Formulation and Standardisation of Banana Peel Extracted Insoluble Dietary Fibre Based Buns

Nidhi Budhalakoti1*

1Centre of Innovative and Applied Bioprocessing, Sector-81 (Knowledge City), PO Manauli, S.A.S Nagar, Mohali-140306, Punjab, India.

Author’s contribution
The sole author designed, analysed, interpreted and prepared the manuscript.

ABSTRACT

Banana peels are the waste products of banana industries which can be the cause of environmental pollution if not utilized properly. These peels are generally used as cattle feed; their utilization in human diet is not studied much. It is a rich source of dietary fibre which is considered to be beneficial in protection against several diseases. It is also rich in other nutrients such as proteins, fats etc. Keeping in view with the importance of dietary fibres in diet, in the present study efforts were made to extract banana peel insoluble dietary fibres and incorporate them into an edible bakery product i.e. buns. Nutritional profile of both the banana peels and the fibres was evaluated. Banana peel insoluble dietary fibres were found to be rich in various nutrients including antioxidants. Buns prepared from the extracted insoluble dietary fibres were subjected to texture analysis. Process of preparing the buns was optimized and further put into testing. Fibres were incorporated up to 40%. Buns containing up to 10% fibres were found to be more palatable and texturally acceptable.

Keywords: Banana peels; banana peel insoluble dietary fibre; nutritional profile; buns; texture analysis.
1. INTRODUCTION

India is the leading producer of bananas in the world with an annual output of about 16,820 mt. There are several by-products of banana plants which can be an excellent source of highly valuable raw materials for various food and non-food applications. Banana peels are the major waste product of the banana industry that causes an environmental problem. Incorporation of banana peels to food products might be an alternative way of value-adding to this waste.

Dietary fibre plays a major role in human diet. Dietary fibre is a type of carbohydrate that cannot be digested by our bodies’ enzymes. It is found in edible plant foods such as cereals, fruits, vegetables, dried peas, nuts, lentils and grains. Fibre is grouped by its physical properties and is called soluble, insoluble or resistant starch. It has a great potential in the preparation of functional foods. It is considered to be beneficial in the prevention of several diseases including cardiovascular diseases, diverticulosis, constipation, irritable colon, colon cancer, and diabetes [1]. The fruit fibre has a better quality than other fibre sources due to its high total and soluble fibre content, water and oil holding capacities, and colonic fermentability, as well as a lower phytic acid and caloric value content [2]. A high dietary fibre content of banana peel (about 50 g/ 100 g) is indicative of a good source of dietary fibre [3]. Usually banana peels are used in feed for animals. According to Wadhwa and Bakshi [4] banana peels can be incorporated at levels of 15 to 30 percent in the diet without affecting palatability and performance of lactating cows. In the present study its applicability to human diet, specifically the extracted dietary fibres has been evaluated with their incorporation into bakery product i.e. buns.

2. MATERIALS AND METHODS

The present investigation was undertaken with the objectives of extraction of banana peel dietary fibers, evaluating the nutritive value of banana peels and banana peel fibers, formulating and standardization of buns incorporated with banana peel fibers.

2.1 Procurement of Raw Materials

Banana peel samples, refined wheat flour and other ingredients were procured from the local market of Mohali (Punjab). Chemicals were procured from Sigma-Aldrich (St. Louis, MO, USA) and Merck Chemicals.

2.2 Processing of Banana Peels

Banana peels were thoroughly cleaned to remove dust, dirt and other foreign materials. Banana peels were washed under running water and then dried in oven at 55°C for 12 hrs. The fully dried peels were subjected to grinding in hammer mill (Polymix Px-MFC 90D by Kinematica). The mesh size of sieve was 1.5 mm.

