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Research article

Development, characterization and shelf-life testing of a novel pulse-based snack bar

Tina Bhakha[1], Brinda Ramasawmy[2], Zaynab Toorabally[3] and Hudaa Neetoo[1,*]

[1] Department of Agricultural and Food Sciences, Faculty of Agriculture, University of Mauritius, Mauritius
[2] Department of Agricultural Production Systems, Faculty of Agriculture, University of Mauritius, Mauritius
[3] Department of Health Sciences, Faculty of Science, University of Mauritius, Mauritius

*** Correspondence:** Email: s.neetoo@uom.ac.mu; Tel: +2304037885; Fax: +2304655743.

Abstract: With the globalization of food trade, we are observing tremendous changes in eating patterns of youngsters. Snack bars represent convenient, appropriately portioned, Ready-To-Eat food items, which not only supply nutrients to the body but also provide a feeling of satiety. The aim of this study was to formulate a novel high-protein, low Glycemic Index and low-fat snack bar that can be eaten on-the-move. Twelve different pulse-based bar formulations were developed and 85.1% of sensory panelists indicated that they particularly liked the taste of formulation M1. Since M1 contained peanuts, a nut-free and date-free equivalent (mM1) was developed to cater for individuals with allergies to these ingredients. A dehydrated mix (DM) based on the mM1 composition, was also developed. The microbiological and sensorial shelf lives of the products were then determined during storage at either ambient (ca. 23 °C) or refrigerated temperatures (ca. 4 °C) by determining counts of aerobic bacteria and yeast and mold. Mean aerobic bacteria and yeast and mold counts of M1 fell in the range of 8.4–9.4 and 4.5–5.4 log cfu/g and 7.5–8.6 and 3.8–4.9 log cfu/g during storage at room and refrigerated temperatures respectively. Aerobic bacteria and yeast and mold counts were consistently higher under ambient storage. Since a microbial population density >7 Log CFU/g usually marks the onset of microbiological spoilage, the bars were estimated to have a microbiological shelf-life of <2 days. Overall this study points to the development of a tasty and nutritious pulse-based Ready-To-Eat snack as well as a dehydrated mix that can be readily reconstituted at home or at work.

Keywords: snack bar; pulses; sensory; microbiology; shelf-life

Abbreviations: RTE: Ready-To-Eat; RTM: Ready-To-Mix; et al.: And others; TVC: Total Viable Counts; cfu/g: Colony Forming Units per gram; RT: Room Temperature; ℃: Degrees Celsius; LABs: Lactic Acid Bacterial species; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein

1. Introduction

Snacks have always been a significant part of the ancient as well as the modern diet [1]. The simplest definition of a snack is a light food item, which is consumed quickly between meals [1]. Due to an increasingly hectic lifestyle with long hours spent at work, snacking has become a common habit in Mauritius as well as worldwide [2]. Moreover, various surveys conducted in developed countries, have shown a decline in energy intake from main meals, while energy from snacks eaten between meals has increased. In fact, almost a quarter of total energy being consumed daily occurs between meals [3]. Unfortunately, it has been increasingly recognized that many snacks can be high in sodium, fat and sugar [4]. Frequent consumption of these types of snack foods can be detrimental to energy balance as well as the nutrient quality of the diet [5]. In addition, uncompensated calories from snacking could contribute to overweight and obesity [6].

There is therefore a need to design and develop alternatives to currently available snacks especially with the growing consumer demand for healthy, natural, minimally-processed and convenient foods. Indeed, changes in the eating patterns of people, competition among food sectors and public awareness of the negative health links between energy-dense snacks and diet-related diseases [7], have all contributed to the increased desire for food products that offer health and wellness [8,9]. Moreover, the combination of a sedentary lifestyle and a change in work conformation, compounded by advancement in technology and replacement of foods rich in essential nutrients, form part of the main reasons for an increased demand for nutritious snacks that can be consumed on-the-go [1].

In recent years, the consumption of cereal bars, as portable snacks, has increased by 11% across the globe [1]. Cereal bars are bar-shaped food products, made by pressing cereals and usually dried fruits or berries, which are in most cases held together by glucose syrup [10]. While cereal bars are popular and convenient snack food items, they are also relatively energy-dense, due to their high fat and high sugar content [10], whilst being relatively nutrient-poor [11]. Indeed, the protein quality of cereals is relatively poor since they are usually deficient in essential amino acid lysine. Fortunately, food bars are amenable for delivering essential elements of the diet, such as protein and fibre, with the inclusion of ingredients such as dried fruits, nuts and pulses. Indeed, the use of legumes/pulses improves the overall protein quality of cereals as they are rich in essential amino acid lysine while cereal proteins complement legume proteins in the essential amino acid methionine [12]. Pulse-based cereal bars could represent an alternative to unhealthy and non-nutritious snacks, thus assisting in promoting healthful eating behaviours among Mauritian individuals.

The aim of the study was therefore to formulate and characterize a nutritious snack available in either a Ready-To-Eat (RTE) portable bar or as a Ready-To-Mix (RTM) dry powder that can be readily reconstituted at home.

2. Materials and methods

2.1. New product development of snack bar

2.1.1. Raw materials

Ingredients used in new product development trials consisted of laird lentils (Mayil Spices Ltd, Mauritius), yellow split chickpeas (Eagle Brand Spices Ltd, Mauritius), oats (Master White Oats), maize powder (Rocket, Veerapen Ltd, Mauritius), beetroot, carrots, dates (Deglet Noor), honey of the Acacia variety (Wescobee, Western Australia) and cardamom (Mayil Spices Ltd, Mauritius) for flavouring. Ingredients used as "add-ons" were coconut powder (Mayil Spices Ltd, Mauritius), peanuts, raisins (Mayil Spices Ltd, Mauritius) or sesame seeds (Mayil Spices Ltd, Mauritius). All ingredients used in the study were purchased from a retail supermarket except for beetroots and carrots which were obtained from a local market and peanuts and dates which were purchased from a wholesaler. These different ingredients were chosen for this part of the study since they are widely consumed in the diet.

2.1.2. Phase 1: Preparation of bar by toasting or steaming

Given the desirable attributes of the snack bar to be low in fat and calories, the cooking methods of choice were steaming (low-fat moist cooking) or toasting (low-fat dry cooking). In order to increase the protein and fiber content, we had proposed cereal bar formulations of two kinds: (i) cereal-legume composites and (ii) cereal-legume-vegetable composites. Oats and maize were the selected cereals while laird lentils and yellow split chick peas were the legumes of choice. Oats are known to contain beta-glucans which are responsible for lowering of low-density lipoprotein cholesterol (LDL) [13] while maize is an essential source of various major phytochemicals such as carotenoids, phenolic compounds, and phytosterols [14,15]. Beetroot was selected as the vegetable as it is highly nutritious and health promoting [16] and also by virtue of its bright red colour.

The cereal-legume composite formulation was cooked by toasting. Briefly, oats (30 g), maize (30 g), laird lentils (40 g) and yellow split chick peas (40 g) were individually toasted in the oven (Bosch) at 130 ℃ for 60 minutes with intermittent turning. The toasted ingredients were then ground in a mixer (Robot Coupe) followed by the addition of cardamom powder (1 g). Ten medium-sized dates were finely chopped and incorporated into the mixture together with 50 mL of warm water and 35 mL of honey. The paste was then uniformly spread in a rectangular pan (20 cm × 8 cm) to a thickness of 2 cm and sprinkled with one of four toppings (raisins, sesame seeds, peanuts or coconut powder) thus yielding four different formulations. The bars were then allowed to cool for 30 minutes and subsequently cut into evenly sized bars measuring 8.5 cm × 2.5 cm.

