

Impact on Incorporation of Purple Yam Flour (*Dioscorea alata* var. *Purpurea*) in Development of Multigrain Nutri-Bar

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Abstract

Consumption of wholesome and healthy diet is the most effective way to combat protein-energy malnutrition and micro-nutrient deficiencies. Present study was carried out to develop a nourishing and cost effective nutri-bar from selected locally available yams, cereals, legumes and oil seeds. Incorporation of purple yam flour at 20% (F1), 15% (F2) and 10% (F3) was performed with changing only the ratio of processed rice flour. The highest mean rank scores for all tested sensory properties of appearance, taste, texture, aroma and overall acceptability were observed in F1. Proximate composition of ash, protein, fat, crude fiber, carbohydrate and calorie contents of products ranged 1.26-1.37%, 11.20-12.02%, 6.57-7.29%, 2.69-3.58%, 73.28-73.41% and 397.57 - 406.81 kcal/100g respectively. The highest values for total sugar, crude-fibre, potassium, sodium, calcium were recorded in F1 with 20.06±0.12%, 3.55±0.08%, 3903±2.8mg/kg, 396.50±10.60mg/kg, 305.00±29.70mg/kg respectively. F1 had the highest TPC- 4.120 mg GAE/g, TFC- 0.179 mg QE/g, anthocyanin content- 0.225 mg/g and antioxidant potential through DPPH assay- 3.142 mg TE/g followed by F2 and F3. The anthocyanin content of three formulas showed significantly positive correlation with antioxidant capacity ($r = 1.00$, $p < 0.05$). In conclusion, nutri-bars are the excellent source of nutrients supplements especially for undernourished population.

Introduction

In Sri Lanka, protein-energy malnutrition is the most prevalent issue of malnutrition. However, Vitamin A deficiency, iron deficiency, over-weight and obesity like issues have been commonly found as a result of deficiencies, excesses or imbalances in the dietary intakes of people [1]. For decades, nutrition has been a major issue for all vulnerable populations in Sri Lanka including elders (>60 years) and children especially aged below 5 years who are at a crucial stage where significant physiological and psychological changes occur [2].

The ongoing economic crisis of the country has made a huge impact on the peoples' capability of accessing a healthy, wholesome meal. The low income families which are found more in rural areas have been unable to fulfil the nutritional needs of those vulnerable groups which are mainly focused in this study. In comparison to students from urban schools, it was found that students from rural schools were significantly more likely to be stunted and underweight ($p > 0.05$) (stunting: 14.8% urban, 30.7% rural; underweight: 35.8% urban, 49.0% rural), and that urban students were also more likely to be overweight

More Information

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(5.3% vs. 1.7%). According to the United Nations International Children's Emergency Fund [3], 21% of Sri Lankan children under the age of five experienced stunting (low height for age) in 2016, as an indication of chronic malnutrition. Additionally, the Sri Lanka Demographic and Health Survey 2016 found that around 21% of children suffered from wasting (low weight for height) and 15.6% were underweight [4]. Also in term of elderly population, malnutrition has been a main cause of mortality and morbidity in the elderly. Some studies have found that how food insecurity influences on the malnutrition of elders and revealed that malnutrition prevalence among elderly ranges from 13% to 54% [5]. The malnutrition risk of elders was 28.0%, compared to 21.7% for food insecurity [6].

To address the locally prevalent malnutrition especially among children and elders, a nutri-bar was standardized and developed in the present research with locally available cost effective ingredients with specific qualities and functionalities. As a major ingredient, purple yam which is considered as underutilized was incorporated to formulate it and its proportion was changed with Kaluheenati rice, a traditional rice variety in Sri Lanka accordingly in all three formulas. Both main ingredients are potential sources of carbohydrates and contain significant amount of anthocyanin like phenolic compounds. According to some researches, purple yam was reported to contain phenolic compounds such as cinnamic acid, sinapic acid, ferulic acid, quercetin and catechin and up to 93.3 mg cyanidin-3-glucoside equivalent (CGE) of anthocyanin per 100g dry material. Also in rice, twelve phenolic acids have been identified with their sum ranging from 7.3-8.7 mg/100g in the endosperm, 177.6- 319.8 mg/100g in the bran, 20.8-78.3 mg/100g in the whole grain and 477.6 mg/100g in husk. For both total proanthocyanidin content (138.15 mg catechin equivalent /100g) and total phenolic content (soluble: 54.49 mg gallic acid equivalents /100g; bound: 24.31 mg gallic acid equivalents /100g) of rice; variety Kaluheenati had the significantly highest value ($P < 0.05$) compared to other rice varieties [7]. Those functional ingredients have enhanced nutri-bar value due to presence of antioxidants which can attribute ability of preventing and controlling chronic diseases in consumers.

The objective of present study was to add value to underutilized local foods like purple yams as ingredients of a ready-to-eat food product which has high potential to be adjusted to modern lifestyle while imparting essential nutrients. The impact of three formulations with changing ratios of main ingredients that purple yams and Kaluheenati rice on sensory, nutritional, physical, and functional properties of final product was assessed. Then the percentages that fulfil

Recommended Dietary Allowances (RDA) of energy, protein, calcium, iron, zinc and magnesium with reference to vulnerable age groups to be nourished, by one serving size of selected nutri-bar formula were evaluated.

