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Preparation and characterization of *Kharode/ Rabadi* from fermented pearl millet flour

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Abstract
Pearl millet (*Pennisetum typhoideum*) also known as *Bajra* or Bulrush millet, is same in height as maize and sorghum. It is the most drought-resistant millet. Pearl millet is known to have a higher protein content and better amino acid balance among all millets. Fermented food products prepared from cereals are an integral part of the diet of the people in many parts of the world. The preparation of cereal based traditional fermented food products and other indigenous beverages remains today as a household art; prepared using relatively simple procedures and equipments.
Kharode is a traditional Indian cereal based fermented food prepared specially the village people of Maharashtra by soaking, lactic acid fermenting and cooking pearl millet grains (Khedkar *et al.*, 2006), which is subsequently thermally gelatinized, hand extruded and dried (Beuchat, 1983). Similar type of food product named *rabadi* had been prepared in the state of Haryana and Rajasthan of India (Dhankher and Chauhan, 2004). *Kharode/rabadi* is a wholesome food, especially for low and average income rural populations in the North-western millet producing region of India. Thus, *Kharode/rabadi* preparation is a simple and inexpensive technique for upgrading the nutritive value of pearl millet through fermentation.

Keywords: pearl millet, *Bajra*, antinutrients, *rabadi* and *Kharode*

Introduction
Pearl millet (*Pennisetum typhoideum*) also known as *Bajra* or Bulrush millet, is same in height as maize and sorghum. It is the most drought-resistant millet. Pearl millet is sown on 26 million ha in Asia. Pearl millet is known to have a higher protein content and better amino acid balance among all millets. Comparative studies of protein quality and mineral constituents of some Indian varieties have shown that the high-protein varieties contain plentiful amounts of essential amino acids and calcium, phosphorus, and potassium (Elyas *et al.*, 2002) [14]. The higher ratio of germ to endosperm is responsible for the higher protein content of pearl millet. Pearl millet has a well-balanced protein, except for its lysine deficiency, with high concentration of threonine and lower (but adequate) leucine than sorghum protein. Tryptophan levels are generally higher in pearl millet than in other cereals. Cereal based fermented products are widely prepared and consumed in India and many Asian countries.
Pearl millet is a staple food for many people in Asian and African countries. The crop is a still principal source of energy, protein, vitamins and minerals for millions of the poorest people in these regions. Pearl millet constitutes a major source of protein and calories in the diet of millions of the Indian population. Starch, a major component of carbohydrates in pearl millet (55-60%), is relatively more resistant to attack by pancreatic amylase. Although pearl millet provides many essential nutrients, the grain contains considerable amounts of antinutrients too. These antinutritive compounds are known to affect mineral availability and digestibility of carbohydrates and proteins in various plant foods. Somewhat high concentrations of these antinutrients in pearl millet may relate for the low nutritive value (Dhankher and Chauhan, 1987a) [11].
Pearl millet is the most important cereal, giving higher and more stable grain yields than sorghum and maize, in the arid and semi-arid tropical areas of Africa and the Indian subcontinent, characterized by low and erratic rainfall, high temperatures, low inherent soil fertility and numerous biotic stresses.
Fermentation is a process by which consumable food products are prepared by use of various micro-organisms. Fermentation has been known to improve protein digestibility as well as starch digestibility of food grains. Fermented food products prepared from cereals are an integral part of the diet of the people in many parts of the world. The preparation of cereal based traditional fermented food products and other indigenous beverages remains today as a household art; prepared using relatively simple procedures and equipments.

Cereal based fermented foods and alcoholic beverages like *idli, dosa, jalebies, kurdi, seera, rabadi*,and *bhaatijaanr, kodokojaanr, ambil* etc. have been a part of Indian traditional fermented foods of varied localities, a practice protected by tradition. Now-a-days fermented foods are receiving world attention due to their disease-preventing and health promoting effects.

Fermented foods also provide variety in the diet. Cereal legume based fermented foods like *idli, dosa, dhokla, khaman, wadi, rabadi, papad* and *kinema* from various parts of India have been well studied and documented; however, there is no proper documentation of similar foods (*kharode*), indigenous to the state of Maharashtra of India.

