

Research Article

Formulation, Process Optimization, and Biochemical Characterization of Cereal-Based Sweet Potato and Mulberry Instant Beverage

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Most of the beverages have a high glycemic index, which is attributed to a sudden rise in blood sugar. The beneficial role of functional foods combination provided the tool to perform and design our study to develop an instant beverage mix (IBM) that might be revealed as the favorable therapeutic potential for the treatment of hyperglycemia and act as a functional beverage. Therefore, resistant fibre-rich ingredients/raw materials were used to formulate the cereal-based instant beverage (CIB). CIB was formulated using black rice flour (40–70%), germinated lentil flour (10–20%), sweet potato flour (10–20%), and mulberry powder (10–20%). The product formulation was optimized with respect to the following responses such as color and appearance, texture, flavor, taste, and overall acceptability using a D-optimal mixture design. The results revealed that the variation in raw ingredients significantly affected the organoleptic properties of trials. The ratio 40 : 20 : 20 : 20 of black rice: germinated lentil: sweet potato: mulberry was found to be optimum for the development of CIB. Optimized CIB had 9.71 ± 0.10 g/100 g of crude protein, 4.73 ± 0.09 g/100 g of fat, and 4.48 ± 0.06 g/100 g of crude fibre. Moreover, the total mineral content and carbohydrate content were found to be 1.08 ± 0.07 g/100 g and 72.45 ± 0.44 g/100 g, respectively, whereas, the energy value was 371.21 ± 4.23 kcal. *In vivo* glycemic index was also performed for the optimized CIB. The findings showed a lower glycemic response (37.70) than the diabetic control group, and blood glucose was found to be lowered (279.67 ± 20.06 to 227.17 ± 13.44 mg/dL) via the hypoglycemic mechanism. Thus, the optimized CIB exhibited a therapeutic effect against diabetic conditions and might be a healthy instant beverage for human consumption.

1. Introduction

Instant beverage mix is referred to as a dry mix which can be blended with water or other liquid foods (i.e., milk) to prepare the instant beverage [1]. The hectic lifestyle of consumers promoted the consumption of instant beverage mixes due to their easy preparation and health benefits. Recently, due to the availability and increasing demand for

“on-the-go” food and beverages, instant beverage mixes have received much demand across the developed countries [2]. Many research studies showed that plant-based diets can prevent the type 2 diabetes [3]. The constituents (fibre, minerals, phytonutrients, and antioxidants) present in plant-based diets play a key role in preventing and treating type 2 diabetes as well as reducing the risk of other complications, such as overweight, abdominal obesity, high

blood pressure, hyperlipidemia, and inflammation. [4]. According to the World Health Organization (WHO) and Food and Agriculture Organization (FAO) [5, 6], diets rich in plant foods are not only effective in reducing health-related complications but also more environmentally sustainable than diets rich in animal products.

Instant foods such as ready-to-eat (RTE), ready-to-drink beverages (RTD), and semicooked or ready-to-cook breakfast and snack items are commonly prepared by using malted or unmalted cereals alone or by mixing with legumes, nuts, fruits and/or vegetables. Among cereals, rice is the second most important cereal in the world, consumed as a staple food by the Asian population, providing energy and other nutrients compared to other staple foods [7]. Black rice is one of the healthiest foods and contains a variety of constituents including antioxidants, essential amino acids, minerals, and dietary fibre. Regular consumption of black rice can help in weight management and detoxification of the body as it gives a feeling of fullness which reduces food intake [8]. Among all colored rice varieties, anthocyanin content is the highest ($327.60 \text{ mg } 100 \text{ g}^{-1}$) in black rice, which is able to prevent the accumulation of bad cholesterol over the heart valves and also reduces the level of cholesterol in the blood. Thus, black rice results in controlling and preventing chronic diseases, such as coronary infarction, cancer, type 2 diabetes mellitus, allergies, and Alzheimer's disease [9, 10]. Many ready-to-serve (RTS) and RTE foods use white rice as the main ingredient; however, it can be replaced with black rice to develop a new food product with increased nutritional status [11]. Among legumes, lentils have the highest content of functional components, such as total phenolic content, than other common legumes [12], which have potential health benefits as complementary medicinal foods, that exert antioxidant, hypolipidemic, cardioprotective, anti-inflammatory, and anticancerous effects [13]. Sweet potato flour contains 2-3% of fibre [14]. Potential benefits of soluble dietary fibre include lowering blood serum cholesterol levels, influencing glucose metabolism, and thus regulating postprandial blood sugar levels and secretion of insulin in diabetic patients [15]. Lund et al. [16] compared 28 varieties of fibre-rich test samples prepared from tropical fruits and vegetables for their capacity to bind cholesterol. On the other hand, the sweet potato-based test sample had the highest cholesterol-binding ability. Therefore, sweet potato genotypes with a high polyphenol content and used as a vegetable, tea, beverage breakfast food, food ingredient, and as a nutritional supplement can be developed to be used for the promotion of health [17]. A natural antioxidant in black mulberry (*Morus nigra*) is the anthocyanin pigment which has excellent free radicals scavenging activity and leads to limit and cure the diseases caused by oxidative damage in human beings [18].

