Optimization of a process for high fibre and high protein biscuit

Parul Singh · Rakhi Singh · Alok Jha · Prasad Rasane · Anuj Kumar Gautam

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Abstract Biscuits are popular and convenient food products due to their ready to eat nature. Biscuits were prepared from sorghum and whole wheat flour with the addition of spirulina (*Spirulina platensis*) powder to produce high fibre and high protein biscuit. Levels of ingredients in biscuits such as spirulina powder, sorghum flour and guar gum were optimized using response surface methodology (RSM) for its sensory, textural and antioxidant attributes. Sensory attributes as colour intensity ($R^2=0.89$, $P<0.0001$), flavor ($R^2=0.98$, $P<0.0001$), sweetness ($R^2=0.97$, $P<0.0001$), graininess ($R^2=0.99$, $P<0.0001$), and crispiness ($R^2=0.94$, $P<0.0001$), textural attributes as hardness ($R^2=0.95$, $P<0.0001$) and fracturability ($R^2=0.96$, $P<0.0001$), antioxidant activity as DPPH inhibition ($R^2=0.87$, $P<0.0001$) and antioxidant activity as ABTS inhibition ($R^2=0.98$, $P<0.0001$) were significantly related to processing parameters of biscuit. Rheological characteristics (TPA and extensograph) of biscuit dough were measured. Studies indicated that amongst all the processing parameters, the composition of spirulina powder and sorghum flour was found to have significant effect on the responses.

Keywords Spirulina · Sensory properties · Textural properties · Antioxidant activity

P. Singh · A. Jha (✉) · P. Rasane
Centre of Food Science and Technology, Banaras Hindu University,
Varanasi 221 005, India
e-mail: alok_ndri@rediffmail.com

R. Singh
Department of Food Science and Technology, National Institute of
Food Technology Entrepreneurship and Management,
Kundli 131 028, India

A. K. Gautam
Department of AH & Dairying, Banaras Hindu University,
Varanasi 221 005, India

Introduction

Spirulina (*Spirulina platensis*) is a photosynthetic, unicellular, microscopic filamentous blue-green alga (Dartsch 2008). It contains 60–70 % crude protein and is a rich source of vitamins (especially vitamin B₁₂), minerals, chlorophyll, carotenoids, carbohydrates, sterols, the pigments phycocyanin and phycocyanobilin, which are mainly responsible for the antioxidant activities rendering health benefits upon regular consumption (Anbarasan et al. 2011). Many clinical studies suggest several therapeutic effects ranging from reduction of cholesterol and cancer to enhancement of the immune system, an increase in intestinal lactobacilli, nephrotoxicity (Mohan et al. 2006).

Extensive research work has been carried out in relation to incorporation of spirulina with nutritional importance to different food products as development of spirulina based biscuits (Sharma and Dunkwal 2012), spirulina enriched pasta (Zouari et al. 2011) and low fat and high protein frozen yoghurt enriched with papaya pulp and spirulina (Dubey and Kumari 2011). Development of spirulina-enriched fermented acidophilus/bifidus-thermophilus (ABT) milk has been reported by Varga et al. (2002).

Sorghum is a staple food for a large section of the people especially in dry land regions but now a days coarse cereals and millets are gaining popularity because of the presence of soluble and insoluble dietary fibre said to be beneficial in various degenerative diseases. Insoluble fraction of dietary fibre in cereal grains contains large proportion of cellulose, which has beneficial effects in the gastrointestinal tract (Riaz 1999). The soluble fractions, which consists mainly pectin, arabinoxylan and β -glucans has the ability to lower the blood serum cholesterol, through its tendency to increase viscosity in the intestine (Mridula et al. 2007).

Most of the bakery products are used as a source for incorporation of different nutritionally rich ingredients for their diversification (Sudha et al. 2007a). Several studies indicated the possibility of incorporating sorghum in wheat flour at various levels for producing bread, biscuits, and other

snacks (Elkhalifa and El-Tinay 2002; Mridula et al. 2007; Serrem et al. 2011; Yousif et al. 2012). The quality of biscuit is governed by the nature and quantity of the ingredients used. Several studies have been done to study effect of ingredients such as influence of finger millet flour (Saha et al. 2011), fibres from different cereals (Sudha et al. 2007a), maltodextrin and guar gum (Sudha et al. 2007b) on the rheological properties of dough and quality of biscuits.

