Preparation of a Low Cost Nutrient Bar Incorporated with Underutilized Seeds as a Convenient and Functional Meal Replacement Alternative

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ABSTRACT

People with busy lifestyles routinely consume fast food as an easy alternative for main meal. Consequently, they prone to suffer from non-communicable diseases such as diabetics. The purpose of this research was to develop a nutritious bar as a meal replacement under the Recommended Dietary Allowances (RDA) using underutilized seeds such as pumpkin, winged beans, watermelon, corn, mung beans, and rice flakes. The proximate analysis, physicochemical properties, sensory attributes and microbiological parameters were evaluated using the Association of Official Agricultural Chemist standards (AOAC), Human Research Ethics Committee standards (Brazil), and Sri Lanka Standards Institution (SLS 516: 1991) respectively. Developed mung based (321), rice flake based (123) and commercial nutrient bar (801) revealed a significant difference ($p \leq 0.05$) in attributes except mouthfeel in the sensory evaluation. A mold and a package was designed with an effective packaging material. The shelf-life was evaluated after adding the permitted preservative (E211-Sodium benzoate) to developed mung based (321), rice flake based (123) bars which had a self-life of one-month. The mung based nutrient bar revealed an average of 1.7±0.1 % moisture, 0.9±0.7% fat, 9.7±0.1% ash, 21.8±2.5% crude fibre, 36.6±0.0 % protein, 29.2±8.3% carbohydrates, and the rice flake bar contained an average of 2.2±0.3% moisture, 2.7±0.2% fat, 7.9±0.2 % ash, 11.4±1.4 % crude fibre, 31.9±0.0 % protein, 43.8±0.0 carbohydrates. The commercial nutrient bar had 1.5±0.1% moisture, 1.5±0.0% fat, 7.8±0.0% ash, 0.5±0.5% crude fibre, 32.5±0.0 % protein, and 56.3±3.9% carbohydrates on average. The energy content (kcal) in rice flakes based, mung based and commercial bars were 327.1, 271.3, and 360 respectively. The finding ascertained that the developed nutrient bars (123, 321) directly fulfill the recommended dietary allowance as a meal replacement concerning European Union Commission Regulations and substantiate that the same nutrient profiles in the commercial nutrition bars could be obtained from the developed samples with a lesser processing cost.

KEYWORDS: *Commercial nutrient bar, meal replacement, recommended dietary allowance, underutilized seeds*

1 INTRODUCTION

The human body digests and absorbs nutrients in food and with metabolism releases energy for mechanical work, and those nutrients are essential to the growth, development and wellbeing. Many people consume fast food instead of a balanced diet and later suffer from non-communicable diseases such as hyperglycemia and hyperlipidemia. Food bars produced under the recommended dietary allowances could offer a healthy alternative to replace popular yet unhealthy fast food (Kelly *et al*, 1997). Nutrient bars are considered as the most functional and convenient food in the market for people with busy lifestyles who often tend to skip their main meals and yet looking for alternatives to boost their energy requirements. Nutrient bars are designed to offer great nutritional profile with all three macro nutrients (proteins, carbohydrates, and fats), dietary fibre, and antioxidants included in them.

In view of producing nutrient bars relatively cheap and with recommended levels of dietary fibres and other essential nutrients, selected grains with proven nutritional values are incorporated. The popular

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choice of grains for nutrient bars included cereals such as rice, corn, wheat, and barley. However, incorporation of underutilized seeds such as winged beans (*Phaseoius aureus*), pumpkin seeds (*Cucurbita maxima*), Mung beans (*Phaseoius aureus*), and watermelon seeds (*Citrullus lanatus*) could bring in some important plant based proteins and dietary fibre to increase the nutritional profile of nutrients bars. Moreover, watermelon seeds and pumpkin seeds are often discarded as waste, yet they are a good source of nutrients.

