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Effect of fermentation and incorporation of *okara* (soybean milk residue) on chemical characteristics of *dhokla*

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Abstract
The present investigation was conducted to see the effect of natural and probiotic (*L. acidophilus*) fermentation on chemical characteristics of *dhokla*. Both types of fermentation significantly (P<0.05) increased the availability of minerals, *in vitro* protein digestibility, total phenolic content and DPPH free radical scavenging activity, while, decreased the phytic acid content of without *okara* and with *okara* supplemented *dhokla* as compared to unfermented control. Supplementation of 10 per cent *okara* powder in *dhokla* whether fermented naturally or with probiotic microorganism significantly (P<0.05) increased the total, soluble and insoluble dietary fibre, total mineral content except iron, total phenolic content and DPPH free radical scavenging activity. The phytic acid content of *dhokla* containing 10 per cent *okara* powder was significantly (P<0.05) higher, while, *in vitro* protein digestibility and availability of minerals were significantly (P<0.05) lower than their respective fermented without *okara* counterparts.

Keywords: Soy residue (*Okara*), *Dhokla*, antioxidant, fermentation, probiotic, chemical

Introduction
Fermented foods are precious as they provide a wide range of nutritious foods having distinct flavors, aromas and textures. Many biochemical changes occur during fermentation, leading to an altered ratio of nutritive and anti-nutritive components and, consequently, affect the products properties, such as bioactivity and digestibility (Zhang *et al*., 2012) [22]. Probiotic fermented foods are recognized as functional foods. Lactic acid bacteria are beneficial microorganisms used as starter cultures for the processing of functional food (Gourbeyre *et al*., 2011; Frick *et al*., 2007) [7, 4]. Scientific reports point to the health benefits of probiotics including improvement intestinal health, reduction in serum cholesterol, immune system stimulation and cancer prevention (Markowiak and Sli´zewska, 2017) [15]. Literature indicates that probiotic foods not only have several potential health benefits but also have nutritional benefits (Sharma and Ghosh, 2006) [18].

Okara is the residue remains after extracting the liquid from soybeans during the manufacture of soymilk or tofu. It is mainly discarded as waste or used as a livestock feed (Li *et al*., 2012) [12]. *Okara* is nutritionally equivalent to soybeans and is high in dietary fiber; therefore, its use as a food component is expected (Kagawa, 2009) [10]. *Dhokla* is one of the most popular indigenous foods of India that is made with a fermented batter from rice or semolina and chickpea and is mainly eaten in breakfast or as a snack which is usually tangy and slightly sweet in taste. Value addition of fermented foods by utilization of byproducts is possible and if such foods are fermented with probiotic microorganism it may enhance the nutritive value of the product. Therefore this study aimed to determine the effect of fermentation and incorporation of *okara* on chemical characteristics of *dhokla*.

Material and Methods
Procurement of Material
The grains of soybean (cv. PS 1347) were procured in a single lot from the Department of Genetics and Plant Breeding, College of Agriculture, CCSHAU, Hisar. The seeds were cleaned and made free of dust, dirt and foreign materials and packed in air tight containers for further use. All the ingredients used for *dhokla* preparation were procured from local market. The culture of probiotic microorganism *Lactobacillus acidophilus* was purchased from the Microbial Culture Collection Centre, NDRI, Karnal.

Extraction of *Okara*

Okara was extracted as per Chinese method. Soybean seeds were soaked overnight, rinsed and ground in a blender by adding water in 1:8 w/v to obtain the soy slurry; the resultant soy slurry was filtered through double layered cheese cloth. When filteration slowed, the remaining liquid was squeezed out by pressing with the hand for 1-2 min and the residue obtained (called *okara*) was freeze dried, ground to fine powder and stored in air tight polythene sheets for further use.

Development of *dhokla*

Dhokla was prepared from Bengal gram flour (100%), while, 10 per cent *okara* powder was supplemented for preparation of *okara* based *dhokla*. For natural fermentation, all the batters of *dhokla* were kept in BOD incubator at 37°C temperature for 8 hours to carry out the fermentation, whereas, for probiotic fermentation, all the batters of *dhokla* were autoclaved at 15 psi for 15 minutes, cooled and then inoculated with probiotic curd containing 10^8 cells/ml and kept in BOD incubator at 37°C temperature for 8 hours. All the *dhokla* batters were steam cooked for 30 minutes in the *dhokla* cooker. The *dhokla* developed without using *okara* and fermentation served as control.