Materials and Methods

Materials

Raw materials

The grains that Kaluheenati rice, green gram, red cowpea, sesame, peanut, coconut, sugar and glucose syrup (dextrose equivalent "DE" 40) were procured from Cargill's super-market of Piliyandala, Sri Lanka.

The fresh tubers of *Dioscorea alata* were purchased from a local yam-shop in Kidelpitiya, Western province, Sri Lanka.

Chemicals

In the current study, analytical reagent-grade and GPR grade chemicals and solvents were used.

1,1-diphenyl-2-picrylhydrazine (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Gallic acid, ferric chloride, Quercetin, and Folin-Ciocalteu phenol reagent were all procured from Sigma-Aldrich, United States.

Methods

Preparation of ingredients

The ingredients of this formulated nutri-bar have to be consisted in processed form with sufficient shelf-life expansion to consume it as ready-to-eat food product [8]. This study was therefore designed to have consisted of a processed powder form of purple yams, Kaluheenati rice, green gram, red cowpea with dehydrated coconut flakes, roasted sesame seeds and crushed peanuts as dry mix ingredients.

Yams (*Dioscorea alata*) processing

Tubers were peeled off and soaked in warm water for 10 min. Then they were sliced in to thickness of 3-4 mm. A special treatment was conducted for yams (*Dioscorea alata*) to retain its colour by inhibiting enzymatic browning.

Three treatments that steam blanching, soaking the slices of peeled tubers in 800 ppm sodium meta-bisulphite solution for 20 min prior to steam blanching and steam blanched with treatment of ascorbic acid were performed. The treatment with ascorbic acid which is a reducing agent that can reduce oxidized substrates (Quinone), was conducted by soaking slices of tubers in 2% ascorbic acid solution for 30 min prior to steam blanch. Then sliced tubers treated with all three methods were steam blanched for 4 min and dehydrated in domestic dehydrator (Nesco- FD-75PR, USA) at 60 °C for an overnight. As the next step, dried yam slices were finely ground and sieved to 0.5 mm mesh size. The moisture content was measured using a digital moisture balance (Shimadzu moisture balance; MOC-120H, Japan).



The impact of those treatments in terms of colour was analysed through chromo-meter. Then the flours were packed in high density polyethylene bags (HDPE) for further analysis [9].

Grain processing

In preparation of grains, they were screened for stones, removed dust and other extraneous substances before being properly washed. Then the cleaned grains were soaked in water for required period of time. That time duration of soaking for Kaluheenati rice was 2 hours while that of cowpea and green gram were for 6 hours. Prior to draining, grains and other particles that floated on water were removed and again washed and cleaned thoroughly. Then drained grains were cooked until the starch was partially gelatinized, just enough time to become tender, but not totally cooked. After roughly 10 -15 min of air drying, those grains were dehydrated using domestic dehydrator (Model: Nesco- FD-75PR, USA) at 60 °C for an overnight. The dry grains were then quickroasted for few minutes over a high flame while being stirred. Then those grains were ground with a grinder and sieved to 0.5 mm mesh size. The moisture content was measured using a moisture balance. (Model: Shimadzu moisture balance; MOC-120H type). Finally, prepared samples were packed in high density polyethylene bags. (HDPE). Peanuts were screened, roasted at 150°C, and their skins were removed. Then crushed and packed in HDPE bags.

Processing of coconut flakes and Sesame seeds

In processing of dehydrated coconut flakes, the coconut kernel was de-shelled and shredded using a grater. Steam blanched for 3-5 minutes to reduce microorganisms, and then dried in a dehydrator for 15 hours at 71°C, till reaching 2.95% moisture content. Grinding followed to achieve the desired size, and finally, the dehydrated coconut flakes were packed in HDPE bags [10].

In terms of processing of sesame seeds, they were washed and soaked briefly to remove defects and other materials. After draining, they were pan roasted at 115°C for 15 minutes, for achieving desired sensory properties. Finally, the seeds were packed in HDPE bags.

Preparation of purple yams incorporated multi-grain nutri-bar

This nutri-bar was prepared with the combination of dry mix ingredients that containing purple yam flour, rice flour (variety: Kaluheenati), green gram flour, red cowpea flour, roasted sesame, roasted crushed peanuts, dehydrated coconut and binder mix which containing-sugar, glucose syrup, vanilla and water.

Nutri-bar was prepared using 60% of dry ingredient mix binding in a 40% of sugar syrup. Three different nutri-bar formulations were performed incorporation of purple yam flour of 20% (F1), 15% (F2) and 10% (F3) of whole mixture while changing only the proportion of

rice flour (variety: Kaluheenati). Other all the ingredients were kept constant except purple-yam and rice-flour. Sugar binder mix melted at 106°C followed by addition of vanilla and dry ingredients mix. Whole mixture was cooked briefly, flattened and cut into 8.5 cm x 3.0 cm pieces. After baking for 3 min at 140 °C, cooled, and packed in a suitable package. Formula was standardized for the nutri-bar after performing several trials. Products (F1, F2 and F3) were evaluated for physicochemical, microbial, sensory properties and for antioxidant properties.

Sensory evaluation

The purple yam and Kaluheenati rice based nutri-bar was prepared in three different formulas that F1, F2 and F3 and the proportions of purple yam flour:Kaluheenati rice flour in those formulas were F1-20%:10%, F2-15%:15% and F3-10%:20% respectively. The sensory evaluation was carried out with the participation of eleven trained panellists from the Industrial Technology Institute. The panellists were instructed to assess three formulas with respect to given sensory attributes such as appearance, colour, taste, texture, aroma and overall acceptability using nine-point hedonic scale. A control sample wasn't taken to assess this test, since commercially available nutri-bars made of local those ingredients weren't available in local market.

