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ORIGINAL ARTICLE

Effect of cooking, fermentation, dehulling and utensils on antioxidants present in pearl millet *rabadi* – a traditional fermented food

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Abstract Effect of cooking, fermentation, dehulling and the use of utensils on flavonoids (quercitin and pelargonidin) – antioxidants present in pearl millet (*Pennisetum typhoideum*) *rabadi,* along with proximate composition and sensory acceptability of the product were studied. Study revealed an increased ash and flavonoids and reduced crude fibre after cooking in all samples, while fermentation enhanced the crude protein and reduced fat and crude fibre after 16 h in fermented-cooked-fermented *rabadi* prepared in steel and earthen pot and cooked-fermented *rabadi* in earthen pot. Enhanced flavonoids were observed in all samples after 16 h fermentation. Fermented-cooked-fermented samples were better with high ($\rho<0.05$) protein in steel pot *rabadi* and high ($\rho<0.001$) ash and quercitin ($\rho<0.1$) in earthen pot *rabadi*. Major nutrients were unaffected after dehulling except the crude fibre, which decreased and quercitin increased significantly ($\rho<0.1$). Remarkable rise in quercitin was observed when *rabadi* was fermented-cooked and fermented in earthen pot. Sensory evaluation showed the acceptance of all samples in the range of liked extremely (fermented-cooked-fermented-steel pot) to liked slightly (fermented-cooked in earthen pot).

Keywords Pearl millet · Rabadi · Cooking · Dehulling · Fermentation · Flavonoids · Utensils

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Introduction

Isoflavones present in soybean, prevent the development of osteoporosis, heart disease and cancer (Choi et al. 2000). Antioxidants can mitigate the consequences of oxidative stress in disease development and aging process (Kalt 2005). Flavonoids, one of the antioxidants are polyphenolic compounds that occur ubiquitously in plants and are also known as vitamin P (Nair and Nagar 1997). These have positive impact in diabetes, oedema, blood anomalies, gout, piles, varicose veins, muscular degeneration, cardiovascular diseases and cancer (Miller 1996). Whole grains, nuts, fruits and vegetables contain flavonoids, terpenoids and other natural antioxidants like carotene, ascorbic acid and tocopherol. As the evidences of chronic diseases are on the rise in India and majority of Indian population consume plant based diet, information of foods rich in antioxidants is very important (Gitanjali et al. 2004). Foods undergo numerous processing before consumption which may alter their nutritional profile (Goyal and Khetarpaul 1994, Negi et al. 2001) including their antioxidants content. (Mitchell et al. 1990, Sato et al. 2006, Turkmen et al. 2006). The present study was carried out to analyze effect of cooking, fermentation, dehulling and use of different utensils on nutritive value and sensory acceptability with special emphasis on flavonoid content (quercitin and pelargonidin) of traditional Rajasthani recipe–*rabadi*, that is prepared from pearl millet in urban as well as rural areas by using utensils made up of steel and earthen pot, respectively.

Material and methods

Pearl millet (*Pennisetum typhoideum*) was procured from local market and was cleaned from dirt, dust and other foreign materials, ground in an electric grinder to prepare whole pearl millet flour. Pearl millet (120 g) was dehulled by moistening it for 5–10 min and ground for 6–7 sec in an electric grinder to remove hull. These dehulled grits were used for *rabadi* preparation to study the effect of dehulling.

Butter milk was bought from the 'Saras' dairy at each time of preparation.

Preparation of rabadi: *Rabadi* was prepared by the modified method of Gupta and Nagar (2008). Pearl millet flour/ grit (100 g) was mixed with butter milk (250 ml). It was diluted with plain water (600 ml) and salt (3 g) was added at the time of cooking. For fermentation, this homogenized mixture was kept in an incubator at 37°C. Samples were cooked by boiling for 5 min followed by simmering for 25 min with constant stirring. Thus 5 types of *rabadi* samples were prepared and analyzed at following stages of preparation:

Pearl millet flour *rabadi* prepared in steel pot following 2 traditional methods: (1) Cooked and fermented (CF) (T_1) having 3 stages for biochemical estimations- a) Raw, b) Cooked and c) Fermented (16 h). (2) Fermented, cooked and fermented (FCF) (T_2) type *rabadi* having 4 stages for biochemical estimations- a) Raw, b) Fermented (4 h), c) Cooked and d) Fermented (16 h). Similarly *rabadi* was prepared in earthen pot also, which were termed as T_3 and T_4, respectively. Pearl millet grits *rabadi* was prepared in steel pot by cooking followed by fermentation (T_5).