Chemical Analysis

Dietary fibre contents were assessed as per the enzymatic method of Furda (1981) [5] and phytic acid content by the method given by Davies and Reid (1979) [3]. The *in vitro* protein digestibility was carried out by using the modified method of Mertz *et al.* (1983) [16]. Total phenolic content was estimated as per Singleton and Rossi (1965) [20]. DPPH free radical scavenging activity was determined by the method of Hatano *et al.* (1988) [8]. For total minerals, the samples were wet acid digested using diacid mixture (HNO_3: $HClO_4$:: 5:1, v/v). The total calcium, potassium, iron and zinc in acid digested samples were determined by atomic absorption spectrophotometer as per the method of Lindsey and Norwell (1969) [13] while phosphorus in acid digested samples was determined colorimetrically (Chen *et al.*, 1956) [1]. The available calcium and zinc were extracted by the method of Kim and Zemel (1986) [11] and available iron was extracted as per the procedure of Rao and Prabhavathi (1978) [17].

Statistical Analysis

The data were statistically analyzed in a completely randomized design using analysis of variance to test the significant differences among treatments (Sheoran and Pannu, 1999) [19].

Results and Discussion

Effect of fermentation on dietary fibre contents of *dhokla*

The data regarding effect of fermentation on total, soluble and insoluble dietary fibre contents of *dhokla* has been presented in Table 1. The total, soluble and insoluble dietary fibre contents of unfermented without *okara* control *dhokla* were 16.36, 2.08 and 14.28 g/100 g, respectively. No significant (P<0.05) difference was observed in total dietary fibre contents of naturally (16.58 g/100 g) and probiotic (16.62 g/100 g) fermented *dhokla* without *okara*, respectively when compared to that of unfermented without *okara* control *dhokla*. Soluble dietary fibre content of naturally (1.43 g/100 g) and probiotic (1.50 g/100 g) fermented *dhokla* without *okara* decreased significantly (P<0.05) i.e. 31.25 and 27.88 per cent, respectively over the unfermented without *okara* control *dhokla*, whereas, insoluble dietary fibre content of naturally (15.15 g/100 g) and probiotic (15.11 g/100 g) fermented *dhokla* without *okara* increased significantly (P<0.05) i.e. 6.09 and 5.81 per cent, respectively over the unfermented without *okara* control *dhokla*. Supplementation of 10 per cent *okara* powder in *dhokla* significantly (P<0.05) increased the total (24.33 and 24.45%), soluble (20.67 and 23.08%) and insoluble (24.86 and 24.65%) dietary fibre contents in both naturally and probiotic fermented *dhokla* over the unfermented without *okara* control *dhokla*. Similarly, decrease in soluble and increase in insoluble fibre content was reported earlier by Marko *et al.* (2014) [14] in cereals.

Table 1: Effect of fermentation on dietary fibre contents of *dhokla* (g/100 g, dry weight basis)

Types of *dhokla*	Total dietary fibre	Soluble dietary fibre	Insoluble dietary fibre
Bengal gram flour (100%) Unfermented Control	16.36±0.16	2.08±0.03	14.28±0.14
Natural fermentation			
Bengal gram flour:*Okara* powder (100:0)	16.58±0.27	1.43±0.01 (-31.25)	15.15±0.28 (+6.09)
Bengal gram flour:*Okara* powder (90:10)	20.34±0.43 (+24.33)	2.51±0.05 (+20.67)	17.83±0.45 (+24.86)
Probiotic fermentation			
Bengal gram flour:*Okara* powder (100:0)	16.62±0.19	1.50±0.02 (-27.88)	15.11±0.21 (+5.81)
Bengal gram flour:*Okara* powder (90:10)	20.36±0.25 (+24.45)	2.56±0.04 (+23.08)	17.80±0.24 (+24.65)
CD (P≤0.05)	0.88	1.00	0.91

Values are mean ± SE of three independent determinations

Values in parentheses indicate per cent increase over unfermented without *okara* control