Kharode is a traditional Indian cereal based fermented food prepared specially the village people of Maharashtra by soaking, lactic acid fermenting and cooking pearl millet grains, which is subsequently thermally gelatinized, hand extruded and dried. Similar type of food product named *rabadi* had been prepared in the state of Haryana and Rajasthan of India. *Kharode/rabadi* is a wholesome food, especially for low and average income rural populations in the North-western millet producing region of India. *Kharode/rabadi* prepared from other cereals like wheat, maize, barley and sorghum mixed with butter milk and allows it to ferment in an earthen or metallic vessel in the open sun. Thus, *Kharode/rabadi* preparation is a simple and inexpensive technique for upgrading the nutritive value of pearl millet through fermentation.

2. Materials and Methods

2.1 Raw Materials

The grains of two pearl millet cultivars, ABPC 4-3 and PPC-6, procured from Pearl Millet Research Station, Aurangabad (under VNMKV, Parbhani).The grains were cleaned and coarsely ground and used for chemical analysis. The raw pearl millet flour (coarsely ground) and autoclaved unfermented pearl millet samples served as the control.

2.1.2 Chemicals

The culture media and chemicals used were obtained from Hi-Media laboratories Pvt. Ltd., Mumbai, India. Ethanol, Dinitrosalicylic acid reagent and potassium sodium tartarate (Rochelle salt) were procured from S.D. Fine Chem. Pvt. Ltd., Mumbai, India. Some chemicals obtained from Sigma Chemical Co. (St. Louis, MO) and chemicals and solvents used were of analytical grade.

2.2 Methods

2.2.1 Physico-Chemical analysis

2.2.1.1 Sample Preparation

Pearl millet (100 g) of two varieties was soaked with 300 ml butter milk; for varied temperature (30, 37.5 and 45^{o}C) and time (5, 10 and15 hr). Natural fermentation of flour at each temperature was carried out in a single batch. The unimbibed liquid was discarded and grains were dried in shade at 30^{o}C for 12 hr and coarsely ground for 15 sec in grinder (Anjalis grinder, Mumbai, India) and packed in air tight containers for further analyses.

2.2.2 Proximate analysis

Determination of moisture

Moisture was determined by hot air oven method by drying the sample in hot air oven at 105 ^{o}C temperature until a constant weight (AOAC, 2010). The moisture content of sample was estimated by the formula:

Moisture content (%) = Initial weight − Final weight /Initial weight ×100

Determination of ash content

Ash content of samples was determined using (AOAC, 2010) method. 5 g of sample was taken in porcelain dish dried previously, weighed and heated at 550±20 ^{0}C till constant weight was achieved.

Determination of crude protein

Crude protein (N×6.2) was estimated using the automatic KEL PLUS instruments, Pelican Equipments, Chennai, India by employing the standard methods of AOAC (2000).

Determination of fat

Fat was estimated using the automatic SOCS PLUS instrument, Pelican Equipments, Chennai, India by employing the standard methods of AOAC (2000).

Determination of carbohydrate content

Carbohydrate content was determined by difference.

Determination of crude fibre

Crude fibre was estimated using the automatic FIBRA PLUS instrument, Pelican Equipments, Chennai, India by employing the standard methods of AOAC (2000).

Thousand kernels Weight

Weight of 1000 kernels of each sample was determined.

2.2.3 Preparation of Buttermilk

Freshly prepared curd (200 g) with desirable consistency was mixed with water (130 ml) to prepare buttermilk. Each day, fresh buttermilk was used immediately for *Kharode/rabadi* fermentation.

2.2.4 Sample preparation for *Kharode/rabadi*

Fermented dough was prepared in the traditional domestic way. Pearl millet (100 g) was soaked with 300 ml butter milk; for varied temperature (30, 37.5 and 45 ^{o}C) and time (5, 10 and15 hr). Natural fermentation of flour at each temperature was carried out in a single batch. The unimbibed liquid was discarded and grains were dried in shade at 30 ^{o}C for 12 hr and coarsely ground for 15 sec in grinder (Anjalis grinder, Mumbai, India) and packed in air tight containers for further analyses.