The cereal-legume-vegetable composite formulation was cooked by steaming. Briefly, laird lentils (40 g) and yellow split chick peas (40 g) were pre-soaked in water for ca. 12 hours. One medium-sized beetroot was washed, peeled and cut into small pieces. Soaked pulses and beetroot (30 g) were dipped in 1500 mL water and cooked under high-pressure in a pressure cooker for 5 minutes (Hawkins). Boiled beetroot and pulses were then ground in a mixer (Robot Coupe) followed by the addition of oats (30 g) and cardamom powder (1 g). Ten medium-sized dates were finely chopped and incorporated into the mixture together with 50 mL of warm water and 35 mL of honey. The paste

was then uniformly spread in a rectangular pan (20 cm × 8 cm) to a thickness of 2 cm and cooked uncovered over a water bath for 30 minutes. Following cooking, the paste was sprinkled with one of four toppings (raisins, sesame seeds, peanuts or coconut powder) thus yielding four different formulations. The cooked paste was then allowed to cool and cut into evenly sized bars measuring 8.5 cm × 2.5 cm. The cereal-legume and cereal-legume-vegetable bars were then tested for overall acceptability with an untrained consumer panel (n = 25) using a 5-point hedonic scale grading "5" for excellent and "1" for highly disliked samples.

2.1.3. Phase 2: Preparation of bar formulation by toasting

Since products cooked by steaming did not receive satisfactory scores during the sensory evaluation (preliminary data not shown), the only cooking method of choice for further product development trials was toasting. In this phase of the product development process, two cereal-legume composites and two cereal-legume-vegetable composites were proposed. Cereal-legume composite bars contained the same fixed ingredients (oats, laird lentils, yellow split chick peas, dates, honey, water), one of two variable cereals (maize or semolina) and one of three add-on ingredients (toasted peanuts and dates, toasted sesame seeds and dates, or oat flakes). Briefly, maize (30 g) or semolina (30 g), oats (30 g), laird lentils (40 g) and yellow split chick peas (40 g) were individually toasted in the oven (Bosch) at 130 °C for 60 minutes with intermittent turning. The toasted ingredients were then ground in a mixer (Robot Coupe) followed by the addition of cardamom powder (1 g). Ten medium-sized dates were finely chopped and incorporated into the mixture together with 50 mL of warm water and 35 mL of honey. The paste was then uniformly spread in a rectangular pan (20 cm × 8 cm) to a thickness of 2 cm and sprinkled with either toasted peanuts and dates, toasted sesame seeds and dates, or oats flakes, thus yielding six different cereal-legume formulations. The bars were then allowed to cool for 30 minutes and subsequently cut into evenly sized bars measuring 8.5 cm × 2.5 cm.

Cereal-legume-vegetable composite bars contained the same fixed ingredients (oats, laird lentils, yellow split chick peas, dates, honey, water), one of two variable vegetables (beetroot or carrot) and one of three add-on ingredients (toasted peanuts and dates, toasted sesame seeds and dates, or oat flakes). Carrots were included in this phase of the study because they are rich in various nutrients [17]. Moreover, carrots have a mild and sweet taste, which is generally liked by consumers [17]. Briefly, a medium-sized beetroot or one medium-sized carrot was washed, peeled and grated. Grated beetroot or carrot (30 g), oats (30 g), laird lentils (40 g) and yellow split chick peas (40 g) were individually toasted in the oven (Bosch) at 130 °C for 30–60 minutes depending on the ingredient. The toasted ingredients were then ground in a mixer (Robot Coupe) followed by the addition of cardamom powder (1 g). Ten medium-sized dates were finely chopped and incorporated into the mixture together with 50 mL of warm water and 35 mL of honey. The paste was then uniformly spread in a rectangular pan (20 cm × 8 cm) to a thickness of 2 cm and sprinkled with one of three toppings (toasted peanuts and dates, toasted sesame seeds and dates, or oat flakes) thus yielding six different formulations. The bars were then allowed to cool for 30 minutes and subsequently cut into evenly sized bars measuring 8.5 cm × 2.5 cm. The six cereal-legume and six cereal-legume-vegetable composite bars are indicated in Tables 1a and 1b respectively.

Table 1a. Cereal-legume composite bars with variable cereal and add-on ingredients.

Cereal / Add-on ingredients	Maize (M)	Semolina (S)
Toasted peanuts & dates (1)	Snack bar-M1	Snack bar-S1
Toasted sesame seeds & dates (2)	Snack bar-M2	Snack bar-S2
Oats flakes (3)	Snack bar-M3	Snack bar-S3

Table 1b. Cereal-legume-vegetable composite bars with variable vegetable and add-on ingredients.

Vegetable / Add-on ingredients	Beetroot (B)	Carrot (C)
Toasted peanuts & dates (1)	Snack bar-B1	Snack bar-C1
Toasted sesame seeds & dates (2)	Snack bar-B2	Snack bar-C2
Oats flakes (3)	Snack bar-B3	Snack bar-C3

A sensory evaluation form was then designed and sensory analysis was conducted with 25 untrained panelists. The sensory parameters (appearance, smell/aroma, colour, taste, texture, attitudes towards product and overall satisfaction) were rated on a hedonic scale of 1–5, where 5 represented the highest score and 1 the lowest. The evaluation form used is available from Supplementary File 1.

2.1.4. Phase 3: Development of a modified snack bar and dehydrated mix

The sensory evaluation conducted during Phase 2 indicated that the cereal-legume composite bar M1, comprising of maize (variable cereal) and peanuts and dates (variable add-on ingredients), earned the highest scores (data not shown). Snack bar (M1) was subsequently modified to formulation mM1 by omitting the addition of peanuts and dates to cater for the needs of individuals with allergies to these specific ingredients. Specifically, mM1 was formulated as described above by mixing toasted laird lentils (40 g), yellow split chickpeas (40 g), maize powder (30 g), oats (30 g), cardamom (1 g), honey (35 mL), and hot water (70 mL) only. The paste was then uniformly spread in a rectangular pan (20 cm × 8 cm) to a thickness of 2 cm. The bars were then allowed to cool for 30 minutes and subsequently cut into evenly sized bars measuring 8.5 cm × 2.5 cm.

For preparation of the dehydrated mix, the procedure described above was followed. Briefly, oats (30 g), maize (30 g), laird lentils (40 g) and yellow split chick peas (40 g) were individually toasted in the oven (Bosch) at 130 ℃ for 60 minutes with intermittent turning. The toasted ingredients were then ground in a mixer to a fine powder (Robot Coupe) followed by the addition of cardamom powder (1 g). The step-by-step procedure is available from Figure 1.

