Quality parameters and shelf stability of millet based ‘Khakhra’

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Abstract
Millets being rich in nutrients, phytochemicals, antioxidants, dietary fiber, along with a number of health promoting properties, are finding place in the market due to increasing awareness of their goodness among the consumers. Due to lack of information on processing and preparation there has been a very slow rise in its acceptance. Value added products based on millets may mend the gap between consumer acceptance and demand. Hence this study was taken up with an objective to develop khakhra, which are thin crackers, incorporating different millets namely foxtail millet (Setaria italica), finger millet (Eleusine coracana), pearl millet (Pennisetum typhoides) and sorghum (Sorghum bicolor) as variants in wholegrain composite flour. The nutritional composition and shelf-stability was analyzed by using standard protocols. The results revealed that products were high in protein content (11.04-17.54 %), dietary fiber (9.35-13.41 %), antioxidant activity (14,691.88 - 2, 14, 115.53 μmoles equivalent to ascorbic acid/g), total phenols and flavonoid contents. Sensory evaluation showed that finger millet incorporated product had low acceptability compared to the rest of the millets used. The free fatty acid, peroxide value and microbial load were well within safe limits for up to 90 days. In conclusion, a shelf-stable traditional ready-to-eat snack can be developed using different millets which can be used as a functional food.

Keywords: Millets, antioxidants, ready-to-eat, functional foods

1. Introduction
In recent times there has been a tremendous shift in food consumption pattern due to increasing awareness of the how refined flours have an adverse effect on health outcome. Consumers are becoming more health conscious and are getting to know more about what type of food is getting into their system by getting acquainted with the nutritional quality of the products consumed [1, 2]. Hence, traditionally consumed cereals, millets, legumes and wholegrains are gaining popularity among consumers. Perspectives towards foods have changed over the decade and acceptance of wholegrain foods as not just nutritious but also as something which improves health and lowers risk of health problems [3]. Wholegrains are rich in nutrients, antioxidants, phytochemicals and dietary fiber which have protective effects against diseases [4]. Very few of the cereals and millets consumed in India such as whole wheat, finger millet, pearl millet, sorghum and brown rice are considered as wholegrains [5]. Phenolics, flavonoids and anthocyanins present in wholegrain cereals, legumes and millets also act as potential antioxidants [6]. These indicate wholegrains have free radical scavenging activity in human body when consumed on daily basis and hence act as protective agents against major non-communicable diseases. A study done by Siddiq & Prakash, [7] found that the antioxidant components were bound to the fiber fraction of the wholegrain foods which were gradually released during the digestion process making them more available to the body.

However, millet and wholegrain based products from either individual grains or in combination are very few. A study on multi millet ready-to-eat extruded snack based on flour blends of finger millet, pearl millet and foxtail millet was reported [8]. Traditional snacks available in India are much more nutritious than those commonly available in the market [9] and Khakhra is one such traditional ready to eat food which originates from the state of Gujarat, India. Khakhras are very thin crackers, crunchy, consumed either as a snack or breakfast item. Gujarat is famous for this snack of course; in recent times it has gained popularity in other parts of the country as well. As these do not require any further processing during consumption, have a long shelf life and are light to weigh, they serve as popular convenient...
snack to be carried during travelling among Indians. These are uniquely hand-made and roasted products, with different varieties available in the market however, most of them do not possess any additional health benefits. Availability of such snack with addition of millets may enhance to the nutritional value and provide health benefits. Thus, in view of this, the present study made an effort to diversify the utilization of millet and wholegrain based composite flour in the production of khakhra, which were nutritionally rich and possessed health promoting properties. It was further investigated for nutritional composition, antioxidant activity, and shelf-life study.

