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Formulation and nutritional evaluation of instant vegetable cereal

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Abstract

Instant vegetable cereal was formulated from red amaranth, corn, barley, and pineapple using the drum drier technique. The mixture design using Minitab for optimization of the instant cereal was also studied in terms of antioxidant, mineral, sensory evaluation, nutritional content and microbiology. The most nutritious preferred instant vegetable cereal was determined using the method of radical scavenging activity 2,2-diphenyl-1-picrylhydrazyl (DPPH), zinc, and calcium content. In general, the optimized instant cereals using Minitab software were acceptable to the consumer with nutritional values as high as those of comparable products on the market. The best formulation (Formulation 1) contained a mixture of raw ingredients of 3.10 mg/100 g of zinc, 89 mg/100 g of calcium and 80% of radical scavenging activity (DPPH). The proximate composition showed that formulation 1 contained 77.85 g/100 g of carbohydrate, 7.64 g/100 g of protein, 371 kcal/100 g of energy, 0.36 g/100 g of fat, 12.91 g/100 g of total dietary fibre (TDF), 2.06 g/100 g of ash, and 3.70 g/100 g of moisture. Microbiological analyses showed that total aerobic plate counts, coliform count and yeast and mould count were 2.5×10^3 CFU/g, < 1.0×10 CFU/g, 1.0×10 CFU/g, respectively. Overall, the best instant vegetable cereal contained vitamin C (110 mg/100 g), vitamin A (4420 µg/100 g), selenium (8.15 µg/100 g), potassium (0.59 g/100 g), calcium (138 mg/100 g) and manganese (2.26 mg/100 g). In conclusion, it can be suggested that the consumption of instant vegetable cereals for daily intake is suitable for maintaining health status.

1. Introduction

Instant cereals are made from grains and it is defined as ready-to-eat food. It was processed into different forms by soaking, swelling, roasting, grinding, rolling or flaking, shredding, or puffing of any cereal. Instant cereal is normally produced from cereals which are the dry seeds of those members of the grass family grown for their grains such as corn, rice, wheat, oats and others. To develop the healthiest instant cereals, the formulation of ingredients is an important factor to be considered during food formulation. The mixture of vegetables in the formulation of instant cereals can increase the nutritional value of cereal products. Besides the selection of ingredients in the formulation, the processing technology of instant cereals is very important in terms of maintaining nutritional values. In this century, drum drying techniques are used to produce dried ingredients or ready-to-eat food that retains its flavour, colour and nutritional value.

Nowadays, most food trends are focusing on functional food, antioxidants, improved health and help in body slimming will be favoured by consumers. Health issues are a major problem all over the world especially among the elderly, as the growing population is hoping for longevity. Vegetables are known to have nutritious elements for human consumption, but to make sure the intake of vegetables is a daily routine it becomes difficult for everybody especially to maintain discipline and long-term consumption for any cholesterol-lowering regime. Other than that, food with high content of dietary fibre has been the focus of many researchers (Alfredo *et al.*, 2009), for example, cereals, tubers, fruits, and algae. However, the formulation of instant cereal with vegetables had been lacking in studies and also less available in the market. The formulation of instant cereal using red amaranth, corn, and barley should maximize the nutritional value. According to Pauziah *et al.* (2017), food-based strategies are needed to combat micronutrient deficiencies and integrate them with other community-

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based nutrition programs to ensure nutritional security. Chronic diseases such as cardiovascular diseases, type 2 diabetes, and cancer could be due to insufficient nutrition and can generally affect the middle-aged and elderly segments of the population (Tontisirin and Kraisid, 2000). Globally, the number of persons aged 60 years or over is expected nearly to triple, increasing from 673 million in 2005 to 2 billion by 2050 (UN, 2007).

The utilization of perishable vegetables is more important because some edible parts are not being consumed or wasted due to a lack of knowledge or food habits. The target should maximize its use in food consumption and minimize its waste. The processing of red amaranth into food formulation and practical approach are to utilize as much edible food material as possible after it has been produced, whether plant, animal or fish. Rather than throw away the stem of amaranth, it should be utilized in food formulation as daily domestic cooked food. In Malaysia, green and red spinach (*Amaranthus tricolor*) are popular for making soup, fried vegetables and noodles. Most of the students consumed only one serving of fruit and vegetable per day, which is below the suggested levels by the Malaysian Dietary Guideline (Sirfan *et al.,* 2020). Thus, to get a beneficial impact on the body, there is a need to develop strategies to optimize the intake of fruit and vegetable and the absorption of nutrients. Reduction in the risk of getting cancers can be linked to the essential nutrient such as vitamins, minerals, proteins, fibres and bio-functional components in fruit and vegetables (Hall *et al.,* 2009) as well as cardiovascular disease, diabetes, stroke, obesity, diverticulosis and cataract (Bogers *et al.,* 2004).

