Development of multigrain premixes—its effect on rheological, textural and micro-structural characteristics of dough and quality of biscuits

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Abstract Four different Multigrain Premixes (MGPs) namely MGP I, MGP II, MGP III, MGP IV were developed to select the best premix for preparation of biscuits based on nutritional value and biscuit quality. The MGPs were prepared using cereals (barley, sorghum, maize, oats), pulses (chickpea dhal, green gram, peas, soya flour), millets (pearl millet, finger millet) and wheat germ each at 20 % level. The MGPs developed had 22.91–27.84 % protein, 16.82–18.72 % dietary fiber and 3.11–3.46 % minerals. The wheat flour was replaced with MGPs separately at different levels of 10, 20, 30, 40 and 50 %. The incorporation of these MGPs significantly ($p \leq 0.05$) decreased the water absorption (56.0–50.9 %), peak viscosity (273.67–154.92 RVU), biscuit spread ratio (10.28–8.15) and increased the pasting temperature (67.10–79.20 °C), dough hardness (311.66–460.26 N) and biscuit breaking strength (13.25–28.68 N). SEM studies showed that incorporation of MGP disrupted the protein matrix. Among the MGPs, MGP III was found to be more suitable even at the 40 % level for obtaining nutritious multigrain biscuits with higher protein, dietary fiber, and mineral content.

Keywords Multigrain premix (MGP) · Multigrain biscuit (MGB) · Consistograph · Rapid visco analyzer (RVA) · Texture profile analysis (TPA) · Scanning electron microscope (SEM)

Introduction

Bakery products have become popular in India as evidenced by two fold increase in their production during the last five years. Among all snack foods, biscuits form the most popular snack item and offer certain advantages such as cheaper than conventional snack items, easy to use during travel or at home, because of their availability in varieties of convenient pack sizes and longer shelf life (Crassina et al. 2012). Though, annual per capita consumption of biscuits is quite low (1.8 kg) as compared to 10–15 kg in developed countries, it has almost doubled from 882 g to 1.8 kg during 1998–2012 (Malhotra and Verma 2015). Total annual production of biscuits both in the organized and unorganized sector is estimated to be around 1.2 million tonnes and the growth rate is around 9 %. Nevertheless, it has been reported that normal biscuits are nutritionally deficient as they are low in protein, dietary fiber as well as vitamins and minerals (Jyotsna et al. 2012; Ritika et al. 2012). Several studies have reported on improving the nutritional quality of biscuits by using protein rich ingredients like oil seed meals (Sridevi and Sarojini 2013), pulses (Jyotsna et al. 2012), industrial by-products like wheat germ (Shivani and Sudha 2011) and milk products (Santiago et al. 2013).

The present day scenario requires exploring the possibility of incorporating novel ingredients in commonly consumed foods rather than developing new products to improve the functional characteristics (Aleem et al. 2012). Recently few studies have been reported on the use of multigrain to improve the nutritional quality of bread and other traditional products. The use of multigrain not only improves the various nutritional parameters, but also reported to improve the quality of protein by mutual supplementation of amino acids (Indrani et al. 2010, 2011).

Some of the grains used in the multigrain premixes are cereals, pulses, oil seeds and millets. The cereals like barley

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and oats are excellent sources of soluble and insoluble dietary fibers, particularly beta-glucan, which is reported to lower cholesterol levels (Izydorczyk and Dexter 2008). Pulses like chickpea, green gram, and pea are rich sources of protein and essential amino acids like lysine, arginine, leucine, isoleucine which are limiting in other grains (Indrani et al. 2010). Soya is known for its rich protein content with high levels of lysine and other micronutrients (Aleem et al. 2012) whereas, millets like pearl and finger millet are rich in minerals (calcium, iron, zinc and phosphorus) and a good source of dietary fiber (Anuradha et al. 2010). Wheat germ, a by-product of wheat flour milling industries is a rich source of protein, dietary fiber, vitamin E and B-group vitamins (Amado and Arrigoni 1992).

Literature survey has clearly shown that there are no study reported on the use of multigrain in biscuits, which is consumed largely by the various sections of the population, particularly children. Hence there is a need to develop suitable multigrain premix for use in biscuits to improve its nutritional quality. The effects of these premixes on the rheological, textural, micro structural characteristics as well as biscuit making qualities were studied.

Materials and methods

Raw materials

The different grains like barley (*Hordeum vulgare* L.), Pearl millet (*Pennisetum glaucum*), maize (*Zea mays* L.), oats (*Avena sativa*), sorghum (*Sorghum vulgare*), finger millet (*Eleusine coracana*), chickpea (*Cicer arietinum*), whole green gram (*Phaseolus aureus* Roxb), whole dry pea (*Pisum sativum*), commercially available wheat flour, cane sugar, Marvo brand bakery shortening (Bunge India Pvt. Ltd., Mumbai, India), skimmed milk powder (Nandini brand, Karnataka Milk Federation, Mysore, India), vanilla essence (Bush Boake Allen Ltd., Chennai, India) were procured from the local market in Mysore, Karnataka, India. The grains were cleaned and stored individually in an airtight container. Defatted toasted soya flour (*Glycine max*) was procured from the Sakthi Soya Company, Pollachi, Tamilnadu, India. Wheat (*Triticum aestivum*) germ was procured from the Yamuna Roller Flour Mills Pvt. Ltd., Thrissur, Kerala, India. The Enzymes like termamyl, pepsin and pancreatin were procured from M/s Sigma Chemical Co., (St. Louis, MO, USA). All other chemicals were of analytical grade.

Preparation of multigrain premixes (MGPs)

All the grains were separately milled in an ultra-centrifugal mill (Retsch ZM 200, Germany) using 200 µm sieve. Wheat germ was roasted using an electric roaster by maintaining the temperature at 130 to 160 °C for about 20 min until it turned to

light brown color and cooled prior to milling. Based on preliminary studies, MGPs containing five different grains were selected and combined in the ratio of 1:1, to get maximum benefit of protein and dietary fiber in all the MGPs without adversely affecting the sensory characteristics of biscuits. The compositions of different MGPs are presented in Table 1.