Effect of fermentation on total mineral contents of *dhokla*

The data presented in Table 2 indicated that the total calcium, phosphorus, potassium, iron and zinc contents of unfermented without *okara* control *dhokla* were 78.68, 270.33, 835.33, 6.52 and 2.89 mg/100 g, respectively. No significantly change was observed in the contents of total calcium, phosphorus, potassium, iron and zinc of both naturally and probiotic fermented *dhokla* without *okara* when compared to those of unfermented without *okara* control *dhokla*. The total calcium, phosphorus, potassium, iron and zinc contents were 104.52, 288.66, 888.17, 6.53 and 3.27 mg/100 g, respectively in naturally and 104.88, 289.17, 889.17 6.57 and 3.29 mg/100 g, respectively in probiotic fermented *dhokla* with *okara* (10%). Supplementation of 10 per cent *okara* powder in *dhokla* significantly (P<0.05) increased the total calcium (32.84 and 33.30%), phosphorus (6.78 and 6.97%), potassium (6.33 and 6.45%) and zinc (2.88 and 3.29%) contents in both naturally and probiotic fermented *dhokla* over the unfermented without *okara* control *dhokla*, whereas, iron contents remained almost similar.

Table 2: Effect of fermentation on total mineral contents of *dhokla* (mg/100 g, dry weight basis)

Types of *dhokla*	Calcium	Phosphorus	Potassium	Iron	Zinc
Bengal gram flour (100%) Unfermented Control	78.68±0.67	270.33±2.32	835.33±6.59	6.52±0.18	2.89±0.02
Natural fermentation					
Bengal gram flour:*Okara* powder (100:0)	77.49±0.48	267.67±1.74	834.50±4.93	6.51±0.10	2.85±0.09
Bengal gram flour:*Okara* powder (90:10)	104.52±1.26 (+32.84)	288.66±0.88 (+6.78)	888.17±8.56 (+6.33)	6.53±0.06	3.27±0.05 (+13.15)
Probiotic fermentation					
Bengal gram flour:*Okara* powder (100:0)	77.95±0.59	266.83±1.17	835.66±3.06	6.54±0.04	2.88±0.06
Bengal gram flour:*Okara* powder (90:10)	104.88±0.95 (+33.30)	289.17±1.76 (+6.97)	889.17±9.33 (+6.45)	6.57±0.05	3.29±0.04 (+13.84)
CD (P≤0.05)	2.68	5.27	21.99	NS	0.19

Values are mean ± SE of three independent determinations
Values in parentheses indicate per cent increase over unfermented without *okara* control

Effect of fermentation on available mineral contents of *dhokla*

The data for effect of fermentation on available mineral contents of *dhokla* has been presented in Table 3. The availability of calcium, iron and zinc of unfermented without *okara* control *dhokla* were 37.24, 30.74 and 28.56 per cent, respectively (Table 4.42). The availability of calcium, iron and zinc improved significantly (P<0.05) i.e. 36.55, 25.44 and 44.47 per cent, respectively in naturally and 38.27, 29.73 and 49.93 per cent, respectively in probiotic fermented *dhokla* without *okara* over the unfermented without *okara* control *dhokla*. Supplementation of 10 per cent *okara* powder in *dhokla* significantly (P<0.05) increased the availability of calcium (29.89 and 32.30%), iron (16.01 and 21.86%) and zinc (37.64 and 42.44%) in naturally and probiotic fermented *dhokla,* respectively over the unfermented without *okara* control *dhokla*. The availability of calcium, iron and zinc were 50.85, 38.56 and 41.26 per cent, respectively in naturally and 51.49, 39.88 and 42.82 per cent, respectively in probiotic fermented without *okara dhokla*, whereas, the availability of calcium, iron and zinc was 48.37, 35.66 and 39.31 per cent, respectively in naturally and 49.27, 37.46 and 40.68 per cent, respectively in probiotic fermented *dhokla* with 10 per cent *okara* powder. The availability of minerals was significantly (P<0.05) higher in probiotic fermented *dhokla* than naturally fermented *dhokla*. Also, the mineral availability was significantly (P<0.05) higher in *dhokla* without *okara* than that of 10 per cent *okara* powder supplemented *dhokla*.

