Effects of Drying on the Physicochemical and Functional Properties of Green Banana (*Musa sapientum*) Flour and Development of Baked Product

S. M. Asif-Ul-Alam, M. Z. Islam^{*}, M. M. Hoque, K. Monalisa

Department of Food Engineering and Tea Technology, Shahjalal University of Science & Technology, Sylhet-3114, Bangladesh *Corresponding author: zohurulislam.engg@gmail.com

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Abstract This study investigated the effects of hot air drying and freeze drying methods on physicochemical and functional properties of green banana (*Musa sapientum*) flour and incorporated biscuits. Both freeze and hot air dried green banana flour was replaced by wheat flour with different degrees of substitutions including 0, 10, 15, 20% and subjected to proximate analysis and sensory evaluation. The results of this study showed that freeze drying method retained high amount of protein, fat, ash and fiber content in green banana flour than hot air drying method. The moisture content was high in hot air dried banana flour than freeze dried flour. That's why the water holding capacity of hot air dried banana flour was high. The freeze dried banana flour had the higher foaming capacity (10.48%) as compared with hot air dried banana flour (8.61%). As the concentration of banana flour increased the spread ratio of biscuits decreased. The addition of increasing level of banana flour had higher ash, fiber, carbohydrate, calcium, phosphorus, iron and zinc content while protein and fat content found lower in the biscuits. In sensory analysis, 5% freeze dried biscuits hold the highest score in color, texture and overall acceptability; and 10% hot air dried secured highest score in flavor.

Keywords: banana flour, supplemented biscuits, freeze drying, hot air drying, functional properties, physicochemical properties, sensory quality

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1. Introduction

Green Banana flour (BF) is an alternative to reducing banana wastes and it is also a low cost material for food industry [1]. According to the available reports, green banana is rich in starch and its flour contains 61.3-76.5 g/100 g of starch (dry weight) and has high fiber content (6.3-15.5 g/100 g) (dry weight) [2]. It has been reported that a high dietary fiber intake has beneficial effects on human health [3]. As a result banana flour can be added into the baked product due to different flavor and texture. Most banana flour, produced from the green unripe fruit is sun dried or dried in crude ovens, under these conditions the quality of the product is very variable [3]. Natural sun drying reduce the qualitative and quantitative value of the dried products. Due to difficulties in controlling the drying conditions, specially temperature and time, "browning effect" (discoloration) was occurred [3]. Currently the market prefers high quality dried products with good reconstitution properties and excellent sensory attributes. Biscuits are one of the most widely consumed food products in all over the world. Biscuits are basically made from wheat flour, sugar, milk powder, baking powder and water. Traditional biscuits are claimed to lack other

essential nutritional components such as dietary fiber, vitamins and minerals which are lost during wheat flour refinement. Thus, biscuits which represent a major enduse of wheat is suitable for enhancing health after incorporating sources of fiber and essential nutrients [5]. The development of new products is a strategic area of the food industry. Consumers are interested in those foods which have the traditional nutritional aspects and which provide health benefits by regular ingestion. The major objectives of this study are i) to evaluate and compare the physicochemical and functional properties of flours produced from unripe banana fruit by the methods of hot air drying and freeze drying. ii) to develop dietary fiberenriched baked product such as biscuit by incorporation of banana flour with wheat flour in the formulations. iii) to study the effect of various levels of banana flour on quality, composition and sensory properties of prepared biscuits.

2. Materials and Methods

The experiment was conducted in the Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet, Bangladesh, 2013.

2.1. Materials

Fresh green bananas (*Musa sapientum*) were collected from local market. Then the sample was dried by using a hot air drier and freeze drier. The dried sample was turned into flour by using a blender. Other materials like refined wheat flour, sugar, fat etc. required for biscuit making were purchased from local markets.

2.2. Biscuit Formulation

The basic formulations used for preparation of biscuits are outlined in Table 1. The biscuits were prepared with the incorporation of hot air dried and freeze dried banana flour in 0, 5, 10, 15 and 20% concentration with wheat flour. The pre-weighed ingredients were mixed properly. The baking chemicals and sugar were dissolved in water. A mixture of raw materials was added to obtain a uniform dough and the dough was allowed to relax for 15 minutes before rolling out. The dough was then kneaded and rolled to a uniform thickness of 3 mm.

