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Determination of glycemic index and chemical composition of formulated *Ipomoea-batatas* based instant beverage mix (IBIBM) and *Morus-nigra* based instant beverage mix (MBIBM)

Vishakha Singh, Mamoni Das and Chandana Choudhury Barua

Abstract
The increasing prevalence of Type 2 Diabetes Mellitus worldwide emphasizes the importance of understanding the role of diet consumption. The foods with low Glycemic Index (GI) value are majorly focused as per the consumer's demand and there are very limited instant foods available with low GI essential for Type 2 diabetic patients. Therefore, this present study was conducted to develop the low GI, *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM) and *Morus-nigra* Based Instant Beverage Mix (MBIBM). And, a comparative investigation was done to estimate the chemical composition, and Glycemic Index (GI) of formulated IBIBM and MBIBM using an animal model. After analysis, the moisture content, crude protein, crude fat, crude fibre and total carbohydrate in IBIBM was found as 6.38 g/100g, 5.36 g/100g, 1.07 g/100g, 4.02 g/100g and 81.91g/100g and in MBIBM this was 7.07 g/100g, 10.96 g/100g, 1.09 g/100g, 7.12 g/100g and 72.65g/100g. A significant ($P< 0.05$) lower glycemic response was observed in both IBIBM and MBIBM in compression to glucose standard. MBIBM was found to be most effective with GI 39.39 than IBIBM that showed GI 41.72. Both IBIBM and MBIBM were found nutritionally superior to that of commercially available instant foods. Therefore formulated IBIBM and MBIBM can be used as a health drink with the ability to manage metabolic disorders like Type 2 Diabetes Mellitus and obesity. This study also opens new avenues for the development of the different types of instant beverage mixes using other functional ingredients.

Keywords: Instant beverage mix, chemical composition, glycemic index, type 2 diabetes mellitus

1. Introduction

Approximate 80% of the populations of a developing country such as India have greater demand for processed food because of increasing urbanization, technology, industrial and economic advances. Among the processed foods, Instant beverage mixtures are very popular among all the age groups in both urban and rural areas. Instant beverages can be fortified with different types of nutraceuticals to enhance their nutrition quality as well as provide better health benefits (Obilana *et al.*, 2018) [28]. Carbohydrate contents present in the food, account for about 50% of total energy intake from a normal diet and carbohydrates that induce a rapid elevation in blood glucose are also majorly responsible for life threatening diseases such as Type 2 Diabetes, obesity and cardiac diseases compared with carbohydrates that elevate blood glucose more slowly (WHO, 2015) [44]. With the concern of health, people are needed to make some significant changes in their normal diets and suppliers are needed to replace free sugars present in the food with other acceptable alternatives which positively support health benefits. Complete removal of sugar from food is a challenging task because of its functionality that goes beyond sweetness.

One well accepted approach to reduce the rapid elevation in blood glucose is the replacement of rapidly digestible carbohydrates to slowly digestible carbohydrates (Lamothe *et al.,* 2017) [19]. Additionally, some non-glycemic nutrients such as fibres, fats and proteins present in the food play a very important role to modulate the Glycemic Index (GI) level (Quek *et al.,* 2016; Owen *et al.*, 2003) [32, 30]. GI is the measure of carbohydrate containing food based on blood glucose rising after a meal (compared with the response of a reference glucose solution or white wheat bread)? Based on GI, food is categorised into three main groups; high-GI foods (> 70), intermediate-GI foods (>55 – < 70) and low-GI foods (< 55) (Venn and Green, 2007). Foods that are rapidly digested, absorbed *and result in marked fluctuations in blood sugar levels* have a high GI (> 70) such as starchy foods, chiefly refined grain products and potatoes, Whereas, foods that are digested more slowly have GI 55 and below are associated with *smaller fluctuations in blood glucose and insulin level as well as* reduce the risks of type-2 diabetes and cardiovascular disease (ADA, 2014). Many studies have reported that low GI food is rich in fibre (especially soluble), protein and carbohydrate content (Eleazu, 2016; Miller *et al.*, 1992) [8, 23] which found to be involved in the management of obesity because of their ability to modulate the nutritional quality of food that is strongly associated with Type-2 Diabetes and other cardiac complications (Simila *et al.*, 2011) [35].

Nowadays beverages with lower GI are being focused on research and nutritionally accepted based on the evidence obtained from observational studies (Mirrahimi *et al.*, 2014) [24] as well as randomized clinical trials (Brand-Miller *et al.*, 2003) [7].