Cereals such as corn and barley knew for their high content of dietary fibre whereas whole corn is loaded with fibre, vitamin C, vitamin B, magnesium and potassium. However, not many studies on the product formulation using red amaranth, pineapples and corn, especially in the production of instant cereal. Corn has been utilized not just for humans but also for animal consumption. For example, grain corn has become one of the new sources of national wealth in Malaysia since 2017 and was given higher priority as animal feed formulation (Hifzan Rosali *et al*., 2019). However sweet corn is mostly cultivated in all areas in Malaysia either for human consumption or fresh use. Overall corn contains vitamin C, vitamin E, vitamin K, vitamin B1 (thiamine), vitamin B2 (niacin), vitamin B3 (riboflavin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), folic acid, selenium, N-p-coumaryl tryptamine and N-ferrulyl tryptamine. Corn is an essential source of various major phytochemicals such as carotenoids, phenolic compounds and phytosterols (Jiang and Wang, 2005; Kopsell *et al.,* 2009; Lopez-Martinez *et al.,* 2009).

The barley and cereal crops are well known for fibre and some phytochemicals such as β–glucan. In general, two forms of barley crop are available in the market, one is hulled and the other is hull-less. Barley is rich in dietary fibre and also a good source of soluble fibre. According to Borderias *et al. (*2005) and Veronese *et al.* (2018) soluble dietary fibre is more functional than insoluble dietary fibre because it is an effective remedy for reducing cholesterol, triglyceride and glucose levels in the blood. Commonly, other cereals have relatively high insoluble dietary fibre and based on literature reported that barley contains 4.73–5.70 g/100 g dm of soluble fibre (Bader Ul Ain *et al.,* 2019). The soluble fibre in other grains ranged 1.4 – 2.3 g/100 g in wheat (De Santis *et al*., 2018), 3.8 g/100 g in oats (Dhingra *et al*., 2012), 3.4-6.6 g/ 100 g in the rye (Hansen *et al*., 2003) and 1.5-3.6 g/100 g in corn (Vitaglione *et al.,* 2008). Many reports showed that soluble dietary fibre is found to have functional properties such as reducing the high cholesterol, triglyceride and glucose levels in the blood (Anderson, 1986). Production of instant cereals with high nutrition values from vegetables, fruits and cereals with improved processing techniques has provided the new potential of functional food.

The incorporation of pineapples in instant cereal gave value-added properties to the products based on the physicochemical composition and nutritional values. Barretto *et al.* (2013) reported that pineapple contains antioxidants, organic acids, bromelain and phenolic compounds. Besides that, Chiet *et al.* (2014) have reported the presence of bioactive compounds and antioxidant capacity for three different varieties of pineapples (Josapine, Morris, and Sarawak) harvested at the commercial maturity stage. Pineapple also contains calcium, potassium carbohydrates, water content, vitamin C and crude fibre (Chaudhary *et al.,*2019). Bromelain in pineapple was reported to reduce swelling in inflammatory cases including sore throat, gout and arthritis (Khalid *et al.,* 2016). In Malaysia, under the 11th Malaysia Plan, the Malaysian Pineapple Industry Board (MPIB) reported pineapple cultivation had more than 11,000 ha of land in 2020. According to MPIB, the market segment for pineapple products was canning, juice and fresh consumption. Pineapples contain nutritional and beneficial values for consumption; thus, it is a golden opportunity for farmers to gain access to domestic and international markets (Malaysian Pineapple Industry Board, 2015).

In product processing, the nutrient value of food is almost worsened or altered during heat treatment processing and is inevitable. It is a challenge for the food

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industry to formulate food products that contain high nutrition content. Several studies have reported the drum drying process could improve protein digestibility and make more amino acids available (Arrage *et al.,* 1992). Besides that, the thermal process of drum drying also stabilizes carotenoids in pure β-carotene (Desobry *et al.,* 1997), improving the quality of the food in terms of digestibility, functionality and nutrient availability (Adepeju *et al*., 2014; Olapade *et al.*, 2015).