Table 1. Basic formulation of biscuit

Ingredients	Quantity (g)
Wheat flour [*]	100
Dalda	40
Sugar	40
Salt	0.5
Milk powder	5
Water	20
Baking powder	5

*Wheat flour was replaced with various levels of hot air dried and freeze dried banana flour by 5%, 10%, 15% and 20% in the formulation.

The rolled out dough was allowed to relax for 5 minutes. Then the biscuits were cut out with round biscuit cutter of 3.5 cm diameter. The cut out biscuits were placed on either greased pans or paper lined pans about 0.5 inches apart and the biscuits were allowed to rest in the pan about 10 minutes and baked at 220°C for 10-15 minutes, cooled to ambient temperature and packed in high density polyethylene bags.

2.3. Proximate Composition

Banana flours were analyzed for their physicochemical and functional properties. Particularly, the functional properties are required for the formulation of value added composite bakery products.

2.4. Physicochemical Properties

Protein (micro- Kjeldahl, \times 6.25), fat (Folch method), moisture, ash and crude fiber were determined by the AOAC (2004) [6] methods. The carbohydrate content was calculated by subtraction method. Calcium (Ca), Phosphorus (P), Iron (Fe) and Zinc (Zn) determined by using Atomic Absorption Spectrophotometer (Thermoscientific iCE 3000) and the calorific value was calculated.

2.5. Functional Properties

2.5.1. Water and Oil Absorption Capacity

The water and oil absorption capacities were determined by the method of Sosulski *et al.*, 1986 [7]. The sample (1.0 g) was mixed with 10 ml distilled water or refined soybean oil, kept at ambient temperature for 30 min and centrifuged for 10 min at $2000 \times g$. Water or oil

absorption capacity was expressed as percent water or oil bound per gram of the sample.

2.5.2. Bulk Density

The bulk density was determined according to the method described by Okaka and Potter [8]. The sample (50 g) was put into a 100 ml graduated cylinder and tapped 20-30 times. The bulk density was calculated as weight per unit volume of sample.

2.5.3. Swelling Capacity

The method of Okaka and Potter [8] with some modifications was used for determining the swelling capacity. The sample filled up to 10 ml mark in a 100 ml graduated cylinder was added with water to adjust total volume to 50 ml. The top of the graduated cylinder was tightly covered and mixed by inverting the cylinder. The suspension was inverted again after 2 min and allowed to stand for further 30 min. The volume occupied by the sample was taken after 30 min.

2.5.4. Foaming Capacity

Foaming capacity and foaming stability were determined as described by Narayana and Narasinga Rao (1982) [9] with slight modifications. Sample (1.0 g) was added to 50 ml distilled water at 30 ± 20 °C in a graduated cylinder. The suspension was mixed and shaken for 5 min to foam. The volume of the foam after whipping for 30 Sec was expressed as foaming capacity

2.5.5. Emulsifying Activity

Emulsifying activity was determined as described by Okaka and Potter [8]. The sample (0.5 g) was added to 10 ml distilled water and shaking the mixture for 3 min in a centrifuge tube. The mixture was made up to 12.5 ml by adding oil and homogenized for 3 min. The resulting emulsion was centrifuged at 2000 RPM for 5 min and then the emulsion volume was measured.

2.6. Physical Analysis of Biscuits

Diameter of biscuits was measured by laying three biscuit edge to edge with the help of a scale rotating them 90° and again measuring the diameter of three biscuits (cm) and then taking an average value. Thickness as measured by stacking three biscuits on top of each other and taking average thickness (cm). Weight of biscuits was measured as average of values of three individual biscuits with the help of digital weighing balance. Spread ratio was calculated by dividing the average value of the diameter by average value of thickness of biscuits. Percent spread was calculated by dividing the spread ratio of supplemented biscuits with a spread ratio of control biscuits and multiplying by 100.

2.7. Sensory Analysis of Biscuits

Biscuit samples were analyzed for sensory characteristics. Sensory quality characteristics were evaluated by a panel of 10 semi-trained members using a 9-point Hedonic scale where 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 =neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely. The biscuits were evaluated for their color, flavor, texture and overall acceptability.

2.8. Statistical Analysis

Data was analyzed using analysis of variance (ANOVA) to determine significant differences among the various samples in triplicate using the software, statistical package for social science (SPSS) version 11.00 SPSS inc., Chicago, IL, USA at the 0.05 level.

