Utilization of Soy Flour and Dehulled Blackgram Flour in the Development of Gluten Free Products

Varsha Rani*, Priyanka Rani and Darshan Punia

Introduction

The term gluten-free product does not refer to the total elimination of gluten rather some residual amount of gluten is allowed. This amount is however strictly regulated by the Codex Alimentarius Standard which recommends that the product not exceeding 20 milligram of gluten per kilogram should be considered a gluten-free product.

In recent years, market for gluten-free products has been increased as the number of patients with celiac disease is growing day by day worldwide and also in India.

Unfortunately the only way out of celiac disease patients is a lifelong elimination of wheat barley, rye, spelt, and kamut or other cereals products which may contain gluten. However, gluten is an essential structure-building protein, contributing to the appearance, crumb structure, and consumer acceptability of many baked products. Never
the less, market survey clearly demonstrated that there is still a major challenge to improve the quality in terms of nutrients, structure, mouthfeel, consumer acceptability and shelf-life of gluten free products (Arendt and Dal Bello, 2008).

Using pulses over gluten free starches can boost the protein, ash, fiber, minerals and vitamins of gluten free products. The high contents of protein in pulses improves the texture of products specially the baked ones. The high content of fibre and moisture in pulses also extends the shelf life of baked products. In last few years various technological efforts have been made to improve the quality of gluten-free products by using flour mixtures (Alvarez-Jubete et al., 2010; Sciarini et al., 2010; Torbica et al., 2010) or non-gluten proteins from egg, milk or soybean (Crockett et al., 2011; Gallagher et al., 2003).

Soybean flour has been an amazing source of protein, unsaturated fatty acids, fiber and calcium. Soy flour can be added up to 50 per cent in any formulation for increasing proteins that lack gluten as it is a leguminous product. In addition to hydrophilic action of soy flour it also inhibits the gluten development while dough preparation; therefore, soy flour should be mixed with other types of flour to get an acceptable volume and structure of bread and other baked products (Sim and Tam, 2001). Soybean proteins isolates and soybean flour have often been used for fortification of bakery products and to improve the protein quality, mechanical behavior and storage life of bakery products (Curic et al., 2007). Dehulled black gram may be considered as another option of increasing nutritional quality of gluten free products.

In gluten-free biscuits the wheat flour needs to be replaced by other ingredients. These ingredients need to replace not only the starch but also the protein fractions. Another advantage in developing gluten-free biscuits is that the formation of a gluten network is not required in a lot of biscuit products. Buckwheat has been used as an important raw material for functional food development because of its functionalities and compounds content, such as proteins, flavonoids and phytosterols (Ötles and Cagindi, 2006). Buckwheat products such as bread, biscuits, snacks, noodles and cookies as well as sprouts have been commercialized and consumed (Hatcher et al., 2008). Keeping in view the above discussed facts and the current demand of market efforts have been made through this research for the development of nutrient dense gluten free snacks.

**Materials and Methods**

**Procurement of material**

The bulk samples of rice, dehulled black gram, soy flour, buck wheat flour, maize flour and other ingredients used in the preparation of different products were purchased from the local market in a single lot. Moisture free and cleaned rice and dehulled black gram were ground to get flour. All the ingredients were stored in the air tight plastic boxes till further use.

**Product development**

Gluten free flour-I was developed using different flour in varying proportions such as rice flour (50%), black gram flour (25%), soy flour (20%) and buckwheat flour (5g) whereas gluten free flour-II was developed using rice flour (80%), maize flour (15%) and buckwheat flour (5g) on fresh weight basis. In both the flours 0.5 per cent of guar gum was also added further to improve the cooking quality of flours. *Matthi, namakpara* and sweet and sweet and salty biscuits were
prepared with gluten free flour-I whereas noodles and macaroni were prepared with Gluten free flour-II, by using standardized recipes.

**Sensory evaluation and shelf life study**

All the products were organoleptically evaluated by 10 celiac patients from Hisar city using employing 9-Point Hedonic Scale. Average of scores for all sensory characteristics, viz., color, appearance, flavor, texture, taste was expressed in terms of overall acceptability.

