

‘Doli Ki Roti’ – An indigenously fermented Indian bread: Cumulative effect of germination and fermentation on bioavailability of minerals

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Received 01.06.10; revised 31.09.10

‘Doli Ki Roti’ – an indigenously fermented product, is prepared by Indian Punjabi Community migrated from Western Pakistan during partition. Earlier, fermentation was carried out in an earthen pot locally known as ‘doli’, therefore, the name derived ‘Doli Ki Roti’. It is a fried *puri* like product having legume stuffing. The unfermented bread (control) contained significant ($P<0.05$) amounts of phytic acid but were reduced to the extent of 5.5 to 15.8% due to cumulative effect of germination and fermentation. This reduction at both the temperatures (35 and 40°C) and time periods (18 and 24 h) caused an enhancement in the bioavailability of calcium and iron up to the extent of 5 to 11.5% and 15.4 to 27.6%, respectively.

Keywords: *Doli ki roti*, Fermentation, Germination, Chickpea, Phytic acid, Mineral bioavailability

IPC Int.Cl.⁸: A47G 19/12, A47J 47/01, A21D 8/06, A21, A23, C22B 26/20, C01B, C01C, C01G, C01G 49/00

Phytic acid acts as an antinutritional factor which is widely prevalent in unrefined cereals and millets. It is a powerful chelating agent for divalent cations and interferes with mineral availability due to formation of insoluble phytases¹. It is known to bind iron, zinc, calcium and magnesium which contributes significantly to poor absorption of dietary essential minerals from cereal based diets².

Hence, it is imperative to reduce the level of this antinutrient through any of the processing and cooking methods so as to improve the nutritional quality of plant foods and fermentation is one such method. In India, various fermented foods including *idli*, *dosa*, *dhokla*, *wadi*, *imarti*, *rabadi*, *doli ki roti*, etc. are prepared and consumed. ‘Doli Ki Roti’ - an indigenously fermented wheat based bread is prepared by the Indian Punjabi community migrated from Western Pakistan at the time of partition. As fermentation is carried out in an earthen pot locally known as ‘doli’, so the name ‘Doli Ki Roti’ is derived. Traditionally, the inoculums of this bread used to be prepared in the temples and distributed to community to prepare this bread on some special occasions like fasts when people used to eat ‘*basra*’ food, i.e. the food

prepared on the previous day and not the fresh hot food prepared on the day of fast. The spices which are added impart flavour and have antimicrobial properties, thereby restricting the growth of pathogenic microflora during natural fermentation. Combination of cereal and legume in this ‘*roti*’ improves the protein quality of vegetarian meal. As there is no documentation about the nutritive value of this indigenous bread, therefore this bread was prepared under standardized laboratory conditions. Moreover, the lack of scientific approach regarding the effect of indigenous fermentation on phytic acid and availability of minerals in this traditional product warranted the significance of the present communication.

Methodology

The poppy seeds, cloves, big cardamom, cinnamon, *jaifal*, jaggery and whole wheat flour were procured from the local market in a single lot and were cleaned of dust and foreign materials.

Procedure for ‘Doli Ki Roti’

For preparation of an indigenous fermented product, ‘Doli Ki Roti’, a procedure was followed as depicted in the flow diagram (Fig. 1)

