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# Nutritional evaluation of multigrain *Khakra*

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ABSTRACT

Multigrain foods are being increasingly considered as an approach to improve the nutritional value of products. *Khakra*, crisp bread from India has huge potential to serve as a nutritional snack beyond local markets. Multigrain *khakra* made using a mix of pearl millet, finger millet, maize, sorghum and whole wheat flour was evaluated for its nutritional qualities. Nutritional aspects based on resistant starch content, fiber content, in vitro protein digestibility and glycemic index were evaluated. It was found that multigrain *khakra* had significantly higher total dietary fiber (2.4 g/100 g) than control whole wheat *khakra* (1.8 g/100 g). It was found that multigrain *khakra* had higher resistant starch (1.2 g/100 g), lower glycemic index (52) and significantly higher protein digestibility (85%) when compared to control whole wheat *khakra* with 0.6 g/100 g resistant starch, 55.2 glycemic index and 70.2% protein digestibility.

## 1. 1. Introduction

Multigrain food products are rich in micronutrients (Ayatse, Eka, & Ifon, 1983; Sahoo, Desai, Kulkarni, Ranveer, & Dandge, 2010) and dietary fibers (Topping, 2007). They are also associated with therapeutic health benefits like retaining gut health, bowel transition, reduction in plasma glucose and lowering cholesterol levels due to their optimum dietary fiber content (Angioloni & Collar, 2011). Multigrain products increased the diversity of fermentable soluble fibers. For example, large amounts of arabinoxylans are provided by pearl millet (Chilkunda and Paramhans, 2002), sorghum (Chilkunda & Paramahans, 2001a, 2001b) and finger millet in *khakra*. *Khakra* is traditional unfermented flat crisp bread. Dietary fibers in multigrain products contribute to improving health in heart patients due to their increased lipid binding properties when compared to single grain products (Angioloni & Collar, 2011). Odes, Lazovski, Stern, and Madar (1993) and Dubois et al. (1993) have reported reduction in lipid levels in subjects who consumed mixed cereal products compared to others.

During processing of starchy foods, the starch molecules undergo several physical modifications depending upon the type of starch and severity of the processing conditions (Goni, Garcia-Dia Manas, & Saura-Calixto, 1996) leading to the formation of resistant starch. The slow digestion of resistant starch has implications for its use in controlled glucose release applications (Sajilata, Singhai, & Kulkarni, 2006) and therefore, a lowered insulin response and greater access to the use of stored fat can be expected (Nugent, 2005). This is clearly important for diabetes and has led to major changes in dietary recommendations for diabetics (Cummings, Edmond, & Magee, 2004).

The whole wheat flour is a rich source of energy 1670 kJ/100 g. Also it is a rich source of macronutrients like vitamin K and the vitamin B complexes (Patel, 2008). The wheat protein gluten is the major factor contributing towards its viscoelasticity and thus affecting its textural properties (Shewry et al., 1999). Maize is a good source of vitamin A, as it contains β-carotene. It is also rich in phytoestrogens, which help in reducing the risks of hormone related cancers such as breast cancer (Ayatse et al., 1983). Studies on maize-wheat blends for formation of extruded products have shown that blends of wheat and maize lead to positive changes in dough and product formation, which is attributed to the zein protein interaction with wheat proteins and strengthening of dough (Yuan et al., 2011). Pearl millet is rich in phosphorous (~650 mg/100 g depending upon the variety) containing 8.5–15% of protein with an average of 11.2% depending on the variety. It is also a good source of fiber (~3.5%). Its calcium content varies from 10 to 80 mg/g, and is also a good source of magnesium (Abdalla, 1998). There are reports which indicate that pearl millet is significantly rich in resistant starch, soluble and insoluble dietary fibers, minerals, and antioxidants (Ragaee et al., 2006). It contains about 92.5% dry matter, 2.1% ash, 2.8% crude fiber, 7.8% crude fat, 13.6% crude protein, and 63.2% starch (Ali et al., 2003). It has been found that the arabinoxylans (soluble fraction of dietary fibers) play a key role in imparting crispness in flat breads (Chikunda & Paramhans, 2002).

