

Formulation and Characterization of Nutrient Bars using Underutilized Seeds: Semolina and Jackfruit Seeds

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Abstract

Background: Fast foods are frequently used as an easy replacement of main meals by people, who lead busy lifestyles. Many nutrient bars are prepared with expensive and popular choices such as sunflower, flax, chia, sesame and hemp seeds. However, underutilized, cheaper alternatives such as semolina and jackfruit seeds could provide the same nutritional background required in a nutrient bar. This study was conducted to develop a nutrient bar from nutrient dense underutilized seeds as a main meal replacement.

Methods: Two distinct nutrient bars were formulated with watermelon seeds, winged bean, and pumpkin seeds as common underutilized seeds. Bar 1 (Treatment 1) was prepared by incorporating semolina seeds and Bar 2 (Treatment 2) was prepared incorporating flour of jackfruit seeds. Nutritional composition, physiochemical properties, microbial parameters and sensory profile of the formulated bars were determined using standard protocols suggested by the United State Department of Agriculture (USDA), Association of Official Agricultural Chemist (AOAC) standards, Sri Lanka Standards Institution (SLSI). The Friedman test was used for statistical analysis.


Results: Samples of Treatment 2 reported significantly highest moisture ($14.4\pm 0.66\%$) and fibre content ($9.5\pm 0.71\%$), while Treatment 1 had the highest fat level ($10.1\pm 0.01\%$). In terms of ash and protein content, there were no significant differences ($P>0.05$), between the developed samples and commercially available nutrient bars. Treatment 1 reported the highest calorific value (388.7 kcal/100g), while the highest phenolic content (8.3 ± 0.30 mg GAE/g) and antioxidant activity were observed in Treatment 2. Based on Sensory evaluation, mean values of colour and mouth feel were highest in Treatment 2, while the mean values for aroma, texture, and overall acceptability were highest in Treatment 1.

Conclusions: Based on overall performance nutrient bar developed under Treatment 1 could be recommended as an excellent source of energy and nutritional component for a daily meal replacement.

Keywords: Daily Meal Replacement, Nutrient Bar, Natural Preservatives, Underutilized Seeds

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INTRODUCTION

Nutrients are compounds in foods, which are essential to maintain bodily functions and a healthy lifestyle. Food provides us with energy, acts as building blocks for repair and growth of cells and provides substances to regulate biochemical processes within the human body. There are six major nutrients: Carbohydrates (CHO), Lipids (Fats and Oils), Proteins, Vitamins, Minerals, and Water [1]. The amount of energy contained in food is indicated by the number of calories in that food. The recommended daily intake of calories depends on age, sex, and the level of physical activity. On average an individual needs to obtain around 2000 calories each day to maintain the basic bodily functions [2].

Many people who live a busy lifestyle tend to overlook the necessity of taking sufficient amounts of daily intake of nutrients coming from a balanced diet. Most people who engage in day jobs in public and private sector skip their main meals due to their busy work schedules [3]. Some find fast food as an alternative to replace the main meals, which could be consumed on the work. However, the majority of fast foods are incapable of providing the goodness coming from a balanced diet. On the other hand, most fast foods are high in calories and unhealthy saturated fatty acids. Regular consumers of fast food face the danger of contacting non-communicable diseases like diabetes and high blood pressure and often suffer from obesity due to high calorie uptake [4].

Nutrient bars could provide a solution for people, who often skip their main meal yet search for a suitable alternative to take daily recommended nutrients. A better formulated nutrient bar could provide a variety of essential macro and micro nutrients, as well as, sufficient amounts of protein and carbohydrates to keep the body running smoothly. Nutrient bars may concentrate on protein and reduced carbohydrates, or they may attempt to serve as a full meal with a higher caloric load (350 kcal), depending on the intent [5].

Most commercially available nutrient bars have been formulated by incorporating expensive ingredients such as sunflower, flax, chia, sesame and hemp seeds. Underutilized seeds, which are often discarded as agro-industrial waste by food and beverage processing companies could provide a cheaper alternative to replace expensive ingredients and at the same time provide the vital nutrients required from a nutrient bar. Production of well-balanced nutrient bar with a reasonable price tag could effectively cut down the consumption of unhealthy fast-foods [6].

This study attempts to develop a nutrient bar incorporating cheap and often wasted ingredients [7], such as Semolina which is a product of wheat milling. It is rich in dietary fibre, while seeds of jackfruit are rich in starch, calcium, vitamins, minerals and antioxidants [8]. Meanwhile, nutrient dense watermelon seeds are rich in protein and fibre [7]. Pumpkin seeds are a rich source of protein, fibre and minerals [8-9].