Determination of physical properties of nutri-bar

Analysis of water activity (aw)

The water activity of the nutri-bars was determined following the operation instructions given in manual of water activity meter (Model: Aqua Lab4TE, USA).

Analysis of pH

25 g of ground and homogenized samples from each nutri-bars were mixed with 250 ml of water using magnetic stirrer at room temperature set at 200 rpm for 1/2 hour to dissolve it properly. Then the pH of the filtrate was measured with calibrated pH meter. (Model: Eutech pH meter, Model no: pH600, Singapore).

Analysis of colour

The colour of the each purple yam flour sample after different treatments implemented to reduce enzymatic browning and nutri-bar from and each formula-were tested using chromometer based on the values of L*(brightness/whiteness), a* (redness/greenness), b*(yellowness/ blueness)(CR-A12, Konica Minolta Sensing Inc. Japan).

Analysis of texture profile

The nutri-bars were evaluated for their textural attributes that adhesiveness (mJ), cohesiveness (mJ), hardness(g), fracturability(mJ), chewiness (mJ), gumminess(g) and springiness(mm). Texture measurements were determined on Brookfield C-3 texture analyser coupled with software texture expert. The test velocity was 1 mm/s. The applied load range to



this analysis was 1000g and trigger load was 2g. Analysis was conducted in triplicates for each samples.

Determination of chemical properties of nutri-bar

Proximate composition analysis

The proximate composition of the nutri-bars was determined according to the AOAC, 2012 methods. Moisture content was determined based on vacuum oven drying method by taking weight loss after 2g of samples were heated in vacuum oven, for 2 hours at 60°C; ash content by incineration of charred samples in a muffle furnace at 550°C; crude fat content by soxhlet extraction method; protein content using the Kjeldahl method and converting to total nitrogen content via a multiplication factor of 6.25; crude fiber content as described in operating manual of FOSS system. Carbohydrate content was calculated using different method as specified by Sompong et al., 2011 through subtracting the summation of moisture, ash, protein and fat by a value of 100. The total energy of the 100g was calculated by factorial method using the below formula;

Energy (kcal) = 4.0 x protein (g) + 4.0 x carbohydrate (g) + 9.0 x fat (g)

All the analysis was performed in triplicate and after getting their means data were expressed as g/100g.

Determination of total sugar content

The total sugar content was analysed following Specification for Jaggery; Sri Lankan Standards 521 [22]. A known amount of sample was hydrolysis with concentrated HCl, and then neutralized with 30% NaOH. The resulting invert solution was adjusted to a final volume of 250 ml. Fehling's solution was titrated against this solution while boiling and adding methylene blue indicator until turning into brick red colour. The total sugar content was calculated using the dextrose factor, dilution factor, and recorded titre in a specific formula with reference to Specification for Jaggery; Sri Lankan Standards 521 [22].

Determination of acid insoluble ash

Following Sri Lankan Standards 1725 Part 1: 2021 which specifies methods of test for cereals, pulses and derived products which incinerated ash in a weighed dish was treated with 5M hydrochloric acid, filtered through ash less filter paper, and washed [23]. Residues were charred, and then ignited at 600°C for an hour. After cooling in a desiccator, final weights were noted.

Determination of mineral composition

Composition of minerals including potassium, calcium, magnesium, iron, zinc and sodium were determined through micro-wave digestion followed the ICP-MS method as specified in AOAC 2012 (999.10) [17].

Determination of anti-oxidant potential of samples

Sample extraction

Extractions were carried out as method described by [11].

Each 2.5 g of properly mixed ground samples of all the three formulations was mixed with 50 ml of volume of 70% ethanol separately and the solutions were shaken overnight at room temperature (25°C) using an orbital shaker (SSL1 11844, Stuart, Sweden). Extracts were then centrifuged at 3000 rpm for 15 minutes and the supernatant was filtered through whatman No.1 filter paper and the filtrates were evaporated using rotary evaporator (Heidolph, Germany). After that resulted solutions were subjected to freeze dry at -40°C for 18 hours at 0.1 mbar using a freeze dryer (Chris-α-1-4/ SC plus, Germany). Residue was kept in freezer until further use.

Determination of total polyphenolic content

The TPC of all three formula of nutri-bars was carried out by using Folin-Ciocalteu reagent as method specified by [20]. Gallic acid standard which was prepared by dissolving 1mg of Gallic acid in 1 ml of distilled water in a vial and its half dilution series of 0.2, 0.1, 0.05, 0.025, 0.0125 and 0.00625 mg/ml was prepared to construct the standard Gallic acid curve. 20 µL of sample, 110 µL of 10 times diluted 2N Folin-Ciocalteu reagent and 70 µL of 10% sodium carbonate (Na₂CO₃) solution were added in a well of 96-well micro plate followed by incubating at 25 ± 2°C for 30 min. Absorbance was measured at 765 nm using a 96-well micro plate reader (SpectraMax Plus³⁸⁴, Molecular Devices, USA) using Gallic acid as the standard. Distilled water was used as the blank.

