ± 0.24[d]
	Fortified	2.21 ± 0.23[c]	2.64 ±0.41[bc]	2.87 ± 0.26[b]	3.45 ± 0.05[a]

Values are expressed as mean ± SD values followed by different letters for eachassay in the same row are significantly different ($P<0.05$)

3.4.8 Colour of the fortified yogurt

Table 4 represents the color of selected fortified yogurt during storage. The L^* valuesof control and fortified yogurts were ranged from 72.67 to 73.86 and from 77.45 to 77.84 respectively. The a^* values of control and fortified yogurts were ranged from -3.96 to -3.48 and from -4.32 to -4.01 respectively. The b^* values of control and fortified yogurts were ranged from 6.12 to 6.65 and from 8.21 to 8.76 respectively. The addition of banana peel extract was not significantly ($p < 0.05$) affected on lightness (L^*), redness (a^*), and yellowness (b^*) of the yogurt during the storage period compared to control yogurt.

Table 4 Mean (±SD) of the Color of Selected Fortified Yogurt during Storage

Yogurt	Code	Storage time (in date)

		1	7	14	21
Control	L*	73.86 ± 0.65^{a}	72.84 ± 1.23^{abc}	72.67 ± 2.12^{c}	73.01 ± 1.45^{bc}
	a*	-3.96 ± 0.57^{bcd}	-3.48 ± 0.34^{a}	-3.53 ± 2.21^{ab}	-3.78 ± 3.45^{bc}
	b*	6.43 ± 0.23^{bc}	6.34 ± 0.21^{c}	6.65 ± 0.12^{a}	6.12 ± 0.21^{d}
Fortified	L*	77.84 ± 0.21^{a}	77.45 ± 2.31^{ab}	$77.67 \pm .23^{bc}$	77.69 ± 0.31^{c}
	a*	-4.28 ± 0.27^{c}	-4.32 ± 1.32^{bcd}	-4.12 ± 2.12^{bc}	-4.01 ± 1.23^{a}
	b*	8.74 ± 0.04^{bc}	8.65 ± 0.32^{c}	8.76 ± 1.23^{a}	8.21 ± 0.76^{ab}

Values are expressed as mean ± SD values followed by different letters for each assay inthe same row are significantly different ($P<0.05$)

3.4.9 Sensory evaluation

The addition of banana peel extract up to 40mg/ 100g yogurt was received a 'like moderate" liking score on appearance, flavor, consistency, after taste and overall acceptability with control yogurts by the panelists (Figure 7).