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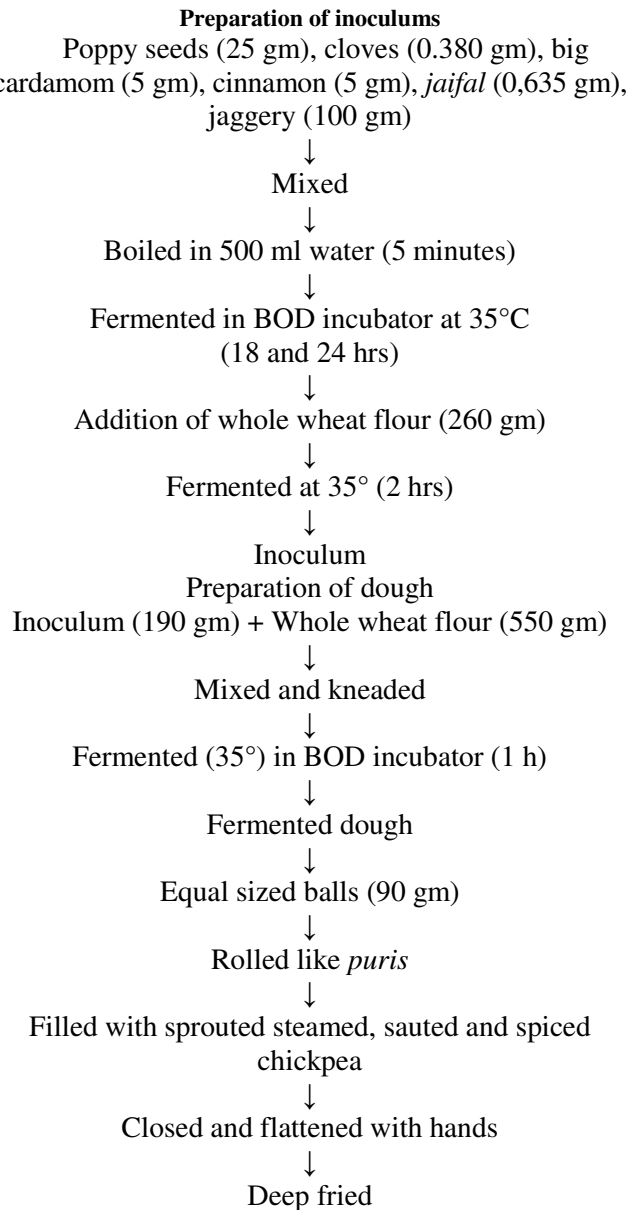


Fig. 1—Development of '*doli ki roti*'

Preparation of inoculums: The poppy seeds (25 gm), cloves (0.380 gm), big cardamom (5 gm), cinnamon (5 gm), *jaifal* (0.635 gm), jaggery (100 gm) were boiled in water (500 ml) for 5 min and kept in BOD incubator at 35°C and 40°C for two different time periods (18 and 24 hrs). After stipulated period of fermentation of the spice-jaggery mixture (500 mg), the whole wheat flour (260 gm) was added to it and again kept for fermentation (2 hrs) at 35°C and 40°C to procure the two types of inocula, i.e. one fermented for 18 h and the other one fermented for 24 hrs.

Preparation of dough: Whole wheat flour (550 gm) was mixed with the inoculum (190 gm) and the

dough of the same was kneaded. It was also kept for 1 h fermentation.

Preparation of *Roti*: Fermented dough was divided into equal sized balls (90 gm) each. These balls were rolled round like '*puris*' having 10.5 cm (diameter) and 3 mm (thickness). It was then filled with sprouted, steamed, sauted and spiced chickpea. After filling the stuffing in the rolled '*puris*', they were again closed in ball shape, flattened with hands (10.5 cm diameter) and deep fried till golden brown.

Chemical analysis: Phytic acid content was determined by the method of Haug and Lantzsch³. For total minerals, the samples were wet acid digested with diacid, i.e. a nitric acid and perchloric acid mixture (HNO₃: HClO₄: 5:1, v/v) in the digestion chamber. The digested samples were dissolved in double distilled water and filtered (Whatman # 42). The filtrate was made to 50 ml with double distilled water and was used for determination of total calcium, iron and phosphorus. Calcium and iron in acid digested samples were determined by Atomic Absorption Spectrophotometer according to the method of Lindsey and Norwell⁴. Phosphorus was determined colorimetrically by the method of Chen *et al.*⁵. Phytate phosphorus was derived by using the following formula⁶.

$$\text{Phytate phosphorus (mg)} = \frac{A \times 28.18}{100}$$

Where A is the phytate content (mg)

Non-phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus.

Available (*in vitro*) calcium was extracted by the method of Kim and Zemel⁷ and available iron was extracted according to the procedure of Rao and Prabhavathi⁸.

