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PROXIMATE COMPOSITION, RESISTANT STARCH AND OTHER PHYTOCHEMICAL CONSTITUENTS OF NATIVE FINGER MILLET CULTIVAR

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Owing to its reported potential health benefits against degenerative and non-communicable diseases, finger millet, yesteryears' rural poor men's diet is currently receiving considerable attention from urban rich. Formulation of functional foods and foods for different age groups demand fitting information regarding nutrient, fiber and other phytochemicals. In the present study, to reap the benefits of native grain, the nutritional quality of natively grown finger millet variety was assessed and the results were presented and discussed.The Percent composition of finger millet flour was analyzed and found to contain moisture, 13.57 ± 0.19%, crude protein 6.12 and 7.08%, crude fat 1.29 and 1.49%, crude fiber 11.95 and 13.93%, crude ash 3.64 and 4.21%, nitrogen free extract (carbohydrates) 63.43 and 73.29% and energy 289.81 Kcal and 334.89 Kcal on as is basis and dry basis respectively. The resistant starch was found to be less than 0.2% however, dietary fiber; IDF, SDF, TDF were estimated to 10.56, 1.06, 11.62% and 12.22, 1.23, 13.44% on as is basis and dry basis respectively. The LC-MS spectral analysis showed the presence of three different carotenes and lutein and the spectrophotometric analysis of finger millet flour revealed the representative concentrations of ascorbic acid, polyphenol, phytic acid and total carotenoids to 0.018%, 3.208% GAE, 0.98% and 0.000512% on as is basis. The above information may help develop foods that may manage, prevent or delay the onset of degenerative and metabolic diseases.

Keywords: Finger millet, Proximate, Polyphenol, Ascorbic acid, Phytic acid, Carotenoids

INTRODUCTION

Small-seeded grains belonging to different variety of annual grasses that are cultivated primarily as grain crops on marginal lands in dry areas in temperate, subtropical and tropical regions are collectively referred to as millets. They are the most important cereals of semi-arid zones of the world and are staple food for millions of people in Africa and Asia. Based on their consumption and cultivation pattern, millets occupy sixth place in the world food after wheat, corn, rice, barley and sorghum. According to Food and Agricultural Organization of United Kingdom (FAO, 2013) report, developing countries, mainly Asia and Africa accounted for 94% of the global millet output, of which India alone has contributed 34%. Of the total 30.3 million tons of global millet produce, pearl millet accounts for about 15 million tons, foxtail millet 5 million tons, proso-millet 4 million tons and finger millet for over 3 million tons. Minor millets offer several health benefits to the consumers as they are good source of dietary carbohydrates, protein, dietary fiber, minerals, and is rich in several other phytochemicals such as, phenols, polyphenols, tannins, flavonoids, antioxidants and phytic acid compared to rice or wheat (Jayaraj *et al.*, 1980; and Hadimani and Malleshi, 1993).

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Among millet crops, finger millet figures prominently; it ranks fifth in importance after great millet (sorghum), pearl millet, foxtail millet and Japanese barnyard millet (Hulse *et al.*, 1980). Finger millet (*Eleusinecoracana L.)* known as ragi, which is grown as subsistence crop is no stranger to the world of millets and accounts for about 10% of the total millet produced. In India, it is cultivated over an area of 1.17 million hectares with total production of about 2.04 million tons. Karnataka State of India is the major producer of finger millet as it alone contributes for more than 60% of the country's land under cultivation of finger millet followed by Uttarakhand (10%), Maharashtra (9.6%), Tamilnadu (6.5%), Odisha (4.8%) and Andhra Pradesh (3.6%). It is considered as poor men's diet is the staple food for millions of inhabitants across Asia and Africa. Despite the decline in the cultivation area of finger millet in India, it is a prevailing crop in southern parts of Karnataka and consequently, the principle food for both the rural as well as urban dwellers in this part of the country.

Since time immemorial, finger millet has been domesticated, intensely cultivated and consumed as principle food in the form of *mudde* (stiff porridge/dumpling), ambli (thin porridge, prepared by boiling flour in water for 15-20 min) and *roti*, unleavened pancake (Malathi and Nirmalakumari, 2007), which has been the dining culture of the large population of south Karnataka. It also occupies an important place in the diets' of the rural population belonging to low-income groups in different states of India like Uttarkhand, Maharashtra, bordering districts of Andhra Pradesh, Tamilnadu, and Kerala and many African countries. Finger millet has received much attention because of its nutritional quality in terms of exceptionally high levels of calcium (0.3%), starch (72 to 79.5%), and appreciable levels of dietary fiber, functional fiber (Hadimani and Malleshi, 1993; Chethan and Malleshi, 2007; and Devi *et al.*, 2011), phenols, tannins, flavonoids, antioxidants (Asharani *et al.*, 2010), phytic acid/phytate and other mineral content (Deosthale *et al.*, 1970; BalkrishnaRao et al., 1973; Pore and Magar, 1979; Hulse *et al.*, 1980; Jayaraj *et al.*, 1980; Joshi and Katoch, 1990; Namiki, 1990; and Bhatt *et al.*, 2003). They are reported to be good source of macronutrients, micronutrients and phenolics thus, may serve as potential natural antioxidants for therapeutic diets (Chandrasekara and Shahidi, 2013; and Shahidi and Chandrasekara, 2013). Polyphenols are reported to possess antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic activities and also implicated in reducing coronary heart disease and improving gut health, (Koshihara *et al.*, 1984; Serrano *et al.*, 1998; Fava *et al.*, 2006; Romier *et al.*, 2009; and Chandrasekara and Shahidi, 2011).