Natural products are always considered excellent sources of biologically active compounds (Ji *et al.*, 2009) [16] and specifically fruits and vegetables have received more scientific attention due to their antioxidant properties as well as their potential to reduce the risk of certain types of metabolic diseases (Ma, *et al.*, 2017; Zhang *et al.*, 2018) [21, 45]. According to the National Potato Council of the United States (2013) [26], *Ipomoea-batatas* are majorly consumed as processed food and are important to reduce the risk of obesity. A study revealed that *Ipomoea-batatas* showed a very low range (23) of glycemic responses because of its nutrient contents, starch structure as well as processing to prepare food (Foster-Powell *et al.*, 2002) [11]. Moreover, in some studies, *Morus-nigra* and products have been investigated and showed lower GI attributes when tested with diabetic subjects and consider as good food for metabolic disorders (Thomas *et al.*, 1993; Jenkins *et al.*, 1983; Jenkins *et al.*, 1981) [39, 14]. Along with, macronutrients such as proteins, fibres and minerals present in *Ipomoea-batatas* and *Morus-nigra*, are found to be valued with their effects to lower blood glucose which are the core of today's food research (Rytz *et al.*, 2019) [33].

Therefore, the present *in-vivo* study was performed to formulate *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM) and *Morus-nigra* Based Instant Beverage Mix (MBIBM), as well as determine their chemical components and GI value using a rat model.

2. Materials and Methods

2.1 Sample procurement

Samples such as *Ipomoea batatas* (sweet potato), *Morus nigra* (black mulberry), *Oryza sativa* (black rice) ad *Lens culinaris* (lentil) were procured from the local market of Jorhat, Assam, India.

2.2 Processing of raw ingredients for the formulation of *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM) and *Morus-nigra* Based Instant Beverage Mix (MBIBM)

Ipomoea batatas flour was obtained by the method outlined by Julianti *et al.*, (2017), *Morus nigra* powder was obtained by the method outlined by Ali *et al.* (2016) [3], *Oryza sativa* L. *indica* flour was obtained by the method outlined by Sutinium *et al.* (2008) [37] and *Lens culinaris* flour was processed according to Hernandez-Aguilar *et al.* (2019) [13] with little modification.

2.2.1 Processing of *Ipomoea batatas* flour

Yellow flashed sweet potato were peeled, washed and dab in a muslin cloth to remove water, then uniform slicing (10x10x5) using stainless steel potato slicer using stainless still, spread in a tray and was oven-dried at 60 C for 10 h and after which it was ground into flour using mixture grinder. The flours were screened through a 100 mesh sieve to get fine flour. Flour was packed in HDPE virgin airtight container and stored at room temperature for further analysis (Julianti, 2017).

2.2.2 Processing of *Morus nigra* flour

Fresh ripe black mulberry was sorted, pulped and cabinet dried at 55 °C for 30 hours and after which it was ground into powder using a mixture grinder. The powder was screened through a 100 mesh sieve to get fine flour and flour were packed in HDPE virgin airtight container and stored at room temperature for further analysis (Ali *et al.*, 2016) [3].

2.2.3 Processing of *Oryza sativa* L. *indica* flour

Black rice was Sorted, cleaned, washed in tap water, drained using Rice draining Colander, soaked in tap water (Black rice: tap water = 1:3 w/w) for 30 min., Rinsing with distilled water for 2 times and removing excess water, then steamed for 50 min by portioning into a cup and adding water (Raw black rice: water = 1:1.5 w/w) using stainless steel perforated colander, then spread it on blotting paper and allow it to be cool at room temperature for 45 min, then spread it on still based Hot air oven tray with a *thickness* of 1x5 cm. and dried for 8 hours at 60 °C using Hot air oven, allow to cool at room temperature for 12 h, dry milling (250μm particle size) in a hammer mill and blended with a mechanical ribbon blender to obtain a homogeneous mixer. Then flour was sieved by 150 mess size to get fine flour and flour was packaged in high density polyethylene (HDPE) virgin airtight containers for future use (Sutinium *et al.*, 2008) [37].

