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Sensory Characteristics, Total Polyphenol Content and *In vitro* Antioxidant Activity of Value Added Processed Barnyard Millet Flour Chapattis

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Abstract

Introduction: Value addition of millet is an important strategy to improve its utilization and the method of processing determines the quality characteristics of the value added products. Utilization of millets in food formulations is increasing worldwide, since they are rich sources of phytochemicals and dietary fiber which offer several health benefits.

Objective: Thus in the present study, an attempt was made to develop roasted and pressure cooked barnyard millet flour incorporated chapatti at different levels and its consumer acceptability to evaluate the total polyphenol content and *in vitro* antioxidant activity.

Materials and methods: Roasted and pressure cooked barnyard millet flour incorporated chapattis were developed at four different levels i.e., 10%, 30%, 50% and 100% millet flour to wheat flour. Sensory evaluation was done for all the developed products using 9 point hedonic scale. For the best variation, the total polyphenol content was estimated using folic ciocalteau method and the *in vitro* antioxidant activity were evaluated on the basis of measuring Ferric reducing ability power (FRAP) and scavenging activity for DPPH radicals by methanolic extracts.

Results: Roasted and pressure cooked barnyard millet flour incorporation in the preparation of chapatti at 10 per cent level was acceptable. The total polyphenol content of best variation (10%) of roasted and pressure cooked barnyard millet flour incorporated chapatti was 6.12 mg/g and 5.38 mg/g respectively. The DPPH radical scavenging activity and Ferric reducing ability power (FRAP) of the same was found to be 59% and 13.42 mg/g and 53% and 11.57 mg/g respectively. The standard chapatti prepared with wheat flour was found to have 4.02 mg/g of polyphenol content, 47% of DPPH radical scavenging activity and 9.84 mg/g of Ferric reducing ability power.

Conclusion: Thus the present study concludes that roasting and pressure cooking enhances the polyphenol content of barnyard millet grains which might also contribute significantly to the management and/or prevention of degenerative diseases associated with free radical damage due to its high polyphenol content and antioxidant activity.

Keywords: Barnyard millet; Roasting; Pressure cooking; Polyphenol; DPPH activity; FRAP assay

Introduction

Millets play very specific role in human nutrition because of their multiple qualities [1]. Barnyard (*Echinochloa frumentacea Link*) is one of the fastest growing crops of all the millets, mature in 90 to 100 days. They are an important source of vital minerals like niacin, magnesium, phosphorus, manganese, iron and potassium. They contain high amounts of protein, fiber, essential amino acid methionine, lecithin, and vitamin E [2]. Recent studies have shown that due to the high content of these nutrients, millets have therapeutic benefits such as control of asthma, migraine, blood pressure, diabetes, heart disease, atherosclerosis and heart attack. Fibre, in millet, prevents gallstones formation. Because of these benefits, millets can be used in functional foods and as nutraceuticals. Hence, they are also called as 'nutri cereals' [3].

At the household level, the common methods of food processing include wet heat treatment such as pressure cooking and dry heat treatment like roasting. These processing methods alter the nutritive value of foods. The nutrient composition and technological properties of minor millet grains offer a number of opportunities for processing and value addition to use as next generation to satisfy the consumers of different culture, location and society. Value addition of millet is an important strategy to improve its utilization and the method of processing determines the quality characteristics of the value added products. Wheat has a unique property of forming an extensible, elastic and cohesive mass when mixed with water. Millet flours lack these properties when used alone. Hence replacement of wheat flour with millet flour in wheat composite flours brings lot of innovative Ready-to-eat or Ready-to-serve minor millet based processed products [4].