The literature reported that black rice (*Oryza sativa* L.), sweet potato (*Ipomoea batatas*), lentil (*Lens culinaris*), and mulberry fruit (*Morus nigra*) have been used for the prevention and treatment of adult-onset diabetes via a specific mechanism of insulin interaction [19]. The beneficial role of these functional food combinations provided us with the tool to think and design our study to develop an instant

beverage mix (IBM) from functional ingredients of plant origin and to determine its efficacy on blood glucose levels of alloxan-induced diabetics [20]. IBM thus might reveal the favorable therapeutic potential for the treatment of hyperglycemia and act as a functional beverage.

There is no research work available on the development of cereal-based instant beverages using the abovementioned functional ingredients. If properly processed, such a product would be novel and could fetch substantial revenue to the beverage processing industry. Apart from this, there is a need to enhance the postharvest processing of these underutilized grains by formulating value-added convenience and healthy products such as instant beverage mixes. This functional beverage meets the consumers' demand by supplying functional components. Thus, the present study was carried out to optimize the ratio of raw ingredients of a CIB, and the effects of the ingredient variations were observed on the organoleptic properties of it as well as the biochemical parameters of the final optimized CIB was also investigated for its therapeutic suitability.

2. Materials and Methods

2.1. Raw Materials. Black rice (*Oryza sativa* L.) and black mulberry (*Morus nigra*) were procured from the counter of the Agricultural Technology Information Centre (ATIC) and the sericulture farm of Assam Agricultural University (AAU), Jorhat, respectively. Both lentils (*Lens culinaris*) and pale yellow-fleshed sweet potato (*Ipomoea batatas*) were purchased from the weekly market of Jorhat town, Assam, India. Soy milk (Life Health Foods, Jorhat, Assam, India) and sucralose with 100 purity (Brand: ProFoods Nutrition, Jaldhara and Co. Jorhat, Assam) were taken as an alternative to sugar in CIB preparation.

2.2. Pretreatment of Raw Materials and Its Processing. Black rice was first cleaned and washed, followed by soaking in water for half an hour. The rice sample was steamed for 45–50 min. and dried using a hot air oven at 60°C for 8 h (Castro Engineering Pvt. Ltd., Howrah, India). Finally, rice was ground into flour using paddy dehuller (Paddy Dehusker, Osaw Industrial Product Pvt. Ltd., Assam, India) and sieved with 150 mesh size. For germinated lentil flour, lentil seeds were first washed, followed by soaking in water by portioning into a cup and adding water (lentil: water = 1 : 2, w/w) for 24 h, and covering with a muslin cloth. After that, legume seeds were washed and allowed to germinate in a muslin cloth at 20°C for 5 days. Germinated samples were steamed for 20 min (a ratio of 1 : 1.5, w/w of lentil and water) and finally, dried at 40°C for 72 h. The germs or sprouts were separated by rubbing. After dehulling, legumes were ground into flour using a paddy dehuller machine and sieved with 150 mesh sieves [21]. Sweet potato was processed according to the method given by Hernandez-Aguilar et al. [22]. In brief, yellow flashed sweet potato was peeled off, washed, and dabbed in a muslin cloth to remove surface water. Thereafter, the washed sweet potato was uniformly sliced in $10 \times 10 \times 5 \text{ mm}$ size. Sweet potato slices were oven-dried at

60°C for 10 h and ground into flour using an electric grinder. The flour was screened with a 100-mesh sieve [22]. Fresh ripe black mulberries were sorted and dried at 55°C for 30 h using a cabinet dryer (XDL315 Dry Cabinet, Hibex India Pvt. Ltd., Assam, India). The dried fruit sample was ground into powder using an electric grinder and sieved with a 100-mesh sieve size. All the flour and powders were separately packed in high-density polyethylene (HDPE) virgin airtight containers and stored at room temperature.

2.3. Experimental Design of Optimization. The selection of the different ingredients for the development of CIB was based on preliminary experiments. The range of flours (independent variables) comprising of black rice (C1: 40–70%), germinated lentil (C2: 10–20%), sweet potato (C3: 10–20%), and mulberry (C4: 10–20%) was taken and experimental formulations were designed using a D-optimal mixture design. The effect of formulations was investigated on responses such as appearance, color, taste, texture, flavor, consistency, and overall acceptability, as shown in Table 1. A total of 20 experiments were performed for the optimization of raw material ratio in CIB, including 4 replicates to obtain an estimate of pure error, as shown in Table 1. The accuracy of fit and the significance of linear and quadratic models were performed by Design-Expert® Version 13 (Stat-Ease, Inc., Minneapolis, MN, USA).

2.4. Preparation of CIB. The raw ingredients (30 g) were taken from each formulation (20 experiments) and added to 100 ml of warm soy milk containing 2 g of sucralose. Thereafter, it was stirred well for proper mixing. The organoleptic evaluation was conducted on the prepared instant beverage (CIB) of each formulation. It was carried out with respect to the following responses such as appearance, color, taste, texture, flavor, consistency, and overall acceptability as shown in Table 1.

2.5. Organoleptic Evaluation. After the preparation of the CIB of each formulation, organoleptic evaluation was conducted in the Department of Food Science and Nutrition, Assam Agricultural University, Jorhat, (India). Organoleptic evaluation of the fresh instant was conducted by a total number of 15 semitrained sensory panelists comprising five males and ten females. The coded CIB samples assigned with three-digit numbers were randomly presented to the trained panelists. All the panelists were asked to rinse their oral cavity with lukewarm water for palate cleansing in between each sample at 20°C and RH of 62%. The organoleptic evaluation was performed using a 9-point hedonic scale ranging from 1 to 9, where 1 depicted “dislike extremely” and 9 depicted “like extremely” [23].