2.3 Determination of Nutrient Composition

In the present study, the banana peels were examined for their dietary fibre content and also for the estimation of their nutritive value. Nutritive analysis included percentages of moisture, ash, fat, protein and carbohydrate, total starch, oxalate content, cellulose content, soluble and insoluble dietary fibres content, estimation of minerals included calcium and phosphorous content besides antioxidant and total phenol content were also estimated. Physico-chemical properties like water and oil holding capacity of banana peels were also estimated. Besides, the extracted banana peel insoluble dietary fibre was estimated for moisture, ash, fat, protein, carbohydrate, starch, phosphorus, calcium, total phenol content, oxalate, water and oil holding capacity. Following methods were used for estimation of the above nutrients:

2.3.1 Moisture

Moisture was determined by using AOAC [5]. Two gram sample was taken in a clean, dried (at 130±3°C for 20 min) and weighed aluminium dish. The content was dried in oven at 130±3°C for 1 hour till a constant weight was obtained and cooled in a dessicator. After cooling, the loss in weight was taken as moisture content and expressed in terms of percentage.

2.3.2 Ash

The ash content was determined using AOAC [5] method. Weighed 5g sample was charred in preheated and pre weighed porcelain dish, cooled in a dessicator and incinerated in muffle furnace at 550°C till the ash became white or grayish white in color and subsequently cooled in a dessicator and weighed soon after reaching
room temperature. Ash content was calculated as the difference between the final and initial weight of porcelain dish and results were expressed in percentage.

2.3.3 Protein

Protein was estimated using Bradford assay. Protein from the sample was extracted using 5% phenol as a solvent. 10g sample was extracted with 5% phenol via stirring for 2 hours. The sample was centrifuged at 8000 rpm for 30 min. The sample was then filtered. The supernatant was then used for the estimation of protein in the sample using Bradford method. Various concentrations of standard protein solutions were also prepared. Absorbance was measured at 595 nm.

2.3.4 Fat

Fat was estimated using hexane as a solvent. The solvent to sample ratio was 6:1. The sample was soaked in hexane for 24 hrs. Then the solvent was evaporated using flash evaporator.

2.3.5 Carbohydrate

Carbohydrate was estimated by using Hedge and Hofreiter [6] method with some modifications. One hundred grams sample was hydrolysed for 3 hrs. in a boiling water bath with 5.0 ml of 2.5 N HCl and cooled to room temperature. Then the sample was neutralized with sodium carbonate until effervescence ceased and the volume was made up to 100ml and centrifuged. After centrifugation the supernatant was collected and 0.2 and 0.1 ml aliquot was used for analysis. Glucose was used as a standard. Four ml of anthrone reagent was added to the sample and standard (0.2-1.0 ml) and heated for eight minutes in a boiling water bath, then cooled rapidly and the absorbance of green to dark green colour was taken at 630 nm.

2.3.6 Oxalate

Oxalates were estimated by the method given by Baker [7] with some modifications. Five gram of well ground sample was added in 100 ml of 2N HCl and the mixture was shaken well for about 2 hours. It was then boiled for about 15 minutes and cooled. This mixture was transferred to 100 ml volumetric flask and diluted to mark with 2N HCl. It was shaken well and filtered. A 25 ml of filtrate was pipetted into a flask and 5 ml phosphoric tungstate reagent was added. It was stirred well and kept overnight. The next day, it was centrifuged and filtered. A 20 ml of filtrate was transferred to a 50ml centrifuge tube and 2 or 3 drops of methyl red was added. The solution was neutralized with ammonia followed by addition of 5 ml of CaCl2 buffer and stirred well. The solution was allowed to stand overnight. The next day, it was filtered through filter paper and washed free from chloride using distilled water (Silver nitrate test). The precipitate along with the filter paper was transferred to the same beaker and 5 ml of 2N H2SO4 was added. The mixture was heated to 80°C and titrated against N/100 KMnO4 solution.

2.3.7 Total phenolic content

Total phenolic content of wheat flour, barley flour, tomato, carrot and spinach powders was estimated by the method given by Singleton and Rossi [8] with some modifications. Dried ground sample (100 g) was extracted with 80 per cent methanol (500ml) in soxhelet. The extract was concentrated by evaporation to yield a gummy concentrate. A 0.5 g of this methanolic extract was taken in 100ml volumetric flask and made up to 100ml with distilled water. From the above solution 0.1 ml was pipetted out into 10 ml tube and added 1.5 ml of Folin-Ciocalteau reagent, 4ml of 20% sodium carbonate solution and made up to 10 ml with distilled water. After 30 min absorbance was measured at 737nm.

