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Formulation of reduced-calorie biscuits using artificial sweeteners and fat replacer with dairy–multigrain approach

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ABSTRACT

Background: Lifestyle modifications related to change in the eating quality and quantity along with mental stress led to the prevalence of non-communicable diseases. Based on the consumers demand, food scientists are now focusing on developing low-calorie/high-fiber functional foods. A biscuit which includes variety of ingredients proved to be a prominent vehicle for incorporating functional ingredients like whole grains, dairy ingredients, fat replacer, artificial sweeteners, etc. Use of highly nutritious, under-utilized minor cereal like pearl millet, use of whole grains flours like wheat and Bengal gram along with dairy ingredients like skimmed milk powder and whey protein concentrate nutritionally complement and counterbalance each other to form a wholesome snack. Further incorporation of artificial sweeteners and fat replacer having functional properties not only decreases the caloric density but also aids in health benefits.

Methods: We investigated the formulation of multigrain flour through the level of substitution of whole wheat flour on (w/w) basis using Bengal gram flour and germinated pearl millet flour based on sensory evaluation. The prepared multigrain flour was then mixed with dairy ingredients to prepare dairy–multigrain flour. The sugar in the product was tried to replace maximally using blends of artificial sweeteners without affecting the sensorial perception. Two types of fat replacer were tried for their suitability in biscuits to maximally replace the fat from the product. The formulated product was evaluated for its proximate analysis and calorie density using bomb calorimetry.

Results: Bengal gram flour (BGF) at 6% and germinated pearl millet flour (GPMF) at 6% were found suitable to replace part of the whole wheat flour (WWF) to make multigrain flour. Skimmed milk powder and whey protein concentrate-70 were added at the rate of 7.8 and 7.0% (on product basis), respectively, to form dairy–multigrain composite. Binary blend of Maltitol and FOS-sucralose in the ratio 3:1 was found suitable to replace 100% of the sucrose in the biscuits. Polydextrose (PD) at 30% was more suitable than Simplese® for partial replacement of fat. The formulated biscuits had 15.98% lower energy and 30% less fat content than that of control.

Conclusion: The study demonstrated that highly acceptable reduced-calorie biscuits can be produced by using dairy–multigrain composite flour with maltitol and FOS-sucralose (as sweetener) and PD (as fat replacer).

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1. Introduction

Overweight and obesity have long been regarded as the main driver of type-2 diabetes and other health-associated risks. Fervent changes in the quality, quantity, and source of food consumed along with a high level of mental stress and sedentary lifestyle have led to an increase in the prevalence of these non-communicable diseases. The recent published IDF Diabetes Atlas (Sixth edition, 2013) reports that there are 382 million people living with diabetes in the world and it is expected

to rise by 55% to 592 million in 2035. There are 175 million people with diabetes still undiagnosed. Coronary heart disease is currently the leading cause of death globally and, together with diabetes, poses a serious health threat. There is robust evidence that lifestyle modification (unhealthy diet rich in calories and saturated fat, physical inactivity) has a sustained effect in expanding these diseases. The relation between the diet and health problems is not a new notion. People are more attentive toward their calorie intake through fat and carbohydrates. In order to limit the prevalence of diabetes and coronary heart diseases, it is recommended to reduce the calorie intake through sugars and saturated fatty acids. The food industry has focused for the last couple of decades on the production of low-fat/low-calorie, high-fiber foods in response to public interest for these functional products. This project had been proposed to formulate a functional biscuit containing valuable dairy ingredients in addition to reduced calories.

Abbreviations: WWF, whole wheat flour; BGF, Bengal gram flour; GPMF, germinated pearl millet flour; PD, polydextrose; FOS, fructo-oligosaccharide.

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Biscuits are the most popular bakery items because of their high nutritive value, ready-to-eat nature, and easy availability in different shapes and sizes at an affordable cost. Biscuits and other sweet baked items are rich in sugar (mainly sucrose) and fat and are usually avoided by calorie-conscious consumers [1]. Functional properties of biscuits can be increased by improving or modifying the major ingredients, namely, flour, sugar, and fat along with supplementation of health-promoting ingredients like whey protein concentrate, skimmed milk powder, dietary fiber, etc. Such modification can be achieved by replacing whole or part of the flour with whole multigrain flour, use of artificial sweeteners and fat replacers.

Incorporation of whole grains increases the nutritional profile of the products as they are a rich source of dietary fiber trace minerals, antioxidants, and phenolic compounds, which play a significant role in protecting against cancer, diabetes, obesity, and cardiovascular diseases [2]. Several workers had studied the effect of partial replacement of wheat flour for biscuit production with defatted soy flour [3] oat, wheat, rice and barley bran [4], chick pea, broad bean or isolated soy protein [5], untreated, roasted, and germinated black gram [6]. Arndt et al. [7] reduced the calorie density and carbohydrate content of biscuits by replacing 20–25% of refined wheat flour with equal weight of whole grain flour. Handa et al. [8] had developed high-fiber cookies providing, respectively, 21% and 12.8% daily value of dietary fiber and iron using whole multigrain flour and fructo-oligosaccharides.