Finger millet contains carbohydrates, proteins, sulphur-containing amino acids, minerals like iron and calcium and various phenolic compounds that contribute to the antioxidant properties of the grains

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(Shimray et al., 2012). It contains about 20% fiber, and is also a good source of iron (2.6 mg/100 g), calcium (293 mg/100 g) and zinc (2.4 mg/100 g). It imparts a dark brown color to the product due to its high iron content. It has been found to be a potent source of antioxidants (Chandra et al., 1996). It has been recognized as an appropriate vehicle against zinc deficiencies prevailing in India (Platel & Tripathi, 2010). Finger millet also is known to have several potential health benefits and some of the health benefits are attributed to its polyphenol contents (Chethan & Malleshi, 2007). Sorghum has shown hypoglycemic effects on consumption of *missiroti and dhokla* (Badgujar, Gaikwad, Sonawane, & Arya, 2016; Lakshmi & Vimala, 1996). Also it has been shown to reduce the glycemic index (GI) when incorporated into wheat based flatbreads (Yousif, Nhepera, & Johnson, 2012). It has been found that sorghum contains arabinoxylans that also play a key role in development of textural crispness.

A range of *khakra* is available in the local market in the form of home-made *khakra* with different flavors. The flavors are essentially a mix of traditional Indian flavorings such as panipuri, pav-bhaji. methi, onion-tomato mix, beetroot based, spicy punjabi flavor and, bitter gourd based. These varieties of flavors are contributing to the popularity of *khakra* beyond the population of Gujarat. There are no big brands producing *khakra*, although there are some export oriented local Gujarat brands in this business. However, there is no nutritional data available on the content of multigrain *khakra*. Thus the present study will examine the nutritional content of this functional food.

## 2. Materials and methods

Whole wheat flour (Aashirwaad, ITC, Mumbai, Maharashtra, India), iodized salt (Tata Salts, Mumbai, Maharashtra, India), and grain flours of maize, sorghum, pearl millet and finger millet (Bhagirathi Products, Mumbai, India) were purchased from local grocery stores (Mumbai, India).

a-Amylase from *Aspergillus oryzae* aqueous solution (≥800 FAU/g), and amyloglucosidase from *Aspergillus niger* (6 U/mg) were purchased from Sigma Aldrich (Mumbai, India). Pepsin (3000 U/g) and protease (25 USP/mg) were a gift of Advance Enzyme India Pvt Ltd. (Mumbai, India). Analytical grade chemicals were used for all the evaluations.

### 2.1. Preparation of control and multigrain khakra

Multigrain flour mixes were designed using statistical software Design expert 7.0.0 (Minneapolis, MN, USA). The optimal mixture design was used, where proportions of whole wheat flour were at (60–80%) and other flours i.e., pearl millet, finger millet, sorghum and maize were at levels ranging between 5 to 10% (w/w of total mix) which yielded 25 different combinations. The optimized formulation contained whole wheat flour (70.71%), maize flour (10%), finger millet flour (7.94%), sorghum flour (6.34%) and pearl millet flour (5.01%). To maintain uniform blending; the flours were mixed and passed through a 36 mesh sieve. Into these flour 70% (v/w) water and 2% (w/w) salt were added to form the dough.

To prepare the *khakra* control; whole wheat flour (100 g), salt (2% w/w dry flour) and water (70 ml/100 g dry weight of flour) were taken and formed into dough. The kneading was done for a span of 10 min and dough was divided into balls of 30 g each. A die (16 cm) was used to provide a uniform circular shape to the dough sheet. The sheet was roasted for 40–45 s on the *khakra* roasting machine (Chef Pro FBM208R, 950-Watt Electric Roti Maker, Mumbai, Maharashtra, India). Freshly prepared *khakra* samples were packed in self-sealable polyethylene pouches of 150 gauges (LDPE, 20 × 15 cm, from the local market, Matunga, Mumbai, India) until the nutritional evaluations were done, a maximum of 3 months.

