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Studies on biochemical characteristics of banana flour and other millet flours

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Abstract
The research aims to evaluate biochemical characteristics of banana flour and other millet flours *viz*. Wheat flour, Ragi flour, Sorghum flour, Foxtail millet flour. Flours were analysed for TSS (°Brix), reducing sugars (%), total sugars (%), non-reducing sugars (%), titratable acidity (%), sugar: acid ratio, ascorbic acid (mg 100 g^{-1}), starch (%), crude protein (%), crude fibre (%), crude fat (%) and potassium (%) were analysed. The results showed that among the flours banana flour shown good at biochemical properties.

Keywords: Banana flour, ragi flour, sorghum flour, foxtail millet flour, biochemical characteristics

Introduction
Banana is one of the most important fruit crop grown in India. It ranks second in area and first in production only after mango in this country. Banana belongs to the family Musaceae and originated from the area between India to Southeast Asia. It was further introduced to Africa during ancient times and taken by European explorers to the Americas and other parts of the world.

Fresh banana is a rich source of potassium. 100 g fruit provides 358 mg of potassium which is 10 per cent of our daily requirement (Menton, 2004) [8]. The American Dietetic Association (ADA) reported that, the present recommended fibre consumption required by adults falls within 20-35 g/day or 10 -30 g/1000 kcal (FAO, 2014). A medium –sized ripe banana provides 12 percent of this requirement (Ching *et al*., 2001). It is also rich in vitamins especially vitamin A, B and vitamin C. Vitamin B6 (pyridoxine) providing 28 per cent of RDA whose bioactivity is high. Green bananas are also good source of vitamins B and C (Alkarkhi *et al.*, 2011) [1].

Post-harvest loss of fresh produce is a major challenge in the post-harvest sector. Fruits are the most perishable agricultural produces facing a tremendous loss from harvest to consumption. The post-harvest losses in banana have been estimated in the range of 4-4.7 quintal from harvesting, disease fruits in 3.16 quintal, storage in 2.08 quintal, transportation 2.5 quintal, to consumption stage (Del Aguila, *et al.*, 2010) [7].

Dehydration of banana and preparation of banana flour could increase the shelf life, availability and utilization of the fruit with reduction in post-harvest losses which would be of great significance to growers and consumers. The nutritional potential and the physiochemical properties of banana flour also make it suitable to be incorporated in various recipes and could be expected to give health benefits.

Millets are a traditional staple food of the dry land regions of the world. In India, millets are grown on about 17 million ha with annual production of 18 mt and contribute 10 per cent to the country's food grain basket. They are nutri-cereals which are highly nutritious and are known to have high nutrient content which includes 7-12% protein, 2-5% fat, 65-75% carbohydrates and 15-20% dietary fibre, carbohydrates, essential fatty acids, B-Vitamins, minerals such as calcium, iron, zinc, potassium, magnesium, phytochemicals with nutraceutical properties.

Materials and Methods
Raw materials
Banana variety Karpura Chakkarakeli was procured from local banana garden while millets grains v*iz*., Wheat, Ragi, Sorghum, Foxtail millet were procured from local market.

Observations Recorded

Total Soluble Solids (^{0}Brix)

The total soluble solids in fruit and flour were recorded by using Erma made hand refractometer. In case of fresh fruit, the pulp was crushed and pulp was extracted through cheese cloth onto the prism of the refractometer. Three readings were taken and the average was expressed as ^{O} Brix. While, in case of flour, 10 g of flour was dissolved in distilled water in 1:4 (product: water) ratio for four hours. Dissolved flour was extracted through cheese cloth on refractometer prism and observations were recorded. These values were multiplied by 5 to represent TSS brix of flour product (Ranganna, 1986) [10, 11].

Reducing sugars (%)

Reducing sugars in the banana were determined by the method enunciated by Lane and Eynon (AOAC, 1965) [2]. Twenty grams of sample was taken in a 250 ml volumetric flask. Two ml of lead acetate solution (45%) was added to the flask for precipitation of colloidal matter and two ml potassium oxalate (22%) was added to this solution to precipitate the excess lead and the volume was made up to 250 ml using distilled water. The precipitate was then filtered through Whatman No. 1 filter paper to obtain lead free filtrate after testing a little of filtrate for its freedom from lead by adding a drop of potassium oxalate. Reducing sugars in the lead-free solution was taken in burette and titrated against 10 ml of standard Fehling's solution mixture of A and B (1: 1) using methylene blue as an indicator till the end point as indicated by the formation of brick red precipitate. The titration was carried out by keeping the Fehling's solution boiling on the heating mantle.