Chemical analysis: Samples were prepared as above and dried in an oven at 80–100°C. They were ground and kept in ziplock polythenes in dessicator to avoid moisture absorption. Moisture (AOAC 1980), ash, fat and crude fibre (Raghuramulu et al. 1983), total N and crude protein contents (N × 6.25) (Hawk et al. 1968) of *rabadi* samples were estimated. Total carbohydrates and energy contents were calculated by the formulae of Gopalan et al. (2002). Total energy in kcal/100 g were calculated by multiplying protein, carbohydrate and fat contents by 4, 4 and 9. For analysis of flavonoids (quercitin and pelargonidin), ethanolic extract of samples was prepared and the flavonoid content was estimated by following the method of Ogbede and Parvez (1991).

Sensory evaluation: Sensory acceptability was assessed by 10 judges for all cooked and fermented samples using 9-point Hedonic scale ranging from like extremely (9) to dislike extremely (1).

Statistical analysis: Estimations were carried out in triplicate. Their mean values with standard error were calculated. Analysis of variance with critical difference was applied to know significant difference among stages. Modified't' test was carried out to compare two types of *rabadies*.

Results and discussion

Cooking: Cooking brought about significant increase in ash content of different *rabadies* (Table 1) due to addition of salt at the time of cooking (Gupta and Nagar 2008). Significant rise in antioxidants was noticed after cooking in all the samples except T_3b. Although T_3 was also prepared in earthen pot as T_4 but increase in antioxidants was found in T_4 only. Initial 4 h fermentation in T_4 might have contributed a rise in antioxidants. It is compatible with the studies of Gitanjali et al. (2004) and Chukwumah et al. (2007). It may be attributed to the dissociation of antioxidants during processing from unavailable state to free form (Kalt 2005), while a significant reduction was observed in antioxidant activity, polyphenols and flavonoids contents on cooking of large number of spices by Bawa et al. (2007). A slight reduction in moisture, crude protein and fat contents was observed after cooking. Crude protein and fat might have dissociated into simpler peptides, amino acids and fatty acids, respectively (Gupta and Nagar 2008). Luo et al. (2009) also observed that amino N was highest at 20 min cooking and decreased with soaking time in *Douchi* prepared from soybean. Crude fibre declined significantly. It might have converted into soluble fibre. This reduction in most of the nutrients led to a decline in energy values in all types of *rabadies*.

Fermentation: Samples T_2 and T_4 have two stages of fermentation- 4 h before cooking and 16 h after cooking, while other samples were fermented for 16 h after cooking only. Table 1 is revealing no significant effect of fermentation on moisture, ash and carbohydrate contents. Crude protein increased significantly after 16 h fermentation in T_2, T_3 and T_4 while 4 h fermentation in T_2 and T_4 and 16 h fermentation in $T_1$and T_5 did not show any change in crude protein content. This proves that earthen pot provided appropriate conditions for fermentation and it was better suited for fermented cooked and fermented types of *rabadi*. High crude protein may be attributed to the microflora developed during the fermentation process (Gupta et al. 2007). Kaur et al. (2009) also observed an increase in protein content in wheat from 11.2 to 21.3%, in pearl millet from 12.2 to 20.3% and in sorghum from 9.2 to 14.7% after fermentation with *Morchella* sp. Fermentation imparted a remarkable rise in quercitin and pelargonidin contents in all types of *rabadies* after 16 h fermentation. Four h fermentation in samples T_2 and T_4 did not contribute any significant change in these antioxidants. Enhancement in antioxidants after fermentation was also observed by Moore et al. (2007) and Zhang et al. (2007) who reported pure culture and solid state fermentation techniques are more beneficial in releasing antioxidants. A significant reduction in fat content was observed that ranged between 4 and 40% in different samples. Significant decrease in crude fibre content in all the samples was observed that may be due to its conversion into soluble form. Energy content was decreased due to decrease in fat content.

Preparation method: Comparison of *rabadies* prepared from 2 methods exhibited the superiority of fermented cooked and fermented samples (T_2d and T_4d) over the cooked and fermented samples (T_1c and T_3c) in terms of higher ash, crude protein and quercitin contents with a decline in crude fibre (Table 1). Better chemical composition in T_2d and T_4d after 16 h fermentation may be due to initialization of fermentation process during 4 h fermentation before cooking that contributed to remarkable

Table 1 Chemical composition (%, dry weight basis) and energy values of pearl millet *rabadi*