The soya flour is used in all the mixes as a source of protein. The ingredient like oats, barley are used as a source of dietary fiber and finger millet, pearl millet is used as a source of minerals and dietary fiber in two mixes each to find out the effect of each on the quality of biscuits. The different flours were mixed in a Hobart mixer (Model N50, Hobart GmbH, Offenburg, Germany) for about 10 min and sieved through 200 µm sieve to get homogenous mixture. The MGPs were packed in an airtight container and stored at 4 °C for further use.

Color analysis of MGPs

The color values of different MGPs were measured using a Hunter color meter (Hunter Associates Laboratory, Inc., Reston, Virginia) with standard D65 day light illuminate and 10° view angle. A higher L* value indicates a brighter or whiter sample. The positive a* value indicates redness and the negative a* value indicates greenness. The positive b* value indicates yellowness and the negative b* value indicates blueness. Numerical values of a* and b* were calculated into hue angle (h°) and chroma value (C*) as reported by Nielsen (1998) by using the following formulas and studies were carried out for sample in triplicates.

$$h^{\circ} = \tan^{-1}(b^{*}/a^{*}); \quad C^{*} = \sqrt{(a^{*})^2 + (b^{*})^2}$$

Proximate composition

The moisture (method 44-16), protein (method 46-10), fat (method 30-10), ash (method 08-01) were analyzed based on AACC (2000) procedures. The soluble and insoluble dietary fibers were estimated according to the methods of Asp et al. (1983). An average of three independent determinations was recorded.

Table 1 MGP formulations

MGP I	MGP II	MGP III	MGP IV
Finger millet	Pearl millet	Barley	Pearl millet
Oats	Finger millet	Sorghum	Barley
Chickpea	Green gram	Chickpea	Maize
Wheat germ	Oats	Pea	Green gram
Defatted soya	Defatted soya	Defatted soya	Defatted soya

MGP multigrain premix, each ingredient at 20 % level

Rheological characteristics of wheat flour

Consistograph characteristics

The effect of incorporation of MGPs on the rheological behavior of wheat flour was determined using Consistograph (Chopin Technologies, Villeneuve-la-Garenne Cedex, France). Consistographic characteristics like (i) water absorption capacity, (ii) dough development time (TprMax), the time to reach maximum consistency with a maximum pressure of 2200 mb (millibar), (iii) maximum tolerance index (Tol), time elapsed from maximum to 20 % of the dough consistency peak and (iv) the extent of decay in pressure, the difference in mb in the dough consistency peak height in 250 s (D250) and 450 s (D450) were determined according to AACC (2000) method 54–50. An average of three independent determinations was recorded.

Pasting characteristics

The pasting characteristics of wheat flour were studied using the Rapid Visco Analyzer (Newport Scientific Pvt. Ltd., Warier Wood, Australia) according to AACC (2000) method 76–21 with modifications. The total program was run for 13 min starting at 50 °C, and heated to 95 °C at a constant rise of 12 °C/min, holding the temperature at 95 °C for 2.5 min and then cooling the system to 50 °C at 12 °C/min for 3 min. The viscosity parameters measured were pasting temperature, peak viscosity, breakdown viscosity, final viscosity and set-back viscosity. An average of three independent determinations was recorded.

Preparation of biscuits

Biscuits were prepared using multigrain premixes according to the AACC (2000) method 10–52 with modification made by Kumar et al. (2015). The biscuits in three replicates were prepared according to following standardized formula.

Wheat flour 300 g, pulverized sugar 105 g, bakery shortening 60 g, sodium chloride 1.5 g, sodium bicarbonate 1.5 g, ammonium bicarbonate 3 g, skimmed milk powder 6 g, dextrose 6 g, vanilla essence 3 ml. The wheat flour was replaced by all the four MGPs separately at 0, 10, 20, 30, 40 and 50 % level respectively.

The method of preparation was as follows: Sugar, bakery shortening, skimmed milk powder, dextrose and vanilla flavor were creamed in Hobart mixer with a flat blade, for 5 min at 61 rpm and to the cream, water containing dissolved sodium chloride, sodium bicarbonate and ammonium bicarbonate were separately added and mixed for 5 min at 125 rpm until homogenous cream. Finally, sifted wheat flour was added and mixed at 61 rpm for 2 min. The dough was sheeted to 3.5 mm thickness using a metal frame of 3.5 mm thickness and cut into

round shape of 55 mm diameter using circular cutter. The baking was done at 200 °C for 10 min. The biscuits were cooled and stored in airtight container.

Texture profile analysis of biscuit dough

The texture of biscuit dough's from all the four mixes were measured with texture profile analysis (TPA) method of Bourne (1978) using a texture analyzer (TA- HD plus, Stable Micro Systems, Surrey, U.K). The hardness, springiness and gumminess of the biscuit dough were measured with 5 Kg load cell. The following conditions were used: biscuit dough thickness, 10 mm (circular disc); diameter, 40 mm; circular probe - 80 mm diameter; crosshead speed, 50 mm/min; compression - 50 % of dough height. The data were collected for three replicates and the mean value was reported.

Quality characteristics of biscuits

Physical characteristics

The diameter (D) and thickness (T) in mm of biscuit were measured by placing six biscuits edge to edge and placing one above the other respectively. The biscuits were rearranged and restacked to get average diameter and thickness. Mean weight of the two biscuits was noted and spread ratio (D/T) of biscuits was calculated.

Texture measurement

The breaking strength of biscuit was determined by texture analyzer using triple beam snap (three point break) techniques as per method described by Gains (1991). The peak force from the resulting curve indicated as the breaking strength of biscuits. The mean of three independent determinations was reported.

Sensory evaluation

Sensory quality of multigrain biscuits (MGBs) were evaluated by 20 panelists of age between 25 and 50 years, including both male and female, who had earlier experience in quality evaluation of bakery products. They were further oriented in four sessions, including two hours of training in each session. Six samples, including control biscuits were evaluated in triplicates by each panelist for crust color, surface character, crumb color, crumb texture, taste, mouth feel and overall acceptability on a 9 point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely) according to the method of Larmond (1997). The samples were identified by code numbers and presented in a random order to the panelist.

Micro structural changes of dough and biscuits

Micro structural studies of dough and biscuits were carried out using scanning electron microscope (SEM) (EVO LS10 SEM, Zeiss, UK). The sample preparation for the study was carried out according to the method of Indrani et al. (2010) with slight modification. The dough was thinly sheeted (thickness 0.5 mm) and cut into 20 × 20 mm size pieces without damaging the structure. The dough pieces and biscuits were defatted using hexane to remove fat and freeze dried. The freeze dried samples were separately sputter coated with gold-palladium alloy and the morphological analysis was carried out at high vacuum using an operating voltage of 10 kV.

Nutritional composition of biscuits

The protein, dietary fiber and mineral content of control and selected experimental biscuits were analyzed using the same method as mentioned earlier.

Statistical analysis

All the experiments were carried out in triplicates and data was statistically analyzed using Duncan's new multiple range tests (DMRT) with different experimental group using statistica software version 7.0 of Stat Soft Incorporation, Tulsa, OK, USA as per the method of Steel and Torrie (1960). The significant level was established at $P \leq 0.05$.

Results and discussion

Color analysis of MGPs

The color values of MGPs are shown in Table 2. There is a significant ($p \leq 0.05$) decrease in lightness value in all the MGPs as compared to wheat flour and found to be 83.19, 79.13, 80.09, 83.8 and 93.55 for MGP I, II, III, IV and wheat flour respectively. The reduction in the lightness (L^*) value resulted in an increase in redness (a^*) value. The decrease in L^* and increase in a^* may be attributed to the presence of dark colored flours like finger millet, pearl millet, wheat germ, etc. The significantly ($p \leq 0.05$) higher increase in redness value in case of MGP I may be due to the presence of wheat germ. The MGP IV had significantly ($p \leq 0.05$) higher positive yellowness (b^*) value of 17.28, may be due to the presence of maize. The chroma which is the measure of purity or intensity of color increased from 10.04 of wheat flour to 16.52, 15.29, 14.78, and 17.31 for MGP I, II, III and IV respectively.

Proximate composition of MGPs

The proximate composition of MGPs is shown in Table 2 and there is a significant ($p \leq 0.05$) decrease in moisture content of all the MGPs (7.27–8.13 %) when compared to wheat flour (12.9 %) which may be due to lower moisture of grains and also moisture loss during milling. The ash content values of MGPs are found to be significantly ($p \leq 0.05$) higher as compared to wheat flour may be due to the use of whole grain flours in the preparation of MGPs. Earlier studies also indicated high ash content in multigrain mixes (Indrani et al. 2010). The wheat flour used in the study contains dry gluten of 9.9 % showing medium hard nature of wheat used for milling. The protein content was found to be higher in MGPs than wheat flour due to the presence of pulses and oil seeds. Among the MGPs, MGP III had highest protein content of 26.28 %. From the Table 2, it is observed that MGP I contained significantly ($p \leq 0.05$) higher fat content than other premixes due to the presence of wheat germ (Shivani and Sudha 2011). The total dietary fiber in MGPs ranged from 16.82 in MGP IV – 18.72 % in the case of MGP I, which is almost 5 to 6 fold higher than wheat flour of 2.93 %, while the soluble dietary fiber ranged from 5.3 in MGP I – 7.4 % in the case of MGP III. The high soluble dietary fiber is due to the presence of either barley or oats in the premix (Izydorczyk and Dexte 2008).

Effect of incorporation of MGPs on rheological characteristics of wheat flour

Consistograph characteristics

The effect of incorporation of MGPs on the rheological characteristics of wheat flour is represented in Table 3. The water absorption capacity significantly ($p \leq 0.05$) decreased from 56.0 in control sample to 50.9 % in the case of MGP II at 50 % of replacement. The reduction in water absorption capacity is attributed to dilution of gluten (Hoseney 1994). There is no drastic increase in dough development time (TprMax) up to 30 % level of incorporation. However, beyond that level, there is a significant ($p \leq 0.05$) increase in TprMax. Earlier, Callejo et al. (2009) also observed an increase in the TprMax while using rye meals in wheat flour.

Maximum tolerance index (Tol), a measure of stability of the dough, increased up to 20 % addition of MGPs and thereafter further addition decreased the tolerance to mixing. Gunathilake et al. (2009), also observed similar results by adding defatted coconut flour to wheat flour and correlated the data to the stabilization of the gluten structure of the dough by coconut protein. Similarly, there could be a stabilization of wheat protein with other proteins present in MGP. The Decay in pressure at 250 s (D250) and 450 s (D450) is greater at any levels of addition of MGP. The decay was decreased consistently as the level of incorporation of MGP increased. Similar

Table 2 Proximate composition and color values of MGPs

Parameter	Wheat flour	MGP I	MGP II	MGP III	MGP IV
Moisture %	12.96 ± 0.1 ^a	7.73 ± 0.06 ^b	7.42 ± 0.05 ^c	8.13 ± 0.09 ^d	7.27 ± 0.06 ^c
Ash %	0.58 ± 0.09 ^a	3.35 ± 0.1 ^b	3.23 ± 0.007 ^{ab}	3.46 ± 0.007 ^b	3.11 ± 0.04 ^c
Total protein %	10.91 ± 0.18 ^a	27.84 ± 0.24 ^b	23.96 ± 0.16 ^c	26.28 ± 0.18 ^d	22.91 ± 0.22 ^e
Dry gluten %	9.94 ± 0.28	—	—	—	—
Total fat %	1.33 ± 0.1 ^a	4.69 ± 0.15 ^b	3.49 ± 0.06 ^c	3.18 ± 0.18 ^d	2.91 ± 0.16 ^d
Total dietary fiber %	2.93 ± 0.24 ^a	18.72 ± 0.39 ^b	18.68 ± 0.22 ^b	17.51 ± 0.03 ^c	16.82 ± 0.18 ^d
Insoluble dietary fiber %	2.32 ± 0.23 ^a	13.41 ± 0.08 ^b	12.53 ± 0.37 ^c	10.13 ± 0.08 ^d	10.03 ± 0.19 ^d
Soluble dietary fiber %	0.62 ± 0.01 ^a	5.31 ± 0.31 ^b	6.51 ± 0.59 ^c	7.38 ± 0.6 ^d	6.79 ± 0.01 ^c
Carbohydrate %	74.22 ± 0.37 ^a	56.39 ± 0.42 ^b	61.9 ± 0.16 ^c	58.95 ± 0.22 ^d	63.8 ± 0.13 ^c
Energy kcal	352.49 ± 0.54 ^a	379.13 ± 0.61 ^b	374.85 ± 0.78 ^c	369.54 ± 0.39 ^d	373.03 ± 0.4 ^c
L*	93.55 ± 0.14 ^a	83.19 ± 0.06 ^b	79.13 ± 0.19 ^c	89.09 ± 0.26 ^d	83.8 ± 0.27 ^b
a*	0.47 ± 0.01 ^a	2.12 ± 0.05 ^b	1.54 ± 0.04 ^c	1.21 ± 0.01 ^d	1.11 ± 0.02 ^c
b*	10.03 ± 0.11 ^a	16.38 ± 0.15 ^b	15.21 ± 0.16 ^c	14.74 ± 0.27 ^c	17.28 ± 0.19 ^d
dE	10.33 ± 0.06 ^a	12.27 ± 0.02 ^b	15.11 ± 0.18 ^c	9.07 ± 0.21 ^d	12.16 ± 0.28 ^b
Hue angle (h°)	87.32 ± 0.01 ^a	82.62 ± 0.13 ^b	84.23 ± 0.21 ^c	86.91 ± 0.09 ^a	86.38 ± 0.05 ^d
Chroma	10.04 ± 0.11 ^a	16.52 ± 0.1 ^b	15.29 ± 0.15 ^c	14.78 ± 0.27 ^c	17.31 ± 0.19 ^d

Values are mean ± standard deviation of three independent determinations. ^a Mean values in the same column within the mix (I, II, III, IV) followed by different superscripts differ significantly ($P \leq 0.05$)

MGPs multigrain premix; L* lightness/darkness; a* redness/blueness; b* yellowness/greenness; dE color difference

observations were made by Callejo et al. (2009) when incorporating rye flour to wheat flour.

Pasting characteristics

The pasting properties of wheat flour as affected by the incorporation of MGPs are shown in Table 4. The pasting temperature significantly ($p \leq 0.05$) increased from 67.10 °C in control sample to 79.20 °C in the case of MGP IV sample at 50 % of replacement. The increase is minimum in MGP III and maximum in MGP IV. This variation could be attributed to the different grains used in MGPs which have different pasting temperatures (Sanaa and El-syed, 2006). Earlier, Khan et al. (2015) also reported increase in pasting temperature with an increase in the level of non-wheat flour due to higher resistance of starch granules to swell. The peak viscosity decreased with the increase in the level of incorporation of MGP. The extent of decrease varied with the type of MGP used. Similar observations were also made by Hamaker and Griffin (1993) while studying the effect of protein on rice starch gelatinization. Breakdown viscosity showed a decreasing trend from 128.83 RVU in control sample to 82.33, 73.67, 65.58, and 63.75 RVU for MGP I, MGP II, MGP III and MGP IV, respectively as the level of incorporation of MGP increased from 0 to 50 %. Final viscosity, which indicate the ability of starch to form a gel on cooling decreased with the increase in the level of incorporation of MGPs. Set back viscosity, a measure of aggregation of amylose fractions leading to the reinforcement of swollen granules and fragments by bonds

between and within them also decreased with the increase in the incorporation of MGP from 0 to 50 % (Khan et al. 2015). A representative graph of RVA at the 30 % level of incorporation of MGPs is depicted in Fig. 1.

Texture profile analysis of biscuit dough

The texture profile analysis of biscuit dough showed that with the increase in the addition of 10–50 % MGP to control biscuit dough, the hardness value increased significantly ($p \leq 0.05$) irrespective of type of MGP confirming that, the dough with MGP is harder than the control biscuit dough. In general, the hardness values were higher for MGP I and II as compared to III and IV at comparable levels. The higher increase in hardness with MGP I and II could be attributed to the presence of finger millet flour in the premix (Crassina et al. 2012). Springiness is the elastic recovery property of dough after removal of first deforming force decreased from 0.21 mm observed for control dough to 0.15 mm in case of IV at 50 % of replacement. A similar reduction in springiness property of biscuit dough by the addition of fiber and protein rich Moringa leaves was reported by Dachana et al. (2010). Gumminess drastically reduced from 52.21 N in control sample to 32.21, 26.09, 24.64 and 23.65 N for MGP I, II, III and IV respectively with an increase in the level of MGPs. Earlier, Nirmala et al. (2011) also reported a reduction in springiness and gumminess value of cookie

Table 3 Effect of incorporation of MGPs on consistograph characteristics of wheat flour and texture profile analysis of biscuit dough's

MGPs	Level (%)	WAC (%)	TPrMax (s)	Tol (s)	D250 (mb)	D450 (mb)	Hardness (N)	Springiness (mm)	Gumminess (N)
MGP I	0	56.0 ^a	180 ^b	332 ^b	59 ^d	423 ^d	311.66 ^d	0.21 ^a	52.21 ^a
	10	54.4 ^b	182 ^b	398 ^a	520 ^a	1002 ^a	403.00 ^b	0.20 ^{ab}	50.36 ^{ab}
	20	53.9 ^{bc}	189 ^{ab}	348 ^b	322 ^b	772 ^b	413.08 ^a	0.19 ^{abc}	48.44 ^b
	30	53.3 ^{bc}	191 ^{ab}	204 ^c	187 ^c	586 ^c	417.72 ^a	0.18 ^{bc}	45.22 ^{bc}
	40	52.9 ^{cd}	272 ^c	174 ^{cd}	157 ^c	206 ^e	419.29 ^a	0.17 ^c	34.88 ^c
	50	51.9 ^d	297 ^a	160 ^d	72 ^d	92 ^f	371.45 ^c	0.17 ^c	32.21 ^c
MGP II	0	56.0 ^a	180 ^b	332 ^b	59 ^d	423 ^d	311.66 ^e	0.21 ^a	52.21 ^a
	10	53.8 ^b	183 ^b	358 ^a	470 ^a	891 ^a	439.77 ^b	0.21 ^a	51.47 ^a
	20	53.3 ^c	192 ^{ab}	337 ^{ab}	338 ^b	774 ^b	446.93 ^b	0.20 ^a	47.78 ^b
	30	52.6 ^d	209 ^c	288 ^c	279 ^c	454 ^c	460.26 ^a	0.19 ^a	40.29 ^c
	40	51.9 ^e	272 ^d	208 ^d	278 ^c	324 ^e	398.22 ^c	0.19 ^a	32.81 ^{cd}
	50	50.9 ^f	309 ^e	103 ^e	185 ^e	269 ^f	372.71 ^d	0.18 ^a	26.09 ^d
MGP III	0	56.0 ^a	180 ^e	332 ^b	59 ^d	423 ^e	311.66 ^e	0.21 ^a	52.21 ^a
	10	55.4 ^b	181 ^e	388 ^a	384 ^a	844 ^a	319.65 ^d	0.21 ^a	36.86 ^b
	20	54.1 ^c	186 ^d	378 ^{ab}	372 ^a	746 ^b	339.69 ^b	0.18 ^b	31.81 ^{bc}
	30	53.6 ^d	193 ^c	280 ^c	313 ^b	716 ^c	358.47 ^a	0.17 ^b	28.73 ^c
	40	53.2 ^e	213 ^b	261 ^d	252 ^c	644 ^d	345.66 ^b	0.16 ^b	27.78 ^c
	50	52.8 ^f	227 ^a	246 ^d	121 ^e	300 ^f	330.76 ^c	0.16 ^b	24.64 ^d
MGP IV	0	56.0 ^a	180 ^f	332 ^b	59 ^d	423 ^d	311.66 ^e	0.21 ^a	52.21 ^a
	10	55.3 ^b	187 ^e	340 ^b	325 ^a	776 ^a	368.66 ^b	0.21 ^a	36.53 ^b
	20	54.8 ^c	197 ^d	300 ^a	292 ^{ab}	539 ^b	382.88 ^a	0.19 ^{ab}	31.32 ^c
	30	54.6 ^{cd}	206 ^c	246 ^{ab}	188 ^b	506 ^c	385.75 ^a	0.19 ^{ab}	24.44 ^{cd}
	40	54.3 ^d	238 ^b	220 ^c	185 ^b	279 ^e	357.87 ^c	0.17 ^{bc}	25.48 ^d
	50	52.8 ^e	259 ^a	115 ^d	133 ^c	162 ^f	325.04 ^d	0.15 ^c	23.65 ^{cd}

Mean values in the same column within the mix (I, II III, IV) followed by different superscripts differ significantly ($P \leq 0.05$)

MGPs multigrain premix; WAC water absorption capacity; TPrMax time in seconds to reach maximum pressure; Tol maximum tolerance index; D250 decay in 250 s; D450 decay in 450 s

dough with the addition of fenugreek and flax seed powder and attributed this to dilution of gluten.

Effect of MGPs on the quality of multigrain biscuits (MGBs)

Color

The surface color values of different MGBs are represented in Table 5. The lightness (L^*) value indicating whiteness of the product decreased from 67.95 in control biscuits to 55.0, 57.78, 59.18 and 60.03 for MGB I, II, III and IV respectively with the addition of MGPs. A similar trend was observed by Shivani and Sudha (2011) for wheat germ based biscuits. The redness (a^*) value of the biscuits increased and yellowness (b^*) value decreased significantly ($p \leq 0.05$) as the level of MGP increased. The increase in redness value of biscuits could be attributed to the presence of high protein in MGPs which resulted in browning due to the Maillard reaction. The chroma and hue angle values which are direct

indicators of color intensity decreased with increase the level of incorporation of MGPs.

Physical characteristics

The biscuits prepared using 0, 10, 20, 30, 40 and 50 % level of incorporation of all the four MGPs separately are evaluated for various physical parameters (Table 5). The weight of the biscuits increased with the addition of MGP due to increase in the density of biscuits (Francine et al. 2011). The diameter of biscuits decreased and thickness increased with the addition of MGP irrespective of the type. This is reflected in the values of the spread ratio, which decreased from 10.28 in control sample to 8.15, 8.55, 9.11 and 8.72 in the case of MGP I, II, III and IV respectively. This is may be due to dilution gluten and disruption of gluten protein matrix. These results are in agreement with earlier studies carried out by Jyotsna et al. (2012) for green gram incorporated biscuits and Crassina et al. (2012) for finger millet flour biscuits.

Table 4 Effect of incorporation of MGPs on pasting characteristics of wheat flour

MGP	Level (%)	Pasting temperature (°C)	Peak viscosity (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Set back viscosity (RVU)
MGP I	0	67.10 ^f	273.67 ^a	128.83 ^a	281.58 ^a	136.75 ^a
	10	67.85 ^e	245.92 ^b	101.08 ^b	274.08 ^b	127.41 ^b
	20	69.35 ^d	235.92 ^c	99.25 ^c	269.00 ^c	123.67 ^c
	30	71.85 ^c	234.67 ^c	98.25 ^d	256.83 ^d	123.25 ^c
	40	76.80 ^b	218.25 ^d	90.58 ^e	240.42 ^e	120.42 ^d
	50	77.70 ^a	201.50 ^e	82.33 ^f	237.92 ^f	118.75 ^e
MGP II	0	67.10 ^e	273.67 ^a	128.83 ^a	281.58 ^a	136.75 ^a
	10	67.05 ^e	241.00 ^b	107.42 ^b	272.00 ^b	138.42 ^a
	20	69.35 ^d	221.83 ^c	98.75 ^c	260.50 ^c	137.42 ^a
	30	75.10 ^c	204.58 ^d	93.58 ^d	251.17 ^d	135.17 ^a
	40	77.50 ^b	182.50 ^e	88.08 ^e	240.92 ^e	130.00 ^b
	50	78.20 ^a	166.58 ^f	73.67 ^f	230.18 ^e	129.26 ^b
MGP III	0	67.10 ^e	273.67 ^a	128.83 ^a	281.58 ^a	136.75 ^a
	10	67.10 ^e	279.67 ^b	125.50 ^b	290.42 ^a	136.25 ^a
	20	68.70 ^d	240.00 ^c	113.87 ^c	249.67 ^c	123.50 ^b
	30	71.90 ^c	220.58 ^d	96.67 ^d	234.17 ^d	121.25 ^c
	40	74.35 ^b	179.83 ^e	68.92 ^e	208.50 ^e	97.58 ^d
	50	75.10 ^a	179.33 ^e	65.58 ^f	206.58 ^f	96.83 ^{de}
MGP IV	0	67.10 ^d	273.67 ^a	128.83 ^a	281.58 ^a	136.75 ^a
	10	69.55 ^c	241.42 ^b	105.33 ^b	269.92 ^b	133.84 ^b
	20	71.05 ^b	222.83 ^c	98.33 ^c	258.02 ^c	133.52 ^b
	30	79.05 ^a	195.00 ^d	89.08 ^d	248.00 ^d	131.83 ^c
	40	78.40 ^a	175.17 ^e	78.58 ^e	240.16 ^e	129.58 ^d
	50	79.20 ^a	154.92 ^f	63.75 ^f	239.25 ^e	129.08 ^d

^a Mean values in the same column within the mix (I, II, III, IV) followed by different superscripts differ significantly ($P \leq 0.05$)

RVU rapid visco unit; MGPs multigrain premix

Texture measurement

Breaking strength of biscuits measured by texture analyzer showed that the force required to break the biscuits increased

significantly ($p \leq 0.05$) from 13.25 in control sample to 28.68, 25.65, 26.69 and 26.4 N in the case of MGP I, II, III and IV respectively at 50 % of replacement. The biscuit hardness is affected by the interaction of proteins and starch by hydrogen

Fig. 1 RVA curve of wheat flour and at 30 % level of incorporation of different MGPs. *a* – Wheat flour; *b*– MGP I; *c*– MGP II; *d*– MGP III; *e*– MGP IV; MGPs multigrain premix

Table 5 Physical properties of multigrain biscuits (MGBs)

Flour	Level (%)	Weight (g)	Diameter (D) (mm)	Thickness (T) (mm)	Spread ratio (D/T)	Breaking strength (N)	L*	a*	b*	dE	h°	Chroma
MGP I	0	7.86 ± 0.32 ^a	57.83 ± 0.41 ^a	5.66 ± 0.51 ^b	10.28 ± 1.02 ^a	13.25 ± 0.26 ^a	67.95 ± 0.01 ^a	10.66 ± 0.14 ^b	35.39 ± 0.16 ^a	3.14 ± 0.05 ^c	36.95 ± 0.19 ^a	73.26 ± 0.11 ^a
	10	8.49 ± 0.57 ^{ab}	57.67 ± 0.52 ^a	5.83 ± 0.41 ^b	9.93 ± 0.72 ^a	16.86 ± 0.3 ^b	64.43 ± 0.12 ^b	11.22 ± 0.33 ^b	34.52 ± 0.09 ^b	3.68 ± 0.14 ^d	36.3 ± 0.19 ^b	72.16 ± 0.25 ^b
	20	8.99 ± 0.57 ^{ab}	57.5 ± 0.54 ^a	5.92 ± 0.2 ^b	9.73 ± 0.41 ^a	17.3 ± 0.37 ^b	62.16 ± 0.4 ^c	11.35 ± 0.14 ^b	34.11 ± 0.07 ^{bc}	5.98 ± 0.39 ^c	35.95 ± 0.11 ^b	71.6 ± 0.17 ^b
	30	9.30 ± 0.36 ^b	57.33 ± 0.52 ^a	6.00 ± 0.63 ^b	9.67 ± 1.04 ^a	19.52 ± 0.55 ^c	59.87 ± 0.11 ^d	11.43 ± 0.16 ^b	34.12 ± 0.04 ^{bc}	8.22 ± 0.13 ^b	35.98 ± 0.01 ^b	71.49 ± 0.26 ^b
	40	9.58 ± 0.27 ^c	56.5 ± 0.55 ^b	6.33 ± 0.82 ^{ab}	9.06 ± 1.33 ^{ab}	24.20 ± 0.79 ^d	60.57 ± 0.18 ^e	11.68 ± 0.43 ^a	33.75 ± 0.16 ^c	8.69 ± 0.01 ^b	35.92 ± 0.08 ^b	72.2 ± 0.39 ^b
	50	9.67 ± 0.27 ^d	55.17 ± 0.41 ^c	6.83 ± 0.75 ^a	8.15 ± 0.85 ^b	28.68 ± 0.88 ^e	55 ± 0.31 ^f	12.52 ± 0.42 ^{ab}	32.93 ± 0.22 ^d	13.32 ± 0.29 ^a	35.23 ± 0.36 ^c	69.2 ± 0.51 ^c
MGP II	0	7.86 ± 0.32 ^a	57.83 ± 0.41 ^a	5.66 ± 0.51 ^c	10.28 ± 1.02 ^a	13.25 ± 0.26 ^a	67.95 ± 0.01 ^a	10.66 ± 0.14 ^b	35.39 ± 0.16 ^a	3.14 ± 0.05 ^c	36.95 ± 0.19 ^a	73.26 ± 0.11 ^a
	10	8.25 ± 0.19 ^b	58.6 ± 0.54 ^a	5.72 ± 0.26 ^{bc}	10.26 ± 0.47 ^a	15.47 ± 0.72 ^b	64.07 ± 0.16 ^b	11.17 ± 0.17 ^b	34.75 ± 0.31 ^a	3.97 ± 0.18 ^a	36.51 ± 0.35 ^a	72.78 ± 0.18 ^{ab}
	20	8.33 ± 0.41 ^b	58.2 ± 0.84 ^a	5.78 ± 0.18 ^{abc}	10.07 ± 0.34 ^a	18.17 ± 0.68 ^c	61.22 ± 0.94 ^c	11.47 ± 0.22 ^{bc}	33.72 ± 0.59 ^b	6.98 ± 0.78 ^b	35.87 ± 0.14 ^a	71.99 ± 0.46 ^{bc}
	30	8.56 ± 0.35 ^b	56.6 ± 0.55 ^b	6.22 ± 0.31 ^{abc}	9.12 ± 0.52 ^b	18.99 ± 0.57 ^c	60.61 ± 0.13 ^c	11.93 ± 0.08 ^c	32.32 ± 0.18 ^c	7.97 ± 0.19 ^c	35.59 ± 0.33 ^{ab}	71.71 ± 0.33 ^c
	40	9.04 ± 0.17 ^c	55.8 ± 0.84 ^{bc}	6.36 ± 0.36 ^{ab}	8.80 ± 0.61 ^b	22.39 ± 0.79 ^d	58.38 ± 0.28 ^d	12.41 ± 0.28 ^{cd}	31.64 ± 0.09 ^c	10.61 ± 0.23 ^d	34.14 ± 0.43 ^b	71.25 ± 0.34 ^c
	50	9.41 ± 0.23 ^c	54.9 ± 0.89 ^c	6.44 ± 0.38 ^a	8.55 ± 0.55 ^{ab}	25.65 ± 0.62 ^e	57.78 ± 0.56 ^d	13.48 ± 0.25 ^d	30.69 ± 0.14 ^d	11.12 ± 0.26 ^d	33.71 ± 0.82 ^b	70.99 ± 0.41 ^c
MGP III	0	7.86 ± 0.32 ^a	57.83 ± 0.41 ^a	5.66 ± 0.51 ^a	10.28 ± 1.02 ^a	13.25 ± 0.26 ^a	67.95 ± 0.01 ^a	10.66 ± 0.14 ^b	35.39 ± 0.16 ^a	3.14 ± 0.05 ^c	36.95 ± 0.19 ^a	73.26 ± 0.11 ^a
	10	8.09 ± 0.29 ^a	57.6 ± 0.55 ^a	5.7 ± 0.27 ^a	10.12 ± 0.42 ^b	13.93 ± 0.21 ^b	65.87 ± 0.17 ^b	10.97 ± 0.11 ^a	35.05 ± 0.25 ^{ab}	3.89 ± 0.09 ^a	36.72 ± 0.48 ^a	72.62 ± 0.2 ^b
	20	8.30 ± 0.20 ^b	57.8 ± 0.45 ^a	5.8 ± 0.27 ^a	9.98 ± 0.44 ^b	15.32 ± 0.29 ^{ab}	63.54 ± 0.38 ^c	11.89 ± 0.11 ^{ab}	34.89 ± 0.39 ^{ab}	4.67 ± 0.13 ^b	36.87 ± 0.01 ^a	71.19 ± 0.02 ^c
	30	8.52 ± 0.15 ^c	57.1 ± 0.45 ^a	5.8 ± 0.27 ^a	9.98 ± 0.37 ^b	16.43 ± 0.27 ^c	61.64 ± 0.23 ^d	12.58 ± 0.24 ^c	34.69 ± 0.28 ^{ab}	7.67 ± 0.29 ^c	35.52 ± 0.29 ^b	70.53 ± 0.16 ^d
	40	8.76 ± 0.15 ^{cd}	57.2 ± 0.74 ^a	5.92 ± 0.19 ^a	9.85 ± 0.31 ^b	19.11 ± 0.15 ^d	61.06 ± 0.29 ^d	13.28 ± 0.18 ^d	34.27 ± 0.16 ^b	8.11 ± 0.17 ^d	35.06 ± 0.26 ^{bc}	70.21 ± 0.16 ^d
	50	8.93 ± 0.30 ^{cd}	55.6 ± 0.55 ^b	6.12 ± 0.38 ^a	9.11 ± 0.51 ^c	26.69 ± 0.65 ^e	59.18 ± 0.27 ^e	13.53 ± 0.07 ^d	34.13 ± 0.13 ^b	9.25 ± 0.03 ^f	34.42 ± 0.09 ^c	69.59 ± 0.12 ^e
MGP IV	0	7.86 ± 0.32 ^a	57.83 ± 0.41 ^a	5.66 ± 0.51 ^c	10.28 ± 1.02 ^a	13.25 ± 0.26 ^a	67.95 ± 0.01 ^a	10.66 ± 0.14 ^b	35.39 ± 0.16 ^a	3.14 ± 0.05 ^c	36.95 ± 0.19 ^a	73.26 ± 0.11 ^a
	10	8.01 ± 0.19 ^a	58.2 ± 0.84 ^a	5.7 ± 0.19 ^{bc}	10.22 ± 0.35 ^a	14.86 ± 0.83 ^b	65.38 ± 0.33 ^b	10.83 ± 0.11 ^a	34.53 ± 0.16 ^b	3.22 ± 0.13 ^a	36.25 ± 0.11 ^b	72.64 ± 0.16 ^b
	20	8.53 ± 0.09 ^b	56.9 ± 0.84 ^b	5.84 ± 0.11 ^{bc}	9.89 ± 0.09 ^{ab}	16.28 ± 0.43 ^c	64.62 ± 0.19 ^c	11.18 ± 0.26 ^a	34.28 ± 0.025 ^b	3.56 ± 0.14 ^b	35.93 ± 0.28 ^{ab}	72.55 ± 0.01 ^b
	30	8.67 ± 0.18 ^b	56.8 ± 0.45 ^b	5.9 ± 0.22 ^{abc}	9.64 ± 0.39 ^{ab}	17.12 ± 0.73 ^c	63.16 ± 0.11 ^d	11.37 ± 0.14 ^{ab}	33.91 ± 0.32 ^b	4.86 ± 0.11 ^c	35.41 ± 0.06 ^c	72.38 ± 0.08 ^b
	40	8.92 ± 0.18 ^{bc}	56.2 ± 0.84 ^b	6.18 ± 0.22 ^{ab}	9.11 ± 0.43 ^{bc}	18.65 ± 0.88 ^d	60.72 ± 0.15 ^e	11.86 ± 0.11 ^b	35.01 ± 0.11 ^c	7.34 ± 0.17 ^d	34.92 ± 0.16 ^d	71.43 ± 0.19 ^c
	50	9.38 ± 0.16 ^c	55.6 ± 0.55 ^{ab}	6.38 ± 0.13 ^a	8.72 ± 0.21 ^a	26.4 ± 0.511 ^e	60.03 ± 0.07 ^f	12.21 ± 0.37 ^c	32.69 ± 0.06 ^d	7.93 ± 0.08 ^f	34.25 ± 0.18 ^e	70.97 ± 0.01 ^d

Values are mean ± standard deviation of three independent determinations. ^a Mean values in the same column within the mix (I, II, III, IV) followed by different superscripts differ significantly ($P \leq 0.05$)

L* lightness/darkness; a* redness/blueness; b* yellowness/greenness; dE color difference; h° hue angle, MGP multigrain premix

bonding. The similar finding of increased breaking strength of biscuits by adding fenugreek seed and flax seed was reported by Nirmala et al. (2011) and attributed to decrease in spread ratio.

Sensory characteristics

The results of sensory analysis indicated that as the level of incorporation of MGP increase from 10 to 50 %, sensory scores of biscuits decreased significantly (Fig. 2a–d). The crust color darkens with the increase in the level of MGPs as indicated by lower scores. This darkening of color may be due to addition of dark color MGPs and also attributed to the Maillard reaction between protein rich MGP and sugars in biscuits (Lee and Brennan 2005). Among the biscuits, the one prepared from MGP I was much darker than other MGPs at comparable levels. The surface of biscuits becomes little rough in MGP incorporated biscuits. The change was similar irrespective of type of MGP used as all premixes are coarser than wheat flour. Crumb color darkened to a varying extent with an increase in the level of MGP. However, the extent of darkening is minimum in biscuits containing MGP

III. The texture of biscuits hardened with increase in the level of MGP. Among the biscuits, MGP III incorporated biscuits is less hard even at the 40 % level of incorporation. The change in texture as a result of incorporation of MGP is also reflected in the breaking strength score. Biscuits made with MGP III had a better wholesome taste as compared with biscuits prepared from other MGPs.

The statistical analysis carried out on different parameters have clearly shown that there is no significant ($p \leq 0.05$) difference in the various quality parameters such as crust color, surface character, crumb color, crumb texture, taste and overall acceptability up to 30 % addition of MGP. However, MGP III could be used even up to 40 % level without any significant ($p \leq 0.05$) change in the above characteristics. Earlier, Crassina et al. (2012) also reported the reduction of the sensory score in the biscuits prepared with above 40 % addition of finger millet flour. This clearly suggests that the formulation of MGP III, containing barley flour, sorghum flour, chick-pea flour, pea flour and defatted soya flour is best suited for preparation of multigrain biscuits with improved sensory characteristics. Hence a biscuit with MGPIII at 40 % level was selected for further studies.

Fig. 2 Sensory evaluations of biscuits. **a** Sensory evaluation of MGP I, **b** MGP II, **c** MGP III, **d** MGP IV

Effect of MGPs on micro-structure of dough and biscuits

The micro structural studies were carried out with the help of SEM to study the internal structural changes occurring in dough development and baking. The changes in structure during dough formation and baking are mainly related to changes of two major components i.e. starch and protein. The SEM images of control dough and MGP III incorporated dough were represented in Fig. 3a and b. From the figure, the large and small starch granules enmeshed in the gluten protein matrix were observed in control dough as earlier reported by Nandeesh et al. (2009). But in MGP III incorporated dough the starch granules are partially enmeshed and number of small protein bodies of multigrain adhering to starch granules is visible. Starch granules are more intact in control dough compared to MGP III incorporated dough. Similar observation of starch granules embed on the protein matrix during dough development is observed by Rojas et al. (2000). Starches play an important role in structuring of dough and biscuits by its interaction with the protein matrix during baking. The protein matrix appears to be very thin in MGP III incorporated dough as the continuity of protein matrix is disrupted by the addition of protein and

fiber rich MGP III as compared to control dough (Pomeranz et al. 1984).

SEM images for control and MGP III incorporated biscuits surface were represented in Fig. 3c and d. In Fig. 3c, the small and large starch granules are gelatinized and gel formation is observed, whereas in Fig. 3d, starch is partially gelatinized and an outline of gelatinized starch is observed. This may be due to the lack of sufficient water in the MGP incorporated dough as most of the water is absorbed by fiber rich MGP (Kulp et al. 1991). The microstructure of MGP III incorporated biscuits showed a severe disrupted gluten protein matrix due to the replacement of wheat flour with high fiber. This is also indicated in the biscuit texture, as the hardness of biscuits increased due to a reduction in the spread ratio (Mc Watters 1977). The permanently disrupted protein matrix with incorporation of green gram flour was reported by Jyotsna et al. (2012). Earlier, Flint et al. (1970), observed openings of various sizes and cavities on the surface of the sweet, semi-sweet biscuits and cream-crackers biscuits. Similar cavities were found in this study. These cavities are formed due to expansion of gas bubbles with increase in baking temperature, resulted in an increase of water vapour pressure leads to rupture of membrane to form holes and tunnels in the biscuits through which gas will escape.

Fig. 3 SEM images of dough and biscuits. **a** Control dough, **b** MGP III at 40 % incorporated dough, **c** Control biscuits, **d** MGP III at 40 % incorporated biscuits. SSG small starch granules, LSG large starch granules, GS gelatinized starch, PGS partially gelatinized starch, PM protein matrix, PB protein bodies

Fig. 4 Effect of MGP on protein, dietary fiber and mineral content of biscuits. *MGP* multigrain premix; Mean values of triplicates; SDs are denoted as *bars*

Nutritional composition of MGB

The nutritional composition of multigrain biscuits developed using MGP III at 40 % is depicted in Fig. 4, clearly shows a significant ($p \leq 0.05$) increase in nutritional parameters like protein, dietary fiber and mineral content when compared to wheat flour biscuits.

Conclusion

All the four multigrain premixes prepared are found to be rich in protein, dietary fiber and mineral content and incorporation of these MGPs at varying proportions significantly ($p \leq 0.05$) decreased the consistograph water absorption, peak viscosity and increased the pasting temperature and biscuit dough hardness. The biscuit making trials showed that biscuits became slightly harder depending on the level and type of premix used. These biscuits had better taste and flavor of multigrain along with increased nutritional characteristics. The MGP III could be incorporated even up to 40 % level indicating its suitability for obtaining biscuits with much higher nutrition and overall acceptability without considerably affecting the other quality parameters. Two fold increases in protein, three fold increases in dietary fiber and one fold increase in mineral content was observed in the multigrain biscuits. The MGPs will give new dimensions in the development of nutritious biscuits and boost the industry to newer heights.

Declaration of conflicting interest This article has not been published previously and neither is under consideration for publication elsewhere. The publication is approved by all authors and tacitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere, including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder.

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