Finger millet was reported to contain phenolic acids, flavonoids, polymeric tannins, anthocyanins and phytates that are involved in regulating postprandial glycemic response by decreasing carbohydrate digestibility by pancreatic amylase and intestinal α-glucosidase (Thompson *et al.*, 1987; Chethan and Malleshi, 2007a; and Shobana *et al.*, 2009). The present investigation deals with the analysis of proximate composition, dietary fiber, resistant starch and other phytochemical constituents like ascorbic acid, polyphenol, tannins, phytic acid and carotenoids of native finger millet grains.

MATERIALS AND METHODS

Collection of Sample

Finger millet (*E. coracana* L., brown cultivar*)* grown by the farmers of Chitradurga District (14.23° North Latitude, 76.4° East Longitude, and 732 M above sea level) was procured from the local market of Chitradurga town of Karnataka State, India. The finger millet samples were brought to laboratory, manually cleaned and preserved in dark and dry place at ambient temperature with passive ventilation. For experimental purpose, where ever required, the samples were ground to fine powder by using pestle and mortar and the flour powder was sieved by 0.6 mm sieve and stored in cool and dry place for further analysis.

Chemicals and Reagents

All the chemicals like acids were purchased from SD fine Chemicals, sodium hydroxide, ammonia, potassium sulfate and other reagents used in this study were of analytical grade and procured from Qualigens fine chemicals. The solvents used were obtained from Spectrochem India and Qualigens India. LC-MS grade solvents were procured from J T Bekker and the termamyl and dietary fiber enzyme kit were purchased from Novozymes and Sigma-Aldrich.

Proximate Composition Analysis of Finger Millet

Proximate composition of a food is the relative proportion of moisture, dry matter, crude protein, crude fat, crude fiber, crude ash and carbohydrate, where in carbohydrate content was calculated by difference method. It gives valuable information about the feed with regard to the levels of various nutrients present in it. The analysis may be done

by *as such basis*, where percentage determined on food as received or consumed or by *dry matter basis* wherein, the percent composition of different constituents were determined with respect to 100% moisture-free samples.

Estimation of Moisture content

The moisture content of the ragi samples were estimated by AOAC Method 930.15. Briefly a 5 g whole finger millet flour samples were transferred to pre-weighed, clean and dry aluminum moisture cups (w_1) and recorded the weight (w_2). The samples containing aluminum moisture cups were transferred to a hot air oven operating at 134 °C for 2 hr. The moisture cups containing samples were removed from the oven and the weight of the moisture cups containing dried sample recorded (w_3). The moisture cups containing dried samples were further transferred to a vacuum desiccator and the percent Loss on Drying (LOD) determined.

Percent moisture content of the sample was calculated by following formula

Percent Moisture = $(w_2\text{-}w_3)/(w_2\text{-}w_1) \times 100$

Estimation of Dry Matter

The dry matter content of the ragi samples were estimated by oven drying method (Method 930.15 AOAC). Pre-weighed clean and dry aluminum cups exposed to 134 °C (w) were added with approximately 5 g flour sample and the weight was recorded (w_1). The cups containing sample were transferred to a forced air oven operating at 134 °C for 2 hr. aluminum cups containing samples were removed and placed in a vacuum desiccator and the weight recorded till the difference of two successive weights was less than 1 mg (w_2). Percent dry matter was then calculated by the formula % Dry matter = $(w_2\text{-}w)/(w_1\text{-}w) \times 100$.

Estimation of Crude Protein

Protein content of finger millet sample was determined by the Kjeldahl method as modified by AOAC method 981.10. Defatted meal samples (0.5 g) were digested by heating with concentrated H_2SO_4 in a digestion block using digestion mixture (K_2SO_4 and $CuSO_4$ 9: 1, w/w) as a catalyst. After digestion, samples were distilled using a steam distillation unit with 10M NaOH. Boric acid (4%) was used to trap ammonia from the distillation and the distillate was titrated with 0.2 NHCl using mixed indicator (Methyl red and bromocresol green in alcohol, 0.1%). Percent nitrogen was used to estimate percent protein concentration by means of a nitrogen-to-protein conversion factor 6.25. The blank reagent was also titrated similarly.