Utilization of millets in food formulations is increasing worldwide, since they are rich sources of phytochemicals and dietary fiber which offer several health benefits [5]. Millets have received considerable attention in the last several decades due to the presence of unique blend of bioactive phytochemicals which are powerful antioxidants. These unique bioactive compounds such as polyphenols in whole-grains are proposed to be responsible for the health benefits of whole-grain consumption such as cardiovascular diseases, cancers, type II diabetes, neurodegenerative diseases or osteoporosis [6,7]. Fortification of diets with millets and cereals rich in phenolic acids was shown to impart many health benefit properties, and this can be exploited in developing healthy foods [8]. Chapatti as a staple food of the Indian subcontinent was selected aptly for enrichment to reach the different sections of the population [9,10]. Chapatti is a flat unleavened, hot plate baked product prepared from whole wheat flour by converting dough with water by

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adding other ingredients like salt and sugar according to the taste. Though wheat flour is a staple food for half of the world population; still it is not a complete diet which lacks in micronutrients [11]. Chapattis prepared with value addition of processed millet flour provide an additional dietary fiber and phytochemicals constituents that could be supportive for diabetic and obese individuals [12]. Therefore, millet polyphenols have received tremendous attention among nutritionists, food scientists, and consumers due to their roles in human health [13]. Thus in the present study, an attempt was made to develop roasted and pressure cooked barnyard millet flour incorporated chapatti at different levels and its consumer acceptability to evaluate the total polyphenol content and *in vitro* antioxidant activity.

Methods and Materials

The barnyard millet was procured from local market of Vellore district, Tamil Nadu, India. The millets were cleaned properly and stored in sealed containers till their use in different processing such as roasting and pressure cooking. Processed foods are usually less susceptible to early spoilage than fresh foods. It is widely accepted that simple and inexpensive traditional processing techniques are effective methods of achieving desirable changes in the composition of grains.

Processing techniques

Roasting: Roasting involves the application of dry heat to grains using a hot pan or at a temperature of 150 to 200°C for a short time and powdered [14].

Pressure cooking: The grains were washed, soaked and pressure cooked in tap water in the ratio of 1:3 (w/v) for two whistles in medium flame. They were solar dried for 2 days and then powdered [15].

Product development: The most acceptable product chapatti was selected for the incorporation of roasted and pressure cooked barnyard millet flour. Totally 9 variations of chapattis containing roasted or pressure cooked barnyard millet flours were developed. Four variations in roasted barnyard millet flour (10%, 30%, 50% and 100%), four variations in pressure cooked millet flour (10%, 30%, 50% and 100%) and one standard product. Wheat is an ideal grain in making chapattis. The use of millet grains as replacement in wheat composite flours seems the best method that can be used for the preparation of nutritional, healthy, safe, high quality and shelf stable food products at house hold and commercial scale to promote the utilization of millet grains. These wheat-millet composite flour blends may have the advantage of being nutritious, economical and health promoting. They have mighty potential to be included in traditional and novel foods. The value added chapatti was prepared by the addition of roasted or pressure cooked barnyard millet flour in different combination with wheat flour as mentioned below.

Preparation of chapattis from processed barnyard millet flours: Barnyard millet incorporated chapattis were prepared by incorporating roasted or pressure cooked barnyard millet flour to wheat flour at 10%, 30%, 50% and 100% levels. Standard chapattis were prepared with wheat flour without the incorporation of processed barnyard millet flour. The various levels of incorporation of roasted and pressure cooked barnyard millet flour for the development of chapattis was given in Table 1.

Preparation of chapatti

The wheat flour was mixed with roasted or pressure cooked barnyard millet flours at various levels of incorporation. The chapattis were prepared from samples according to the procedure (Table 2 and Figure 1).

Variations	Wheat Flour (g)	Processed Barnyard Millet Flour (g)	
		Roasted	Pressure cooked
Standard	100	-	-
V1	90	10	10
V2	70	30	30
V3	50	50	50
V4	-	100	100

Table 1: Variations for the preparation of chapattis from processed barnyard millet flour.

Ingredients	Weight (g)
Wheat flour	At different levels
Processed Barnyard millet flour	At different levels
Oil	1 tsp.
Salt	To taste

Table 2: Ingredients used in the preparation of chapatti.

Wheat flour
↓
Mix thoroughly with required amount of salt and water
↓
Mix well for few minutes
↓
Made into dough
↓
Kept for 10-15 minutes
↓
Divide into balls
↓
Thin it in roll board and pin
↓
Heat the tawa and cook till it was done
↓
Reverse the chapatti with ladle till it cook

Figure 1: Method for preparation.

