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Full Paper

Development of Banana Flour Incorporated Biscuit and Evaluation of its Physicochemical Properties

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Abstract

The Postharvest loss of Embul banana (*Musa* spp.) is very high due to its surplus production in Sri Lanka. This work aims towards the development of nutritionally rich biscuits by partial replacement of wheat flour with banana flour and candied citrus peel (*Citrus aurantifolia*). During the preliminary studies, the appropriate proportion of wheat flour, banana flour, and citrus peel was selected through a sensory evaluation using 9-point hedonic scale by 20 untrained panelists. The final product was prepared using the selected composition of banana and wheat flour 50:50 and 10% citrus peel. Significant differences were observed in all the sensory properties ($p<0.05$) except appearance ($p>0.05$) during the storage period. Ascorbic acid content was significantly reduced ($p=0.000$) with the storage period. The product was microbiologically safe for consumption for up to 2 months period. This study investigated the potential of the development of *Embul* banana-based biscuits with improved functional and nutritional properties.

Keywords: banana, biscuit, citrus peel, flour, value addition

Introduction

Due to consumers' demand for healthier foods, the food industry is directing new product development towards the area of functional food and ingredients. The term functional food was first introduced in Japan and refers to processed foods containing ingredients that aid specific functions of the body in addition to being nutritious [1]. Functional foods are foods that have a potentially positive effect on health beyond basic nutrition. Functional foods promote optimal health and help to reduce the risk of disease [2]. At present, the consumption of functional foods has become a trend.

Biscuits are the most popular consumed bakery item worldwide. Some of the reasons for such wide popularity are their ready-to-eat nature, affordable cost, good nutritional quality, availability in different tastes, and longer shelf life [3].

Banana is a tropical herb of the genus *Musa* and of the family Musaceae. Banana is one of the most popular fruits in the world [4]. Banana which serves as an instant energy booster is one of the most widely consumed fruits in the world. Bananas are an excellent source of potassium which also contains dietary fibres. Banana contain lots of minerals and other nutrients as well. Bananas are high in potassium, vitamins A and B_6, and carbohydrates. They are low in fat, cholesterol, and salt [5]. The soluble fibre tends to slow down digestion and keep you feeling full for a longer time [6]. Hence, there are many health benefits such as controlling blood sugar levels, improving digestive health, helping with weight loss, supporting heart and kidney health, reducing the risk of cancer, etc. [7]. Banana is a climacteric fruit. Due to the climacteric nature of the banana, its shelf life is short, spoilage is most ensured, and waste is therefore abundant [8]. However, in Sri Lanka, post-harvest losses are higher than losses at harvesting in *Embul* banana. Post-harvest practices such as handling, transportation, and storage are time-consuming. Therefore, significant loss of weight, visual quality, and nutrients can occur when fresh produce reaches the consumer [9]. The high perishability of the banana due to its high moisture content (74.3%), together with its abundant production, oversupply, and low cost, has prompted the search for stable food products made from bananas to increase production utilization and take advantage of the nutrients that bananas supply [10]. Therefore, this research was done to develop a functional food using banana.

Citrus (*Citrus* L. from Rutaceae) is one of the most popular world fruit crops and contains active phytochemicals that can protect health [11]. Citrus peel is a major by-product obtained during the processing of citrus products. Recently, fruits and vegetables have received much attention as a source of biologically active substances because of their antioxidant, anti-cancer, and anti-mutagenic properties [12]. Citrus peels contain immune-boosting vitamin C, bone-building calcium, and anti-inflammatory, antioxidant bioflavonoids. They also provide potassium, which helps to keep blood pressure in check, and limonene, a phytochemical that may have anti-cancer effects and can help with heartburn [13].

The aim of the present study was the improvement of the functional and nutritional properties of fruit-based biscuits. Specifically, it was conducted in main steps of preparation of dehydrated banana powder and evaluation of its physicochemical properties, evaluation of organoleptic, nutritional, microbial, and physicochemical characteristics of banana powder supplemented biscuit, to utilize fruit, by-products effectively, and to utilize the excess banana production for value addition.

