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Effect of fermentation and processing on in vitro mineral estimation of selected fermented foods

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Abstract

The bioavailability of minerals, Zn, Fe, Ca and Mg from cereals, is low because it is present as an insoluble complex with food antinutrient component such as phytic acid. The commonly consumed fermented foods in Khandesh region of Maharashtra, India was investigated for some minerals and anti nutritional factor, Phytate. We study the effect of fermentation with an aim to reduce the content of phytic acid, while maintaining sufficient levels of minerals, in the expectation of increasing its in vitro availability. Fermentative reduction of phytate increase the amount of Fe, Zn, Ca and Mg several fold. In all process products phytate: Zn, phytate: Fe, phytate: Ca and phytate: Mg ratio of the fermented and processed products was lower than the raw foods indicating increased availability of minerals from fermented foods than raw foods.

Introduction

Cereals and pulses are important substrates for fermented foods and are staple in the Indian subcontinent (Hurell, 2004). Such plant based diets are, often associated with micronutrient deficits, exacerbated in part by poor micronutrient availability. Diet related factors in plant foods that affect availability of minerals include: the chemical form of nutrient in food and zinc, than on macronutrients. The absorption of Ca, Fe and Zn is affected. The anti interactions between nutrients and other organic components that is antinutritional factors (Cheng and Bray, 1951). These factors have a greater influence on the bioavailability of the micronutrients in plant food, particularly calcium; iron nutritional factors have adverse effect on nutrition and physiology. Many of these interfere with digestion thereby preventing competent utilization of the legume protein (Gercis-Villanova *et al*., 1982). Sometimes they are capable of precipitating harmful effects in man and animals, with obvious toxicity ranging from severe reduction in food intake and nutrient utilization to profound neurological effects culminating in death (Davies and Olpin, 1979). Plant based diets contains substantial amount of phytate that reduces dietary minerals like Zn, Fe, Ca and Mg absorption (Irving and McMullen, 1980). Minerals form an integral part of functionally important organic compounds (Gibson, 1994). They are essential for the normal functioning of muscles, heart, nerves and in the maintenance of body fluid (Morris and Ellis, 1985).

Due to the world wide prevalence of phytate and its adverse effects on health, there has been growing attention in the development of practical strategies to reduce phytate content of staple grains. Traditional food processes such as soaking, germination and fermentation (AOAC, 1999) by activating phytase which hydrolyses/degrades phytate into lower inositol phosphate have been reported to substantially reduce or degrade phytic acid (Mohite *et al*., 2011). In present study commonly consumed steamed, baked, fried and sun dried fermented food prepared in Khandesh region (Maharashtra) and other parts of India was subjected to improve the *in vitro* mineral availability from fermented foods.

Materials and Methods

Food materials

Fermented foods consumed in Khandesh region used for this study were *kurdai*, *Bibde*, *Gulgule*, *Papad*, *Dhinde*, *Chikani* and *Nagali Papad*. Other fermented foods were, *Idli*, *Dhokla*, *Jalebi* and *Dosa*.

Sample collection

The grains, Black gram (*Phaseolus mungo*), rice (*Oriyza sativa*), Sorghum (*Sorghum moench*), wheat (*Triticum aestivam*), Bengal gram (*Cicer arietinum*), Nagali (*Eleusine coracana*), Chikani (*Poaceae* Family), used in this study were purchased from a local market and household located in Jalgaon city and nearby. Each food sample was prepared by removing

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inedible husks, seeds, rind orpits, where necessary using local utensils and traditional methods.

Each sample of raw food type was then combined and ground to a fine homogeneous powder to form one composite sample in a labeled, sealed trace element free polyethylene bags. This unfermented (dry matter) (UFDM) was used for analysis. Each food sample was then subjected to the traditional fermentation employed by many khandesian women in food preparation and the sample was used as fermented batter (FB) for analysis. After further processing either by steaming, frying or drying the final processed product (PP) was prepared and used for analysis. The chemical analysis was done in triplicates. The detail for the formulation of various food products was given in Table 1.