Sweetener plays an important role in providing flavor, appearance, color, taste, and dimension to the finished product. Due to prevalence of diseases like diabetes and obesity, the use of artificial sweeteners as a sucrose substitutes for the development of low-calorie products has been the focus of R&D in the recent past. Polyols and other bulk sweeteners are used in food products such as sugar-free candies, cookies, and chewing gum in which the volume and texture of sugar, as well as its sweetness are important. Several workers had studied the effect of replacement of sugar on cookies with artificial sweeteners like sorbitol, mannitol, lactitol, maltitol, fructose and xylitol [1], stevia [3], sucralose and maltodextrin [9], and inulin and erythritol [10].

Reducing fat in products like cookies and other bakery good is an arduous job for the bakery industry. Fat, apart from being responsible for soft and crisp texture of biscuits, also imparts flavor, lubricity, mouth-feel, aeration, and taste to the product. At present, a wide variety of ingredients are employed to mimic the unique properties and qualities of fat in bakery products. Polydextrose (PD) and Simplese® are two of the most popular carbohydrate and protein-based fat replacer, respectively. Polydextrose is a complex carbohydrate made from glucose, citric acid, and sorbitol, which forms a highly viscous gel-like matrix contributing to creaminess and mouth-feel [11]. Simplese® is micro-particulated whey protein manufactured from whey protein concentrate by simultaneous heating and shearing resulting in a particle size of 0.1–10 µm. Such small particles have the ability to provide creaminess sensation associated with fat. Sudha et al. [12] studied the implication of fat replacement on dough rheology and biscuit quality using polydextrose and maltodextrin. Several attempts had already been made to replace fat in cookies by use of fat mimetics like polydextrose), maltodextrin, dairytrim, pectin, and Simplese® [13], combination of fat replacer like corn fiber, maltodextrin or lupine extract [14], maltodextrin and guar gum [15] etc. In most cases, formulation of products with acceptable properties could only be achieved when partial, instead of full, replacement of fat was used. Reduction in fat beyond 30–40% resulted in higher hardness, firmness, and breaking strength of cookies [13,12].

The aim of the present investigation was to formulate a biscuit using whole multigrain flour [comprising of whole wheat flour (WWF), Bengal gram flour (BGF), and germinated pearl millet flour (GPMF)] by replacing 100% of the refined wheat flour. In order to reduce the calorie content of the above formulated biscuit, two types of artificial sweeteners (maltitol and FOS-sucralose liquid sweetener) were tried singly and/or in combination to replace all of the sucrose. Attempts

were also made to partially replace the fat in the biscuit using fat replacers (Simplese® and polydextrose).

2. Material and methods

2.1. Raw materials and ingredients

The various ingredients used for biscuit formulation were procured from different sources. These include (1) wheat (Var. HD 2967) (Agriculture Science Centre, NDRI, Karnal), (2) pearl millet (Var. 9444) (Jind, Haryana), (3) Bengal gram (Var. BG 1103) (IARI, New Delhi) for the preparation of multigrain flour, (4) refined wheat flour suitable for biscuit preparation (Rajdhani group, New Delhi) (composition data provided by the supplier: 9.8% protein, 74% carbohydrates, 0.8% fat, 0.4% minerals, and 15% moisture), (5) bakery shortening (MarvoPride, Masterline Bakery Service, Bangalore) (composition data provided by supplier: 100% fat, 0.10% moisture, and 0.10% free fatty acid), (6) Polydextrose (Litesse®, DuPont Danisco, Denmark), (7) Simplese® (CP Kelco, Finland), (8) Maltitol (Roquette, France), (9) FOS-sucralose liquid sweetener (Sweetos) (Ensigns Health Care Pvt. Ltd. Pune, Maharashtra), (10) Cane sugar (local market, Karnal), (11) Soya lecithin (Pharmaceutical grade, Sonic Biochem Extraction Limited, Madhya Pradesh), (12) Cardamom flavor (International Flavors and Fragrances, USA), (13) Ammonium bicarbonate and Sodium bicarbonate (SRL, India), (14) Spray dried skimmed milk powder (SMP) and whey protein concentrate-70 (WPC) (Modern Dairies Ltd., Karnal).

2.2. Preparation of germinated pearl millet flour (GPMF)

The raw, clean pearl millet seeds were soaked in 2% sodium carbonate solution [alkali treatment to reduce the levels of tannins and phytates before germination [16] in the ratio 1:3 for 12 h at room temperature (~25 °C). The soak water was changed after every 6 h interval. The soaked grains were washed and immersed in 0.1% formaldehyde solution (to prevent mold growth) in the ratio 1:2 for 12 h at room temperature (~25 °C). They were then placed between the folds of muslin cloth in a tray and allowed to germinate at 25 °C in incubator with continuous watering (spraying) for 48 h. Seeds were dried at 55–60 °C for 8 h to a moisture content of 11–12%. The rootlets of germinated and dried seeds were removed by scrubbing manually. The separated vegetative parts were removed by winnowing. Germinated, roasted pearl millet grains were finally milled to flour, which was sieved through 52 mesh sieve to form fine flour of particle size of 355 µm or less.