Organoleptic evaluation of the developed value added chapattis

Selection of any food products depends upon its perceived taste, nutritional labelling, family and cultural preference and prior purchase habits. Taste is generally regarded as the predominant reason for selecting a food followed by health and other considerations [16]. Organoleptic quality evaluation of the product plays an important role in the acceptance and preference of foods. The consumer acceptability of each product was carried out by panel of members through organoleptic evaluation using 9 point hedonic scale with score card of scores ranging from 9 to 1, where 1=dislike extremely, 5=neither like nor dislike and 9=like extremely was used. The parameters assessed for chapattis were colour, flavour, taste, texture, foldability, breakability and over all acceptability. A ballot sheet was prepared to evaluate sensory attributes of developed value added processed barnyard millet flour chapatti.

Selection of best variation of value added chapatti

Based on the organoleptic evaluation results, the best and highly accepted variation from each processing techniques (roasted and pressure cooked) of barnyard millet flour incorporated chapattis and the standard chapatti were selected for the determination of total polyphenol content and *in vitro* anti-oxidant activity.

Determination of total polyphenol content and *in vitro* antioxidant activity

Several studies have shown that 80% methanol is an effective solvent in extracting phenolics and other polar substances in cereals [17,18]. In this study, methanolic extract of the standard chapatti and the selected best chapatti of each processing techniques was used for the determination of total polyphenol content and antioxidant activity.

Extraction procedure

For methanolic extract of each selected product, 50g of powdered sample was macerated and extracted with 500 ml of 80% methanol in a glass jar for 48 hours at room temperature in an orbital digital agitator (Rotatest 560VIT. 15-300 T/MIN). After filtering with filter paper and the methanol solvent was removed under reduced pressure at low temperature (40°C to 45°C) using a rotary vacuum evaporator [19].

Determination of total polyphenol content

The total polyphenol content of the methanolic extract of selected products was carried out according to the Folin-Ciocalteu colorimetric method [20].

In vitro anti-oxidant activity

DPPH radical scavenging activity: The free radical–scavenging activities of extracts and ascorbic acid were measured by using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) as procedure proposed by Burits [21]. The DPPH solution (0.3 ml) was added to 2.5 ml of extracts. The mixture was shaken and left to stand at 20°C for 30 minutes at room temperature. Then, the absorbance was measured at 517 nm. Ascorbic acid was used as positive control. The inhibition percentage of the DPPH radical was calculated using the following formula,

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

Where,

A_0 indicate absorbance of the control and

A_1 indicate absorbance of extract sample.

Ferric reducing ability power (FRAP): The ferric ions (Fe^{3+}) reducing activity power was measured according to the method described by [22]. The method was based on the chemical reaction of Fe^{3+} to Fe^{2+}. The working solution was prepared by mixing 1 ml of extracts with 2.5 ml phosphate buffer (0.2M; pH 6.6) and 2.5 ml $N_6C_6FeK_3$ (1%; m/v). The mixing was incubated at 50°C for 30 minutes. The Fe^{2+} was monitored by measuring the formation of ferrous complex at 700 nm. The reducing power of the extracts was represented as ascorbic acid equivalent (mg AAE/g).

Statistical analysis

The final data was compiled and analyzed by using statistical methods. The results were represented as Descriptive statistics such as mean, standard deviation followed by Duncan's multiple comparison tests and correlation p values < 0.05 were considered significant.

Results and Discussion

Organoleptic evaluation of the roasted and pressure cooked barnyard millet flour incorporated chapatti

In a product development, there is a need to state the descriptive characteristics of the product along with a mere comparison of scores to assess the product quality. Descriptive characteristics of any product are very important to assess the acceptability of the product. The acceptability of the developed value added products varied with the incorporation levels [23]. The mean scores of the organoleptic evaluation of all the developed value added products using nine point hedonic scales were given in Tables 3 and 4. The pooled score of standard chapatti in all the attributes such as colour, flavour, taste, texture, foldability, breakability and overall acceptability was 8.6. Among the four variations developed, the variation 1(V1) of both roasted and pressure cooked barnyard millet flour incorporated chapatti at 10% level has got highest mean score of 8 in all the attributes respectively (Figures 2 and 3). As the level of incorporation of processed barnyard millet flour increases, the acceptability range of the developed product decreases. The chapattis prepared by the addition of 10% cereal bran showed better performance and were quite comparable with whole-wheat flour regarding the proximate components and sensory attributes [24]. Results of the Duncan's test revealed that there was significant difference for all the variations in response to all the organoleptic