Materials and Methods

Materials

Fresh *Embul* bananas, lime, and sweet orange were purchased, transported to the laboratory, and stored in room temperature conditions. Other materials purchased were wheat flour, margarine, milk, sugar, baking powder, milk powder, preservatives, and eggs.

Dehydrated Banana Flour Formulation

Banana flour was produced using the oven-drying method. Two maturity levels of *Embul* banana were selected as full yellow (FY) and yellow with trace green (YG) based on their peel colour index [7]. The bananas were sorted and washed thoroughly with tap water and all the dirt was removed. Bananas were peeled and sliced. Banana pieces were dipped in water with 1.5 M Citric acid and 1.5 M Ascorbic acid for 15 minutes to avoid the browning reaction and then drained. Slices were spread on trays. The trays were kept in the air oven. Banana slices were dried in an air oven at 55 °C till moisture reached 7±1%. Dried banana chips were powdered using a laboratory scale grinder and sifted through a sieve. The flour samples were sealed and packed in polythene for further analysis.

Evaluation of Physicochemical Properties of Banana Flour

Moisture, ash content, crude protein, crude fibre, and free fat of the dehydrated banana flour samples were determined according to the standard methods of AOAC [14]. Ascorbic acid was determined using the titration method. Water activity and dispersibility of the two powders were also determined as physical characteristics.

Determination of Crude Protein

The crude protein of samples was measured through the determination of total nitrogen content using the Kjeldahl method [14]. Protein percentage was calculated using the following equation which is a modification of AOAC official method 920.87.

$$\text{Nitrogen\%} = \frac{(\text{Sample titre-Blank titre}) \times \text{Molarity of HCl} \times 14 \times 100}{\text{Weight of the sample taken}}$$

Protein % = Nitrogen percentage × 5.70

Determination of Free Fat

Free fat content was determined using the Soxhlet method and it was estimated to express as a percentage which is a modification of AOAC official method 920.39 [14]. The process of drying, cooling, and weighing was repeated until a constant weight was taken.

$$\text{Percentage of the fat} = \frac{X - F}{W} \times 100\%$$

W = Weight of the sample
F = Weight of the empty flask
X = Weight of the flask with fat

Determination of Crude Fibre

The crude fibre content of the sample was determined and expressed as a percentage (w/w) which is a modification of AOAC official method 962.09 [14].

Determination of Total Ash

The total ash of the sample was determined and the ash percentage was calculated using the following equation, which is a modification of AOAC official method 923.03 [14].

$$\text{Ash\% (Wet Basis)} = \frac{W_2\text{-W}}{W_1} \times 100\%$$

W_2 = mass of the dish with ash in grams
W = mass of sample in grams
W_1 = mass of dish in grams

Determination of Moisture

Moisture was determined by using an OHAUS MB45 moisture analyzer. About 5 g of sample was placed in the moisture analyzer and then it was allowed to count moisture at 105 °C.

Determination of Ascorbic Acid

Ascorbic acid was determined using titration method and calculation was done according to the following formula which is a modification of AOAC official method 967.21.

$$\text{mg of ascorbic acid per 100 g or m} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{A} \times \text{W}}$$

A = Aliquot of extract taken for estimation
W= Weight or volume of sample taken for estimation

Determination of Dispersibility

Hundred milliliters of distilled 20 °C water was poured into 250 mL beakers. The agitation of water was done by a magnetic stirrer. 10 g of powder was gently poured onto the surface of the water and time for the powder to be well dispersed was recorded. Well-dispersed means no particles settling on the bottom and no particles floating on the surface [15] [16, 17].

Preparation of Candied Citrus Peel Pieces

Citrus aurantifolia (Lime) and *Citrus sinensis* (Sweet orange) were selected as raw materials. They were thoroughly washed with tap water, peeled, and sliced into small pieces. The weight of the peels was measured. Citrus peels were blanched for 5 minutes at 60 °C. The sugar solution was prepared as follows.