All criteria should be reviewed in order to optimize the nutritional and product quality of instant cereal. Selection of raw materials, processing technology and nutritional availability are only a few examples. In the search for the best formulation, the main objective is to determine the optimum levels of the components or key ingredients. The mixture design of the experiment with Minitab represents one of the optimization techniques currently in use in food science and technology. Minitab's mixture procedures may be used with the smallest amount of experimental resources while maintaining efficiency and simplicity.

Thus, the aims of this study were to produce instant vegetable cereal with optimized antioxidant and nutritional content. The optimized instant vegetables cereal was also studies in terms of microbiology, mineral and sensory evaluation. The mixture design using Minitab for optimization of the instant cereal was also investigated and discussed as well.

2. Materials and methods

2.1 Raw materials and sample preparation

Red amaranth and pineapple were bought from Pasar Borong Selangor. Corn (Variety hybrid P4546) was supplied by MARDI Seberang Perai, Pulau Pinang. Natural pearled Australian barley bought at the hypermarket. The vegetables were sorted for any defects like bruising, discolouration of leaves, or mould. Morris pineapple (*Ananas comosus* L.) pulps were used to produce the pineapple puree. The red amaranth, corn, barley and pineapple pulp were washed in filtered water. Then weighed according to the mixture experimental design as referred to in Table 1 (Minitab), and then ground using a waring blender. After that, the mixture samples were dried into a coarse flake using a double drum dryer (R. Simon Ltd. Nip Feed Test Machine, Model 4766, Nottingham, UK) (Zuwariah *et al*., 2019).

Table 1. Parameters studied in physical optimization of instant cereals

Parameter	Low level	High level
Corn	0.3	0.4
Red amaranth	0.3	0.4
Barley	0.3	0.4

Then, the dried flakes were stored in the aluminium pack at room temperature until further analysis.

2.2 Design of experiment

Mixture designs were used to optimize instant cereal (Minitab Inc., Pennsylvania, USA). The sum of all the elements in a mixture is 100%. Red amaranth, corn and barley were chosen as the raw components. Three of them were used as variables as they may affect the responses in antioxidant, 2,2-diphenyl-1- picrylhydrazyl (DPPH). The remaining ingredient, which is pineapple was left constant.

$$0 \leq xi \leq 1, 1 + x2 + x3 = 1$$

where i = 1, 2, 3

2.3 2,2-diphenyl-1- picrylhydrazyl (DPPH) assay

The antioxidant capacity of each sample was assessed using the method proposed by Alothman *et al.* (2009). The free radical scavenging effect was determined based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The DPPH assay was used to measure the free radical scavenging effect. About 1.0 mL of sample extracts were added with 2.0 mL of freshly prepared methanolic DPPH solution (20 ppm). The mixture was then thoroughly vortex-mixed and left to stand for 30 min in the dark. The absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Hitachi U-2800 Japan) against methanol as a blank for auto-zero, and the percentage of inhibition of the DPPH radical was expressed as the antioxidant activity and calculated as follows:

$$\%\ \text{Scavenging activity} = \frac{\text{Abs control} - (\text{Abs sample} - \text{Abs blank})}{\text{Abs control}} \times 100$$

Where Abs blank is the absorbance of sample extracts without DPPH solution, whereas Abs control is the absorbance of DPPH solution without sample extracts.

2.4 Mineral

The zinc, calcium, selenium, and potassium were determined according to the in-house method based on the Association of Official Analytical Chemists (2012) (Chapter 9, method 9.1.09 and Chapter 50, method 50.1.14). The procedure entails decomposing organic stuff using heat and intense hydrochloric acid, then dissolving the inorganic residue in an adequate proportion of diluted nitric acid. The organic matter is first destroyed by dry ashing at 600°C for 2 hrs until whitish or greyish ash is obtained. The ash is dissolved in hydrochloric acid and filtered before being diluted to volume with nitric acid and read directly on an Inductively Coupled Plasma-Optical Emission Spectrometry system (ICP-OES). The elemental

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composition was determined using an ICP-OES model Optima 5300DV (PerkinElmer Inc., USA) with axial viewing. The following conditions were used to set up the ICP-OES:

RF Power: 1300 Watt

Plasma gas rate (Ar): 15 mL/min

Auxiliary gas rate: 0.2 mL/min

Nebulizer gas rate: 0.6 mL/min

Pump flow rate: 1.5 mL/ min

2.5 Proximate composition

The moisture, crude protein, crude fat and ash content of instant cereals were measured in triplicates using the Association of Official Analytical Chemists' (AOAC) authorized procedures 925.10, 923.03, 920.85, and 920.87 (AOAC, 2000). Carbohydrate was estimated by different. Total dietary fibres were determined using the AOAC Official Method of Analysis 985.29 (45.4.07) (AOAC, 2012).