Determination of Total Flavonoid Content

The TFC of all three formula of nutri-bars was carried out as method specified by [18] using Quercetin as a standard. The Quercetin standard solution (1mg/ml) was prepared diluting in methanol. 250 µL was taken and diluted it in 750 µL methanol to prepare 0.25 mg/ml solution followed by preparing half dilution series of 0.125, 0.0625, 0.03125, 0.0156 and 0.0078 mg/ml. Then 100 µL of sample/extracts and 100 µL of 2% aluminium chloride were added into a well of 96-well micro plate while covering it with a slip. Prior to getting absorbance readings, micro-plate was incubated at room temperature 25 ± 2°C for 10 min and then respective absorbance readings were recorded at 415 nm using Quercetin as the standard using micro plate reader (SpectraMax Plus³⁸⁴, Molecular Devices, USA). All the procedures were carried out in triplicates.

Determination of antioxidant potential through DPPH assay

The DPPH radical scavenging activity of nutri-bar samples was determined as method described by [15]. Having carried out DPPH screening, 90 µL of methanol, 60 µL of DPPH radical (20mg/100 ml) and 50 µL (2 mg/ml) of sample/standard were mixed in a well and incubated at 25 ± 2°C for 10 min. Concentrations that 37.5, 75, 150, 300, 600 µg/ml of each extracted sample were run for the dose response studies. Trolox acts as



the standard antioxidant and extracts were subjected to dose response studies to find IC_{50} values. The activity was calculated using below equation and finally activity was expressed as mg Trolox equivalents (TE)/ g of the each nutri-bar sample.

$$DPPH \text{ radical scavenging activity (\%)} = [(Ac - As) / Ac] \times 100$$

Where;

Ac - absorbance of the control

As - absorbance of the sample

Determination of anthocyanin content

The differential pH method was used to determine the total anthocyanin content as method described by [12]. The sample was dissolved in two distinct buffer solutions, each containing 1 mL of buffer. The sample was first diluted in Potassium chloride buffer (pH 1) until it had a volume of 10 mL, and then it was diluted in Sodium acetate buffer (pH 4.5). After being left to stand for 15 minutes, absorbance measurements were taken via spectrophotometry, using each wavelength read at a length of 520 and 700 nm.

$$\text{Anthocyanin content (mg/100g)} = \frac{A \times MW \times DF \times V \times 100\%}{\varepsilon \times L \times W}$$

Where;

A = Absorbance [A520 - A700] pH 1 - [A520 - A700] pH 4.5,

ε = extinction coefficient (Cyanidin-3-glycoside: 26900 L / mol cm)

L = width of cuvette (1 cm)

MW = molecular weight of cyaniding-3-glycoside 448.8 g / mol,

DF = factor of dilution.

W = Sample weight

V = Volume of the extract

Statistical Analysis

The results were statistically analysed using SPSS software [16]. The calculations were performed by one-way analysis of variance (ANOVA) and significant differences between the results were reported as at 95% confidence level ($P < 0.05$). Mean separation was performed using the Turkey's multiple comparison.

Results and Discussion

The processing methods for yam performed by using several treatments were shown in Table 1.

Three treatments that steam blanching, soaking the slices of tubers in 800 ppm sodium meta-bisulphite solution for 20 min prior to steam blanching and ascorbic acid treatment prior to steam blanching were performed to select the best pre-processing method. The sliced tubers treated and non-treated were steam blanched for 4 min followed by drying and grinding to 0.5mm sieve size. They were prepared into flour, the impact of those treatments in terms of colour was analysed through chromo-meter as shown in Table 1.

Table 1: Colour Value of Purple Yam Flour Prepared Using 4 Different Methods

Treatment	Colour			Colour of the flour	Colour
	L	a	b		
Control	13.76±1.39 ^b	4.47±0.83 ^c	5.51±0.79 ^a		Dark blackish gray
Steam blanch	16.63±1.14 ^a	8.83±0.37 ^b	-0.50±0.06 ^c		Dark grayish violet
SMS + Steam blanch	14.38±0.50 ^b	9.07±0.47 ^b	-0.82±0.20 ^c		Dark violet
Ascorbic + Steam blanch	18.35±1.08 ^a	11.12±1.14 ^a	3.52±0.12 ^b		Reddish brown

Note: Values were presented in mean ±SD

Means within a column with the same letters are not significantly different ($P > 0.05$) by one-way ANOVA

The steam blanch with treatment of sodium meta-bisulphite was identified as the best treatment since samples were retained with distinct purple colour.

Sensory evaluation

The nutri-bar samples based on three formulas (F1, F2 and F3) were evaluated for sensory attributes in terms

of appearance, colour, aroma, texture, taste and overall acceptability with respect to nine-point hedonic scale. The Table 2 shows the results for the mean rank scores of sensory evaluation test.