An equal amount of sugar as the weight of citrus peel was taken. Half of the sugar amount was heated with water up to 70 °Brix. The mentioned Brix value was obtained when measured using a Brix meter. Citric acid was added to the solution as a preservative. Citrus peel pieces were added to the heated sugar solution and kept for 6-8 hours. Then the peels were filtered and again other sugar amount was added to the filtered water and heated up to 70 °Brix as above and kept for 6-8 hours. This step was done for the whole amount of sugar. Then sugar was allowed to absorb to the citrus peels by osmosis. Peels were washed with tap water to remove surface sugar and dried in an air oven at 55 °C for about 4 hours. Candied citrus peel pieces were sealed and packed in polythene.

Determination of Moisture

The same method was followed as described above for the determination of moisture of banana flour.

Product Development

Preliminary experiments were conducted to select a suitable ratio of banana flour and wheat flour in developing biscuits as value-added products.

Formulation of Embul Banana-incorporated Biscuit

Banana flour, wheat flour, 125 g of sugar, 75 g of Margarine, 1 g of Milk powder, baking powder, Vanilla essence, and an egg were used as ingredients for biscuit preparation.

Table 1 shows the flour combinations (banana and wheat) used as treatments to prepare the biscuits.

Table 1. Treatment arrangements to select the best flour composition

Treatments	Banana flour	Wheat flour
Treatment 1	25% (75 g)	75% (225 g)
Treatment 2	50% (150 g)	50% (150 g)
Treatment 3	75% (225 g)	25% (75 g)

Figure 1 shows the procedure for the preparation of biscuits.

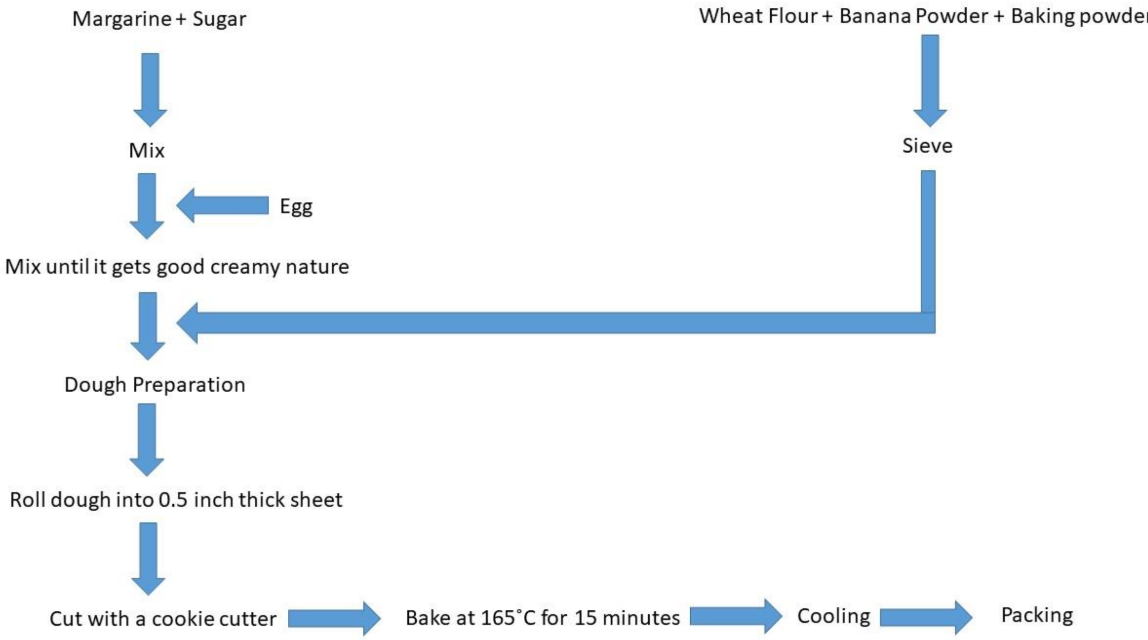

Figure 1. The procedure of preparation of biscuits

Evaluation of Acceptability of the Developed Product

Product quality was checked according to SLS standard (SLS 251:1991) and sensory analysis. Biscuits were tested for appearance, taste, texture, aroma, and overall acceptability by using a sensory evaluation panel consisting of 30 untrained panelists and a 9-point hedonic scale.