2.6 Vitamin C and vitamin A (β-*carotene*) *analyses*

Methods of vitamin A (β-carotene) and vitamin C were based on Ismail and Fun (2003). The samples were analyzed using High-Performance Liquid Chromatography (HPLC) by Waters Alliance HPLC System. The waters symmetry C18 column (4.6 × 250 mm × 5 µm) was used. The mobile phase consists of 0.1 M potassium acetate, pH 4.9(2) Acetonitrile-water (50:50) for vitamin C while for vitamin A, acetonitrile-methanol- ethyl acetate (88:10:2) was used.

2.7 Microbiology analyses

Microbiological analyses were carried out on raw materials and final products as quality and safety control. The analyses done were total plate count (TPC), yeast and mould (Y&M) and total coliform (TC) according to the Association of Official Analytical Chemists (AOAC) method; using the pouring plate technique carried out in the biohazard cabinet. For TPC analysis, the media used is Total Plate Agar (Oxoid, United Kingdom), Potato Dextrose Agar (Oxoid, United Kingdom) added with 10% tartaric acid for yeast and mould, while for TC , Violet Red Bile Agar (VRBA) was used. A total of 10 g of sample was weighed and diluted with 90 mL of Ringer's (Sigma Aldrich, USA) solution. A 10^1 to 10^5 dilution was carried out. A total of 1 ml of each diluent was transferred into a petri dish before the media were poured in (agar temperature set at 50°C) and the agar allowed to solidify at room temperature. All petri dishes for TPC and TC were incubated at the temperature of 37°C for 24 to 48 hrs while for Y&M at the temperature of 32°C for approximately 72 hrs. Observation of the microbial colonies growth was done using a colony counting tool.

2.8 Sensory evaluation

An acceptance test was carried out on the sensory evaluation of instant vegetable cereals in the matter of colour, aroma, after taste, taste and overall acceptance. Sixty untrained panelists were invited to participate in this evaluation. All of them were MARDI staff and practical students from various universities and colleges with ages ranging from 21 to 58. Panelists must possess good health and non-smokers. The evaluation was conducted in Food Sensory Laboratory at Food Science and Technology Research Center in MARDI under ambient temperature and fluorescent light. Tissue and plain water were given to all the panelists on a tray. Then, each of the samples was served to them in plastic cups with 3-digit random numbers labelled to them. Panelists were required to rinse their mouths after each sample evaluation before went on for the next sample. Panelists then would have to answer a sensory evaluation form which had a 7-point hedonic scale for each sample for the attribute mentioned. The evaluation was based on their degree of like (scale of 1-7) where 1 = dislike extremely and 7 = like extremely. Samples with the mean scores of more than 5.00 for overall acceptability were considered acceptable.

2.5 Statistical and data analysis

Minitab software was used to create and analyze the experiments (version 16). The full cubic models were fitted to the experimental data in this study:

where Y is the predictive dependent variable (zinc, calcium and DPPH); β1, β2, β3, β12, β13, β23, β123, δ12, δ13 and δ23 are the equation coefficients. The

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \delta_{12}X_1X_2(X_1 - X_2) + \delta_{13}X_1X_3(X_1 - X_3) + \quad (1)$$

statistical analysis package STATISTICA (Release 5.0 stat soft Inc. Talsa, OK) was used to test the ten instant cereal formulations using the one-way ANOVA and Duncan's test at the level of $P<0.05$.

3. Results and discussion

Three criteria were defined in the optimization technique: maximize the amount of zinc, calcium and DPPH. Red amaranth, corn and barley must be consumed in large amounts to achieve the maximum antioxidant levels. Red amaranth, corn, and barley were chosen to be the variables toward the responses: antioxidant capacity (DPPH), zinc, and calcium (Tables 1 and 2). The effect of variables on responses was

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studied using statistical analysis using Minitab 16 software, as shown in Table 2. Table 3 shows the effect of corn, red amaranth, and barley on the minerals and antioxidants of instant cereals. The best model has a low standard deviation as well as a high R-squared value (Table 3). It was showed the predicted responses gave the highest composite desirability, which are 0.9533 for zinc, 0.9874 for calcium, and 1.000 for DPPH. The predicted responses and the composite desirability near one indicate that the optimum zinc, calcium, and DPPH were met. Zero composite desirability denotes that one or both responses are outside their acceptable limits. The regression coefficients for all the terms in the models are shown in Table 3. The coefficients of the equation which correspond to zinc, calcium and DPPH, are shown together with their statistical significance and full cubic model. Corn, barley and red amaranth combinations have synergistic effects because the interaction of all substances boosts the DPPH and mineral content. It was discovered that all raw materials, including corn, barley, and red amaranth, had a significant effect on calcium content ($P<0.01$).