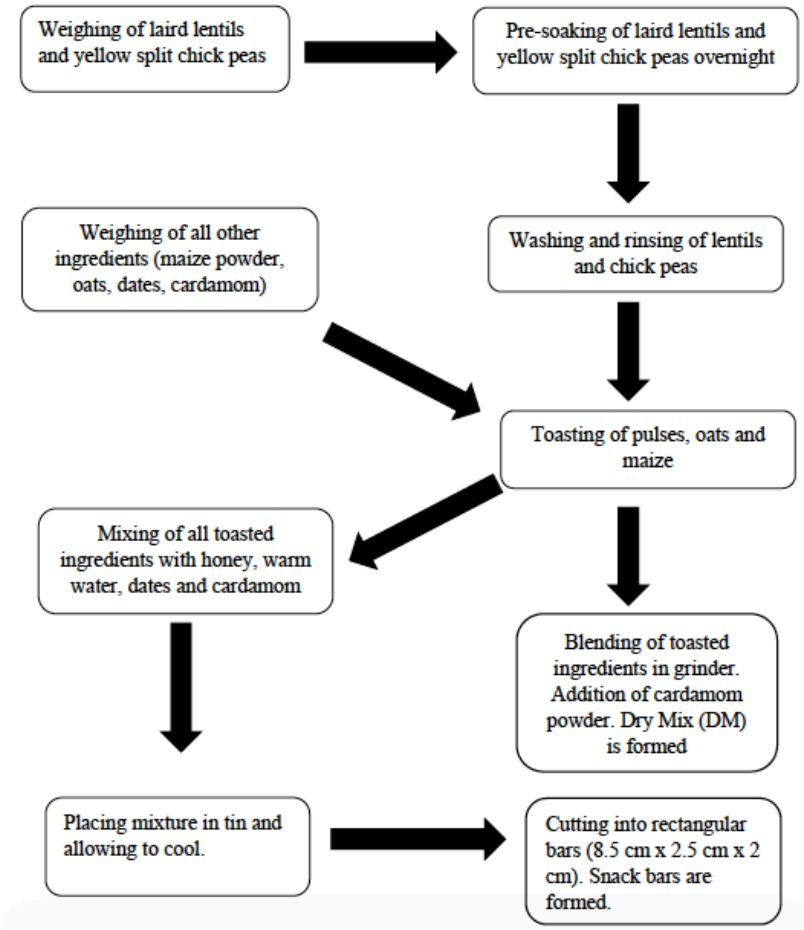

Figure 1. General flowchart for the manufacture of snack bars and dry mix.

2.2. Physicochemical and proximate analysis

The snack bars (M1 and mM1) were prepared as described earlier. They were then vacuum-packaged in 18 ounce-standard bags (Nasco, Whirl-Pak®) using a vacuum-packaging machine (Multivac) and kept at ambient temperature (ca. 23 ℃) or at refrigerated temperature (4 ℃) for a period of five days and subjected to physicochemical analyses daily. Similarly, the dehydrated mix was prepared, air-packaged and then stored at ambient or refrigerated temperature for a period of four weeks and analyzed weekly. For determination of pH, a 10% (m/v) suspension of the samples was prepared by mixing 5 g samples into with 45 mL of distilled water. The pH of the mixture was measured in triplicate using a digital pH meter (Mettler Toledo) [18]. Moisture was estimated following the AOAC official method of analysis [19]. Briefly, snack bar samples were ground in a coffee mill (Robot Coupe) for one minute. Five grams of the ground sample were placed in moisture dishes and dried in an oven set at 105 ℃ for 3 hours. Samples were held in the dessicator approximately 45 minutes before weighing. Samples were placed in the oven again and dried to constant weight. For determination of water activity, a water activity meter (Novasina) was used under controlled temperature. Samples were crumbled in a coffee mill and placed in a plastic sample dish (4 cm diameter by 1 cm high) to the fill line (about ½ full). Samples were kept covered prior to testing to prevent moisture loss. The instrumental surface colour of the comminuted samples was

also determined using a chromameter (Minolta CR-410, Konica Minolta, Japan) with a measurement area of 8 mm in diameter, observation angle of 10 ℃ and standard illuminant C. All the physicochemical analyses were carried out in three independent replicates and their measurements were taken in triplicates.

For proximate analyses, snack bars and dehydrated mix were prepared as described above. The crude protein N × 6.25 was determined by the Kjeldahl method [19]. Briefly, 0.5 g of each bar sample was weighed and transferred in a digestion tube. One gram of Kjeltab was then weighed and transferred in the tube followed by 15 mL of concentrated sulfuric acid. The digestion tube was placed in the digestion apparatus operating at 350–400 ℃. After digestion, the sample was transferred into a 100 mL-distillation flask to which distilled water was added to the 100 mL mark. The distillation apparatus was allowed to heat up and 5 mL of 5% boric acid and indicator were added to a conical flask. Five mL of 40% sodium hydroxide was added in the apparatus and was released slowly in the conical flask. Five mL of digested sample was then added into the apparatus and was released slowly in the conical flask. Water was then added and a green colour was observed as ammonia produced was trapped. The solution in the conical flask was titrated with 0.01 M hydrochloric acid. A colour change from green to pink was seen. Two independent replicates were carried out. The titre value was noted and the crude protein was calculated using the following formulae:

$$\text{Total Nitrogen (\%)} = \frac{(\text{Sample Titre} - \text{Blank Titre}) \times \text{Molarity} \times 14 \times 100}{\text{Sample Mass} \times 1000}$$

$$\text{Protein (\%)} = \text{Total Nitrogen (\%)} \times \text{Relevant factor (6.25)} \quad (1)$$

Total lipids were analysed by the Soxhlet method following the AOAC official method of analysis [19]. Briefly, 2 g of sample was weighed and transferred into a Whatman filter paper. The filter paper was then folded in the bottom of an extraction thimble. A Quickfit boiling flask was weighed and was fit into the bottom of the Soxhlet extractor. The thimble was then placed into the Soxhlet extractor and petroleum spirit was then slowly added into the apparatus. The reflex condenser was then connected over the extraction. The apparatus was allowed to heat until the fat solvent boiled gently. The Soxhlet apparatus was run for 8 hours. Following this time period, the petroleum spirit was removed from the extractor and the boiling flask was heated up until it was almost dried. The flask was placed in the oven and allowed to dry at 100–105 ℃ and the boiling flask was then allowed to cool and its weight determined. Two independent replicates were carried out.

The % crude fat was then calculated using the formula below:

$$\text{\% Crude Fat} = \frac{(W_3 - W_2)}{W_1} \times 100 \quad (2)$$

where W_1 = Weight of sample, W_2 = Weight of Quickfit boiling flask and W_3 = Weight of Quickfit boiling flask + Crude fat.

2.3. Microbiological and sensorial shelf-life testing

Snack bars (M1 and mM1) and dehydrated mix (DM) were prepared, packaged and kept at 23 ℃ or 4 ℃ as described previously. For microbial shelf-life estimation, microbiological analyses of snack bars and dry mix were carried out daily and weekly respectively. Briefly, a sample of each product weighing 25 g was aseptically placed in a sterile stomacher bag to which 225 mL of sterile

Buffered Peptone Water (BPW) was added. The mother sample was then serially diluted and plated on Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) for enumeration of Aerobes [20] and Yeast and Mold [21] respectively. The plates were incubated at 30 ℃ for 48 hours. All the colonies were then enumerated with a colony counter.

For sensorial shelf-life estimation, a sensory evaluation questionnaire (Supplementary File 2) was designed. Sensory analysis of the snack bars was conducted with 25 untrained panelists daily for a period of 5 days while analysis of the dry mix was carried out weekly for a period of 4 weeks. Samples were withdrawn at defined intervals and rated for sensory parameters such as appearance, colour, texture, taste and smell/aroma on a hedonic scale of 1–5, where 5 represented the highest score and 1 the lowest. The sensorial assessment was conducted in a single replicate given the complex logistics associated with a sensory evaluation.