2. Materials and Methods
The raw materials selected for the study were brown rice (Oryza sativa, Navara), finger millet (Eleusine coracana), pearl millet (Pennisetum typhoides) and sorghum (Sorghum bicolor), foxtail millet(Setaria italica), grain amaranth (Amaranthus cruentus, Rajgeera), soyabean (Glycine max), flaxseeds (Linum usitatissimum) and black cumin (Nigella sativa), spices like turmeric, red chilli powder, carom seeds, cumin seeds and salt. These ingredients were purchased from local certified organic shop in one batch, cleaned of extraneous matter. Grains used for composite flour formulation was individually oven dried at 60°C for 30 mins. Composite flours were prepared by blending ingredients in different proportions namely brown rice at 50%, soyabean 15%, amaranth seeds 8%, while flax and black cumin each at 1%. Four different variations were produced by altering the different millets at 25 % namely pearl millet, finger millet, sorghum, and foxtail millet. Spices were added at 2.5 gm each, while 7.5 gm of salt was added for 100gm of flour used. These ingredients were purchased from local certified organic shop in one batch, cleaned of extraneous matter, grains were oven dried separately and milled. All chemicals used for the study were of analytical grade and purchased from Sd Fine Chemicals, Himedia laboratories, Qualigens Chemicals Ltd., Mumbai, India. Glass double-distilled water was used and all experiments were carried out in triplicate.

2.1 Preparation of flour and product
Khakhras were prepared according to fig 1. Four variations were produced by varying the different millet namely pearl millet /bajra khakhra (BK), finger millet/ragi khakhra (RK), sorghum khakhra (SK), and foxtail millet khakhra (FMK).

2.2 Analysis of nutritional composition
The formulated products were analyzed for moisture by oven drying method, fat by solvent extraction by Soxhlet method, protein by Kjeldahl nitrogen distillation and multiplying the nitrogen value with 6.25, total ash by direct incineration, dietary fiber consisting of both insoluble and soluble fibers was estimated by rapid enzymatic assay method, the carbohydrate content was calculated by difference.

2.3 Analysis of antioxidant activity and Bioactive components
2.3.1. Total antioxidant activity by Phosphomolybdenum method
This assay is based on the formation of a reduced complex MO (V) by the sample in different extraction media at an acidic pH. The results were expressed as μmoles of total antioxidant activity per g of sample.

2.3.2 Analysis of bioactive components
The different extraction media selected for estimation of polyphenols and flavonoids were water, and methanol. A known amount of sample was mixed with extracting media and shaken for 3h which was then filtered through Whatman filter paper No.1. Polyphenols were estimated with Folin–Ciocalteuo reagent and values expressed as mg of tannic acid equivalents/100 g of sample. The flavonoid content was estimated using the Dowd method as adapted by Arvouet-Grand and others was expressed as mg of quercetin equivalents per 100 g of sample.

2.4 Sensory evaluation
All the developed products were evaluated by a semi trained panel (n=20). The products were evaluated for Appearance, Texture, Aroma, Taste and Overall acceptability on a nine-point hedonic scale.
2.5 Shelf-life study
The khakhras were packed in Low-Density Polyethylene (LDPE) and High-Density Polyethylene (HDPE) bags, heat sealed and stored at ambient temperature for a period of 90 days. The storage quality of khakhras were evaluated for moisture content, free fatty acid (FFA), peroxide value (PV) [17] and microbial load (pour plate method) [18].

2.6 Statistical Analysis
Data presented represents means ± standard deviation of triplicate measurements. The significant difference comparisons were done by one way analysis of variance (ANOVA), followed by the T-test in SPSS 27.0 and the statistical significance was defined as P < 0.05. Students “T” test was used to determine the level of significant difference between two different solvents used in the experiments.

3. Results and Discussions
3.1 Nutritional composition
The nutritional composition and mineral contents of four experimental varieties of khakhras are presented in Table 1. The analyzed products with moisture content of 6 - 7% showed nearly 12 ~19% protein content, 11- 14% fat, 10-14% dietary fiber and 49-57% carbohydrates content. Each product variant differed significantly in terms of protein, fat, carbohydrate, total dietary fiber and mineral contents. Higher protein content was found in SK (18.79%) and BK showed a lower content of 11.76%. Similar findings were obtained in a study where extruded snacks were prepared using flour blend [20]. The nutritional composition and mineral contents were found mostly in pearl millet-based product with calcium (127mg), phosphorus (169mg), zinc (2.67) and magnesium (160 mg/100g). Composite flour developed by Bolarinwa and others using malted sorghum and soy flour showed an increase in minerals such as iron, calcium, zinc and phosphorus content upon substitution at varied levels [21].