Pumpkin seed meal is high in crude protein, vitamin B complexes, pectin, dietary fibres, and essential antioxidants such as carotenoids, lutein, zeaxanthins, and other polyphenolic compounds (Yadav et al, 2010). Watermelon seeds are a rich source of protein, lipids, carbs and minerals such as magnesium, potassium, phosphorus, sodium, iron, zinc, manganese, and copper (Collins et al, 2007). Watermelon seeds contain chemical compounds such as alkaloids, and phenolic compounds such as lactones, tannins, and flavonoids which are proven to be cancer preventing.

This study aims at developing a ready- to- eat nutrient bar using two formulations: flaked rice-based and mung bean based, incorporated with a multitude of grains and underutilized seeds to improve the nutritional profile and health benefits. Use of underutilized seeds like pumpkin and watermelon seeds would bring down the production cost of the nutrient bar in the competitive market and would be a good way of popularizing the consumption of these often wasted seeds. The study also deals with determining the nutritional value, shelf-life, and the sensory profile of the final product.

2 METHODS AND MATERIALS

2.1 Materials

Table 1. Materials of the Nutrient Bars (123,321)

Types of ingredients	Developed rice flakes based Nutrient bar (123)		Developed mung based Nutrient bar (321)	
	Quantity (g)	**Percentage (%)**	**Quantity (g)**	**Percentage (%)**
Rice flakes	30	26.08 %	-	-
Mung bean	-	-	30	26.08%
Pumpkin seeds	10	8.69 %	10	8.69%
Watermelon seeds	5	4.34 %	5	4.34%
Corn flour	15	13.04 %	-	-
Pop corn	-	-	15	13.04%
Winged bean	15	13.04 %	15	13.04%
Desiccated coconut	40	34.77 %	40	34.77%
Salt	0.03	0.02 %	0.03	0.02%

The desiccated coconut and binding agent were produced and, the collected seeds were precooked, and ground into fine particles. The commercial nutrient bar was purchased from the market and it contained rice, soya, glucose, vegetable oil, mung beans, soy lecithin, salt, citric acid, and plant extraction (Rosemary) as the ingredients according to information in the label.

2.2 Preparation of food binder

A significant amount of proximal sugar caramel and glucose syrup were combined to create a food binder. Then the mixture was heated in a food-grade stainless steel container where the binder was homogenized at a Total Soluble Solid (TSS) content of 71± 1 after methods described by Carla et al, 2020.

2.3 Preparation of desiccated coconut

Mature and good quality coconuts were selected and cracked into halves. The coconut halves were then placed in an oven at 65 °C for 10-15 minutes to separate the meat from the shell and the soft brown skin was peeled off. Next, the coconut meat was chopped into small pieces and blended until a fine consistency was obtained. After that it was dried in an oven at 180 °C for 10-15minutes

2.4 Preparation of Nutrient bar

Two formulations were developed: flaked rice-based nutrient bar (123) and mung-based nutrient bar (321). The Commercial nutrient bar (801) served as the control.

As the First step, all of the seeds (Flaked rice/mung beans, winged beans, pumpkin seeds, and watermelon seeds) were cooked at low heat of 65 °C. Then, corn flour, desiccated coconut, and salt were added and thoroughly mixed. After that, vanilla flavor and artificial preservatives were added and remixed well. Sugar caramel and liquid glucose binder were put into the mixer in I 1:3 ratio. Finally, mixture was compressed in a mold to produce nutrient bars with dimensions of 2.5 cm × 2.5 cm × 5.6 cm and weight of 35 g and kept for 30 minutes to set. Then all nutrient bars were placed in an aluminum metalized bag and stored at 25 ± 2 °C temperature for further analysis.

3 ANALYSIS OF NUTRIENT BARS

3.1 Microbial evaluation

The microbial count was recorded (SLS 516: 1991) just after preparation and time intervals of 3,7,14 and 30 days from preparation. The nutrient bars were kept sealed in an aluminum metalized bag at 25 ± 2°C room temperature until the evaluation of the microbial count. The evaluated microbiological parameters included the total viable plate count in Nutrient Agar medium, yeasts and molds development in Potato Dextrose Agar.