2.4. Statistical design and analysis

All experiments were conducted in at least two independent trials except for the sensory evaluation which was carried out only once for each formulation trial. Microbiological, physicochemical and proximate composition data were analysed by a single factor analysis of variance (ANOVA), and Tukey pairwise comparisons using Minitab® Release 17. Statistical significance was attributed to P values of <0.05.

Sensory data was analysed using the IBM Software Package for the Social Science (SPSS) Statistics 22 by making relevant cross tabulations and computing percentage frequencies to determine the most preferred formulation. Relevant column charts for the questions were drawn using Microsoft Excel 2013.

3. Results and discussions

3.1. Formulation of cereal-legume and cereal-legume-vegetable bars

Cereals and legumes are ancestral staple foods that are rich in carbohydrates, fibre and micronutrients [22]. They also provide a fair amount of proteins with protein content of 9–18% and 20–40% respectively [22]. When combined, protein efficiency is improved thanks to complementary essential amino acid profiles [23]. Phase 1 of the product design and development process involved formulating different cereal-legume and cereal-legume-vegetable snack bars with one of four toppings (coconut powder, raisins, peanuts or sesame seeds). In all formulations trials, the two pulses were equally abundant, i.e., at a ratio of 1:1. Moreover, in the cereal-legume composite bars, the cereals (oats and maize) and legumes were also abundant at a ratio of 1:1. Laird lentils and yellow split chickpeas are pulses that are frequently consumed in Mauritius given their nutritive properties. For instance, lentils have a high protein, fiber, and iron content [24]. Moreover, their low Glycemic Index (GI) makes them suitable for individuals suffering from diabetes [24]. The high fiber content of yellow split chickpeas [25] also has the potential to lower the level of cholesterol in blood, thus reducing the risk of cardiovascular diseases [25]. Additionally, peas are rich in essential vitamins, minerals and isoflavones that help lower the risks of prostate and breast cancer [25].

The preparation of the snack bars was also carefully executed to remove any anti-nutritional factors in the ingredients as well as to retain beneficial nutrients. For instance, pulses used in the formulation

were pre-soaked overnight to reduce the amount of phytate, an important anti-nutritional factor [26]. Pre-soaking also enhances the digestibility of nutrients [26] and separates physical contaminants such as minute particles of dirt, grits and other debris [27]. Moreover, beetroot that was incorporated in the cereal-legume-vegetable formulation was first thermally processed by pressure-cooking. In fact, pressure-cooking is known to enhance the retention of nutrients such as vitamin A and C [28].

Steamed snack bars were poorly rated by sensory panelists compared to their toasted counterparts (data not shown). Ravi et al. [29] also noted different odor profiles for steamed vs toasted chickpea dhal which in turn affected the sensory quality of the snack (boondi) produced from lentils cooked by steaming or toasting. Moreover, the presence of beetroot imparted an overpoweringly earthy aroma and a texture deemed too moist. Indeed, Bach et al. [30] also showed that boiled beetroot was strongly associated with earthy flavours. On the other hand, panelists preferred the bars formulated with maize powder and cooked by toasting due to the increased grittiness and crunchiness of the bar. In fact, toasting can enhance the taste, appearance, aroma and texture of food [31].

3.2. Formulation of new variants of bars cooked by toasting

Since bars cooked by toasting earned higher sensory scores, six new cereal-legume bars and six new cereal-legume-vegetable bars were formulated using different ingredients and cooked by toasting. Of the twelve snack bar variants, all panelists indicated a higher preference for formulation M1, incorporating maize powder, and topped with peanuts and dates. The snack bar, M1 got the highest scores as far as appearance, taste, texture and attitude of the consumer were concerned (data not shown). In fact, 78.3% of the panelists found the bar M1 to taste good or very good. Moreover, 91.3% of the panelists were satisfied or very satisfied with the product and expressed their willingness to purchase this product. The formulations incorporating beetroot or carrots did not earn a high score due to the reportedly “bland taste”. This is in contradiction with findings of Olsen et al. [32] who indicated that carrots tend to impart a mild and sweet taste which is generally liked by children. Moreover, beetroot has also been reported to be relatively high in sugar [33]. It is likely that the participants were not used to the taste of beetroot or carrot in a sweet snack. Indeed, in the Mauritian cuisine, root vegetables such as beetroot and carrot are customarily cooked and consumed as a savoury dish. The formulation with semolina was also less well received due to a perceived “raw-like” taste.

Snack bar formulation M1 was modified to a date- and peanut-free formulation mM1, to cater for people who are allergic to peanuts and dates. As a matter of fact, about three in every 500 people across the world are affected by the commonly occurring food allergen in peanuts, with children being the most prone to peanut allergies [34]. In addition, a study carried out by Kwaasi et al. [35] had shown that dates were also potent allergens. A dehydrated mix (DM) incorporating the same basic ingredients as in mM1, that can be readily reconstituted with water or milk, was additionally developed to cater for time-pressured individuals. In fact, 46% and 31% of the population aged between 18 to 34 years and 35 to 54 years of age respectively have the highest incidence of skipping breakfast in the morning due to lack of time or for being too busy [36].

3.3. Composition and physicochemical analyses

The centesimal composition of the snack bars (M1 and mM1) and dry mix are presented in Table 2. Mean moisture content of the two bars were comparable and ranged from 41.15–41.63%

while that of the dry mix was significantly lower, with a water content of 13.36%. The significantly higher water content of the bars reflects the volume of water that was exogenously added to the mixture during preparation of the bars compared to the dry mix where no water was added. However, moisture values recorded for snack bars M1 and mM1 were considerably higher than counterparts developed by other authors with moisture values ranging from 4.35–23.46% [18,37,38]. Similar to our work, Ryland [13] also developed a snack bar incorporating (micronized and flaked) lentils with a relatively high moisture content (32.5–32.8%) although it was still lower than our product. One advantage of a higher water content in our snack bars is that the product can offer greater satiety with lower energy density since water adds more weight and volume without carrying any calories. According to Rolls [39], satiation and satiety are tied to the volume of a food consumed. Using water to add volume appears to promote satiation [40,41]. It is worth noting that the moisture content of the dry mix was also higher than comparable "flour-type" products developed by other authors. For instance, Ezeokeke and Onuoha [42] found that the moisture content of maize, soybean and banana flours ranged from 7.43–10.57%. Similarly, the moisture content of pineapple peel flour developed by Damasceno et al. [18] was found to be ca. 6.78%. Higher moisture content of the snack bars brought about a correspondingly higher water activity (0.917–0.923). Indeed, the water activity for snack bars developed by other researchers was generally lower and ranged from 0.50–0.72 [13,43].

The % crude protein of M1 (9.8%) was higher than mM1 (8.7%) and DM (6.4%) due to the addition of peanuts which represents a rich source of protein [44]. Moreover, M1 has a higher protein content than that of a generic fruit and nut cereal bar that contains approximately 1.8 g of protein per serving, i.e., 6.4%. Similarly, the protein content of M1 was superior to that of bars developed in other studies. For instance, Rafiu et al. [25] and Torres et al. [28] both noted considerably lower protein content for their cereal bars with estimated values of 0.05% and 3.38–4.04% respectively.