In previously reported studies; Nadeem *et al.* (2018) has combined dates with cereal and legumes to make a date bar [10], while Kumar *et al.* (2018) has created a protein bar with added spirulina for children suffering from malnutrition [11]. However, these products could not suffice the full nutritional requirements of a full meal. Use of often wasted seeds of agricultural produce to formulate a nutrient bar could bring in a commercial value to them and provide farmers the opportunity to increase their income.

METHODOLOGY

Study Setting

All the studies covered under this research were conducted at Uva Wellassa University, Badulla from 28th of September 2020 to 31st of May 2021.

Preparation of Dry Ingredients

Winged bean seeds, watermelon seeds, and pumpkin seeds were washed, dried and then

roasted under low flame (45 – 82 °C) until they were light brown in colour. Then they were grounded to obtain a fine powder.

Preparation of Nutrient Bars

Precooked seeds, desiccated coconut, corn flour, semolina, jackfruit seeds flour and salt were combined in a stainless-steel mixing bowl and mixed well under low heat. Sugar caramel was prepared and vanilla flavour and citric acid were added to the mixture. The resulting mixture was molded out (2.5×2.5×4 cm) and packed in a heat sealable aluminium foil package. Treatment plan of nutrient bars is given in Table 1.

Table 1: Formulations of Nutrient Bars

Ingredients (in gram)	Treatment 1 (T 1)	Treatment 2 (T 2)
Semolina	160g	0g
Jackfruit seeds	0g	160g
Corn flour	40g	40g
Watermelon seeds	40g	40g
Pumpkin seeds	40g	40g
Winged bean seeds	80g	80g

Proximate Analysis

Moisture content was measured using a moisture analyzer (DW-110MW laboratory Halogen Moisture Analyzer, China). The ash Content (AOAC, 942), crude fibre (AOAC, 978.10), crude fat (AOAC, 2003.05), crude protein (AOAC, 2001.11) were measured using AOAC standard methods [12-13]. To measure the Carbohydrate content, the moisture, ash, fibre, fat and protein content were totalled and then reduced by 100. The formula that was used to calculate the carbohydrate content is as follows.

$$\begin{aligned} \text{Carbohydrate \%} &= 100 - (\text{moisture \%} \\ &+ \text{ash \%} \\ &+ \text{crude fibre \%} \\ &+ \text{crude fat \%} \\ &+ \text{crude protein \%}) \end{aligned} \quad (1)$$



Figure 1: Samples Obtained for Treatment 1



Figure 2: Samples Obtained for Treatment 2

Gross Energy Value

Gross energy values of nutrient bars were calculated using standard factors for energy in the form of kcal/g as 4, 9 and 4 kcal/g for protein, lipid and carbohydrate, respectively. The energy contents were summed up to result total or gross energy [14].

Physico-chemical Analysis

Water activity (a_w) was measured using AQUALAB 4TE Water activity meter, while pH value was measured with a portable pH meter after calibration. Brix determination was done using Mettler Toledo Refracto 30GS Portable Handheld Refractometer. Following

calculations were used to calculate the Brix value (Equation 2 and 3).

$$\text{Degree of Factor} = \frac{1 + \text{volume of water}}{\text{weight of sample}} \quad (2)$$

$$\text{Brix value} = \text{reading of refractometer} \times \text{degree of factor} \quad (3)$$

Phyto-chemical Analysis

Antioxidant activity was measured using DPPH Radical Scavenging Assay [15]. One gram of each powdered nutrient bar sample was taken into a small beaker and 9 ml of distilled water was added into it to prepare the solution. From each prepared powder solutions, 1.2 g was taken and it was mixed with 20 ml of 80% methanol. The solutions were added into screw cap tubes and were centrifuged at 11,000 rpm for 10 minutes. Around 1 ml of 80% methanol was filled into a set of test tubes and 20 µl of the sample was transferred in to the first test tube, 40 µl sample was put into the second test tube and 60 µl sample was added into the third test tube. All the tubes were vortexed using a vertex mixer for 2 minutes. DPPH solution was prepared mixing 3.94 mg of DPPH powder with 100 ml of absolute methanol. From the sample, 0.5 ml was taken into another tube and 2.5 ml of DPPH solution was added into each tube. The test tubes were kept in a dark room for 20 minutes and absorbance was determined at 517 nm using a UV spectrophotometer (N-6000 Model, Yoke Instruments, China). The inhibition % was calculated using the following formula.