2.3. Preparation of whole wheat flour (WWF) and Bengal gram flour (BGF)

Wheat grains were cleaned, washed, dried, and milled to flour. Bengal gram were cleaned, washed, soaked in water (1:3 ratio of grains to water) for 12 h, roasted (60 °C/6 h) for easy removal of outer coating and then milled to flour. Both the flours were then sieved through 52 mesh sieve to obtain a particle size of 355 µm or less.

2.4. Incorporation of dairy components

Skimmed milk powder and whey protein concentrate were mixed with refined wheat flour as dry ingredients for the preparation of control biscuits and with multigrain flour (WWF, BGF, and GPMF) for the preparation of high-fiber, reduced-calorie biscuits at the levels indicated in Table 1.

2.5. Preparation of control biscuits

Control biscuits were prepared using the creaming method adopted by [17] with slight modification. Bakery shortening was creamed using Hobart Mixer at high speed (259 RPM) until its volume doubled.

Table 1
Ingredient levels for control and formulated biscuits with sequential replacement of refined wheat flour, sugar, and fat.

Ingredients	Control formulation	Formulation with replacement of		
		Step 1: Refined wheat flour	Step 2: Sugar	Step 3: Bakery shortening [#]
Refined wheat flour (%)	40	–	–	–
Whole wheat flour* (%)	–	22–34 (28)	28	28
Bengal gram flour* (%)	–	5–8 (6)	6	6
Germinated pearl millet flour* (%)	–	1–10 (6)	6	6
Sucrose (%)	23	23	–	–
Maltitol (%)	–	–	17.25	17.25
FOS-Sucralose (%)	–	–	5.75	5.75
Polydextrose (%)	–	–	–	5.95
Bakery shortening (%)	17	17	17	11.05
Skimmed milk powder (%)	7.8	7.8	7.8	7.8
Whey protein concentrate (%)	7	7	7	7
Lecithin (%)	2	2	2	2
Ammonium bicarbonate (%)	0.5	0.5	0.5	0.5
Sodium bicarbonate (%)	0.5	0.5	0.5	0.5
Salt (%)	0.2	0.2	0.2	0.2
Cardamom flavor (%)	2	2	2	2
Water (mL/100 g dry ingredients)	12	16–18	18.5	20

* Multigrain flour ingredients; values in parenthesis show the optimized formulation of multigrain flour.

Final formulation.

Ground sugar was then mixed to the foamed cream along with the lecithin and cardamom flavor. Refined wheat flour, skimmed milk powder, whey protein concentrate, and sodium bicarbonate were homogenously mixed, added to the above creamed mixture and mixed to a crumbly texture. Required amount of water was divided in two parts to dissolve salt in one and ammonium bicarbonate in the other, before adding to the above mixture to prepare dough. The dough was then fed in to the cookie drop machine and biscuits of spiral shape with a thickness of 3 mm were collected on baking tray and baked at 165 °C for 16 min followed by cooling at room temperature for 30 min. Biscuits were packed in metallized LDPE pouches (20.87 µm thick) and stored at 37 °C for further analysis.

2.6. Selection of the level of BGF and GPMF for multigrain flour

To prepare multigrain flour for the preparation of optimized biscuit, BGF and GPMF were tried at different levels (GPMF at 1, 4, 6, and 10%; BGF at 5, 6, 7 & 8%) to partially replace the WWF. Based on the sensory score obtained (9-point Hedonic Scale), the best levels were selected

Table 2
Sensory score (9-point Hedonic Scale) for selection of Bengal gram flour and germinated pearl millet flour based on the level of fortification.

Treatment	Color	Texture	Sweetness	Flavor	Overall
Attributes	acceptability				
Multigrain flour (replacement of WWF on w/w basis)					
BGFat5%	7.6 ± 0.40 ^A	6.4 ± 0.19 ^A	7.4 ± 0.24 ^A	6.5 ± 0.22 ^A	6.6 ± 0.29 ^A
BGFat6%	7.9 ± 0.10 ^A	7.6 ± 0.19 ^B	7.6 ± 0.19 ^A	7.5 ± 0.22 ^B	7.7 ± 0.20 ^B
BGFat7%	7.6 ± 0.24 ^A	6.8 ± 0.25 ^{AB}	7.2 ± 0.37 ^A	6.8 ± 0.25 ^{AB}	6.9 ± 0.24 ^{AB}
BGFat8%	7.5 ± 0.16 ^A	7.1 ± 0.19 ^{AB}	7.3 ± 0.20 ^A	6.8 ± 0.25 ^{AB}	7.1 ± 0.12 ^{AB}
GPMF flour (replacement of WWF on w/w basis)					
GPMFat1%	6.8 ± 0.34 ^X	6.8 ± 0.40 ^{XZ}	7.0 ± 0.28 ^X	6.7 ± 0.30 ^X	6.9 ± 0.24 ^X
GPMFat4%	7.1 ± 0.29 ^X	6.2 ± 0.25 ^{XY}	7.0 ± 0.27 ^X	6.6 ± 0.24 ^X	6.8 ± 0.25 ^X
GPMFat6%	7.2 ± 0.15 ^X	7.4 ± 0.27 ^Z	7.2 ± 0.12 ^X	7.3 ± 0.18 ^X	7.4 ± 0.22 ^X
GPMFat10%	6.4 ± 0.37 ^X	6.6 ± 0.29 ^{XZ}	7.3 ± 0.12 ^X	6.7 ± 0.20 ^X	6.8 ± 0.25 ^X