% Protein= (Vol. Acid – Vol. Blank) X 1.4007 X 0.2 N X 6.25/g sample

Estimation of Crude Fat

The crude fat in the finger millet sample was estimated by following extraction of fat by soxhlet extraction method as described in AOAC Method 920.39C. A 3 g flour sample (w) was subjected to drying (AOAC Method 934.01) and the dried sample was transferred to clean and dry extraction thimble, which was packed with glass wool to permit the free flow of solvent. A pre-weighed clean and dry heating flask (w_1) was filled with petroleum ether (BP 40 °C to 60 °C, AOAC Method 960.39) and fat was extracted at a condensation rate of 5 to 6 drops per second for 8 hours. After the extraction of fat, the ether was removed by Buchi type vacuum evaporator and the residual ether was dried in oven at 100 °C for 30 min. The flask was then cooled in vacuum desiccator for 2 hr. and recorded the weight (w_2).

Percent crude fat was calculated as $[(w_2\text{–}w_1)/(w_1\text{–}w)] \times 100$

Estimation of Crude Fiber

The estimation of crude fiber in finger millet meal was performed by AOAC Method 978.10. The defatted meal (2 g, w_0) was hydrolyzed by boiling with 150 ml of 0.25 N H_2SO_4 for 30 min. The suspension filtered, washed twice with hot distilled water and the residue obtained was hydrolyzed by boiling with 150 ml of 0.313 N NaOH for 30 min. The residue was washed with hot water and acetone. The crucible containing sample was dried in oven for 3 hr at 103 °C and the weight recorded (w_1). The sample was then ignited in a Muffle furnace at 600°C for 3 hr and was held in an oven at 103 °C for 1 hr and weight recorded (w_2). Percent crude fiber was calculated as $[(w_1\text{-}w_2)/(w)] \times 100$.

Estimation of Crude Ash

The crude ash in the finger millet powdered sample was estimated by AOAC Method 942.05. To an empty pre-weighed silica crucible (w), a 3 g of finger millet flour sample was added and the weight of the crucible containing sample was recorded (w_1). The sample was dried in a forced air oven at 100 °C for 5 hr (AOAC Method 934.01) and further ignited in a muffle furnace for 3 hr at 600 °C. After cooling, the crucible containing white or light grey ash was transferred to a desiccator for 2 to 4 hr. the weight of the crucible was checked over time and the lowest weight recorded (w_2). Percent crude ash was calculated by the formula $[(w_2\text{-}w_1)/(w_1\text{-}w_2)] \times 100$.

Determination of Nitrogen Free Extract

Nitrogen Free Extract (NFE) or soluble carbohydrates was determined by difference method (*as is basis*)

%NFE = 100 – (% moisture + % crude protein + % crude fat +% crude ash + % crude fiber) or

%NFE = % dry matter – (% crude protein + % crude ash + % crude fat + % crude fiber)

The conversion of NFE from *as is basis* to *dry matter basis* and vice versa is done by the following formula

% *as is basis* (% dry matter x % dry matter basis)/(100)

% *dry matter basis* – (% as is basis x 100)/(% dry matter)

Determination of Total, Soluble and Insoluble Dietary Fiber

The soluble, insoluble and total dietary fiber was estimated by using total dietary fiber assay kit (TDF 100-A and TDF 10-C, Sigma, St Louis) based on enzymatic gravimetric analysis by AOAC Methods 985.29, 991.42, 991.43 and 993.19. The Total Dietary Fiber (TDF), Soluble Dietary Fiber (SDF) and Insoluble Dietary Fiber (IDF) were estimated by following the procedure described in Sigma Kit.

Estimation of Resistant Starch

RS was estimated by the method of Mangala *et al.* (1999) using treatment of Finger millet meal by Termamyl for 45 min in water at boiling temperature followed by treatment of residue by protease (Megazyme) at 60 °C for 30 min in phosphate buffer (0.1 M, pH 7.5) and centrifuged. The residue was treated with amyloglucoside in acetate buffer (0.1 M, pH 4.6) at 60°C for 35 min. The insoluble residue (RS) was washed with water, suspended in 2ml of 2M KOH solution and solubilized by stirring for 30 min in ice bath. The suspension neutralized, made to 9 ml by acetate buffer and treated with amyloglycoside for 35 min at 60 °C. Glucose released was determined by glucose oxidase peroxidase method (Trinder, 1969) and was calculated as glucose released x 0.9.

