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ORIGINAL ARTICLE

Plantain peel - a potential source of antioxidant dietary fibre for developing functional cookies

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Abstract Plantain cultivar *Nendran* is popular as a staple food in many parts of India and deep fried chips made from raw matured *Nendran* are one of the popular snack items in India. This study aims to utilize peel from *Nendran* variety- the main byproduct of banana chips industry- to develop high fibre cookies with enhanced bioactive content. Proximate analysis indicated that peels are rich in total dietary fibre (64.33 g/100 g), vitamins (Folic acid- 33.12 mg/100 g) and minerals (Potassium- 35.61 mg/100 g). *Nendran* Peel Flour (NPF) was extracted with hexane, ethyl acetate and methanol. Phenolic and flavonoid content was high for ethyl acetate extract (15.21 and 9.39 mg QE/g dry weight). Methanol extract was more potent in reducing Copper ion (2.36 μM TR/g dry weight) and scavenging NO (IC_{50}-381.71 μg/mL). Ethyl acetate extract was capable of scavenging DPPH and hydroxyl radical. HPLC profiling showed presence of gallic acid, protocatechuic acid, rutin hydrate and quercetin in ethyl acetate extract and gallic acid, chlorogenic acid and vanillic acid in methanol extract. Cookies prepared with NPF possess higher total dietary fibre content. There was a decrease in spread ratio, breaking strength and browning index of cookies as the percentage of NPF increased. NPF incorporation gradually increased the phenolic content from 4.36 to 5.28 mg GAE, compared to control cookie (3.21 mg GAE). DPPH scavenging activity also increased with increase in NPF. Hence NPF is a very good source of antioxidant dietary fibre and acceptable cookies can be produced by replacing wheat flour with 10 % NPF.

Keywords Nendran peel · Nutrition · Antioxidant activity · Cookies · Total phenolic content · DPPH scavenging

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Introduction

Fruits and vegetable processing waste is highly perishable and seasonal, and are a problem to the processing industries and pollution monitoring agencies. This problem can be recovered by utilizing its high value compounds, including the dietary fibre fraction that has a great potential in functional foods preparation. The increasing interest to find new sources of dietary fibres with specific bioactive constituents, may add new beneficial properties to the traditionally commercialized products. In this aspect the concept of antioxidant dietary fibre is gaining importance. Currently peels of a variety of fruits get focused as natural source of antioxidants and dietary fibre. With these grounds, banana peel has attracted attention as recent reports suggest it as a very good source of dietary fibre and antioxidants.

Banana/plantain (*Musa paradisiaca*) is one of the major fruit crops in India which is the largest producer of bananas in the world. These cultures occupy the fourth world rank of the most significant foodstuffs after rice, corn and milk (INIBAP 2002). Plantain is similar to unripe bananas in outward appearance, larger and their flesh is starchy rather than sweet, used mostly unripe, and require cooking. Peel-the main by-product of the banana processing industry represents approximately 30 % of the fruit. This by-product constitutes an environmental problem because it contains large quantities of nitrogen and phosphorus and its high water content makes it

susceptible to modification by microorganisms (Tchobanoglous et al. 1993). The banana fruits are consumed at different stages of maturity and the amount of peels is expected to increase with the development of processing industries that utilise the green and ripe banana. Banana peel flour potentially offer new products with standardised compositions for various industrial and domestic uses (Emaga et al. 2007). Various studies have been conducted to investigate possible value addition to banana peel including the production of banana peel flour (Ranzani et al. 1996), the effects of ripeness stage on the dietary fibre components and pectin of banana peels (Emaga et al. 2008).

The Flour prepared from matured plantain, the Indian variety '*Nendran*' (*Musa paradasiaca*) is very popular as a weaning food in Southern regions of India. Deep fried chips made from raw matured *Nendran* are one of the popular snack items in India. Peel constitutes 30 % of the *Nendran* (Kachru et al. 1995) which is an important issue of environmental pollution as waste disposal is a major problem in the region. *Nendran* is one of the important agricultural crops of the region and if the peel can be proven as a source of antioxidant dieatry fibre, there is a great potential to develop an array of value added health products form peel. Hence, the purpose of this study was to profile the bioactive compounds in plantain peel and assess its antioxidant activities. The study also aims to develop cookies utilizing plantain peel flour which would have potential benefits in the management and prevention of life style associated diseases.

Materials and methods

Sample preparation

Plantain peel, from the variety *Nendran*, identified as *Musa paradaisica*, was collected from one of the local banana chips making industry, located at Trivandrum district of Kerala, India. The peels were washed, pre treated with cold water containing 0.5 % citric acid for 10 min, drained and sliced into small pieces. It was then dried in an oven at 50 °C for 16–24 h. The peel was then ground in a blender (Ultra centrifugal mill ZM200, Retsch, Germany) and sieved through a sieving machine with mesh size 20 mm (Vibro Sifter-PVS30, Prism Pharma Machinery, India) to get *Nendran* peel flour (NPF).