2.3 Processing of *lens culinaris* flour

Lentil seed was washed using tap water and then soaked inside a glass bottle with distilled water (1:2 w/w) for 24 hours and covered with a cotton cloth. After this hydration and imbibition process, the seeds were washed again and the excess water was left to drain from the seeds for 10 min using a perforated colander. Next, the seed was allowed to germinate in muslin cloth, washing them with purified water every 24 h for 5 days, then steamed for 20 min by Portioning into a cup and adding water (lentil: water = 1:1.5 (w/w) using stainless still perforated colander, then spread it on blotting paper and allow it to be cool at room temperature for 45 min., then spread it on still based hot air oven tray with a *thickness* of 1x5 cm. and dried for 72 h at 40°C using hot air oven, allow to cool at room temperature for 12 hours, sprout removed by hand abrasion, peeled removed and ground (250 μm particle size) using paddy de-husking machine and sieve through 150 mess size to get fine flour. Flour was packed in HDPE virgin airtight container and stored at room temperature for further analysis (Hernandez-Aguilar *et al.* (2019) [13].

2.3.1 Formulation of *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM):

The formulation of *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM) was done using the method outlined by Sengev *et al.* (2012) [34]. For the present study *Ipomoea batatas*, *Oryza sativa* L. *indica*, *Lens culinaris*, and *Morus nigra* of varying proportions were blended to obtain five test samples namely TS1, TS2, TS3, TS4 and TS5 for each formulation as shown in Table 1

Table 1: Formulation of *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM)

Blend	*Ipomoea batatas* (Sweet potato flour)	*Lens culinaris* (Germinated lentil flour)	*Oryza sativa* L. *indica* (Black rice flour)	*Morus nigra* (Mulberry powder)
TS 1	35.00	21.66	21.66	21.66
TS 2	40.00	20.00	20.00	20.00
TS 3	45.00	18.33	18.33	18.33
TS 4	50.00	16.66	16.66	16.66
TS 5	55.00	15.00	15.00	15.00

2.3.2 Formulation of *Morus-nigra* Based Instant Beverage Mix (MBIBM): The formulation of *Morus-nigra* Based Instant Beverage Mix (MBIBM) was done using the method outlined by Sengev *et al.* (2012) [34]. For the present study *Morus nigra*, *Ipomoea batatas*, *Oryza sativa* L. *indica* and *Lens culinaris*, and of varying proportion were blended to obtain five test samples namely TS1, TS2, TS3, TS4 and TS5 for each formulation as shown in Table 2.

Table 2: Formulation of *Morus-nigra* Based Instant Beverage Mix (MBIBM)

Blend	*Morus nigra* (Mulberry powder)	*Lens culinaris* (Germinated lentil flour)	*Ipomoea batatas* (Sweet potato flour)	*Oryza sativa* L. *indica* (Black rice flour)
TS 1	35.00	21.66	21.66	21.66
TS 2	40.00	20.00	20.00	20.00
TS 3	45.00	18.33	18.33	18.33
TS 4	50.00	16.66	16.66	16.66
TS 5	55.00	15.00	15.00	15.00

2.4. Organoleptic evaluation of formulated *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM) and *Morus-nigra* Based Instant Beverage Mix (MBIBM)

For each test sample, 30gm of instant beverage mix was mixed in 200ml of soya milk and were subjected to organoleptic evaluation. The sensory evaluation of the formulated *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM) and *Morus-nigra* Based Instant Beverage Mix (MBIBM) was performed using the method of I we, M.O., (2002). The scoring was based on a 9- point hedonic scale ranging from 9 (extremely like) to 1 (extremely dislike) and 5 (neither like nor dislike). It was done by a panel of the 21semi-trained person in the department of food science and nutrition, Assam agricultural university, Jorhat.

2.5 Determination of chemical composition

The chemical composition (i.e., moisture, protein, fat, fibre, ash) of the formulated instant beverage mixes (IBIBM and MBIBM) was estimated according to the standard analytical methods (AOAC, 2000). The carbohydrate content was determined by the calculated difference method and the energy value was determined by multiplying the proportion of protein, fat and carbohydrate by their respective physiological energy values and taking the sum of the products (Farzana *et al.*, 2015) [10].

2.6 Determination of *in-vivo* Glycemic Index (GI)

The animals used for this experiment were eighteen adult healthy male Wistar rats (between 150 and 250 grams) from Chakraborty Enterprises, Kolkatta. All the procured rats were raised in the Dept of Pharmacology and Toxicology, College of Veterinary Sciences, AAU Khanapara. Rats were housed in polypropylene cages in small groups of 6 rats per cage. The rats were kept in a controlled condition, 12 hours light and dark cycle for acclimatization. Deionized distilled water was offered ad libitum. The animals were divided into three groups namely Group A (Control group), Group B and Group C respectively. Each group consists of 6 animals, notably, with no statistical differences. Control Group (Group A) was fed with rat ration along with standard glucose. Group B was fed with IBIBM and Group C were fed with MBIBM.

