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Full Length Research Paper

Isolation and use of bitter gourd polysaccharide in formulating dietetic soft drinks

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This study was conducted to determine the polysaccharide components of bitter gourd and dietetic soft drinks developed using crude bitter gourd polysaccharides. Both water insoluble materials (WIMs) and alcohol insoluble materials (AIMs) were prepared from bitter gourd flesh and AIMs were fractionated for pectin, hemicelluloses and cellulose. Major parts of bitter gourd flesh were found cellulose (27.5%), followed by hemicelluloses (13%) and the lowest parts were pectin (12%). Hot water extraction method was used to isolate crude bitter gourd polysaccharides and heating for 60 min at 90°C, resulting in the highest solid release (1.73%). In case of formulating dietetic soft drinks, optimum condition for hot water extraction as well as the best acid-sweetener ratio was used to formulate the dietetic soft drinks. Three samples were prepared using crude polysaccharides isolated by heating of 0, 5, 7.5 and 10% bitter gourd slices at optimum condition. Significant differences were observed among the samples in terms of color, flavor, taste and overall acceptability. Samples prepared using 7.5% bitter gourd slices into optimized solution secured the highest scores (60%).

Key words: Bitter gourd, water extraction, polysaccharides, dietetic drinks, high sweeteners.

INTRODUCTION

The modern busy and competitive life blessed people with comfort but affected the normal life with various complicated diseases like hypertension, obesity and diabetes. In Bangladesh, there has been an increase in overweight and obesity in different population groups, as well as an increase in mortality caused by diabetes mellitus (Ministry of Health Bangladesh, 2000). Due to costly and prolong allopathic treatment of these diseases, people frequently looked for cheap alternative natural remedy. Since ancient times, people used a portion of plants as medicine, which is associated with the treatment of several ill fated diseases including diabetes. Thus, herbal plants are considered a useful means to prevent certain disorders (Khattak et al., 2005). Usefulness of bitter gourd as food and medicine has long been known to people (El-Bartan et al., 2006). Bitter gourd *(Momordica charantia L.)* is a popular vegetable in Asia and is used to prepare several dishes. They are highly nutritive and are relatively high in proteins, minerals and vitamins. Bitter gourd has already been reported as a good source of phenolic compounds, which possessed potent antioxidant activity (Budrat and Shotipruk, 2008; Aminah and Anna, 2011).

It has some medicinal properties and is recommended for curing blood diseases, rheumatism**,** diabetes and asthma. It is very effective in inhibiting the growth of HL60 human leukaemia cells and this effect probably contribute in cancer prevention (Kobori, 2003). The aqueous juice of bitter gourd fruit has been shown to possess hypoglycaemic activity (Sitasawad et al., 2000). All parts of bitter gourd, including the fruit, taste bitter. It is frequently observed that many diabetic patients squeeze bitter gourd into juice and drink in the morning for natural remedy. The fresh squeeze bitter gourd juice is extremely bitter and tasteless and thus very difficult to drink. Therefore, developing a suitable formulation as

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dietetic drink or as powder form from bitter gourd will be very convenient and cheap for consumers to take and control body weight and health related problems such as diabetes and hypertension. Besides, vegetable beverages are low caloric, nutritious and rich in antioxidants.

On the other hand, bitter gourd (*M. charantia*) is one of the most important vegetables grown in Bangladesh during rabi season. Production of bitter gourd has been increased from 19 thousand tons in 1990 to 1991, to 25 thousand tons in 2007 to 2008 (DAE, 2007). It is estimated that a loss of nearly 40 to 50% of vegetables occur due to improper post-harvest handling and transportation during harvesting period (Sing and Chanda, 1990). Therefore, the research will contribute substantially to reduce post-harvest losses of this vegetable and to get fair price by the poor farmers. Studies so far carried out mostly concerning antioxidant activity of bitter gourd extract and their medicinal properties (Wu et al., 2008; Zhu et al., 1990; Harinantenaina et al., 2006). Li et al. (2010) reported antioxidant activity of polysaccharides extract of bitter gourd as affected by different cultivars. They found that all the cultivars had strong scavenging activity. However, report on water and organic soluble polysaccharides as well as bitter gourd based soft drinks are scarce. Therefore, the purpose of the study is to characterize bitter gourd polysaccharides and to develop soft drinks using crude bitter gourd polysaccharide extract.