Data are presented as mean (±SEM) (n = 15). ABC; XYZ: Means within a column with at least one similar superscript do not differ significantly (p > 0.05). WWF = whole wheat flour; BGF = Bengal gram flour; GPMF = germinated pearl millet flour; w/w = weight by weight.

and used for preparation of the optimized biscuit for further research (Table 1).

2.7. Selection of type and level of artificial sweetener

Two sweeteners, maltitol and FOS-sucralose, were used for the study. Maltitol is a white-color, odorless, sweet, and cool crystalline powder which replaces sugar on weight-by-weight basis and provides 2.40 kcal/g [18]. FOS-sucralose is a liquid bulk sweetener which imparts sweetness equivalent to sugar. It substitutes sugar on weight-by-weight basis and contributes less than one calorie/g (as per manufacturer's report) [19]. These two sweeteners were evaluated individually (maltitol and FOS-sucralose at 25, 50, 75, and 100 w/w on sucrose basis) and their binary blends (maltitol:FOS-sucralose at 1:3, 1:1, and 3:1 for 100% replacement of sucrose on weight basis) at different levels to compensate the sweetness level in the product. Based on the sensory score obtained (9-point Hedonic Scale), the best sweetener or their blend was selected and used for preparation of the optimized biscuit for further research (Table 1).

2.8. Selection of type and range of fat replacer

Two types of fat replacer [Polydextrose (PD) and Simplesse®] were tried individually to maximally replace the fat in the product without significantly affecting the sensory properties. Previous studies indicated that maximum fat replacement could be done in the range of 10–40% [13,12]. PD is a bulking and fat sparing agent, bland in taste and provides only 1 kcal/g. It is suitable for baked goods, confectionaries, frozen dairy desserts, etc. [20]. Simplesse® has a low-calorie value (2 calories/g), as its protein particles are hydrated [21]. Fat replacers were added in the form of powder as a part of dry ingredients. Based on the sensory score obtained (9-point Hedonic Scale), the best type of fat replacer was selected and used for preparation of the optimized biscuit for further research (Table 1).

2.9. Preparation of optimized product

The calculated amount of bakery shortening was creamed using Hobart Mixer at high speed (259 RPM) until its volume doubled. Maltitol and FOS-sucralose were then mixed to the foamed cream along with the lecithin and cardamom flavor. Multigrain flour, skimmed milk powder, whey protein concentrate, polydextrose, and sodium bicarbonate were homogenously mixed, added to the above creamed mixture until a final crumbly texture was achieved. The required

Table 3
Sensory score (9-point Hedonic Scale) for selection of artificial sweetener based on the level of fortification.