Table 2: Mean Rank Scores for Three Formula of Nutri-Bar

Sensory attributes	Formula		
	F1	F2	F3
Appearance and color	8.27±0.65 ^a	7.82±0.60 ^a	6.18±0.99 ^b
Aroma	7.55±0.52 ^a	7.45±0.52 ^a	6.09±0.70 ^b
Texture	8.27±0.65 ^a	7.45±0.52 ^b	6.18±0.60 ^c
Taste	7.82±0.60 ^a	7.73±0.65 ^a	6.55±0.69 ^b



Overall acceptability	8.36±0.51 ^a	7.45±0.52 ^b	6.45±0.52 ^c
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Note: Mean followed by different superscript letters in the same row are statistically difference ($p < 0.05$)

F1- Nutri-bar with 20% purple yam and 10% kaluheenati rice

F2- Nutri-bar with 15% purple yam and 15% kaluheenati rice

F3- Nutri-bar with 10% purple yam and 20% kaluheenati rice

It was seen that the nutri-bar based on F1 formula had the highest mean rank scores for all the tested sensory characteristics followed by F2 and F3 respectively. A significant difference ($p < 0.05$) was found among sensory attributes for all three samples. There was no significant difference ($p > 0.05$) observed between F1 and F2 for appearance and colour, aroma and taste while significant difference ($p < 0.05$) was observed

between texture and overall acceptability. All the sensory parameters analysed in F3 were significantly differed ($p < 0.05$) from other two F1 and F2 formulas.

Analysis of chemical properties of nutri-bar

Proximate composition

The results of the proximate analysis of all three samples have been presented in Table 3.

Table 3: The Nutritional Composition of Three Formula of Nutri-Bar

Parameter	Formula		
	F1	F2	F3
Moisture content (%)	7.56±0.36 ^a	6.93±0.17 ^a	6.02±0.24 ^b
Protein content (%)	11.20±0.02 ^c	11.47±0.04 ^b	12.02±0.08 ^a
Crude fat content (%)	6.57±0.09 ^c	6.81±0.05 ^b	7.29±0.04 ^a
Crude fiber content (%)	3.55±0.08 ^a	2.91±0.07 ^b	2.66±0.06 ^c
Ash content (%)	1.26±0.09 ^a	1.37±0.01 ^a	1.39±0.03 ^a
Total CHO content (%)	73.41±0.38 ^a	73.42±0.19 ^a	73.28±0.22 ^a
Total sugar (%)	20.06±0.12	19.16±0.22	18.43±0.24
Energy(kcal/100)	397.57	400.85	406.81

Note: Values were presented in Mean ± SD of triplicate results CHO: Carbohydrate

Values with different superscript letters within one row denote statistically significant differences by the Turkey test at $P < 0.05$

F1- Nutri-bar with 20% purple yam and 10% kaluheenati rice

F2- Nutri-bar with 15% purple yam and 15% kaluheenati rice

F3- Nutri-bar with 10% purple yam and 20% kaluheenati rice

There was seen that highest content of protein, crude fat, minerals and energy has been recorded in F3 formula followed by F2 and F1 respectively. This may be due to higher incorporation of Kaluheenati rice flour (20%) which has relatively inherent higher values for those nutrients of protein, minerals, fat than purple yam, in F3 compared to other two formulas F1 and F2. Inversely, moisture content and crude fiber content were high in F1 formula followed by F2 and F3 respectively. Moisture content of nutri-bars ranged from 7.56-6.02% and significant difference was not found in F1 and F2.

All three samples were significantly varied ($p < 0.05$) in terms of protein contents and the highest protein content was observed in F3 followed by F2 and F1. According to the recent reported studies conducted with reference to proximate composition of selected Sri Lankan traditional rice varieties, Kaluheenati had the highest protein content (11.0±0.4%) and the lowest crude fiber content (0.92±0.03%) (Kariyawasm et al., 2013). Those results were further proved by this

study through showing the lowest crude fiber content in F3 formula which was incorporated highest amount of Kaluheenati rice flour. Crude fat content was significantly higher in F3 formula than F2 and F1 and their fat content ranged from 7.29-6.57%. Among three formulas, F3 showed the highest ash/mineral content of 1.39% however a significant difference ($p < 0.05$) wasn't observed among three formulas. The highest carbohydrate content was recorded in F2 formula that 73.42%. Due to the relatively higher protein and fat contents, the energy value in F3 is higher than the other two formulas and energy contents varied from 397.57 kcal to 406.81 kcal per 100g. The total sugar content was significantly varied ($p < 0.05$) among three formulas and F1 had the highest content that 20.06±0.12% (w/w) followed by F2 and F3 showing 19.16±0.22% and 18.43±0.24% (w/w) of total sugar content respectively.

Mineral composition

Mineral contents of nutri-bars based on three formulas in terms of Fe, Zn, Ca, Mg, Na and K were presented in Table 4.



Table 4: The Mineral Composition of Three Formula of Nutri-Bar

Formula	Iron	Zinc	Calcium	Magnesium	Sodium	Potassium
F1(mg/kg)	14.30±0.85 ^a	14.20±0.00 ^a	305.00±29.70 ^a	743.50±2.12 ^c	396.50±10.60 ^a	3903±2.83 ^a
F2(mg/kg)	14.95±1.49 ^a	14.45±1.20 ^a	282.50±17.68 ^a	847.00±8.49 ^b	278.00±15.56 ^b	3624±8.49 ^b
F3(mg/kg)	15.10±1.84 ^a	15.75±0.64 ^a	258.50±10.60 ^a	941.00±1.41 ^a	255.50±13.44 ^b	3076±3.54 ^c

Note: Values were presented in Mean ± SD of triplicate results

Values with different superscript letters within column denote statistically significant differences by the Turkey test at P<0.05)

F1- Nutri-bar with 20% purple yam and 10% kaluheenati rice

F2- Nutri-bar with 15% purple yam and 15% kaluheenati rice

F3- Nutri-bar with 10% purple yam and 20% kaluheenati rice

The mineral contents of all tested nutri-bar formulas significantly varied in terms of magnesium, sodium and potassium contents. The significantly highest sodium content was found in F1 formula and a significant difference wasn't observed between F2 and F3 formulas. The highest iron, zinc and magnesium contents were recorded in F3 formula followed by F2 and F1. The potassium, sodium and calcium contents were higher in F1 formula followed by F2 and F3. The studies have revealed that purple yam contained significantly higher amount of potassium and sodium. The sodium to potassium ratio should be comply with

the requirement issued by WHO that the ratio should be equal or less than one. In this study its ratios were recorded as 0.10, 0.076, 0.083 for F1, F2 and F3 respectively indicating their consumption would not cause for hypertension [24].