All the products were stored for a period of two months and these were evaluated for fat acidity, peroxide value and total microbial count at an interval of fifteen days.

**Nutritional evaluation**

Protein, fat, crude fibre, ash were determined by the standard method of AOAC (2000). A factor of 6.25 was applied to convert the amount of nitrogen to crude protein. Carbohydrates were calculated by difference method, whereas energy value was calculated by factorial method using factors of 4, 4 and 9 for protein, carbohydrates and fat, respectively. For the determination of minerals, samples were digested in di-acid mixture. Calcium, iron and zinc were determined by atomic absorption spectrophotometer according to the method of Lindsey and Norwell (1969), whereas phosphorus was determined colorimetrically by the method of Chen et al., 1956. Phytic acid was determined colorimetrically using a spectrophotometer at 465 nm (Davies and Reid, 1979). The amount of phenolic compounds was estimated as tannic acid equivalent according to Folin-denis reagent (Swain and Hills, 1956). β-carotene in sample was separated by column chromatography and estimated colorimetrically (AOAC, 2000).

Gluten content was analysed through hand washing method (AACC, 2000).

**Statistical analysis**

Means of standard deviation of different variables/parameters were calculated. Statistically data were analyzed using SPSS statistical package (version 16.0) for windows (SPSS Inc., Chicago IL, USA).

**Results and Discussion**

The overall mean scores given to sensory characteristics of gluten free products showed that sweet biscuits, sweet and salty biscuits, macaroni, noodles and matthi were adjudged as “liked moderately” to “liked very much” while namakpara was adjudged as liked slightly by the celiac patients. However, all the products were found to be acceptable by the celiac patients (Table 1). The overall acceptability which was the mean score of colour, appearance, aroma, texture and taste of gluten free products decreased with the advancement of storage period of two months. However, all the products were found to be acceptable up to two months by the celiac patients.

The nutrient composition of two types of flour was compared and it was found that gluten free flour-I contained significantly higher ($p<0.05$) contents of protein, crude fibre and energy, iron, zinc and calcium that the gluten free flour-II (Tables 2 and 3). Since the gluten free flour-I was developed using as rice flour (50%), black gram flour (25%), soy flour (20%) and buckwheat flour (5g), the black gram flour and soy flour contributed a fair amount of nutrients. However, the carbohydrates and β-carotene contents were higher ($p<0.05$) in gluten free flour-II since rice is a dominating source of starch and maize had higher content of β-carotene as compared to other flours. When the nutrient
composition of products was compared it was found that the nutrient composition of macaroni and noodles did not differ significantly as the more or less same ingredients were used in their preparation and both of these were extruded. Similarly, the nutrient composition of matthi and namakpara also did not differ significantly as both of these were deep fried products and were having significantly higher contents of fat and energy. Sweet biscuits had slightly higher contents of fat, crude fibre, ash, energy and iron than the sweet and salty biscuits.

Irrespective of extrusion, frying and baking matthi and namakpara had significantly higher amount of nutrients followed by biscuits and noodle and macaroni. In a previous study the influence of soy flour on gluten-free dough rheological properties and bread quality was determined. Soy flour was added at 45, 50 and 60 per cent to yellow maize flour basis. No significant differences in the influence of soy flour on dough rheological properties and bread quality were found between the samples with various added amounts of soy flour (45%, 50%, or 60%) (Vilmane and straumite, 2014). Wójtowicz et al., (2013) showed a good acceptability of buckwheat enriched snacks, at a level of 30 per cent corn-buckwheat incorporated snacks as an attractive type of appetizer with increased nutritional properties. The biscuits enriched with buckwheat had increased spread ration, hardness and fracturability (Filipčev et al., 2011).

With advancement of storage period the values of fat acidity and peroxide value of flour and products were increased significantly. Increase in fat acidity and peroxide value with advancement of storage period might be due to the breakdown of triglycerides and oxidation of unsaturated fatty acid (Table 4).