Enrichment of Biscuit by Using Candied Citrus Peel

Candied citrus peel pieces were incorporated into the best type of biscuit which was above selected. They were added using 2 ratios 10% and 20%. The enriching ratios of citrus peel to the previously prepared biscuits are mentioned in Table 2.

Table 2. Enriching ratio of citrus peel

Treatment	Variety
Treatment 1	Lime 10%
Treatment 2	Lime 20%
Treatment 3	Sweet orange 10%
Treatment 4	Sweet orange 20%

Evaluation of Acceptability of the Developed Product

Product quality was evaluated according to SLS standard (SLS 251:1991) and sensory analysis. Biscuits were tested for appearance, taste, texture, aroma, and overall acceptability by using a sensory evaluation panel consisting of 20 untrained panelists and a 9-point hedonic scale. The results were analyzed by the Friedman test of the MINITAB 17 statistical package.

Physicochemical Analysis of the Biscuit

Total ash, free fat, crude protein, and crude fibre content were determined using the same AOAC (2000) methods that were followed as described above for the determination of these contents in banana flour. The acidity of extracted fat and acid-insoluble ash was determined according to Sri Lankan standards specifications for biscuits [18, 19]. Ascorbic acid content was also determined. Moisture was determined by using the OHAUS MB45 moisture analyzer by the United States of America.

Determination of Acidity of Extracted Fat

Powdered biscuits were transferred to the extraction thimbles. The end of the thimbles were plugged with cotton wool. Extraction thimbles with samples were placed in the Soxhlet apparatus. Clean-dried 250 mL round bottom flasks were fixed. 200 mL of extracting solvent (Pet.Ether) was added to the pre-dried weighed. Flasks were connected to the Soxhlet extractor and fitted with the condenser. They were refluxed for 5 hours.

Once the refluxing was over solvent was distilled off and the flask was oven-dried at 105 °C for 2 hours. Then it was desiccated and weighed, and fat was estimated and expressed as a percentage. 50 mL of ethanol was measured and added to the flask containing fat to dissolve fat samples in ethanol.

The solution was boiled and titrated with the potassium hydroxide solution to a distinct pink. The colour changes from yellow to light red when titrated with potassium hydroxide solution. The blank one is in light purple.

Blank titration was carried out and blank titre was subtracted from the titre of the fat [19].
Calculation:

$$\text{Acidity of extracted fat (as oleic acid), percent by mass} = 1.41 \times V \times \frac{c}{m_1 - m_0}$$

V = volume, in mL, of potassium hydroxide solution required for the titration (corrected for the blank)
c = concentration in mol / l, of the potassium hydroxide solution
m_1 = mass, in g, of flask containing fat
m_0 = mass, in g, of the empty flask

Determination of Acid Insoluble Ash

5 g of sample was weighed and placed in a pre-cleaned, dried, and weighed crucible. Then it was ignited slowly over a Bunsen flame until no more fumes evolved. The dish was transferred to a muffle furnace (Model- JTM S1000) at 600 °C incinerated until white ash was obtained. The crucible was removed and desiccated before weighing. 25 mL of hydrochloric acid was added and covered with a watch glass and heated in the water bath for 10 minutes. Contents were mixed with a glass rod and filtered through an ashless filter paper. The filter paper was washed with water until washings were free from acid when tested with blue litmus paper. The washed filter paper was placed in a dish and then incinerated in the muffle furnace.

The dish was cooled in a desiccator and weighed. The process of heating, cooling, and weighing at 30-minute intervals was repeated until the difference between two successive weighings did not exceed 1 milligram [18].