The fat content determined for the products were 3.6, 1.9 and 2.7 g/kg Dry Mass for M1, mM1 and DM respectively. The fat content of M1 was higher than the other samples primarily due to the presence of peanuts which is quite high in beneficial fats (~49%) [45]. According to Appel et al. [46], peanuts comprise of >50% monounsaturated and 30% polyunsaturated fat. In a study carried out by Kris-Etherton et al. [47], the author demonstrated that total cholesterol and LDL of subjects was lowered by 11 and 14% respectively, while the HDL remained constant following a peanut-based diet high in monounsaturated fat. Besides, omega-6 fatty acids found in peanuts have been shown to protect against cardiovascular diseases [48]. Compared to a generic fruit and nut cereal bar containing 2.6 g of fat per 28 g serving [49], M1 contained 3.6 g per 30 g serving. However, the fat content was generally lower than other similar products developed by other researchers. In a study carried out by Padmashree et al., [50] the fat content of the formulated bars ranged from 7.32% to 10.72%. It is also worthwhile mentioning that the fat content of mM1 was slightly lower ($P > 0.05$) than DM where it should have been approximately the same, since the dehydrated mix consisted of similar ingredients as the mM1 formulation except for water. Nevertheless, the variation in the fat content can also be attributed to natural batch-to-batch variation of ingredients used for the development of the cereal bars [25].

Table 2. Composition and water activity of M1, mM1 and DM.

Parameters	Formulations		
	M1	mM1	DM
Moisture (%)	41.15 ± 1.15^{a}	41.63 ± 0.95^{a}	13.36 ± 0.71^{b}
Water activity	0.917 ± 0.051^{a}	0.923 ± 0.029^{a}	0.772 ± 0.022^{b}
Protein (%)	9.8 ± 0.2^{a}	8.7 ± 0.2^{b}	6.4 ± 0.2^{c}
Fat (%)	3.6 ± 0.4^{a}	1.9 ± 0.2^{b}	2.7 ± 0.3^{ab}

Note: Values represent the means of at least two replicates. Different superscript letters indicate values within the same row that were significantly different ($p < 0.05$).

3.3.1. pH

The pH of M1 and mM1 stored at room or chilled temperatures are presented in Table 3a. pH of snack bars M1 and mM1 decreased from 7.43 to 6.25, and 7.42 to 6.80 respectively during ambient storage although the difference was not statistically significant ($P > 0.05$). This is probably due to the production of lactic acid by hetero-fermentative spoilage LABs [25]. Similarly, the pH of DM stored at RT and 4 ℃ decreased from 7.47 to 6.25 and 7.47 to 6.33 respectively over the 4-week period (Table 3b). The drop in pH is highly conducive for yeast and mold growth in these products since these organisms are relatively more acid-tolerant [51].

Table 3a. pH of snack bars during ambient and refrigerated storage.

Storage duration	pH			
(days)	M1		mM1	
	RT	4 ℃	RT	4 ℃
1	7.43 ± 0.06^{Aa}	7.43 ± 0.06^{Aa}	7.42 ± 0.02^{Aa}	7.42 ± 0.02^{Aa}
2	7.28 ± 0.15^{Aa}	7.43 ± 0.14^{Aa}	7.38 ± 0.03^{Aa}	7.40 ± 0.06^{Aa}
3	7.01 ± 0.13^{Aa}	7.39 ± 0.05^{Aa}	7.33 ± 0.01^{Aa}	7.41 ± 0.05^{Aa}
4	6.47 ± 0.42^{Aa}	7.34 ± 0.03^{Aa}	6.94 ± 0.20^{Aa}	7.35 ± 0.05^{Aa}
5	6.25 ± 0.38^{Aa}	7.12 ± 0.10^{Aa}	6.80 ± 0.01^{Aa}	7.07 ± 0.31^{Aa}

Note: Values represent the means of three replicates. Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row for the same formulation that were significantly different ($P < 0.05$).

Table 3b. pH of Dry Mix during ambient and refrigerated storage.

Storage duration	pH	
(weeks)	RT	4 ℃
1	7.47 ± 0.08^{Aa}	7.47 ± 0.08^{Aa}
2	6.36 ± 0.06^{Aa}	6.58 ± 0.02^{Aa}
3	6.30 ± 0.01^{Aa}	6.34 ± 0.02^{Aa}
4	6.25 ± 0.02^{Aa}	6.33 ± 0.05^{Aa}

Note: Values represent the means of three replicates. Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row that were significantly different ($P < 0.05$).

3.3.2. Colour measurement

The instrumental colour (L*, a*, b*) values of snack bars (M1 and mM1) and DM are summarized in Tables 4–6. The lightness (L*) value of M1 and mM1 stored at RT decreased from 64.6 to 52.4, and from 74.9 to 54.8 respectively over the five days, hence indicating that the bars had darkened slightly (Table 4a). This is probably due to the presence of reducing sugars taking part in Maillard browning [52]. Maillard browning is a complex series of non-enzymatic browning reactions between amino acids and reducing sugars that often leads to an unpleasant change in the colour and nutritional value of food [53,54]. On the other hand, the L* values of DM did not vary considerably over the four-week storage period at either room or refrigerated temperature (Table 4b).

Table 4a. Changes in L* of M1 and mM1 during ambient and refrigerated storage.

Storage duration	L*			
(days)	M1		mM1	
	RT	4 ℃	RT	4 ℃
1	64.6 ± 0.02^{Aa}	66.9 ± 1.00^{Ab}	74.9 ± 0.50^{Aa}	70.2 ± 0.03^{Ab}
2	62.9 ± 8.81^{Aa}	61.0 ± 8.51^{Aa}	65.5 ± 5.80^{Ba}	65.1 ± 7.31^{Aa}
3	55.0 ± 0.05^{Aa}	55.0 ± 0.05^{Ba}	56.2 ± 1.20^{Ca}	57.6 ± 1.60^{ABa}
4	52.3 ± 0.19^{Ba}	53.2 ± 0.73^{Ba}	55.6 ± 2.23^{Ca}	56.2 ± 1.20^{ABa}
5	52.4 ± 1.09^{Ba}	51.5 ± 0.56^{Ba}	54.8 ± 1.26^{Ca}	54.4 ± 2.56^{Ba}

Note: Values represent the means of three replicates. Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row for the same formulation that were significantly different ($P < 0.05$).

Table 4b. Changes in L* of DM during ambient and refrigerated storage.

Storage duration	L* values	
(weeks)	RT	4 ℃
1	78.6 ± 0.49^{Aa}	79.0 ± 0.54^{Aa}
2	75.7 ± 0.50^{Ba}	78.6 ± 0.49^{Ab}
3	75.1 ± 0.01^{Ba}	77.0 ± 0.01^{Ba}
4	76.2 ± 1.93^{Aa}	76.5 ± 0.32^{Ba}

Note: Values represent the means of three replicates. Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row that were significantly different ($P < 0.05$).

As expected, the redness (a*) of snack bar M1 was slightly higher than that of mM1 due to the presence of dates (Table 5a) although the difference was not statistically significant ($P > 0.05$). Over the course of the storage period, there was no considerable change in the redness of the snack bars (Table 5a). However, in the case of the dry mix, the redness intensity had increased over the three weeks and then decreased sharply in the last week of chilled storage. It is not clear what biochemical processes could be responsible for the changes in redness undergone but it could likely be due to non-enzymatic oxidation.

Table 5a. Changes in a* values of snack bars during ambient and refrigerated storage.