3. Results and Discussion

3.1. Proximate Composition of Banana Flours

Table 2 shows that the proximate composition of the banana flour samples. A noticeable difference was observed in moisture, ash, crude fiber, carbohydrate, protein, calcium, phosphorus, iron and zinc content of hot air dried and freeze dried banana flour. Freeze dried banana flour showed the highest value in all examined parameters except in moisture. In addition, textural quality, chemical and biochemical reactions, as well as microbial growth rates are greatly affected by the moisture content of food products [10]. The research article [11] concluded that banana pseudo-stem flour is a potential functional food ingredient for products containing high dietary fiber. According to [12] moisture, fat and ash content of modified banana flour were significantly lower than banana flour. This is due to heat, moisture, and autoclave treatment of modified banana flour that had an impact on the physicochemical properties of flour.

Component	Hot air dried	Freeze dried banana
Component	banana flour	flour
Moisture (%)	14.31±0.6	9.33±0.06
Protein (%)	3.19±0.08	3.95±0.2
Fat (%)	0.50±0.05	1.31±0.07
Ash (%)	1.20±0.09	2.55±0.07
Fiber (%)	4.2±0.1	4.9±0.3
Carbohydrate (%)	80.80±0.5	82.86±0.4
Calcium (mg)	32±0.2	35±0.1
Phosphorus (mg)	93±0.4	94±0.3
Iron (mg)	2.6±0.1	2.7±0.1
Zinc (mg)	0.18±0.1	0.19±0.1

Table 2. Proximate composition of banana flours

Results are expressed as mean values and standard deviation of three replicates

3.2. Functional Properties of Flours

The functional properties of flours play an important role in the manufacturing of bakery products. The hot air dried banana flour and freeze dried banana flour were analyzed for their functional properties. Table 3 shows the various functional properties of flours. The water holding capacity of freeze dried flour was found lower than that of hot air dried flour. The oil absorption capacity (OAC) of hot air dried flour was higher than that of freeze dried flour. The oil absorption capacity (OAC) of flour is equally important as it improves the mouth feel and retains the flavor. The swelling capacities of hot air dried flour and freeze dried flour are 2.6 and 6.01 (g paste\g dry sample) respectively. The foaming capacity of freeze dried flour was higher than that of hot air dried flour. Foaming capacity is assumed to be dependent on the configuration of protein molecules. Flexible proteins have good foaming

capacity, but a highly ordered globular molecule gives low foam's ability [13]. Emulsifying activity was also higher in hot air dried flour. The bulk density of hot air dried flour was 0.53 g/ml, higher than that of the freeze dried flour (0.36 g/ml). The yield of freeze dried flour (25.22%) was higher than that of hot air dried flour (22.21%).

Table 3.	Functional	properties	of flours
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Table 5. Functional properties of nours								
Property	Hot air dried banana flour	Freeze dried banana flour						
Water holding capacity (g water\ g dry sample)	3.01±0.28	2.26±0.11						
Oil holding capacity (g oil\ g dry sample)	0.75±0.08	0.52±0.06						
Swelling capacity (g paste\g dry sample)	2.6±0.45	6.01±0.09						
Foaming capacity%	8.61±0.28	10.48±0.08						
Emulsifying activity%	1.67±0.06	0.8±0.04						
Bulk density (g\ml)	0.53±0.03	0.36±0.03						
Yield%	22.21±0.80	25.22±0.22						