3.2 Proximate composition

The Association of Official Analytical Chemists (AOAC) procedures were used to determine the proximate composition of the nutrient bars. Total carbohydrates quantification was performed according to the Phenol-sulfuric acid method in AOAC 923.09, ash according to the AOAC 1942, and moisture were quantified according to methods AOAC 2002, crude fibre content according to the AOAC 978.10, fat content according to the AOAC 2003.05 respectively. The sum of all the components; total carbohydrates, moisture, ash, crude fibre and crude fat were summed and subtracted from 100 to calculate the crude protein content.

3.3 Phytochemical Composition

The pH of the nutrient bar was determined using the SLS 280 (2009) method, and the color of the nutrient bar was measured using the Royal Horticultural Society Color Charts Edition V. The Association of Official Analytical Chemists (AOAC) procedures were used to determine the water activity, Brix range, total phenolic content, and antioxidant activity. The total phenolic content of nutrient bars was determined using Folin-Ciocalteu reagent (Singleton & Rossi, 1965).

3.4 Stability during storage

The freshly prepared nutrient bars were placed in metalized bags at room temperature (25± 2 °C). The color, moisture, and microbiological attributes (Yeast and molds, and total plate count at 25± 2 °C) were analyzed after 3, 7, 14, and 30 days of storage.

3.5 Analysis of sensory properties

The sensory attributes were evaluated for the purpose of monitoring stability, and for market acceptance. The sensory evaluation was conducted with the participation of a consumer-type panel that included 30 untrained panelists (university students and staff) ranging in age from 20 to 40 years old. The 9-point hedonic scale was used for the sensory evaluation.

3.6 Cost analysis

The production cost of the two nutrient bars that were developed included cost of ingredients, cost of packaging material, and cost of treatment and miscellaneous costs.

3.7 Statistical analysis

All results in the paper were reported as mean and Standard Deviation (SD) from three replicates (n = 3). A one-way analysis of variance (ANOVA) with post-hoc Tukey HSD test was used to compare mean values between groups, and all tests were evaluated for statistical significance at $p \leq 0.05$ using MINITAB 17 software. (Montgomery, 2009)

4 RESULTS AND DISCUSSION

4.1 Proximate composition of prepared nutrient bars and commercially available nutrient bar

The moisture level of 321, 123, and 801 nutritional bars were measured according to the FDA standard for cereal flours and related products (Food and Drug Administration, 2017). The moisture content of the 123,321 and 801 type nutrient bars were 2.23±0.3%, 1.66±0.1%, and 1.45±0.1% respectively. 123,321 and 801 samples have a significant difference in moisture ($P < 0.05$). The desirable texture of dried snack food items and crispness are sensitive to moisture uptake by the food from the atmosphere. The P-value related to the moisture parameter versus storage is 0.028, and there was a significant difference ($P < 0.05$) in moisture against the storage.

The P-value in the analysis of the variance in ash content is 0.004, and there was a significant difference ($P < 0.05$) between 123,321 and 801 Samples. 321 and 801 nutrition bars belong to the same group when result was grouped using the tukey technique at 95% confidence. Samples with high ash content may have a high concentration of various mineral elements able to speed up metabolic processes and promote growth and development. As a result, it is possible to aggregate a 321 nutrition bar with various mineral elements. (Zamora-Gasga *et al*, 2014)

The crude fibre content of the 123, 321, and 801 bars were 11.4 ±1.4%, 21.8 ± 2.5%, and 0.5±0.5%, respectively where the P-value is 0.007, and there was a significant difference ($P < 0.05$) between 123,321 and 801. The 321 (Mung based nutrient bar) nutrient bar has a higher fibre content as compared to the fibre content of commercial available nutrient bar. While fibre-rich foods are known to expand the inside walls of the colon, allowing waste to move effortlessly and reducing the risk of various cancers, they also lower cholesterol levels in the blood and reduce the risk of numerous cancers (Bello et al, 2008). However, the importance of keeping low fibre intake in the feeding of infants and weaning children has been emphasized, as high fibre levels in the weaning diet can irritate the mucosa of the intestine. (Bello et al, 2008).