Analysis of functional properties

Three formulas were tested for determination of total phenolic content, total flavonoid content, anthocyanin content and antioxidant activity or DPPH free radical scavenging activity and those results have been presented in Table 5 and Figure 1.

Table 5: Anti-Oxidant Potential of Extracts of Three Formulas

Formula	TPC	TFC	DPPHactivity	Anthocyanin
F1	4.13±0.10 ^a	0.18±0.05 ^a	3.14±0.70 ^a	0.22±0.00 ^a
F2	3.23±0.15 ^b	0.15±0.03 ^a	2.61±0.18 ^a	0.13±0.00 ^b
F3	2.03±0.10 ^c	0.12±0.04 ^a	2.21±0.94 ^a	0.05±0.00 ^c

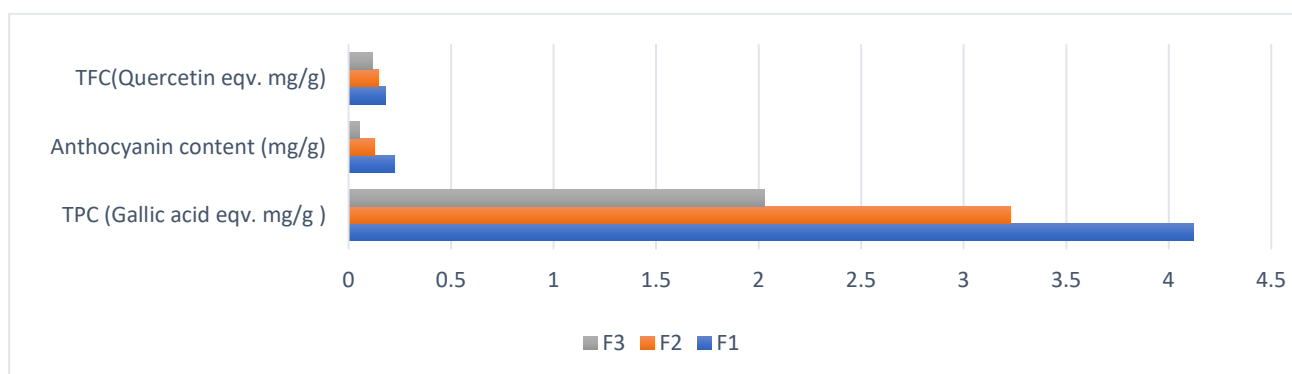
Note: Values were expressed as Mean± SD TPC: mg gallic acid equivalents (GAE) /g, TFC: mg quercetin equivalents (QE) /g, DPPH mg Trolox Equivalents (TE)/g, Anthocyanin content ; mg cyaniding-3-glucoside/g

Values with different superscript letters within one column denote statistically significant differences by the Turkey test at P<0.05

F1- Nutri-bar with 20% purple yam and 10% kaluheenati rice

F2- Nutri-bar with 15% purple yam and 15% kaluheenati rice

F3- Nutri-bar with 10% purple yam and 20% kaluheenati rice

**Figure 1: Bioactive Compound Composition of Nutri-Bars**

Note: F1- Nutri-bar with 20% purple yam and 10% kaluheenati rice

F2- Nutri-bar with 15% purple yam and 15% kaluheenati rice

F3- Nutri-bar with 10% purple yam and 20% kaluheenati rice



The phenolic content of all three formulas significantly differed and ranged from 2.03 ± 0.10 to 4.12 ± 0.10 mg (GAE) / g. The total flavonoid content of tested three formulas varied from 0.12 ± 0.04 to 0.18 ± 0.05 mg (QE) /g without showing a significant difference. The anthocyanin content was significantly varied in the range from 0.05 ± 0.00 to 0.22 ± 0.00 mg/g. In comparison of those three functional components, F1 formula which had the highest incorporation of purple yams has showed the maximum content of phenolic, flavonoid and anthocyanin and it was followed by F2 and F3 respectively. This may be due to substantially high amounts of phenolic acids that 7.3-12.7 (GAE)/g, anthocyanin that 0.1-0.9 mg cyaniding-3-glucoside/g and flavonoid that 6.2-7.0 mg QE/g in purple yam

according to the recent studies [13]. Some studies have revealed that the contents of some antioxidants including anthocyanin decreased in the processing of purple yam when become flour and the products. Other than purple yam, functional components which existed in final product were contributed mainly originated with antioxidant rich cereals of Kaluheenati rice and legumes of mung-bean and red cowpea.