Results are expressed as mean values and standard deviation of three replicates

3.3. Proximate Composition of Biscuits

The proximate composition of biscuits is shown in Table 4. In the present study several types of biscuits, one containing 0% banana flour and the other containing different amount of hot air and freeze dried banana flour were prepared and analyzed for their composition. Moisture content of freeze dried banana flour incorporated biscuits was less than the hot air dried banana flour biscuits, due to less moisture content of freeze dried banana flour. Protein content of banana flour incorporated biscuits was less than the control (0% banana flour), because banana flour contain less amount of protein. According to Bonazzi C. & Dumoulin E [14] the biological value of dried proteins varies with the drying procedure. Prolonged exposures to high temperatures can affect the functional properties or render the protein less useful in the diet. The fat content of control biscuits was 20.5% and it decreased to 17.21% in 20% hot air dried banana flour biscuits and 18.51% in 20% freeze dried banana flour biscuits. The fiber content of control biscuits was 0.97%, which increased to 2.86% in 20% hot air dried banana flour biscuits and 3.05% in 20% freeze dried banana flour biscuits. This was due to the high fiber content in banana flour, and freeze dried banana flour contain higher amount of fiber than hot air dried flour. According to [15] total dietary fiber content of the wholemeal bread with 7% banana peel flour was higher (14.4%) than the control sample (11.3%). The carbohydrate content was determined by difference method was found to be higher in 20% hot air dried banana flour biscuits. Beside this, Calcium (Ca), Phosphorus (P), Iron (Fe) and Zinc (Zn) increased with increasing % of banana flour incorporation. Freeze dried banana flour biscuits showed higher amounts of observing minerals than hot air dried. From the standpoint of nutritional quality the sample with 0% banana flour had higher protein and fat content. But the fiber content was high in 20% freeze dried banana flour biscuits. Results showed that 5 % freeze dried banana flour biscuits had the highest amount of energy (488.58 Kcal), and 20% hot air dried banana flour biscuits had the lowest amount of energy (473.37 Kcal). The moisture, ash, protein, fat and total carbohydrate contents of biscuits were more or less

similar to those reported by [16]. Banana flour and modified banana flour was found to be acceptable in bread

formulations at 20% substitution [12].

			Table 4. Pr	oximate compo	osition of bisc	uits			
Parameter	Control	Bisc	uit with hot air	dried banana fl	our	Bisc	uit with freeze	dried banana f	lour
Parameter	SO	S1	S2	S3	S4	S5	S6	S7	S8
Moisture (%)	3.22 ± 0.2	2.71±0.2	2.50±0.1	2.15±0.1	2.00±0.0	2.11±0.2	1.96±0.15	1.74±0.2	1.66±0.1
Protein (%)	9.37±0.01	9.03±0.2	8.75±0.1	8.31±0.2	7.98±0.4	9.12±0.01	8.95±0.00	8.41±0.14	8.13±0.24
Fat (%)	20.5±0.1	19.09±0.15	18.78±0.04	18.11±0.08	17.21±0.1	20.10±0.29	19.85±0.1	19.03±0.02	18.51±0.5
Ash (%)	0.60±0.01	0.79±0.04	0.98±0.3	1.10±0.2	1.17 ± 0.08	0.87±0.04	1.04±0.25	1.21±0.31	1.30±0.1
Fiber (%)	0.97±0.01	2.01±0.05	2.39±0.04	2.71±0.1	2.86±0.16	2.21±0.2	2.57±0.25	2.90±0.4	3.05±0.17
Carbohydrate (%)	66.31±0.1	68.38±0.01	68.99±0.3	70.33±0.17	71.64±0.2	67.8±0.04	68.2 ± 0.08	69.61±0.01	70.4±0.03
Energy (Kcal)	486.94±2.0	481.45±2	479.98±3	477.55±2	473.37±2	488.58±4	487.25±4	483.35±3	480.71±2
Calcium (mg)	18.35±0.02	25±0.03	28±0.05	31±0.04	34±0.04	26±0.03	28±0.03	33±0.02	34±0.03
Phosphorus (mg)	113.65±0.2	170±0.4	212±0.3	290±0.5	365±0.4	177±0.2	215±0.3	299±0.3	371±0.3
Iron (mg)	1.50 ± 0.01	2.01±0.01	2.52 ± 0.02	3.00±0.01	3.5±0.01	2.02±0.01	2.52 ± 0.01	3.02±0.01	3.62±0.01
Zinc (mg)	0.84 ± 0.01	1.23±0.01	1.72±0.02	2.11±0.01	2.54 ± 0.01	1.25±0.01	1.74 ± 0.01	2.12±0.01	2.55±0.01

 $S_0=0\% \text{ BF (control)}, S_1=5\% \text{ ABF}, S_2=10\% \text{ ABF}, S_3=15\% \text{ ABF}, S_4=20\% \text{ ABF}, S_5=5\% \text{ FBF}, S_6=10\% \text{ FBF}, S_7=15\% \text{ FBF}, S_8=20\% \text{ FBF}.$ The values are mean ±S.D of three independent determinations.