Fats are essential because they provide a larger portion of energy (9 kcal/g) to the body. The crude fat content of the 123, 321, and 801 nutrient bars were 2.7±0.2%, 0.9±0.7% and 1.5±0.0% respectively where the P-value is 0.166 and there were no any statistically significant difference ($P > 0.05$) between 123,321 and 801 samples.

The 123 type nutrient bar provides 43.8±0% useable carbohydrates, whereas the 321 type nutrient bar has 29.2± 8.3% and the 801 type nutrient bar has 56.3± 3.9%.Since the P-value is 0.080, there was no any significant difference in carbohydrate content which nearly gives half of the energy content by fats (4 kcal/g) among 123,321 and 801 samples.

The 123, 321, and 801 bars had a protein content of 31.9%, 36.6%, and 30.33%, respectively. The P-value for analyzing protein content variance is 0.808, and there were no statistically significant differences ($P > 0.05$) between 123,321 and 801. Mung beans are a rich source of proteins. Consequently sample 321, which is the mung bean based nutrient bar, had the highest protein content.

Table 2. Proximate Content of the Nutrient Bars (123,321,801)

Component analyze	321 Sample	123 Sample	801 Sample
Protein %	36.6±0.0	31.9±0.0	30.3±0.0
Carbohydrates %	29.2±8.3	43.8±0.0	56.3±3.9
Fat %	0.9±0.7	2.7± 0.2	1.5± 0.0
Ash %	9.7±0.1	7.9± 0.2	7.8± 0.0
Fibre %	21.8±2.5	11.4±1.4	0.5± 0.5
Moisture %	1.7±0.1	2.2±0.3	1.5±0.1

Values are mean ± Standard deviation of 3 replicates (n=3). Treatment means having significant difference between samples ($P \leq 0.05$)

Figure 1. Mung based nutrient bar (321), Flake rice based nutrient bar (123) and Commercial nutrient bar(801).

The 123,321 and 801 nutrition bars have a calorific value of 327.1, 271.3, 360 kcal per 100g. (calorific value of food by employing the 4-9-4 method). According to the European Union Commission Regulation, meal replacement' products (shakes, bars) are considered to have calorific value 200-250 kcal energy, protein of 25-50% of total energy content, and fat of ≤ 30% of total available energy content. The prepared energy bars (123,321) were in the standard range of calorific value.