Samples	Moisture	Ash	Crude protein	Fat	Crude fibre	Carbo-hydrate	Energy, Kcal/ 100 g	Quercitin mg / 100 g	Pelargonidin mg/ 100 g
T_1a	3.9 ± 0.14[a]	3.0 ± 0.09[a]	12.0 ± 0.42[a]	7.6 ± 0.26[a]	0.85 ± 0.03[a]	72.7 ± 0.67[a]	407.0 ± 1.38[a]	10.2 ± 0.63[a]	3.3 ± 0.52[a]
T_1b	3.7 ± 0.13[a]	5.2 ± 0.18[b]	11.2 ± 0.35[a]	7.4 ± 0.21[a]	0.70 ± 0.02[b]	71.8 ± 0.90[a]	398.4 ± 0.28[a]	14.3 ± 0.45[b]	5.3 ± 0.70[b]
T_1c	3.2 ± 0.11[a]	5.4 ± 0.19[b]	13.5 ± 0.33[a]	4.5 ± 0.16[b]	0.55 ± 0.01[c]	72.8 ± 0.81[a]	385.5 ± 0.49[b]	19.8 ± 0.64[c]	8.1 ± 0.80[c]
	NS	ρ<0.01	NS	ρ<0.01	ρ<0.05	NS	ρ<0.01	ρ<0.05	ρ<0.05
T_2a	3.9 ± 0.14[a]	3.0 ± 0.09[a]	12.0 ± 0.42[a]	7.6 ± 0.26[a]	0.85 ± 0.03[a]	72.7 ± 0.67[a]	407.0 ± 1.38[a]	10.2 ± 0.63[a]	3.3 ± 0.52[a]
T_2b	3.8 ± 0.12[a]	3.0 ± 0.08[a]	13.9 ± 0.42[a]	7.4 ± 0.23[a]	0.80 ± 0.02[b]	71.1 ± 0.86[a]	406.4 ± 0.31[a]	10.2 ± 0.77[a]	3.4 ± 0.30[a]
T_2c	3.3 ± 0.11[a]	6.4 ± 0.22[b]	13.6 ± 0.41[a]	6.6 ± 0.22[a]	0.55 ± 0.02[b]	69.7 ± 0.98[a]	392.0 ± 0.28[b]	19.6 ± 0.87[b]	7.1 ± 0.45[b]
T_2d	3.1 ± 0.11[a]	7.0 ± 0.24[b]	17.8 ± 0.49[b]	4.6 ± 0.16[b]	0.25 ± 0.01[c]	67.4 ± 1.00[a]	381.6 ± 0.64[b]	29.0 ± 0.64[c]	9.4 ± 0.46[c]
	NS	ρ<0.001	ρ<0.05	ρ<0.01	ρ<0.001	NS	ρ<0.001	ρ<0.001	ρ<0.01
T_3a	3.9 ± 0.14[a]	3.0 ± 0.09[a]	12.0 ± 0.42[a]	7.6 ± 0.26[a]	0.85 ± 0.03[a]	72.7 ± 0.67[a]	407.0 ± 1.38[a]	10.2 ± 0.63[a]	3.3 ± 0.52[a]
T_3b	2.9 ± 0.10[b]	4.0 ± 0.14[b]	11.4 ± 0.29[a]	6.0 ± 0.18[b]	0.50 ± 0.01[b]	75.2 ± 0.72[a]	400.6 ± 0.13[b]	10.4 ± 0.53[a]	3.0 ± 0.12[a]
T_3c	2.8 ± 0.08[b]	4.8 ± 0.14[b]	15.1 ± 0.39[b]	5.8 ± 0.11[b]	0.35 ± 0.01[b]	71.1 ± 0.73[a]	397.4 ± 0.40[b]	15.1 ± 0.60[b]	7.9 ± 0.36[b]
	ρ<0.05	ρ<0.05	ρ<0.05	ρ<0.05	ρ<0.01	NS	ρ<0.05	ρ<0.05	ρ<0.01
T_4a	3.9 ± 0.14[a]	3.0 ± 0.09[a]	12.0 ± 0.42[a]	7.6 ± 0.26[a]	0.85 ± 0.03[a]	72.7 ± 0.67[a]	407.0 ± 1.38[a]	10.2 ± 0.63[a]	3.3 ± 0.52[a]
T_4b	3.9 ± 0.12[a]	3.0 ± 0.09[a]	13.7 ± 0.30[a]	6.3 ± 0.15[b]	0.75 ± 0.02[a]	72.4 ± 0.68[a]	400.9 ± 0.19[ab]	9.6 ± 0.48[a]	3.9 ± 0.64[a]
T_4c	3.4 ± 0.12[ab]	6.0 ± 0.21[b]	12.4 ± 0.25[a]	6.1 ± 0.16[b]	0.70 ± 0.02[a]	71.4 ± 0.77[a]	389.7 ± 0.60[b]	28.9 ± 1.05[b]	9.2 ± 0.32[b]
T_4d	2.9 ± 0.10[b]	6.8 ± 0.19[b]	16.6 ± 0.56[b]	5.8 ± 0.15[b]	0.25 ± 0.01[b]	67.6 ± 1.01[a]	389.2 ± 0.44[b]	34.1 ± 0.42[b]	11.9 ± 0.92[b]
	ρ<0.05	ρ<0.001	ρ<0.05	ρ<0.05	ρ<0.001	NS	ρ<0.001	ρ<0.001	ρ<0.01
T_5a	2.4 ± 0.08[a]	2.9 ± 0.08[a]	11.0 ± 0.34[a]	8.0 ± 0.24[a]	0.70 ± 0.01[a]	75.1 ± 0.75[a]	415.8 ± 0.52[a]	13.1 ± 0.66[a]	4.2 ± 0.20[a]
T_5b	2.2 ± 0.07[a]	5.2 ± 0.13[b]	10.9 ± 0.33[a]	7.6 ± 0.21[a]	0.55 ± 0.01[ab]	73.6 ± 0.76[a]	406.2 ± 0.21[b]	16.9 ± 0.98[b]	5.9 ± 0.21[b]
T_5c	2.1 ± 0.07[a]	5.3 ± 0.18[b]	11.8 ± 0.30[a]	6.2 ± 0.18[b]	0.40 ± 0.01[b]	74.2 ± 0.74[a]	399.8 ± 0.16[b]	28.4 ± 0.54[c]	9.2 ± 0.65[c]
	NS	ρ<0.01	NS	ρ<0.05	ρ<0.01	NS	ρ<0.001	ρ<0.01	ρ<0.01