Table 5. Physical properties of biscuits

Parameter	Control	Biscuit with hot air dried banana flour				Bis	Biscuit with freeze dried banana flour			
Parameter	S_0	S_1	S_2	S ₃	S_4	S ₅	S_6	S ₇	S_8	
Diameter (cm)	4.38±0.01	4.21±0.06	4.21±0.03	4.27±0.18	4.28±0.16	4.09±0.06	4.14±0.16	4.17±0.02	4.19±0.15	
Thickness (cm)	0.45±0.15	0.51±0.04	0.52±0.07	0.58±0.02	0.59±0.02	0.42±0.01	0.44±0.01	0.48±0.02	0.49±0.02	
Weight (g)	4.52±0.08	5.74±0.59	6.05±0.60	6.23±0.38	6.45±0.49	4.40±0.06	4.54±0.24	5.08±0.05	5.5±0.09	
Spread ratio	9.73±0.21	8.25±0.75	8.09±0.62	7.36±0.12	7.25±0.43	9.73±0.33	9.40±0.29	8.68±0.42	8.55±0.61	
% spread	100	84.78	83.14	75.64	74.51	100	96.60	89.20	87.87	

 $S_0 = 0\% BF (control), S_1 = 5\% ABF, S_2 = 10\% ABF, S_3 = 15\% ABF, S_4 = 20\% ABF, S_5 = 5\% FBF, S_6 = 10\% FBF, S_7 = 15\% FBF, S_8 = 20\% FBF. The values are mean <math>\pm S.D$ of three independent determinations

Table 6. Mean sensory scores of formulating biscuits	g biscuits	lating	formul	of f	scores	sensory	Mean	ole 6.	Ta
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Parameter	Biscuit type	Original order of mean	Biscuit type	Ranked order of mean	Sig. (p<0.05)
	S ₀	7.30ª	S ₀	7.30 ^a	
	S1	7.10 ^a	S ₁	7.10 ^a	
	S_2	6.60 ^{ab}	S ₂	6.60 ^{ab}	0.006
	S ₃	6.30 ^b	S ₃	6.30 ^b	
C 1	S_4	6.20 ^b	S_4	6.20 ^b	
Color	S_0	7.30 ^a	S_0	7.30 ^a	
	S ₅	7.20 ^a	S ₅	7.20 ^a	
	S_6	7.10^{a}	S_6	7.10 ^a	0.000
	S ₇	6.60^{ab}	S ₇	6.60 ^{ab}	0.008
	S ₈	6.20 ^b	S ₈	6.20 ^b	
	S_0	7.80^{a}	S ₀	7.80 ^a	
	S1	7.00 ^b	S ₂	7.20 ^{ab}	
	S_2	7.20 ^{ab}	S1	7.00 ^b	0.001
	S ₃	6.70 ^{bc}	S ₃	6.70 ^{bc}	
Flavor	S_4	6.20 ^c	S_4	6.20 ^c	
Flavor	S_0	7.10 ^a	S_0	7.81 ^a	
	S ₅	7.10^{a}	S ₆	7.30 ^a	
-	S_6	7.30 ^a	S ₅	7.10 ^a	0.888
	S ₇	7.00^{a}	S ₇	7.00 ^a	
	S ₈	7.00^{a}	S ₈	7.00 ^a	
	S_0	7.80^{a}	S ₀	7.81ª	
	S1	6.60 ^b	S1	6.60 ^b	
	S_2	6.50 ^b	S_2	6.50 ^b	0.003
	S ₃	6.30 ^b	S ₃	6.30 ^b	
Texture	S_4	6.20 ^b	S_4	6.20 ^b	
Texture	S_0	7.81 ^a	S ₀	7.81ª	
	S ₅	7.54 ^a	S ₅	7.54ª	
	S_6	6.62 ^b	S ₆	6.62 ^b	0.002
	S ₇	6.20 ^b	S ₇	6.20 ^b	
	S ₈	6.62 ^b	S ₈	6.62 ^b	
	S_0	7.60^{a}	S ₀	7.63 ^a	
	S1	6.20 ^c	S ₂	7.20 ^{ab}	
	S ₂	7.20 ^{ab}	S ₃	$6.80^{ m abc}$	0.013
	S ₃	6.80 ^{abc}	S_4	6.70 ^{bc}	
Overall	S_4	6.70 ^{bc}	S ₁	6.20 ^c	
Acceptability	S ₀	7.60^{a}	S_0	7.60^{a}	
-	S ₅	7.30 ^{ab}	S ₅	7.30 ^{ab}	
	S_6	6.70 ^{bc}	S_6	6.70 ^{bc}	0.001
	S ₇	6.20 ^c	S ₇	6.20 ^c	1
	S ₈	6.20 ^c	S ₈	6.20°	7