MATERIALS AND METHODS

The fresh bitter gourd used in the study was collected from the local market. High sweeteners like aspartame, neutame and saccharine were kindly provided by Nahid Hasan Jony, Production Manager, Givency Foods and Essential, Hotapara, Dhaka.

Characterization of different parts of bitter gourd

Firm bitter melons having solid, light green color and without blemishes and any signs of yellow or orange color on the skin were selected. Selected fresh bitter gourds were washed thoroughly. All the bitter melons were cut open lengthwise and all the seeds were collected. Then outer skins of the pieces separated carefully and the fleshes were chopped into small pieces. All the separated portions were collected, weighed and stored at -20°C. Previously stored frozen samples was bought to room temperature and analyzed for their chemical compositions.

Preparation of fresh water insoluble materials (WIM)

The insoluble flesh materials (WIM) were prepared by washing bitter ground pulp with deionized water at room temperature. About 50 g pulps were mixed with water at a ratio of 1 to 4 (w/w), homogenized and finally stirred for 1 h. The resultant suspension was centrifuged at 4200 rpm for 30 min. The supernatant was discarded and the pallet was collected, oven dried and finally weighed. The weighed samples were expressed as percentage of total samples taken.

Preparation of alcohol insoluble materials (AIM)

The flesh alcohol insoluble materials (AIM) were obtained by washing bitter gourd pulp with 95% alcohol. About 50 g pulps were mixed with 50 ml 95% alcohol and heated at 60°C for 60 min, cooled and centrifuged at 4200 rpm for 30 min. The supernatant was decanted carefully and pellet was collected. AIMs were prepared by washing the pellets in alcohol two times and finally oven dried. The dried samples were weighed and expressed as percentage of total samples taken.

Sequential extraction of alcohol insoluble materials (AIM)

Sequential extraction of AIM was carried out according to the method of Fugel et al. (2003). Little modification was included only in centrifugation and pectic fraction isolation. Among the four pectic fractions isolated by Fugel like water, hydrochloric acid, oxalate and alkali soluble, only alkali soluble pectin fraction was isolated in our case. Instead of ultrafiltration, centrifugation at 10,000 rpm for 30 min was used to separate the fraction.

Extraction of crude polysaccharides from bitter gourd

All soluble compounds including polysaccharides of bitter gourd were extracted by heating bitter gourd slices at different temperatures and time and termed as crude polysaccharides. Heating time and temperature were optimized conducting a series of experiments.

Time effect

Slices of bitter gourd (5 mm) were taken in conical flask. Water was added at the ratio of 2:1 (water: bitter gourd =2:1, v/w) and the top opening of flask were tightly closed with cotton bar. Three flasks with samples were heated at 30, 60 and 90 min, respectively, at fixed temperature (50°C). Then the samples were bought to the room temperature and filtered by cheese cloth. After filtration, 10 ml filtrate was taken separately in three centrifuge tubes and was centrifuged at 3000 rpm for 30min. The pellets were collected, dried and weighed. The weighed samples were expressed as percentage of total weight.

Temperature effect

Same as time effect, three flasks containing samples were heated at 50, 70 and 90°C respectively at a fixed time. Then, the samples were bought to room temperature and filtered with cheese cloth. After filtration, 10 ml filtrate was taken separately in three centrifuge tubes and was centrifuged at 3000 rpm for 30 min. The pallets were collected, dried and weighed. The weighed samples were expressed as percentage of total weight.