2.6. Optimization Using D-Optimal Design. The ratio of raw ingredients/materials was optimized using organoleptic response values on the basis of better-set goals which were maximum for each response (appearance, color, taste, texture, flavor, consistency, and overall acceptability).

The criterion of the set goal for optimization was determined according to Pérez-Báez et al. [24]. The lower and upper limits for black rice flour (C1: 40–70%), germinated lentil flour (C2: 10–20%), sweet potato flour (C3: 10–20%), and mulberry powder (C4: 10–20%) were fixed on the basis of our preliminary trials. For carrying out the optimum solution of multiple responses, the individual goals were combined into an overall composite function called the desirability function [25]. The desirability of optimized CIB was found to be closer to one. Contour plots for all responses showed better results with respect to the used ingredient levels. Moreover, optimized CIBs were further subjected to proximate, free radical scavenging activity, and biochemical analysis (*in vivo*).

2.7. Proximate Analysis of Optimized CIB. Standard AOAC analytical methods [26] were used to analyse the proximate composition i.e., moisture, protein, fat, fibre, and ash of optimized CIB. The carbohydrate content was determined by the differential method and the energy value was determined by multiplying the proportion of protein, fat, and carbohydrate with its respective physiological energy value.

2.8. Determination of Free Radical Scavenging Activity of Optimized CIB. Free radical scavenging activity was estimated using the DPPH method given by Vani et al. [27]. Analysis was carried out in the analytical laboratory of the Department of Food Science and Nutrition and Department of Food Science and Technology, Assam Agricultural University, Jorhat, Assam.

2.9. Biochemical Analysis of Optimized CIB

2.9.1. In Vivo Glycemic Index (GI). Twelve adult healthy male Wistar rats (150–250 g) from Chakraborty Enterprises, Kolkata were used to determine the glycemic index. All the animals were reared in the Department of Pharmacology and Toxicology, College of Veterinary Sciences, Assam Agricultural University, Khanapara, Assam. The rats were housed in polypropylene cages (five rats per cage). The rats were incubated in a controlled condition with a 12 h light and dark cycle for acclimatization. Deionized distilled water was offered *ad libitum*. The animals were divided into two groups, namely, group A (control group) and group B (optimized CIB as a feed). Each group consists of 10 animals. The control group (group A) was fed with rat ration along with standard glucose and group B was fed with optimized CIB. The guidelines of the Institutional Animal Ethics Committee had been followed during animal experimentation (Approval no. 770/GO/Re/S/03/CPCSEAFVSc/AAU/IAEC/18-19/709 dated 28.12.2018).

(1) Glucose Tolerance Test (GTT). GTT was performed using blood samples taken from overnight fasting animals. Fasting blood sugar was taken from Group A before administration of 0.15 g of standard glucose solution (dissolved in distilled water). Blood glucose was determined after feeding at the

TABLE 1: Organoleptic responses of different CIB formulations.

Samples	Cereal (C ₁)	Pulse (C ₂)	Fruit (C ₃)	Vegetable (C ₄)	Responses						Overall acceptability
					Appearance	Color	Flavor	Taste	Texture	Consistency	
TS1	53.83	20	10.40	15.77	7	7.20	7.10	7.30	7.30	7.3	7.30
TS2	40	20	20	20	7.90	7.90	7.60	8	8	7.80	7.60
TS3	66.21	10.00	13.78	10	6.60	6.50	6.70	6.60	6.90	6.70	6.70
TS4	62.86	17.14	10	10	6.70	6.70	6.80	6.50	6.80	6.80	6.90
TS5	54.79	10.00	15.34	19.87	7.10	7.20	7	6.90	7.50	7.30	7.20
TS6	50.02	19.99	19.99	10	7.70	7.60	7.30	7.20	7.60	7.40	7.20
TS7	45.44	19.92	15.23	19.41	7.40	7.50	7.50	7.70	8.20	7.70	7.50
TS8	53.29	15.65	16.35	14.71	6.70	6.80	7.20	7.70	7.00	7.20	7.10
TS9	66.46	10	10.01	13.53	6.80	6.70	6.60	6.70	6.70	6.60	6.70
TS10	57.39	17.98	13.20	11.42	6.70	7	7	7	7.20	7.30	7
TS11	57.79	11.36	20	10.84	7.10	7.10	6.90	7.20	6.90	7.20	6
TS12	46.22	18.55	20	15.23	6.90	7.60	7.70	7.90	6.50	7.60	7.50
TS13	53.83	20	10.40	15.77	7	7.20	7.10	7.30	7.30	7.30	7.30
TS14	59.05	12.98	10.52	17.44	7.20	7.30	7	7	7.30	7.30	7.10
TS15	62.86	17.14	10	10	6.70	6.70	6.80	6.50	6.80	6.80	6.90
TS16	57.79	11.36	20	10.84	7.30	7.10	7	6.80	7.20	6.80	7.10
TS17	51.09	16.18	12.72	20	7.40	6.90	7.10	7.20	6.80	7.10	7.30
TS18	49	11.01	19.99	20	7.80	7.60	7.50	7.40	7.50	7.40	7.30
TS19	40	20	20	20	7.90	7.90	7.60	8	8	7.80	7.60
TS20	50.02	19.99	19.99	10	7.70	7.60	7.30	7.20	7.60	7.40	7.20

TS1 to TS20, test formulations 1 to 20 based on the D-optimal mixture design.

interval of 15, 30, 45, 60, 90, and 120 mins. The same method was performed with Group B (feed supplied as optimized CIB) after determining their fasting blood sugar level according to the method proposed by Thannoun [28].