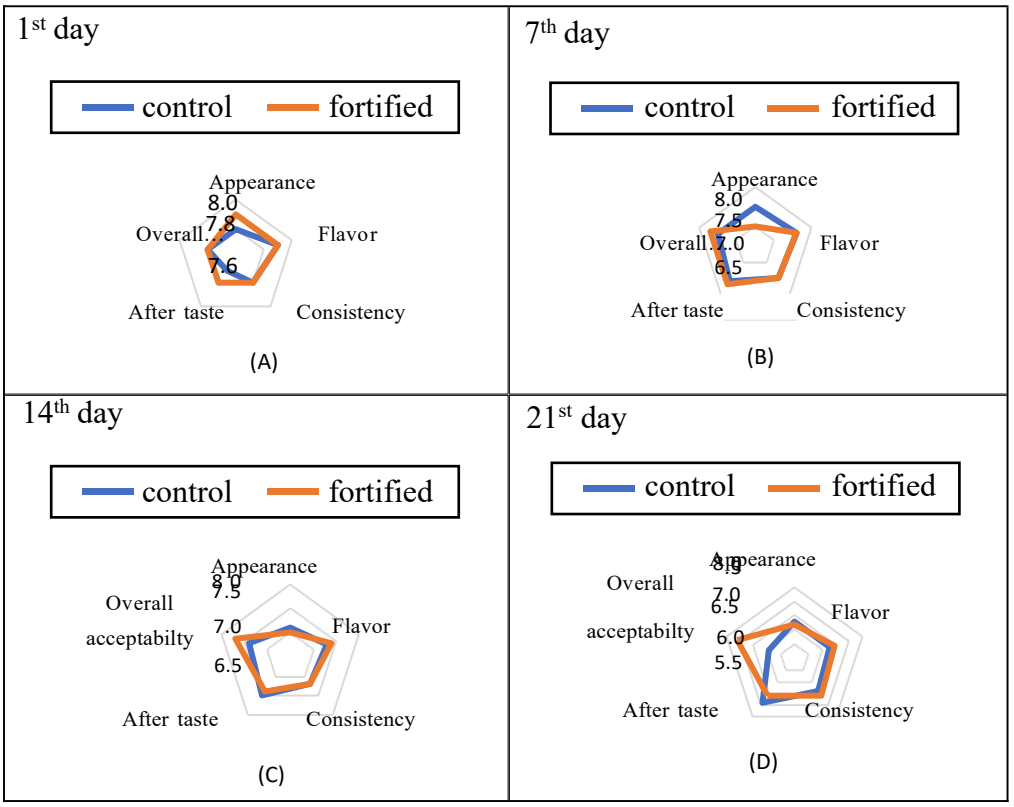

Figure 7 Influence of Banana Peel Extracts on the Sensory Properties of Yogurts (Panelist n = 30) during Storage Period

A = 1st day, B = 7th day, C = 14th day, D = 21st day. A 9 points hedonic scale: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like or dislike, 4 =dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely. Fortified: 40mg/ 100 g yogurt and control: 0mg/ 100 g yogurt

3.5 Method Validation

Calibration curves and linearity tests

The correlation coefficients (r^2) for the calibrations curves were within a range of 0.93-1.00, which was acceptable and ensured that the selected regression models satisfactorily fit the data.

4 DISCUSSION

4.1 The Ethanolic Banana Peel Extracts

4.1.1 Total phenolic content (TPC)

Previous research also has revealed a higher TPC for the *Ambul* banana peel compared to the *Seeni* and *Kolikuttu [2]*. Specifically, Daundasekara and Rajapaksha revealed TPC in the methanolic extract of *Ambul, Anamalu, Kolikuttu,*

Seeni banana peels as 113.52 µg GAE/ mL extract, 117.87 µg GAE/ mL extract, 52.00 µg GAE/ mL extract and -6.48 µg GAE/ mL extract respectively[2]. Different extract solvents and different procedures adopted for TPC analysis, even when using gallic acid as reference are likely to give different results. The current study results are higher than these values. But, it was unable to conduct a direct comparison of these findings with current study results due to differences in extraction method and the solvent used to get the banana peel extract. The variation of TPC in banana peel might be influenced by variety, growth environment, harvesting time, sample preparation, extraction solvent, extraction method and methods of determination.

4.1.2 Total flavonoid content (TFC)

Phenolic acids such as hydroxycinnamic acids, flavonoids like quercetin, catecholamines, dopamine and L-dopa and anthocyanine are the major polyphenols in the banana peel [14]. They exhibited antioxidant activity in antioxidant mechanisms like radical scavenging ability assays. TFC of banana peel extracts was not possible to compare with the literature data due to limited studies on TFC in banana peels. TFC analysis, even when using rutin as a reference is likely to give different results. The variation of TFC in banana peel might be influenced by variety, growth environment, harvesting time, sample preparation, extraction solvent, extraction method and methods of determination.

4.1.3 Determination of antioxidant activity by DPPH assay and ABTS+assay

As reported in the literature, the strong antioxidant properties of the banana extract could be due to different antioxidant components present in the banana peels. The concentration of phenolic compounds or the degree of hydroxylation of the phenolic compounds increases, the DPPH radical scavenging activity increases [1]. Specifically, Daundasekara and Rajapaksha revealed DPPH and ABTS+ % inhibition in the methanolic extract of *Ambul, Anamalu, Kolikuttu, Seeni* banana peels as 53.77%, 51.85%, 54.26%, 54.65% and 7.39%, 58.72%, 58.08% and 67.14% respectively[2]. Different extract solvents (methanol) were adopted for DPPH and ABTS+ assays. The current study results are higher than these values. But, it was unable to conduct a direct comparison of these findings with current study results due to differences in extraction method and the solvent used to get the banana peel extract. The variation of TPC in banana peel might be influenced by variety, growthenvironment, harvesting time, sample preparation, extraction solvent, extraction method and methods of determination.