Variations	Colour	Flavour	Taste	Texture	Foldability	Breakability	Overall acceptability
Standard	8.6 ± 0.54^a	8.6 ± 0.54^a	8.4 ± 0.54^a	8.6 ± 0.54^a	8.6 ± 0.54^a	8.6 ± 0.54^a	8.6 ± 0.54^a
V1	8.2 ± 0.44^a	8 ± 0.00^a	8 ± 0.00^a	7.6 ± 0.54^b	7.8 ± 0.44^b	7.8 ± 0.44^b	8 ± 0.00^a
V2	6.6 ± 0.54^b	6.4 ± 0.54^b	6 ± 0.7^b	6 ± 0.00^c	5.8 ± 0.44^c	6 ± 0.00^c	6.2 ± 0.44^b
V3	4.6 ± 0.54^c	4.4 ± 0.89^c	3.4 ± 0.54^c	3.6 ± 0.89^d	3.8 ± 0.44^d	3.4 ± 0.54^d	4 ± 0.7^c
V4	2.4 ± 0.89^d	2 ± 0.7^d	1.6 ± 0.54^d	1.8 ± 0.83^e	1.8 ± 0.44^e	1.8 ± 0.44^e	2 ± 0.00^d

a-e values in the same column with different superscripts are significantly different at (p<0.05) in Duncan's multiple range test.

Table 3: Organoleptic evaluation of roasted barnyard millet flour incorporated chapatti.

Variations	Colour	Flavour	Taste	Texture	Foldability	Breakability	Overall acceptability
Standard	8.6 ± 0.54^a	8.6 ± 0.54^a	8.4 ± 0.54^a	8.6 ± 0.54^a	8.6 ± 0.54^a	8.6 ± 0.54^a	8.6 ± 0.54^a
V1	8 ± 0.44^a	8.2 ± 0.44^a	8 ± 0.44^a	7.8 ± 0.54^b	7.6 ± 0.54^b	7.8 ± 0.54^b	8 ± 0.44^b
V2	6.2 ± 0.83^b	6.2 ± 0.44^b	5.6 ± 0.54^b	5.8 ± 0.44^c	5.6 ± 0.54^c	5.8 ± 0.44^c	5.8 ± 0.44^c
V3	4.4 ± 0.54^c	4.2 ± 0.83^c	3.4 ± 0.54^c	3.4 ± 0.54^d	3.6 ± 0.54^d	3.4 ± 0.54^d	3.6 ± 0.54^d
V4	2.4 ± 0.89^d	2 ± 0.7^d	1.6 ± 0.54^d	1.8 ± 0.83^e	1.8 ± 0.44^e	1.8 ± 0.44^e	2 ± 0.00^e

a-e values in the same column with different superscripts are significantly different at (p<0.05) in Duncan's multiple range test.

Table 4: Organoleptic evaluation of pressure cooked barnyard millet flour incorporated chapatti.

parameters evaluated for acceptability. Hence from the above results it was clear that millet incorporation in the preparation of chapatti at 10 per cent level was acceptable. Thus the variation 1 from each processing techniques of barnyard millet flour incorporated chapattis with their standard product were chosen for the estimation of total polyphenol content and antioxidant activity.

Total polyphenol content of methanolic extract of selected value added chapattis

Polyphenol is an antioxidant phytochemicals containing several hydroxyl group, they tend to prevent or neutralize the damaging effects of free radicals. It was described that alcoholic extracts of several species of millets are rich sources of phenolic compounds and show antioxidant activities, metal chelating, and reducing powers [25]. The total polyphenol content of the methanolic extract of selected value added chapattis and the standard chapatti were summarized in the Table 5.

Phenolic compounds exhibit significant pharmaceutical activities such as anticancer, anti-inflammation and anti-oxidative effects [26]. In this study, the total polyphenol content of best variation (10%) of roasted and pressure cooked barnyard millet flour incorporated chapatti was 6.12 mg/g and 5.38 mg/g respectively. The standard chapatti was found to have 4.02 mg/g of total polyphenol content. The highest levels of polyphenols were observed in roasted and pressure cooked barnyard millet flour incorporated chapatti compared to the standard chapatti. The highest content of phenolic compounds is known to have direct antioxidant property due to presence of hydroxyl groups which can function as hydrogen donor [27].