$$\text{Reducing sugars (\%)} = \frac{\text{Factor} \times \text{volume made up}}{\text{Titre value} \times \text{Weight of the sample}} \times 100$$

Total sugars (%)

Total sugars were determined following the method described by Lane and Eynon (AOAC, 1965) [2]. A quantity of 50 ml lead free filtrate was taken in a 100 ml volumetric flask to which, 5 ml of concentrated HCl was added, mixed well and then kept for 24 hours at room temperature. Acid was then neutralized with NaOH using a drop of phenolphthalein as an indicator till the pink colour persisted for at least few seconds. Then volume was made up to 100 ml. Total sugars were then estimated by taking this solution in a burette and titrating it against standard Fehling's solution mixture of A and B (1:1) using methylene blue as an indicator taking brick red colour as an end point.

$$\text{Total sugars (\%)} = \frac{\text{Factor} \times \text{Volume made up}}{\text{Titre value} \times \text{weight of sample}} \times 100$$

Non-reducing sugars (%)

Non-reducing sugars were estimated using the formula.

Non-reducing sugars = total sugars – reducing sugars.

Titratable acidity (%)

Ten grams of sample was taken, ground well and transferred to volumetric flask and volume was made up to 100 ml with distilled water. The contents were filtered through Whatman No.1 filter paper. An aliquot of 10 ml was taken into conical flask to which 2-3 drops of phenolphthalein indicator was added and titrated against 0.1 N NaOH till pink colour was obtained and persists at least for 15 seconds, as an end point. (Ranganna, 1986) [10, 11].

$$\text{Titratable Acidity (\%)} = \frac{\text{Titre value} \times \text{Normality of NAOH} \times 0.067}{\text{Volume of aliquot taken}} \times 100$$

Sugar: acid ratio

To determine the sugar/acid ratio the sugar concentration (^{O}Brix) was divided by the acid concentration.

$$\text{Sugar: acid ratio} = \frac{\text{TSS } (^O\text{Brix})}{\text{Titratable Acidity (\%)}}$$

Ascorbic acid (mg 100g^{-1})

The ascorbic acid content in banana was determined by 2, 6-dichlorophenol indophenol visual titration method as given by Ranganna (1986) [10, 11] and expressed in mg 100 g^{-1}.

Preparation of 2, 6- dichlorophenol indophenol dye solution

Fifty milligrams of sodium salt of 2, 6 dichlorophenol indophenol dye was weighed into a beaker and 42 mg of sodium bicarbonate and dissolved in 150 ml hot distilled water. The volume was made up to 200 ml with distilled water.

Preparation of 3% metaphosphoric acid

Thirty grams of metaphosphoric acid was dissolved in a small quantity of distilled water and the volume was made up to 1000 ml.

Procedure

Ten grams of freshly ground sample was blended with 3 per cent metaphosphoric acid and made up to 50 ml with 3 per cent HPO_3. The contents are filtered through Whatman No.1 filter paper. Ten millilitres of the HPO_3 extract were taken and titrated against standard 2, 6-Dichloro phenol indophenol dye to a pink end point (Ranganna, 1986) [10, 11].

$$\text{Ascorbic acid (mg /100 g}^{-1}) = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up}}{\text{Volume taken} \times \text{Weight of the sample}} \times 100$$

Estimation of starch

Starch content in Banana was estimated as per the method developed by Sadasivam and Manickam (2008) [13].

Preparation of standards

Standard glucose: Prepared by dissolving 100 mg of glucose in 100 ml water (Stock solution).

a) Working standard: Prepared by diluting 10 ml of stock in to 100 ml of water.
b) Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold 95% sulphuric acid.
c) 80% ethanol: Take 80 ml of ethanol and dilute to 100 ml.
d) 52% perchloric acid: Take 52 ml of perchloric acid and dilute to 100 ml.

Procedure
Homogenize 0.1 to 0.5g of the sample in hot 80% ethanol to remove sugars. Centrifuge and retain the residue. Wash the residue repeatedly with hot 80% ethanol till the washings do not give colour with anthrone reagent. Dry the residue well over a water bath. To the residue add 5.0 ml of water and 6.5 ml of 52% of perchloric acid. Extract at 0 °C for 20 min. Centrifuge and save the supernatant. Repeat the extraction using fresh perchloric acid. Centrifuge and pool the supernatants and make up to 100 ml. Pipette out 0.1 or 0.2 ml of the supernatant and make up the volume to 1 ml with water. Prepare the standards by taking 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard and make up the volume to 1 ml in each tube with water. Add 4 ml of anthrone reagent to each tube. Heat for eight minutes in a boiling water bath. Cool rapidly and read the intensity of green to dark colour at 630 nm with spectrophotometer (Model UV-1800), at Central Instrumentation Cell (CIC), PJTSAU, Hyderabad.