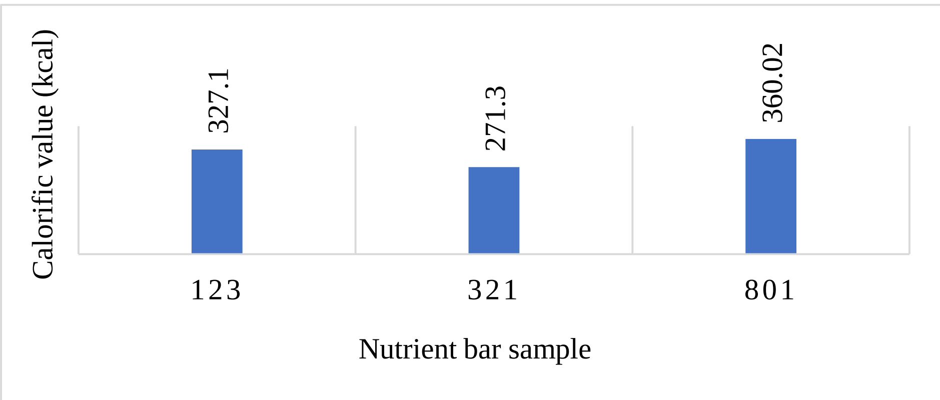

Figure 2. Calorie (kcal) content in 123,321,801 Nutrient bars

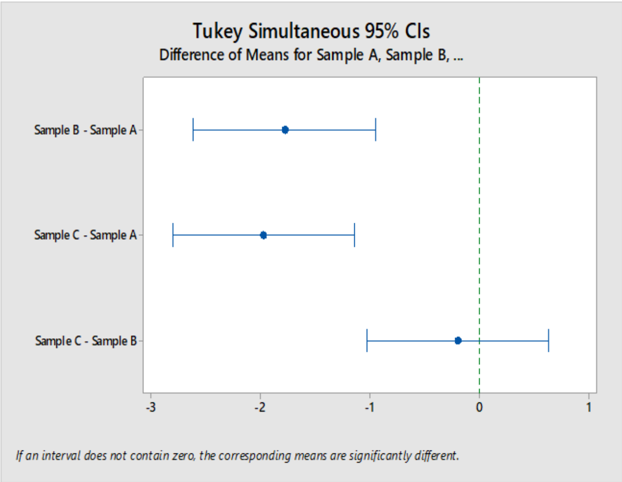

Figure 3. Comparative difference of mean Ash content in Nutrient bar (123,321, 801sample) using Tukey simultaneous 95%.

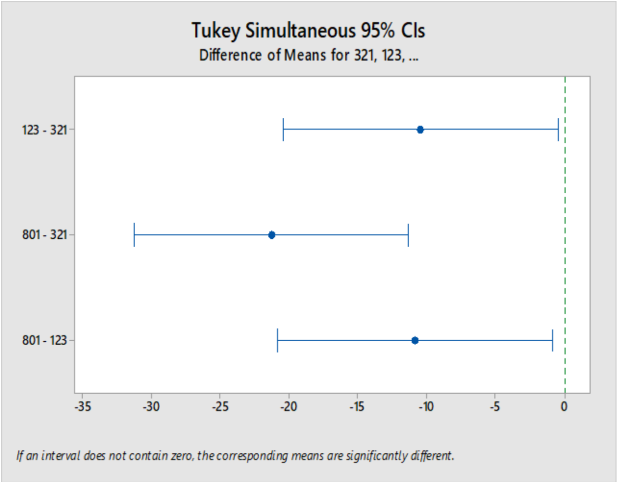

Figure 4. Comparative difference of mean crude fibre content in Nutrient bar (123, 321, 801 sample) using Tukey simultaneous 95%

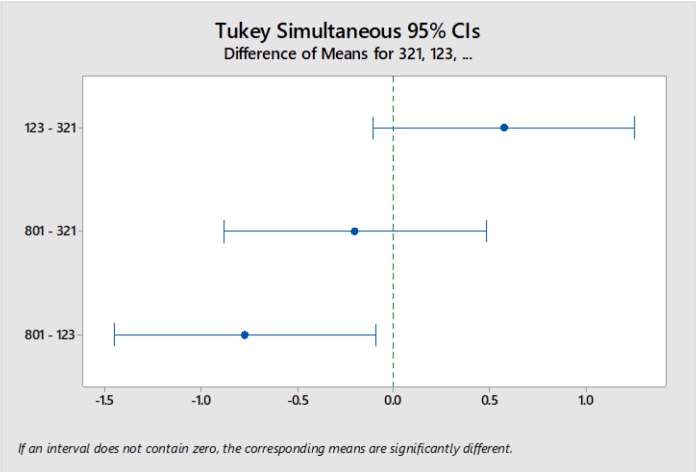

Figure 5. Comparative difference of mean moisture content in Nutrient bar (123,321, 801 sample) using Tukey simultaneous 95%

4.2 Microbial characteristics of prepared nutrient bars and commercially available nutrient bar

The limited total viable plate count in food was (CFU 10^{-1}) 100-10000 due to the SLS normative standards limits. In analyzing the variance in total plate count, the P-value is 0.040 and they have significant differences ($P < 0.05$) between 123,321 and 801 when grouping data using the tukey method at 95% confidence, samples 123, 321, 801 nutrition bars belong to the same group. Sample 801 nutritious bar had the higher Total viable plate count.

According to the Sri Lankan Institute of standardization, the food items categorized under the instant meal replacements must have their yeast and mold count under the stipulated limits of 10-1000 (CFU 10^{-1}). For yeast and mold, the P-value is 0.219 and, they have no significant difference ($P>0.05$) between 123,321 and 801. Sample 801 nutrient bar had higher yeast and mold count. There were no any statistically significant differences ($P > 0.05$) among the three different nutrient bars after 30 days of shelf life when the yeast and mold count were analyzed.

