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Nutrient and antioxidant analysis of raw and processed minor millets

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ABSTRACT

The nutrient composition of processed minor millets was investigated by various cooking methods like wet heating (boiling, blanching, soaking and germination) and dry-heating (roasting) and to compare the antioxidant activity of processed minor millets in relationship with their total phenolic content. Foxtail and proso millet were subjected to different processing methods; samples were dried and powdered into fine flours, respectively. Standard methods were used to evaluate the flours for moisture, ash, protein, fibre, iron, phosphorus, calcium, magnesium and zinc. The antioxidant activity was estimated with respect to DPPH radical scavenging activity; FRAP assay and reducing power method. The soaked samples of foxtail and proso millet showed higher scavenging activity which was found to be (51.06% and 52.12%) respectively. The antioxidant power of roasted foxtail millet and blanched proso millet had significant increase ranging about (317.5 µ mol and 236.8 µ mol) respectively using FRAP assay. The blanched samples had higher reducing power indicating enhanced antioxidant activity i.e. (0.426 and 0.418) respectively. The phytochemical content was determined qualitatively. The blanched and germinated millet samples possessed higher antioxidant activity.

Introduction

The under utilized food grains have a vast scope for not only supporting the commercially grown crops by reducing pressure on their availability but they are cheap source of nutrients and can be raised at low management cost (Sankhala, et al., 2004). Minor millets are a group of grassy plants with short slender stalks and small grains possessing remarkable ability to survive under severe drought. The nutritional significance of minor millets lies in their richness in micronutrieints like calcium, iron, phosphorus, vitamins and sulphur containing amino acids. Soluble fibre content of minor millets varies from around 3.4% in foxtail and proso millets to 6.5% in little, barnyard and finger millets. For these superior properties of minor millets, they have been recently designated as "NUTRITIOUS MILLETS" by Prof. M.S. Swaminathan. (Malik, et al., 2002). Antioxidants in foods have recently emerged as the biomolecules of utmost interest of human health. Dietary antioxidants inactivate reactive oxygen species (ROS), reduce oxidative damage, lead to improved immune functions and reduce risk of infectious diseases. (Kaur and Kapoor, 2007). Antioxidants found in whole grain foods are minerals (Ca, Mg, K, P, Na and Fe) and phytochemicals (Phytates and phenolic compounds) which are responsible for the high antioxidant activity of whole grain foods (Sridevi, et al., 2008). Phenolic compounds do not have any known nutritional function but they may be important to health, because of their antioxidant potency (Hertog, et al., 1995; studies have indicated that antioxidant activity of whole grains is highly correlated to their phenolics (Chitra and Pillai 2002). Phenolic levels in foods vary and are influenced by storage and type of extent of processing. The present investigatin was undertaken to measure the nutrient content and antioxidant activity of selected minor millets; to consolidate the importance of traditional cooking methods and its health benefits.

Materials And Methods

Sources of Raw materials: The millet samples (foxtail and proso) were purchased from local stores assuring the best quality. About three samples was procured in a period of two weeks, pooled, homogenized which was later used for analysis.

Preparation of samples

Wet-heating

Boiling: Samples were boiled in 200 ml of water that was just sufficient to be absorbed by grains during boiling for 30 min. The whole content after boiling was dried in hot air oven at 50°C for 30 min. (Mallik, et al., 2002).

Blanching: Blanching was done by using distilled water and bringing it to boiling at 98°C in an aluminium container. The grains were subjected to blanching (seeds to boiling water ratio 1:5) for 15 min and dried at 50°C for 45 min using hot air oven (Singh, et al., 2006).

Soaking: The sample were allowed to soak in sufficient amount of distilled water for two hours and then roasted for 3 min in shallow pan, so that the hydrated millet samples get dried (Majumdar and Premavalli, 2006).

Germination: The foxtail millet was soaked in double the amount of water for 12 hours. Then the water was decanted and the grains were tied in muslin cloth to allow germination for 24 hours and proso millet was allowed to germinate for 36 hrs.

ii. Dry heating:

Roasting: The grains were roasted in a shallow pan for 3 min. the pan was maintained at that temperature for the purpose of uniform roasting. After roasting the samples were brought to room temperature and processed further (Mallik, et al., 2002).

Boiled, blanched, soaked, roasted and germinated grains were ground to fine powder. The resultant flours were packed in air tight plastic containers and stored in refrigerator at 5°C until use for analysis.

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Sample Extraction

About 1g of powdered sample is taken and added 20ml of 50% ethanol shake well, vigorously and allow soaking for 48 hrs. Then centrifuge the sample at 2000 rpm for 15 min and the supernatant is taken for analysis. The tubes are covered with aluminium foil and stored at 4°C till the antioxidant analysis.

Proximate analysis of processed millet flour:

The crude powder (Kjeldahl, N*5.85), crude fiber (Fibra plus), ash and moisture (Sartorius moisture analyzer) were determined according to AOAC (2001) methods.

Determination of mineral composition:

The Mg and Zn content was determined using atomic absorption spectrophotometer (AAS, Model Shimadzu, AA - 6300). The iron and phosphorus was estimated colorimetrically by the Wong's method and ammonium oxalate method.