Calculation
Find out the glucose content in the sample using standard graph. Multiply the value by a factor 0.9 to arrive the starch content.

Crude Protein (%)
The crude protein content was estimated according to the Kjeldahl's method as described in AOAC (2005) method at Central Instrumentation Cell (CIC), PJTSAU, Hyderabad. 0.5 grams of biscuits sample was weighed and put into the digestion tubes and 5.0 g of digestion mixture (98 g of potassium sulphate + 2 g copper sulphate) plus 10 ml of concentrated H_2SO_4 was carefully added. The samples were placed in the digestion unit for one and half hour at 375ºC. In a 100 ml conical flask, 40 ml of 4% boric acid was taken and few drops of mixed indicator (1.0 ml of 0.2% bromocresol green + 3.0 ml of 0.2% methyl red) were added. Distillation was done for 10 minutes in the Kjeldhal distillation apparatus (KELPLUS; Model- KES 8LRTS) after adding 10 ml of distilled water, 15 ml of 40% NaOH and steaming for 10 seconds. The distillate collected in conical flask was blue in colour. Titration was done adding indicator with standard 0.1 N HCl till the contents of the flask turned to pink colour. A blank was run simultaneously.

Calculation
From the nitrogen content of the sample, the protein content was calculated by multiplying with a factor of 6.25

Protein (%) = % N x 6.25

Crude fat (%)
Fat was estimated as crude ether extract of the dry material using automatic Soxtherm extraction unit (AOAC, 1997), at Central Instrumentation Cell (CIC), PJTSAU, Hyderabad.

Procedure
The extraction beaker was cleaned, dried for an hour at 103 °C in the drying chamber and was cooled off in the desiccator to room temperature. The empty beaker was weighed (W_1). 2.0 g of sample were weighed into thimbles and covered with fat-free cotton. The thimbles were inserted into the thimble holders and put into the beakers. About 150 ml of petroleum benzene (60-80 °C B.P.) solvent was added. The weighed extraction beaker with samples were kept into the Soxtherm instrument (SOCSPLUS; Model- SCS-8) and fitted properly to the sealing rings of the PTFE cylinders to avoid any leakage of solvent and extracted for one and a half hour. Petroleum benzene was evaporated in the apparatus and the flask was dried with the residue in the hot air oven at 100ºC for 1 hour, cooled in a desiccator and weighed (W_2).

Calculations

$$\text{Fat content (\%)} = \frac{W_2\text{-}W_1}{\text{Sample weight (g)}} \text{X100}$$

Where,
W_1 = Weight of empty cup (g)
W_2 = Weight of dried extraction cup after fat extraction (g)

Crude fibre (%)
The crude fibre content of samples was determined by boiling with 1.25% dilute H_2SO_4, washed with water, further boiled with 1.25% dilute NaOH and the remaining residue after digestion was taken as crude fibre (AOAC, 1995) [3], at Central Instrumentation Cell (CIC), PJTSAU, Hyderabad.

Procedure
1.0 gram of moisture and fat free sample was weighed and placed in the fibre bag. A glass spacer was kept in the bag. The bags were loaded in the sample carousel at the previewed positions of 1-6. The sample carousel was put into the glass container carefully and 500 ml of 1.25% dilute H_2SO_4 was added. The glass container axial was heated for 30 min. After completion of the time, the bags were washed by boiling with 500 ml distilled water for 30 min and then 500 ml of 1.25% NaOH was added and left for another 30 min for heating. Later again 500 ml distilled water was added and boiled for further 30 min. The residue was transferred to empty crucible and weighed as (W_1), then dried at 100 °C for 4 hrs in hot air oven, transferred to desiccator for cooling and weighed (W_2). The crucible was incinerated in a muffle furnace at 600 °C for 3 hrs. Then crucible was cooled in desiccator and weighed (W_3).

Calculations

$$\text{Crude fibre (\%)} = \frac{W_2\text{-}W_3}{W_1} \text{X100}$$

Where,
W_1 = Weight of the sample (g)
W_2 = Weight of the crucible + sample after heating at 100 °C (g)
W_3 = Weight of the crucible + sample after heating at 600 °C (g)
W_2-W_3 = Weight of crude fibre (g)

Estimation of potassium
Principle: When liquid samples containing potassium were burnt in the flame, it emitted proportionately photons characteristics of its wave length and intensity.