Formulation of dietetic bitter gourd juice using crude polysaccharides

Three bitter gourd dietetic juices were prepared using crude polysaccharide extracts isolated from 5, 7.5 and 10% bitter gourd slices by hot water extraction method. For hot water extraction, the optimized time and temperature was used. The best sweeteners and the best acid-sweeteners ratio optimized earlier were used in the final formulation. Optimization of acid-sweeteners ratio was carried out based on sensory data obtained from sensory evaluation of different acid sweeteners solution. A control sample

Table 1. Composition of 100 g of soft drinks from bitter gourd.

Ingredients	Formulations			
	S_1	S_2	S_3	C
Bitter gourd slices (%)	5	7.5	10	0
High sweeteners (%)	0.065	0.0655	0.065	0.065
Citric acid (%)	0.3	0.3	0.3	0.3
Water (%)	94.63	92.135	89.635	99.635
Potassium meta-bisulfate, KMS (%)	0.06	0.06	0.06	0.06

Table 2. Weight of different parts of bitter gourd.

Bitter gourd	Content (g)	Percent of whole weight (%)
Skin	29.6±0.02	27.05±0.2
Flesh	73.6±0.1	67.27±0.01
Seeds	6.2±0.04	5.67±0.15

Table 3. Major compositions of different parts of bitter gourd.

Components	Biter gourd		
	Skin	Seed	Flesh
Moisture (%)	87.4	87.2	87.5
Ash (%)	0.66	0.65	0.65
Vitamin-C (mg/100 mg)	108.66	31.32	120.22
Fiber (g/100 g)	1.9	1.7	1.8
Reducing sugar (%)	0	0.62	0.618
Non-reducing sugar (%)	0	0	0
Total sugar	0	0.62	0.618
AIM (%)	-	-	5.54
WIM (%)	-	-	7.03

was prepared without bitter gourd slices. The samples were coded as S_1, S_2, S_3 and C. The ingredients required for different formulation are shown in Table 1.

Preparation of dietetic bitter gourd juice

The firm and fresh bitter gourd were washed thoroughly and cut into 5 mm thick slices. Then sliced bitter gourds were taken in three conical flasks according to the formulation given in Table 1. All other ingredients were mixed separately and added to a flask containing bitter gourd slices. The top opening of the flasks were tightly closed with cotton bar. Then the samples were heated at previously optimized time (60 min) and temperature (90℃). After heating, the samples were brought to the room temperature and filtered using cheese cloth. After filtration, the samples were homogenized at high pressure (170 bar) for 3 passes. Potassium meta-bisulfate (KMS) at the rate given in Table 1 was added to the finished product. Finally the products were bottled, hot sealed and stored.

Chemical analysis

The fresh bitter gourds were analyzed for moisture, ash, vitamin-C, total soluble solids, fiber, reducing sugar, non-reducing sugar and total sugar content as per the methods of Ranganna (1992).

Sensory evaluation of bitter gourd juice

The consumer acceptability of bitter gourd was evaluated by a taste testing panel of 10 judges. Prepared bitter gourd juices were evaluated for color, flavor, texture and overall acceptability. The panelists were trained and selected from among the student, teachers and employees of the Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh. Prepared bitter gourd juices were marked as 1, 2, 3, 4 and 5 to make the evaluation unbiased. A small amount of bitter gourd juice was given to the panelists and asked to give marks of each sample according to their preference. The scoring was done on a continuous scale marked from 0 to 100%.

Statistical analysis

Data were analyzed to assess statistical differences among the treatments by using analysis of variance (ANOVA). Analyzed samples were ranked using Duncan's multiple range test (DMRT). All analyses were carried out in triplicates.