Storage duration	a* values			
(days)	M1		mM1	
	RT	4 ℃	RT	4 ℃
1	3.4 ± 1.97^{Aa}	3.4 ± 1.97^{Aa}	2.9 ± 1.48^{Aa}	3.1 ± 1.75^{Aa}
2	4.5 ± 0.71^{Aa}	4.1 ± 0.13^{Aa}	3.1 ± 0.02^{Aa}	3.2 ± 0.24^{Aa}
3	4.1 ± 0.33^{Aa}	4.1 ± 0.38^{Aa}	2.5 ± 0.34^{Aa}	2.8 ± 0.64^{Ba}
4	5.0 ± 0.91^{Aa}	4.6 ± 1.15^{Aa}	2.7 ± 0.10^{Aa}	3.2 ± 1.39^{Aa}
5	5.5 ± 0.01^{Aa}	5.7 ± 0.01^{Aa}	4.0 ± 0.01^{Aa}	4.9 ± 0.02^{Aa}

Note: Values represent the means of three replicates. Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row for the same formulation that were significantly different ($P < 0.05$).

Table 5b. Changes in a* values of DM during ambient and refrigerated storage.

Storage duration	a* values	
(weeks)	RT	4 ℃
1	1.1 ± 0.01^{Ba}	1.1 ± 0.01^{Ba}
2	2.4 ± 0.13^{Ca}	2.1 ± 0.03^{Ca}
3	3.4 ± 0.22^{Da}	3.1 ± 0.03^{Da}
4	0.6 ± 0.03^{Aa}	0.3 ± 0.04^{Aa}

Note: Values represent the means of three replicates. Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row that were significantly different ($P < 0.05$).

As far as b* values of snack bars and DM are concerned, we did not observe any significant differences ($P > 0.05$) between product formulations (M1 vs mM1) or storage conditions (4 vs 23 ℃) since all samples contained maize powder (Table 6a and 6b). The predominant yellow colour of maize could have masked any subtle differences among the different treatments. It is worth mentioning that the yellow colour of maize is attributed to presence of pigments such as carotene and xanthophyll [55].

Table 6a. Changes in b* values of snack bars during ambient and refrigerated storage.

Storage duration	b* values			
(days)	M1		mM1	
	RT	4 ℃	RT	4 ℃
1	28.2 ± 0.09^{ABa}	28.2 ± 0.09^{ABa}	28.5 ± 0.43^{ABa}	28.5 ± 0.43^{Ba}
2	25.3 ± 0.04^{Ba}	26.0 ± 0.41^{Ba}	26.8 ± 1.08^{Ba}	28.6 ± 0.55^{Ba}
3	26.2 ± 1.18^{Ba}	26.1 ± 0.45^{Ba}	26.3 ± 1.23^{Ba}	26.9 ± 0.83^{Ba}
4	30.2 ± 3.04^{Aa}	29.8 ± 3.42^{Aa}	29.9 ± 1.03^{Aa}	31.1 ± 3.51^{Aa}
5	28.7 ± 0.01^{ABa}	30.5 ± 0.01^{Aa}	31.8 ± 0.52^{Aa}	34.1 ± 0.50^{Ab}

Note: Values represent the means of three replicates.

Table 6b. Changes in b* values of DM during ambient and refrigerated storage.

Storage duration (weeks)	b* values RT	4 ℃
1	26.3 ± 0.01^{Aa}	26.3 ± 0.01^{Ba}
2	26.6 ± 0.02^{Aa}	25.9 ± 0.01^{Ba}
3	24.9 ± 0.64^{Ba}	24.9 ± 0.52^{Ca}
4	27.1 ± 0.01^{Aa}	28.1 ± 0.75^{Aa}

Note: Values represent the means of three replicates. Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row that were significantly different ($P < 0.05$).

3.4. Microbiological shelf-life determination

3.4.1. Aerobic Plate Counts

The development of aerobes in M1 and mM1 bars stored at either room or refrigeration temperature are shown in Table 7a. Aerobic Plate Counts for M1 and mM1 significantly ($P < 0.05$) increased from <2 log cfu/g to a maximum population density of 9.4 log cfu/g and 9.1 cfu/g respectively after five days of storage at room temperature. Similarly, the population of aerobes for the dehydrated mix also significantly ($P < 0.05$) increased from <2 log cfu/g to a population density of 8.9 cfu/g after four weeks of storage at room temperature ($P < 0.05$) (Table 7b). However, the microbial development of the bars (M1 and mM1) and dehydrated mix (DM) by the end of the refrigerated storage (8.6 log cfu/g (M1), 8.4 log cfu/g (mM1) and 8.6 log cfu/g (DM)) period was considerably lower than their unrefrigerated counterparts (9.4 log cfu/g (M1), 9.1 log cfu/g (mM1) and 8.9 cfu/g (DM)), due to slower growth of microorganisms at reduced temperatures [56].

Table 7a. Population density of aerobes (log cfu/g) for M1 and mM1 bars during ambient and refrigerated storage.

Storage duration (days)	Aerobic Plate Counts			
	M1		mM1	
	RT	4 ℃	RT	4 ℃
1	<DL	<DL	<DL	<DL
2	8.4 ± 0.97^{Aa}	7.5 ± 0.02^{Aa}	8.1 ± 0.59^{Aa}	7.5 ± 0.22^{Aa}
3	8.5 ± 0.73^{Aa}	7.6 ± 0.18^{Aa}	8.1 ± 0.04^{Aa}	8.0 ± 0.50^{Aa}
4	8.3 ± 0.99^{Aa}	8.3 ± 0.09^{Aa}	9.0 ± 0.43^{Aa}	8.1 ± 0.51^{Aa}
5	9.4 ± 0.11^{Aa}	8.6 ± 0.16^{Aa}	9.1 ± 0.38^{Aa}	8.4 ± 0.12^{Aa}

Note: Values represent the means of three replicates. <DL = < limit of detection by the plating methodology (<2 log cfu/g). Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row that were significantly different ($P < 0.05$).

The microbial status of the snack bars and dry mix partly depends on the microbial quality of its individual ingredients including the cereals and legumes. The microflora of cereal ingredients such as corn and oats likely reflect that of the growing, storage and processing environments of these commodities [57]. In general, the background microbiota of cereals is quite varied and includes

bacteria of different groups including lactic acid bacteria, rope-forming bacteria (e.g., *Bacillus* spp.), coliforms and enterococci that can have psychrotrophic, mesophilic or thermoduric tendencies [57]. This may therefore explain the relatively high bacterial load in both the bars and dry mix stored at either ambient or refrigerated temperatures. Ryland [13] indicated that a pH below 4.6, temperature below 21 ℃ or above 38 ℃, and low water activity (a_w) will inhibit microbial growth. However, since the pH of the bars was close to neutrality and water activity exceeded the minimum threshold required for bacterial growth (>0.91), the product readily supported bacterial growth. The rapid increase in aerobic counts in the dry mix stored at either room or refrigerated temperatures could be attributed to the growth of microorganisms other than bacteria since the water activity of the dry mix was < 0.91 (0.772).

Table 7b. Population density of aerobes (log cfu/g) for DM during ambient and refrigerated storage.

Storage duration (weeks)	Aerobic Plate Counts	
	RT	4 ℃
1	<DL	<DL
2	8.1 ± 0.50^{A}	<DL
3	8.6 ± 0.15^{A}	<DL
4	8.9 ± 0.10^{Aa}	8.6 ± 0.10^{Aa}

Note: Values represent the means of three replicates. <DL = < limit of detection by the plating methodology (<2 log cfu/g). Different uppercase superscript letters indicate values within the same column that were significantly different (P < 0.05). Different lowercase superscript letters indicate values within the same row that were significantly different (P < 0.05).