Treatment / Attributes	Color	Texture	Sweetness	Flavor	Overall acceptability
25M+75S (T-1)	6.7 ± 0.30 ^{AB}	6.9 ± 0.29 ^A	7.3 ± 0.25 ^{AB}	7.1 ± 0.24 ^{AB}	7.1 ± 0.19 ^{AB}
50M+50S (T-2)	6.6 ± 0.39 ^{AB}	6.4 ± 0.29 ^A	6.6 ± 0.43 ^{AB}	6.8 ± 0.25 ^{AB}	6.6 ± 0.29 ^{AB}
75M+25S (T-3)	6.5 ± 0.24 ^{AB}	6.6 ± 0.29 ^A	6.8 ± 0.30 ^{AB}	6.8 ± 0.30 ^{AB}	6.8 ± 0.25 ^{AB}
100M (T-4)	6.7 ± 0.30 ^{AB}	6.6 ± 0.43 ^A	6.7 ± 0.51 ^{AB}	6.6 ± 0.48 ^{AB}	6.8 ± 0.34 ^{AB}
25S+25F+50M (T-5)	7.6 ± 0.19 ^A	6.4 ± 0.19 ^A	7.3 ± 0.12 ^{AB}	7.5 ± 0.16 ^A	7.1 ± 0.29 ^{AB}
25S+25M+50F (T-6)	7.6 ± 0.33 ^A	7.1 ± 0.10 ^A	7.5 ± 0.22 ^A	7.5 ± 0.16 ^A	7.5 ± 0.16 ^A
25F+75S (T-7)	7.0 ± 0.32 ^{AB}	6.6 ± 0.29 ^A	7.0 ± 0.27 ^{AB}	7.0 ± 0.27 ^{AB}	7.0 ± 0.27 ^{AB}
50F+50S (T-8)	7.1 ± 0.20 ^{AB}	7.2 ± 0.34 ^A	7.2 ± 0.15 ^{AB}	7.3 ± 0.12 ^{AB}	7.3 ± 0.12 ^A
75F+25S (T-9)	6.3 ± 0.20 ^{AB}	6.9 ± 0.58 ^A	6.8 ± 0.34 ^{AB}	6.6 ± 0.19 ^{AB}	6.7 ± 0.25 ^{AB}
100F (T-10)	6.0 ± 0.28 ^{BC}	6.4 ± 0.43 ^A	6.0 ± 0.27 ^B	6.2 ± 0.34 ^B	6.0 ± 0.27 ^B
50F+50M (T-11)	6.3 ± 0.25 ^{AC}	6.5 ± 0.20 ^A	6.9 ± 0.41 ^{AB}	6.6 ± 0.24 ^{AB}	6.5 ± 0.20 ^{AB}
25F+75M (T-12)	7.1 ± 0.24 ^{AC}	7.1 ± 0.24 ^A	7.2 ± 0.28 ^{AB}	7.1 ± 0.13 ^{AB}	7.4 ± 0.13 ^A
75F+25M (T-13)	5.9 ± 0.13 ^{BC}	6.3 ± 0.14 ^A	6.6 ± 0.47 ^{AB}	6.5 ± 0.29 ^{AB}	6.4 ± 0.24 ^{AB}
100S (T-14)	7.3 ± 0.25 ^A	7.3 ± 0.14 ^A	7.4 ± 0.24 ^{AB}	7.1 ± 0.13 ^{AB}	7.5 ± 0.20 ^A

Data are presented as mean (±SEM) (n = 15). ^{ABC} Means within a column with at least one similar superscript do not differ significantly (p > 0.05). M = maltitol; S = sucrose; F = fructo-oligosaccharide–sucralose; w/w = weight by weight; T (1–14) = Treatment for sweeteners.

amount of water was divided in two parts to dissolve salt in one and ammonium bicarbonate in the other, before adding to the above mixture to prepare dough, which was then processed to biscuits by the same procedure as for control biscuits.

3. Physico-chemical analysis

3.1. Sensory evaluation

The freshly baked biscuits (four treated samples at a time for replacement of each ingredient) were presented to fifteen members to evaluate their sensory attributes. Each treatment was evaluated for sweetness, overall quality, flavor, color, and texture attributes as perceived by the panelists (hard, crisp, porous, uniform). The panelists were subjected to an orientation session to improve their reproducibility

Table 4
Sensory score (9-point Hedonic Scale) for selection of fat replacer based on the level of fortification.

Treatment / Attributes	Color	Texture	Sweetness	Flavor	Overall acceptability
Fat replacer (%)					
10SP+90IETFS	8.0 ± 0.16 ^A	7.5 ± 0.27 ^A	8.0 ± 0.16 ^A	7.3 ± 0.34 ^A	7.7 ± 0.24 ^A
20SP+80IETFS	7.8 ± 0.25 ^A	7.2 ± 0.12 ^A	7.8 ± 0.12 ^A	7.3 ± 0.25 ^A	7.4 ± 0.10 ^A
30SP+70IETFS	7.9 ± 0.24 ^A	7.4 ± 0.19 ^A	7.8 ± 0.20 ^A	7.4 ± 0.27 ^A	7.4 ± 0.22 ^A
40SP+60IETFS	7.6 ± 0.19 ^A	7.0 ± 0.00 ^A	7.8 ± 0.25 ^A	7.1 ± 0.19 ^A	7.1 ± 0.10 ^A
10PD+90IETFS	8.3 ± 0.25 ^a	8.3 ± 0.12 ^a	8.1 ± 0.19 ^a	8.0 ± 0.27 ^a	8.2 ± 0.20 ^a
20PD+80IETFS	7.8 ± 0.41 ^a	7.9 ± 0.48 ^a	7.5 ± 0.39 ^a	7.2 ± 0.25 ^a	7.5 ± 0.27 ^{ab}
30PD+70IETFS	8.3 ± 0.30 ^a	8.2 ± 0.15 ^a	8.1 ± 0.10 ^a	8.1 ± 0.10 ^a	8.1 ± 0.10 ^a
40PD+60IETFS	7.5 ± 0.16 ^a	6.9 ± 0.19 ^b	7.1 ± 0.29 ^a	7.3 ± 0.25 ^a	6.8 ± 0.20 ^b

Data are presented as mean (±SEM) (n = 15). ^{ABC, abc} Means within a column with at least one similar superscript do not differ significantly (p > 0.05). PD = polydextrose; IETFS = inter-esterified trans free shortening; SP = Simplex; w/w = weight by weight.

and accuracy. For each sample, panelists scored their liking of these characteristics using the 9-point Hedonic Scale (1 – dislike extremely, 2 – dislike very much, 3 – dislike moderately, 4 – dislike slightly, 5 – neither like nor dislike, 6 – like slightly, 7 – like moderately, 8 – like very much, and 9 – like extremely) [22].