Table 1. Ingredients and methods used for preparation of various products

Product	Ingredients	Quantity	Recipe
Dhokla	Bengal gram dhal flour Curd Sodium carbonate Oil	100g 50g 2g 25g	All the ingredients were mixed to ferment for 10 -12 h and poured into a greased pan and steamed for 20min.
Jalebi	Refined wheat flour Curd	60g 60g	Curd and water were mixed with refined wheat flour to make a thin batter and fermented for 10 -12h. The batter was poured through a 0.5cm hole of cloth into hot oil/ghee to fry till golden brown and soaked into sugar syrup
Gulgule	Whole wheat flour Jaggery Sodium carbonate	110g 100g 1/3 tsp	Ingredients were mixed to get consistency and allowed to stand for 4 -5 h then fried in oil by adding circular lumps
Kurdai	Wheat grain salt	100g To taste	Wheat grains were soaked in water for 3 days. Then Ground and filtered through muslin cloth. The filtrate was collected and cooked in hot water with adding salt up to thick consistency and sun dried for 5 days
Bibde	Sorghum Wheat salt cumin seed Chilly powder Coriander powder	100g 50g To taste 2g 4g 2g	Sorghum grains were soaked for 5 days and wheat grain for 3 days. It was ground and filtered separately to stand it for overnight. Grind and filter separately. All the ingredients were mixed and cooked in hot water to bring thick consistency. Then it was placed on cloth for sun drying.
Dosa	Black gram dal Rice Salt	100 g 300g To taste	Soak dhal and rice overnight, ground to paste with water and ferment for 10-12 h.
Papad	Sorghum	100 g	Sorghum grains were soaked for 3-4 days and sun dried. Sun dried grains were grinded. This flour was mixed in hot water and cooked. A small fraction of this dough is rolled over plate and sun dried.
Dhinde	Sorghum Fenu greek	100 g 20 g	Sorghum grains were soaked overnight, divided into two equal parts. One part was mixed with Fenugreek and mixture was grinded. Other of sorghum was cooked and mixed with Fenugreek-sorghum flour mixture. This complex dough was fried.
Chikani Papad	Chikani	100 g	Chikani grains were soaked overnight, then grinded. All the ingredients were mixed in boiling water and cooked to thick consistency.
Nagali Papad	Nagali	100 g	Nagali grains were soaked overnight, then grinded. All the ingredients were mixed in a boiling water and cooked to thick consistency

Analytical methods

The raw, fermented and processed food samples were analyzed for phytate and mineral analysis.

Phytate analysis

Phytic acid was determined by the complexometric titration method (White *et al*. 1973), as modified by Garcia Estepa *et al*. (Delvin, 1997) was used. The brief protocol was as follows: 5 g of food sample was extracted under magnetic agitation with 40 ml of extraction solution composing 10 g/100 g Na_2SO_4 in 0.4 M/L HCl for 3 hour at room temperature which was centrifuged at 5000 rpm for 30 min. Supernatant was collected. 10 ml of supernatant mixed with 10 ml of 0.4 M/L HCl, 10 ml, 0.02 M/L $FeCl_3$ and 10 ml, 20 g/100 g sulphosalicylic acid. It was boiled in water bath for 15 min and centrifuged for 10 min at 5000 rpm. Further it was filtered and residue washed several times with distilled water. Supernatant of the filtrate was diluted to 100 ml. Aliquot of the filtrate (20 ml) adjusted to pH = 2.5 with 0.5 M by glycine and diluted to 200 ml and further it was heated at 70-80^{o}C and titrated with 50 mM/L EDTA till color changes from red, maroon to clear yellow. Phytic acid content was calculated using Fe: P (4:6) ratio (Delvin, 1997). Following formula was used for calculation of phytic acid percentage.

$$\% \text{ phytic acid} = 0.66\ (10\text{-}v)\ /m \qquad (1)$$

where, v = volume of EDTA (ml) and m = sample mass (gram).

Fe and Zn analysis

Preparation of sample and analysis

5 g of food sample was weighed into ashing vessel and dry-ashed at 450^{o}C in muffle furnace. Cake was broke with stirring rod and dissolved in 10 ml concentrated HCl solution was boiled and evaporated nearly to dryness. Residue was re-dissolved in 20 ml 2N HCl, gently boiled. It was filtered through fast paper into 100 ml volumetric flask. Paper and residue were thoroughly washed with deionised water. Diluted up to 100 ml. Absorption of solution was directly measured using atomic absorption spectrophotometer. Fe and Zn were analysed in food samples by atomic absorption spectrophotometer (Chemito, Mumbai) (Jackson, 1997).