A blood sample was collected from the experimental animals by the tail-tipping method. Blood glucose was determined by using the Accu-Check Roche blood glucose

meter (Active blood glucometer kit, Pillbox Pharmacy, Assam, India).

The glycemic index (GI) for each diet was determined by calculation of the incremental area under two hours of blood glucose response or curve (iAUC) for each diet and compared with iAUC for glucose solution standard according to the method of Jenkins [29] by using the following equation:

$$GI = \left[\left(\frac{\text{Incremental Area Under 2 h blood glucose Curve for food}}{\text{Incremental Area Under 2 h blood glucose Curve for glucose}} \right) \times 100 \right]. \quad (1)$$

2.9.2. Supplementation Study. Sixty male Wistar rats (150–250 g) were used to study the effect of optimized CIB supplementation on blood glucose levels. The animals were divided into five groups: alloxan free, control group fed with rat ration, group A (diabetic control) fed with rat ration, group B treated with metformin and fed with rat ration, and group C (C1, C2, and C3) fed diet loaded with 50%, 60%, and 70% of optimized CIB, respectively (Table 2). Diabetes was induced by a single intraperitoneal administration of alloxan monohydrate (160 mg/kg) with 4% saline (average 0.90 ml per specimen) after overnight fasting. Animals were left undisturbed for 48 h. After 48 h, blood samples were collected from the surviving rats by retro-orbital puncture and blood glucose level was checked. The rats that had a value above 200 mg/dl were considered as diabetic.

(1) Determination of Blood Glucose Level. Blood samples were collected from the experimental animals on days 0, 2, 7, 14, and 21 by retro-orbital puncture and centrifuged at 3000 rpm for 20 min. Serum was collected in a micro-centrifuge and glucose level was determined using a commercially available assay kit with an auto analyser. The standard laboratory method was used for blood glucose estimation by using a spectrophotometer (iCE 3000 series, Systronics, India).

2.10. Statistical Analysis. In the present study, a D-optimal mixture design was applied to design the experiments and to analyse the data of organoleptic observations. All experiments were performed independently with at least three determinations. Mean values \pm standard deviations for all

TABLE 2: Proportion of diets for assessing the hypoglycemic effect of optimized CIB.

Experimental group	Proportion of diet	Number of animals
Control	Rat ration (100%)	10
A (diabetic control)	Alloxan + rat ration (100%)	10
B (standard metformin)	Alloxan + rat ration (100%)	10
Group C (CIB)		
C1	Alloxan + rat ration (50%) + CIB (50%)	10
C2	Alloxan + rat ration (40%) + CIB (60%)	10
C3	Alloxan + rat ration (30%) + CIB (70%)	10

CIB, cereal-based instant beverage; C1 to C3, group C subgroups.

quantitative parameters were calculated using Microsoft Excel® 2016 (Microsoft Co., Ltd., Washington, USA). Paired “*t*” test, analysis of variance, and *F*-test were used to compare the effect of a specific treatment, the ratio of between-group variability to within-group variability, and the equality of the different treatments on the time of the interval using IBM® SPSS® Statistics version 22.0 (New York, USA). A probability value ≤ 0.05 was considered to be significant.

3. Results and Discussion

3.1. Effect of Ingredients Ratio on Organoleptic Properties of CIB. The role of the different raw ingredients on organoleptic properties was determined by the second-order polynomial model which examined the effect of the significant difference between the independent variable (linear effect) and combined variable (interactive effect) on the organoleptic responses of the developed products. The linear and interactive effects of all the ingredients on the individual organoleptic parameters of the formulated CIB are shown in Table 3.

The independent variables showed a significant ($p < 0.05$) effect on the organoleptic properties of the product. Organoleptic evaluation of the 20 test samples obtained through a D-optimal mixture design revealed that the appearance of CIB formulation ranged from 6.6 to 7.9 in different test samples. Appearance and color of the product were linearly and significantly ($p < 0.05$) improved by pulse, fruit, and vegetable. The texture was linearly and significantly ($p < 0.05$) improved by cereal, pulse, and fruit, while consistency was linearly and significantly ($p < 0.05$) improved by pulse and vegetable. Moreover, the texture ranged from 6.7 to 8.2. The flavor, taste, and overall acceptability of the product were linearly and significantly ($p < 0.05$) improved by all the independent variables.

The interactive effect between pulse and fruit and fruit and vegetable significantly ($p < 0.01$) improved the appearance. Similarly, the interactive effect between cereal and pulse, pulse and fruit, as well as fruit and vegetable significantly improved the color. It ranged from 6.5 to 7.9. The effect between cereal and pulse, pulse and fruit, as well as fruit and vegetable significantly improved the flavor (6.6 to 7.7) and consistency (6.6 to 7.8) of the product. The interactive effect between cereal and pulse, cereal and fruit, pulse and fruit, pulse and vegetable, as well as fruit and vegetable significantly ($p < 0.01$) improved the taste of the product. Similarly, the interactive effect between cereal and

fruit as well as pulse and fruit significantly ($p < 0.01$) improved the texture of the CIB. All the independent combined variables (interactive effect) significantly ($p < 0.05$) raised the overall acceptability of the product. The organoleptic responses including appearance, color, flavor, taste, texture, and consistency of CIB together contributed to 89% overall acceptability of the product. Sensory evaluation indicated that the overall acceptability of the different test samples ranged from 6.7 to 7.6 as shown in Table 1. The 2D representation of the D-optimal mixture design in relation to the independent variables involved in the organoleptic properties of CIB is shown in Figures 1(a)–1(n).