Table 1: Nutritional composition of khakhras (per 100 g)

| Nutrient composition       | Pearl millet (BK) | Finger millet (RK) | Foxtail millet (FMK) | Sorghum (SK) |
|---------------------------|------------------|--------------------|----------------------|--------------|
| Moisture (%)              | 6.03±0.1       | 6.67±0.26         | 5.77±0.21           | 6.67±0.15    |
| Protein (g)               | 11.05±0.49     | 13.96±0.15        | 14.64±0.08          | 17.54±0.97   |
| Fat (g)                   | 11.94±0.28     | 10.31±0.27        | 13.76±0.36          | 12.03±0.51   |
| Total Ash (g)             | 3.62±0.59      | 3.33±0.31         | 3.59±0.87           | 3.24±0.46    |
| Soluble Dietary Fiber (g) | 0.96±0.01      | 0.99±0.01         | 1.16±0.06           | 1.1±0.18     |
| Insoluble Dietary Fiber (g)| 9.8±0.09      | 12.39±0.08        | 8.22±0.14           | 11.28±0.01   |
| Carbohydrate (g)          | 56.55           | 52.41             | 52.82                | 48.51        |
| Iron (mg)                 | 11.08±0.01     | 7.02±0.01         | 9.14±0.04           | 9.11±0.01    |
| Calcium (mg)              | 119±0.07       | 176±0.01          | 127±0.28            | 126±0.02     |
| Phosphorus (mg)           | 159±0.51       | 164±0.46          | 162±0.71            | 180±1.04     |
| Sodium (mg)               | 116.40±0.44    | 121.30±0.4        | 108.39±0.83         | 116.59±0.43  |
| Zinc (mg)                 | 2.50±0.13      | 3.47±0.4          | 2.74±0.33           | 3.64±0.0     |
| Magnesium (mg)            | 151±0.53       | 178±0.98          | 170±0.53            | 186±1.08     |

Values are mean ± standard deviation. Figures in parenthesis represent values in dry matter basis. Values in different superscripts indicate significant difference among samples on application of Tukey’s Test. NS=Not Significant.

3.2 Antioxidant components and total antioxidant activity
According to recent development in research understanding suggest that the synergistic effect of the fiber along with phenolic acids in wholegrains may contribute to the many health effects [23-26]. Phenolic compounds are well researched due to their bioactive properties and possible health benefits. The antioxidant components (AC) and total antioxidant activity (TAA) of all samples were analyzed in different solvent extracts to understand their potential antioxidant capacity and data are presented in Table 2. There was a wide variation in total phenol content among different types of samples and extraction media. Higher level of total phenols was seen in methanolic extract with BK (237.78mg/100g). Although cereals and millets are not a good source of flavonoids, the prepared products showed a good amount of flavonoids ranging 85.75 to 553.23 mg/100g. This may be due to the presence of nigella sativa and other spices present in the product [27]. Also, a review by Yao and others done to understand effects on flavonoids in food on health showed that cumin and peppermint were good sources of flavonanes namely naringenin and eriodictyol [28]. According to Nambiar and others, flavonoids act as powerful antioxidants exerting greater effects than vitamin C, E, zinc and selenium by preventing oxidized cholesterol formation and preventing from free radical damage [29]. Total antioxidant activity also varied among samples in different extraction medium. With highest activity found in BK [2,14,115.53 μmole equivalent to ascorbic acid/g] following the same trend as in total phenols and flavonoids. In the methanolic extract there was not much difference statistically among the other three samples, yet significant differences from one another was seen among samples in...
water as extraction media. A study done to understand the antioxidant activity in pearl millet cultivars, showed that processing and heat treatments increased the flavonoid content and higher antioxidant activity was observed [30].