For HCl-extractability minerals, the phosphorus in the samples was extracted with 0.03 N HCl by shaking the contents at 37°C for 3 hrs. The clear extract obtained after filtration with Whatman # 42 filter paper was oven-dried at 100°C and wet acid digested. The amount of the HCl-extractable phosphorus in the digested samples was determined by methods just described for the estimation of total phosphorus. The HCl-extractability of minerals in foods is an index of their bioavailability from the foods^{9,10}.

Statistical analysis

The data were statistically analyzed in complete randomized design for analysis of variance and coefficient of correlation¹¹.

Results and discussion

Phytic acid is known to be a major storage form of phosphorus in legumes. The phytic acid content of the unfermented bread stuffed with sprouted chickpea contained 632.3 mg of phytic acid per 100 gm (Table 1). The fermentation at 35°C for 18 and 24 hrs slashed the phytic acid content, i.e. varying from 5.5 to 15.2% over the control while such reductions were further increased, i.e. 8.6 to 15.8% due to an increase in the fermentation temperature and duration.

Inherent phytase activity in cereals and legumes¹² and also microbial phytase activity responsible for the hydrolysis of phytic acid¹³ account for lowering the phytate content. The optimum temperature (35-45°C) has been recognized to be the best for phytase action from plants and microbial source. Hence, the indigenous breads fermented at both 35 and 40°C had less phytic acid contents.

The unfermented bread with sprouted chickpea had 57.7 mg calcium per 100 gm (Table 1). The influence of temperature and period of fermentation did not bring any significant ($P>0.05$) change in calcium contents of bread as values for 35°C vs 40°C were 58.0 and 57.9 mg/100 gm (18 h) and 60.0 and 61.9 mg/100 gm (24 hrs), respectively. The calcium availability (*in vitro*) in sprouted chickpea bread was 80.0 to 84.7% (35°C) and 81.9 to 85.3% (40°C) when fermentation was carried out for 18 and 24 hrs. Such figures revealed a per cent improvement of 4.5 and 10.7 over the control (76.5%) in 35°C fermented breads for 18 and 24 hrs, respectively (Table 1). The corresponding values for breads fermented at 40°C were 7.0 (18 hrs) and 11.5% (24 hrs).

The iron contents of bread stuffed with sprouted chickpea ranged from 8.7 to 9.5 mg/100 gm when fermented for 18 and 24 hrs at 35 and 40°C (Table 1). The iron availability (%), which improved due to 35°C fermentation in relation to control (36.2) was 41.7 (18 hrs) and 46.1 (24 hrs). Fermentation at 40°C further increased the bioavailability of iron for two experimental periods in sprouted chickpea breads. Higher temperature and prolonged fermentation period improved the availability (*in vitro*) of minerals (Table 1).

The increased minerals' bioavailability may be ascribed to reduced content of phytic acid mediated

through fermenting microbial hydrolysis by phytase. Fermentation of cereal-legume blends has been reported to be effective in reducing the antinutrients like phytic acid¹⁴ and sequentially exerting beneficial effects on calcium and iron bioavailability. There exists a negative correlation between phytic and bioavailability of minerals¹⁵. Previous studies have also reported that fermentation of cereal flour and *tef* increased bioavailability of iron^{16,17}.

The unfermented bread with sprouted chickpea had 306.8 mg total phosphorus per 100 gm. The total P content did not differ significantly ($P>0.05$) when the bread was fermented at 35 and 40°C for varying time periods (Table 2).

The data presented in Table 2 showed significant ($P<0.05$) reduction in phytate phosphorus, viz. 168.4 (18 hrs) and 1521.0 (24 hrs) mg/100 gm for 35°C fermented breads having sprouted chickpea in relation to unfermented one (178.1 mg/100 gm). Further, increase in fermentation temperature led to more ($P<0.05$) decrease in phytate phosphorus. There were concomitant increases in non-phytate phosphorus as a result of fermentative processes. When the breads were fermented at 35°C for 18 and 24 hrs, the values for non-phytate phosphorus were 143.3 and 159.3 mg/100 gm. These values were further significantly ($P<0.05$) increased to 143.3 and 182.6 mg/100 gm as a result of 40°C fermentation for two respective periods.