3.2. Optimization of Formulation and Verification. Optimization of the independent variables was performed for the organoleptic responses based on better-set goals for each response, which were “in range” for all ingredients and “maximum” for all responses. The results of the optimization process showed that a maximum desirability could be obtained at a level of 40% incorporation of cereals and 20% incorporation of pulses, fruits, and vegetables. At this optimal level, the values of the predicted responses were found to be appearance (7.90), color (7.90), flavor (7.60), taste (8.00), texture (8.00), consistency (7.80), and overall acceptability (7.60). For verification of the optimized level of variables (40% of cereal, 20% of pulse, 20% of fruit, and 20% of vegetable), organoleptic evaluation was again performed at this level and observed values for each response were found to be closer to predicted values. The variation was found to be 1% in all the organoleptic parameters. Thus, the ratio of raw ingredients with 40:20:20:20 (cereal: pulse: fruit: vegetable) was termed as “optimized CIB” formulation and later it was subjected to proximate, free radical scavenging activity and *in vivo* studies.

3.3. Proximate of Optimized CIB. The proximate composition of optimized CIB is shown in Table 4. The moisture content of the optimized CIB was 7.55 ± 0.16 g/100 g, which was within the recommended limit given by the Food Safety and Standards Regulation of India, 2011 [30]. The protein content of the optimized CIB formulation was 9.71 ± 0.10 g/100 g. The optimized CIB contained a range of crude protein levels, varying from 13.92g/100g to 22.12g/100g, attributable to the inclusion of germinated lentils [31]. This could be related to the germination and malting process, in which lentil seeds absorb water by imbibition process to initiate

TABLE 3: Linear and interactive effect of each variable on organoleptic responses of CIB formulations.

Variables	Appearance	Color	Flavor	Taste	Texture	Consistency	Overall acceptability
<i>Independent variables</i>							
Linear effect (individual effect)							
β_1 (cereal)	5.99	6.20	6.59*	7.23*	7.18*	6.84	6.67*
β_2 (pulse)	7.10*	7.40*	7.91*	7.82*	6.43*	7.72*	7.40*
β_3 (fruit)	7.80*	8.31*	8.62*	8.54*	7.29*	6.66	7.87*
β_4 (vegetable)	7.23*	7.52*	8.10*	8.23*	6.16	7.77*	7.50*
<i>Combined variables</i>							
Interactive effect (combined effect)							
$\beta_1 \beta_2$ (cereal and pulse)	5.82	6.87*	5.72	6.22*	5.74	5.40	5.82*
$\beta_1 \beta_3$ (cereal and fruit)	6.29	6.00	7.20*	7.10*	6.43*	6.89*	6.80*
$\beta_1 \beta_4$ (cereal and vegetable)	5.94	6.32	5.89	6.12	6.12	6.34	6.12*
$\beta_2 \beta_3$ (pulse and fruit)	7.23*	7.43*	8.10**	8.42**	7.83*	7.21*	7.70*
$\beta_2 \beta_4$ (pulse and vegetable)	6.23	6.40	6.82*	6.55*	6.92	7.22*	6.69*
$\beta_3 \beta_4$ (fruit and vegetable)	6.89*	7.12*	7.72*	7.92**	6.43	7.22*	7.22*
ANOVA value							
P value	0.01	0.01	0.01	0.001	0.05	0.01	0.001
R ²	0.80	0.81	0.81	0.86	0.83	0.80	0.89

*Significance level at $p < 0.05$; **Significance level at $p < 0.01$; R² = coefficient of regression.