Table 2: Antioxidant components and antioxidant activity in different extraction media

| Extraction media | Pearl millet (BK) | Finger millet (RK) | Foxtail millet (FMK) | Sorghum (SK) |
|------------------|------------------|-------------------|---------------------|--------------|
| Methanol         | 237.7±8.17 b     | 163.02±4.37 b     | 159.65±4.84 b       | 180.64±4.6 b |
| Aqueous          | 62.3±2.53 b      | 64.06±1.84 b      | 74.32±4.72 b        | 65.29±5.37 b |

Total Flavonoids (mg quercetin equivalents/100 g)

| Extraction media | Pearl millet (BK) | Finger millet (RK) | Foxtail millet (FMK) | Sorghum (SK) |
|------------------|------------------|-------------------|---------------------|--------------|
| Methanol         | 553.25±3.04 a   | 208.65±2.98 b     | 204.67±13.04 b      | 187.44±4.14 c |
| Aqueous          | 115.54±13.12 b  | 118.19±0.99 a     | 85.72±10.2 b        | 98.64±3.04 a |

Total Antioxidant Activity (μmoles equivalent to ascorbic acid/g)

| Extraction media | Pearl millet (BK) | Finger millet (RK) | Foxtail millet (FMK) | Sorghum (SK) |
|------------------|------------------|-------------------|---------------------|--------------|
| Methanol         | 2.14,115.53±2380.27 a | 1,41,028.51±11331.36 b | 1,22,274.35±203.11 b | 1,20,175.51±2742.66 b |
| Aqueous          | 22.071.67±521.15 b | 14.691.88±293.17 b | 15.423.78±1141.48 b | 17.366.21±902.66 b |

Values are mean ± standard deviation. Values in different superscripts indicate significant difference among samples on application of Tukey’s Test.

3.3 Sensory evaluation

Table 3 depicts the mean organoleptic scores of khakhra for the characteristics of appearance, colour, texture, aroma, taste and overall acceptability. The difference in sensory scores was found to be statistically significant at 5% level. Among the four varieties, SK scored high for appearance (7.70), colour (8.17), texture (7.5), and aroma (7.7). While similar scores were seen in sample BK for taste and aroma as SK. Scores for texture were found to be nearly same among samples FMK and SK. Overall acceptability of the khakhra showed no significant difference among samples FMK, SK and BK while RK was least accepted and was significantly different from the rest of the samples. A study on organoleptic, nutritional characteristics and storage stability of value added khakhra using kodomillet, showed that up to 60% incorporation of kodo millet flour was highly acceptable [21]. While another study on khakhra using barnyard millet, wheat flour, soyabean flour and green gram dal flour, concluded significantly higher (p<0.05) values for all the sensory parameters in 10 % pulse incorporated khakhra [31].

Table 3: Mean Organoleptic Scores of Khakhras

| Sensory quality | BK | RK | FMK | SK |
|-----------------|----|----|-----|----|
| Appearance      | 8.06±0.37 a | 7.15±0.34 b | 8.00±0.62 b | 7.70±0.48 a |
| Colour          | 7.65±0.39 b | 6.85±0.34 b | 7.78±0.44 b | 8.17±0.41 a |
| Texture         | 7.4±0.49 b  | 6.70±0.48 b | 7.50±0.47 b | 7.50±0.41 a |
| Aroma           | 7.70±0.4 a  | 6.80±0.26 b | 7.30±0.42 b | 7.70±0.42 b |
| Taste           | 7.75±0.34 b | 6.75±0.26 b | 7.55±0.5 b  | 7.17±0.33 b |
| Overall Acceptability | 7.30±0.46 a | 6.75±0.26 b | 7.38±0.46 b | 7.44±0.44 a |

Values are mean ± standard deviation. Values in different superscripts indicate significant difference among samples on application of Tukey’s Test (Horizontal rows).