3.2. Proximate analysis

Raw materials (WWF, BGF, and GPMF), control biscuits, and optimized biscuits were evaluated for their moisture, fat, protein, ash content [23], and carbohydrate content (by difference method) [24].

3.3. Energy value

To measure the gross energy of the optimized and control biscuits, 1 g moisture-free sample of optimized and control biscuits in the form of pellets were taken respectively. The pellets were combusted using the Adiabatic CC01/M3 Microprocessor Bomb Calorimeter (Toshniwal Technologies Pvt. Ltd., India). Pure and dry benzoic acid (Merck, Germany) was run as standard. Energy content of pellets was calculated as follows: $W = [(H \cdot M) + E_1 + E_2]/T$, where W = Energy equivalent of calorimeter in calories per degree centigrade, H = Heat of combustion of standard benzoic acid in calories per g, M = Mass of standard benzoic acid sample in g, T = Corrected temperature rise in degrees centigrade, E_1 = Correction for heat of combustion of threads in calories, and E_2 = Correction for heat of combustion of firing wire in calories. Each sample was run in triplicate, taking the mean of all runs as final energy content. The metabolizable energy of the control and optimized biscuits was also calculated applying general factors to macronutrients (fat: 9 kcal/g, carbohydrate: 4 and protein: 4) as specified in the Code of Federal Regulations [25].

3.4. Statistical analysis

The results obtained in the present study were subjected to one-way analysis of variance (ANOVA) with Tuckey post-test and two-tailed paired t test using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California, USA (www.graphpad.com), to evaluate the statistical significance of the data.

4. Results and discussion

4.1. Selection of BGF and GPMF level in multigrain flour

The effect of different levels of BGF and GPMF on descriptive sensory parameters is presented in Table 2. It can be observed that there was no significant difference (p > 0.05) in color and sweetness among the different treatments of BGF. Flour at 6% scored highest for all sensory parameters. Scores were significantly different (p < 0.05) from 5% in terms of the texture, flavor, and overall acceptability, but non-significantly different from 7 and 8% levels. Increasing the amount of BGF from 6% decreased the sensory scores non-significantly (p > 0.05) due to increase in beany flavor. Among different treatments of GPMF, there was no significant difference (p > 0.05) in color, sweetness, flavor, and overall acceptability, whereas the difference was significant in texture attributes (p < 0.05). As the level of GPMF increased above 6%, sensory score decreased due to increase in deviation from golden brown color, non-uniform surface (cracks), and increased millet flavor. Similar investigations by partial replacement of wheat flour with chickpea and broad bean flour as well as isolated soy protein [5], dehulled pigeon pea (*Cajanuscajan L*) flour or pigeon pea by-product flour [26], black gram flour [6], sunflower protein isolate [27], and soy flour [28,29] were reported for biscuits. Descriptive sensory parameters scores presented in Table 2 indicate that the fortification by partial substitution of WWF by BGF (6%) and GPMF (6%) resulted in the best sensory scores among all the levels of treatment.

Table 5
Sensory score (9-point Hedonic Scale) for the comparison of control and optimized biscuit.

Parameters	Color	Texture	Sweetness	Flavor	Overall acceptability
Control	8.41 ± 0.12 ^A	8.25 ± 0.15 ^A	8.54 ± 0.15 ^A	8.37 ± 0.14 ^A	8.35 ± 0.15 ^A
Optimized	8.27 ± 0.18 ^A	8.10 ± 0.23 ^A	8.26 ± 0.35 ^A	8.10 ± 0.20 ^A	8.15 ± 0.23 ^A

Data are presented as mean (±SEM) (n = 15). Means within a column with at least one similar superscript do not differ significantly (p > 0.05).

4.2. Selection of type and level of artificial sweetener

Sensory scores pertaining to replacement of sucrose (on w/w basis) with maltitol and/or FOS-sucralose liquid sweetener are shown in Table 3. The texture scores of the different treatments did not vary significantly (p > 0.05). Quantity of sweeteners to be added had a significant role in altering the appearance, flavor, and texture [30,31]. Pasha et al. [32] showed that 50% sucrose along with 50% fructose improved the sensory characteristics, i.e. color, taste, texture, etc. of the cookies. In the current study, the biscuit containing both the artificial sweeteners along with sucrose (T-5 and T-6) had higher color scores, and as the level of sucrose decreased, color score also decreased. Table 3 also shows that, as the level of FOS-sucralose liquid sweetener increased, color score decreased. The least score for color, texture, and sweetness was for T-13 followed by T-10. A similar result was observed by [33] where the color, browning, and texture began to degrade when inulin percentage was increased in pound cakes and cupcakes. Savitha et al. [9] found that sweetness of the biscuits containing 0.05% sucralose and 30% maltodextrin was similar in intensity to that of the control biscuits. Zoulias et al. [1] concluded that maltitol, lactitol, and sorbitol could replace sugar in low-fat cookies of acceptable properties with lower sweetness. On the basis of overall acceptability score, T-14 (control) and T-6 scored maximum followed by T-12 non-significantly (p > 0.05). In order to plummet the calories by replacing 100% of the sucrose without comprising the sensory attributes, T-12 was considered appropriate for selection.