Resistance starch was also estimated by enzymatic method as described in AOAC official Method 2002-02 section 45.41.5. Finger millet meal (100 mg) was treated with 4 ml of pancreatic α-amylase (10 mg/ml) containing amyloglucosidase (3 U/ ml) for 16 hr at 37 °C and 200 strokes/ min. A 4 ml of -20 °C ethanol (95%) was added to the tubes, mixed vigorously and the precipitate formed was recovered by centrifugation. The ethanol washing of the residue was repeated twice and the insoluble Resistant Starch (RS) was recovered by centrifugation. The obtained RS dissolved in 2 M KOH (2 ml) by stirring for 30 min in an ice-water bath, neutralized by 4 M HCl and subjected to amyloglucosidase (330 U) digestion in acetate buffer (0.1 M, pH 3.8) for 30 min at 50 °C. RS was estimated by measuring the glucose released by Glucose oxidase- peroxide method and calculated as glucose released x 0.9.

For products containing <10% RS:RS (g/100 g sample) = ΔA x F x (10.3/0.1) x (1/1000) x (100/W) x (162/180) = ΔA x F/ W x 9.27

Determination of Total Carotenoids

Total carotenoids in the Finger millet sample were estimated by the method as described by Asharani *et al.* (2010) and Ranganna S (1986). A 5 g flour sample was extracted repeatedly with acetone. The pooled acetone extract was further extracted with petroleum ether/water mixture (1:8 v/ v). The petroleum ether extract was washed with water, dried over anhydrous sodium sulfate and the absorbance of the extract measured at 453 nm. The total carotene content was calculated as β-carotene equivalents by using molar extinction co-efficient of β-carotene.

Carotenes and lutein was also detected by LC-MS (Shimadzu UPLC Prominence synchronized to Q TRAP 4000 MDS SCIEX, LC-MS/MS Applied Biosystems). The neutral extract of flour sample was enriched by an OasisR HLB Solid Phase Extraction (SPE) cartridge (Waters, Milford, USA) and the eluent concentrated under vacuum in a rotovap (Ependeroff). The residue dissolved in methanol, filtered by a 0.22 μm syringe filtre (Supelco) and a 10 μL of sample was injected into the HPLC column (C_{18}, 2.3 × 100 mm, 3.4 μ particle size, Waters) held at 40 °C (CTO 20 AC) for separation at a solvent flow rate of 100 μL per min over 50.01 min. The LC system consisted of (A) 0.1% formic acid in acetonitrile-methanol (70:30 v/v, J T Bakers, HPLC grade) and (B) 0.1% aqueous ammonium acetate. Chromatography was initiated by a binary gradient solvent system of 5:95 of A:B for 10 min which was gradually changed to 75:25 of A:B at 35 min, the flow was maintained at 75:25 of A:B till 40 min, it attains initial condition of 5:95 of A:B at 43 min and continues till chromatography stops at 50.01 min. The UPLC eluent was directed into mass spectrometer fitted with APCI interface. The Total Ion Chromatogram (TIC) of Enhanced Mass Scan (EMS) and Q1 Mass Scan (Q1MS) data acquisition was performed in full scan mode from 100 amu to 1,200 amu. The

mass spectrometer was operated in both positive and negative polarity with ion spray voltage: 4000 and 2750, source temperature: 450 °C and 275 °C, vacuum: 4.6^{-5} Torr, curtain gas: 20, GS1 50, GS2 50, CE 5, and declustering potential of 40. The turbo ion source was set at 1000 amu/ sec with the interface heater on, dynamic LIT fill time on and 967 scans in a period. The EMS and Q1 MS of TIC was generated.

Determination of Phytic Acid

Phytic acid was determined according to the method described by Wheeler and Ferrel (1971) using $Fe(NO_3)_3.9H_2O$ standard. The phytate phosphorus was calculated from the ferric ion concentration assuming 4:6 (iron: phosphorus) molar ratio. The phytic acid content was calculated on the assumption that it contains 28.20% phosphorus by weight (Deshpande *et al.*, 1982).

Extraction and Determination of Polyphenols

Extraction of polyphenols from Finger millet samples was performed by the method as suggested by Tajoddin *et al.* (2010) with little modification. A 2-g of defatted finger millet meal was refluxed by methanol for two hours, supernatant was decanted in a RBF and the extraction of residue was repeated. The residue remained after extraction with methanol was subjected to reflux extraction twice with acid methanol (1% HCl in methanol) for 2 h each time. The supernatants of methanol extract and acid methanol extracts were concentrated individually using vacuum evaporator (Buchi Type) were analyzed for polyphenol content. The concentration of polyphenols was expressed in milligram of Gallic Acid Equivalents (GAE) per 100 g of sample.

Extraction and Estimation of Ascorbic Acid

The ascorbic acid from the flour samples were extracted by TCA (5%) as described by Thippeswamy *et al.* (2015) and was estimated by the method of Schaffert and Kingsly (1955).

RESULTS AND DISCUSSION

The results on the proximate composition, resistant starch, total polyphenols, phytic acid carotenoids, lutein, and ascorbic acid content of native finger millet (brown variety) grown in Chitradurga District of Karnataka State of India are presented in the following paragraphs.