3.4 Peroxide value, free fatty acid and moisture content

Free fatty acids, peroxide value and moisture content of developed products from initial to 90 days of storage in different packaging material were analyzed. These parameters help determine the keeping quality of the developed product. While moisture is one of the important factors responsible for the deterioration of quality of the products during storage, it was found that there was not much difference among the varieties, while very slight difference appeared among the packaging material. Free fatty acid value (FFA) of developed products from initial to 90 days of storage are as seen in the Fig 2. Free fatty acids increased with increase in number of days, it varied nearly about 0.01 to 1.05% in low density polyethylene (LDPE) and 0.01 to 0.99% in high density polyethylene (HDPE). Highest free fatty acid value was seen in sample BK (1.06%) stored in LDPE. Between LDPE and HDPE pouches, BK showed significantly higher values on 90th day, 1.05 and 0.99% respectively. There was no significant difference observed in FFA between the two types of packaging material.
Peroxide value of the developed products from initial to 90 days of storage in different packaging material are as seen in Fig 3. Peroxide value increased with increase in days, it varied from 2.28 to 7.12 in LDPE and 2.28 to 6.4 (Meq O₂/Kg) in HDPE. Significant difference among packaging material on 90 days of storage was seen in sample FMK and BK (P<0.01). However, peroxide values in both the storage material were within acceptable range. Similar values for peroxide value (0.59-7.4 Meq O₂/Kg) were recorded in a shelf-life study of homemade extruded products formulated using pearl millet based composite flour [32].

Table 4. denotes the microbial load of different varieties of khakhra stored in LDPE and HDPE for a duration on 90 days. There was no growth of any kind of microorganisms on initial to 30th day of storage of all samples packed in HDPE. But bacteria started to grow on 60th day in products stored in LDPE. Highest bacterial count was observed at 90 days in sample SK (LDPE) i.e., 86 CFU, which was well within the permissible limits. Significant difference among packaging material was found (P<0.05). However, E. coli was observed to be nil, and a bare minimum count of salmonella was seen. This may be attributed to the fact that khakhra have a low moisture content. Similar findings were observed in a study done on finger millet khakhra [33]. Thus, indicating that the khakhra could be safely stored in LDPE and HDPE for a period of 90 days.
Table 4: Microbial load of stored khakhras

| Organism         | Variation | Duration in days |
|------------------|-----------|-----------------|
|                  |           | 0               | 60           | 90           |
| TBC X10^7 CFU    |           |                 |              |              |
| BK(LDPE)         | 2         | 7.34a           | 81.67a       |
| BK(HDPE)         | 0         | 0               | 34.34b       |
| SK(LDPE)         | 0         | 0               | 36e          |
| SK(HDPE)         | 0         | 0               | 26.34b       |
| RK(LDPE)         | 0         | 11ab            | 82.34*       |
| RK(HDPE)         | 0         | 0               | 35.67b       |
| FMK(LDPE)        | 0         | 14.67ab         | 77*          |
| FMK(HDPE)        | 0         | 0               | 44.34b       |
| E. coli X10^6CFU |           |                 |              |              |
| BK(LDPE)         | 0         | 0               | 0            |
| BK(HDPE)         | 0         | 0               | 0            |
| SK(LDPE)         | 0         | 0               | 0            |
| SK(HDPE)         | 0         | 0               | 0            |
| RK(LDPE)         | 0         | 0               | 0            |
| RK(HDPE)         | 0         | 0               | 0            |
| FMK(LDPE)        | 0         | 0               | 0            |
| FMK(HDPE)        | 0         | 0               | 0            |
| Salmonella X 10^6 CFU |   |                 |              |
| BK(LDPE)         | 0         | 0               | 0            |
| BK(HDPE)         | 0         | 0               | 0            |
| SK(LDPE)         | 0         | 0.5             | 0.34         |
| SK(HDPE)         | 0         | 0               | 0            |
| RK(LDPE)         | 0         | 0               | 0            |
| RK(HDPE)         | 0         | 0               | 0            |
| FMK(LDPE)        | 0         | 0.17            | 0.17         |
| FMK(HDPE)        | 0         | 0               | 0            |

Values are mean values of triplicate determination. Values in different superscripts indicate significant difference among samples on application of Tukey’s Test (column).

4. Conclusion
Millets being nutritionally superior, climate compliant crops can be utilized in development of value added ready to eat products such as khakhra. From this study incorporation of different millets influenced the nutritional composition. Pearl millet incorporated khakhra showed highest antioxidant components and antioxidant activity. The storage quality parameters and microbial load were well within the permissible limit making the product shelf stable for up to 90 days. However, it is necessary to popularize these traditional ready-to-eat products among all sectors of the population and also as a functional food.

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