4.3. Selection of type and range of fat replacer

Table 4 presents the different sensory scores obtained from the different treatments of fat replacers. As the level of Simplese® increased, color score decreased non-significantly (p > 0.05). The highest score for textural attribute corresponded to lowest level of Simplese® and vice versa. There was no significant difference in the sweetness and flavor

score at different levels of Simplese®. The highest score for overall acceptability was for the lowest level of Simplese® and lowest for the highest level. It is also evident that, as the level of Simplese® increased, tenderness in the biscuits also increases resulting in the lower textural score. Zoulias et al. [13] found that cookies prepared with Simplese® were the tenderer among samples prepared with different fat mimetics. Gallagher et al. [34] prepared biscuits of high standard by replacing fat with Simplese® at the level of 25.02% along with 14% Novelose (% flour), 14.51% sodium caseinate (% flour), and 25% Raftilose (% sugar).

PD was used to replace fat content partially in the range of 10–40%. It can be seen from Table 4 that replacement at 10% got the highest score in terms of color, texture, sweetness, and overall acceptability, whereas 30% got highest for flavor attribute. There was no significant (p > 0.05) difference among the treatments for different levels of PD in the sensory attributes except for the overall acceptability between 10 and 40%. As the level of PD increased, sensory score decreased up to 20% followed by an increase at 30% replacement before decreasing again. Sudha et al. [12] found that biscuit dough properties can be enhanced by replacing fat with PD or maltodextrin. Chugh et al. [15] reduced the fat level in the biscuits by 62.5% using maltodextrin and guar gum. Zoulias et al. [13] found that PD was the most acceptable fat mimetic at 35% replacement. Increasing the fat replacer content increased the hardness, thereby decreasing the texture score. Increasing the Simplese® content deviated the goldenbrown color of biscuits toward darker hues. When PD was used at 30%, higher sensory scores comparable to other levels were obtained and therefore selected for the formulation of optimized biscuits.

4.4. Sensory evaluation of control and optimized biscuit

Sensory evaluation is very crucial parameter in evaluating the acceptability of the product. Table 5 summarizes the results of sensory analysis of the control and optimized biscuit and gives the mean scores for all the sensory attributes. It was observed that there was no significant difference (p > 0.05) in the sensory scores for all the sensorial

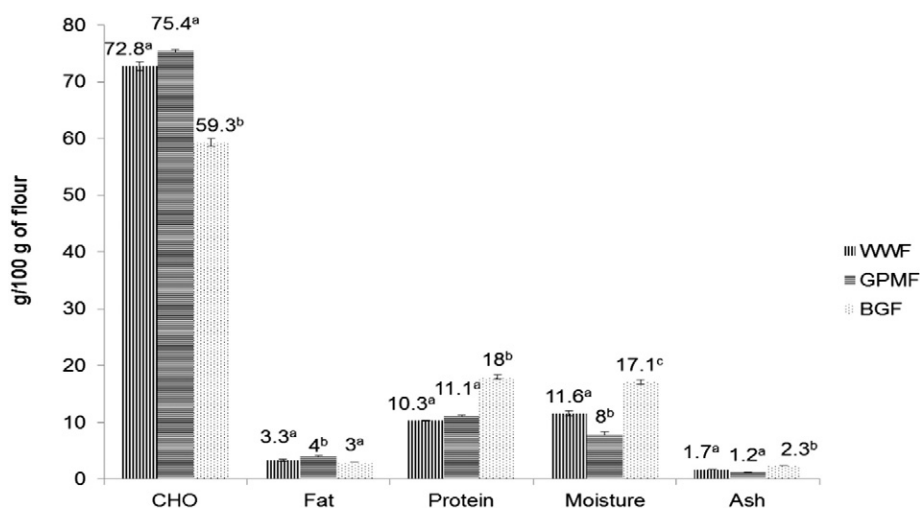


Fig. 1. Proximate analysis of whole wheat flour (WWF), germinated pearl millet flour (GPMF), and Bengal gram flour (BGF). CHO = carbohydrate. Data are presented as mean (±SEM) (mean of four replicates). ^{abc} means within a column for individual composition with at least one similar superscript do not differ significantly (p > 0.05). Data are presented on % (w/w) basis.