Chemicals and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), trolox and curcumin were purchased from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India. Copper (II) chloride and neocuproine were purchased from Alfa Aesar, Heysham, England. Ascorbic acid, gallic acid and Folin–Ciocalteau reagent were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. The solvents used were purchased from Spectrochem Pvt. Ltd. (Mumbai, India). All other reagents were of analytical grade.

Nutritional composition and physico-chemical analysis

The proximate analysis was determined by the procedure of Association of Official Analytical Chemist (Association of Official Analytical Chemists (AOAC) 2000). The methods for analyzing the various parameters are - moisture 934.01 (4.1.03), crude protein 955.04 (2.4.03), crude fat 920.39 (4.5.01), ash 942.05 (4.1.10), crude fibre 962.09 (4.6.01) and carbohydrate by difference. For mineral analysis (968.08), and vitamins viz., thiamine (953.17), riboflavin (981.15), niacin (975.41) and folic acid (944.12) were estimated using AOAC methods (1990). Ascorbic acid content was analysed using 2, 6 Dichlorophenol-Indophenol colorimetric method (Ranganna 1997). Water holding capacity was determined as described by Ruperez and Saura-Calixto (2001).

The insoluble and soluble dietary fibre from NPF was isolated according to Bureau of Indian Standard Method - IS: 11062 (1984).

Determination of hemicelluloses, cellulose and lignin content

The Van Soest method (Van Soest and Wine, 1967; Van Soest, 1987) was used to measure cellulose, hemicelluloses and lignin.

Determination of pectin

The pectin was determined according to Koubala et al. (2008) with slight modifications. 5 g sample treated with 1 M H_2SO_4 (pH-1.5) was stirred continuously for 1 h at 85 °C. The residues were removed by filtering and pectins were precipitated with 96 % ethanol. The precipitated pectins were centrifuged, collected and washed with 96 % ethanol and oven dried at 50 °C.

Evaluation of antioxidant efficacy

One-hundred grams NPF was sequentially extracted with hexane, ethyl acetate, methanol, 70 % methanol and finally with water at room temperature. After extraction, extracts were concentrated in a rotary evaporator (Heidolph Laborota 4010, Schwabach, Germany) at 40 °C and made up to 100 mL (with the respective solvents) and stored under refrigeration until further study.

Antioxidant activities of different extracts were evaluated using total phenolic content (TPC), total flavonoid content (TFC), DPPH, hydroxyl and NO radical scavenging activity and CUPRAC assays. The TPC, TFC, DPPH, and hydroxyl scavenging activity were determined as described by Suresh et al. (2011). NO scavenging activity and CUPRAC assay was

measured as described by Marcocci et al. (1994) and Ozyurek et al. (2007) respectively.

Analysis of phenolic compounds

The active extracts and the reference compounds solutions (1 mg/mL) were prepared in methanol and filtered through 0.45 μm PTFE filter; 20 μL was injected into the HPLC system. The analysis was performed on a Shimadzu (Kyoto, Japan) HPLC system containing two LC-8A preparative liquid chromatography pump units, a reversed-phase Phenomenex (Torrance, CA, USA) Luna® C18 column (250×4.6 mm i.d.; 5 μm) with an extended guard column of the same material, a column oven (CTO-10 AC *VP*), a system controller (SCL-10A *VP*), a Rheodyne injector (Idex Health & Science, Oak Harbor, USA) with a loop of 20 μL volume and a diode array detector (DAD; SPD-M10A *VP*).

The HPLC analysis was performed according to Rodriguez-Delgado et al. (2001) with some modifications. The mobile phase used was methanol-acetic acid-water (10:2:88, v/v) as solvent A and methanol-acetic acid-water (90:2:8, v/v) as solvent B with the gradient program 0–15 min 15 % B, 16–20 min 50 % B, 21–35 min 70 % B, 36–50 min 100 % B and finally the column was regenerated in 10 min. The flow rate was 1 mL/min, the injection volume was 20 μL and column was at room temperature. The eluted fractions were monitored at 280 nm. Phenols were identified by comparing retention times of peaks for the experimental and standard samples. Data acquisition and analysis were carried out using SHIMADZU-CLASS-VP version 6.14 SP1 computerized chromatography analysis software.

Cookies preparation

Cookies were prepared according to AACC method- 10-50D (2000). Briefly 100 g wheat flour/ blend of wheat flour and NPF, 56 g sugar, 23.5 g shortening, 1.1 g glucose, 1.1 g $NaHCO_3$, and 0.89 g NaCl were mixed with 12 mL of water. Control cookies were prepared using only wheat flour. Other cookies were prepared by replacing wheat flour with NPF in the ratio of 5, 10 and 15 % without altering the total flour content of the preparation.

Chemical characteristics

Moisture, fat, crude fibre, ash, protein, and carbohydrate of the cookies were evaluated as per the procedure described elsewhere (AOAC 1990). The total, soluble and insoluble dietary fibre content was estimated as per the procedure of Bureau of Indian Standard Method (1984) as described earlier.