2.2.5 Preparation of *Kharode/Rabadi*

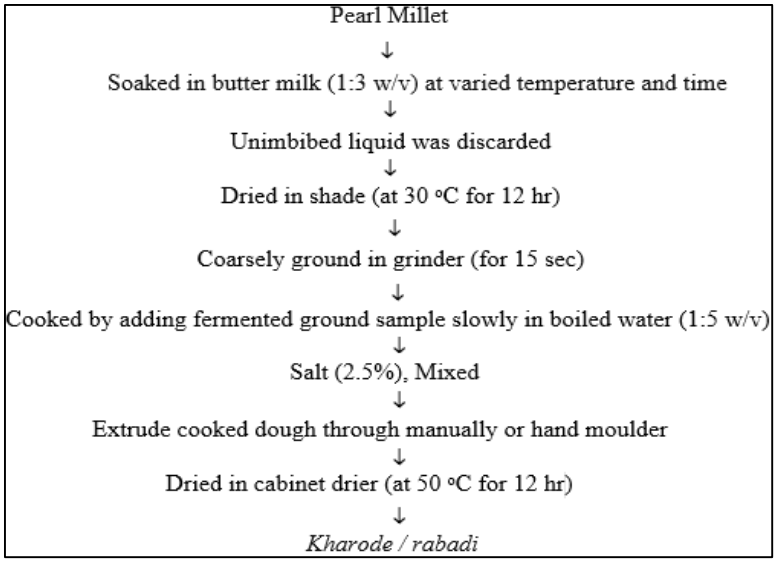

Fig 4.1: Flow chart of preparation of *kharode/rabadi*

2.2.6 Determination of IVPD and IVSD

In vitro protein digestibility was determined according to the method of Maliwal (1983), as modified by Manjula and John (1991). *In vitro* starch digestibility was assessed by the method of Singh *et al.*, (2010).

2.2.7 Estimation of Total Minerals

2.2.7. 1 Preparation of sample

Defatted cereal grains were dried in oven at 70 °C for 12 hr. The oven dried samples were ground in electrically operated grinder with stainless steel blades. The ground samples were stored in polythene pouches with proper labelling and used for further determination.

2.2.7. 2 Estimation of minerals

To 1g of ground moisture-free sample (unfermented and fermented) was added 25ml of di acid mixture (HNO_3:$HCLO_4$: 5:1 v/v), and the mixture was kept overnight. The next day it was digested by heating until clear white precipitates settled at the bottom. The large crystals were dissolved by diluting in double-distilled water, and the contents were filtered through Whatman No. 42 filter paper. The filtrate was made up to 50ml with double-distilled water and was used for the determination of total iron, copper, zinc, and manganese by atomic absorption spectrophotometry (Perkin Elmer Model 3300, USA) according to the method of Lindsey and Norwell (1969).

2.2.8. Determination of phytic acid content

Phytic acid content was determined according to AOAC (2004).

2.2.9 Nutritional properties

2.2.9.1 Physical Parameters

The diameter of the *Kharode* was measured at two diametrical positions to an accuracy of 0.02 mm using a Verniercaliper (Mitutoyo, Kawasaki, Japan). Each *Kharode* was weighed in a monopan analytical balance (AEG-220, Shimadzu, Tokyo, Japan) to an accuracy of 0.0001 g.

2.2.9.2Moisture content

It was determined as referred under 2.2.4.1

3. Results and Discussion

3.1 Physico- chemical composition of pearl millet

The unprocessed pearl millet flour from ABPC4-3 and PPC-6 cultivars was evaluated for physico-chemical composition and the results are presented in Table 4.3.

Table 4.3: Physico-chemical quality of pearl millet

Variety	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbo- Hydrate (%)	Fiber (%)	1000 Kernel wt (g)
ABPC 4-3	11.20 ± 0.03	11.56 ± 0.21	7.82 ± 0.29	1.18 ± 0.01	68.50 ± 0.25	3.70± 0.06	12.80 ± 0.12
PCC – 6	10.85 ± 0.14	11.30 ± 0.14	7.45 ± 0.21	1.08 ± 0.01	69.20 ± 0.22	3.52± 0.08	12.00 ± 0.14

*Values are means ± (S.D.).

Unprocessed pearl millet flour contained 11.2% moisture, 11.56% protein, 7.82% fat, 1.18% ash, 3.70% fiber and 12.8 g thousand kernel weight respectively for ABPC 4-3 cultivar, whereas, 10.85% moisture, 11.30% protein, 7.45% fat, 1.08% ash, 3.52% fiber and 12.0 g thousand kernel weight respectively for PPC-6 cultivar. The ABPC 4-3 cultivar showed higher levels of physico-chemical constituents than the PPC-6 cultivar except carbohydrates. The findings of the study are in agreement with those of previous workers (Sharma and Khetarpaul, 1997).