Total viable count of gluten free flour and products were determined up to two months of storage on three different types of media (LB, PDA and YEPDA) at 30°C under stationary conditions. No total viable count was found in sweet and sweet and salty biscuits at the storage of 15 days whereas it was ranged from 1.0 x10^1 to 5.6 x10^1 cfu/g within matthi, namakpara, noodles, macaroni and gluten free flour being minimum in matthi and maximum in flour and that increased with time. Maximum counts were observed at 45 days in all samples ranged 1.0 x10^4 to 3.3x10^4. Yeast colonies were also observed in samples of noodle, macaroni and gluten free flour at 45 days of storage. Fungi were not detected in any of the samples except in gluten free flour at 45 days of storage period (Table 5).

**Table 1 Consumer acceptability of wheat free (gluten free) products**

|           | Colour | Appearance | Aroma | Texture | Taste | Overall acceptability |
|-----------|--------|------------|-------|---------|-------|-----------------------|
| Noodles   | 8.2±0.2| 7.6±0.2    | 7.0±0.2| 7.1±0.3 | 7.1±0.2| 7.4±0.4               |
| Macaroni  | 8.0±0.3| 7.8±0.4    | 7.6±0.3| 7.2±0.2 | 7.4±0.2| 7.6±03                |
| Sweet biscuit | 8.0±0.2| 8.4±0.2    | 8.4±0.4| 8.0±0.3 | 8.2±0.4| 8.2±0.5               |
| Sweet and salty biscuit | 8.2±0.1| 8.4±0.3    | 8.2±0.3| 8.3±0.4 | 8.4±0.3| 8.3±0.3               |
| Namakpara | 7.0±0.2| 7.0±0.1    | 7.0±0.2| 6.6±0.2 | 6.4±0.3| 6.8±0.2               |
| Matthi    | 7.4±0.1| 7.2±0.1    | 7.4±0.2| 7.0±0.3 | 7.0±0.2| 7.2±0.2               |
| CD (P<0.05) | 0.29   | 0.38       | 0.22  | 0.37    | 0.37  | 0.24                  |

Values are mean ± SD of responses from a panel of 10 judges.
Table 2: Proximate composition of wheat free (gluten free) products (% dry matter basis)

| Products           | Moisture | Protein   | Fat     | Ash     | Crude fiber | Carbohydrates | Energy   |
|--------------------|----------|-----------|---------|---------|-------------|---------------|----------|
| Gluten free flour-I | 4.80 ± 0.2 | 21.00 ± 1.3 | 1.51 ± 0.3 | 1.50 ± 0.2 | 2.60 ± 0.3 | 72.10 ± 1.2 | 378 ± 9.5 |
| Gluten free flour-II | 8.30 ± 0.5 | 10.53 ± 0.7 | 0.60 ± 0.1 | 1.09 ± 0.1 | 1.15 ± 0.2 | 78.56 ± 1.9 | 370 ± 8.2 |
| Noodles            | 6.97 ± 1.2 | 9.27 ± 0.8 | 1.15 ± 0.3 | 0.98 ± 0.2 | 1.94 ± 0.5 | 81.63 ± 2.3 | 374 ± 10.8 |
| Macaroni           | 6.77 ± 1.8 | 9.38 ± 1.5 | 1.10 ± 0.5 | 0.95 ± 0.3 | 1.98 ± 0.8 | 79.82 ± 3.1 | 367 ± 13.5 |
| Sweet biscuit      | 1.80 ± 0.3 | 18.42 ± 1.8 | 21.6 ± 2.2 | 3.05 ± 0.8 | 2.38 ± 0.5 | 55.55 ± 3.8 | 488 ± 14.5 |
| Sweet and salty biscuit | 1.52 ± 0.2 | 19.21 ± 2.2 | 20.8 ± 2.6 | 2.88 ± 0.6 | 2.21 ± 0.4 | 53.41 ± 3.2 | 478 ± 13.9 |
| Namakpara          | 1.33 ± 0.1 | 20.32 ± 2.1 | 22.4 ± 1.9 | 4.03 ± 0.9 | 2.58 ± 0.3 | 51.92 ± 3.1 | 491 ± 14.8 |
| Matthi             | 1.50 ± 0.2 | 20.00 ± 1.7 | 24.6 ± 2.2 | 4.45 ± 0.5 | 2.96 ± 0.5 | 49.45 ± 2.5 | 499 ± 11.5 |