Physical properties of cookies

Spread ratio

The spread ratio of cookies was determined by AACC method 10–52 (AACC International 2000). Six cookies were laid edge to edge and total diameter was measured using a scale, then cookies were rotated and re-measured again and average of two measurements was calculated. For the thickness determination, six cookies were laid one over the other one, and thickness was measured using a calliper, cookies were rearranged and thickness was measured again, and average was taken. Spread ratios were calculated from the formula

$$\text{Spread ratio} = \text{Diametre} \Big/ \text{Thickness}$$

Colour characteristics

Surface colour of cookies was measured using Minolta colour analyser (Minolta, Carriers Sur Seine, France) on the basis of CIE L*, a* and b* colour system. The instrument was standardized against a white tile before use. Browning Index and the Total colour change of the cookies were calculated from the following formulas:

$$\textit{Browning Index} = \frac{[100 \times (x - 0.031)]}{0.17}$$

where,

$$x = \frac{(a1.75L)}{[5.64L + \alpha - 3.012b]}$$

$$\text{Total Colour change, } \Delta E = \sqrt{(L_o - L)^2 + (a_o - a)^2 + (b_0 - b)2}$$

Texture measurements

The fracture force test was conducted on cookies using texture analyzer (TA–HDi, Stable Microsystems, UK) by a 'measure force in compression' test with a sharp blade cutting probe (SMS HBP/ BS). The analyzer was set at a 'return to start' cycle, a speed of 1 mm/s and a distance of 3 mm. A force/penetration distance plot was made for every test. Hardness and brittleness of the cookies can be estimated by the maximum force/breaking strength (N).

Sensory analysis

A semi-trained panel of ten members comprising of staff and students (in the age group 20–50 years) evaluated the sensory properties of the cookies. The panelists were asked

to rate each sensory attribute using the control cookie as the basic for evaluation of surface color, appearance, texture, taste/flavor, interior color and overall quality on a 9-point hedonic scale (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). Water was provided to rinse the mouth between evaluations. The samples were coded with letters and served to the panelists at random to guard against any bias.

Antioxidant and radical scavenging activity of cookies

The cookies were ground in a laboratory grinder (Ultra centrifugal mill ZM200, Retsch, Germany). Ten grams powdered samples were extracted with ethyl acetate and methanol. Then the extracts were concentrated at 40 °C and made up to 100 mL (with their respective solvents) in standard flask and stored under refrigeration until further study. TPC, TFC and DPPH radical scavenging activity were determined as described earlier.

Scanning electron microscopy (SEM)

SEM analysis of baked cookies was carried out using a JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan). Prior to examination, samples were sputter coated with gold–palladium to render them electrically conductive by using HUMMLE VII Sputter Coating Device (Anatech Electronics, Garfield, N.J., USA). The freeze-dried pieces of the cookies were fractured into sizes of about 1×1×0.5 cm using a knife. The interior surface of the samples was exposed to gold sputtering. The micrographs were taken at magnification of 1500× for the baked cookie samples.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of triplicate measurements. The data were analyzed by one way ANOVA with one factor using SPSS software version 11.5. The level of significance was set at $p<0.05$.

Results and discussion

Evaluation of nutritional composition of NPF

NPF possessed the following proximate composition: Moisture 5.84 g/100 g, protein 5.89 g/100 g, fat 5.12 g/100 g, ash 7.83 g/100 g and carbohydrates 11.03 g/100 g. Proximate composition of banana peel is reported to vary according to its maturity stages, variety as well as geographical conditions. Crude protein, fat and ash content of raw banana peel are reported to vary from 5.5–9.9, 2.2–13.1 to 4.6–15.25 % respectively depending on the variety and maturation (Lee et al. 2010). Variations are there from earlier reports of Emaga et al. (2007) [Protein-8.4, Fat-3.7, ash-7.5] and Ighodaro (2012) [Protein-6.89, fat-3.67, ash-17.59, carbohydrates-48.18]. These variations can be attributed to the change in variety, geography, climate, soil fertility, application of fertilizers and even time of harvesting. The content of vitamins viz., ascorbic acid, thiamine, riboflavin, niacin and folic acid in NPF were 9.2, 0.08, 0.065, 0.12 and 33.12 mg/100 g respectively. It was found that potassium (35.61 mg/100 g) is the most abundant mineral in NPF followed by calcium (28.63 mg/100 g), sodium (14.49 mg/100 g) and iron (6.96 mg/100 g).