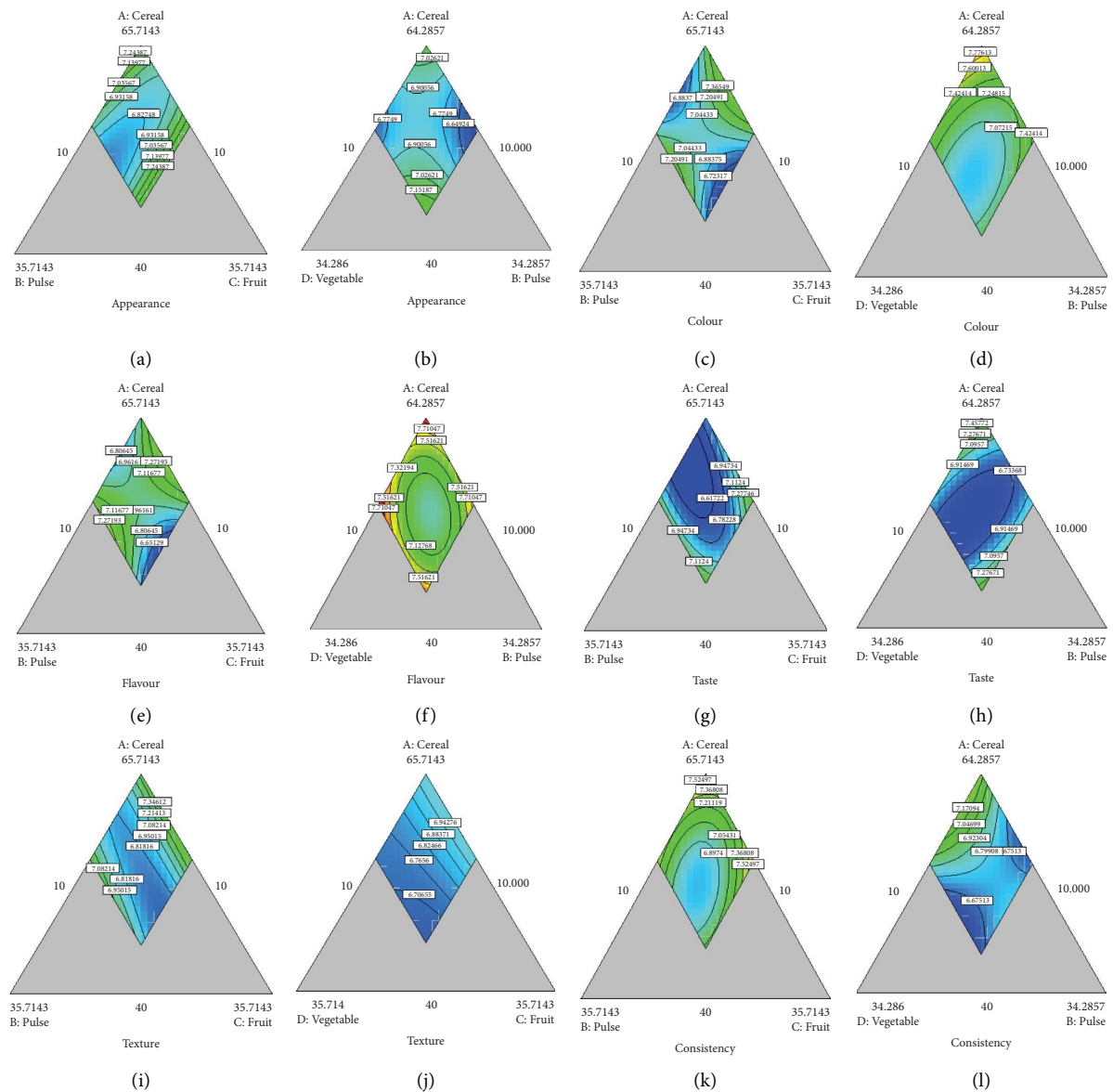


FIGURE 1: Continued.

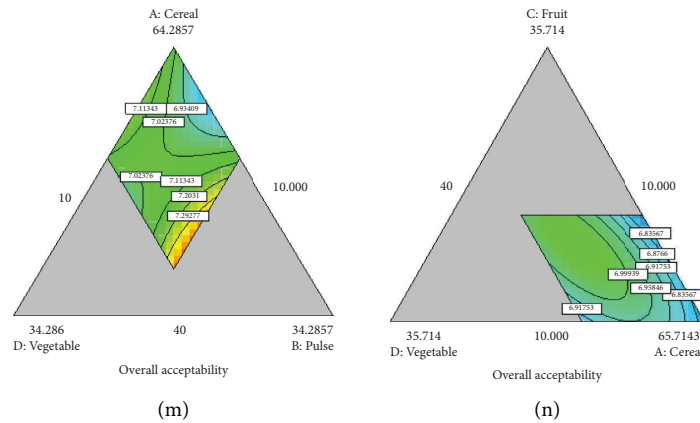


FIGURE 1: Effect of independent variables on sensory attributes of cereal-based instant beverage (CIB). Appearance (a and b), color (c and d), flavor (e and f), taste (g and h), texture (i and j), consistency (k and l), and overall acceptability (m and n).

TABLE 4: Proximate composition of optimized CIB.

Parameters	Nutrients (per 100 g of dry weight basis)
Moisture (g)	7.55 ± 0.16
Crude protein (g)	9.71 ± 0.10
Crude fat (g)	4.73 ± 0.09
Crude fibre (g)	4.48 ± 0.06
Total mineral content (g)	1.08 ± 0.07
Total carbohydrate content (g)	72.45 ± 0.44
Energy (kcal)	371.21 ± 4.23

Data are expressed as the mean ± standard deviation (SD).

sprouting [32]. Sprouting increases the crude protein content, reduces the phytate level of legumes, and promotes the activity of protease and phytase enzymes, which makes solubilization of phytates easier and releases soluble protein and minerals that increase protein content [33]. The fat content of optimized CIB was 4.73 ± 0.09 g/100 g, which was higher than the fat value (1 g/100 g) of the healthy drink mix available in the market. This could be due to the use of fat-rich lentil flour in optimized CIB formulation. A similar range of fat content from 3.75 g/100 g to 7.26 g/100 g was reported by Qiu et al. [34]. The total mineral content of optimized CIB was 1.08 g/100 g. This was in agreement with Santos et al. [35], who reported a total mineral content of 1.9 g in black rice flour. Chen et al. [36] reported that black rice bran had a higher mineral content than other cereal brans. The crude fibre content of CIB was 4.48 g/100 g. Cereal's outer bran layer is rich in crude fibre content. According to the World Health Organization (WHO), dietary fibre promotes metabolism and prevents non-communicable diseases [37]. The total carbohydrate content of the optimized CIB was 72.45 g/100 g and the energy value was 371.21 kcal/100 g as shown in Table 4. Similar energy content from 309 kcal/100 g to 350 kcal/100 g in different samples of sprouted legume-based composite diet was reported by Okorie et al. [38]. Thus, an increased metabolic process and degradation of starch into simple sugar provide energy during the germination process [39].