Antioxidant-rich foods can help cells stabilize and protect themselves from oxidative stress and vitamin C, in particular, can help prevent or delay cancer and promote healthy ageing. Thus, the DPPH radical scavenging analysis was employed to evaluate the free radical scavenging activity of hydrogen donating antioxidants in many plant extracts or foods. Radical scavenging is the main mechanism by which antioxidants act in foods. Table 4 shows that the DPPH radical scavenging amount increased significantly ($P < 0.05$) in Run 1 when the addition of 0.35 corn, 0.35 red amaranth and 0.3 barley compared with other samples. However, the lowest DPPH was found in run 8, when using corn, red amaranth, and barley combinations in the proportion of 0.3, 0.3, and 0.4 respectively. Apart from that, all other samples contained a significant amount of DPPH radical scavenging in a range of 68.34 - 77.56% of samples. On the other hand, the highest zinc content was also found in run 1, with 30.7 mg/kg even though the remaining run set has a considerable amount of zinc in a range of 25.75 - 29.15 mg/kg samples. Run 6 had the highest calcium level, at 88.95 mg/100 g, but all other samples had a considerable quantity of zinc, ranging from 62.15 to 88.95 mg/100 g. According to a previously studied by Zuwariah *et al.* (2021), they found that DPPH radical scavenging in barley was the lowest if compared with germinated corn, purple sweet potato, green spinach, and red amaranth.

Table 2. The composition of instant cereals (1.0)

Run Order	Corn	Red amaranth	Barley
1	0.35	0.35	0.3
2	0.316667	0.316667	0.366667
3	0.35	0.3	0.35
4	0.366667	0.316667	0.316667
5	0.4	0.3	0.3
6	0.3	0.35	0.35
7	0.3	0.4	0.3
8	0.3	0.3	0.4
9	0.316667	0.366667	0.316667
10	0.333333	0.333333	0.333333

The lowest values of DPPH content were located in the corn-barley edge and the centre (Figure 1a.). However, the highest values were given in the red amaranth and corn edge and also in the interaction of barley, corn, and red amaranth edge. This could be related to a synergy between these three-component. In a recent study, Zuwariah *et al.* (2021) discovered that

Table 3. Effect of corn, red amaranth and barley on minerals and antioxidants of instant cereals.

Coefficient of equation 1	Zinc, mg/kg	Calcium, mg/100 g	DPPH, % scavenging
R2	0.83	0.99	0.87
β_1 (Corn)	-1614	-55629	9240
β_2 (Red Amaranth)	3999	-57483	12629
β_3 (Barley)	-1008	-10884	-48910
β_{12}	-3673^{NS}	240789^{*}	-13241^{NS}
β_{13}	2647^{NS}	169989^{*}	72371^{NS}
β_{23}	-5713^{NS}	177629^{*}	69529^{NS}
β_{123}	8576^{NS}	-647576^{*}	-140760^{NS}
δ_{12}	17200^{NS}	-68600^{*}	106760^{NS}
δ_{13}	-8600^{NS}	142000^{*}	-191800^{NS}
Predicted responses	30.65	88.56	79.74
Desirability	0.9533	0.9874	1.000
Composite Desirability		0.9800	
Global solution		Corn = 0.351515 Red amaranth = 0.348485 Barley = 0.3	

*Significant at $p<0.01$, NS: Non significant coefficient

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Table 4. Response values for zinc, calcium and DPPH of instant cereal formulations

Run Order	Parameter			Response		
	Corn	Red amaranth	Barley	Zinc, mg/kg	Calcium, mg/100 g	DPPH (% scavenging)
1	0.35	0.35	0.3	30.7±0.28^{a}	88.95±0.07^{b}	79.63±1.18^{a}
2	0.317	0.317	0.367	25.9±0.14^{e}	63.15±0.21^{h}	75.58±1.79^{c}
3	0.35	0.3	0.35	26.8±0.28^{d}	74.1±0.14^{f}	68.34±1.33^{f}
4	0.367	0.317	0.317	27.9±0.14^{c}	76.8±0.28^{e}	65.34±0.75^{g}
5	0.4	0.3	0.3	26.8±0.28^{d}	86.15±0.21^{c}	77.56±0.90^{b}
6	0.3	0.35	0.35	25.75±0.35^{e}	90.2±0.28^{a}	74.02±0.80^{cd}
7	0.3	0.4	0.3	27.75±0.35^{c}	72.35±0.21^{g}	70.55±0.27^{e}
8	0.3	0.3	0.4	26.15±0.21^{e}	80.95±0.07^{d}	67.75±1.60^{f}
9	0.317	0.367	0.317	26.15±0.21^{e}	77.1±0.14^{e}	63.69±0.96^{g}
10	0.333	0.333	0.333	29.15±0.21^{b}	62.15±0.21^{i}	72.12±1.13^{de}