Moisture Content

The results on the moisture content and dry weight of the finger millet samples are presented in Table 1. The percent moisture was calculated by the ratio of difference in the weight of dry and raw (*as is*) samples to that of the raw weigh of samples and the result multiplied 100. The Losses on Drying (LOD) of the samples were found to be 13.57± 0.19%. The dry matter was calculated by the ratio of dry weight of the sample to that of the raw weight and the resultant multiplied 100. The dry matter in the Finger millet samples were estimated to 86.43% (Table 1).

Moisture content of the grain seeds depends up on the varietal difference of the seed coat, post-harvest age of the grain and meteorological factors such as climate, weather, moisture level, temperature of the region and the storage conditions. Our results on the moisture content in finger millet are in accordance with Shobana *et al.* (2013), Gupta *et al.* (2014) and Desai *et al.* (2010) who reported 13.1%, 12.58% and 12.67% of moisture in finger millet grains. The reports on moisture content of finger millet seeds in the literature have been shown to be in the range of 6.99% to 13.1%. Saldivar (2003), Bisoi *et al.* (2012), Shobana *et al.* (2013), David *et al.* (2014), Gupta *et al.* (2014) and Devi *et al.* (2011) reported 7.7%, 8.51%, 10%, 13.1% 6.99%, 12.58 (*E. coracana* DFM1) and 11.89%, (*E. coracana* HR374) and 7.7% of moisture content respectively in finger millet seeds.

Crude Protein

Crude protein was determined by estimating percent nitrogen in the samples by Kjeldahl method (AOAC method 981.10, 1990) and the resultant multiplied by conversion factor 6.25. The concentration of the crude protein in the whole finger millet sample on *as is basis* and *dry basis* were estimated to 6.12± 0.15% and 7.08% respectively (Table 1).

Dietary protein is remarkable nutrient distributed in plants, animals, microorganisms and their products. A survey of the studies relating to the concentration of protein in finger millet by different investigators reveals the underlying differences in the protein content of finger millet seeds. The results of our investigations on the concentration of protein (Table 1) and the reported values in the literature suggest that the finger millet is the moderate source of protein. The literature citing crude protein content in finger millet was in the range of 0.4% to 12%. Bachar *et al.* (2013) studied 30 accessions of finger millet found in Oasis of Gabes, Tunesia and reported that majority of accessions possess protein content between 4% and 6% however, three accessions GH3, GH4 and GH5 showed 0.79%, 8.2% and 11.88% protein respectively similarly, David *et al.* (2014),

Table 1: Proximate Composition Analysis of Finger Millet Flour

Component	g/100 g Flour *(as is basis)*	g/100 g Flour *(dry basis)*
Moisture	13.57 ± 0.19	--
Dry matter	86.43 ± 0.19	100
Crude protein	6.12 ± 0.15	7.08
Crude fat	1.29 ± 0.08	1.49
Crude fiber	11.95 ± 0.99	13.93
Crude ash	3.64 ± 0.01	4.21
Acid insoluble ash	0.64 ± 0.03	0.74
NFE	63.43 ± 1.32	73.29
Total K Cal	289.81	334.89

Saleh *et al.* (2013) and Gupta *et al.* (2014) showed 10.28%, 9.8% and 12.25 and 11.6% protein in raw finger millet flour. Mwikya *et al.* (2002), Sildivar (2003), Chethan and Malleshi (2007a), Bisoi *et al.* (2012), Desai *et al.* (2010) and Devi *et al.* (2011) reported the protein content of finger millet around 3% to 7%. Gunashree *et al.* (2014) reported that different processing techniques exhibited 3.1 to 13.5% of crude protein, which otherwise showed 6.8% of crude protein in unprocessed finger millet flour. On the contrary, 8.7% of crude protein in unprocessed floor has been shown to decrease after different processing techniques (Shobana *et al.*, 2013) like refining (3.6%), malting (4.5%), hydrothermal processing (8.3%), decortication (6.5%), expanding (4.7%), popping (6.4%), flacking (6.5%), toasting (6.2%) and roller drying (5.8%).

Crude Fat

The crude fat in the samples were estimated by (AOAC Method 920.39C, AOAC Method 934.01 and AOAC Method 960.39). The percent crude fat was calculated by ratio of weight of petroleum ether extracted material to that of the weight of the sample (*as is basis* or *dry basis*) and the resultant multiplied by 100. The crude fat content in the whole Finger millet meal was estimated and found to be 1.29 ± 0.08% and 1.49% on *as is basis* and *dry basis* respectively (Table1). Our results on the fat content in finger millet seeds are in agreement with that of the earlier reported values. Crude fat content of 1.5% and 1.8% in finger millet flour was shown to reduce till 0.6% by subjecting grains to different processing techniques (Shobana *et al.*, 2013; and Gunashree *et al.*, 2014). The crude fat in the finger millet was reported in the range of 0.8 to 2.1% (Gupta *et al.*, 2014; Devi *et al.*, 2011; Mwykya *et al.*, 2002; Desai *et al.*, 2010; Bhatt *et al.*, 2003; Glew *et al.*, 2008; and David *et al.*, 2014) whereas, Sridhar and Lakshminarayana (1994) reported total lipid content at much higher side, 5.2% including free lipids 2.2%; bound lipids 2.4%; and structural lipids 0.6% and also suggested that 74% of the total lipid content in finger millet is unsaturated.