According to the Figure 2, pearson correlation coefficient analysis was showed that there was a strong linear relationship ($R^2=0.9997$) between the two variables of DPPH activity and anthocyanin content (mg/g) of the formula. Since the obtained P-value is 0.012 (i.e. $p < 0.05$), this correlation was statistically significant.

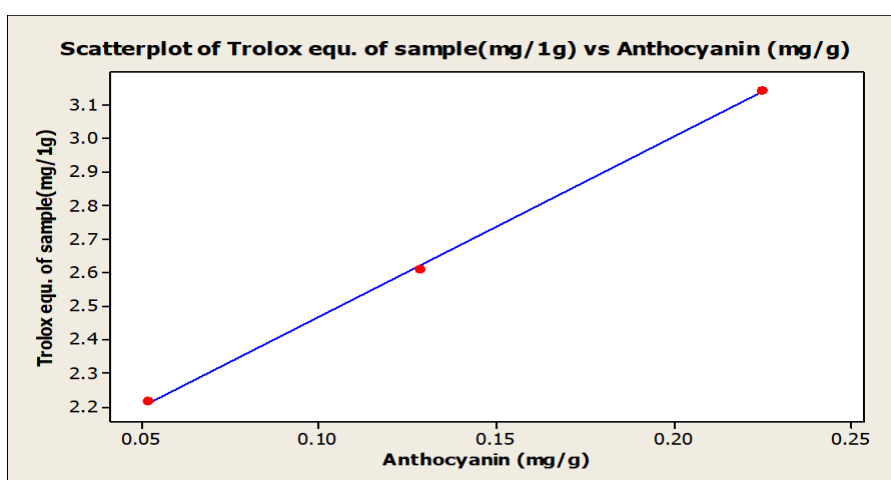


Figure 2: Correlation between DPPH Activity and Anthocyanin Content of the Sample

Analysis of physical and textural properties

Texture profile analysis

The influence of different proportions of purple yam: Kaluheenati rice in formulation of F1, F2 and F3 on adhesiveness, cohesiveness, hardness, springiness,

gumminess, fracturability and chewiness of prepared nutri-bars are presented in Table 6. Textural properties are very important factors that are needed to control the acceptability of the product [14].

Table 6: Textural Attributes of Different Formulas of Nutri-Bar

Form ula	Texture attributes						
	Adhesiveness (mJ)	Cohesiveness (mJ)	Hardness (g)	Fracturability (mJ)	Chewiness (mJ)	Gumminess (g)	Springiness (mm)
F1	2.41 ± 0.25^b	0.28 ± 0.09^a	5783.33 ± 935.43^b	112.83 ± 3.27^a	838.64 ± 86.2^a	3055.7 ± 499.44^a	56.06 ± 3.48^a
F2	2.89 ± 0.33^b	0.16 ± 0.02^a	7309.33 ± 629.72^b	68.18 ± 6.02^b	787.65 ± 29.4^a	2084.6 ± 189.30^b	38.59 ± 10.20^b
F3	3.66 ± 0.54^a	0.15 ± 0.03^a	8740.00 ± 304.46^a	51.79 ± 6.30^c	19.37 ± 3.42^b	941.97 ± 144.65^c	0.73 ± 0.02^c

Note: Values were expressed as Mean $\pm$ SD; Values with different superscript letters within one column denote statistically significant differences by the Turkey test at $P < 0.05$

F1- Nutri-bar with 20% purple yam and 10% kaluheenati rice

F2- Nutri-bar with 15% purple yam and 15% kaluheenati rice

F3- Nutri-bar with 10% purple yam and 20% kaluheenati rice



The nutri-bar based on F1 formula was recorded the least force to stick/adhere two or more different molecules together indicating its highest tendency to adhere. There was no significant different between F1 and F2 formulas in terms of adhesiveness. The force related with cohesiveness was higher in F1 followed by F2 and F3. Hardness or the resistance of material to be deformed lowest in F1 showing lesser hardness. As F1

needed highest amount of force it had significantly lowest tendency to be fractured. The other three textural parameters of chewiness, gumminess and springiness were highest in F1 followed by F2 and F3.

Analysis of physical properties

The colour of nutri-bars based on each respective formulas produced in this study were presented in Table 7.

Table 7: Physical Properties of Different Formulas of Nutri-Bar

Physical parameters	Formula		
	F1	F2	F3
Colour L value	23.36±1.39 ^b	26.97±1.62 ^a	28.92±2.84 ^a
a value	6.72±0.51 ^b	7.19±0.30 ^b	8.62±0.45 ^a
b value	2.24±0.26 ^a	2.50±0.49 ^a	3.94±0.82 ^b
pH	6.10 ± 0.04 ^a	5.78 ± 0.00 ^b	5.66 ± 0.01 ^c
a _w	0.604±0.01 ^a	0.576±0.02 ^b	0.554±0.02 ^c
Acid insoluble ash	0.052± 0.0015	0.080± 0.0019	0.09± 0.0032

Note: Values were expressed as Mean± SD

Values with different superscript letters within one column denote statistically significant differences by the Turkey test at P<0.05

F1- Nutri-bar with 20% purple yam and 10% kaluheenati rice

F2- Nutri-bar with 15% purple yam and 15% kaluheenati rice

F3- Nutri-bar with 10% purple yam and 20% kaluheenati rice

Colour is one of quality indicators which represent a consumer's preference. The results of Table 7 express the colour of each formula with three dimensional representation of the value of L*, "a*" and "b*". As the b value was increasing, the concentration of purple yam flour decreased. Hence F1 has recorded the lowest b value as the lower b value indicates the most intensive purple colour. F3 had the highest b value among three formulas and it was significantly different (p<0.05) with F2 and F1 formulas. Also, F3 formula has recorded the significantly highest value for a* that 8.6283±0.45 showing higher intensity in terms of red colour while there was no significant difference between F2 and F1 formulas with respect to a*. It may be due to the incorporation of highest concentration that 20% of Kaluheenati rice flour for F3 formula. L value which indicates lightness was highest in F3 formula followed by F2 and F1 as with the increase in intensity of purple colour imparted by anthocyanin, the darkness of the samples also increased giving low L values.