The total dietary fibre (TDF), insoluble (IDF) and soluble dietary fibre (SDF) content of NPF was found to be 64.33, 56.88 and 7.45 g/100 g, respectively. Studies by Emaga et al. (2007) on peels from five different varieties of plantain reported that TDF vary from 32.9 to 49.9, IDF 27.3 to 36.5 and SDF varied from 5.6 to 13.6 %. A study by Lee et al. (2010) revealed that TDF, IDF and SDF from peels of yellow green stage of ripening were 55.46, 36.23 and 18.91 % respectively. NPF contain higher levels of TDF with a proportionate increase in the level of IDF. The capacity of dietary fibre to take up and hold water has been used to explain their faecal bulking properties (McConnell et al. 1974). There is already a report that the ripe banana peel powder increases the fecal bulk of rats (Ranzani et al. 1996). The cellulose (13.08±1.85 g/100 g), hemicelluloses (2.23±0.89 g/100 g) and lignin (7.65± 1.54 g/100 g) were estimated in NPF. The neutral detergent fibre (NDF), acid detergent fibre (ADF) and Pectin was found to be 22.97, 20.74 and 8.5 g/100 g respectively. The results have variations from earlier report of Emaga et al. (2011). According to Emaga et al. (2011) the cellulose (7.1 g/100 g) content is less whereas the hemicelluloses (4.5 g/100 g) and lignin (12 g/100 g) content is higher when compared to our results. The variations may be due to change in maturity, geographical and climatic condition. The water holding capacity of peel was found to be 8.095± 1.07 g water/g of sample. This result proposed that NPF will definitely aid for faecal bulking.

The above studies suggest that NPF is a rich source of dietary carbohydrates and minerals and other nutrients. Since they are good source of fibre, especially, insoluble dietary fibre, they can play a major role in intestinal regulation by increase faecal bulk and decrease intestinal transit. As most of the bioactive phytochemicals are bound to dietary fibre (Perez-Jimenez et al. 2009), further studies were carried out to establish the bioactive potential of NPF. NPF was assayed for various in vitro antioxidant studies and the phytochemicals extracted in appropriate solvents were profiled by HPLC.

Biochemical profiling and antioxidant activities of NPF

Plant foods contain an array of bioactive compounds which are gaining lot of attention for their health care potential. Plant polyphenols can scavenge free radicals and inactivate other pro-oxidants, and can also interact with a number of biologically relevant molecules. Since different antioxidant assays give widely diverging results, no single method can be used for evaluating antioxidant activity of foods (Frankel and Meyer 2000). A major part of the antioxidant compounds that are produced by plants, especially phenolic compounds, are reported to bind to the fibre (Perez-Jimenez et al. 2009). Therefore NPF was sequentially extracted with hexane, ethyl acetate and methanol, and analysed for its antioxidant activity using various assays.

The TPC of different extracts of NPF was evaluated in terms of Gallic acid equivalents and follow the order Ethyl acetate (15.21)>Methanol (9.41)>Hexane (2.63). The TFC of the NPF extracts was evaluated in terms of Quercetine equivalents and follow the order Ethyl acetate (9.39)>Methanol (1.71)>Hexane (0.91). The high phenolic content of ethyl acetate extract may be due to phenolic compounds such as polymerized prodelphinidins, flavonol glycosides and B-type procyanidin dimers and monomeric flavan-3-ols as reported earlier by Rebello et al. (2014).

Antioxidant assays

Antioxidant activity is important in view of the free radical theory of many life style associated diseases. The potential of antioxidants has prompted investigators to search for natural compounds with convincing antioxidant activity and less cytotoxicity. Phytochemicals from plant sources have received a great deal of attention because of their antioxidant activity. The antioxidant potential of NPF was assessed by its ability to reduce copper ion and to scavenge DPPH, hydroxyl and NO radicals.

The copper reduction method can detect changes of total antioxidant capacity, which may not be perceived through the measurement of individual antioxidants. The copper ion reducing potential of extracts is calculated based on trolox equivalents. The results showed that the NPF methanol extract possessed higher cupric ion reduction potential (2.36 μM TR/g dry weight) followed by ethyl acetate extract (1.41 μM TR/g dry weight). The hexane extract did not exhibit any activity. The cupric reducing ability measured for a food extract may indirectly but efficiently reflect the total antioxidant power of the sample.

DPPH scavenging assay has been used to assess the antioxidant activity of various plants and food products with reliable and reproducible results. Among the extracts the NPF ethyl acetate fraction was found to be more active (IC_{50}-55.23 μg/mL) followed by methanol fraction (IC_{50}-70.72 μg/mL). The IC_{50} of the standard gallic acid was 3.01 μg/mL. The hexane extract does not possess any activity.

Hydroxyl radical, an extremely reactive free radical formed in biological system causing hydroxylation of various biomolecules by reduction of unsaturated bonds present in their structures. The double bonds present in polyphenols are exposed to the same reductive principle, making them scavenge hydroxyl radicals. Among the NPF extracts, the ethyl acetate fraction exhibited promising hydroxyl radical scavenging potential (IC_{50}-3.43 μg/mL) followed by methanol (147.70 μg/mL), which is more active than the standard mannitol used (567.20 μg/mL), but the activity is less when compared to the activity of polyphenol catechin (8.1 μg/mL). The hexane extract does not exhibit hydroxyl radical scavenging activity.