### 2.2. Proximate analysis of multigrain and whole wheat khakra

The proximate composition i.e., moisture (AACC, method 44-15A), fat (AACC, method 30-25), ash (AACC, method 08-01), protein multiplying with a factor of 6.25 (AACC, method 46-13) and carbohydrate content (by difference) of control whole wheat *khakra* and multigrain *khakra* were evaluated (AACC, 2000).

### 2.3. Total, soluble and insoluble dietary fiber in control and multigrain khakra

Total, soluble and insoluble dietary fiber contents were determined using an enzymatic method (Marpalle, Sonawane, & Arya, 2015). The sum of the insoluble dietary fiber and soluble dietary fiber contents were calculated as total dietary fiber. Samples were defatted (AACC, method 30-25) using petroleum ether and oven dried at 60 °C. In 1*g* of *khakra* sample, 40 ml of 0.05 M MES-TRIS blend buffer solution of pH 8.2 was added and mixed using a magnetic stirrer. While stirring at low speed; heat stable α-amylase solution (50 µl) was added. Samples were covered with aluminum foil and incubated for 35 min in a shaking water bath at 95–100 °C. *Khakra* samples were removed and 10 ml water was added and cooled to 60 °C. To this solution, 100 µl of protease solution was added and incubated for 30 min at 60 °C. Samples were removed and pH of solution was adjusted to 4.1- 4.8 with 0.5 N HCl using a pH meter (EUTECH, Mumbai, Maharashtra, India). Then 200 µl of amyloglucosidase solution was added and incubated at 60 °C for 30 min and digested solution was centrifuged at 5000 × *g* (Remi, Thane, Maharashtra, India) for 5 min. The residue was washed twice with hot (70 °C) water (10 ml), and all the supernatants were pooled for determination of soluble dietary fibers (SDF). The insoluble residue was washed with 15 ml 95% ethanol and acetone (twice) and centrifuged at 5000 × *g* for 5 min between washes. The insoluble residue was oven dried at 100–105 °C. The dried residue obtained was evaluated for ash and protein contents. The insoluble dietary fiber (IDF) was calculated as.

Insoluble dietary fiber (IDF) = weight of insoluble residues - weight of ash - weight of protein.

For determination of SDF, 4 times the volume of 95% ethanol was added in the supernatants and kept for 12 h to precipitate the SDF. The supernatant was discarded and the residue was washed twice with 15 ml 95% ethanol and acetone and centrifuged at 5000 × *g* for 5 min. Similar to IDF, residues were oven dried at 100–105 °C. SDF content was calculated as

$$SDF = weight\ of\ insoluble\ residues - weight\ of\ ash - weight\ of\ protein.$$

### 2.4. Determining the resistant starch (RS) content

The RS content of control whole wheat *khakra* and multigrain *khakra* was determined using the method of Kim, Chung, Kang, Kim, and Park (2003), a modification of the AOAC enzymatic-gravimetric method (AOAC, 1990) briefly described below. The fresh insoluble dietary fiber (IDF) residue prior to being dried was obtained from 1g *khakra*. A freshly prepared 2 M KOH solution (6 ml) was added to the IDF residues, mixed thoroughly and shaken continuously for 30 min at room temperature (25 ± 2 °C) on a continuous shaker. Further, 3 ml of acetate buffer (1 M pH 4.2) was added and the pH was adjusted to 4.2 using 2 N HCl. 40 µl of amyloglucosidase was added and the solution mixed and incubated for 30 min at 40 °C, with shaking. Samples were centrifuged for 15 min at 5000 × *g* and the supernatants were collected. The pellet was re-suspended in 10 ml distilled water and the centrifugation repeated. The supernatants were combined with the water washings and adjusted to a final volume of 100 ml with distilled water. Total glucose in the sample was estimated by using the dinitrosalicylic acid (DNSA) method (Miller, 1972) described below.

An aliquot of sample was prepared in 1 ml distilled water. Then 1 ml