RESULTS AND DISCUSSION

Characterization of different parts of bitter gourd

All soluble compounds of fruits or vegetables usually play important roles in preparing juices. The distribution of these soluble compounds is not same in different parts like skin, flesh and seeds. Whole bitter gourd were divided carefully into its skin, flesh and seeds and expressed as percentage of its total weight. The results are presented in Table 2. According to Table 2, flesh is the highest part of bitter gourd, followed by skin and the lowest part are seeds. On an average, 67.27% of bitter gourd is flesh. Usually, most of the soluble polysaccharides like pectin, hemicelluloses and cellulose are located in the flesh, whereas skins are the good source of plant color like chlorophylls in case of bitter gourd. For preparing dietetic soft drinks, it is necessary to know details about the sugar content and fiber content of different parts of the raw materials used (bitter gourd). The major chemical compositions therefore, in different parts like skin, flesh and seeds of bitter gourd were analyzed and the results are presented in Table 3. Next

Table 4. Sequential extraction of alcohol insoluble materials.

Samples	Amount taken (mg)	Extracted (mg)	Recovery (%)	Percent based on recovery (%)
Alcohol insoluble materials (AIS)	100	-	-	-
Pectin	-	12±0.02	12	24
Hemicellulose	-	13±0.2	13	26
Cellulose		27.5±0.4	27.5	55
Total	100	52.5±0.03	52.5	

Table 5. Effect of heating time in releasing soluble solids at 50°C.

Extraction time (min)	Weight of solid (mg)	Percent of total weight (%)
30	6±0.02	0.6
60	10±0.3	1.0
90	8±0.25	0.8

to moisture, fiber is the major constituent of bitter gourd. Bitter gourd also contain a small amount of sugar and vitamin C. Skin contained the highest fiber (1.9%) and the lowest fiber was found in seed.

However, the variation in fiber content in skin, seeds and flesh of bitter gourd are much close. In case of total sugar, both seeds and flesh contained almost a similar amount of sugar and no sugar was found in the skin. It is interesting that bitter gourd did not contain any reducing sugar. Bitter gourd contained a fair amount of vitamin C and the highest amount was found in the flesh, followed by skin and the lowest amount was found in seeds. It is noticeable that bitter gourd is a good source of minerals and it was found almost the same (~0.65) in all the parts of bitter gourd. The result is similar to those obtained by Yuwai et al. (1991). The small variation may be due to varietals difference, agro-ecological condition and methods of analyses. As the major bitter gourd juice derives from flesh, the composition of the flesh especially that of the polysaccharide constituents plays an important role in extracting juice. Water insoluble materials (WIM) and alcohol insoluble materials (AIM) were considered as suitable preparations to describe flesh polysaccharides (Femenia et al., 2007). The WIM and AIM of bitter gourd flesh were prepared and the results are presented in Table 3. The yield of AIM isolated from bitter gourd flesh was 1.27 times less than that of WIM. The content of 5.54% AIM in bitter gourd flesh is relatively low compared to other fruits like pineapple, grape and pumpkin (Aziz et al., 2011; Femenia et al., 2007; Li et al., 2010).

Sequential extraction of AIM of bitter gourd pulp

AIM of the bitter gourd flesh was prepared and fractionated into pectin, hemicellulose, and cellulose as shown in Table 4. As shown in Table 4, 100 mg AIS was fractionated among which pectin, hemicellulose and cellulose contributed 12, 13 and 27.5%, respectively. The absolute recovery of total fraction corresponds to only 52.5%, among which the highest fraction was cellulose and its recovery was 27.5%. On the other hand, the pectic fraction showed the lowest recovery (12%). The yield of hemicelluloses and pectic fraction remained very close and each showed almost half of cellulose fraction. During sequential extraction, the yield of pectic and hemicelluloses fraction were less than that of cellulose fraction, which indicated that the soluble polysaccharides content in bitter gourd pulp was low. Overall yield of each fraction was only 52.5%. This might be explained by the loss during extraction and purification steps (Aziz et al., 2010).