NO at higher concentration has been implicated in many diseased states. The NO scavengers inhibit nitrite formation by competing with oxygen to react with NO. Among the NPF extracts, only methanol extract possessed NO scavenging activity. The IC_{50} was found to be 381.71 μg/mL. The IC_{50} for the standard ascorbic acid was 92.8 μg/mL.

Analysis of phenolic compounds

In view of the fact that antioxidant activity is mostly a function of the constituent phenolics, the qualitative analysis of phenolic acids in the active NPF extracts was carried out by HPLC. The phenolic acid profile of the NPF ethyl acetate and methanol extracts is shown in Fig. 1.

Gallic acid, protocatechuic acid, catechol, chlorogenic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid, rutin hydrate, ellagic acid and quercetin in 1 mg/mL concentration were feed initially. The retention times were 5.035, 8.309, 10.293, 15.403, 17.173, 19.947, 24.352, 25.739, 27.840, 28.341 and 32.085, respectively. In the NPF ethyl acetate extract four polyphenols were identified- Gallic acid, protocatechuic acid, rutin hydrate and quercetin. Gallic acid, chlorogenic acid and vanillic acid were identified in the NPF methanol extract. From the HPLC chromatogram it is clear that the ethyl acetate fraction contains more polyphenols, some of them are not identified, which may be correlated to its better antioxidant activity.

Development of cookies

Proximate analysis of cookies

Table 1 summarises the proximate composition of the control and high fibre cookies prepared using wheat flour replaced with 5, 10 and 15 % NPF. Moisture content is a critical parameter as far as the texture, acceptability and shelf life of cookies is concerned. As can be seen, moisture content of the cookies increased with an increase of NPF in the cookie. This may be due to the higher water absorption and water