3.2 *In vitro* protein digestibility and *in vitro* starch digestibility of pearl-millet

The effect of fermentation temperature and period on IVPD and IVSD are shown in Table 3.1. A significant increase ($P \leq 0.05$) was first observed at 5 hr fermentation period at all temperatures and further significant increase at 10 and 15 hr in the IVPD of both millet cultivars. The increase was from 52.30 to 84.30% for ABPC4-3 cultivar and from 49.45 to 82.40% for PPC-6 cultivar. The IVPD of ABPC4-3 cultivar was higher than the IVPD of PPC-6 cultivar. Protein digestibility was highest in both the cultivars fermented for 15hr at 45°C.This may also contribute to the improvement in protein digestibility as a significant ($P \leq 0.05$) negative correlation coefficient existed between phytic acid and protein digestibility (*in vitro*) of pearl millet. Thus, fermentation offers unique nutritional advantages for making protein of coarse-grained pearl millet more digestible, possibly by

significantly reducing its phytate content and decreasing the level of polyphenols. This improved IVPD caused by fermentation could be attributed to the partial degradation of complex storage proteins to more simple and soluble products (Chavan *et al.*, 1988); it could also be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes. Soaking decreases the anti-nutrients that interfere with the IVPD.

Table 3.1: Effect of fermentation temperature and period on *in vitro* protein digestibility (%) and *in vitro* starch digestibility (mg/maltose/gm/2hr) of pearl-millet*

Variety		ABPC 4-3		PPC - 6	
Fermentation Temp (0C)	**Fermentation Time (hr)**	**IVPD**	**IVSD**	**IVPD**	**IVSD**
Control	0.0	52.30 a ± 0.14	18.60 a ± 0.28	49.45 a ± 0.21	16.80 a ± 0.14
30.0	5.0	58.25 b ± 0.21	72.95 b ± 0.78	56.60 b ± 0.28	68.80 b ± 0.14
	10.0	61.80 d ± 0.14	86.40 d ± 0.28	60.75 d ± 0.07	81.80 c ± 0.14
	15.0	64.40 f ± 0.14	94.40 h ± 0.28	62.80 e ± 0.14	92.85 g ± 0.07
37.5	5.0	60.85 c ± 0.07	78.45 c ± 0.21	60.30 c ± 0.14	84.80 d ± 0.14
	10.0	63.35 e ± 0.21	89.30 e ± 0.14	62.65 e ± 0.21	90.30 e ± 0.14
	15.0	76.50 i ± 0.42	93.35 g ± 0.21	75.01 g ± 0.01	92.30 f ± 0.28
45.0	5.0	65.30 g ± 0.14	90.45 f ± 0.21	64.30 f ± 0.14	94.70 h ± 0.14
	10.0	75.20 h ± 0.14	98.60 i ± 0.28	74.80 g ± 0.14	99.70 i ± 0.14
	15.0	84.30 j ± 0.28	111.30 j ± 0.14	82.40 h ± 0.28	108.45 j ± 0.21

*Values are means ± (S.D.).Means not sharing a common letter in a column are significantly different at $P \leq 0.05$, as assessed by Duncan's multiple range test.

High molecular weight polyphenols are known to precipitate proteins, reduce protein digestibility (Hulse *et al.*, 1980). Fermentation is found to increase the IVPD (Dhankher and Chauhan, 1987) [11].

The soaking treatment positively affected the IVSD. The IVSD of ABPC4-3 cultivar was higher than of PPC-6 cultivar. Drastic and significant increase ($P \leq 0.05$) was first observed at 5hr fermentation period at all temperatures and further significant increase at 10 and 15hr in the IVSD of the two pearl millet cultivars (Table 4.12).

Improvement in starch digestibility during fermentation may be due to breakdown of starch to oligosaccharides. Reduction in the phytate content of pearl millet during fermentation (Dhankher and Chauhan, 1987a) [11] due to the enzymatic hydrolysis of phytic acid by microbial phytase may also contribute to better starch digestibility. A significant ($P \leq 0.05$) negative correlation coefficient was found between the phytic acid and starch digestibility (*in vitro*) of pearl millet. observed while preparing *rabadi* by fermenting pearl millet flour with country buttermilk, a gradual increase in the starch digestibility (*in vitro*) with increase in the temperature and period of fermentation. Improvement was most pronounced when fermentation was performed at 45 °C for 9 hr.