3.4.2. Yeast and Mold Counts

Yeast and Mold Counts (YMC) for M1 and mM1 bars increased to a maximum population density of 5.4 log cfu/g and 5.5 cfu/g after five days of storage at room temperature (Table 8a). This is likely due to the presence of osmophilic yeasts such as *Saccharomyces rouxii* or osmotolerant yeast strains such as *Zygosaccharomyces bailii*, *Debaromyces hansenii* or *Torulaspora delbrueckii* typically present in ingredients or foods which have a high sugar content [58]. Indeed, the products contained honey (M1 and mM1) and dates (M1), both of which have a high sugar content. Tokuoka et al. [59] and Jermini et al. [60] reported the presence of these yeast species in various high-sugar food products. Moreover, we noted that although the population of YMC in bars stored at refrigerated temperatures was consistently lower than their counterparts stored at ambient temperature, the difference was not always statistically significant (P > 0.05). Indeed, spoilage yeasts are equally adapted to thrive at cold temperatures [57]. *Candida*, *Debaromyces*, *Saccharomyces* and *Torulopsis* are all psychrotolerant yeasts. With respect to DM, YMC increased to a maximum population density of 5.1 log cfu/g after four weeks of storage at Room Temperature (Table 8b) and this may be attributed to xerophilic molds, which are often isolated from low or intermediate-moisture foods. It is worth mentioning that the population of YMC also increased under refrigerated storage albeit slower (P < 0.05). According to Abdullah [61], a moisture content of <10.5% or water activity of <0.65 for corn flour, is needed to prevent growth of fungi. Since the moisture content (13.36%) and water activity (0.772) were higher than the threshold, fungal growth could be observed.

Table 8a. Population density of yeasts and molds in snack bars during ambient and refrigerated storage.

Storage duration (days)	Yeast and Mold Counts			
	M1		mM1	
	RT	4 ℃	RT	4 ℃
1	<DL	<DL	<DL	<DL
2	4.5 ± 0.48^{Aa}	3.8 ± 0.10^{Aa}	4.8 ± 0.01^{Aa}	4.5 ± 0.48^{Aa}
3	4.9 ± 0.60^{Aa}	3.8 ± 0.14^{Ab}	5.2 ± 0.22^{Aa}	4.9 ± 0.60^{Aa}
4	5.3 ± 0.16^{Aa}	4.6 ± 0.50^{Aa}	5.3 ± 0.12^{Aa}	5.3 ± 0.16^{Aa}
5	5.4 ± 0.08^{Aa}	4.9 ± 0.41^{Aa}	5.4 ± 0.08^{Aa}	5.4 ± 0.08^{Aa}

Note: Values represent the means of three replicates. <DL = < limit of detection by the plating methodology (<2 log cfu/g) Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row that were significantly different ($P < 0.05$).

Table 8b. Population density of yeasts and molds in DM during ambient and refrigerated storage.

Storage duration (weeks)	Yeast and Mold Counts	
	RT	4 ℃
1	<DL	<DL
2	4.8 ± 0.08^{A}	<DL
3	5.0 ± 0.09^{Aa}	4.2 ± 0.37^{Ab}
4	5.1 ± 0.10^{Aa}	4.3 ± 0.46^{Ab}

Note: Values represent the means of three replicates. <DL = < limit of detection by the plating methodology (<2 log cfu/g). Different uppercase superscript letters indicate values within the same column that were significantly different ($P < 0.05$). Different lowercase superscript letters indicate values within the same row that were significantly different ($P < 0.05$).

Taken together, since the onset of microbial spoilage of food products is usually marked by aerobic plate counts exceeding 7 log cfu/g [56], the bars were considered spoilt after five days of storage at room or refrigerated temperatures. The microbiological shelf-life of the snack bars (M1 and mM1) was conservatively estimated as <2 days when kept at RT or 4 ℃. On the other hand, the shelf life of DM was estimated to be <4 weeks when kept at 4 ℃ and <2 weeks when kept at RT. According to Perchonok [62], shelf life is the point at which the product no longer represents its initial definition. Specifically, the IFST Guidelines [63] indicate that shelf-life is defined as the time during which the food product will remain safe, be certain to retain desired sensory, chemical, physical and microbiological characteristics and comply with any label declaration of nutritional data. Hence in addition to microbiological assessment, sensorial evaluation should also be done to determine the sensorial shelf life.

3.5. Sensorial shelf-life determination

Sensory panelists found the snack bar M1 to be satisfactory or very satisfactory overall after up to 1 day at room temperature or 5 days at refrigeration temperature (Figure 2). For snack bar mM1, it was deemed satisfactory overall after up to 1 day at room temperature or up to 2 days at refrigeration temperature. With regard to visual appearance, 100% of panelists found M1 and mM1 to have a good

or very good visual appeal after up to 2 days or 1 day of refrigerated storage respectively (Figure 3). As far as the taste of snack bars was concerned, 90–100% of panelists found the taste of M1 and mM1 to be good or very good after up to 2 days and 1 day of refrigerated storage respectively (Figure 4). With regard to the texture of the bars, 90–100% of panelists found it to be acceptable even after 5 days of refrigerated storage (Figure 5). However, bars stored at ambient temperature earned low scores and were described as "hard", "tough" or "unpalatable" after more than one day of storage. Padmashree et al. [50] also observed a change in texture of formulated protein bars during storage. According to Simon et al. [64], the hard texture which developed in protein bars might be due to the migration of moisture and formation of ordered secondary structures as well as lower surface hydrophobicity of particles of polypeptides. Taken together, the sensorial shelf-life of M1 and mM1 snack bars was determined to be ≤5 days and ≤1 day when stored at 4 °C. Moreover, M1 bars were preferred to mM1 due to the presence of dates and peanuts which likely enhanced both the taste and texture of the bars. For the DM, they had an overall higher acceptability during storage at refrigerated than at room temperature with a sensorial shelf-life of up to 4 weeks when chilled (data not shown).

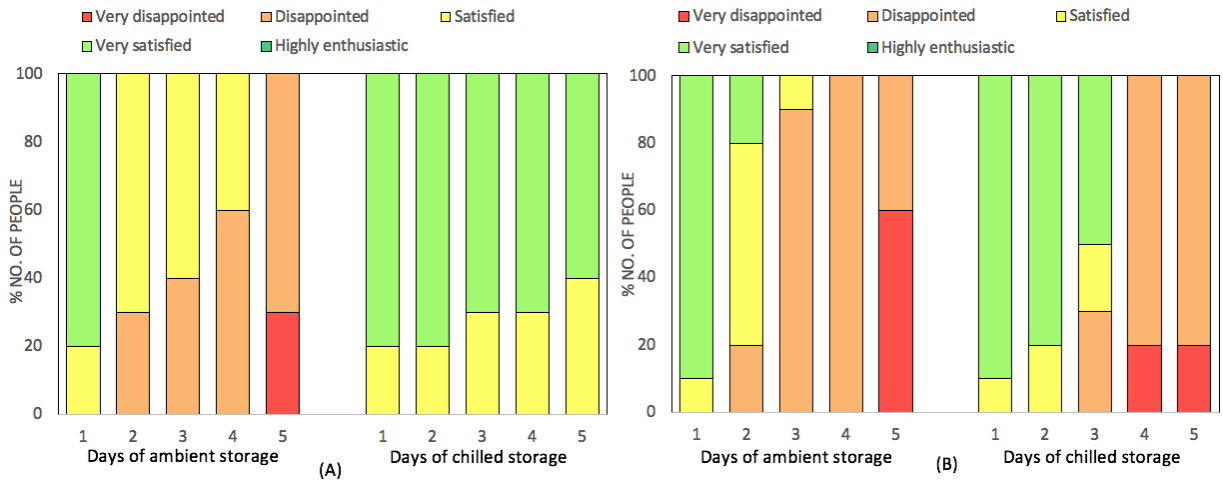

Figure 2. Overall satisfaction with snack bars M1 (A) and mM1 (B) stored at ambient or chilled temperature.