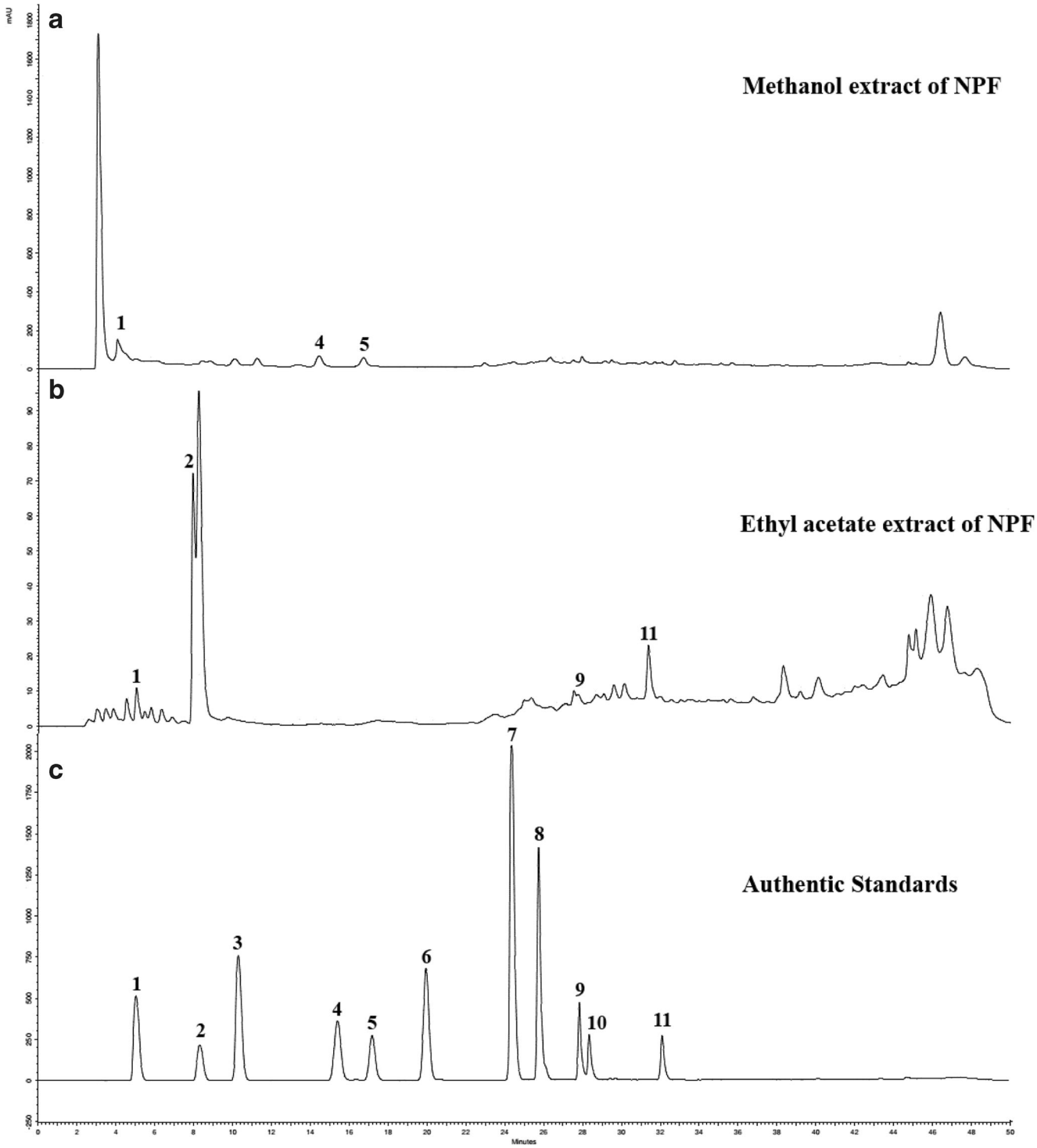

Fig. 1 HPLC chromatogram (280 nm) of (A) methanol extract of NPF; (B) ethyl acetate extract of NPF; (C) authentic standards – 1. Gallic acid, 2. protocatechuic acid, 3. catechol, 4. chlorogenic acid, 5. vanillic acid, 6. syringic acid, 7. p-coumaric acid, 8. ferulic acid, 9. rutin hydrate, 10. ellagic acid, 11. Quercetin

holding capacity of NPF fibre. It is reported that moisture content of cookies increases with the increase in fibre content (Chung et al. 2014; Sharma and Gujral 2014). Protein content of the cookies decreases as wheat flours was replaced with NPF. There was no significant difference in the fat content of the cookies. Small but statistically significant differences were obtained between the ash contents of the samples. Dietary fibre content increased significantly when the flour was partly replaced with NPF. Fibre has been associated with physiological effects such as increased fecal bulk, reduced

Table 1 Proximate composition of cookies

	Control	5 % NPF	10 % NPF	15 %NPF
Moisture (%)	5.57±0.23[a]	6.25±0.34[b]	6.37±0.11[b]	6.55±0.05[b]
Ash (%)	1.08±0.04 [a]	1.23±0.03 [b]	1.78±0.05 [c]	1.90±0.01 [d]
Fat (%)	23.22±0.10 [a]	22.94±0.14[a]	22.99±0.08[a]	23.16±0.05 [a]
Protein (%)	11.32±0.06 [a]	10.25±0.04 [b]	9.61±0.05 [c]	8.99±0.06 [d]
Carbohydrate (%)	58.41±0.08 [a]	56.25±0.13 [b]	52.0±0.09 [c]	48.16±0.11 [d]
TDF	13.26±0.01 [a]	15.78±0.08 [b]	24.88±0.11 [c]	36.74±0.03 [d]
IDF	8.43±0.01 [a]	10.54±0.03 [b]	17.24±0.33 [c]	26.87±0.10 [d]
SDF	4.83±0.01 [a]	5.25±0.06 [a]	7.63±0.44 [c]	9.87±0.07 [d]

[a-d] Values in same row with different alphabets (superscript) are significant and values with same alphabets (superscript) are not significant, P 0.05

levels of plasma cholesterol and reduced glycemic response to meals (Frape and Jones 1995). Fibre promotes glucose attenuation and laxation, and can reduce the risk of coronary heart disease, colon cancer and obesity (Threapleton et al. 2013; Papathanasopoulos and Camilleri 2010; Aune et al. 2011). As there is an increase in demand for foods with increased dietary fibre content, NPF can be a potential source for dietary fibre in these products.

Physical properties of cookies

Diameter, thickness, spread ratio and breaking strength The effect of incorporation of NPF on the physical properties of cookies such as diameter, thickness and spread ratio are presented in Table 2. There was no significant difference in the diameter of control and fibre enriched cookies. On the other hand, the thickness of the cookies differ significantly, where, average thickness of control cookies was 5.8 mm and that for the cookies with 5, 10 and 15 % of NPF was 7.0, 7.1 and 7.2 mm respectively. This difference in the thickness is reflected in spread ratio of control as well as cookies with NPF. As can be seen, spread ratio of cookies with NPF is significantly lower as compared to the control cookies. Since the water content was kept constant for the dough preparation for all the formulations in the present study, there is an increase in the number of hydrophilic sites in the dough with NPF due to increase in fibre content for the limited water available in the cookie dough as the fibre hold more water. This leads to the partitioning of free water and higher concentration of sugar in the available water phase, resulting in more viscous dough. Since, the dough with NPF was already in an elastic solid form and more viscous due to reduced water content, its spreading during baking would have been reduced resulting in cookies with an increased thickness and thus reduced spread ratio. It is reported that spread ratio of cookies is affected by the viscosity of the dough (Zucco et al. 2011). Tangkanakul et al. (1996) reported that the spread factor decreases with increase in fibre content in cookies. A study by Kawai et al. (2013) reported that lower water activity resulted in lower spread ratio of the cookies.