2.3.8 Total antioxidant activity

Antioxidant activity was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method of Brand-Williams et al. [9] with some modifications. Ten gram of sample was homogenized with 15 ml of 80% methanol. The homogenate was filtered through four layers of cheesecloth and the residue was treated with 15 ml of 80% methanol for two successive extractions. The filtrate was combined and centrifuged at 4000 rpm for 10 minutes. The supernatant of methanol extract was collected and diluted to various concentrations (1%, 2.5%, 5%, 7.5% and 10%) for measurement of total antioxidant activity. After the samples at various concentrations were studied, the concentration representing 5g of original sample weight was chosen as an appropriate concentration for assessing antioxidant activity of the sample. A 0.1 mM solution of DPPH in methanol was prepared and 4 ml of this solution was treated with 0.2 ml of diluted extract. A control was treated with 0.2 ml of
distilled water instead of the extract. The mixture was left to stand for 60 minutes before the decrease in absorbance at 517 nm was measured.

2.3.9 Total dietary fibre

Dietary fibre in cereal flours and vegetable powders was determined by the method given by Asp and Johansson [10] with some modifications. The sample (800 mg) was added to 50 ml distilled water and boiled for 15 minutes in order to gelatinize the starch molecules. Then 50 ml of 0.2 N HCl was added to the flask. Physiological enzyme pepsin (100 mg) was added to the sample and incubated for 18 hours at 40°C. After incubation was completed, neutralization with NaOH was done to bring pH to 7. Then 50 ml of 0.1M sodium phosphate buffer was added to maintain the pH up to 6.8 and 3ml of DMSO was added to the sample followed by incubation and agitation with pancreatin (100 mg) for 1 hour at 40°C. The acidification of the mixture up to the pH of 4-5 was done with the help of HCl. The sample was then centrifuged for 30 minutes at 3000 rpm and filtered using gooch crucibles. The sediment was first washed with water three times and then with acetone. The residue with acetone was transferred to weighed crucibles and dried overnight at 105°C. The crucible was weighed for the insoluble dietary fibre content of the sample.

The supernatant and the first water wash was collected and concentrated to 150 ml. The aliquot was precipitated with 4 volume of 95% ethanol. The sample was then centrifuged at 3000 rpm. The sediment was washed 3 times with 50 ml acetone. The sample was oven dried at 50-60 °C for 1 hour. Thus soluble dietary fibre was obtained by weighing the crucible.

2.3.10 Phosphorus

Estimation of phosphorus was done by method of Fiske and Subbarow as given in Ranganna [11] with some modifications. To 5 ml of ash solution obtained by dry ashing, 5 ml of molybdate reagent was added and mixed. Two ml of aminonaphtholsulphonic acid solution was added, mixed and the volume was made up to 50 ml. This was allowed to stand for 10 min. Similarly, a blank was prepared using water in place of sample. Thereafter the color was measured at 650 nm in a spectrophotometer.

2.3.11 Calcium

Calcium content in the sample was determined titrimetrically by method of AOAC [5]. A 10 ml of aliquot of ash solution was taken in 250 ml conical flask to which 8 to 10 drops of bromocresol green indicator was added. Thereafter sufficient 20 per cent sodium acetate was added until the solution was blue (pH at this point is 4.8 -5.0). The solution was heated and 3 per cent oxalic acid was added drop wise to bring the pH to 4.4 to 4.6 (as indicated by green color of the solution), which is optimum for the precipitation of calcium oxalate. The solution was again heated for 1 minute and allowed to stand until clear or overnight and filtered through filter paper. Individual conical flask precipitate was filtered and filter papers were washed with about 50 ml of dilute ammonium hydroxide solution using small portions at a time. Finally, the filter paper was washed with 125ml distilled water and 5 ml concentrated H2SO4. The tip of the filter paper was broken and the washings were collected in original flask, heated at 90°C and titrated while hot with 0.1 N potassium permanganate solution. The filter paper was also added to conical flask, and titration was completed at the end of a stable faint pink colour. A blank determination was also carried out using distilled water in place of ash solution and corrections were made.