Values are presented as mean±standard deviation, n = 3. Values with different superscripts within the same column are significantly different ($p<0.05$)

DPPH radical scavenging in barley was the lowest if compared with germinated corn, purple sweet potato, green spinach, and red amaranth. The effect of the operating conditions in a double-drum dryer on the content of bioactive compounds and antioxidant activity was studied by many researchers. Córdova *et al*. (2020), found that antioxidant activity and vitamin C in dried broccoli showed high dependence on the drying condition of the drum dryer. They also reported that antioxidant activity varied unevenly with the different drying conditions, probably because antioxidant activity reflects the contribution of several compounds that exhibit different antioxidant mechanisms. Aside from that, Chia and Chong (2015) reported that the radical scavenging activity of drum-dried peel powder was significantly lower than fresh peel. This could be related to heat processes that cause the degradation of phytochemical substances. As a result, the breakdown of phytochemicals, component migration, and various chemical reactions (Davey *et al*., 2000).

When examining contour plots for calcium (Figure 1b), we concluded the same tendency as DPPH with the lowest values in the centre. However, the highest values were given in the corn vertexes and the red amaranth and barley edge. Aside from that, the contour plots in Figure 1c indicated an increasing tendency for zinc values in the corn edge, while the lowest zinc values were seen as the percentage of barley increased. According to Sandström (1989), processed cereals have a moderate zinc content, typically between 10 to 25 mg/kg. The mineral content of basil had lost after drying, according to Özcan e*t al*. (2005). The decrease in mineral content such as calcium and zinc and the quality of the dried product could be due to the high processing temperature used.