Crude Fiber, Total Dietary Fiber, Insoluble Dietary Fiber and Soluble Dietary Fiber

CF is the dry weight of the sample residue (retained after digesting the meal with acid followed by base) subtracted weight of the ash. The percent crude fiber was calculated by the ratio of dry weight of the residue (retained after acid and alkali digestion of meal) to that of the weight of the sample (*as is basis* or *dry basis*) and the resultant multiplied by 100. DF is an estimate of residue remained after complete enzymatic digestion of digestible matter present in the sample. The results on the crude fiber and dietary fiber content of finger millet are presented in Table 1 and Table 2 respectively. The concentration of crude fiber in the Finger millet flour sample was estimated to 11.95 ± 0.99% (*as is basis*) and 13.93% (*dry basis*). The IDF and SDF in the meal were estimated to 10.56% and 1.06% and the TDF was calculated to be 11.62% for *as is basis*, whereas, the concentration of IDF and SDF on *dry basis* was found to be 12.2% and 1.2% respectively and the TDF was calculated to 13.44%.

Finger millet was reported to contain high levels of fiber as it is compared to that of the cereal grains like rice, wheat and most other millets. Our results on the fiber content of

Table 2: Dietary Fiber and Resistant Starch Contents of Finger Millet Flour

Component	g/100 g Flour *(as is basis)*	g/100 g Flour *(dry basis)*
Insoluble dietary fiber	10.56	12.22
Soluble dietary fiber	1.06	1.23
Total dietary fiber	11.62	13.44
Resistant starch	< 0.15	< 0.15

finger millet (Tables 1 and 2) evidences that the concentrations of CF matches well with that of the TDF of native finger millet tested. Considerable amount of literature is available on the fiber content of finger millet, which reports large disparities in the concentration of fiber in finger millet from 0.93% to 22.5%. Ramulu and Rao (1997) reported 11% IDF, 2% SDF and 12% TDF, Mangala *et al.* (1999) 17, 1 and 18%; Roopa and premavalli (2008) 18.1, 0.7 and 19.1%; Dharmaraj and Malleshi (2010) 15.7, 1.4 and 17.1%; Shobana *et al.* (2013) reported 16.1, 3.5 and 19.6% IDF, SDF and TDS in finger millet. Chethan and Malleshi (2007a), Gopalan *et al.* (2009) and Mathangi and Sudha (2012) reported 15-20%, 11.5% and 18% DF respectively in finger millet. Contrary to this various studies reports CF content of finger millet in the range of 3.1 to 4.3%, for example, Joshi and Katoch (1990) observed 3.7%, Ravindran *et al.* (1991) 4.3%, Desai *et al.* (2010) 3.62 to 3.8%, Devi *et al.* (2011) 3.5%, Saleh *et al.* (2013) 4.3 to 3.6%, David *et al.* (2014) 3.1%, Gunashree *et al.* (2014) 3.17%, Gupta *et al.* (2014) 3.27 and 3.58% of CF in finger millet. However, Bachar *et al.* (2013) observed large variations in the NDF (22.92 to 57.25%), ADF (10.67 to 14.79) and CF (0.93 to 10.01%) content of thirty finger millet accessions.

In the present investigation the concentrations of CF (13.93) and DF (13.44) matches well, which is in contrast to the results obtained by Kamat and Belvadi (1980) and Saldivar (2003) who reported large differences in the CF (3.6%) and DF (18.6 and 19.1%) content of finger millet.

Crude Ash and Acid Insoluble Ash

The percent crude ash (acid soluble) obtained after igniting finger millet meal (*as is basis*) in a muffle furnace at 600 °C was estimated to 3.64±0.01% and the concentration of acid insoluble ash was found to be 0.64±0.03% whereas, the total ash content and acid insoluble ash content of sample on *dry basis* were estimated to 4.21% and 0.74% respectively (Table 1). The mineral ash (soluble ash) content was calculated by subtracting acid insoluble ash from total ash and was estimated to 3% and 3.47% for *as is basis* and for *dry basis* respectively. The ash content of the finger millet is reported in the range of 1.7 to 4.13% however, most studies showed it to be between 2.1 to 2.7% (Singh and Raghuvanshi, 2012). Singh and Srivastava reports 1.47 to 2.52% of ash content of 16 varieties of finger millet and Bachar *et al.* (2013) showed the variation in total ash content of 30 accessions of finger millet ranging from 2.77 to 3.86% and Amadou *et al.* (2013) reported 2.7% total ash.