2.3.12 Cellulose

Cellulose content was estimated using anthrone method given by Updegraff [12]. Three ml nitric acid was added to 1 g sample in a test tube and mixed in a vortex mixture. The test tube was then placed in a water bath at 100°C for 30 min. The test tube was then cooled and the contents were centrifuged for 15-20 min. The supernatant was discarded and the residue was washed with distilled water. Ten ml of 67% sulphuric acid was then added to the sample and this mixture was then allowed to stand for 1 hr. One ml of the same solution was then diluted to 100 ml with distilled water. To 1 ml of the diluted solution 10 ml anthrone reagent was added and mixed well and this was kept in a boiling water bath for 10 min. A blank was set with water. Cellulose was used as a standard and series of volumes was made and anthrone was used for colour development. Absorbance was taken at 630 nm.

2.3.13 Total starch

Anthrone method was used for total starch analysis, for this about 0.1 to 0.5 g of the sample
was homogenised in hot 80% ethanol to remove sugars for 1 hr. This was centrifuged and the residue was retained. The residue was repeatedly washed with 80% ethanol till the washings do not give colour with anthrone reagent. The residue was dried over a water bath. To the residue 5.0 ml of water and 6.5 ml of 52% perchloric acid was added. The residue was extracted for 20 min at 0 °C and was centrifuged (10,000 rpm) and the supernatant was saved. The extraction was repeated using fresh perchloric acid and centrifuged. The supernatants were pooled and the volume was made up to 100 ml. About 0.1 or 0.2 ml of the supernatant was pipetted out and the volume was made up to 1 ml with water. The glucose standards were prepared and 4 ml of anthrone reagent was added to each tube. This was heated for eight minutes in a boiling water bath and was cooled rapidly and the intensity of green to dark green colour was read at 630 nm.

2.3.14 Water and oil absorption capacity

Water and oil absorption capacity was determined by method given by Smith and Circle [13] with some modifications. Five gram sample was mixed with 30 ml distilled water or oil and mixture was allowed to stand for 10 minutes. It was centrifuged at 2000 rpm for 5 minutes to decant the supernatant. The volume of supernatant was determined.

2.4 Extraction of Dietary Fibre

Gravimetric method with water as solvent was used for the extraction of dietary fibre. Banana peels were washed thoroughly and water was added to the sample. The entire mixture was then placed in water bath at 90°C for 4 hrs with constant stirring. The mixture was then strained with the help of a muslin cloth. The residue consisted of insoluble dietary fibres while the supernatant was used for the precipitation of soluble dietary fibres with the help of ethanol.

The insoluble dietary fibre was used for nutrient analysis namely protein%, carbohydrate%, phosphorus, calcium (mg/100 g), water and oil holding capacity etc. It was also used to substitute refined wheat flour for the development of dietary fibre based buns. Water was used for the extraction of the dietary fibre as it is a neutral solvent.

2.5 Product Formulation

Buns were prepared by substituting banana peel fibres with refined wheat flour. Blends substituting refined wheat flour (30 g) with banana peel fibre up to 40 and 10% were prepared. Other ingredients included sugar 5 g, yeast 5 g, milk powder 5 g, water 20 ml and oil 10 ml or 5 g butter, 50 mg salt. Refined wheat flour and banana peel fibre were sieved and a uniform blend was made. Yeast was dissolved in a little amount of luke warm water and little amount of blend mixture was added to it. This mixture was kept for proofing at 30°C for 1½ hours. Sugar was added to it. Remaining mixture was added to it and kneaded to soft smooth dough. Dough was again punched for few minutes. Dough was sheeted, rolled and moulded. Then it was placed in greased sheet, covered with a wet cloth and allowed to rise in tin under 30°C. Water was sprayed on the bun surface before putting it on oven. Bun was baked at 200°C for 10 minutes. After 10 minutes, bun was taken out from oven and allowed to cool.

2.6 Texture Analysis

Texture analysis of the buns was conducted on TA.HD plus texture analyser by stable micro systems with load cell 5 kg. Pre-Test Speed, test speed, post test speed, target mode, distance, time and trigger force of the cylindrical probe was 5.00 mm/sec, 2.00 mm/sec, 5.00 mm/sec, 5.00 sec and 10g respectively.

2.7 Statistical Analysis

Two-way ANOVA without replication was applied in order to measure the statistical differences using a Microsoft Excel Programme package.