The water activity (a_w) of three formulas ranged from 0.6043±0.01 to 0.5433±0.02 and it is seen that statistical significant difference (p<0.05) among a_w all the formulas. F1 has recorded the highest value followed by F2 and F3.

pH values of all three formulas were significantly different (p<0.05) as presented in Table 07 and F1 has recorded the highest value followed by F2 and F3. pH value is important to select the suitable preservative for the finally selected formula where each preservative have a specific range of pH to be active. Calcium propionate, a preservative is added for grainy food was selected considering its active pH range.

Requirement of conformity for grain based products in Sri Lankan scenario

Some specifications have been prescribed in the Sri Lanka Standards for the grain based products [23] based on the following parameters which should be complied with the requirements that 6-12% for moisture (w/w)%, maximum value of 0.1 for the acid insoluble ash (w/w)% and minimum value of 6.0% for protein (w/w)%. According to the results taken for moisture, protein (Table 3) and acid insoluble ash (Table 7) contents of three formulas has been complied with Sri Lanka Standards requirements.

Comparison of selected nutri-bar formula (F1) with Recommended Dietary Allowances (RDA)

Comparison of selected nutrient contribution of selected nutri-bar formula (F1; per size of 50g) to the Recommended Dietary Allowances (RDA) was presented in Table 8.



Table 8: Comparison of selected nutri-bar formula (F1) with Recommended Dietary Allowances (RDA)

	Energy (kcal)	Protein (g)	Calcium (mg)	Iron (mg)	Zinc (mg)	Magnesium (mg)
AVG RDA*	2000	55	800	22	7	224
AVG RDA**	1075	26	500	12	4	60
Nutri-bar (F1) 50g of serving size	198.78	5.6	15.25	0.72	0.71	37.18
% of fulfilment per 50 g of nutri-bar						
% of fulfilment*	9.94%	10.18%	1.91%	3.27%	10.14%	16.60%
% fulfilment**	18.49%	21.53%	3.05%	6%	17.75%	61.96%

Note: *Recommended Dietary Allowances for Sri Lankan elders at age of above 60 Yrs (RDA, 2007)

**Recommended Dietary Allowances for Sri Lankan children at age below 5 Yrs (RDA, 2007)

One serving size (i.e. 50g) of the selected formula provides 18.49% of energy contributes to the RDA for children under 5 years while 9.94% of energy contributes to the RDA value for elders. Around half of the protein requirement to RDA for children under 5 yrs (43.08%) will be fulfilled by 2 servings size of nutri-bar (F1) per day. The contribution of calcium to the RDA will be 3.05% for children while 1.91% for elders from 1 serving size. As well as the contribution of iron to the RDA made by 1 serving will be 6% and 3.27% for children and adolescents respectively. The contribution of zinc, a mineral plays a vital role in brain development of children, will be 17.75% for children while and 10.14% for elders. 1 serving size of nutri-bar will fulfil 61.96% of per day magnesium requirement of children under 5 years while fulfilling 16.60% RDA for magnesium of elders. In considering above comparison, the selected nutri-bar formula has been able to fulfil nutrient requirement of those vulnerable groups in substantial percentages.

Conclusion

In the present study, the ready-to-eat nutri-bar based on three different formulas of F1, F2 and F3 were developed with acceptable sensory properties. The flour proportions of purple yams: rice in those formulas were F1- 20%: 10%, F2- 15%: 15% and F3-10%: 20% respectively. The highest mean rank scores for all sensory attributes in terms of appearance and colour, taste, texture, aroma and overall acceptability were observed in the F1 formula and it was selected as the best formula. The fat contents and fiber contents significantly ($p < 0.05$) varied and the highest contents of fat (7.29 g/100g) and crude fiber (3.55 g/100g) were obtained for F3 and F1 formulas respectively. The highest protein content was observed in F3 followed by F2 respectively. The highest carbohydrate content was obtained for F2 formula and no significant difference ($p < 0.05$) was observed among those three formulas in terms of carbohydrates.

The highest TPC, TFC, anthocyanin and antioxidant activity were observed in F1 formula where the highest incorporation of purple yam flour. The most desirable

values for chewiness, gumminess, springiness and less hardness were found in F1 formula and calcium, sodium, potassium contents were also highest in F1. Considering those factors, especially preferable sensory characteristics, F1 was selected as the best formula.

The caloric value of nutri-bar formulas ranged from 397.57-406.81 kcal/100g while protein contents of bars ranged from 11.20 g to 12.02 g per 100g. Since substantial amount of protein and energy requirement to RDA for children less than five years will be fulfilled through F1, this product will be more suited for the consumption of children below 5 years.

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Declaration of Interest

The authors report there are no competing interests to declare.

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