Nitrogen free extract

The NFE is the measure of total carbohydrates by difference method. It is the sum of percent crude protein, crude fat, crude fiber, crude ash and moisture subtracted by 100. The results on the total carbohydrates are shown in Table 1. The concentrations of total carbohydrates in whole finger millet flour were estimated to 63.43% and 73.39% on *as is basis* and *dry basis* respectively. Total carbohydrate content of finger millet has been reported to be in the range of 65 to 79.5% (Pore and Magar, 1979;Hulse *et al.*, 1980; Joshi and Katoch, 1990; and Bhatt *et al.*, 2003). Our results on the total carbohydrate content in finger millet are in accordance with earlier reports (Wankhede *et al.*, 1979; Malleshi *et al.*, 1986; Saldivar, 2003; Shobana and Malleshi, 2007; Gopalan *et al.*, 2009; Mathangi and Sudha, 2012; Bisoi *et al.*, 2012; and Gunashree *et al.*, 2014) However, Gupta *et al.* (2014) reported 79%, Desai *et al.* (2010) 76.51% and David *et al.* (2014) 76.43% of carbohydrates in finger millet.

Resistant Starch

The results on RS are presented in Table 2. The RS in the Finger millet meal was found to be 0.18% and 0.11% by the method of Mangala *et al.* (1999) and AOAC method (2002-02 section 45.41.5) respectively. Mangala *et al.* (1999) reported the RS content of processed and unprocessed finger millet (Indaf) to be 0.02 and 0.12% respectively whereas, Roopa and Premavalli (2008) found 0.8 to 1% among base and hilly finger millet varieties.

Determination of Total Carotenoids

The washed petroleum ether extracts of acetone soluble fractions of Finger millet flour were dissolved in methanol and used to estimate total carotenoids by reading the absorbance at 453 nm in a spectrophotometer (Shimadzu, UV 2600). The total carotene content was calculated as β-carotene equivalents by using molar extinction co-efficient of â-carotene. The total carotenoids in the Finger millet flour were estimated to 512 µg per 100 g or 0.000512% (Table 3).

Table 3: Total Polyphenol, Total Carotenoid and Ascorbic Acid Content of Finger Millet Flour (*as is basis*)

Component	g/ 100 g Flour
Total polyphenol (acid methanol extract)	3.2
Phytic acid	0.98
Ascorbic acid	0.018
Carotenoids	512 µg

Table 4: LC-MS Analysis Showing the R_t and Mass of Carotenoids Extracted from Finger Millet

Component	Retention Time (R_t)	Q1 Positive MS *amu* [M*] H+	Ttention Time (R_t)	Q1 Negative MS *amu* [M*] H-
α-Carotene	29.261	537.41	29.26	535.41
γ-Carotene	29.44	537.41	29.471	535.41
δ-Carotene	29.716	537.41	29.723	535.416
Lutein	24.46		24.46	568.44

The results on detection of carotenes and lutein by LC-APCI-MS are presented in Table 4. The results evidences that the spectrum generated from TIC of Q1-EMS positive scan and Q1-EMS negative scan of the extracts of whole finger millet flour could resolve three different carotenes into different peaks at R_t values of 29.26, 29.48 and 29.71 and lutein at R_t of 24.461. Mass spectrum generated from TIC of EMS positive ions and TIC of EMS negative ions showed the molecular mass of $M^*[H^+]$ 537.4 amu and $M^*[H^-]$ 535.4 amu in APCI positive and negative ionization modes respectively. The spectrum of lutein peak, that was resolved at R_t 24.461 min in EMS negative mode showed molecular mass $M^-[H]$ 568.4 amu which corresponds well with that of lutein.

Carotenoids are essential antioxidants that prevent atherosclerosis and maintain immune functions and vision (Dwyer *et al.*, 2001; and Beatty *et al.*, 1999). The literature on the carotenoid content of finger millet is rather scarce, however, Gunashree *et al.* (2014) reported 43.82 µg total carotenoids per gram of finger millet flour whereas Asharani *et al.* (2010) conducted studies on 14 finger millet cultivars and estimated 78 to 316 µg total carotenoids per 100 g of whole grain samples. Our results on the total carotenoid content are superior to Asharani *et al.* (2010) but, inferior to Gunashree *et al.* (2014), who have determined it by HPLC method