3. RESULTS AND DISCUSSION

The nutrient composition of banana peels and banana peel insoluble dietary fibre has been presented in Tables 1, 2 and 3. Singanusong et al. [14] reported the water retention and oil retention capacity of Banana Peel Cellulose to be 2.91 g water/g and 0.08 g oil/g dried sample respectively which is lower than the value reported in the present study. Oxalate content in banana peels was found to be 0.51 mg/g [15]. Happi Emaga et al. [3] reported the cellulose content of banana peel to be 7 to 12 g/100 g. In the present study overall soluble and insoluble dietary fibre content of banana peel was found to
be 18.75 and 42.5 g/100 g respectively. The total dietary fibre content was 61.25 g/100 g. Banana peel are a good source of lignin (6-12%), pectin (10-21%), cellulose (7.6-9.6%), hemicelluloses (6.4-9.4%) and galactouroninc acid [16]. Wachirasiri et al. [17] reported that the total dietary fibre content of banana peel was 50.25±0.2 g/100 g. Gonzalez-Montelongo et al. [18] reported TPC of 12–19 mg GAE/g-dw in the methanolic extracts obtained from banana peels for two different varieties. The total phenolic content of banana peels and banana insoluble dietary fibre in the present study was 19.82 and 9.25 mg GAE/100 g.

The moisture%, ash % and calcium mg/g, of banana peels was reported to be 6.70%, 8.50% and 19.20 mg/g respectively [15]. The moisture, ash and calcium content of banana peel in the present study were 3.25%, 10% and 1950 mg/100g respectively. The phosphorus content of various varieties of banana peel fibres were estimated to range from 212.00 to 140.00 mg/100g [19]. In the present study the phosphorus content of banana peel was estimated to be 579 mg/100 g. Romelle et al. [20] estimated the carbohydrate content of banana peel to be 43.40 ± 0.55%. In the present study the carbohydrate content was 56.1±3.50%. Non-significant difference was found in the nutrient composition of banana peel and banana peel fibre in terms of moisture, ash, fat, carbohydrate% and starch% content. Significant difference was found in the protein and oxalate content of banana peel and banana peel fibre. Water holding capacity of banana peel and banana peel fibre was 5.27 and 7 g/g dried sample respectively. Fibres in general have higher swelling and water retention capacity or hydration properties. These properties are responsible for inducing fermentation process in the gut and also accounts for physiological effect such as fecal bulking of minimally fermented

### Table 1. Nutrient composition of banana peels and banana peel fibres

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Banana peel</th>
<th>Banana peel fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture%</td>
<td>3.25</td>
<td>5</td>
</tr>
<tr>
<td>Ash%</td>
<td>10</td>
<td>9.11</td>
</tr>
<tr>
<td>Fat%</td>
<td>5.1</td>
<td>0.403</td>
</tr>
<tr>
<td>Carbohydrate%</td>
<td>56.1</td>
<td>49.91</td>
</tr>
<tr>
<td>Starch%</td>
<td>6.81</td>
<td>0.99</td>
</tr>
<tr>
<td>Protein (mg/100 g)</td>
<td>479.5</td>
<td>90.90</td>
</tr>
<tr>
<td>Phosphorus (mg/100 g)</td>
<td>579</td>
<td>136</td>
</tr>
<tr>
<td>Calcium (mg/100 g)</td>
<td>1950</td>
<td>1950</td>
</tr>
<tr>
<td>Oxalate (mg/100 g)</td>
<td>139.5</td>
<td>0</td>
</tr>
<tr>
<td>Total phenolic content</td>
<td>19.82</td>
<td>9.82</td>
</tr>
<tr>
<td>Water holding capacity (g/g dried sample)</td>
<td>5.27</td>
<td>7</td>
</tr>
<tr>
<td>Oil holding capacity (g/g dried sample)</td>
<td>1.86</td>
<td>3.05</td>
</tr>
<tr>
<td>Cellulose (mg/100 g)</td>
<td>21.1</td>
<td>-</td>
</tr>
<tr>
<td>Total dietary fibre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insoluble (g/100 g)</td>
<td>42.5</td>
<td>-</td>
</tr>
<tr>
<td>Soluble (g/100 g)</td>
<td>18.75</td>
<td>-</td>
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### Table 2. ANOVA table (Two-way ANOVA without replication)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Moisture%</th>
<th>Ash %</th>
<th>Fat%</th>
<th>CHO%</th>
<th>Starch%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana peel</td>
<td>3.25</td>
<td>10</td>
<td>5.1</td>
<td>56.1</td>
<td>6.81</td>
</tr>
<tr>
<td>Banana peel fibre</td>
<td>5</td>
<td>9.11</td>
<td>0.403</td>
<td>49.91</td>
<td>0.99</td>
</tr>
</tbody>
</table>