The values are mean \pm S.D of three independent determinations. The means with different superscripts in a row differ significantly (p \leq 0.05). S₀= 0% BF (control), S₁= 5% ABF, S₂= 10% ABF, S₃= 15% ABF, S₄= 20% ABF, S₅= 5% FBF, S₆=10% FBF, S₇=15% FBF, S₈= 20% FBF.

3.4. Physical Properties of Biscuits

The physical properties of biscuits prepared from hot air dried banana flour and freeze dried banana flour are shown in Table 5. Among the biscuit samples, the control biscuit had the highest diameter. Freeze dried flour biscuits had less diameter than hot air dried flour biscuits. The diameter of biscuit sample decreased from 4.38-4.28 cm with 0-20% hot air dried banana flour respectively. The diameter of biscuit sample decreased from 4.38-4.19 cm with 0-20% freeze dried banana flour respectively. The thickness of biscuit ranges from 0.42-0.59 cm. The hot air dried flour biscuits were thicker than freeze dried flour biscuits. Biscuits which contained 20% hot air dried flour had a higher thickness (0.59 cm), and 5% freeze dried flour biscuits had a lowest thickness (0.42 cm). The change in diameter and thickness were reflected in spread ratio and % spread of biscuits. The spread ratio and percent spread of control biscuits were 9.73 and 100 respectively. Spread ratio and percent spread decreased with the addition of banana flour. But these parameters decreased highly in hot air dried flour biscuits than freeze dried flour biscuits. Other researchers also reported reduction in the spread ratio when soy flour and fenugreek flour were substituted for wheat flour [17]. At 0-20% addition hot air dried flour, the weight of the biscuit increased gradually from 4.52 to 6.45 g. But freeze dried flour biscuits were lighter in weight than hot air dried flour biscuits. 5% freeze dried flour biscuit had less weight (4.40 g) than control biscuits (4.52 g).

3.5. Sensory Characteristics of Biscuits

The sensory characteristics of biscuits prepared from hot air dried banana flour and freeze dried banana flour are shown in Table 6. The analysis indicated that there were significant differences in color, flavor, texture and overall acceptability between control biscuits and biscuits containing hot air and freeze dried banana flour ($p \le 0.05$) except flavor in freeze dried biscuits. With the increasing level of banana flour in the formulation, the sensory score for color, texture and overall acceptability of biscuits decreased. The texture of control biscuits and 10% freeze dried banana flour biscuits were statistically similar. By comparing the parameters (color, flavor, texture and overall acceptability) between freeze dried banana flour and hot air dried banana flour biscuits 5% freeze dried banana flour biscuits was superior in color and texture, and 10% freeze dried banana flour biscuits were superior in flavor. In overall acceptability 10% hot air and 5% freeze dried banana flour containing biscuits attained higher score.

4. Conclusion

The present study demonstrated that different drying techniques for the preparation of green banana flour noticeably change the nutritional and functional properties of banana flour. Nutritional composition was slightly higher in freeze dried banana flour than hot air dried banana flour. The study showed that green banana flour can be successfully incorporated in wheat flour biscuits up to a level of 20% to yield biscuits of enhanced nutritional quality with acceptable sensory attributes. However the hot air dried green banana flour incorporated biscuits had less nutritional and sensory attributes than freeze dried green banana flour incorporated biscuits. This was due to high retention of nutrient in freeze dried banana flour. Hence, development and utilization of such functional foods will not only improve the nutritional status of the population, but also helps those suffering from digestion disorder. The finding of this study may help generate technology to diversify the use of green banana flour by the food processing enterprises, specially baking industries. More studies should be conducted to investigate the possibility of using green banana flour as an ingredient in other food products in order to increase applications of such value-added food ingredient. To improve the nutritional quality of green banana flourbased biscuits, high protein soy flour or nut pieces or flavor can be incorporated in the formulation.

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