3.3. Total mineral content of pearl millet

Kharode/Rabadi was prepared from a soaked pearl millet and buttermilk mixture. This mixture was fermented at different temperatures, i.e., 30, 37.5**,** and 45 °C for varying periods, viz. 5, 10 and 15 hr. Tables 3.2 and 3.3 shows the micro elements contents *i.e*., iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) of two varieties of pearl millet before and after treatments. No apparent changes in the concentrations of Fe, Mn, Cu, and Zn were observed during the fermentations of pearl millet blends.

3.3.1. Iron

Fermentation significantly ($P \leq 0.05$) reduced the Fe content of the two cultivars sequentially 188.30 to 142.80 µg/g for ABPC 4-3 and 178.20 to 140.20 µg/g for PPC-6. Longer the fermentation period and higher the temperature of fermentation, the greater was the decrease in fermentation of the minerals in wheat.

Same results were reported earlier in pearl millet. Fermentation of pearl millet caused appreciable changes in the chemical composition (moisture, ash, fiber, protein, and fat contents), but markedly reduced the mineral contents like Na, K, Mg, Cu, Fe, Mn, and Zn (Ahmed and others 2009) [2]. Stated that natural fermentation insignificantly affected Fe content. Abdalla *et al.*, (1998) [1] observed a significant reduction in Fe content of three pearl millet genotypes.

3.3.2 Manganese

Non-fermented pearl millet flour had initial manganese (Mn) content 19.2µg/g for ABPC4-3 cultivar and 18.80 µg/g for PPC-6 cultivar. However, a significant decrease in Mn content was observed during fermentation when the pearl millet was soaked for 15 hr at 45 ^{0}C. However, Ahmed (1999) confirmed that natural fermentation had no effect on Mn content of pearl millet.

Table 3.2: Effect of Fermentation temperature and fermentation time on mineral content*

ABPC 4-3					
Fer. Temp (^{0}C)	**Fer. Time (hr)**	**Fe (µg / g)**	**Mn (µg / g)**	**Cu (µg / g)**	**Zn (µg / g)**
Control	0.0	188.30 i ± 0.28	19.20 h ± 0.03	14.20 f ± 0.01	68.60 h ± 0.14
30.0	5.0	168.10 h ± 0.28	19.00 h ± 0.14	14.00 f ± 0.00	53.20 g ± 0.01
	10.0	153.30 g ± 0.28	18.20 g ± 0.14	13.50 e ± 0.14	52.70 f ± 0.14
	15.0	147.40 d ± 0.28	17.30 f ± 0.14	12.20 c ± 0.03	50.40 e ± 0.28
37.5	5.0	152.20 f ± 0.28	18.40 g ± 0.14	13.40 e ± 0.14	52.40 f ± 0.31
	10.0	148.90 e ± 0.28	16.60 e ± 0.14	12.50 d ± 0.14	49.70 d ± 0.13
	15.0	145.40 c ± 0.28	13.20 c ± 0.14	11.10 b ± 0.14	47.60 c ± 0.14
45.0	5.0	147.30 d ± 0.28	14.40 d ± 0.14	12.60 d ± 0.14	49.30 d ± 0.28
	10.0	144.20 b ± 0.28	12.10 b ± 0.14	11.30 b ± 0.14	47.10 b ± 0.00
	15.0	142.80 a ± 0.28	10.30 a ± 0.14	10.20 a ± 0.14	45.30 a ± 0.27

*Values are means ± (S.D.).Means not sharing a common letter in a column are significantly different at $P \leq 0.05$, as assessed by Duncan's multiple range test.

3.3.3. Copper

Fermentation significantly ($P \leq 0.05$) reduced Cu content of both the cultivars (viz.ABPC4-3 and PPC-6) from 14.20 to 10.20 µg/g and 12.60 to 09.20 µg/g respectively, which in line with results of Abdalla *et al.* (1998) [1].