Analysis of Polyphenols, Phytic Acid and Ascorbic Acid

The results on the estimation of total polyphenols, phytic acid and ascorbic acid are presented in Table 3. The results on the investigations of total polyphenols, phytic acid and ascorbic acid content in finger millet were estimated to 3.21% GAE, 0.98% and 0.018% respectively. Finger millet variety studied in the present investigation showed highest polyphenol content as it is compared to that of the values available in the literature. The extraction solvent, temperature and method have a profound influence on the extractability of polyphenols. Extraction by methanol and acidified methanol at ambient temperature extracted 19.6 and 35.5% of polyphenols whereas, the same solvents by reflux at boiling temperature extracted 53.9 and 100% polyphenols (Chethan and Malleshi, 2007a). Ramachandra *et al.* (1977) reported variations in the polyphenol content of 26 finger millet varieties from 0.08% to 2.44% whereas, Shankara (1991), Chethan and Malleshi (2007a) and Siwela *et al.* (2010) studied eighty five, five and sixteen varieties of finger millet and extracted 0.19 to 3.37%, 1.3 to 2.3% and 0.34 and 1.84% of polyphenol. McDonough *et al.* (1986) reported 0.55 to 0.59% polyphenol while Thompson (1993) and Saldivar (2003) reported 0.61% and 0.1% of polyphenols.

Polyphenols, tannins and phytic acid are considered as antinutrients as they are reported to decrease mineral bioavailability, protein and carbohydrate digestibility (Thompson *et al.*, 1987, Hag *et al.*, 2002; and Chethan and Malleshi, 2007). The discovery of range of beneficial activities associated with polyphenols, tannins and phytates transformed their nutritional status from anti-nutrients to nutra-ceuticals. Polyphenols are reported to have different beneficial activities like antioxidant, anti-mutagenic, anti-oestrogenic, antiviral, anti-carcinogenic, anti-inflammatory, and platelet aggregation inhibitory activity (Sripriya *et al.*, 1996; and Viswanath *et al.*, 2009). They are also reported to possess antimicrobial activity Viswanath *et al.* (2009) and Chethan and Malleshi (2007b) *invitro*antidiabetic (Shobana *et al.*, 2009; and Hegde *et al.*, 2005), anti-cardiovascular properties and also found to inhibit aldose reductase thus prevents sorbitol accumulation Chethan *et al.* (2008) and also prevents glycation and cross linking of collagen Hegde *et al.* (2002). Due to the potential benefits polyphenols are also useful in management of several physiological disorders such as diabetes mellitus, hypertension, vascular fragility, hypercholesterolemia, prevention of oxidation of Low-Density Lipoproteins (LDLs) and also improvement of the health of gastrointestinal tract (Scalbert *et al.*, 2005).

Phytic acid is a pervasive molecule that is distributed in the plant kingdom. It is implicated to possess antioxidant, antidiabetic, and anticancer activity (Thompson, 1993). Different studies have reported its abundance in legumes, cereals and millets and also estimated its levels in different finger millet accessions in the range of 0.15 to 0.69% however, Sridevi *et al.* (2008) reported 3.9% phytic acid in one brown

cultivar. Our results on phytic acid content of native finger millet are in accordance with Gunashree *et al.* (2014), who reported 0.68 to 0.7% and 0.685% phytic acid. On the other hand, Thompson (1993), Rao (1994) and Mwykya *et al.* (2000) reported 0.48, 0.15 and 0.36% phytic acid in finger millet cultivars.

Rane *et al*. (2014) analyzed the concentrations of ascorbic acid in raw and processed finger millet meal. They have estimated an initial concentration of 0.006% of ascorbic acid in unprocessed finger millet flour which was increased to 0.013 and 0.016% after 4 hr and 24 hr of germination of finger millet seeds. Our results on ascorbic acid content (0.018%) in native finger millet appeared superior to that of the results reported by Rane *et al*. (2014) however, the unprocessed finger millet seeds contain low levels of ascorbic acid as compared to yellow variety mung bean seeds (Thippeswamy *et al.*, 2015). Ascorbic acid is a potential antioxidant molecule, deficiency of which is reported to cause scurvy, blood vessel fragility and connective tissue damage. It is reported to quench potentially damaging free radicals thus prevent oxidation of biomolecules (Arrigoni and Tullio, 2002). At adequate to high dosage it is reported to trigger immunostimulation, anti-inflammatory response and also decrease the levels of inflammatory cytokines IL-1α, IL-2, IL-8, TNF-α, and C- reactive protein in cancer patients and the people with rheumatoid arthritis (Naidu, 2003).

CONCLUSION

Karnataka State of India is the largest producer of finger millet, which has been staple food for millions of poor people. Body of evidence suggests that, finger millet contains high levels of antioxidants and minerals and thus, may manage, prevent, or delay the onset of non-communicable and degenerative diseases. Currently, finger millet is finding place in the production of value added foods such as, malt, biscuits, bakery products, functional foods and various complementary foods. The information regarding the proximate analysis, fiber and various other phytochemical constituents of native finger millet studied in the present investigation may help explore its potentials for the benefit of consumers' and farmers too, which in turn restore or increase its cultivation.

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