Table 2. Phytate: Zn, phytate: Fe, phytate: Ca, phytate: Mg ratios of fermented products

Sample	Phytate : Zn			Phytate : Fe			Phytate : Ca			Phytate : Mg		
	UFDM	FB	PP	UFDM	FB	PP	UFDM	FB	PP	UFDM	FB	PP
Steamed												
Idli	15.31	1.595	0.728	4.804	0.296	0.208	1.310	0.063	0.013	1.092	0.078	0.062
Dhokla	16.46	2.694	1.176	17.77	1.541	0.255	0.961	0.107	0.042	3.215	0.235	0.149
Fried												
Jalebi	27.41	3.179	2.449	4.534	0.462	0.231	2.027	0.094	0.019	1.126	0.110	0.067
Gulgule	26.96	3.101	0.969	2.717	0.462	0.231	0.303	0.029	0.019	0.675	0.060	0.038
Dosa	15.00	1.274	0.702	4.703	0.218	0.112	1.283	0.033	0.012	1.069	0.054	0.036
Dhinde	14.17	1.665	0.809	4.124	0.352	0.133	0.582	0.059	0.018	1.828	0.127	0.079
Sun Dried												
Kurdai	16.59	1.722	0.889	1.987	0.282	0.098	0.826	0.033	0.040	2.699	0.206	0.094
Bibde	10.36	0.722	0.375	10.30	0.185	0.062	0.713	0.041	0.024	0.795	0.078	0.041
Papad	08.81	0.546	0.025	2.161	0.091	0.005	0.285	0.020	0.0009	0.773	0.058	0.002
Chikani Papad	08.90	0.520	0.180	2.185	0.065	0.035	0.288	0.017	0.0075	0.632	0.041	0.018
Nagali Papad	12.59	1.104	0.233	2.436	0.192	0.042	0.467	0.022	0.005	1.354	0.088	0.016

UFDM- unfermented (dry matter), FB- fermented batter, PP- processed product
Values are the mean of three replicates

Ca and Mg analysis

Calcium and magnesium were analyzed by titrimetric method. 5 ml of sample prepared as used above was pipette out in a 250 ml conical flask. 5 ml of ammonium buffer was added and diluted upto 100 ml with de-ionized water. Pinch of Erichrome black T was added in it and the solution was warmed to 600C. It was titrated against EDTA until the red color changed to blue. The end point was noted as 'A'. The amount of calcium in sample (mg/L) was calculated as follows,

$$\text{Calcium (mg/L)} = F \times B \times 1000 / \text{Volume of sample} \quad (2)$$

where B is ml of consumption of EDTA by Ca alone and Factor value for calcium (F) is 2.

In another flask 5 ml sample was taken and 5 ml of NaOH solution was added. The sample was diluted to 100 ml with deionised water and a pinch of murexide indicator was added. It was titrated against EDTA until the pink color turned to blue and the end point was noted as 'B'. The amount of magnesium in sample (mg/L) was calculated as follows,

$$\text{Magnesium (mg/L)} = F \times (A - B) \times 1000 / \text{Volume of sample} \quad (3)$$

where A is ml of consumption of EDTA by Ca and Mg while B is ml of consumption of EDTA by Ca alone and Factor value for magnesium (F) is 1.2 (Ihekoronye and Ngoddy, 1985).

Results and Discussion

The phytic acid content of investigated food samples was shown in Figure 1. The level of phytate in unfermented dry matter sample was ranged from 5.34 mg/100 g to 8.91 mg/100 g. Unfermented dry matter of *Gulgule* was found to contain the lowest level of phytate, while *Jalebi* had the highest level of phytate. The fermented and processed product of all sample investigated had relatively lower levels of phytate as compared with the unfermented samples.