3.3.4. Zinc

The effect of fermentation temperature and period on Zn content is shown in Table 4.13 and 4.14. A significant decrease ($P \leq 0.05$) in Zn content was first observed at 15hr of fermentation period at 30 ºC temperature and further significant decreases at 37.5 ºC and 45 ºC temperature for 15hr of fermentation period of the two millet cultivars. The decrease was from 68.60 to 45.30 µg/g for ABPC4-3 cultivar and 66.40 to 48.20 µg/g for PPC-6 cultivar, respectively. Bookwalter *et al.*,(1987) found that 50% extraction rate reduced Zn content of pearl millet from 23 to 18µg/g. Abdalla *et al.*,(1998) [1] reported that iron, manganese, copper and zinc content ranged from 70-180 µg/g, 18-23 µg/g, 10-18 µg/g and 53-7O µg/g respectively.

Table 3.3: Effect of Fermentation temperature and fermentation time on mineral content*

PPC - 6					
Fer. Temp. (^{0}C)	**Fer. Time (hr)**	**Fe (µg / g)**	**Mn (µg / g)**	**Cu (µg / g)**	**Zn (µg / g)**
Control	0	178.20 i ± 0.14	18.80 g ± 0.14	12.60 f ± 0.14	66.40 g ± 0.14
30	5	165.30 h ± 0.14	18.70 g ± 0.28	12.40 f ± 0.14	54.10 f ± 0.14
	10	152.90 g ± 0.00	17.40 f ± 0.14	12.10 e ± 0.00	53.40 e ± 0.14
	15	147.40 e ± 0.14	16.50 e ± 0.14	11.20 c ± 0.14	51.04 d ± 0.02
37.5	5	149.80 f ± 0.14	17.30 f ± 0.14	11.90 e ± 0.00	54.20 f ± 0.14
	10	146.20 d ± 0.01	16.60 e ± 0.42	11.60 d ± 0.14	52.70 e ± 0.14
	15	142.40 b ± 0.01	14.20 c ± 0.14	10.50 b ± 0.14	50.40 c ± 0.14
45	5	146.40 d ± 0.03	15.30 d ± 0.13	11.20 c ± 0.14	51.30 d ± 0.14
	10	143.10 c ± 0.00	13.40 b ± 0.28	10.40 b ± 0.14	49.60 b ± 0.14
	15	140.20 a ± 0.01	10.10 a ± 0.00	9.20 a ± 0.14	48.20 a ± 0.14

*Values are means ± (S.D.).Means not sharing a common letter in a column are significantly different at $P \leq 0.05$, as assessed by Duncan's multiple range test.

3.4 Phytic acid content

Phytic acid (phytate), myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) is one of the widespread occurring compound in plant seeds and/or cereal grains, roots and tubers and it is major inhibitor of iron and zinc absorption from human diets. The nutritionally important minerals, such as calcium, magnesium, copper, iron (Fe^{2+} and Fe^{3+}), and others form complexes with phytic acid resulting in reduced solubility of the metals. Its influence on iron and zinc absorption is of great public health importance. Synergistic effects of divalent metal ions in the formation of metal-phytate complexes have also been reported. The contribution of fiber and other food constituents capable of binding with minerals and the proteins undoubtedly have effects on phytate-mineral interactions. In mature cereal grains, legumes, and oil seeds, the major portion of the total phosphorus is present in the form of phytic acid (phytate). Phytase hydrolyzes phytate into inositol and phosphates or phosphoric acid. The availability of phosphorus when present in the form of phytate depends on the species, the age of the experimental animal, and the level of phytase activity in the intestinal tracts of the specific species. Low absorption of these nutrients from plant-based diets in developing countries is considered to be a major factor in the etiology of iron deficiency after 4 to 6 months of age. Although pearl millet provides many essential nutrients, the grain contains considerable amounts of phytic acid and polyphenol too. These antinutritive compounds are known to affect mineral availability and digestibility of carbohydrates and proteins in various plant foods. Also, dietary phytate is known to inhibit proteolytic and amylolytic enzymes. Relatively high concentrations of these antinutrients in pearl millet may account for the low nutritive value (Dhankher and Chauhan 1987a) [11] of this coarse grain. The nutritional value of pearl millet can be considerably improved if the levels of phytic acid and polyphenols in the grains are significantly decreased. Fermentation has been reported to decrease phytic acid and polyphenols.

The data on the effect of soaking for a period of 5, 10, and 15hr and soaking temperature of 30.0, 37.5 and 45.0 ^{0}C on phytic acid content of two cultivars of pearl millet, ABPC4-3 and PPC-6 were presented in Table 3.4.