In the use of animals for experimentation, the guidelines of the Institutional Animal Ethics Committee were followed (Appendix no.1).

2.7 Glucose tolerance test (GTT)

GTT was conducted using blood samples collected from overnight fasted animals. Fasting blood glucose was drawn from Group A (control group) before giving 0.15 g standard glucose dissolved in distilled water. Blood glucose was determined after feeding at 15, 30, 45, 60, 90 and 120 minutes. The same method was performed with Group B and Group C by taking their fasting blood glucose and then giving them test food according to the method outlined by Thannoun *et al.* (2010) [38].

2.8 Blood sampling

The blood sample was collected from the experimental animal with tail tipping method and blood glucose was determined by using glucose tester device Accu check Roche.

2.9 Measurement of the glycemic index

Glycemic Index (GI) for each diet was determined by calculation of Incremental Area Under two hours of blood glucose response or Curve (iAUC) for each diet and compared with the iAUC for glucose solution standard according to the method of Wolever *et al.* (1991) [43] which also reported by FAO/WHO (1998) [9] using the following equation

$$GI = \frac{\text{Incremental Area Under 2h blood glucose Curve for food}}{\text{Incremental Area Under 2h blood glucose Curve for glucose}} \times 100$$

The GI calculated for the test samples were classified by using the GI scale (Gordillo *et al.*, 2016) as given; Glycemic index scale: Low: 55 or low; Medium: 56-69; High: 70 or more than.

3. Results and Discussion

3.1 Organoleptic evaluation of *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM) and *Morus-nigra* Based Instant Beverage Mix (MBIBM): The result of sensory

evaluation reveals that out of the 5 formulated test samples of *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM), TS1 (35.00% Ipomoea batatas flour and 21.66% of *Morus nigra powder*, *Oryza sativa L. indica flour* and *Lens culinaris* flour) had the highest significant organoleptic score in terms of appearance (8), colour (8), taste (8), flavour (7), texture (7), and consistency (8) and overall acceptability (8) in comparison to other test samples similarly for *Morus-nigra* Based Instant Beverage Mix (MBIBM) out of 5 test samples, TS3 (45.00% of *Morus nigra* powder and 18.33% of *Ipomoea batatas* flour, *Oryza sativa L. indica* flour and *Lens culinaris* flour) had the highest significant organoleptic score in terms of appearance (8), colour (8), taste (7), flavour (7), texture (8), and consistency (7) and overall acceptability (8) in comparison to other test samples.
Based on its highest organoleptic score the sample was further analyzed for chemical properties and blood glucose response.

3.2 Chemical composition of formulated *Morus-nigra* Based Instant Beverage Mix (MBIBM) and *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM)

The moisture content of the developed IBIBM was 6.38g/100g which was within the permissible limit recommended by Food Safety and Standards Regulations, 2011. The protein content of formulated IBIBM was 5.36±0.08g/100g which was supported by several researchers where they found crude protein content from 4.60gm/100gm to 8.42 gm/100gm in sweet potato based food mixes (Farzana *et al.*, 2017; Omoniyi *et al.*, 2016) [29].
Whereas lower protein content was observed when compared with the commercially available health drink powders like Horlicks (33g/100g). It was probably due to the use of protein powder in commercially available health drink products. The fat content of IBIBM was 1.07g/100g which was higher than the commercially available health drink powders (1g/100g). This may be due to the use of pulse flour in the formulation which contained a higher amount of fat. The crude fibre content of IBIBM was recorded at 4.20±0.12 g/100g. Some researchers observed crude fibre content in instant flour ranged from 2.90 g/100g to 6.62 g/100g (Antia *et al.* 2006; Omoniyi, 2016; Srivastava *et al.*, 2012) [5, 29, 36]. The total carbohydrate content and energy value of the IBIBM was 81.91g/100g and 358.14 ±2.1kcal/100g respectively. Similarly, Adetola *et al.* (2020) [2] also reported a range from 349.52 kcal/100gm to 368.75 kcal/100gm of energy value for *Ipomoea-batatas* based food mix. Similarly MBIBM was investigated with all proximate analysis i.e. moisture, protein, fats and total carbohydrates were recorded as 7.07±0.31g/100g, 10.96±0.17 g/100g, 1.09±0.11 g/100g, 7.12±0.08 g/100g, and 72.65±0.38g/100g respectively which directly support the finding of (Benitez *et al.* 2013 and Thomas *et al.* 2013) [6, 40].