Determination of total phenolic content:

Total phenolic content (TPC) was determined in sample extracts using the Folin-Ciocalteau reagent (Bray Thorpe, 1954).

DPPH radical scavenging activity

This method is based on the ability of the antioxidant to scavenge the DPPH cation radical. Briefly, 200 µL of aliquot of sample extract or standard was added to 2800 µL of DPPH reagent (0.1mM methanol) and vortexed vigorously. It was incubated in dark for 15min at room temperature and the discoloration of DPPH was measured against the control. The absorbance was measured at 517nm using spectrophotometer (Systronics reference model – 2201). (Williams, et al., 1995).

FRAP assay (Ferric Reducing Antioxidant Power):

This method is based on the ability of the sample to reduce Fe^{+3} to Fe^{+2} ions. To 200µL of aliquot of sample extract or standard added of sample extract or standard added 2800 µL of FRAP reagent to the appropriate concentration. After incubation for 6 min, the absorbance was measured at 593 nm against blank using spectrophotometer (systronics reference model – 2201). (Sreeramulu, et al., 2009).

Reducing Power:

The reducing power of the samples was determined, as discussed by (Oyaizu, 1986). About 50µL of phosphate buffer (0.2M, PH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated for 20 min at 50°C.

2.5ml of 10% TCA was then added and centrifuged at 1000 rpm for 10 min. The resultant supernatant (2.5 ml) was mixed with an equal volume of distilled water and 0.5 ml of ferric chloride was added. Absorbance was measured spectrophotometrically at 700nm against blank and higher absorbance of sample indicates greater reducing power.

Determination of phytochemical content:

The phytochemical content of the samples was detected qualitatively such as alkaloids, flavonoids, saponins, carbohydrates, steroids, tannins, glycosides, resins and thiols. (Sadasivam, et al., 2004).

Statistical Analysis:

Data collected were subjected to analysis of variance (ANOVA) using the SPSS 10.0 Statistical Package. Correlation between TPC and antioxidant activities of the sample was also analysed.

Results And Discussion

Nutrient Evaluation:

Moisture: The moisture content of foxtail and proso millet ranged from 11.2% to 5.63% and 11.9% to 5.34% respectively. The table-1 shows that the moisture content of the germinated millet was found to be higher in both the samples indicating a lower shelf life when compared with the rest. Similarly the low moisture content in boiled, blanched and soaked samples may be due to drying after their processing. The reduction in moisture content of roasting could have been due to the high temperature.

The high temperature has been reported to result in vaporization of water in the grains. (King and Parwastin, 1987).

Ash: The ash content of processed millet flours ranged from 3.0g to 4.5g and 3.5g to 4.0g of foxtail and proso millet respectively. From the table-1 it is evident that the germinated millet has higher ash value which denotes increased content of minerals in the sample due to the bran. The lowest value was shown by raw sample of foxtail millet and soaked sample of proso millet (1.5g) respectively. Fasasi (2009) in his study on proximate antinutritional factors and functional properties of processed pearl millet flours ranged from 1.9% in fermented millet flour to 2.7% in roasted millet flour. The ash content indicates a rough estimation of the mineral content of product.

Protein: The table-1 shows that the protein content of germinated proso millet is higher when compared with the rest (7.86g). The protein content of boiled sample (11.4g) was higher in foxtail millet and germinated sample had the subsequent higher content (11.13g). Fasasi (2009), in his study of processed pear millet flours reported that the highest increase was observed in germinated millet flour (18.7%). The increase in protein content of germinated and fermented may be due to protein synthesis. Mauron (1982) reported that fermentation and germination may be a desirable processing teachnique to increase the protein content of millet seed. The protein quality of minor millets was higher and the content of lysine was lower when compared to major millet (Kalinova and Moudry, 2006).

Crude Fibre: The roasted foxtail millet showed remarkably low fibre content (0.5g) and the germinted millet in both the samples had increased content (7g and 4g) respectively. The processed proso millet sample contains no fibre content except the germinated millet because of the removal of bran. The high crude fibre content in germinated millet flour may be due to sugar utilization in the seed for metabolic sprouting activity leaving fibrous seeds. (Ikenbomah, et al., 1986). Similar observations have been reported for cow pea (Padmashree, et al., 1987).

Iron: The iron content of processed flour was significantly when higher compared to raw millet in both the samples. The table-2 reveals that the germinated foxtail millet and roasted proso millet contains higher iron content (13mg) within the samples. Malik et al (2002) showed in his study nutrient composition of pearl millet as influenced by cooking methods, a considerable increase in iron content after roasting of pearl millet grains. It may be concluded that on the basis of limited number of samples that roasting process may be beneficial in increasing the iron content.

Calcium, Phosphorous and Magnesium: The table-2 shows the mineral content of processed millet flour samples. The calcium content in roasted foxtail and soaked proso millet was greatly reduced (7.5mg) when compared with raw sample (31mg and 14mg) respectively. The decrease in calcium content of soaked millet may be due to leaching of mineral during soaking done prior to germinatin (Singh, et al., 2006).