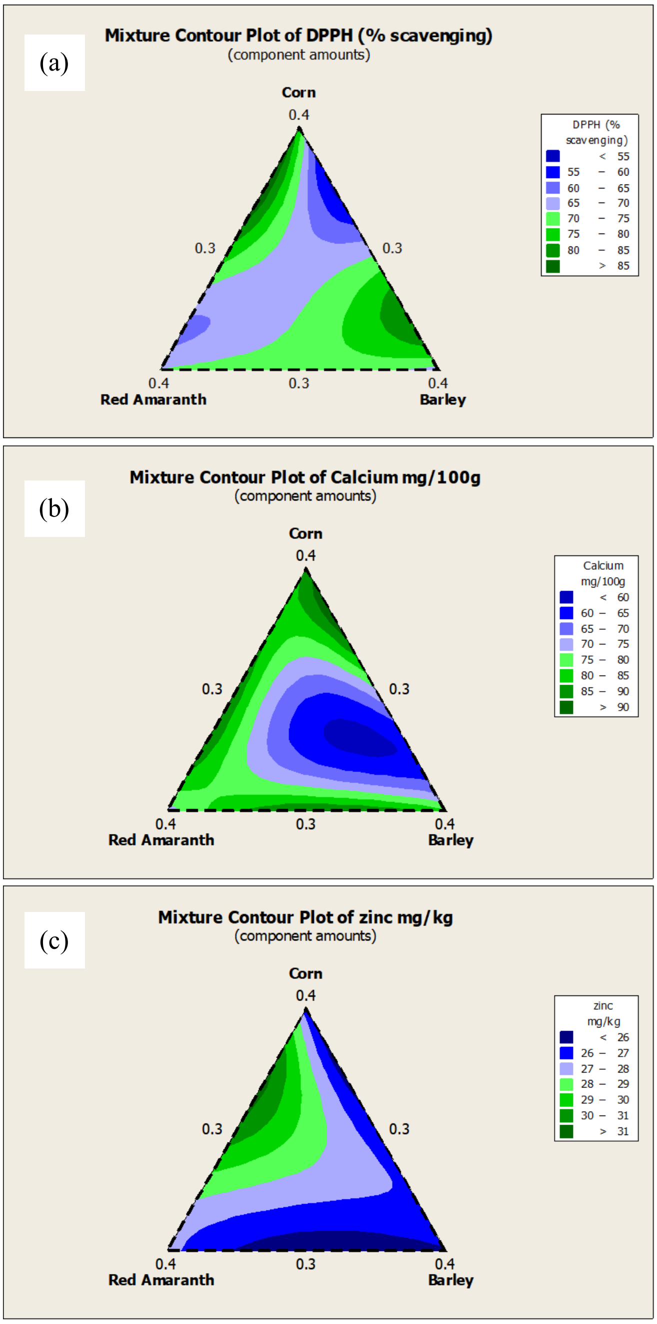

Figure 1. Contour plots for predicted instant cereals: DPPH content (a), calcium content (b) and zinc content (c) of instant cereals formulated with corn, red amaranth and barley.

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3.2 Nutritional analysis and microbiology analysis of optimized instant cereals.

The nutritional parameters of optimized instant cereals showed in Table 5. Formulation 1 contained 77.85 g/100 g of carbohydrate, 7.64 g/100 g of protein, 371 kcal/100 g of energy, 0.36 g/100 g of fat, 2.06 g/100 g of ash, and 3.70 g/100 g of moisture. Instant cereal also contained high dietary fibre (12.91 g/100 g), soluble fibre (0.65 g/100 g) equal to barley, 1.8 times more vitamin C (99.2 mg/100 g) than orange and 14 times more vitamin A (11.89 mg/100 g) if compared with carrots. Besides that, it also contained zinc (3.1 mg/100 g), selenium (8.15 µg/100 g), calcium (76.54 mg/100 g), potassium (0.59 g/100 g), other minerals and also vitamin B. It could provide enough Recommended Nutrient Intake (RNI) for the elderly to maintain a healthy life (RNI, 2017). Dietary nutrients are responsible for the synthesis of antioxidant enzymes. Margaritelis *et al*. (2018) reported that poor diet habits such as vitamin C intake could reasonably explain the deficiencies observed in the "low" antioxidant groups. Thus, subsequently reversed by the targeted antioxidant supplementation. On the other hand, vitamin B is also reported as an active coenzyme mainly in the metabolism of amino acids, nucleic acids, glycogen, porphyrin and lipids. In addition, vitamin B6 may indirectly play an antioxidant role by serving as a coenzyme in the glutathione antioxidant defence system (Wu *et al.,* 2004). Aside from that, zinc was identified as a co-factor for essential enzymes that help the antioxidant defence system function properly. Zinc was discovered to play a role in the control of glutathione peroxidase and as a co-factor for superoxide dismutase (Marreiro *et al.,* 2017). The effect of instant cereal on blood enzymatic antioxidants in old rats was studied by Syahida *et al*. (2020). Serum antioxidant content in rats treated with instant vegetable cereal was higher than the control group in glutathione reductase (GR) (24.712.39 nmol/min/mL) and glutathione peroxidase (GPx) (322.19 nmol/min/mL), while superoxide dismutase (SOD) levels were comparable. GR, GPx, and SOD are very important antioxidant defenses against oxidative stress in the body. It is also shown to be a good therapeutic agent against reactive oxygen species, thus can prevent many diseases such as cancer, anti-inflammatory, and oxidative stress-related diseases (Inal *et al.,* 2001; Yasui and Baba, 2006). It is possible to conclude that the formulation of instant cereals is scientifically proven as a nutritious food that is rich in antioxidants and improved blood antioxidant levels in aged rats.

Microbiological analyses showed that instant cereals are within acceptable limits until the end of shelf life study. Microbiological quality of food studies is varied according to the type of food and the processing it has undergone. The microbial growth count can be a valuable means of observing trends by comparing the results obtained through out the study. In this study, microbial growth had shown no significant increase along the shelf life storage. TPC for optimized instant cereal (Formulation 1) at the end of the study was observed at 2.5×10^3 CFU/g, indicating that the product is still within the acceptable limits for Powdered Dietary Food for Special Medical Purposes according to Australian Standards. According to the Food Standards Australia New Zealand for Microbiological Reference Criteria for Food (2001); which the maximum count for the total viable count is 1.0×10^4 CFU/g. Yeast and mould count for the instant cereals is 2.0×10 CFU/g and is also within the acceptable limits as referred to by the same Australian Standards.

Table 6 shows that the TPC count is $<1.0\times10$ CFU/g, which indicates no growth detected. According to Australian Standards, the presence of coliform microorganisms in ready-to-eat food over 1.0×10 CFU/g

Table 5. Nutritional analysis of optimized instant cereals.