$$\text{Inhibition \%} = \frac{\text{control} - \text{sample}}{\text{control}} \times 100 \quad (4)$$

Shelf-Life Evaluation

Total plate count of nutrient bars was determined according to the procedure given in SLS 516: part 1: 1991 [16]. Colony forming units were calculated using the following formula.

$$\text{Colony Forming Units/ ml} = \frac{\text{No. of colonies} \times \text{total dilution factor}}{\text{volume of culture plated in ml}} \quad (5)$$

Sensory Evaluation

Sensory characteristics of the nutrient bars such as colour, aroma, flavour, texture, taste and overall acceptability at room temperature were evaluated with a panel of 30 untrained panellists on a 9- point Hedonic Scale. The scale ranged from “Extremely Like” (1) to “Extremely Dislike” (9). During sensory evaluation a commercially available nutrient bar was used as the control.

Statistical Analysis

All the analysis were conducted in triplicate to verify accuracy of all results. The Mean ± Standard Deviation (SD) values were calculated for all the parameters, except for the sensory attributes. Data obtained from the proximate, physico-chemical, phytochemical analysis were subjected to the Analysis of Variance (One Way ANOVA), while the sensory attributes were subjected to Friedman analysis using Minitab 17. Significant differences of means (P<0.05) were further determined using the Tukey’s pairwise comparison at a confidence level of 95%.

RESULTS AND DISCUSSION

Proximate Analysis

Results obtained for the proximate analysis of the nutrient bars are shown in the Table 2. The moisture content varied significantly amongst three types of nutrient bars (P<0.05 at 5% level of significance). The highest moisture content (14.4±0.66%) was observed in Treatment 2, while the lowest moisture content was reported from the commercially available nutrient bar (1.3±0.27%), as shown in Table 2. The chemical, physical, and microbial stability of foods are affected by the properties of water. Even a slight increment in moisture content of low and intermediate moisture containing foods can significantly reduce their shelf life. In addition, moisture content influences the textural properties of low moisture foods [17]. Therefore, the short shelf life of Treatment 2 could be related to its

higher moisture content than the other samples.

There were no significant differences in the mean ash contents among the three types of nutrient bars ($P > 0.05$ at 5% levels of significance). However, Treatment 2 had the high ash content ($8.7 \pm 1.14\%$), which could be related to the presence of jackfruit seed flour that contain around 3087 mg/kg calcium, 130.74 mg/kg iron is, 1478 mg/kg potassium, 60.66 mg/kg sodium, 10.45 mg/kg copper, and 1.12 mg/kg manganese [18]. Generally, a high ash content means that the food product is a rich source of minerals [19].

The fibre content denoted significant differences among the three types of nutrient bars ($P < 0.05$). Treatment 2 had the highest fibre content ($9.5 \pm 0.71\%$), while the lowest fibre content was observed in the commercially available nutrient bar ($0.5 \pm 0.71\%$), as shown in Table 2. Presence of high amount of dietary fibre makes it an excellent bulk laxative. The presence of high fibre content in jackfruit seeds flour prevents constipation and contributes towards smooth bowel movements [19].

The fat content denoted a significant difference among three types of Nutrient bar ($P < 0.05$). The highest fat content was observed in the Treatment 1 ($9.9 \pm 0.01\%$), while the lowest fat content was observed in the commercially available nutrient bar ($1.7 \pm 0.01\%$). Treatment 2 contained $7.1 \pm 0.01\%$ of fat. According to the United State Department of Agriculture (USDA), a nutrient bar should contain 10% (w/w) fat. The prepared nutrient bars in this study recorded the required amount of fat compared to the commercial nutrient bar. The Dietary Reference Intake (DRI) for fat in adults is 20% to 35% of total calories from fat. That accounts to about 44 g to 77 g of fat per day, if the total intake of calories per day is 2,000. It is recommended to eat more of monounsaturated fat (15% to 20%), polyunsaturated fat (5% to 10%) and less

saturated fat (less than 10%), because they provide health benefits. Further, it is recommended to eat less of trans fat (0%) and cholesterol (less than 300 mg per day), due to the negative impacts on health [20].