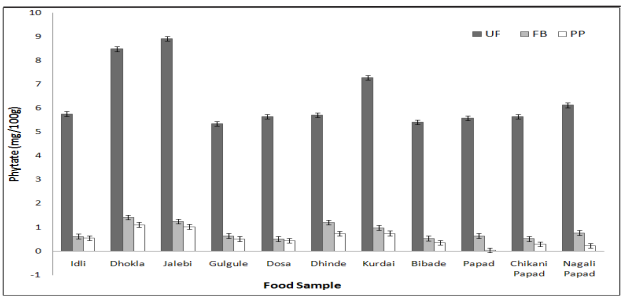

Figure 1. Effect of fermentation and processing on Phytate of food samples

Similar results were reported by (Khan *et al*., 1986). The phytate contents of unfermented *Dhokla* batter was 8.48 ± 0.01 mg/100 g which was reduced to 1.412 mg/100 g in fermented batter, further the content reduced to 1.112 mg/100 g in processed product. Marfo *et al*. (1990) also reported reduction in phytate content during fermentation of bread and other wheat products (Marfo *et al*., 1990). Fermentation increased the activity of nature phytase enzyme leading to high phytate degradation (Abebe *et al*., 2007). Similar results were reported by (Oboh *et al*., 2003) in fermented cassava products. The reduction in phytic acid content after processing was also significant.

Phytate: Zn, phytate: Fe, phytate: Ca and Phytate: Mg molar ratios for unfermented dry matter, fermented batter and processed food samples are shown in Table 2. The zinc content of the raw food sample varied the least, values ranging from 0.198 mg/100 g for *Gulgule* to 0.751 mg/100 g for *Chikani Papad*. Zinc was reported to be minimum in fermented *Gulgule* batter 0.207 mg/100 g and maximum in fermented

dough of *Papad* (1.180 mg/100 g). The zinc content increased after fermentation which could be due to reduction of phytate on processing in each food sample (Figure 2).

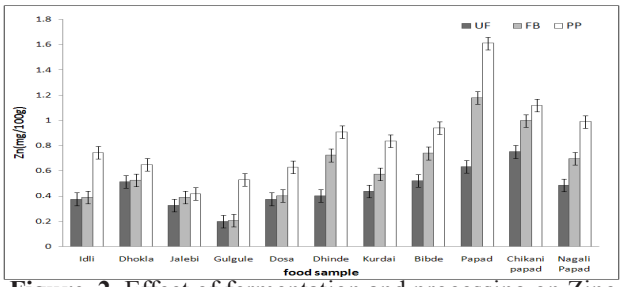

Figure 2. Effect of fermentation and processing on Zinc of food samples

Most of the raw foods had phytate: zinc molar ratio above 15, with the exception of *bibde*, *Dhinde*, *Papad*, *chikani* and *Nagali Papad*. Phytate: Zinc molar ratio of 10 or less is usually associated with adequate Zn availability in animal and above it associated with clinical or chemical evidence of Zn deficiency in rats (Osagie, 1998). In the present study phytate: Zn molar ratio of unfermented *Idli*, *Dhokla*, *Jilebi*, *Gulgule*, *Kurdai*, *Dhinde* was 15 or more showing them poor source of zinc. Phytate: zinc molar ratio in fermented batter and processed product was less than 15 indicate that adequate Zn availability.

Fermented sample had a low Phytate: Fe ratio as compared to the unfermented sample, with *bibde* dough having lowest (Figure 1). Most of the fermented and processed foods analyzed consequently phytate: iron molar ratio 1:0, with the exception of unfermented dry matter of *Dhokla* (Phy: Fe 1.5414). Phytate begins to lose its inhibitory effects on iron absorption when phytate: iron molar ratio less than 1.0. The unfermented dry matter batter had phytate: iron ratio above 1.0, a level said to compromise iron absorption (Garcia-Villanova *et al*., 1982). The values for Fe were reported to be minimum in fermented *Dhokla* batter (0.916 mg/100 g) and maximum in *Chikani Papad* batter (7.880 mg/100 g). After processing Fe was again reported to be maximum in *Chikani Papad* products (8.121 mg/100 mg) and minimum in *Idli* (2.596 mg/100 g). The increased in Fe content after processing might be due to the cooking in Fe utensils and further degradation of phytate. Similar results were reported for *bhalla*, *bhatura* and bread and other foods by (Riat and Sadana, 2009). Higher Fe content after fermentation may be ascribed to decreased contents of phytic acid which has a significant correlation with minerals. The Fe content was higher in sun dried processed products than steamed food samples (Figure 3). Maximum Ca was found in unfermented dry matter of *Papad* (19.537 mg/100 g) and minimum in *Idli* and *Jalebi* (4.395 mg/100 g). After fermentation the contents of Ca increased with maximum in *Nagali* fermented batter (34.120 mg/100 g) and minimum Idli (9.79 mg/100 g) (Figure 4). The calcium content of the unleavened corn bread was lower than that of fermented tef injera (Parveens and Hafiz, 2003). The increased Calcium content in fermented samples is due to the breakdown of Ca-phytate complex with the fermentation (Riat and Sadana, 2009). The phytate to Ca ratio is fermented batter samples was > 0.2, which is undesirable for good Ca availability (Sandberg, 2002). The phytate: Ca ratio is maximum in *Chikani Papad* fermented batter of *Dhokla* (0.1070) while minimum in *Chikani Papad* fermented batter (0.017) which further reduces after processing of fermented batter to processed batter indicates that good availability of Ca.