Parameter	Value	Vitamin mg/100 g	
Energy, kcal/100 g	371±1.15	Vitamin A	11.89±0.36
Fat, g/100 g	0.36±0.08	Vitamin C	99.225±6.72
Protein, g/100 g	7.64±0.02	Vitamin B1	1.434±0.29
Moisture, g/100 g	3.70±0.32	Vitamin B2	0.138±0.01
Ash, g/100 g	2.06±0.04	Vitamin B3	2.708±0.12
Carbohydrate, g/100 g	77.85±0.29	Vitamin B5	1.187±0.14
Total dietary fibre, g/100 g	12.91±0.37	Vitamin B6	1.23±0.38
Insoluble dietary fibre, g/100 g	12.63±0.05	Vitamin B7	53.42±2.07
Soluble dietary fibre, g/100 g	0.61±0.05	Vitamin B9	66.871±1.08
Potassium, mg/100 g	577.59±7.41		
Calcium, mg/100 g	76.54±0.27		
Zinc, mg/100 g	3.10±0.01		
Selenium, µg/100 g	8.15±1.21		

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is considered exceeding the acceptable limits. Coliform contamination in food products can relate to the mishandling of raw material, cross-contamination that might occur after heat treatment, failure of heat treatment, or inadequate sanitation process. Overall, the microbiological quality of instant cereal in this study is considered safe for ready-to-eat foods.

Table 6. Microbiology analysis of optimized instant cereals

Microbiology Test	CFU/g
Total Viable Count (PCA)	2.5×10^3
Yeast and Mould (PDA)	1.0×10
Total Coliform count (VRBA)	$<1.0\times10$

Results given are averaged from duplication

3.3 Sensory evaluation of optimized instant cereals.

Based on Table 7 the sensory evaluation of instant cereal and commercial healthy drinks was determined and tabulated. There was no significant difference between instant cereals and commercial drinks in the aspect of colour. Besides, the scores for aroma, aftertaste, and taste were not significant between instant cereal and commercial, in which the range is 5 and 6 respectively. It can be concluded that most panellists 'slightly liked' to 'moderately liked' the samples. The presence of bitterness in vegetables as a dominant flavour might be the main cause, thus contributing to after taste of instance cereals.

However, in overall acceptability of instant cereal had the nearest score (5.65) with a commercial drink (5.83) even though it had almost to 'moderately liked' score by panellists. This study revealed that the formulation of instant cereal with red amaranth, barley and corn are very new among panellist. Aside from that, panellists evaluated scores based on their lack of understanding of nutritional advantages. According to several researchers, nutritional and nutritious food products are usually given a lower ranking (Oyeyinka *et al.*, 2015; Lee *et al.,* 2018). According to Lee *et al.* (2018), the formulation of instant cereal beverages incorporated with 30% corncob powder had lower mean scores (6.00) than commercial instant cereal beverages in the current market. This result also suggested that it was possible to incorporate high amounts of nutritional raw material such as red amaranth, barley, corncob powder and others in product formulation while maintaining its palatability.

4. Conclusion

Instant vegetable cereal has the potential to be used as a daily cereal routine consumption because it is complementary feeding due to nutritional, vitamin, mineral, antioxidant and sensory attributes being comparable to that of commercial health beverages. According to the mixture design, red amaranth, corn, and barley affected DPPH radical scavenging percentage, zinc, and calcium content. The best mixture design gives optimal nutritional values of instant cereal based on antioxidants, vitamin C, vitamin A, minerals, and fibre. Microbiological analyses showed that instant cereals are within acceptable limits and safe for consumption. The formulation of instant cereal with red amaranth, barley, and corn had the highest score by panellists and was not significant with commercial samples. In future work, the effect of taking instant vegetable cereal on blood enzymatic antioxidant, cholesterol, and glucose level in the animal study will be investigated.

Conflict of interest

The present study was performed in absence of any conflict of interest.

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Table 7. Sensory evaluation scores of optimized instant cereals versus commercial product.

Samples	Colour	Aroma	After taste	Taste	Overall acceptability
Instant cereal	5.22 ± 1.48^a	5.43 ± 1.16^a	5.60 ± 1.47^a	5.83 ± 1.20^a	5.65 ± 1.27^a
Commercial health drinks	5.43 ± 1.20^a	5.52 ± 1.24^a	5.87 ± 1.40^a	5.69 ± 1.06^a	5.83 ± 1.03^a

Values are presented as mean±standard deviation, n = 3. Values with different superscripts within the same column are significantly different ($p<0.05$)

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