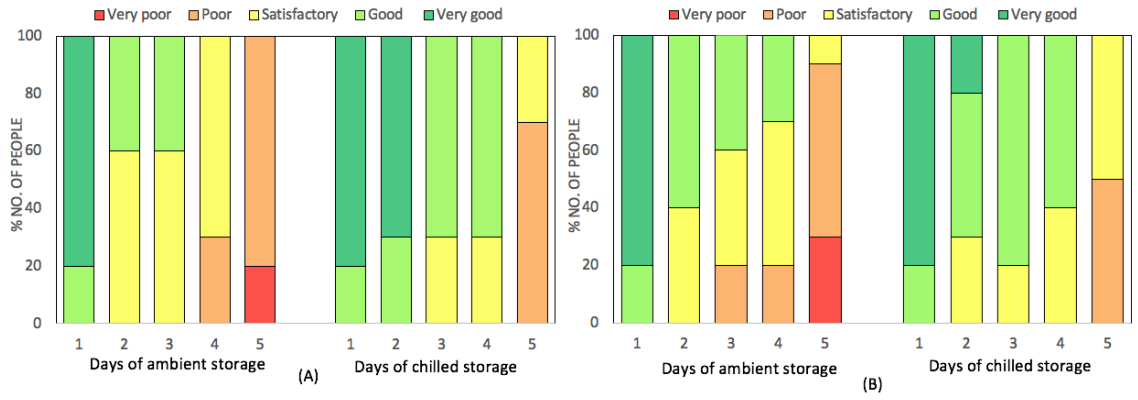

Figure 3. Visual appearance of snack bars M1 (A) and mM1 (B) stored at ambient or chilled temperature.

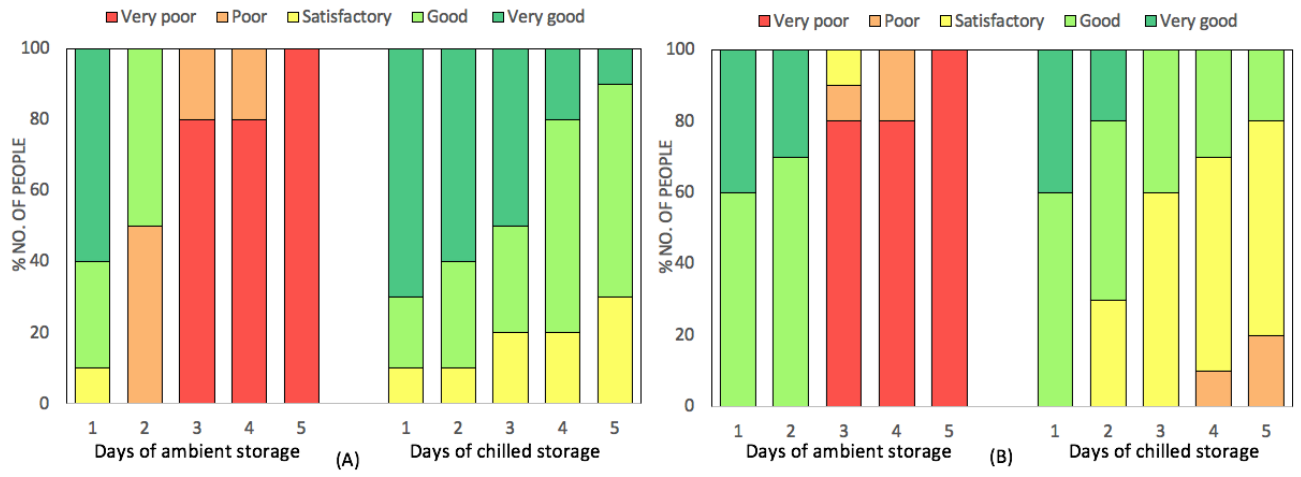

Figure 4. Taste of snack bars M1 (A) and mM1 (B) stored at ambient or chilled temperature.

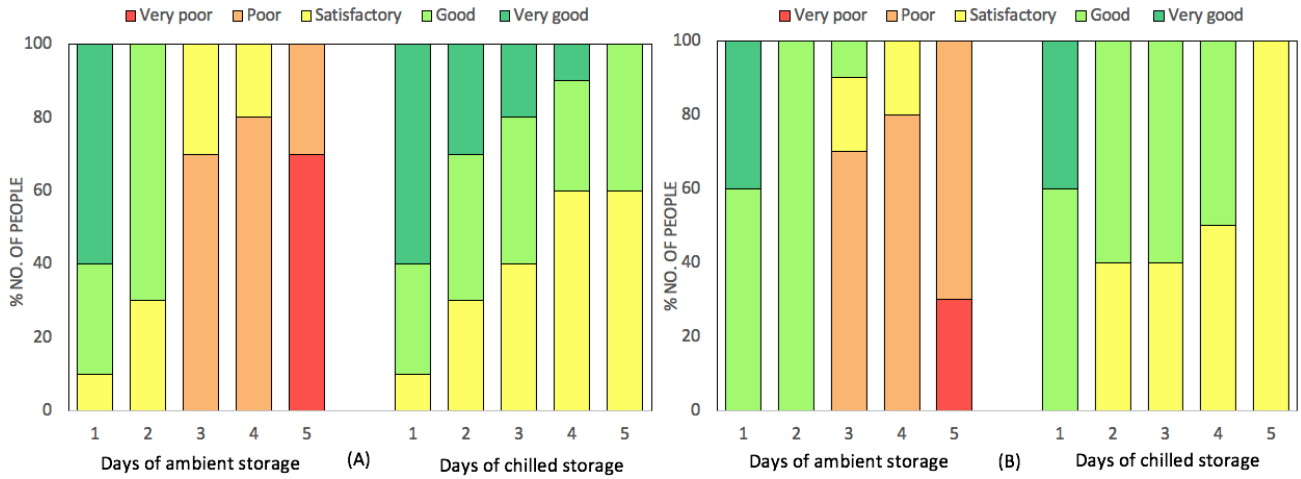

Figure 5. Texture of snack bars M1 (A) and mM1 (B) stored at ambient or chilled temperature.

4. Conclusions

This study highlights the design and development of a novel pulse-based cereal in the form of Ready-To-Eat bars and a ready-to-reconstitute dry mix. The cereal bars developed may constitute a snack that can be consumed "on-the-go" while the dehydrated mix can be readily reconstituted at home or even at work and customized with add-ons such as nuts, dried fruits and sweetened to taste. The products were formulated with locally available and healthful ingredients such as laird lentils, yellow split chickpeas, oats and maize powder which complement each other in terms of essential nutrients. Moreover, snack bars developed can be considered more nutritious than commercial counterparts due to their higher crude protein content and absence of artificial flavorings and chemical preservatives. However, the storage study indicated a relatively short shelf-life of <2 days when kept at 4 °C. These working prototypes have now successfully passed the proof-of-concept stage. Future research will involve optimizing product formulation and package design for improved shelf-life.

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Conflict of interest

The authors declare no conflicts of interest in this paper.

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