Table 3.4: Effect of Fermentation period and temperature on Phytic acid content of pear millet

Fer. Period (hr)	**Fer. Temperature (^{0}C)**	**Phytic Acid (mg/100 gm)**	
		Variety ABPC 4-3	**Variety PPC - 6**
0	Control	772.40 j ± 1.41	762.40 j ± 1.41
5.0	30.0	757.40 i ± 1.41	747.45 i ± 0.92
	37.5	747.25 h ± 1.2	733.95 h ± 1.2
	45.0	731.15 g ± 0.49	716.10 g ± 0.42
10.0	30.0	716.30 f ± 1.56	700.80 f ± 0.99
	37.5	701.90 e ± 0.71	685.10 e ± 1.41
	45.0	688.40 d ± 1.41	668.25 d ± 1.63
15.0	30.0	667.50 c ± 1.13	651.90 c ± 0.71
	37.5	652.00 b ± 0.14	636.45 b ± 0.92
	45.0	635.50 a ± 1.98	617.60 a ± 1.27

*Values are means ± (S.D.).Means not sharing a common letter in a column are significantly different at $P \leq 0.05$, as assessed by Duncan's multiple range test

Table 3.4 shows that phytic acid content of both the cultivars was decreased significantly at all soaking periods on increase in the temperature from 30.0 to 45.0 ^{0}C. The reduction was from772.4 to 635.5 mg /100 g (17.8% reduction) for ABPC4-3 cultivar and from 762.4 to 617.6 mg/100 g (18.9% reduction) for PPC-6 cultivar. Natural fermentation at 30.0, 37.5 and 45.0 °C for 15hr brought about a significant reduction in phytic acid content of pearl millet. The ABPC4-3 cultivar showed higher levels of phytic acid than the PPC-6 cultivar.

Generally, fermentation (soaking in whey) is known to cause a greater reduction in phytic acid than other anti-nutrients and this could be due to the low pH of fermented dough, which is considered to be optimum for phytase activity.

Microbial phytase, as reported in several microorganisms (Daniels and Fisher 1981; Lopez *et al.*, 1983), may hydrolyse phytic acid during fermentation of flour and account for reduction in phytic acid content in the fermented product. While studying the natural fermentation of raw pearl millet, Mahajan and Chauhan (1987) reported that endogenous phytase of pearl millet contributed significant reduction of the phytate content of fermented pearl millet which was dependent upon pH and temperature of fermentation. Fermentation was found to decrease antinutrients of pearl millet by decreasing the phytic acid. Results obtained in this study were similar to those obtained by Marfo *et al.*, (1991). Kheterpaul and Chauhan (1989) [22] reported that mixed fermentation for 72 hr at 30°C reduced phytic acid by 56%. Microbial phytase, was reported to be present in several microorganisms; which hydrolyses phytic acid during fermentation of flour, accounting for a reduction in phytic acid content in the fermented product.

3.5. Nutritional profile of Kharode/Rabadi

3.5.1 Moisture content of kharode/rabadi

The moisture content of *Kharode* had a positive impact on its texture and it mostly depends on the carbohydrate constituents of the batter. The *Kharode* made in the traditional way (15hr fermentation) from pearl millet var. ABPC4-3 and var. PPC-6 exhibited a moisture content of 7.32% and 7.65% respectively (Table 3.5). The moisture content was slightly less in *kharode* with an increase in time of fermentation. It was observed that moisture content of *kharode* made from var. ABPC4-3 cultivar showed less value compared to that of var. PPC 6 and was found to be more fragile to handle. As per the Bureau of Indian Standards (BIS, 1972), moisture content in *kharode* should be between 12.0 and 15.0%. Several other reports also indicated the variation in moisture content of *kharode* prepared from different cereal blends (Shurpalekar *et al.*, 1970; Arya 1992). *Kharode* become brittle and break if the moisture content is very low and prone for spoilage if moisture is more than the desired level (Kulkarni *et al.*, 1996).

Table 3.5: Nutritional profile and frying characteristics of *kharode/rabadi**

Parameter	ABPC 4-3	PPC-6
Moisture (%)	7.32 ± 0.21	7.65 ± 0.26
Protein (%)	13.25 ± 0.21	13.56 ± 0.24
Fat (%)	3.62 ± 0.09	3.76 ± 0.09
Ash (%)	4.03 ± 0.07	4.25 ± 0.09
Crude Fiber (%)	1.83 ± 0.10	1.98 ± 0.09
Oil uptake (%)	12.12 ± 0.28	12.67 ± 0.32
Expansion (%)	15.01 ± 0.98	15.26 ± 1.01
Frying oil Temp (ºC)	174.8 ± 1.5	174.9 ± 1.8

*Values are means ± (S.D.).