4.3 Sensory evaluation of prepared nutrient bars and commercially available nutrient bar

Table 3. Results of sensory evaluation

Parameter	P value	123	321	801	Highest sum of rank
Aroma	0.00	7.08	6.91	5.75	49.0
Color	0.00	7.50	7.25	5.25	49.0
Texture	0.01	6.50	7.91	6.08	53.5
Taste	0.01	7.00	8.00	6.00	52.0
Mouth feel	0.45	7.50	7.50	7.25	44.0
Overall acceptability	0.00	7.33	8.50	6.66	56.50

The results were statistically analyzed using Friedman test by Minitab 17 version.

In Hypothesis determination,

Null Hypothesis (H_0): There is no any significant difference within each ingredient combination of nutrient bar samples.

Alternative Hypothesis (H_1): There is a significant difference within each ingredient combination of nutrient bar samples.

Reject H_0 if,

Statically tested "P" value < Critical α value (0.05).

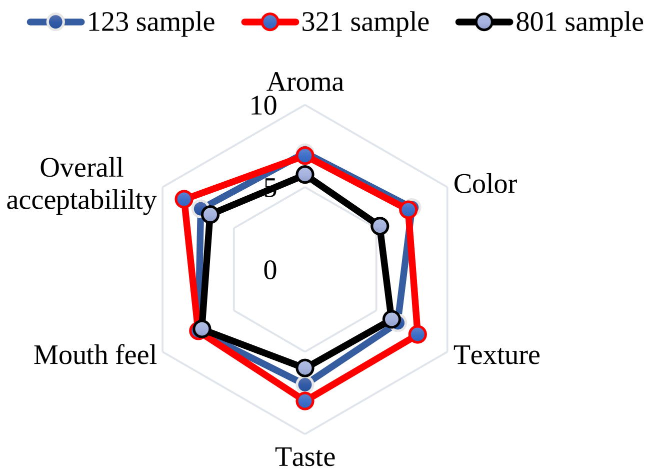

Figure 6. Mung based nutrient bar (321), Flake rice based nutrient bar (123) and Commercial nutrient bar(801).

4.4 Physiochemical characteristics of prepared nutrient bars and commercially available nutrient bar

The pH value of a food items are used to determine the acidity of the particular food items. Any pH value below pH 7 is considered acidic, whereas values above pH 7 are considered basic. The more acidic food is in lower pH reading. They must achieve pH equilibrium in a reasonable amount of time (Rushing, 1999). The mean pH value of the 123, 321, and 801 nutrient bars were 6.2 ± 0.0%, 6.3 ± 0.0% and 6.9 ± 0.0% respectively. The P-value in the pH parameter during the shelf life analysis is 0. 073. There was no any significant difference (P > 0.05) in the pH value with respect to shelf life.

Water activity (aw) is the amount of water in a food product and is usually related to its moisture. That amount of water is used (free water) for microorganisms to grow. The water activity of food such as honey, bread, and cookies are 0.9 or less and can be considered as shelf-stable without refrigeration

(Pittia *et al*, 2016). In the nutrient bar samples of 123, 321 and 801, the water activity was 0.6±0.0%, 0.6±0.0%, and 0.6±0.0% respectively. When it comes to the evaluation of water activity against shelf life, the P-value is 0. 434 suggesting that there was no any statistically significant difference (P > 0.05) in water activity with shelf life. (P > 0.05)

The salinity of the 123, 321 and 801 nutrient samples were 1.7 ± 0.0%, 1.4±0.0%, and 1.4 ±0.0%, respectively. In the salinity parameter versus shelf life, the P-value is 0. 008. Therefore, a statistically significant difference (P < 0.05) is present for salinity with respect to shelf life and with the time salinity increased in all three samples.

The 123, 321, and 801 have mean Brix value of 45.4±0.3%, 46.9±0.3%, and 55.3±0.2%, respectively. The P-value for Brix value versus shelf life is 0.461. As a result, there was no statistically significant difference (P > 0.05) in Brix value within the shelf life among the three samples.