3.4. Free Radical Scavenging Activity of Optimized CIB.

The free radical scavenging activity of optimized CIB was 75.65%. The free radical scavenging activity was observed to be significantly ($p < 0.05$) higher in CIB than in PIB. This could be due to the presence of black rice flour as the main constituent which has a potent source of phytochemicals, comprising of anthocyanins, flavones, flavonoids, glycosides, carotenoids, and tocopherols. The study also reported that the most abundant anthocyanin present in black rice extract is cyanidin 3-glucoside which prevents diseases associated with hyperlipidemia and hyperglycemia by regulating the hepatic lipogenic enzyme activities, inhibiting α -glucosidase activity, and inducing pancreatic beta cells regeneration, thereby causing an increase in blood insulin level and reducing blood glucose level [40, 41].

3.5. Glycemic Index of Optimized CIB.

Group A animals fed with glucose standard had 75 mg/dl of initial fasting blood glucose level, followed by 92 mg/dl at 60 min and again decreased gradually to 77 mg/dl after 120 min. Group B animals fed with optimized CIB had 85 mg/dl of initial fasting blood glucose level, followed by 98 mg/dl at 60 min and again gradually decreased to 79 mg/dl after 120 min. In experimental groups, the peak blood glucose level was observed at 45 min and 60 min after consumption of optimized CIB, which significantly ($p < 0.05$) decreased within 120 min, indicating slow digestion and absorption of the optimized CIB (Figure 2). Comparatively and significantly ($p < 0.05$) minimum rise in blood glucose level was observed in group B fed with optimized CIB, and this may be due to the higher dietary fibre presence in optimized CIB whereas the glucose standard contains no dietary fibre. Several studies have reported that the soluble dietary fibre promotes the satiety feeling which decreases the quantity and frequency of food intake and exerts hypoglycemic effects as well as reduces the regulatory systems stress related to glucose homeostasis through the activation of endocrine L cells in the colon by their physiological ligands and short chain fatty acids. They promote proglucagon expression and GLP-1 secretion, thereby controlling insulin secretion and

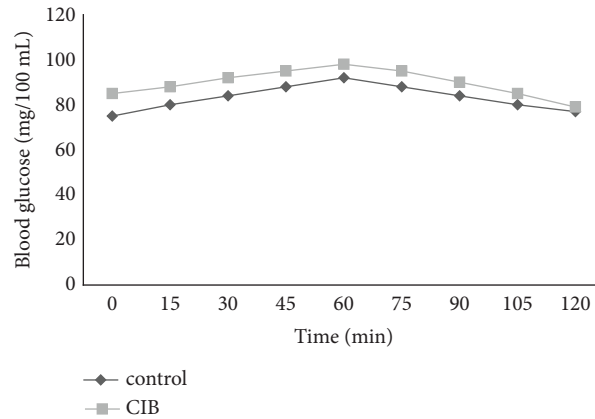


FIGURE 2: Blood glucose response for rats fed with control and CIB. Data are expressed as the mean ($n = 3$).

maintaining glucose homeostasis. Thus, the optimized product (CIB) is able to combat and maintain the higher blood glucose level. Hence, this could be suggested for the treatment of diabetes mellitus [42].

3.5.1. Incremental Area under Blood Glucose Response Curve (iAUC) and Glycemic Index of Optimized CIB. The data of the blood glucose response were used to calculate the glucose response incremental area. The iAUC value of the reference and sample are shown in Table 5. The mean iAUC of glucose standard and optimized CIB was 1132 and 427, respectively. The iAUC values expressed that CIB had a lower level of glucose than orally given glucose. The glycemic index is an important parameter when considering diet as a treatment for metabolism-related disorders, such as adult-onset diabetes mellitus, an improvement in postprandial blood glucose concentration after having a meal [43]. The glycemic index of optimized CIB was calculated from their iAUC and iAUC of glucose standard. The glycemic index of CIB was 37.70. The studies reported that the low glycemic index of foods, such as black rice and germinated lentil seed, are rich in dietary fibres, proteins, and phytonutrients. Fibers and protein play crucial roles in producing short chain fatty acids (SCFAs) and amino acids (AAs). These SCFAs and AAs, once produced, stimulate insulin signaling and activate the insulin secretion channels. Consequently, a diet rich in dietary fiber and amino acids helps in maintaining stable blood glucose levels [44]. Several studies have reported that a water-soluble dietary fibre is low or resistant to digestion and absorption in the small intestine. It may be fermented by gut microflora in the large bowel or as such excreted through feces, thus maintaining the blood glucose levels [45].

3.6. Effect of Supplementation of Optimized CIB on Blood Glucose Level. At the end of the supplementation period, the diabetic control experimental group A showed a significant ($p \leq 0.05$) increase in the blood glucose level from the initial value of 284.17 ± 16.36 mg/dl to 293.83 ± 20.80 mg/dl, after induction of alloxan and fed with rat ration, whereas, the

TABLE 5: Mean incremental area under the curve (iAUC) and glycemic index for formulated CIB¹.