### Table 3. ANOVA table (Two-way ANOVA without replication)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Phosphorus (mg/100 g)</th>
<th>Calcium (mg/100 g)</th>
<th>Total phenol content (mg/100 g)</th>
<th>Oxalate (mg/100 g)</th>
<th>Protein (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana peel</td>
<td>579</td>
<td>1950</td>
<td>19.82</td>
<td>139.5</td>
<td>479.5</td>
</tr>
<tr>
<td>Banana peel fibre</td>
<td>136</td>
<td>1300</td>
<td>9.25</td>
<td>0</td>
<td>90.9</td>
</tr>
</tbody>
</table>

*p<0.05*
dietary fibre. Buns containing 40 per cent dietary fibre were very bulky while those containing 10 per cent fibre were lighter. Foods are complex multicomponent systems. The majority of structural components are below the 100 μm range, and therefore it is problematic to detect them in their natural or transformed state [21]. However, application of special image acquisition technique such as Scanning Electron Microscope presents potential solutions to the problems for observation of micro structural features of food products. Scanning Electron Microscope images were taken from JCM-6000 bench top SEM (Jeol, Japan). The sample was gold coated for 0.5 minutes using sputtering chamber (Smart coater) at 220 volts. The SEM images of banana peel fibre incorporated buns taken at 20 and 100 μm resolution (x850 and x270) show starch globules along with gluten strands (Fig. 1). Microstructure changes in the bread during baking as affected by composition have been studied [22].

Significant difference was found between the antioxidant content (Table 4) of banana peels and banana peel fibres (p<0.05). Baskar et al. [23] noticed a high percentage inhibition (98.19%) of banana peel extract in scavenging free radicals at 10 mg·ml⁻¹. Sundaram et al. [24] studied the antioxidant activity and protective effect of banana peel against oxidative hemolysis of human erythrocyte. Percent radical scavenging activity of banana peel and banana peel fibre at various concentrations is as follows:

**Table 4. Antioxidant content of banana peel and banana insoluble peel fibre**

<table>
<thead>
<tr>
<th>Conc. (mg/ml)</th>
<th>%SCV</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.665</td>
<td>0.122%</td>
<td>0.816</td>
</tr>
<tr>
<td>1.33</td>
<td>2.69%</td>
<td>0.795</td>
</tr>
<tr>
<td>2.66</td>
<td>5.63%</td>
<td>0.771</td>
</tr>
<tr>
<td>3.99</td>
<td>9.17%</td>
<td>0.742</td>
</tr>
<tr>
<td>5.32</td>
<td>14.44%</td>
<td>0.699</td>
</tr>
<tr>
<td>6.65</td>
<td>19.46%</td>
<td>0.658</td>
</tr>
<tr>
<td>Control</td>
<td>0.817</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 1. Images and SEM images of banana peel fibre incorporated buns**
The results of texture profile analysis showed that there was a significant difference between the control and insoluble dietary fibre based buns (Table 5). Hardness and chewiness of the dietary fibre based buns was relatively higher than the control buns. This might be due to the insoluble dietary fibres which were substituted for refined wheat flour.

4. CONCLUSION

Dietary fibres are considered to be an important part of diet. Dietary fibres as such are not digested by our body, rather are utilized by our gut microflora. Banana peels can therefore, be a useful source for extracting these dietary fibres and utilizing them for preparing various nutritionally rich bakery products. Optimization and further evaluation can set some other important basis of utilizing this product. In the current study, up to 10% banana peel fibre incorporated buns were found be suitable for consumption without causing any alteration in the palatability and taste.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES


