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Formulation and physico-chemical properties of dietary fiber enhanced low glycemic multi-grain Cracker for adults using locally available cereals and legumes

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Abstract

Crackers are popular among all types of age group including younger and older. They are considered as healthy snacks due to their low levels of salt, sugar and moderate content of fat but they are in low content of Dietary Fiber (DF). Since there is an inverse relationship between intake of DF and risk for developing Non-Communicable Diseases (NCDs) in adults, the present study was conducted with an objective of formulating a cracker with enhanced DF content by incorporating selected locally available whole grain cereals and legumes. Flour composition of a normal cracker formulation (i.e. 100% (W/W) wheat flour) was substituted at 50%, 40% and 30% levels with a multi-grain flour containing Finger Millet (FM), Brown Rice (BR) and legumes; either Green Gram (GG), Chick Pea (CP) or Soya Bean (SB) in different ratios. Since 50% substitution (BR: FM: legume=1.5:1.5:2) was not be able to produce the required rheological properties in the dough, studies were continued with 40% and 30% substitutions. In 40% substitution of wheat flour with the multi-grain flour was only feasible with CP. The formulation of cracker with 40% substitution was repeated at 2:1:1, 1:2:1 and 1:1:2 ratios of BR: FM: CP. The 30% substitution was possible to formulate crackers with all types of selected legumes when the ratios of multi-grain flour had 1:1:1(BR: FM: legume). Proximate composition and the DF content of both raw materials and final products, Glycemic Index (GI; in-vitro) and sensory attributes of formulated crackers were carried out. Of the raw materials, SB had the highest DF content (24.88%) while CP, GG, FM and BR had a DF content of 11.27%, 10.58%, 9.57% and 2.99%, respectively. Of the formulated crackers, the highest DF content of 3.91% was obtained for the 40% substitution where the composition of mixed-grain flour was 1:1:2 in BR: FM: CP (F1) and it was significantly higher than DF of wheat cracker ($p \leq 0.05$). From the formulated crackers, the lowest predicted Glycemic Index of 57.28% was obtained for F1 and it was lower than that obtained for wheat cracker (70.23%). The mean ranks for appearance, color, flavor, crispiness, creaminess, mouth feel and overall acceptability in sensory evaluation obtained by F1 were not significantly different from ranks obtained by the wheat cracker ($p>0.05$). In cracker formulation, 40% of mixed-grain flour was the optimum level of substitution and the cracker thus formulated with 1:1:2 ratio in BR: FM: CP was the best formulation.

Keywords: Cereals, legumes, cracker, dietary fiber, glycemic index.

Introduction

The health sector of Asian countries continues to face challenges from the epidemic of nutrition and diet related NCDs including diabetes, Cardio Vascular Diseases (CVDs), hypertension, obesity, stroke, cancers and gastrointestinal disorders[1]. NCDs have become the leading cause of deaths globally[2] and similarly, it has become the most prominent health issue in Sri Lanka during last two decades[3]. Registrar General's data[4] indicates that NCDs had accounted for 71% of total deaths in Sri Lanka. It is widely believed that the global NCD epidemic is associated with recent lifestyle changes of people, especially inaccurate dietary factors.

A high intake of less fibre carbohydrate diets eventually results in developing NCDs. A generous intake of DF fiber has a protective effect against NCDs and reduces risk of developing NCDs[5,6]. The average DF intake per day of Sri Lankan adults is 18.1g which is less than the recommendation level of 28-36 g per day[7].

DFs are naturally found in the plant sources including vegetables, fruits, legumes and whole grains[8,9]. Cereal grains are important sources of both digestible and unavailable carbohydrates[10]. Presently, the consumption of traditional cereals; millet, sorghum, brown rice has decreased while attracting to more refine wheat and polished rice. Wheat and rice grains have become the global grains and recent years there is a clear shift from coarse grains to more polished grains[11]. Legumes are high in nutrient especially protein than cereal grains. The resistant starch in legumes also behaves like a fiber component and unavailable oligosaccharides present in legumes serve as a probiotic for intestinal micro-flora[12].

Since 1970's, it has been seen that an enhanced awareness of the benefits of DF among the consumers. Consequently, consumer's interest in fiber rich food products had shown to increase. These fiber rich food products include breakfast cereals, laxative beverages and cereal bars with added bran fiber. Simultaneously consumer desired healthy snacks are increasing with diversification of products such as low-fat, low sugar, low-salt and low or no cholesterol.

Although the snack products are highly popular among majority of worldwide population, they are high in carbohydrate and fat thereby contained high calorie value but, low in fiber, vitamin and mineral which make less healthy for daily use especially for adults. Crackers contain little or no sugar, moderate levels of fat and relatively low level of salt and they are good substitutes for deep fried or sweeter snacks[13]. Crackers fall to the type of biscuits which show typical flaky inner layers[14] and having higher demand and acceptability among all age group, longer shelf-life and a better taste. Due to their nature as a snack, they can be used as a source for incorporation of different nutritionally rich ingredients for product diversification and nutritional improvements. Further, they can be modified as a functional food to improve health or well-being by providing benefit beyond that of the traditional nutrients it contains[15,16].

Diversified food products with special features could be prepared by making extensive use of cereals and legumes that are widely available in Sri Lankan local market. Incorporation of legume flour improves the protein quality of cereal based products without causing significant difference in the acceptability of the developed product[17].

GI is a useful nutritional concept that gives links between the physiological effects of carbohydrate-rich foods and human health. The rate of digestion and absorption is influenced by a number of factors including DF, type of starch, protein-starch interaction, anti-nutrients and physical form of food[18]. The extent to which the carbohydrate in a food can raise the blood glucose concentration after ingestion is measured as the GI. The GI is measured calculating the Area Under Curve (AUC) of blood glucose level (glycemic response), following ingestion of a test food containing 50g available carbohydrate and comparing the same with a reference food with equal amount of available carbohydrate[19]. Basically, GI is a ranking of foods based on their glycemic response compared with a reference food either glucose or white bread[20].

The present study was conducted with the aim of formulating a DF enhanced cracker especially for adults by incorporating selected locally available cereal and legume flours.

Materials and methods

Materials: AT-362 rice variety was obtained from the Rice Research Institute, Ambalanthota. FM variety Oshda and legume samples of SB and GG were purchased from Grain Legumes and Oil Seed Crops Research and Development Centre (GLOSCRDC), Angunakolapellessa. CP was purchased from local market.

Refined wheat flour (WF) was purchased from local market in Sri Lanka with brand name of "Prima" (Prima, Ceylon Agro-Industries Limited, Seeduwa, Sri Lanka). Other ingredients of cracker formulation salt, sugar, shortening, instant and dry yeast were purchased from the local market. All the chemicals used were of analytical grade except ethanol used for DF analysis (commercial grade) purchased from SIGMA- ALDRICH CHEMIE GmbH, Steinheim, Germany.

Sample preparation: Legumes seeds and cereal grains were cleaned by removing foreign material using sieves. Immature and damaged seeds were removed by visual inspection. Homogeneous bulk samples of cereals and legumes were taken.

Paddy sample At 362 was de-husked using a roller mill (Type-THU35B, Satake, Japan). Those BR and FM were rinsed several times under running water, drained for 1h, dried at 60^0C for 6h and ground into flour using cross beater mill in the presence of 0.5 mm sieve. The milled samples were sealed in polyethylene bags separately.

CP, SB and GG were rinsed several times under running water, soaked for 12h except GG for 8h while covered with 3 inches of water, drained for 1h, dried at 60^0C. Samples were powdered using cross beater mill in the presence of 0.5mm sieve. The milled samples were sealed in polyethylene bags separately.

Methods: Proximate analysis: Proximate composition analysis was done for the flour samples of raw materials (BR, FM, GG, CP and SB).

Proximate composition of flour samples of BR, CP, GG and SB were carried out according to the methods described in AOAC[21]. Parameters were determined in triplicates. Moisture contents of flours were determined according to the oven drying method[21] applying gravimetric principal. Crude protein content of flours was determined by Kjeldahl method[21] using Kjeldahl heating digestion unit (VELP Scientifica DK 20) and Kjeldahl semi- automatic distillation unit (VELP Scientifica DK 139). Crude fat content was determined by Soxhlet extraction method[21] using Automatic extraction systems Soxtherm (C. GERHARDT GGGH and CO. KG Analytical Systems). Crude fibre content was determined[21] using Fibertec™ M6 Fibre Analysis System (FOSS-1020 HOT EXTRACTOR). Ash content was determined by dry ashing method[21] with gravimetric principal. Total carbohydrate content was determined according to the method described by Sompong et al[22].

Determination of total DF content: Total DF contents of flour samples were determined by the enzymatic gravimetric method as described by Asp et al[23]. In this method, starch and protein are digested with enzymes into small fragments. Ethanol is added to the filtrate to precipitate the soluble fiber and both soluble and insoluble fiber is recovered by filtration.

Cracker formulation: The wheat flour content of typical cracker formulation (i.e. 100% WF) was substituted with cereal and legume flour mixture (multi-grain flour) at 50%, 40% and 30% substitution levels (W/W %) as shown in the Table-1.

Table-1: Flour compositions used in cracker formulation.

Flour Composition (WF: BR: FM: Legume)	Percentage ratio in composite flour
WF: BR: FM: CP/GG/SB (Wheat Flour : Brown Rice : Finger Millet : Chick Pea/ Green Gram/Soya Bean)	50:15:15:20
	60:20:10:10
	60:10:20:10
	60:10:10:20
	70:10:10:10

Note: For single trial of preparation of cracker, only one kind of legume flour portion was taken from each legume flours used either CP or GG or SB. Control sample was prepared from 100% WF.

Using above compositions as shown in Table-1 the possible compositions of cracker formulation were identified.

Preparation of cracker: Crackers were produced under laboratory conditions. Initially dry mixing of composite flour mixture, sugar, salt and instant dry yeast was carried out for ten minutes in the Hobart mixture (Model CE.100, England). Shortening followed by sufficient water were added gradually while continuing the mixing until obtain a non-sticky dough. The dough was kneaded manually for about five minutes to make it soft dough. Allow the dough to rest for one hour after covering it with a plastic bowl.

Laminating dust which is normally used for spread between two layers was prepared using ingredients of WF (100g), shortening (35g) and salt (1.5g) by mixing in a Hobart mixer. Weigh 10% of dough weight and divide it in to two portions.

The dough was sheeted with a rolling pin in one direction up to 1.7mm thickness. Then it was divided in to three parts and half of the dust portion was spread to the middle part as a layer. One part of sheeted dough was folded over it and rest of the dust was sprinkled over folded sheet. Finally other part of dough was folded over it. Sheeting was continued until it reaches 1.7mm thickness. Again it was divided into three parts and folding was done as previously done. It was rotated by 90^o and sheeted to 1.2-1.5mm thickness. Small holes were formed on the sheet using a pin containing roller. Cut into 3×4cm squares and transferred in to a baking tray. Crackers were baked in a pre-heated oven at 180^OC for 20minutes. Crackers were allowed to cool and packaged in suitable pouches in separately according to different formulations with proper labeling.

Determination of *in-vitro* starch digestibility of cracker: *In-vitro* starch digestibility of selected formulated crackers and control samples were determined using the modification of the method described by Thompson et al[24].

The cracker samples were crushed in to coarse powder using motor and pestle. (The particle size should be approximately equal to the size which can be broken down by teeth). Available carbohydrate content of crackers was calculated using the 'by difference' method.

Available carbohydrate content=100 - (% crude protein + % crude fat + % moisture + % ash + % total dietary fiber).

***In-vitro* starch digestion:** Portion of available carbohydrate (1.000g) of the sample, human saliva (5ml) and distilled water (10ml) were placed in to a dialysis bag (13cm length, 4.5cm width, 4.8nm pore diameter with molecular weight cut off: 10,000-12,000 Daltons). Dialysis bags were previously warmed in 40% ethanol solution at 60^oC for 10min and they were stored in 0.001M $NaHCO_3$ solution at 4^oC prior to dialysis. Contents of dialysis tubing were gently massaged to mix. Then dialysis tubing was suspended in 800mL of distilled water at 37^oC with continuous agitation. While agitation, 1mL of dialysate was pipetted into screw cap tubes in triplicates at time intervals 1, 2, and 3h after hydrolysis. Same procedure was followed for white bread which was used as the reference food.

Reducing Sugar Assay: Reducing sugar was assayed in solutions as methods described by Miller[25] and Saqib et al.[26].

1mL of sugar solution from series of standard D-Glucose solutions of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7mg/mL concentrations was added to screw cap tubes containing 4mL of DNS reagent and mixed well. Tubes were placed in boiling water bath for 5min, transferred to crushed ice to cool down rapidly and brought to room temperature by placing them in water bath. Absorbance was measured at 540nm, using spectrophotometer (UV-1601, Japan). Standard curve for standard D-glucose was plotted concentration *vs*. absorption.

1mL of dialysate from *in-vitro* digested samples of developed crackers and control were pipetted out in to a screw cap tube containing 4mL of dinitrosalicylic (DNS) reagent and the same procedure at 1h, 2h and 3h was followed as previously described for standard D-glucose. The reducing sugar content with respect to each absorbance value was obtained from a standard curve which was previously prepared for D-glucose. Hydrolysis curve was plotted with % starch hydrolysis *vs* time for the products.

Estimation of GI: GI calculations were done according to Goni et al.[27] and Germinie et al.[28]. The area under the hydrolysis curves (AUC) of the test food and the reference food were calculated and Hydrolysis Index for products was calculated.

$$\text{Hydrolysis Index (HI)} = \frac{\text{Area under the curve of test food}}{\text{Area under the curve of } reference \text{ food}} \times 100$$

Predicted GI value (GI_{HI}) = 39.71 + (0.549 × HI)

Since above equation is developed for values reported based on the GI of glucose=100, it should be converted to a value based on bread by multiplying by 0.7.

Adjusted predicted GI value = $GI_{HI} \times 0.7$

Sensory evaluation: Sensory evaluation was conducted using 15 trained panelists who were asked to score color, mouth feel, crispiness, flavor, creaminess and overall acceptability on 9 point hedonic scale based on their preferences. Samples were coded with three digits of random numbers and served in random order for the panelists.

Physical properties of Crackers: Physical properties of crackers were determined by method described by Baljeet et al[29].

Diameter: Diameter of crackers was measured by laying six crackers edge to edge, and then the diameter was measured again after rotating by 90^{o}. Average diameter was calculated.

Thickness: Six crackers were stacked on top of each other and thickness was measured. Average thickness was calculated.

Weight: Average weight of four individual crackers was taken as the weight of a cracker.

Spread ratio: Spread ratio was calculated using following equation.

Spread ratio = (Average diameter/Average thickness)

Puffiness: Puffiness of sample was calculated by following equation as described by Nammakuna et al.[30].

% puffiness = (Thickness of baked cracker – Thickness of cracker dough) x 100
Thickness of cracker dough

Statistical analysis: Obtained data were analyzed using Microsoft Office Excel (Microsoft Corporation®) and IBM SPSS 19 (SPSS Institute, Chicago, USA), and MiniTab 14 software. Three replicates were taken in each analysis except for DF (two replicates) and Mean and Standard Deviations were calculated. Difference between the formulations was evaluated by one way analysis of variance (ANOVA) at 5% significant level. Kruskal Walli's test and mean separation technique were used to analyze sensory evaluation results.

Results and discussion

Present requirement has been increased to create an availability of healthy snack with good nutrition profiles and enjoyable sensory perceptions. For this purpose healthy cracker was formulated using local available raw materials of cereals and legumes.

Cereals; BR variety AT-362 and FM variety 'Oshda' and legumes; CP, GG and SB were selected for the study as they are commonly cultivated and local availability in Sri Lankan market. Whole grain cereal and legume based diets are very healthy due to the increased DF content and protein quality of the end product.

According to the present study, it was found that SB contains significantly ($p \leq 0.05$) higher amounts of 33.05 % of protein, 14.5 % of fat, 5.81% of minerals and 24.88% of DF on dry basis where as BR and FM had high carbohydrate content (75.18% and 76.37% respectively). The protein, fat, ash and DF contents were comparatively higher in legumes than cereals. As expected the protein content of pulses was found to be 2-3 folds greater than that of the cereals.

Table-2: Proximate composition of cereal and legume flours.

Commodity	Ash (%)*	Fat (%)*	Protein (%)*	Carbohydrate (%) **	Dietary Fiber (%)*
Finger millet	3.03±0.02^{c}	1.89±0.04^{c}	9.42±0.06^{e}	74.60	9.57±0.22^{c}
Brown rice	1.34±0.01^{d}	2.45±0.02^{c}	10.43±0.11^{d}	73.42	2.99±0.40^{d}
Chick pea	3.09±0.04^{c}	6.85±0.38^{b}	22.54±0.12^{c}	58.29	11.27±0.04^{b}
Mung bean	3.48±0.03^{b}	0.89±0.06^{d}	26.55±0.09^{b}	61.50	10.58±0.13^{c}
Soya bean	5.81±0.04^{a}	14.50±0.43^{a}	33.05±0.07^{a}	41.60	24.88±0.79^{a}

Values are expressed as 'Mean ± SD' of three independent determinations, *Values are given in dry basis, **Calculated by difference, Different superscripts in a column represent significantly difference samples.

The most interested constituent in the present study was the total DF content. Legume contains higher total DF content than the selected cereals. Out of analyzed varieties, SB had the highest total DF content of 24.88% where as the lowest was 2.99% found in BR. There were limited previous studies available for total DF content and majority of studies were done to estimate crude fiber content.

The highest protein and fat content were obtained in SB. For the total mineral content expressed as ash content, 3.03%, 1.34%, 3.09%, 3.48% and 5.81% were obtained for FM, BR, CP, GG and SB respectively.

Exploring the possibility of incorporating novel ingredients in commonly consumed foods with the means of improving functional properties and nutritional profile had become a novel trend in food industry[16]. In composite flour mixtures legume proteins are low in methionine and cysteine (i.e. sulfur containing amino acids) while cereal proteins are deficient in lysine. So the combination may improve the protein quality of end product.

Previous studies on bakery products, composite flour and fiber were utilized for substitution in wheat flour at levels ranging from 5% to 40%[31]. A study done by Sudha et al.[16] reported that preparation of biscuits from different cereals were found to be crispy and palatable at incorporation level of maximum up to 40% with a slight increase in hardness.

Possibility of cracker formulation: The possibility of formation of cracker with different combination of multi-grain flour and wheat flour in the composite flour mixture is summarized in Table-3. The flour compositions of formulated crackers at 30%, 40% and 50% substitution levels are shown in Table-3. In the present study replacement of WF by 50% of multi-grain flour in cracker formulation was not possible due to the inadequacy of gluten protein to form the structure of dough.

Table-3: Possibility of cracker formulation.

Flour compositions (%) WF: BR: FM: Legume	Possibility of forming cracker		
	Chick Pea	Green Gram	Soya Bean
50:15:15:20	Not possible	Not possible	Not possible
60:20:10:10	Possible	Not possible	Not possible
60:10:20:10	Possible	Not possible	Not possible
60:10:10:20	Possible	Not possible	Not possible
70:10:10:10	Possible	Possible	Possible

WF: Wheat Flour BR: Brown Rice FM: Finger Millet.

The formulation of cracker in 40% substitution of multi-grain flour was only possible when incorporation of CP. The reason may be the soluble fiber content present in CP (~5.5%) are high and more capable of absorbing water to form a gel than SB (~3.5%) and GG[33] and thereby contributing to flexible dough structure. At 30% level of substitution, preparation of cracker was possible with all three types of legumes, but practically dough quality was decrease as CP>SB>GG.

Table-4: Flour composition of formulated Crackers.

Formula	Compositions			
	Wheat	Legume	Brown rice	Finger millet
F1	60 :	20 (CP) :	10 :	10
F2	60 :	10 (CP) :	20 :	10
F3	60 :	10 (CP) :	10 :	20
F4	70 :	10 (CP) :	10 :	10
F5	70 :	10 (SB) :	10 :	10
F6	70 :	10 (MB) :	10 :	10
Control	100	-	-	-

CP- Chick Pea SB- Soya Bean GG-Green Gram.

When grain flour substitution was increased in the preparation of cracker, it was difficult to be achieved sheeting and folding due to the low dough strength. Reasons for the reduction of dough strength were lack of gluten content, delaying hydration and gluten development due to increased fiber content. A similar formulation had been done by Eissa et al. 2007[33] explained that legume flours could be incorporated up to 10% level in the formation of biscuits without affecting their sensory quality. Guadagni and Delpha[34] indicated that up to 50% of some legume flours could be added to some products without significant loss in palatability. In the present study, although normal cracker could be produced using 34% of water, in the novel formulations about 41-44% of water had to be used with varying composition.

Proximate composition of crackers: The proximate composition of formulated crackers which made from different compositions of multi-grain flours were compared with the proximate composition of 100% wheat cracker (Table-5). In the nutritional point of view ash, fat, protein and DF contents of crackers are increased with the use of composite flour than the wheat cracker. The moisture content of formulated crackers was lower than 4%, i.e the maximum moisture content that can be present in cracker[35]. The lower moisture content will increase the shelf life of the product. The highest total DF content was in 20% chick pea added cracker at 40% level of substitution (F1). Protein and fat contents of SB incorporated cracker (F5) were the highest amounts which showed significantly different ($P<0.05$) from wheat cracker.

Sensory properties of formulated crackers: The average ranks obtained for appearance, color, mouth feel, crispiness, flavor, creaminess and overall acceptability of formulated crackers are presented in Table-6 and 7. The crackers with two substitutions of 30% (F4, F5 and F6) and 40% (F1, F2 and F3) were separately subjected to sensory evaluation and then selected formulas from those two substitutes were compared with the sensory properties of 100% wheat cracker.

The ranks obtained for F5 sample (Table-6) among all tested attributes in sensory evaluation were the highest than F4 and F6 samples. Therefore F5 was selected as the best sample from sensory evaluation at 30% substitution level.

Table-5: Proximate composition of formulated crackers and wheat cracker.

Formulation	Ash (%)*	Fat (%)*	Protein (%)*	Carbohydrate (%) **	Dietary Fiber (%)*
Control	1.57±0.05^{c}	14.24±0.29^{b}	10.17±0.05^{d}	70.58	1.87±0.50^{c}
F1	2.52±0.01^{b}	14.80±0.18^{b}	11.80±0.14^{b}	67.79	3.91±0.26^{a}
F2	2.46±0.04^{b}	14.36±0.44^{b}	11.16±0.04^{c}	68.51	3.48±0.13^{b}
F3	2.75±0.00^{a}	13.54±0.04^{c}	10.54±0.62^{d}	70.73	3.76±0.22^{a}
F4	1.45±0.08c^{d}	14.51±0.17^{b}	10.98±0.06^{c}	69.37	3.69±0.20^{a}
F5	2.74±0.02^{a}	15.33±0.29^{a}	12.42±0.10^{a}	66.37	3.74±0.47^{a}
F6	2.42±0.02^{b}	13.46±0.10^{c}	11.47±0.07^{c}	68.91	3.63±0.04^{a}

Values are presented as 'Mean ± SD' of three independent determinations. *Values are given in dry basis, **Calculated by difference, ND-Not determined. Different superscripts in a column represent significantly difference samples.

Table-6: Sensory evaluation of 30% substituted crackers.

Sensory attribute	Mean rank of F4	Mean rank of F5	Mean rank of F6	*P* value
Appearance	18.1	33.5	17.4	0.001
Color	19.3	28.4	21.3	0.137
Mouth feel	16.1	31.1	21.8	0.007
Crispiness	17.9	28.4	22.7	0.09
Flavor	14.8	32.0	22.2	0.002
Creaminess	15.7	32.7	20.6	0.001
Overall acceptability	14.8	33.4	20.8	0.000

Table-7: Sensory evaluation of 40% substituted crackers.

Sensory attribute	Mean rank of F1	Mean rank of F2	Mean rank of F3	*P* value
Appearance	36.2	15.3	17.5	0.000
Color	34.3	19.7	15.0	0.000
Mouth feel	32.5	17.3	19.1	0.002
Crispiness	29.0	18.4	21.6	0.077
Flavor	33.5	15.4	20.1	0.000
Creaminess	30.8	15.2	23.0	0.005
Overall acceptability	35.4	15.3	18.3	0.000

The ranks obtained for F1 sample (Table-7) among all tested attributes in sensory evaluation were the highest than F2 and F3 samples. Therefore F1 was selected as the best sample from sensory evaluation at 40% substitution level.

According to the mean separation results, F1 sample was significantly different and highly preferred over F5 in relation to appearance, color, mouth feel, crispiness, flavor, creaminess and over all acceptability (Table-8). Crackers produced substituting wheat flour content with 40% of multi-grain flour in F1 sample (WF: FM: BR: CP: 60:10:10:20) was selected as the best cracker with respect to sensory attributes.

Table-8: Sensory evaluation of F1, F5 and wheat cracker.

Sensory attribute	Mean rank of Control	Mean rank of F1	Mean rank of F5	*P* value
Appearance	27.2	28.8	13.0	0.001
Color	28.6	26.8	13.6	0.003
Mouth feel	29.6	26.8	12.5	0.001
Crispiness	30.8	23.6	14.6	0.003
Flavor	25.9	28.9	14.2	0.005
Creaminess	30.8	26.9	11.3	0.000
Overall acceptability	30.8	27.0	11.2	0.000

According to the mean separation results, the significantly different sample was F5 where no significant different ($p>0.05$) was found among control and F1 samples in relation to all tested sensory attributes. Therefore appearance, color, mouth feel, flavor, creaminess and overall acceptability of F1 sample were not significantly different ($p>0.05$) from control. Thus substitution of wheat flour with multi-grain flour containing ratio of BR: FM: CP at 40% level of substituted crackers had no any adverse effect to the sensory properties with compared to 100% wheat cracker.

It was seen that, in the study of sensory characteristics of crackers, when incorporation of non wheat flour was increased hardness of crackers enhanced than control. The similar changes in hardness of biscuits, cakes and breads with various legume flours addition have been reported[36-39]. The increase in hardness of biscuits might have caused by disruption of the well-defined protein-starch complex of the dough due to reduction in the wheat structure which forming proteins and starch complex. Color characteristic is a major criterion that affects the quality of the food products. The 20% CP incorporated cracker (40% substitution) had the most favorable color which is light yellowish brown color. The color of cracker biscuits was progressively darker as the crackers contained higher levels of CP flour (at 20 and 30 %) with a significant reduction in taste, odor and overall acceptance of final products due to higher intensity of leguminous taste and odor[40]. The color of the top surface of cracker biscuits was generated in the baking process possibly due to non-enzymatic browning (Maillard reactions) between reducing sugars and amino acids and also possibly due to starch dextrination and sugar caramelisation[38].

Predicted Glycemic Index (GI_{HI}): The Predicted Glycemic Index (GI_{HI}) of the tested cracker samples were presented in Table-9.

The lowest starch digestibility was noted in 20% of CP incorporated cracker at 40% substitution level (F1) than all other formulated crackers. F1 cracker was compared with the wheat cracker (control) and white bread as the reference food. According to the results shown in Table 9 formulated crackers had Intermediate GI values where as F1 has the lowest GI value of 57.28%.

In the case of glycemic Index 20% chick pea incorporated cracker (40% substitution) showed the lowest predicted glycemic index (GI), which means that it increases post prandial blood glucose level at a comparatively slower rate over others. High DF, fat and protein contents and low available carbohydrate content may collectively generate this result[27].

Table-9: Predicted Glycemic Index values of samples, control and white bread.

Sample	Area under curve	Hydrolysis Index (%)	Predicted Glycemic Iindex (%)	Adjusted predicted Glycemic Iindex (%)
F1	81.05	76.72	81.83	57.28
F2	101.60	96.17	92.51	64.75
F3	89.85	85.04	86.40	60.48
F4	105.32	100.34	94.12	65.35
F5	93.85	88.83	88.48	61.93
F6	108.55	102.74	96.12	67.28
Control	112.45	106.44	98.14	69.70
White bread	105.65	100	94.61	70.23