As reported in the literature, Jude Awele Okolie, O lamide Emmanuel Henry was used both ethanolic and methanolic banana peel extracts of two different veritiesof banana peels as *Musa omini* and dwarf cavendish[1]. They showed, even though they were quite low in terms of TFC and TPC their antioxidant activities were higher compared to that of the ethanolic extracts of both varieties of banana peels. In this study also showed the antioxidant behavior of the banana peel extracts. Even thoughSeeni variety was ranked quite low in terms of TPC and TFC, its antioxidant activities were higher compared to that of the ethanolic extracts of both *Ambul* and *Kolikuttu* verities of banana peels. The organic compounds present in banana peel may extract with an organic solvent (ethanol) used.

4.1.4 Survey

As the result, 81%, 17% and 2% of the selected population prefer to consume yogurt, bakery products and milkshakes respectively. Therefore yogurt is used for the formulation with banana peel extract. As reported in the literature, seaweed extracts were used as the potential functional ingredients in yogurt and the effect of grape pomace dried by different methods on physicochemical, microbiological and bioactive properties of yogurt[20].

4.2 Formulated Yogurts with Different Concentrations of Seeni Banana Peel Extracts

4.2.1 TPC

The TPC of fortified yogurts were increased along with increased extract concentrations. A similar study was also observed the TPC of fortified yogurts were increased along while increasing the concentrations of the extracts fortified with grape pomace in yogurts[20].

4.2.2 Determination of antioxidant activity by DPPH assay and ABTS+assay

The concentration of phenolic compounds or the degree of hydroxylation of the phenolic compounds increases, the DPPH radical scavenging activity increases [1]. The increase in seaweed extract and apple pomace amount led to an increase in the antioxidant activity of yogurt which agreed with thisstudy.

4.2.3 Sensory evaluation of yogurts

The addition of banana peel extract up to 40mg/ 100g yogurt was received “like verymuch” liking score on appearance, flavor, consistency, color and overall acceptability. But the panelist claimed the slightly acidic taste and watery mouthful at 50mg/ 100 g yogurt. It might be the probable reason to receive a lower score on it. For comparison with previous studies indicated the use of higher concentration of grape pomace and seaweed extract in yogurt was received a lower score on color, flavor and texture.

4.3 The Selected Yogurt (40mg/ 100ml Milk) Was Formulated with Seeni Banana Peel Extract during the Storage Period

4.3.1 TPC

This study indicated the banana peel extracts were more effective in maintaining TPC in yogurts during storage. As reported in the literature, they noticed that TPC fell most rapidly in the first week and stabilized after the second week[20]. This study also showed a slight reduction in the TPC of yogurts during the storage period. Therefore a slight decrease in the TPC of yogurt could bedue to the decomposition of phenolic compounds in the presence of Lactic acid

bacteria during storage under refrigeration conditions.

4.3.2 Antioxidant activity by DPPH assay and ABTS+ assay

The concentration of phenolic compounds or the degree of hydroxylation of the phenolic compounds increases, the DPPH radical scavenging activity increases [1]. In ABTS+ assay, the radical scavenging ability in fortified yogurts was slightly dropped after 7 days of storage, but stable during storage. These findings indicated that the ethanolic banana peel extract was stable ingredients as an antioxidant in yogurts as the seaweed extract stabled in yogurt. But fortified yogurts did not show the same antioxidant activity. The previous study noticed that the milk protein in yogurts, radical scavenging and antioxidant activities of many polyphenolic compounds were decreased due to the formation of a polyphenolic-milk protein complex [14].

4.3.3 Peroxide value

Lipid oxidation is one of the major concerns in food quality deterioration. These results indicated the delaying of lipid oxidation in fortified yogurt with banana peel extract compared to control yogurt during the storage period.