Table 3: Effect of fermentation on available mineral contents of *dhokla* (%, dry weight basis)

Types of *dhokla*	Available calcium	Available iron	Available zinc
Bengal gram flour (100%) Unfermented Control	37.24±0.32	30.74±0.22	28.56±0.19
Natural fermentation			
Bengal gram flour:*Okara* powder (100:0)	50.85±0.57 (+36.55)	38.56±0.36 (+25.44)	41.26±0.39 (+44.47)
Bengal gram flour:*Okara* powder (90:10)	48.37±0.30 (+29.89)	35.66±0.19 (+16.01)	39.31±0.33 (+37.64)
Probiotic fermentation			
Bengal gram flour:*Okara* powder (100:0)	51.49±0.42 (+38.27)	39.88±0.23 (+29.73)	42.82±0.46 (+49.93)
Bengal gram flour:*Okara* powder (90:10)	49.27±0.28 (+32.30)	37.46±0.32 (+21.86)	40.68±0.29 (+42.44)
CD (P≤0.05)	1.26	0.88	1.10

Values are mean ± SE of three independent determinations
Values in parentheses indicate per cent increase over unfermented without *okara* control

Effect of fermentation on phytic acid content and *in vitro* protein digestibility of *dhokla*

The data on effect of fermentation on phytic acid content and *in vitro* protein digestibility of *dhokla* has been presented in Table 4. The phytic acid content decreased significantly (P<0.05) in both naturally (208.03 and 197.37 mg phytic acid/100 g) and probiotic (230.25 and 215.97 mg phytic acid/100 g) fermented without and with 10 per cent *okara* supplemented *dhokla* over the unfermented without *okara* control *dhokla* (343.67 mg of phytic acid/100 g). The decrease in phytic acid content over the unfermented without *okara* control *dhokla* was 42.57 per cent in without *okara* and 37.16 per cent in *okara* powder (10%) supplemented probiotic fermented *dhokla* and 39.47 (without *okara*) and 33.00 per cent (with *okara*), respectively in naturally fermented *dhokla*. A significant (P<0.05) difference was observed between without *okara* and 10 per cent *okara* powder supplemented *dhokla* in both naturally and probiotic fermented *dhokla*. In contrast, no significant difference was observed between naturally and probiotic fermented without *okara* and 10 per cent *okara* powder supplemented *dhokla.* Reduction in the phytic acid content during *L. acidophilus* fermented composite dairy-cereal substrate has been reported earlier by Ganguly *et al*., 2014 [6].

The unfermented control *dhokla* had 63.75 per cent *in vitro* protein digestibility which was improved significantly (P<0.05) i.e. 27.48 and 24.19 per cent in naturally fermented without *okara* and 10 per cent *okara* powder supplemented *dhokla,* respectively and 29.74 and 25.96 per cent in probiotic fermented without *okara* and 10 per cent *okara* powder supplemented *dhokla,* respectively over the unfermented without *okara* control *dhokla*. No significant (P<0.05) difference was observed in *in vitro* protein digestibility of probiotic (82.71%) and naturally (81.27%) fermented without *okara dhokla*. Also, probiotic (80.30%) and naturally (79.17%) fermented *dhokla* containing 10 per cent *okara* powder supplemented *dhokla* did not differ significantly from each other. In both naturally and probiotic fermented *dhokla* the *in vitro* protein digestibility of 10 per cent *okara* powder supplemented *dhokla* was significantly (P<0.05) lower than that of *dhokla* without *okara*. Improvement in protein digestibility as a result of fermentation is mainly because of that fermenting microflora may produce some proteolytic enzymes during fermentation. Similar results were reported earlier by Ganguly *et al*., 2014 [6] for *L. acidophilus* fermented composite dairy-cereal substrate.