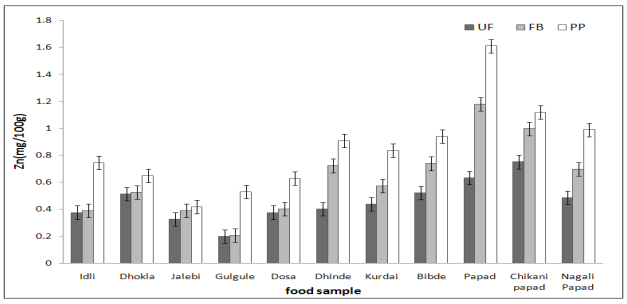

Figure 3. Effect of fermentation and processing on iron of food samples

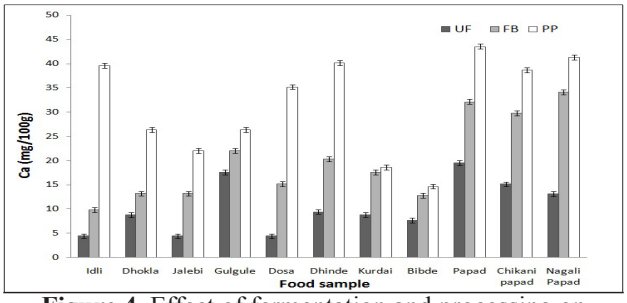

Figure 4. Effect of fermentation and processing on calcium of food samples

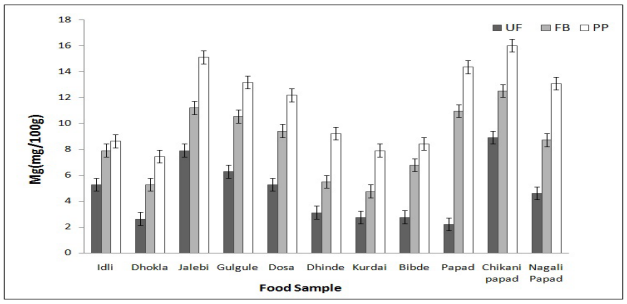

Figure 5. Effect of fermentation and processing on magnesium of food sample

The magnesium content of fermented food samples varied, the least values ranging from 2.637 mg/100 g for *Dhokla* to 12.512 mg/100 g for *Chikni Papad*. Mg values for *bibde*, *kurdai Dhinde* were

much lower (4.765 – 6.790 mg/100 g). The Mg content increased from unfermented dry matter to fermented batters, which again increase in processed product food. Maximum increase in Mg content observed in processed *Chikani Papad* (16.009 mg/100 g) and minimum in *Dhokla* (7.461 mg/100 g) (Figure 5). The critical molar ratio above which magnesium absorption is compromised by Phytate is uncertain.

Conclusion

Fermentation and processing resulted in a decrease in phytic acid content of the products with increase in the Zn, Ca, Fe & Mg contents. The removal of antinutritional factor by fermentation technology enhances the nutritional value of foods. Sun drying resulted in better availability of Zn, Fe & Ca while frying increase the availability of Mg. The phytate: mineral molar ratio of fermented and food processed product are lower that of the raw foods. Thus indicating the Zn, Ca, Fe and Mg are more available from products than from the raw foods. Therefore, fermentation as being a simple and cheapest method should be promoted at the household level to reduce the level of nutrient inhibitors (like Phytate) and thus improve the availability of mineral from the diet.

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