The protein content did not change significantly in all three types of nutrient bars. The highest protein content was observed in Treatment 2 ($29.5 \pm 3.26\%$), while Treatment 1 reported the lowest protein content ($20.9 \pm 2.31\%$), as shown in Table 2. Main protein contributions are coming from the seeds used in the formulations. Reports indicated that Jackfruit seeds contain 13.50% protein [18], Pumpkin Seeds has 30.23% [21], Winged bean seeds contain 34.18-40.30% protein [8] and Watermelon seeds contain 16.33- 17.75% of protein [7]. Proteins are required for the growth and maintenance of tissues and could also serve as a valuable energy source, but only in situations of fasting, exhaustive exercise or inadequate calorie intake.

The carbohydrate content had significantly changed among three types of nutrient bars ($P < 0.05$ at 5% levels of significance). A typical energy bar supplies 20-40% (w/w) of carbohydrate. The commercially available nutrient bar had $66.4 \pm 0.01\%$ of carbohydrate. However, the prepared nutrient bars had the required quantity of carbohydrates. Generally, the nutrient bars that contain a concentrated source of carbohydrates for quick energy and a source of protein for muscle repair and growth are formulated to cater the needs of sports and fitness enthusiasts.

Gross Caloric Value

Caloric values of different nutrient bars were calculated as shown in the Table 3. According to the USDA [22], a nutrient bar provides 350 kcal/100 g. The Treatment 1 was reporting the highest amount of calories, indicating that it may be utilized as a meal replacement, because it provides sufficient energy for the human body, compared to Treatment 2.

Table 2: Proximate Analysis of the Nutrient Bars

Parameter	Treatment 1	Treatment 2	Commercially Available Nutrient Bar
Moisture	3.5±1.18 ^b	14.4±0.66 ^a	1.3±0.27 ^c
Ash	7.3±2.53 ^a	8.7±1.14 ^a	7.8±0.01 ^a
Crude fibre	4.5±0.71 ^b	9.5±0.71 ^a	0.5±0.71 ^c
Crude fat	9.9±0.01 ^a	7.1±0.01 ^b	1.7±0.01 ^c
Crude protein	20.9±2.31 ^a	29.5±3.26 ^a	22.5±0.71 ^a
Carbohydrate	53.9±0.00 ^b	30.9±0.01 ^c	66.4±0.01 ^a

Note: Treatment 1: Semolina incorporated Nutrient bar, Treatment 2: Jackfruit seeds flour incorporated Nutrient bar. Values are mean ± standard deviation of replicates. Different superscript letters in each column denote significant differences at 5% significant level in each row, as suggested by the One-Way ANOVA test followed by the Tukey's pairwise comparison.

Table 3: Caloric Value of Different Nutrient Bars

Sample	Gross Energy Value (kcal/100g)
Treatment 1	388.6±0.00 ^a
Treatment 2	305.0±0.00 ^c
Commercially Available Product	370.1±0.00 ^b

Note: Treatment 1: Semolina incorporated Nutrient bar Treatment 2: Jackfruit seeds flour incorporated Nutrient bar. Values are mean ± standard deviation of replicates. Different superscript letters in each column denote significant differences at 5% significant level in each row, as suggested by the One-Way ANOVA test followed by the Tukey's pairwise comparison.

Physico-Chemical Analysis

Average values of physico-chemical characteristics of the three types of nutrient bars are shown in Table 4. For any sort of bacteria, the minimum a_w value required for growth is of 0.75, while osmophilic yeast and xerophilic fungi are capable to develop in a_w of 0.61 and 0.65, respectively. Therefore, commercially available nutrient bar presented a_w with values below 0.60, while Treatment 1 presented a_w value below 0.75. Treatment 2 reported a high a_w value than the Treatment 1. This could be the reason for the short shelf life obtained for Treatment 1 and 2 compared with the commercial product.

Foods without adequate acidity may allow the growth of microorganisms (bacteria, molds, parasites), which causes food spoilage and food-borne illnesses. Citric acid can be used to acidify the foods. Low acidic foods have the pH value greater than 4.5. For caramels it is in the 4.5 – 5.0 pH. Vegetables with a more neutral pH are in the 4.6 to 6.4 range [23]. Since the both prepared

samples had been incorporated with the citric acids and caramels, Treatment 1 showed 5.74±0.09 as the mean pH value, while 5.83 ± 0.27 was reported as the mean pH of Treatment 2, which can be considered as low pH values, compared to the commercially available nutrient bar.

Sugar content is an important determinant of the nutritional value, since refined sugar acts as a quick and simple source of energy and provide taste characteristics of processed foods. The ability to rapidly measure sugar content during food production and processing is critical in ensuring consistent high product quality. Brix is a method that has been widely used to rapidly verify the sugar content [24]. The highest Brix value was shown in Treatment 1 (56.4±7.36%), while the lowest brix value was reported in Treatment 2 (51.9±7.73%). It did not denote significant difference among three types of nutrient bars ($P>0.05$ at 5% levels of significance).