Values with different superscripts differ significantly at the indicated level of significance. In T_1 (Pearl millet flour- steel), T_3 (Pearl millet flour-earthen pot) and T_5 (Pearl millet grit- steel) – a= Raw, b =Cooked, c= Fermented (16 h). In T_2 (Pearl millet flour –steel) and T_4 (Pearl millet flour-earthen pot) – a= Raw, b = Fermented (4 h), c = Cooked, d = fermented (16 h). NS =Not significant (n=3)

improvement in the above nutrients during 16 h fermentation after cooking.

Dehulling: Dehulled sample (T_5c) had higher fat, energy and quercitin contents and a significant (ρ<0.1) lower crude fibre content (Table 1). Higher fat in T_5c may be attributed to higher germ and endosperm part on 100 g basis than T_1c that includes bran also in similar weight. High fat contributed to higher energy values in dehulled sample. A rise in quercitin in T_5c may be due to disruption of bran. It provides better conditions for fermentation and cooking to release flavonoids from bound to free state (Saharan et al. 2002).

Utensils: Comparison of T_1c and T_2d were made with T_3c and T_4d respectively. Effect of utensil was not observed in fermented- cooked–fermented *rabadi* for any nutrient except quercitin. It was found significantly (ρ<0.1) higher in T_4d than T_2d. In cooked and fermented samples, fat and energy contents were higher in T_3c with reduced crude fibre (ρ<0.05) content as compared to T_1c. No significant difference was noticed in any other nutrients due to the use of different utensil (Table 1). Higher quercitin in T_4d may be due to appropriate conditions for fermentation in earthen pot because of its porous structure.

Table 2 Sensory overall acceptability scores of pearl millet *rabadi*

T_1b	8.3 ± 0.14 [a]
T_1c	8.1 ± 0.30[ab]
T_2c	8.3 ± 0.20[a]
T_2d	8.5 ± 0.16[a]
T_3b	6.8 ± 0.37[bc]
T_3c	7.4 ± 0.32[abc]
T_4c	6.2 ± 0.46[c]
T_4d	7.5 ± 0.16[ac]
T_5b	8.1 ± 0.26[ab]
T_5c	7.9 ± 0.26[ab]

T_1–T_5: As in Table 1. Means ± SE with different superscripts differ significantly (ρ<0.001) (n = 10 panelists)

Acceptability: Sensory overall acceptability scores ranged between 8.5 (T_2) and 6.2 (T_4), i.e., liked extremely to liked slightly as shown in Table 2. Among all samples, pearl millet flour sample prepared in steel pot (T_1 and T_2) and pearl millet grit sample (T_5) were scored higher as compared to *rabadi* prepared in earthen pot (T_3 and T_4).

Conclusion

Pearl millet flour *rabadi* prepared after fermentation (4 h), cooking and fermentation (16 h) in steel (T_2) and earthen pot (T_4) obtained higher ash and crude protein with lower crude fibre content. These 2 samples showed enhanced flavonoids also followed by dehulled sample (T_5). Besides this, T_2 scored highest for acceptability followed by T_1 and T_5. Therefore the traditional methods of *rabadi* preparation may be considered better than others both in terms of nutritive value and sensory acceptability.

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