Values are mean ± SD of three independent determinations

Table 3: Micronutrient and anti-nutrient profile of wheat free (gluten free) products

| Products           | Iron (mg/100g) | Zinc (mg/100g) | Calcium (mg/100g) | Phytic acid (mg/100g) | Polyphones (mg/100g) | B-carotene (µg/100g) | Gluten (g/100g) |
|--------------------|----------------|----------------|-------------------|----------------------|---------------------|----------------------|------------------|
| Gluten free flour-I | 4.50 ± 0.2     | 3.20 ± 0.2     | 104.1 ± 2.0       | 695.0 ± 19.97        | 449.0 ± 16.6        | 8.58 ± 1.5          | ND               |
| Gluten free flour-II | 1.54 ± 0.1     | 1.18 ± 0.1     | 25.5 ± 1.9        | 254.7 ± 8.6          | 211.7 ± 6.2         | 15.67 ± 2.1         | ND               |
| Noodles            | 2.50 ± 0.3     | 1.58 ± 0.5     | 95.5 ± 2.2        | 420.3 ± 10.2         | 346.4 ± 5.8         | 28.10 ± 3.6         | ND               |
| Macaroni           | 2.61 ± 0.2     | 1.41 ± 0.4     | 93.6 ± 3.2        | 410.3 ± 8.9          | 350.7 ± 5.2         | 26.53 ± 3.1         | ND               |
| Sweet biscuit      | 1.34 ± 0.2     | 1.15 ± 0.4     | 26.5 ± 2.6        | 204.7 ± 6.3          | 171.5 ± 3.2         | 6.58 ± 1.6          | ND               |
| Sweet and salty biscuit | 1.29 ± 0.1     | 1.18 ± 0.5     | 28.4 ± 2.8        | 184.6 ± 5.4          | 175.4 ± 3.6         | 6.41 ± 1.8          | ND               |
| Namakpara          | 1.60 ± 0.2     | 1.22 ± 0.3     | 22.3 ± 2.4        | 225.3 ± 6.3          | 191.5 ± 4.1         | 8.67 ± 2.1          | ND               |
| Matthi             | 1.54 ± 0.2     | 1.18 ± 0.4     | 27.5 ± 3.1        | 219.7 ± 7.1          | 186.4 ± 3.9         | 10.58 ± 3.2         | ND               |
| CD (P<0.05)        | 1.38           | 1.05           | 4.36              | 6.37                 | 5.68                | 2.26                |                  |

Values are mean ± SD of three independent determinations
Table 4: Fat acidity (mg KOH/100g) and peroxide value (meq/100 g) in wheat free (gluten free) flour and products

| Products                        | 0 days   | 15 days  | 30 days  | 45 days  | 60 days  |
|---------------------------------|----------|----------|----------|----------|----------|
| Gluten free Flour-I             | 10.92 ± 1.2 | 18.26 ± 1.2 | 26.45 ± 2.1 | 35.60 ± 2.4 | 42.75 ± 1.9 |
| Gluten free Flour-II            | 9.61±1.1 | 16.53±2.1 | 24.13±3.5 | 32.25±3.3 | 38.15±3.4 |
| Noodles                         | 11.40 ± 0.9 | 17.59 ± 1.1 | 25.30 ± 1.4 | 38.12 ± 1.5 | 45.60 ± 2.1 |
| Macaroni                        | 12.05 ± 1.1 | 17.80 ± 2.2 | 24.90 ± 2.4 | 36.40 ± 2.4 | 46.12 ± 2.6 |
| Sweet and salty biscuit         | 30.24 ± 2.7 | 42.40 ± 3.2 | 50.00 ± 4.0 | 66.48 ± 2.8 | 72.10 ± 3.0 |
| Sweet biscuit                   | 28.61 ± 2.4 | 37.58 ± 2.2 | 45.40 ± 2.0 | 57.18 ± 3.1 | 60.08 ± 3.2 |
| Namakpara                       | 50.67 ± 2.2 | 62.03 ± 2.3 | 70.80 ± 2.5 | 85.73 ± 3.8 | 92.10 ± 2.7 |
| Matthe                          | 48.54 ± 1.9 | 59.40 ± 2.1 | 67.90 ± 2.3 | 83.10 ± 3.4 | 90.38 ± 3.6 |