Extraction of crude polysaccharides from bitter gourd

Though water insoluble material in bitter gourd pulp was more than that of alcohol insoluble materials, water extraction of polysaccharide components were preferred considering the cost of overall process. Water insoluble compounds of bitter gourd were extracted by heating at different temperature and time. The effect of heating time and temperature in releasing soluble compounds were optimized by conducting a series of experiments. The effect of heating time in releasing solid compounds is shown in Table 5. Table 5 indicated that the release of soluble solids increased with increasing heating time up to 60 min. Additional heating resulted in decrease of releasing solids. The accelerated rate of solid release up to 60 min as depicted in Table 5 was because of increasing solubility of water insoluble materials due to prolong heating. The decelerated rate of solid released with increasing heating beyond 60 min, might be due to

Table 6. Effect of heating temperature in releasing soluble solids for 60 min.

Extraction temperature (°C)	Weight of solid (mg)	Percent of solid (%)
50	10±0.01	1.00
70	15.3±0.4	1.53
90	17.3±0.01	1.73

Table 7. Mean sensory score of prepared bitter gourd drinks.

Responses	Sensory attributes*			
	Colour	Flavor	Taste	Overall acceptability
S_1	47±3[a]	47±14[a]	49±5[a]	50±7[a]
S_2	61±5[b]	58±11[b]	61±11[b]	60±6[b]
S_3	54±2[b]	57±9[b]	54±9[a]	57±5[a]
S_0	55±7[b]	55±0[b]	59±7[b]	57±7[a]

S_1 (extract from 5% slices), S_2 (7.5%) S_3 (10%) S_0 (0%), *having different suffix indicates statistically different at $P<0.05$.

high loss of bitter gourd cell tissues, which resulted in reverse migration of released solids. The effect of heating temperature in releasing soluble solids of bitter gourd flesh is shown in Table 6.

As shown in Table 6, with increasing heating temperature, solid content of the solution was also increased. The highest solid released was 1.73% at 90°C, followed by 1.53% at 70°C and the lowest was 1% at 50°C. The heating temperature was selected at 90°C, even though the optimum heating temperature was not achieved. The optimum point might be obtained if heating was continued further. Due to hydrolyzing tendency of soluble polysaccharides of fruits and vegetable at high temperature, heating temperature was not increased at or beyond 100°C.

Sensory evaluation of dietetic juices

Bitter gourd juices containing various levels of crude polysaccharides extracted from bitter gourd slices were subjected to sensory evaluation. The optimized heating conditions and acid-high sweeteners ratio were used to formulate drinks. The scores given by the panelists during sensory evaluation was described as the higher the scores the better was the color, flavor, taste and overall acceptability. The ANOVA results revealed that there were significant differences in colour acceptability among the bitter gourd drinks S_1 (extract from 5% slices), S_2 (7.5%), S_3 (10%) and S_0 (0%). The multiple range test results on mean values of each preferences showing statistical significance difference among the samples are shown in Table 7.

As shown in Table 7, it was observed that drinks prepared using 7.5% (S_2), secured the highest score concerning the color preference, followed by drinks containing no bitter gourd extract (S_0) and then followed by drinks with 10% slices (S_3), and the drinks of 5% slices (S_1) secured the lowest score. Except sample S_2, the obtained scores of all other samples were poor in comparison to the entire scale (100%). According to the scores, most of the panelists were confused in preferring the color of these samples. In case flavor preference, sample S_1 secured the lowest score and was not preferred by the panelists. The other samples were almost similar. Except sample S_1, there was no significant difference among these samples. As shown in Table 7, sample S_2 secured the highest score concerning the taste preference followed by samples S_0 and then followed by S_3 and the sample S_1 secured the lowest score.

Conclusion

Dietetic drinks containing crude polysaccharides extracted from 7.5% bitter gourd are well accepted and can be one possibility to increase the consumption of bitter gourd as anti-diabetic vegetable. However, the effectiveness of the drink still needs to be proven.

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