Antioxidant Value in Nutrient Bars

The IC_{50} values, which correspond to the concentration of nutrient bar samples that can scavenge 50% of the free radicals present with in the human body once they ingest the food with antioxidants. High IC_{50} values imply low antioxidant activity. The IC_{50} values for samples 123, 321, and 801 were 113.54 μg/ml (0.01%), 261.12 μg/ml (0.01%) and 138.23 μg/ml (0.01%) respectively.

In analyzing the variance in phenolic content, the P-value is 0.345. Due to that, there was no significant difference (P< 0.05) among 123,321 and 801.When data were grouprd using the tukey method at 95% confidence, samples of 123,321 and 801 nutrition bars belonged to the same group. However, sample 123 comparatively recorded a lower IC50 value which indicates that rice-based nutrient bar had a higher antioxidant activity than 321 and 801.

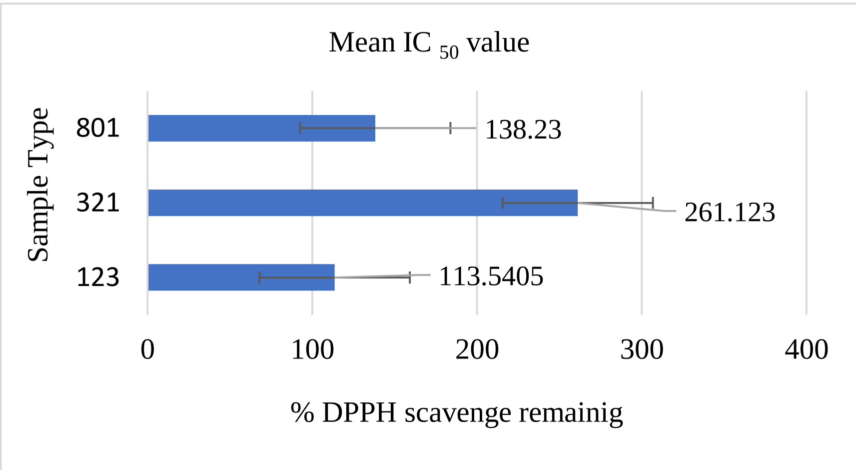

Figure 7. Radical DPPH scavenging activity of the 123, 321, and 801 nutrient bars.

Total Phenolic Content

The plant based food items are rich in phenolic compounds and as a result they are associated with high profile of sensory characteristics (Chi-Tang, 1992). The phenolic values in the 123, 321, and 801 samples were 0.006%, 0.003 % and 0.005% respectively.

P-value for the total phenolic content, is 0.012. Due to that, they have significant differences (P< 0.05) between 123,321 and 801.

The results showed that the samples had reduced total phenolic compound levels. It has been reported that total phenolic components could be get destroyed significantly during food preparation (Perret & Yu, 2002). Folin-Ciocalteu method is widely used to determine the total phenolic levels in botanical and biological samples although it has its own limitations. Other reducing agents, such as L-ascorbic acid and sulfur dioxide, might react with the Folin-Ciocalteu agent and add to total absorbance, causing total phenolic levels to be overstated (Singleton & Rossi, 1965).

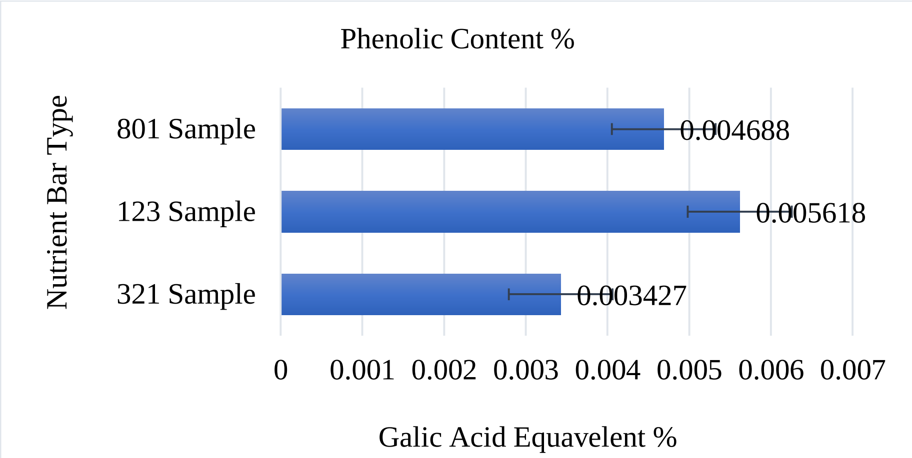

Figure 8. Total phenolic contents of the Nutrient Bars 123,321,801.