A significant reduction in total phosphorous and magnesium contents was observed when compared with raw millet flour samples. The phosphorus content ranged between 156.8mg to

37.2mg and175.2mg to 204 mg. The magnesium content decreased during soaking and boiling treatments.

Zinc: The table-2 shows that blanched foxtail and germinated proso millet flours (18.07 mg and 19.2mg) had higher amount of zinc content. There was greater increase in the zinc content of processed samples when compared with the raw sample, which may be due to heat treatments during processing.

Total Phenols: The high antioxidant activity of any food samples can be correlated to the high phenolic content among the food sample (Thippeswamy and Naidu, 2005). The phenolic content of germinated millet in both the samples had significant increase which was found to be 1.69mM and 7.52mM respectively.

Antioxidant activity (AOA)

The AOA as determined by the three different methods showed a wide range of values. The DPPH radicals scavenging activity of soaked millets of both the samples was higher which was found to be 51.06% and 52.12% respectively. The increase is due to the lower heat treatment provided to soaked samples after processing. The FRAP values of roasted sample of foxtail millet and blanched proso millet had highest antioxidant power ranging from 317.5 µmol and 236.8 µmol respectively. The FRAP values depends on the part of samples used for determination. The blanched millet of both the samples had higher reducing power i.e., 0.426 and 0.418 respectively which determines higher antioxidant activity of these millets. It was observed that ethanol had the higher antioxidant activity when compared with other solvents. The correlation between TPC and AOA among foxtail and proso millet was determined which showed 44.6% significant difference in foxtail millet with respect to DPPH, FRAP assay and reducing power; whereas proso millet showed 56.46% significant difference with respect to AOA. Thus TPC was significantly correlated with AOA as determined by the three methods.

Phytochemical content

The phytochemical content was determined qualitatively indicating their presence or absence in the samples. The foxtail millet showed phytochemicals such as alkaloids, proteins, carbohydrates, phenols in high amounts whereas glycosides found in trace amounts, whereas resins and thiols are absent. Similarly in proso millet alkaloids, carbohydrates, protein, phenols are present in high amounts ; whereas glycosides, tannin, steroids, thiols and resins are absent.

Conclusion

The presence of anti-nutritional factors is removed after subjecting to processing treatments. Roasting of millet reduces the anti-nutritional factors that have promoted the health benefits of millets. Germinated millet has been found to significantly increase the crude protein content of millet flour. The presence of phenols and phytochemicals had further enhanced antioxidant activity which helps to neutralize and counteract the effects of free radicals. Thus minor millets are significantly nutritious even after processing methods.

Proximate composition of processed millet flours Table: 1

Variation	Moisture (g)		Ash(g)		Protein(g)		Fibre(g)	
	Foxtail millet	Proso millet	Foxtail millet	Proso millet	Foxtail millet	Proso millet	Foxtail millet	Proso millet
Raw	11.2	11.9	1.5	2.0	12.3	12.5	8.0	2.2
Boiled	3.15	2.37	3.0	3.5	11.46	6.87	5	0
Blanched	1.31	1.67	2.5	2.5	9.5	4.58	3	0
Roasted	2.53	2.06	2.0	3.0	5.56	5.89	0.5	0
Soaked	2.43	1.55	3.5	1.5	9.82	4.91	2	0
Germinated	5.63	5.34	4.5	4.0	11.13	7.86	7	7

Mineral composition of processed millet flours Table: 2

Variation	Iron (g)		Calcium(g)		Phosphorous (mg)		Magnesium (g)		Zinc(g)	
	Foxtail millet	Proso millet	Foxtail millet	Proso millet	Foxtail millet	Proso millet	Foxtail millet	Proso millet	Foxtail millet	Proso millet
Raw	2.8	0.8	31	14	290	206	81	153	2.4	1.4
Boiled	5	8	225	15	156.8	175.2	37	36.7	5.4	6.2
Blanched	5	10	33	30	203.2	148	37.3	36.6	18.07	2.4
Roasted	10	13	7.5	22.5	232	196.8	38	36.6	16.9	7.8
Soaked	5	5	15	7.5	36.9	187.2	36.9	36.4	7.8	7.1
Germinated	13	5	31.5	45	37.2	204	37.2	37.9	11.8	19.2

Antioxidant activity and phenolic content of processed millet flours Table: 3

Variation	DPPH (%)		FRAP (µmol of trolox)		Reducing power		Phenolic content (mM of GAE)	
	Foxtail millet	Proso millet	Foxtail millet	Proso millet	Foxtail millet	Proso millet	Foxtail millet	Proso millet
Boiled	31.52	31.04	197.4	115.2	0.151	0.394	4.56	1.44
Blanched	39.02	50	214.1	236.8	0.426	0.418	4.16	5.88
Roasted	36.79	42.45	317.5	103.6	0.357	0.357	8.36	4.36
Soaked	51.06	52.12	237.5	207.6	0.341	0.402	9.44	5.4
Germinated	37.81	41.97	258.1	153.8	0.400	0.392	16.92	7.52

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