Calculation:

$$\text{Acid insoluble ash, percent by mass (On dry basis)} = \frac{m_1 - m_0}{(m_2 - m_0)} \times \frac{1000}{(100 - M)}$$

m_0 = mass of empty dish in g
m_1 = mass of the dish containing ash, in g
m_2 = mass of the dish containing the sample in g
M = percentage of moisture

Evaluation of Spread Ratio

Three rows of five biscuits each were formed, and the height was measured. The biscuits were arranged in a horizontal manner keeping edge to edge. Then the sum of the diameter was measured with the height. The spread ratio was calculated by using the following formula.

$$\text{Spread ratio} = \frac{\text{Average diameter}}{\text{Average height}}$$

Microbiological Quality

Microbiological quality is a common criterion used to determine the acceptability and shelf life of developed products. Microbial counts of the developed products mainly depend on product characteristics, resident micro-flora of the product, and handling quality of utensils used during the processing, storage, and distribution [20]. The total plate count of the biscuit was evaluated as described by AOAC official methods 2000.

Total Plate Count

Plate count agar solution was prepared as follows. 6 g of plate count agar was measured to two 250 mL conical flasks and volumerise up to 200 mL. Required test tubes, conical flasks, pipette tips, and plate count agar solutions were sterilized using a pressure cooker for 20 minutes. Required petri plates and mortar and pestle were sterilized in the oven at 105 °C for 2 hours.

10 g of sample was weighed, and 90 mL of distilled water was added. 1 mL from the initial suspension was taken and it was added to 9 mL of distilled water. Like that serial dilutions were made up to 10^{-6}. 1 mL of each dilution series was poured into Petri dishes with 15 mL of plate count agar. Then plates were incubated at 37 °C for 48 hours. The cultured samples for microbiological analysis are shown in plate 3.18. Colonies were counted and expressed as colony-forming units per gram of food. After the incubation at 37 °C for 48 hours, the colonies were counted in each dish containing not more than 300 colonies. Dishes were retained containing less than 300 colonies at two consecutive dilutions. One of these dishes needed to contain at least 15 colonies. The number of microorganisms (N) per milliliter or gram of product was calculated, using the the following equation.

$$N = \frac{\Sigma C}{(N_1 + 0.1N_2)d}$$

ΣC = Sum of the colonies counted on all the dishes retained
N_1 = Number of dishes retained in the first dilution
N_2 = Number of dishes retained in the second dilution
d = Dilution factor corresponding to the first dilution

Storage Quality Evaluation

Biscuits with lime peel were stored under ambient conditions for seven weeks. The biscuits were packed in two packaging materials as follows.
Combination of metalized material : 30 BOPP / 25 Met. CPP
Combination of transparent material : 20 BOPP / 25 CPP
Moisture content, ascorbic acid content, and microbiological analysis (total plate count) were conducted each week.

Data Analysis

The experimental design was Completely Randomized Design. Data was analyzed by MINITAB 17 statistical software. The data of the experiment was analyzed using ANOVA and means were separated using the Tukey test. Non-parametric data was analyzed by using MINITAB 17 computer package. Appearance, taste, texture, aroma, and overall acceptability results were analyzed by the Freidman test of the MINITAB 17. The results of the storage study were analyzed using two factor factorial experimental design.

Results and Discussion

Evaluation of Physicochemical Properties of Powder

According to the proximate analysis of the FY powder and YG powder, it is evident that the crude fibre content has reduced with the ripening (Table 3). A clear understanding of moisture content is important for a product, as it affects the quality and stability of foods, which makes it an important and often used analytical parameter in the food industry [21]. The results of proximate analysis (Table 3) show that the crude protein and moisture content have increased with the ripening.

Table 3. Proximate analysis of banana flour

Proximate constituent	Banana powder from FY stage	Banana powder from YG stage
Crude fibre (%)	0.17* ± 0.01	0.45 ± 0.06
Crude protein (%)	0.005 ± 0.001	0.002 ± 0.001
Moisture (%)	15.86 ± 0.01	6.28 ± 0.01
Free fat (%)	0.19 ± 0.01	0.19 ± 0.01
Ash (%)	2.68 ± 0.10	2.48 ± 0.10

* Values are mean ± standard deviation of triplicate (n=3)

Ascorbic acid content was equal for 2 powders (54.5 mg/100 g). The water activity of FY powder and YG powder were 0.57 ± 0.01 and 0.42 ± 0.01 respectively. Since p (0.000) < α (0.05), there is a significant difference in water activity between the two powders at 95% confidence level. Dispersibility of FY powder and YG powder were 41.33 ± 2.52 and 11.33 ± 1.15 respectively. Since p value (0.000) is less than α (0.05), there is a significant difference in dispersibility between the two powders at 95% confidence level.