Sample preparation

0.5 g of sample were added with 10 ml of di-acid mixture of HNO_3 and $HClO_4$ in the ratio of 9:4. The mixtures were placed on hot plate until all the fumes disappeared and the solution became light coloured. It was made up to 100 ml in a standard volumetric flask and filtered.

Preparation of working standard

1000 ppm potassium was prepared by dissolving 1.907 g of AR grade KCl in 1 lit of distilled water. From these different working standards were prepared by diluting 0, 1, 2, 3, 4 and 5 ml of the stock solution to 100 ml to obtain 0, 10, 20, 30, 40 and 50 ppm potassium working standards.

Procedure

5 ml of extract was pipetted out into a 25 ml volumetric flask and diluted with distilled water. The standards and the sample extract were aspirated to the flame photometer (Model-CL378), at Central Instrumentation Cell (CIC), PJTSAU, Hyderabad. Meter reading was noted and potassium content calculated.

Calculations

$$\text{Potassium (\%)} = \frac{\text{Reading} \times 50}{\text{Weight of sample} \times 10^6} \times 100$$

Results and Discussion

The observations recorded on biochemical characteristics of banana flour and other millet flours were presented in Table. 1

TSS of the flours Banana, Wheat, Ragi, Sorghum and Foxtail millet were 1.63º Brix, 1.67º Brix, 1.17º Brix, 1.87º Brix and 1.13º Brix respectively. Ravinder Singh (2017) reported that TSS content was unripe banana flour (1.6) and cooked banana flour (1.9); reducing sugars was 10.48%, 2.07%, 1.54%, 1.55% and 1.85% respectively; total sugars 18.14%, 6.85%, 4.52%, 6.09% and 5.45% respectively; non-reducing sugars 7.66%, 4.78%, 2.97%, 4.54% and 3.61% respectively.

Titratable acidity for the flours Banana, Wheat, Ragi, Sorghum and Foxtail millet was 0.25%, 0.14%, 0.05%, 0.05% and 0.05% respectively; sugar: acid ratio 6.65, 12.41, 25.39, 36.16 and 20.98 respectively; ascorbic acid content 13.04 mg, 4.80 mg, 3.57 mg, 6.61 mg and 5.19 mg respectively; starch 51.43%, 73.25%, 61.03%, 78.03% and 66.19 respectively. Similar results were reported by Mota *et al*. (2000) [9].

Crude protein for the flours Banana, Wheat, Ragi, Sorghum and Foxtail millet was 4.36%, 18.43%, 7.27%, 10.37% and 12.47% respectively; crude fat content 0.94%, 2.14%, 1.91%, 2.86% and 6.33% respectively; crude fibre 1.11%, 1.56%, 2.50%, 1.14% and 0.75% respectively; potassium content 1.63%, 0.52%, 0.55%, 0.58% and 0.63% respectively. Similar results were reported by Suntharalingam and Ravindran (1993) [14] in green banana flour.

Table 1: Biochemical characteristics of banana flour and other millet flours

Characteristics	Banana flour	Wheat flour	Ragi flour	Sorghum flour	Foxtail millet flour
TSS (^{O}Brix)	1.63	1.67	1.17	1.87	1.13
Reducing sugars (%)	10.48	2.07	1.54	1.55	1.85
Total sugars (%)	18.14	6.85	4.52	6.09	5.45
Non-reducing sugars (%)	7.66	4.78	2.97	4.54	3.61
Titratable acidity (%)	0.25	0.14	0.05	0.05	0.05
Sugar: acid ratio	6.65	12.41	25.39	36.16	20.98
Ascorbic acid (mg)	13.04	4.80	3.57	6.61	5.19
Starch (%)	51.43	73.25	61.03	78.03	66.19
Crude protein (%)	4.36	18.43	7.27	10.37	12.47
Crude fat (%)	0.94	2.14	1.91	2.86	6.33
Crude fibre (%)	1.11	1.56	2.50	1.14	0.75
Potassium (%)	1.63	0.52	0.55	0.58	0.63

Conclusion

Based on the results, it can be concluded that banana flour recorded highest reducing sugars, total sugars, non-reducing sugars, titratable acidity, ascorbic acid and potassium. So, it was concluded that banana flour shown better biochemical characteristics in all aspects compared to other flours.

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