Table 4: Physico-Chemical Characteristics of Nutrient Bars

Types of Nutrient Bars	Water Activity (a _w)	pH	Total Soluble Solids/ TSS (Brix) (%)
Treatment 1	0.70±0.06 ^a	5.74±0.09 ^b	56.4±7.36 ^a
Treatment 2	0.76±0.03 ^a	5.83±0.27 ^b	51.9±7.73 ^a
Commercially available nutrient bar	0.54±0.00 ^b	6.89±0.02 ^a	55.7±0.90 ^a

Note: Treatment 1: Semolina incorporated Nutrient bar, Treatment 2: Jackfruit seeds flour incorporated Nutrient bar. Values are mean ± standard deviation of replicates. Different superscript letters in each column denote significant differences at 5% significant level in each row, as suggested by the One-Way ANOVA test followed by the Tukey's pairwise comparison.

Phytochemical Analysis

In the present study, the total phenolic content of the Treatment 2 was reported the highest value compared with the other nutrient bars. Inclusion of jackfruit seeds could be the reason for this high phenolic content, since jackfruit seeds contain lignans, isoflavones, saponins, and many phytonutrients. The health benefits of these phytochemicals are wide-ranging from anti-cancer to anti-hypertensive. These antioxidants are also useful as anti-ulcer and anti-aging tonics [11].

The antioxidant activity denoted significant differences among three types of nutrient bars ($P < 0.05$). The %DPPH inhibition measures the free radical scavenging property of a particular substance and is a measure of its antioxidant potential. The DPPH radical scavenging activity depends on the phenolic compounds present in the sample, and the samples that are rich in phenolics, exhibit high DPPH inhibition [18].

Treatment 2 showed a high antioxidant activity (low IC₅₀ value).

Microbial Analysis

Microbial analysis was done in order to ensure the product is safe for human consumption throughout the storage period. Total plate count was detected at 7 day time intervals for 28 days of storage time, for nutrient bars along with the heat sealable aluminium foil package. The total Plate Count was lower than the standard limits given by SLSI (less than 1×10^4 CFU/g) for Treatment 1 for 21 days and for Treatment 2 for 14 days, without adding any artificial preservatives. The commercially available nutrient bar's shelf life was noted as one month in their label.

Sensory Evaluation

In terms of sensory attributes, the estimated median score for colour and mouth feel were highest in Treatment 2, but the estimated median score for aroma, texture, and overall acceptability were highest in Treatment 1.

Table 5: Phytochemical Analysis Results of Nutrient Bars

Types of Nutrient Bars	Total Phenolic Content (mg GAE/g)	Antioxidant Activity IC 50 (mg/ml)
Treatment 1	2.9±0.39 ^c	350.7±5.49 ^a
Treatment 2	8.3±0.30 ^a	211.9±0.58 ^b
Commercially Available Product	4.2±0.46 ^b	219.6±1.33 ^b

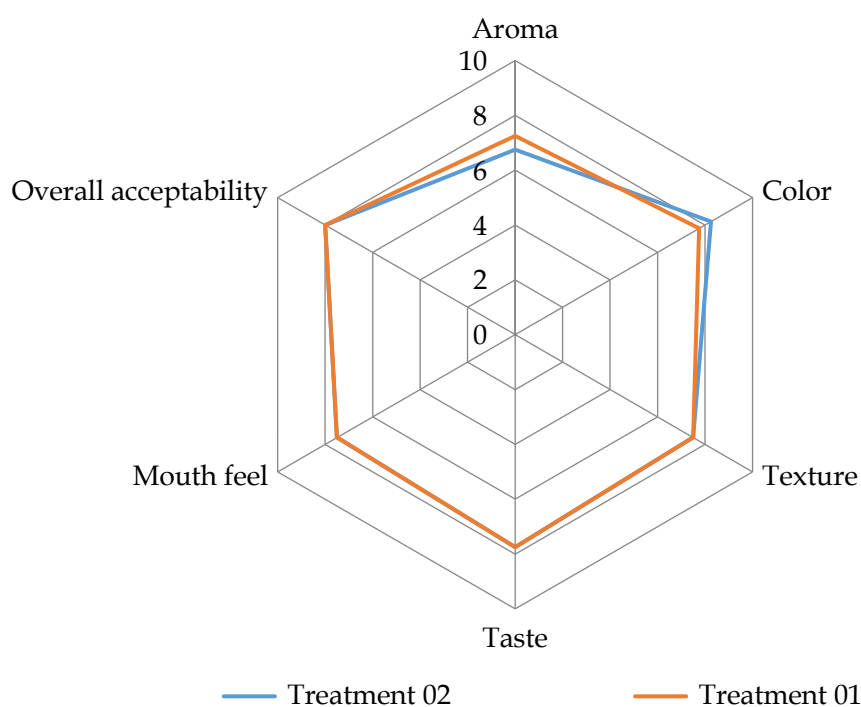
Note: Treatment 1: Semolina incorporated Nutrient bar, Treatment 2: Jackfruit seeds flour incorporated Nutrient bar.

