Suman and Neelam Khetarpaul

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 Sliżewska, 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 filtration 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 10⁹ 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) [3] 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 (HNO₃: HClO₄: 5:1, v/v). The total calcium, potassium, iron and zinc in acid digested samples were determined colorimetrically (Chen et al., 1956) [1]. The available calcium and zinc were extracted by the method of Kim and Zemel (1986) [15] 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 Panu, 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)

<table>
<thead>
<tr>
<th>Types of dhokla</th>
<th>Total dietary fibre</th>
<th>Soluble dietary fibre</th>
<th>Insoluble dietary fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal gram flour:Okara powder (100%)</td>
<td>16.36±0.16</td>
<td>2.08±0.03</td>
<td>14.28±0.14</td>
</tr>
<tr>
<td>Natural fermentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (90:10)</td>
<td>20.34±0.43 (+24.33)</td>
<td>2.51±0.05 (+20.67)</td>
<td>17.83±0.45 (+24.86)</td>
</tr>
<tr>
<td>Probiotic fermentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (100%)</td>
<td>16.62±0.19</td>
<td>1.50±0.02 (-27.88)</td>
<td>15.11±0.21 (+5.81)</td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (90:10)</td>
<td>20.36±0.25 (+24.45)</td>
<td>2.56±0.04 (+23.08)</td>
<td>17.80±0.24 (+24.65)</td>
</tr>
<tr>
<td>CD (P&lt;0.05)</td>
<td>0.88</td>
<td>1.00</td>
<td>0.91</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Types of dhokla</th>
<th>Calcium</th>
<th>Phosphorous</th>
<th>Potassium</th>
<th>Iron</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal gram flour (100%) Unfermented Control</td>
<td>78.68±0.67</td>
<td>270.33±2.32</td>
<td>835.33±6.59</td>
<td>6.52±0.18</td>
<td>2.89±0.02</td>
</tr>
<tr>
<td>Natural fermentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (100:0)</td>
<td>77.49±0.48</td>
<td>267.67±1.74</td>
<td>834.50±4.93</td>
<td>6.51±0.10</td>
<td>2.85±0.09</td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (90:10)</td>
<td>104.52±1.26 (+32.84)</td>
<td>288.66±0.88 (+6.78)</td>
<td>888.17±8.56 (+6.33)</td>
<td>6.53±0.06</td>
<td>3.27±0.03 (+13.15)</td>
</tr>
<tr>
<td>Probiotic fermentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (100:0)</td>
<td>77.95±0.39</td>
<td>260.83±1.17</td>
<td>835.66±3.06</td>
<td>6.54±0.04</td>
<td>2.88±0.06</td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (90:10)</td>
<td>104.88±0.95 (+33.30)</td>
<td>289.17±1.76 (+6.97)</td>
<td>889.17±9.33 (+6.45)</td>
<td>6.57±0.05</td>
<td>3.29±0.04 (+13.84)</td>
</tr>
<tr>
<td>CD (P&lt;0.05)</td>
<td>2.68</td>
<td>5.27</td>
<td>21.99</td>
<td>NS</td>
<td>0.19</td>
</tr>
</tbody>
</table>

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 dhokla were 37.24, 27.4 and 10.26 per cent, respectively (Table 4.2). The availability of calcium, iron and zinc improved significantly (P<0.05) i.e. 36.55, 25.40 and 44.47 per cent, respectively in naturally and 38.27, 31.97 and 59.93 per cent, respectively in probiotic fermented dhokla, 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 per cent), iron (16.01 and 21.86 per cent) and zinc (37.64 and 42.44 per cent) in naturally and probiotic fermented dhokla, respectively over the unfermented without okara control dhokla. The availability of calcium, iron and zinc was 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 calcium 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)

<table>
<thead>
<tr>
<th>Types of dhokla</th>
<th>Available calcium</th>
<th>Available iron</th>
<th>Available zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal gram flour (100%) Unfermented Control</td>
<td>37.24±0.32</td>
<td>28.84±0.19</td>
<td>28.56±1.79</td>
</tr>
<tr>
<td>Natural fermentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (100:0)</td>
<td>50.85±0.57 (+36.55)</td>
<td>38.56±0.36 (+25.44)</td>
<td>41.26±0.39 (+44.47)</td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (90:10)</td>
<td>48.37±0.30 (+29.89)</td>
<td>35.66±0.19 (+16.01)</td>
<td>39.31±0.33 (+37.64)</td>
</tr>
<tr>
<td>Probiotic fermentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (100:0)</td>
<td>51.49±0.42 (+38.27)</td>
<td>39.88±0.23 (+29.73)</td>
<td>42.82±0.46 (+49.93)</td>
</tr>
<tr>
<td>Bengal gram flour:Okara powder (90:10)</td>
<td>49.27±0.28 (+32.30)</td>
<td>37.46±0.32 (+21.86)</td>
<td>40.68±0.29 (+42.44)</td>
</tr>
<tr>
<td>CD (P&lt;0.05)</td>
<td>1.26</td>
<td>0.88</td>
<td>1.10</td>
</tr>
</tbody>
</table>

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 29.73 and 10.26 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
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 (110.51 130.42 mg gallic acid equivalent/100 g) and probiotic (111.92 and 133.01 mg gallic acid equivalent/100 g) fermented with and with okara (10%) dhokla, respectively over the unfermented without okara 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) [8,9].

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) [31].

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

<table>
<thead>
<tr>
<th>Types of dhokla</th>
<th>Phytic acid (mg/100 g)</th>
<th>In vitro protein digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal gram flour (100%) Unfermented Control</td>
<td>343.67±7.90</td>
<td>63.75±0.45</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Types of dhokla</th>
<th>Total phenolic</th>
<th>DPPH free radical scavenging activity (mg GAE/100 g) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal gram flour (100%) Unfermented Control</td>
<td>85.50±0.54</td>
<td>12.77±0.17</td>
</tr>
</tbody>
</table>

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 (+76.98) | 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) | 16.94 | 1.61 |

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

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