The *kharode* prepared from pearl millet var.ABPC4-3 found to have nutritional profile such as protein (13.25%), fat (3.62%), fiber (1.83%) and ash (4.03%). Whereas, *Kharode* prepared from pearl millet var.PPC-6 had protein (13.56%), fat (3.76%), fiber (1.98%) and ash (4.25%). It was observed that nutritional profile of *kharode* made from var.ABPC 4-3 cultivar showed less values compared to that of *kharode* prepared from pearl millet var. PPC-6. These results indicated that protein and ash levels of *Kharode* prepared from both the varieties of pearl millet increased after soaking and fermentation, thus fermentation and soaking could be regarded as viable means for improvement of the nutritional quality of pearl millet. It was also observed that the proximate analysis showed that *Kharode* made from fermented flour of PPC-6 had higher nutritional quality attribute compared to *Kharode* of ABPC4-3. Protein content was 13.25% that was found to be lower than that of wheat flour supplemented *papads* (Garg and Dahiya, 2003).

Fig 3.1: *Kharode* prepared from fermented pearl millet variety ABPC 4-3

Fig 3.2: *Kharode* prepared from fermented pearl millet variety PPC - 6

4. Conclusions

Natural fermentation (soaking) was found to cause appreciable changes in the chemical composition viz. moisture, ash, fibre, fat and protein of the two cultivars of pearl millet tested. Fermentation significantly ($P \leq 0.05$) increased the ash and protein content, but significantly ($P \leq 0.05$) reduced the fibre and fat content. On the other hand, soaking significantly ($P \leq 0.05$) enhanced the IVPD, IVSD, but mineral contents of the two cultivars were markedly reduced during fermentation. Fermentation of pearl millet caused significant reduction in phytic acid for both cultivars. Although soaking slightly increased protein content, it resulted in significant reduction in phytic acid for both cultivars. Natural fermentation at 45 ºC had the most pronounced effect on lowering phytate followed by that at 37.5 and 30 °C; the higher the temperature, the greater the loss of phytic acid during fermentation. This was accompanied by significant improvement in protein digestibility (*in vitro*). An improvement in protein

digestibility *(in vitro*) was noticed at all the temperatures of natural fermentation, the highest being at 45.0 °C. Fermentation and soaking could be regarded as viable means for improvement of the nutritional quality of pearl millet. Fermentation offers unique nutritional advantages for making the starch and protein of coarse-grained pearl millet more digestible, possibly by drastically reducing its phytate content. Processing techniques reduce the levels of antinutritional organic factors and enzyme inhibitors by releasing exogenous and endogenous enzymes such as phytase enzyme formed during processing. It may be concluded that by indigenous fermentation with natural organism of indigenously developed food mixtures is a potential process for developing food products of improved nutritional quality. This type of fermented food product not only offers unique nutritional value but also has therapeutic value.

The consumption of such food mixtures may be useful in controlling some infections induced by pathogens or antibiotics. An indigenous *kharodi/rabidi* can be prepared from the naturally fermented flour of pearl millet without addition of any chemical additives to improve the textural quality of *kharodi/rabadi*. The instrumental and sensory analysis showed that *kharodi/rabadi* made from fermented flour of pearl millet of PPC-6 has 'very good' quality attributes within a very short period of fermentation than the one made from traditional method. Traditional methods of preparation of *kharodi/rabadi* thus, seem to have certain nutritional advantages. *Kharodi/rabadi* offers unique dietary reward of not only improving the amino acid profile of pearl millet but also of making the starch and the resultant protein more digestible. Reduction in the phytic acid and polyphenol content of pearl millet through *kharodi/rabadi* fermentation may imply improve digestibility of proteins and carbohydrates and enhanced bioavailability of minerals in the fermented product. Hence, this study provides an option to improve the process of making more nutritious, a popularly consumed traditional fermented food, *kharodi/rabadi*. So, there is a thrust to introduce such easily adoptable, profitable and nutritious indigenous technologies for making *kharodi/rabadi* there in the market.

5. References

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