The P extractability of the unfermented bread containing sprouted chickpea was 61.0% (Table 2). Due to fermentation, extractability increased to 66.4 and 69.8% (35°C) as opposed to 67.7 and 71.1% (40°C) for two fermentation periods, i.e. 18 and 24 h, respectively.

An enhanced extractability of phosphorus may be attributed to the hydrolytic cleavage of phosphorus from the phytic acid by microbial phytases during fermentation. This is substantiated by significant decrease in phytic acid besides phytate phosphorus and correspondingly increases in non-phytate phosphorus. Decrease in phytic acid content may indicate that the divalent phosphorus cations are freed from the phytate mineral complex, which may, thereby, increase HCl-extractable phosphorus in fermented product.

The results are consistent with the earlier findings reported in *rabadi*¹⁹, fermentation of finger millet¹⁶ and lactic acid bacteria fermentation of *tef*¹⁷.

Conclusively, this indigenously fermented bread is not only an ideal blend of cereal and legume but

Table 1—Effect of fermentation on phytic acid (mg/100 gm), total calcium and iron (mg/100 gm) and availability (*in vitro*) of calcium and iron (%) of indigenously fermented bread stuffed with sprouted chickpea (on dry matter basis)

Temperature of fermentation (°C)	Period of fermentation (h)	Phytic acid	Total calcium	Available calcium	Total iron	Available iron
35	0	632.3±1.53	57.7±3.44	76.5±3.55	9.1±0.20	36.2±3.31
	18	597.5±1.02 (5.5)	58.0±3.55	80.0±2.74 (4.5)	9.5±1.73	41.7±3.06 (15.4)
	24	535.9±6.69 (15.2)	60.0±1.44	84.7±1.45 (10.7)	8.7±0.03	46.1±2.63 (27.3)
40	0	632.3±1.53	57.7±3.44	76.5±3.55	9.1±0.20	36.2±3.11
	18	577.3±2.37 (8.6)	57.9±1.80	81.9±4.03 (7.0)	8.7±0.18	43.4±5.19 (19.8)
	24	532.3±2.32 (15.8)	61.9±3.10	85.3±2.33 (11.5)	8.9±0.56	46.2±3.67 (27.6)
CD (P<0.05)						
Temperature		5.21	NS	0.67	NS	0.26
Period		7.61	NS	3.95	NS	6.09
Temp. x period		1.07	NS	NS	NS	NS

Values are means±SE of three independent determinations

Figures in parentheses indicate percentage

NS = Non-significant

Table 2—Effect of fermentation on phytate and non-phytate phosphorus (mg/100 gm) and phosphorus extractability (%) of indigenously fermented bread stuffed with sprouted chickpea (on dry matter basis)

Temperature of fermentation (°C)	Period of fermentation (h)	Total phosphorus	Phytate phosphorus	Non-phytate phosphorus	Phosphorus extractability
35	0	306.8±1.74	178.1±1.04	128.7±0.97	61.0±1.73
	18	311.7±1.73	168.4±1.15	143.3±0.98	66.4±2.04
	24	310.3±4.50	151.0±1.03	159.3±0.88	69.8±1.10
40	0	306.8±1.74	178.1±1.04	128.7±0.97	61.0±1.73
	18	306.0±1.17	162.7±0.95	143.3±0.98	67.7±2.15
	24	332.6±1.83	150.0±0.97	182.6±1.01	71.1±0.78
CD (P<0.05)					
Temperature		NS	1.75	3.85	0.51
Period		NS	9.00	9.92	4.31
Temp. x period		NS	8.95	8.85	NS

Values are means±SE of three independent determinations

NS = Non-significant

also is nutritious due to the cumulative effect of germination and fermentation. It contains less amount of phytic acid and higher availability of dietary essential minerals. This traditional product should be promoted due to its better nutritive value and people should be educated not to forget it and get it vanished from the meals under the influence of modern dietary habits and changing life style patterns.

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