Figure 2: Roasted barnyard millet flour incorporated chapatti.

Figure 3: Pressure cooked barnyard millet flour incorporated chapatti.

Methanolic extracts of selected value added products	Total polyphenol content (mg/g)
Standard chapatti	4.02
RBMF incorporated chapatti at 10% level (V1)	6.12
PCBMF incorporated chapatti at 10% level (V1)	5.38
RBMF: Roasted barnyard millet flour PCBMF: Pressure cooked barnyard millet flour	

Table 5: Total polyphenol content of methanolic extract of selected products.

It is a well-known fact that millets are rich in polyphenols. The increase in total polyphenol content of roasted barnyard millet flour could result from release of bound polyphenols or from maillard reaction products formed during roasting that have been reported to possess scavenging activity on reactive oxygen species [28,29]. Maillard reaction involves condensation reactions between sugars and amino acids and has been found to be linked to polyphenols [30] via inhibition of polyphenol oxidase [31]. The longer the cooking time, the greater losses of the total phenolic compound measured. This could be due to the breakdown of phenolics or losses (leached out) during cooking as most of the bioactive compounds are relatively unstable to heat and easily solubilized [32]. Thus pressure cooked millets showed high total phenolic content due to its short cooking period.

In vitro antioxidant activity of methanolic extract of selected value added chapattis

Table 6 shows the finding of *in vitro* antioxidant activities of selected products. Antioxidants properties were evaluated on the basis of measuring Ferric reducing ability power (FRAP) and scavenging activity for DPPH radicals by methanolic extracts. The model of scavenging DPPH radical is a widely used method to evaluate the free radical scavenging activities of antioxidants [33]. In the DPPH assay, the colored stable DPPH radical (Purple) is reduced in the presence of an antioxidant or a hydrogen donor in to the non-radical form (Yellow). The DPPH scavenging activities of antioxidants are attributed to their hydrogen donating abilities [34]. In the present study, it should be noted that roasted and pressure cooked barnyard millet flour incorporated chapatti showed potent DPPH radical scavenging activity at 59% and 53% respectively when compared with the standard chapatti (47%).

Furthermore, FRAP assays was used to determine antioxidant activity of selected products. Methanolic extracts of roasted and pressure cooked barnyard millet flour incorporated chapatti showed better effect (13.42 mg/g and 11.57 mg/g respectively). These data marked that the roasted and pressure cooked millet flour incorporated chapatti has higher significant activities than standard (wheat flour) chapatti to reduce Fe^{3+} to Fe^{2+} by measuring the absorbance at 700 nm. Significant correlations were observed between total polyphenol content and DPPH scavenging activity (r=0.92) and ferric reducing ability power (r=0.87) indicating the role of polyphenol compounds in inhibiting free radicals and radical cations.

Conclusion

Based on the obtained results, the incorporation of 10% roasted and pressure cooked barnyard millet flour to wheat flour in the preparation of chapatti showed better sensory acceptability. This indicates that millet based products of good sensory qualities could be produced from 10% incorporation of processed millet flour. The total polyphenol content and *in vitro* antioxidant activity of the barnyard millet flour was influenced by both the processing methods. The chapatti made

Methanolic extracts of selected value added products	% inhibition of DPPH (mg/ml)	Antioxidant activity FRAP (mg AAE/g)
Standard chapatti	47%	9.84
RBMF incorporated chapatti at 10% level (V1)	59%	13.42
PCBMF incorporated chapatti at 10% level (V1)	53%	11.57
RBMF: Roasted barnyard millet flour PCBMF: Pressure cooked barnyard millet flour		

Table 6: Properties of the different antioxidant process of DPPH free radical scavenging activity and ferric reducing ability power of the selected products.

from barnyard millet flour subjected to roasting and pressure cooking exhibited higher antioxidant activity (DPPH scavenging activity and FRAP) mainly due to its high polyphenol content. Thus, the present study concludes that roasted and pressure cooked barnyard millet flour could be utilized in value addition and might also contribute significantly to the management and/or prevention of degenerative diseases associated with free radical damage due to its high polyphenol content and antioxidant activity.

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