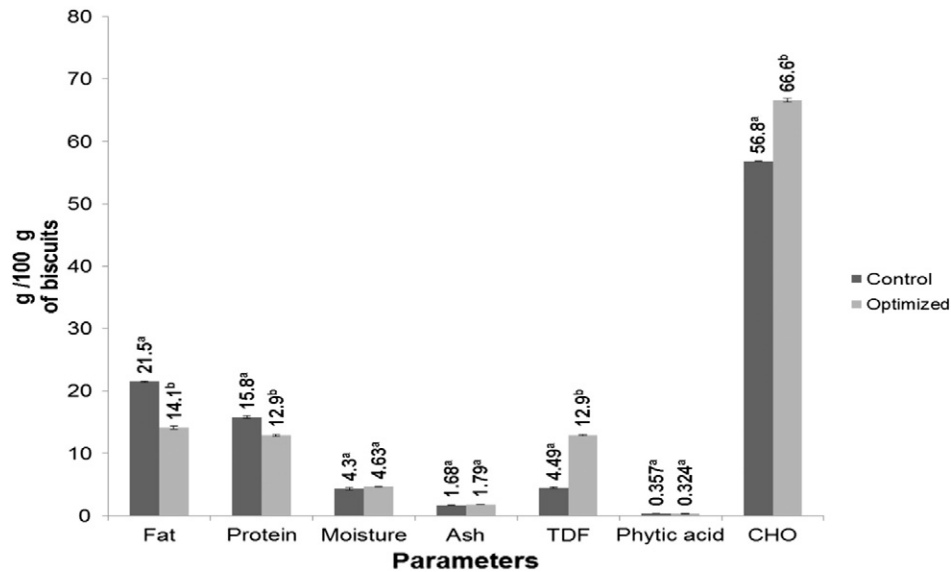


Fig. 2. Proximate analysis of control and optimized biscuits. CHO = Carbohydrate, TDF = Total dietary fiber. Data are presented as mean (\pm SEM) (mean of four replicates). ^{ab} means within a column for individual composition with at least one similar superscript do not differ significantly ($p > 0.05$). Data are presented on % (w/w) basis.

attributes. However, control biscuit showed higher values than the optimized biscuit.

4.5. Proximate analysis

WWF, BGF, and GPMF were analyzed for their moisture, ash, protein, fat, and carbohydrate content. The results obtained are shown in Fig. 1. Proximate analysis of optimized and control biscuits are presented in Fig. 2. The values obtained for carbohydrate content, fat, protein, moisture, and ash content for control product were 56.8%, 21.5%, 15.8%, 4.30%, and 1.68%, respectively, whereas for optimized product were 63.32%, 15.81%, 14.77%, 4.16%, and 1.93%, respectively. It can be observed that carbohydrate and fat of the optimized product differed significantly ($p < 0.05$) from the control. The increase in carbohydrate content in the optimized product was due to the addition of maltitol and PD which was calculated as part of carbohydrate (by difference). Replacement of fat with PD resulted in 30% reduction of fat in the optimized product. The non-significant decrease in protein content of the optimized product was due to the replacement of refined wheat flour with multigrain flour as the former contains more amount of gluten protein than WWF.

4.6. Energy value

Bomb calorimetry directly measures the heat of combustion of a food and thus gives values for gross energy whereas metabolizable energy is the total calorie content minus calories that are presumably

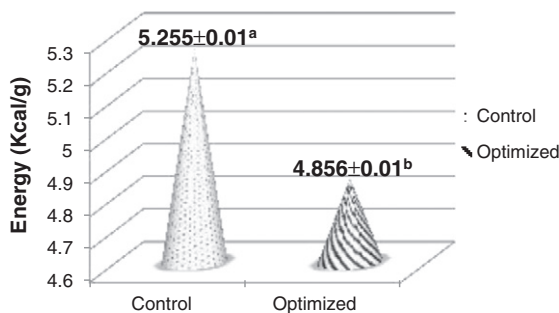


Fig. 3. Gross energy value of control and optimized biscuits. Data are presented as mean (\pm SEM) ($n = 3$). ^{ab} means for individual composition differ significantly ($p < 0.05$).

not absorbed and excreted as waste by the body [35]. Fig. 3 represents the gross calorific value for control and optimized product. The mean values of control and optimized biscuits were 5.25 and 4.85 kcal/g, respectively, which was significantly different ($p < 0.05$) from each other. The metabolizable energy was 4.83 kcal/g and 4.06 kcal/g of control and optimized biscuits, respectively, which were significantly different ($p < 0.05$) from each other. The metabolizable calorie content in the formulated biscuits was 15.98% lower than that in control.

5. Conclusion

It can be concluded from the above study that multigrain flour consisting of 88% WWF, 6% BGF, and 6% GPMF can be used successfully to replace 100% of the refined wheat flour to formulate healthy reduced-calorie biscuits having the additional benefit of dairy nutrition. Binary blend of maltitol and FOS-sucralose in combination (3:1) were proven successful to replace 100% sugar in the biscuits. PD found to be more suitable as a fat replacer for biscuit-like products than Simplese®. PD can be used up to 30% to partially replace the fat in the product without significantly affecting the sensorial attributes. The formulated functional biscuits had 15.98% lower energy content than the control product. The study demonstrated that highly acceptable reduced-calorie biscuits can be produced by using dairy-multigrain composite flour with maltitol and FOS-sucralose (as sweetener) and PD (as fat replacer).

Conflict of Interest

The authors of this manuscript declare that they have no conflict of interest.

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