Table 2 Diameter, Thickness, Spread Ratio and Breaking Strength of Cookies

Sample	Diameter(D), (mm)	Thickness(T), (mm)	Spread ratio (D/T)	Breaking strength (N)
Control	38.0±0.2[a]	5.8±0.11[a]	6.55±0.089[a]	68.11±0.8[a]
5 % NPF	38.0±0.41 [a]	6.5±0.09[b]	5.85±0.017[b]	67.26±0.35[a]
10 % NPF	38.5±0.73 [a]	6.9±0.13[c]	5.58±0.001[c]	58.7±0.91[c]
15 % NPF	39.0±0.13 [a]	7.2±0.07[d]	5.42±0.034[d]	52.67±1.02[d]

[a-d] Values in same row with different alphabets (superscript) are significant and values with same alphabets (superscript) are not significant, P 0.05

Colour measurements

Colour plays an important role in the acceptance of a food product by the consumer. Colour, texture and taste are very important as far as the acceptability of cookies is concerned. The colour of cookies was significantly affected by the substitution of wheat flour by NPF (Table 3). There was significant decrease in 'L' (lightness), 'a' (redness) and b (yellowness) with increase in the percentage of fibre in the cookies. The Maillard reaction and caramelization of sugar is considered to be responsible for the production of brown pigments in baked foods. These reactions are influenced by many factors like pH, temperature, available water, sugar, protein and amino acids etc. It is reported that the Maillard reaction between reducing sugars and protein during baking mainly determine the colour of the cookie (Chevallier et al. 2000). In the present study, it was observed that the protein content of the cookies with NPF is significantly lower than the control cookies suggesting that Maillard reaction played a major role in the decrease of 'a' and 'b' values. However, the cookies were darker (decrease in L) may be due to the natural pigments of NPF such as polyphenols and chlorophyll. Earlier report on incorporation of brown rice in bread and noodles made the colour darker without having much effect

Table 3 Colour measurements of cookies

Sample	L*	a*	b*	Browning Index	Total Colour Change
Control	53.8±0.14[a]	13.06±0.09[a]	29.30±0.41[a]	94.01±7.01[a]	55.89±2.84[a]
5 % NPF	52.86±0.054[b]	9.44±0.13[b]	25.97±0.23[b]	78.83±2.10[b]	54.21±0.74[a]
10 % NPF	49.43±0.61[c]	8.71±0.17[c]	22.78±0.07[c]	86.0±3.28[c]	60.39±1.41[b]
15 % NPF	46.18±0.22[d]	5.41±0.24[d]	18.30±0.18[d]	58.08±1.20[d]	56.60±0.50[a]

[a-d] Values in same row with different alphabets (superscript) are significant and values with same alphabets (superscript) are not significant, P 0.05

on 'a' and 'b' values (Chung et al. 2012). Similar results were reported by Sharma and Gujral (2014) who showed that the higher fibre addition in cake and biscuit formulation promoted severely the non-enzymatic browning reactions as evidenced by the low L* value.

Texture

Texture, as in the case of colour, is one of the most important sensory parameter which determines the consumer acceptability of foods. It determines the eating quality of foods. Texture of the cookies was measured using texture analyzer TA- HDi (stable Microsystems) and is represented in terms its force required to break/snap the cookies. The results are represented in Table 3. It can be seen that the force required to break/snap the cookies decreased significantly on incorporation of fibre. The samples become softer giving less resistance to the applied force during analyses. Similar results are reported for cookies made from flour substituted with different sources of fibre (Sharma and Gujral 2014). Hardness of the cookies depends on the composite matrix of the protein aggregates, lipids, and sugars, which are embedded in some of ungelatinized starch granules (Chevallier et al. 2000). As the wheat flour was replaced with NPF, the protein content decreases significantly affecting the formation of gluten net work. This decrease in gluten may be one of the reasons for the decrease in hardness of the cookies with increase in NPF content. It is reported that reduction of gluten in cookie dough by substituting with rice flour retarded the formation of gluten matrices, resulting in substantial decrease in hardness (Chung et al. 2014). Also, the moisture content of the cookies with NPF was higher than the control as the fibre retains more moisture, which may also made the cookies softer.

SEM analysis

SEM images of the cookies (A-control, B- 5 %, C-10 % NPF and D-15 % NPF) containing different proportions of NPF (A-control, B- 5 %, C-10 % NPF and D-15 % NPF) can be seen in Fig. 2. The swollen starch granules can be seen embedded a continuous layer of gluten. Starch granules seemed to be trapped in a protein network containing wheat gluten. Occurrence of some cavities which increased with the percentage of NPF was also observed in the cookies. This may be due to the fact that continuity of the gluten matrix was disrupted when more NPF was incorporated in the dough. Also, fibre holds more water which expands and evaporates during baking leading the formation of cavities with different sizes. This increased porosity with increase in the percentage of fibre, may be correlated with the decrease in breaking strength.