Evaluation of Acceptability of the Product

The result of sensory evaluation exhibited significant differences (p<0.05) between sensory attributes at 95% confidence level and biscuits with 50:50 flour combination scored higher rating for all sensory attributes at 95% confidence level.

The result of sensory evaluation exhibited significant differences (p<0.05) in sensory attributes at 95% confidence level and biscuits with equal amounts of banana and wheat flour and 10% lime peel scored higher ratings for all sensory attributes at 95% confidence level.

Physicochemical Analysis of Biscuits

According to the results of the proximate analysis, crude fibre content and moisture content increased with the incorporation of lime peel. But moisture%, acid insoluble ash% and acidity of extracted fat% are in the range according to SLS specifications number for biscuits. The crude protein is reduced in fortified biscuits (Table 4).

Table 4. Proximate analysis of biscuits

Proximate constituent	Biscuit without peel	Biscuit with peel
Crude fibre (%)	0.013* ± 0.01	1.06 ± 0.35
Crude protein (%)	0.004 ± 0.001	0.0002±0.00
Moisture (%)	3.65 ± 0.01	5.55 ± 0.01
Free fat (%)	13.06 ± 0.85	11.18 ± 0.43
Ash (%)	1.40 ± 0.01	1.41 ± 0.20

* Values are mean ± standard deviation of triplicate (n=3)

The spread ratio is an important character of biscuits. It depends on the ingredients used and the degree of softening of gluten. This should be a constant for a particular type of biscuit. The spread ratio of biscuits in this study was 5.

Storage Study

Two different treatments (packing materials), 30 BOPP / 25 Met .CPP and 20 BOPP / 25 CPP were used. Since p value (0.000) is less than α, we can conclude that there is an interaction effect between packing material and time interval at 5% significance level.

Moisture content of biscuits which is packed in 20 BOPP / 25 CPP is higher than the biscuits packed in 30 BOPP / 25 Met.CPP (Figure 2). 30 BOPP / 25 Met. CPP is an industrial level applying packing material for biscuits due to its superior barrier properties, low permeability for water vapour, low oxygen transmission, excellent sealing strength etc. Moisture content in two materials showed increments and reductions as a result of environmental conditions.

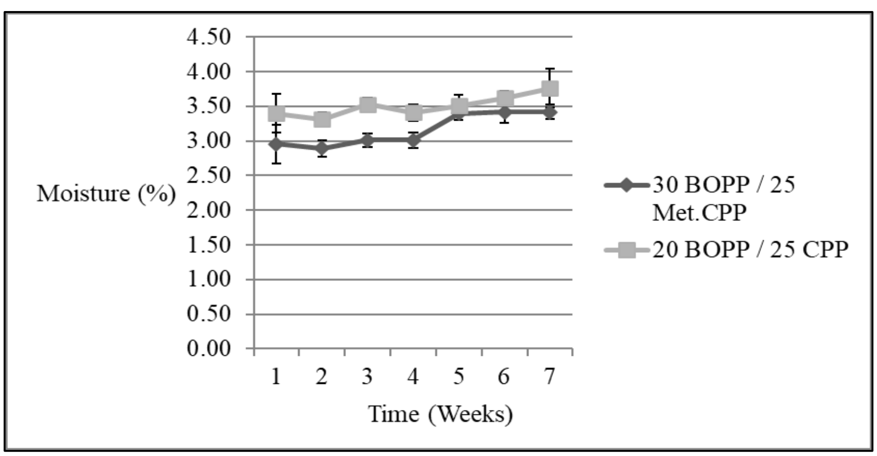

Figure 2. Changes in moisture % during storage

Ascorbic acid content of biscuits which packed in two packing materials has gradually decreased with the storage period as a result of internal chemical reaction (Figure 3).