Values are mean ± standard deviation of replicates. Different superscript letters in each column denote significant differences at 5% significant level in each row, as suggested by the One-Way ANOVA test followed by the Tukey's pairwise comparison.

Table 6: Results of the Total Plate Count

Type	Just after Preparation (CFU/g)	After 3 Days (CFU/g)	After 7 Days (CFU/g)	After 14 Days (CFU/g)	After 21 Days (CFU/g)	After 28 Days (CFU/g)
Treatment 1	0	0	30	330	670	TMTC
Treatment 2	0	70	300	500	TMTC	TMTC

Note: Treatment 1: Semolina incorporated Nutrient bar, Treatment 2: Jackfruit seeds flour incorporated Nutrient bar; TMTC: Too Much To Count.

**Figure 3:** Spider-Web Diagram for Sensory Evaluation of Two Products**Table 7:** Cost Analysis of the Developed Nutrient Bars

Ingredients	Price/ 100g of Mixture (T 1) / Rs:	Price/ 100 g of Mixture (T 2) / Rs:
Common raw seeds	50.00	50.00
Semolina	12.25	-
Jackfruit seeds	-	15.00
Sugar	2.00	2.00
Other ingredients (Salt, Glucose syrup, Citric acid Vanilla)	10.00	10.00
Others	20.00	20.00
Total	94.25	97.00

Note: T 1: Semolina incorporated Nutrient bar T 2: Jackfruit seeds flour incorporated Nutrient bar

According to the estimated median values, Treatment 2 had the highest value for colour while the Treatment 1 had the highest value for aroma. In case of the overall acceptability, Treatment 1 had the highest acceptance, compared with the Treatment 2.

Cost Analysis

Cost of production for a commercially available nutrient bar in Sri Lankan market is about Rs. 100.00 per 100 g. The processing cost of 100 g of nutrient bars developed from Treatment 1 and 2 were Rs. 94.25 and Rs. 97.00, respectively.

CONCLUSIONS

The developed nutrient bars meet the recommended dietary allowances, according to proximate analysis. Foods that fall within the meal replacement product compositional criteria should have energy contents between 200-250 kcal and 25.5% of that energy should come from protein, followed by 30-35% from fat. Therefore, the prepared nutrient bars met these requirements and ensure that both of these nutrient bars can be used as a meal replacement.

Product developed from Treatment 2 demonstrated a lower IC₅₀ value (highest antioxidant activity), than Treatment 1 and the commercial nutrient bar. Meanwhile, the Treatment 1 had a longer shelf life (21 days) than Treatment 2 (14 days). Based on the sensory evaluation, Treatment 1 scored the highest mean values for overall acceptability. Moreover, nutrient bar developed using first treatment had the least cost of production based on the cost analysis. With the above scientific reasoning, nutrient bar developed using Treatment 1 could be commercialized as an effective, convenient, nutritional daily meal replacement substituting junk and fast foods.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

HJS: Carried out the investigations, data collection, statistical analysis, and prepared

the first draft of manuscript; TC: supervised the study and revised the manuscript; KW: supervised the study. All authors read and approved the manuscript.

FUNDING

Authors received no funding for the present research work.

ACKNOWLEDGEMENTS

Authors wish to acknowledge Prof. K.G.C. Senarathna, Head of the Department of Biosystems Technology, Faculty of Technological Studies, Dr. A.P. Henagamage, Head of the Department of Science and Technology, Faculty of Applied Sciences, Prof. A.M.W.K. Senavirathne, Head of the Department of Export Agriculture, Faculty of Animal Science and Export Agriculture and Prof. W.A.J.P. Wijesinghe, In charge of Advanced Food Science Laboratory for providing laboratory facilities, equipment, instruments, and chemicals to successfully complete this research.

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