Sensory evaluation

Table 4 indicates the sensory profile of the cookies prepared from flours of different composition studied. It can be noticed that Cookies with 10 % NPF scored higher for all the parameters evaluated. However there was no significant difference in the colour, taste and texture of control cookies and cookies with 5 and 10 % NPF and were significantly different for cookie with 15 % NPF as compared to other cookies. There was no significant difference in the overall acceptability of all the cookies.

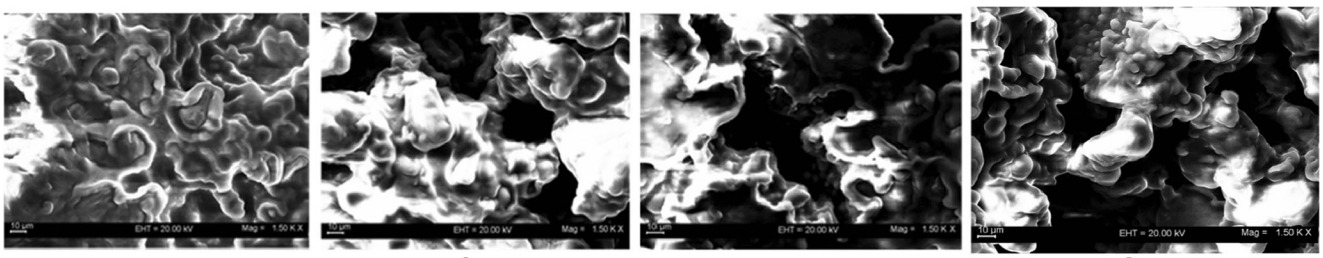

Fig. 2 SEM images of the cookies (A-control, B- 5 %, C-10 % NPF and D-15 % NPF)

Table 4 Sensory analysis of Cookies

	Control	5 %	10 %	15 %
Colour	7.214±0.487[a]	7.571±0.535 [a]	7.785±0.487 [a]	6.5±0.408 [b]
Taste	7.285±0.698 [c]	7.571±0.449 [c]	7.714±0.393 [c]	6.0±0.763 [d]
Texture	7.071±0.607[e]	7.428±0.672[e]	7.5±0.408[e]	6.5±0.816[e]
Overall acceptability	7.142±0.377 [f]	6.785±0.566 [f]	7.357±0.475 [f]	6.357±0.899[f]

[a-f] Values in same row with different alphabets (superscript) are significant and values with same alphabets (superscript) are not significant, P 0.05

Antioxidant and radical scavenging potential of cookies

The incorporation of NPF gradually increased the phenolic content in cookie from 5 % (4.36), 10 % (4.87) and 15 % (5.28 mg GAE), as compared to the phenolic content of control cookie (3.21 mg GAE). Previous reports suggest that replacement of wheat flour with other sources rich in dietary fibre improve the phenolic content of cookies (Sharma and Gujral 2014). Radical scavenging potential of the cookies were measured in terms of DPPH radical scavenging activity. As can be noticed (Fig. 3), the incorporation NPF progressively increased the radical scavenging activity of the cookies. This may be correlated with the increase in phenolic content of the cookies with increase in NPF content. It is also reported that the Maillard reaction products formed during baking can also contribute to antioxidant activity (Vitali et al. 2009). Thus incorporation of NPF improves the health benefits by increasing antioxidant activity and its dietary fibre content.

Conclusion

The study concludes that NPF is a very good source of dietary fibre and antioxidant phytochemicals. Acceptable cookies were produced by replacing wheat flour with NPF at 10 % level. Incorporation of NPF increased fibre content as well as antioxidant potential of the cookies. There is increasing interest to find new sources of dietary fibre with specific bioactive constituents that may add new healthy properties to the traditionally commercialized products. NPF could be used as a suitable source of dietary fibre with associated bioactive compounds and could be incorporated as ingredients in a large variety of food products such as making biscuits, cakes etc. Since functional foods are an effective way to deliver beneficial agents aimed at reducing disease risk, the value addition of NPF in the form of a functional food ingredient can bring remarkable socio-economical change in the region.

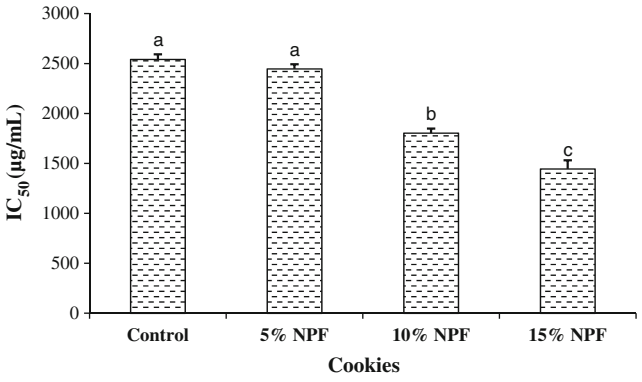

Fig. 3 IC_{50} values of different Cookies for scavenging DPPH. Results are mean±SD of triplicate measurements (n=3) and the significance accepted at P ≤0.05. [a-c] Means with different letters are significantly different

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