The GI is about foods high in carbohydrates. The starches in food decompose in to glucose by digestive enzymes (more precisely alpha-amylase and additionally pancreatic amylase), absorb and enter in to blood stream. Foods high in fat or protein don't cause blood glucose level to raise much. Starch granules are made up of two types of molecular components: amylose and amylopectin. Starches with lower amylose content have higher GI. Inversely, starches with higher amylose content are less susceptible to gelatinization (break down into glucose) which makes for low GI. Cereal starches normally contain 15-28% amylose. Starch in pulses contains much more amylose (33-66%) and less susceptible to digestion. The fiber contains in foods block the amylase action and thereby contributing to reduce glucose absorption. Basically, the fibers directly or indirectly contribute to reducing intestinal glucose absorption[40].

Considering low Glycemic Index (*in-vitro*), high DF content and acceptable sensory results of 20% chick pea incorporated cracker at 40% level of substitution (F1) was selected as the best out of prepared crackers.

Physical properties of crackers: The physical properties of F1 cracker were compared with the physical properties of 100% wheat cracker (control) as shown in Table-10. With regards to the diameter and thickness there was no any significant difference ($p>0.05$) between the F1 and control cracker. There is a significant difference ($p \leq 0.05$) in spread ratio, thickness and puffiness of the two crackers at 5% significant level. Spread ratio and weight were significantly higher in F1 whereas puffiness was significantly lower than the control.

Table-10: Physical properties of F1 and control.

Parameter	F1	Control
Diameter (D) (cm)	5.29 ± 0.05^a	5.35 ± 0.17^a
Thickness (T) (cm)	0.65 ± 0.12^a	0.72 ± 0.20^a
Spread ratio (D/T)	8.14 ± 0.32^a	7.43 ± 0.21^b
Weight (W) (g)	4.01 ± 0.07^a	3.87 ± 0.00^b
Puffiness $(T-T_0)/T_0$	4.42 ± 0.11^b	5.00 ± 0.07^a

Values are 'Mean ± SD' of three independent determinations. Different superscripts in a row represent significantly difference samples.

In preparation of dough, it was difficult to sheet up to the required thickness (~1.2 mm) when non wheat flour was incorporated. Puffing and blister forming ability of wheat cracker were high thus final thickness could be high in control than the formulated crackers. Previous studies[33] showed increasing in thickness and reducing width of crackers supplemented by chickpea flours. It has been observed that addition of chickpea flour at higher levels (20-30%) significantly affected density, width, thickness and spread ratio of final products. Spread ratio was affected by the composition of ingredients and water content of dough. The weight of cracker was high, probably due to the ability of composite flour to retain oil. But the puffiness was low in F1 because of the low gluten content which introduces less extensibility. In general, thickness, width and spread ratio were affected by the increase in the level of non WF in the crackers. As the level of replacement increased above 10 %, it was showed remarkable impact on the thickness of crackers.

Nutritional composition of F1 and control: Nutritional composition of F1 and control was presented in Table-11.

Table-11: Nutritional composition of F1 and control.

Parameter	F1 (per 100 g)	Control (per 100 g)
Moisture /g	3.09^b	3.45^a
Ash /g	2.52^a	1.57^b
Fat /g	14.80^a	14.24^a
Protein /g	11.80^a	10.17^b
Carbohydrate /g	67.79^b	70.58^a
Dietary fiber /g	3.91^a	1.87^b
Energy /kCal Energy/kJ	451.56 1889.33	451.16 1887.65

Different superscripts in a raw represent significantly difference samples.

F1 had significantly higher ($p \leq 0.05$) amounts of ash, protein and DF with compared to control, while moisture and carbohydrate contents were significantly lower in F1 than control (Table-10). There was no significant difference in fat content ($p>0.05$). Thus the incorporation of mix grain flour has increased the nutritional properties and energy value of the cracker significantly. Moreover, F1 cracker contained DF content more than twice present in control.

Conclusion

According to the results obtained, maximum possible incorporation of multi-grain flour (non wheat flour) to cracker formulation was 40% without affecting to the sensory properties. The product with 60% WF, 10% BR,10% FM and 20% CP, had the highest DF content, best textural properties, low GI and it was the most sensory preferable product among the developed products. So it was selected as the best formula (F1) to develop DF enhanced multi-grain cracker.

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