Peroxide value (meq/100 g of sample)

| Products                        | 0 days   | 15 days  | 30 days  | 45 days  | 60 days  |
|---------------------------------|----------|----------|----------|----------|----------|
| Gluten free Flour-I             | 0.11 ± 0.01 | 0.28 ± 0.02 | 0.39 ± 0.01 | 0.46 ± 0.01 | 0.58 ± 0.01 |
| Gluten free Flour-II            | 0.09±0.02 | 0.25±0.03 | 0.36±0.03 | 0.41±0.02 | 0.46±0.05 |
| Noodles                         | 0.28 ± 0.01 | 0.46 ± 0.02 | 0.60 ± 0.01 | 0.75 ± 0.01 | 0.88 ± 0.01 |
| Macaroni                        | 0.30 ± 0.01 | 0.44 ± 0.01 | 0.65 ± 0.01 | 0.78 ± 0.01 | 0.85 ± 0.01 |
| Sweet and salty biscuit         | 0.30 ± 0.32 | 0.68 ± 0.42 | 0.89 ± 0.40 | 1.05 ± 0.48 | 1.20 ± 0.50 |
| Sweet biscuit                   | 0.26 ± 0.40 | 0.60 ± 0.50 | 0.85 ± 0.45 | 0.95 ± 0.40 | 1.16 ± 0.50 |
| Namakpara                       | 0.42 ± 0.06 | 0.60 ± 0.08 | 0.90 ± 0.05 | 1.15 ± 0.05 | 1.40 ± 0.04 |
| Matthe                          | 0.35 ± 0.05 | 0.50 ± 0.04 | 0.80 ± 0.05 | 1.06 ± 0.04 | 1.38 ± 0.08 |

Values are mean ± SD of three independent determinations

Table 5: Total microbial count of wheat free (gluten free) flour and products at different storage period

| Products                        | Total viable count (cfu/g) |
|---------------------------------|---------------------------|
|                                | 15 days | 30 days | 45 days |
| Gluten free Flour-I            | 5.6 x10⁴ | 6.3x10³ | 1.3x10⁴ |
| Gluten free Flour-II           | 5.3 x10⁴ | 6.0x10² | 1.2x10⁴ |
| Noodle                         | 1.2 x10⁴ | 1.6x10² | 1.2x10⁴ |
| Macaroni                       | 1.4 x10⁴ | 1.6x10² | 3.2x10⁴ |
| Sweet and salty biscuit        | -       | 1.1x10² | 1.5x10³ |
| Sweet biscuit                  | -       | 1.2x10⁴ | 1.0x10⁴ |
| Namakpara                      | 1.5 x10⁴ | 1.1x10³ | 1.6x10⁴ |
| Matthe                         | 1.0 x10⁴ | 1.0x10² | 3.3x10⁴ |
It may be concluded from this research that soy flour, dehulled black gram flour, maize flour, rice flour and buckwheat flour can be successfully used in the development of gluten free biscuits, noodle, macaroni and fried snacks without affecting the sensory qualities of products along with good shelf life. Addition of soy flour and dehulled black gram flour has improved the nutrient profile of gluten free products specially the protein and mineral contents. All the products were liked and acceptable by the celiac patients. Adding variety to the limited food choice may be the cost effective way to improve the health and nutritional status of celiac patient.

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