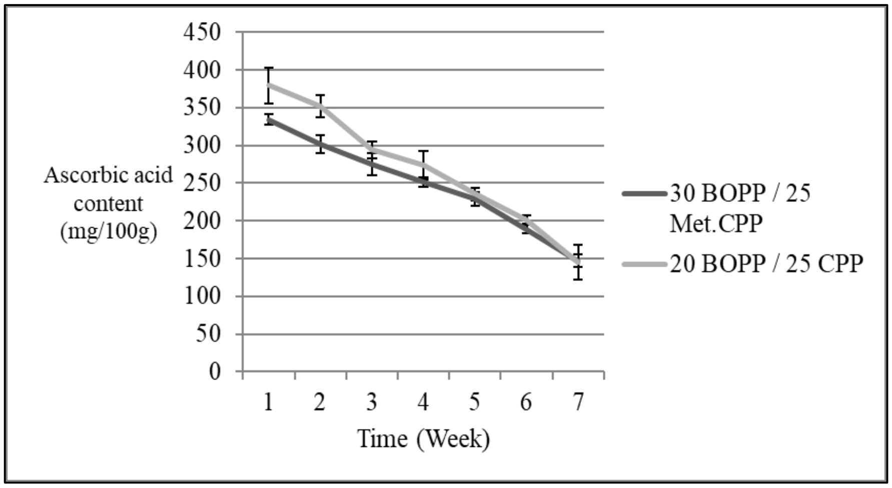

Figure 3. Changes in ascorbic acid content during storage

Biscuits in 30 BOPP / 25 Met.CPP packing material contains a low amount of total plate count compared with biscuits in 20 BOPP / 25 CPP (Figure 4).

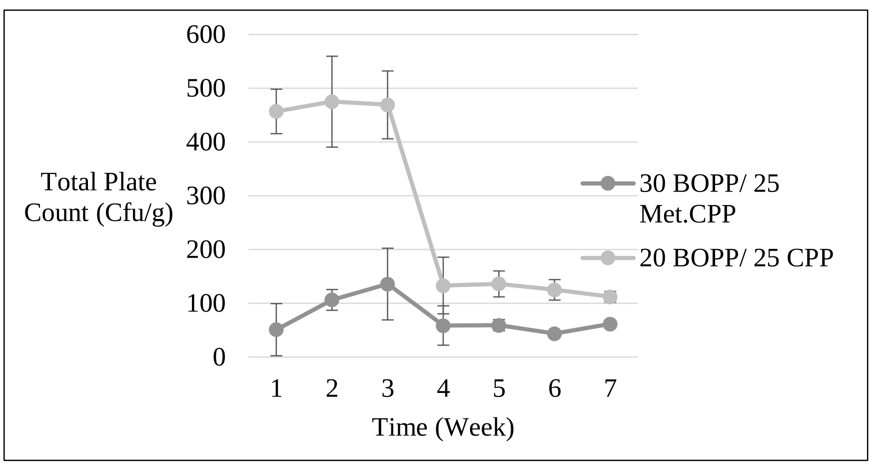

Figure 4. Changes in total plate count during storage

Here the reason for having low total plate count in metalized packing material can be the excellent sealing property and superior barrier properties.

Significant differences were observed in all the sensory properties ($p<0.05$) except appearance ($p>0.05$) during the storage period. Ascorbic acid content was significantly reduced ($p=0.000$) with the storage period. The product was microbiologically safe for consumption for up to 2 months period.

Conclusion

Dehydrated banana flour with the YG stage has more acceptable physicochemical properties than flour with the FY stage. Citrus peel, a fruit by-product, could be effectively utilized for the preparation of biscuits. YG stage banana powder could be incorporated in a 1:1 ratio with wheat flour and 10% candied lime peel in the formulation of biscuits without affecting their overall quality. Banana powder and lime peel-supplemented biscuits have acceptable organoleptic, nutritional, microbial, and physicochemical properties. It can be concluded that 30 BOPP / 25 Met. CPP was the best packing material for biscuits when compared with 20 BOPP / 25 CPP. The major conclusion arising out of this study was the potential of fruit-based biscuits with improved functional and nutritional properties.

Conflicts of Interest

The authors report there are no competing interests to declare.

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