4.3.4 pH

The pH values of fortified yogurts were slightly higher than control yogurts. But it was not statistically significant ($p < 0.05$). It could be due to the higher pH value ofthe *seeni* banana peel extract (5.87). The pH value of fortified yogurt was dropped up to 7 days and it was raised from the second to end of the storage time. They noticed the reduction of pH during the storage of yogurts. This reduction can be explained by the high rate of production of lactic acidand galactose at the initial 7 days of storage due to high bacterial metabolic activity with the consumption of lactose.

4.3.5 Titrable acidity

A similar result was reported that the titrable acidity of fortified yogurt containing olive leave extract gradually increased during storage[21]. Titrable acidity may be influenced by the level of nonfat solid substances such as citrates, protein and phosphates[21]. This study also showed the titrable acidity was increased during the storage period.

4.3.6 Microbial count

4.3.6.1 Lactic acid bacteria count

The addition of banana peel extracts in yogurt has not influenced lactic acid formation. The pH value of banana peel extract was 5.2. And the optimum pH value of *Lactobacillus rhamnosus* for growth is in the range from 6.4 to 4.5[22]. Therefore they can survive in the yogurt.

4.3.6.2 Yeast and mold count

The addition of banana peel extracts in yogurt does not influence yeast and mold counts. It is the same as the results obtained from the other studies. The microbial count in yogurt was unaffected by seaweed extract addition.

4.3.7 Colour

The storage time was unaffected ($p < 0.05$) on the color of the yogurt during 21 days of refrigeration storage. The L*, a* and b* values of the fortified yogurts were slightly darker, redder and less yellow during storage. Like previous research, they reported that the L* and b* values of yogurt on fortification of olive leaf extract were decreased during[21].

4.4 Sensory

The sensory properties of fortified yogurt with the storage time were determined. The appearance, flavor, consistency, after taste and overall acceptability, were positively correlated with storage time. It indicated the likeness of selected quality attributes. The fortified yogurt received a liking score “like slightly” on flavor and overall appearance after 21 days of storage. These results indicated the sensory attributes of fortified yogurt decreased after 14 days of storage. The lower sensory score might be associated with the reduction of pH due to the formation of lactic acid by lactic acid bacteria.

5 CONCLUSION

In the current study, the antioxidant potential of ethanolic banana peel extract of three different varieties of Sri Lanka was evaluated. Banana peel extract was successfully applied for the formulation of bioactive compounds rich in yogurts. ‘*Seeni’ (ABB), ‘Kolikuttu’ (AAB)* and *‘Ambul’ (AAB)* were used as the three varieties of banana. It was proved that the antioxidant potential was higher in ethanolic ‘*Seeni’* banana peel extract compared to the other two varieties. The preliminary consumer survey showed the acceptability for yogurt to incorporate with banana peel extract. The ethanolic banana peel extract of ‘*Seeni’* was incorporated with yogurt within the range of 0-1000 µL/100 mL milk. The extract incorporated up to 800 µL/100g in yogurts received the highest rating in the 9 points hedonic scale in terms of appearance, flavor, consistency, and overall acceptability. It exhibited a higher TPC, DPPH, $ABTS^{+}$ and lower peroxide value compared to control yogurt after 21 days. The color, pH, titrable acidity, the microbial count did not show any significant differences at refrigeration storage. The study was demonstrated that banana peel extract has the potential to be used as a functional food ingredient for promoting the storability of yoghurt.

6 SUGGESTIONS AND RECOMMENDATION

Further research is needed to understand the mechanism of antioxidants of yogurts with added banana peel extracts to profile the change of bioactive compounds during the storage period. The vacuum package and the low oxygen environment packing can be used to collect the banana peels to reduce the browning of banana peels during the drying

period. It is essential to standardize the fat, solid nonfat, sugar content along with the proximate composition of yogurts. Further research is needed to identify the changes in antioxidant potentials of banana peels in different ripening stages. As a suggestion, the natural banana flavor can be added to the yogurts. Depending upon the application, antioxidants can be recommended in yogurt manufacture.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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