Table 3: Chemical composition of formulated Instant Beverage Mixes

Raw ingredients	Nutrients (per 100 g of dry weight basis)						
	Moisture (g/100g)	Crude Protein (g/100g)	Crude Fat (g/100g)	Crude Fibre (g/100g)	Total mineral content (g/100g)	Total carbohydrate content (g/100g)	Energy (Kcal)
IBIBM	6.38±0.22[d]	5.36±0.11[d]	1.07±0.06[d]	4.20±0.06[d]	1.08±0.10[d]	81.91±0.45[a]	358.71±3.81[b]
MBIBM	7.07±0.31[c]	10.96±0.17[b]	1.09±0.11[c]	7.12±0.08[b]	1.11±0.09[c]	72.65±0.38[b]	321.67±4.61[c]

Values are means ±standard deviation of triplicate analysis.

3.3 Estimation of Glycemic Index (GI) of the formulated *Ipomoea-batatas* Based Instant Beverage Mix (IBIBM) and *Morus-nigra* Based Instant Beverage Mix (MBIBM)

The blood glucose level was monitored at the fasting period and after feeding at an interval of 15 min, 30 min, 45 min, 60 min, 90 min and 120 min, respectively. The blood glucose response for reference food and test foods are presented in Table 4.

Table 4: Mean blood glucose response for formulated *Ipomoea-batatas* and *Morus-nigra* Based Instant Beverage Mixes fed to normal healthy rats

Experimental group	Sample	Mean blood glucose level at different time intervals (blood glucose mg/100ml)						
		0 min	15 min	30 min	45 min	60 min	90 min	120 min
Group A (control group)	Glucose standard	75	83	85	89	92	83	77
Group B	IBIBM	77	83	86	91	93	84	68
Group C	MBIBM	80	85	88	94	89	84	78

3.4 Blood glucose response for formulated *Ipomoea-batatas* and *Morus-nigra* Based Instant Beverage Mixes

The blood glucose response of formulated IBIBM and MBIBM were evaluated and presented in Table 4. The peak blood glucose level in experimental groups after consumption of IBIBM and MBIBM was obtained at 45 and 60 min which decreased significantly ($p<0.05$) till the observation period of 120 min (2 hours) indicating slow digestion and absorption of the Instant Beverage Mixes (Fig.1).