Samples	iAUCmg.min/100 ml	GI
Glucose standard	1132.50	—
CIB	427.00	37.70

GI, glycemic index.

experimental group B showed a significant decrease ($p < 0.05$) in the blood glucose level (279.17 ± 36.60 mg/dl to 167.23 ± 10.35 mg/dl) which was injected with standard metformin and fed with rat ration. At the end of the supplementation period, the average reduction in blood glucose level was observed to be 111.94 mg/dl. Under group C, the subgroup C3 fed with 70% of optimized CIB showed the highest significant decrease ($p < 0.05$) in blood glucose level from 279.67 ± 20.06 mg/dl to 227.17 ± 13.44 mg/dl than subgroups C1 and C2 after the supplementation. At the end of the supplementation period, the highest average reduction in blood glucose level was observed in subgroup C3 (52.50 mg/dl), followed by subgroup C2 (50.34 mg/dl) and subgroup C1 (42.33 mg/dl) as shown in Table 6. This might be due to the presence of crude fibre, which binds with water to form a viscous gel and passes through the small intestine. This is relatively unchanged until it reaches the colon, where it is fermented by the gut microflora and produces short-chain fatty acids, resulting in the decrease of serum-free fatty acids. This reduces blood glucose levels through competition in insulin-sensitive tissues, which is most beneficial for diabetic patients [46]. Furthermore, the presence of phytonutrients in black rice and mulberry powder reduces the blood sugar level as it decreases the intracellular production of H_2O_2 , resulting in the reduction of apoptosis and β -cells' destruction thus maintaining the insulin secretion in the blood [47, 48]. Franco San et al. [49] reported that anthocyanin compounds in functional foods help in re-growth and maintenance the function of β -cells. These cells are responsible for the synthesizing and secreting of insulin into the bloodstream, helping to control the blood glucose level during diabetic complications. Rathna et al. [50] reported that the whole grain black rice is digested slowly and is thereby important to slow releasing of glucose in the blood

TABLE 6: Effect of optimized CIB supplementation on blood glucose levels (mg/dL) of alloxan-induced diabetic rats and mean decrease in blood glucose level (mg/dL) after supplementation¹.

Experimental groups	Day 0	2-day diabetic rat	Supplementation period (days)			Mean
			7	14	21	
Group A	87 ± 12.14	284.17 ± 16.36	284.67 ± 20.63	288.50 ± 20.43	293.83 ± 20.80	287.79 ± 83.53 ^A
Group B	76 ± 14.42	279.17 ± 36.60	248.99 ± 30.51	215.50 ± 14.47	167.23 ± 10.35	227.72 ± 76.59 ^{CDE}
<i>Group C</i>						
C1 (50%)	89.83 ± 13.42	270 ± 24.53	261.33 ± 20.05	247.17 ± 19.18	227.67 ± 19.17	251.54 ± 71.30 ^{BCD}
C2 (60%)	79.67 ± 13.72	258.17 ± 31.67	245.33 ± 36.27	231.33 ± 35.49	207.83 ± 32.02	235.66 ± 71.71 ^{EF}
C3 (70%)	85.67 ± 10.71	279.67 ± 20.06	259.83 ± 16.76	249.50 ± 17.20	227.17 ± 13.44	254.04 ± 73.14 ^{BCD}
Mean time	83.13 ± 14.34 ^a	271.14 ± 30.82 ^b	256.10 ± 30.26 ^c	242.58 ± 30.27 ^d	218.56 ± 33.32 ^e	

Data are expressed as the mean ± standard deviation (SD). The mean values on the same column with different lower-case superscripts and row with different upper-case superscripts represent significant differences ($p < 0.05$) based on analysis of variance (ANOVA) and Duncan's multiple range tests. CIB, cereal-based instant beverage; C1 to C3, group C subgroups, group A (diabetic control) fed with rat ration, group B treated with metformin and fed with rat ration, group C (C1, C2, and C3) fed diet loaded with 50%, 60%, and 70% of optimized CIB, respectively.

as well as also promoting obesity reduction which is a main cause of type 2 diabetes mellitus. Several studies documented that zinc from sweet potato has the ability to raise adiponectin hormone levels in the blood that appears to play a crucial role in protecting against insulin resistance and minimizing the chance of developing adult-onset diabetes mellitus [51, 52].

4. Conclusion

The present study explored the utilization of different functional food matrix formulations to prepare cereal-based instant beverage that has a lower GI value of 37.70. Supplementation study found that the incorporation of optimized CIB in animal diets at 50%, 60%, and 70% significantly decreased the level of glucose in blood up to 42.33 mg/dl, 50.34 mg/dl, and 52.50 mg/dl, respectively. The findings of this study provide evidence for the selection of suitable ingredients for the formulation of CIB that can manage and control the blood glucose and lipid profile, thereby exerting additional health benefits in the control and treatment of several noncommunicable diseases. The technology for the production of instant beverage mixes with enhanced nutritional and functional properties can be transferred for commercialization to local food, health and wellness industries, and entrepreneurship. Thus, it can be accessible to people to have safe nutritious and convenient ready-to-serve food. The obtained findings will hopefully provide crucial supporting data for further study on other noncommunicable diseases. The effect of a specific amount of optimized CIB can also be investigated for liver diseases, CVDs, and obesity.

Data Availability

The data used to support the findings of the study are available from the corresponding authors upon request.

Ethical Approval

Ethical approval for the involvement of animal subjects in this study was granted by Institutional Animal Ethics Committee, Approval no: 770/GO/Re/S/03/CPCSEAFVSc/AAU/IAEC/18-19/709, dated 28.12.2018.

Consent

Verbal informed consent was obtained for experiments involving human voluntary participation.

Conflicts of Interest

The authors declare that they have no conflicts of interest that could have appeared to influence the work reported in this paper.

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