Table 4: Effect of fermentation on phytic acid and *in vitro* protein digestibility of *dhokla* (dry weight basis)

Types of *dhokla*	Phytic acid (mg/100 g)	*In vitro* protein digestibility (%)
Bengal gram flour (100%) Unfermented Control	343.67±7.90	63.75±0.45
Natural fermentation		
Bengal gram flour:*Okara* powder (100:0)	208.03±2.84 (-39.47)	81.27±0.32 (+27.48)
Bengal gram flour:*Okara* powder (90:10)	230.25±3.79 (-33.00)	79.17±0.81 (+24.19)
Probiotic fermentation		
Bengal gram flour:*Okara* powder (100:0)	197.37±4.23 (-42.57)	82.71±0.39 (+29.74)
Bengal gram flour:*Okara* powder (90:10)	215.97±6.17 (-37.16)	80.30±0.42 (+25.96)
CD (P≤0.05)	16.94	1.61

Values are mean ± SE of three independent determinations

Values in parentheses indicate per cent increase/decrease over unfermented without *okara* control

Effect of fermentation on antioxidant activity of *dhokla*

The data regarding effect of fermentation on total phenolic content and DPPH free radical scavenging activity of *dhokla* has been presented in Table 5. The total phenolic content of unfermented *dhokla* was 85.50 mg gallic acid equivalent/100 g, which increased significantly (P<0.05) in naturally (110.51 and 130.42 mg gallic acid equivalent/100 g) and probiotic (111.92 and 133.01 mg gallic acid equivalent/100 g) fermented without and with *okara* (10%) *dhokla*, respectively over the unfermented without *okara* control *dhokla*. The total phenolic content improved significantly (P<0.05) i.e. 29.25 and 30.90 per cent in without *okara*, 52.54 and 55.57 per cent in 10 per cent *okara* powder supplemented *dhokla* in both naturally as well as probiotic fermented *dhokla*, respectively over the unfermented control *dhokla*. In both naturally and probiotic fermented *dhokla* the total phenolic content of 10 per cent *okara* powder supplemented *dhokla* was significantly (P<0.05) higher than that of *dhokla* without *okara*. Increase in total phenolic content as a result of fermentation was reported earlier for soybean (Juan and Chou 2010; Chonkeeree *et al*., 2013) [9, 2].

The DPPH free radical scavenging activity improved significantly (P<0.05) i.e. to the extent of 50.90 and 68.44 per cent in naturally fermented without and with *okara* (10%) *dhokla*, respectively and 61.79 and 76.98 per cent in probiotic fermented without and with *okara* (10%) *dhokla*, respectively when compared to that of unfermented without *okara* control *dhokla* (12.77%). The DPPH free radical scavenging activity was significantly (P<0.05) higher in probiotic fermented *dhokla* (20.66 and 22.60%) than naturally fermented (19.27 and 21.51%) *dhokla*. Supplementation of 10 per cent *okara* powder in both naturally and probiotic fermented *dhoklas* significantly (P<0.05) increased the DPPH free radical scavenging activity over the fermented without *okara dhokla*. Improvement in antioxidant activity of soybean by lactic acid bacteria was reported by Wang *et al.* (2006) [21].

Table 5: Effect of fermentation on antioxidant activity of *dhokla* (dry weight basis)

Types of *dhokla*	Total phenolic content (mg GAE/100 g)	DPPH free radical scavenging activity (%)
Bengal gram flour (100%) Unfermented Control	85.50±0.54	12.77±0.17
Natural fermentation		
Bengal gram flour:*Okara* powder (100:0)	110.51±0.68 (+29.25)	19.27±0.10 (+50.90)
Bengal gram flour:*Okara* powder (90:10)	130.42±0.82 (+52.54)	21.51±0.27 (+68.44)
Probiotic fermentation		
Bengal gram flour:*Okara* powder (100:0)	111.92±0.54 (+30.90)	20.66±0.19 (+61.79)
Bengal gram flour:*Okara* powder (90:10)	133.01±0.66 (+55.57)	22.60±0.25 (+76.98)
CD (P≤0.05)	2.10	0.65

Values are mean ± SE of three independent determinations

Values in parentheses indicate per cent increase over unfermented without *okara* control

Conclusion

It may be concluded from the present study that supplementation of *dhokla* with *okara* resulted in utilization of this byproduct for nutritional enrichment and fermentation by natural as well as probiotic microorganism not only enhanced the antioxidant activity, digestibility and mineral availability but also decreased the phytic acid content. Thus, consumption of such foods not only offers unique nutritional value but may also have therapeutic properties.

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