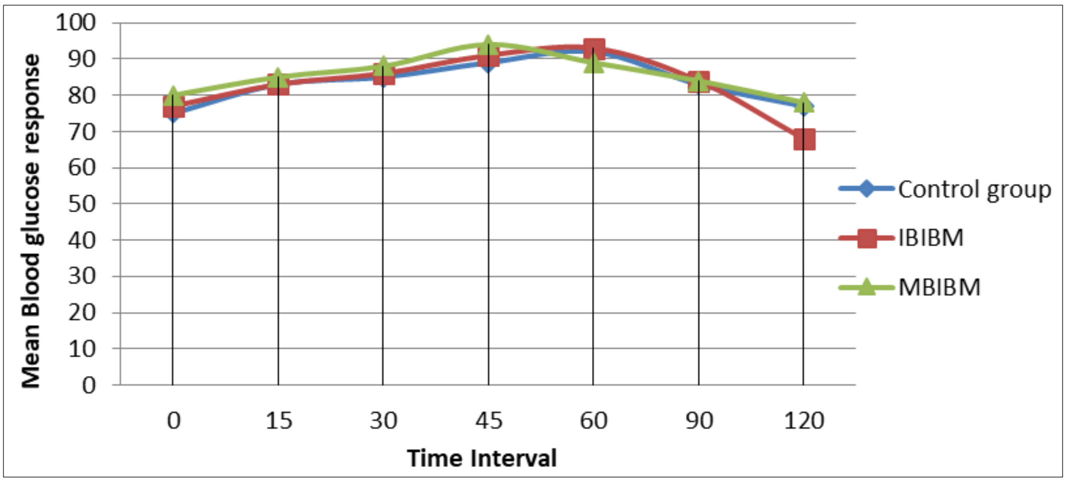

Fig 1: Blood glucose response for formulated IBIBM and MBIBM

From figure 1 it was observed that the initial mean fasting blood glucose level of animals in Experimental Group A fed with glucose was 75mg/dl which rose to the peak level of 92mg/100ml at 60 minutes and gradually decreased to 77mg/dl after 120 minutes. Significantly ($P<0.05$) lowest fluctuation of blood glucose was recorded in MBIBM. This result is attributed to the presence of higher dietary fibre content in MBIBM in compression to IBIBM and glucose standards. Researchers reported that the soluble dietary fibre may exert hypoglycemic effects by promoting the feeling of satiety, which reduces food intake associated with hyperglycemia as well as reducing the stress on regulatory systems related to glucose homeostasis through the activation of endocrine L cells (G-protein coupled receptors, GPR43 and 41) in the colon by their physiological ligands and short chain fatty acids, they promote proglucagon expression, GLP-1 (Glucagon-like peptide 1) secretion, and could thereby control insulin secretion and glucose homeostasis and hence it should be recommended for the prevention and management of Diabetes Mellitus (Miao *et al.* 2015; Tolhurst *et al.*, 2012) [22, 41].

3.5 Incremental area under blood glucose response curve (iAUC) of formulated *Ipomoea-batatas* and *Morus-nigra* Based Instant Beverage Mixes

From the above glucose response, incremental area under the blood glucose response curve was calculated (Wolover *et al.*, 1991) [43]. The (iAUC) value of reference food and test food are presented in Table 5. The mean (iAUC) of glucose standard was 1132, IBIBM was 472.50 and MBIBM was 457.50 respectively. From the mean (iAUC) values it can be observed that the blood glucose levels for the test products were markedly lower than orally administered glucose.

3.6 Estimation of Glycemic index of formulated *Ipomoea-batatas* and *Morus-nigra* Based Instant Beverage Mixes

Glycemic index of formulated *Ipomoea-batatas* and *Morus-nigra* Based Instant Beverage Mixes (IBIBM, MBIBM) were calculated from their iAUC and iAUC of glucose standard. The GI of test samples, IBIBM and MBIBM are presented in Table 5. The glycemic index of IBIBM was 41.72 in and MBIBM was 39.39, this was due to the higher fibre and protein content of MBIBM. Literature has shown that soluble fibre content that is carbohydrate low or resistant to digestion and absorption and makes it pass the small intestine. It is either fermented in the large bowel or excreted as faeces and thus controls the glucose levels in the bloodstream (Nikhta *et al.*, 2020; Muir, 2019) [27, 25].

Movarek *et al.* (2018) reported that low-glycemic index (GI) foods, such as black rice, sweet potato, malted lentil and mulberry release glucose very slowly into the bloodstream, helps to keep the pancreas not overworking and make feel satiated for a longer period because of the presence of different nutritient components such as dietry fibres, proteins, phytonutrients etc. in such type of foods. Where, fibers are important for short chain fatty acids (SCFAs) production and protein for amino acids production which stimulate signaling for insulin (Kim, 2018) and trigger the channels for insulin secretion (Newsholme *et al.*, 2005) respectively to control blood glucose level. As well as phytonutrients act as antioxidants and enhance the secretion of insulin by inhibiting the destruction of β-cells of the pencrease (Lee *et al.*, 2015; Posuwan *et al.*, 2013) [20, 31] to control glucose in the bloodstream.

Table 4: Mean incremental area under the curve (iAUC) and glycemic index for formulated *Ipomoea-batatas* and *Morus-nigra* Based Instant Beverage Mixes (IBIBM and MBIBM)

Samples	iAUC mg. min/100ml	GI
Glucose standard	1132.50	-
IBIBM	472.50	41.72
MBIBM	457.50	39.39

4. Conclusion

From the present study it can be stated that instant beverage mix can be made with the incorporation of functional ingredients like *Ipomoea batatas* (sweet potato), *Morus nigra* (Mulberry), *Oryza sativa* L. *indica* (black rice) and *Lens culinaris* (germinated lentil). These Instant Beverage Mixes are nutritionally superior to that of commercially available instant foods. Therefore formulated IBIBM and MBIBM can be selected to manage metabolic disorders like diabetes and obesity as well as this study also opens new venues for the formulation of different types of instant beverage mixes using other functional ingredients such as *Oryza sativa L.* indica (black rice) and *Lens culinaris* (germinated lentil).

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