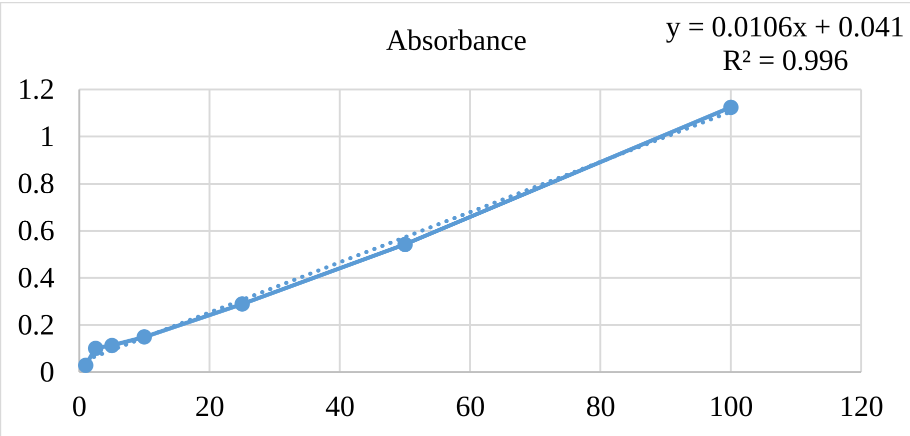

Figure 9. Standard Gallic Acid Curve (Genwali et al, 2013)

4.5 Cost Analysis of prepared nutrient bar and commercially available nutrient bar

Total cost analyses contained material cost and other expenses (labor, energy, and equipment cost).

Table 4. The cost of 1 nutrient bar (35g)

	Developed rice flakes based Nutrient bar (123)	**Developed mung based Nutrient bar (321)**
Material cost	Rs.16.28	Rs.16.32.
Other Expenses (Energy Cost, Equipment cost, Labour cost)	Rs. 12.76	Rs. 12.76
Total Cost	Rs.29.04.	Rs.29.08.

The commercially nutrient bar of 10 g costs LKR 10. Accordingly, production of a 35 g commercial nutrient bar would cost LKR 35.00. Production cost of 35g of 123 and 321 nutrient bars was LKR 12.76 and 12.76 respectively and could be available for a market value of LKR 30.00.

5 CONCLUSION

Proximate analyses have revealed that the developed nutrient bars 123 and 321 meet the Recommended Dietary Allowances. Since foods categorized under the compositional criteria for "meal replacement" products (shakes, bars) energy content should be in the range of 200-250 kcal, energy from protein should be at 25-50%, and energy from fat ≤ 30% of total available energy content. Except for the attribute 'mouthfeel', all other sensory attributes have shown significant differences among samples 123,321 and 801. In the physicochemical analysis, only the water activity remains constant among the three nutrient bars. There was no any significant difference in phenolic content or antioxidant activity between samples. In shelf-life evaluation revealed that the developed bars (123,321) can be kept for 30 days at 25 ± 2 °C with aluminum metalized packaging material. According to the European Union Commission Regulations (EUCR), a meal replacement product should have 200-250 kcal energy composition. Accordingly, the prepared 123, 321 type bars could be considered to fulfill the calorie requirements set forth by EUCR with less effort and at a lower cost than commercially available nutritional bars (801). The lowest cost of nutrient bar on the Sri Lankan market costs Rs.10.00 (0.050 USD) for 10g. The 123,321 nutrient bars could be processed at 10g for around LKR 8.50 (0.042 USD). As 123 and 321 nutrient bars are made entirely of plant-based ingredients, vegans and vegetarians could use it as a meal replacement due to its rich nutritional profile.

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