The objectives of this study were to develop high fibre and high protein biscuits and optimization of process by standardizing the levels of spirulina powder, sorghum flour, and guar gum. Another objective was to determine their effects on various parameters such as sensory (colour intensity, flavour, sweetness, graininess and crispiness), textural (hardness and fracturability) and functional (antioxidant activity) attributes that influences the eating quality of biscuits.

Materials and methods

Materials

Sorghum (*Sorghum vulgare*) flour, whole wheat flour and guar gum were procured from the local market of Varanasi,

Uttar Pradesh (India). Spirulina (*S. platensis*) powder was procured from Green Agro Foods, Pune (India). Other materials used in this study such as powdered sugar, butter, baking powder, baking soda, egg were also procured from the local market of Varanasi, Uttar Pradesh (India).

Processing of biscuits

For experimental biscuit, spirulina powder, guar gum, sorghum flour and whole wheat flour were mixed in the required proportion as per the central composite rotatable design (Table 1) and sugar 34 g, butter 45 g, egg 7 g, baking powder 4 g, baking soda 1 g and vanilla essence 1 ml were also weighed for each formulation. The various blends of spirulina powder, sorghum flour and guar gum were sieved together with other dry ingredients as mentioned above. Butter and sugar powder were mixed for 4 min using MultiMix Hand Mixer (INALSA, Spain), and then 7 g beaten egg along with 1 ml of vanilla essence was incorporated to the cream. The fluffy flavoured cream was thoroughly mixed with the sieved ingredients and kneaded gently with hot water to produce soft and non-sticky dough. The oven was pre-heated to 200 °C. The dough was then rolled on a platform and cut into round shape. Cut biscuit doughs were then lined upon food grade steel mesh on an oven

Table 1 Central composite rotatable design (CCRD) for the optimization of the high fibre and high protein biscuit

Factors			Responses								
SP (g/100 g)	SF (g/100 g)	GG (g/100 g)	H (g)	F (mm)	AA (% DPPH inhibition)	AA(% ABTS inhibition)	CI	F	S	G	C
5	22.5	1.5	1921.68	0.493	15.24	16.36	7.1	8.82	7.66	6.9	7.64
5	22.5	1.5	1921.65	0.493	15.51	16.72	7.3	8.8	7.64	6.88	7.63
5	22.5	1.5	1921.62	0.492	15.32	16.49	7.5	8.78	7.62	6.86	7.62
7	30	2	2109.91	0.568	19.95	19.97	8.2	7.98	6.78	7.2	7.94
3	30	2	2109.88	0.568	11.87	13.55	6.8	8.98	8.02	7.18	7.93
5	9.88	1.5	1545.14	0.341	15.23	16.25	7.7	8.76	7.6	4.8	6.65
5	22.5	1.5	1921.55	0.491	13.41	16.69	7.9	8.74	7.58	6.84	7.61
5	22.5	1.5	1921.58	0.491	15.17	16.73	6.9	8.72	7.56	6.82	7.6
5	22.5	0.65	1920.98	0.489	15.31	16.65	7.2	8.7	7.54	6.8	7.58
5	22.5	2.34	2121.99	0.569	11.81	16.21	7.4	8.68	7.52	6.78	7.57
1.63	22.5	1.5	1920.78	0.488	7.78	10.34	5	9.02	8.12	6.76	7.56
3	15	2	1734.44	0.417	11.89	13.51	6.6	8.96	8	5.7	7.24
7	15	1	1732.34	0.417	19.95	19.92	8.4	7.96	6.76	5.68	7.22
5	35.11	1.5	2298.14	0.644	15.55	16.62	7.6	8.66	7.5	7.4	8.15
3	15	1	1733.45	0.417	11.76	13.56	6.4	8.94	7.98	5.66	7.2
3	30	1	2109.67	0.568	11.72	13.22	6.2	8.92	7.96	7.16	7.91
5	22.5	1.5	2021.6	0.525	11.25	15.28	6.7	8.62	7.48	6.74	7.98
7	30	1	2109.78	0.568	19.84	19.88	8.6	7.94	6.74	7.14	7.89
7	15	2	1843.45	0.447	19.98	19.43	8.8	7.92	6.72	5.64	7
8.36	22.5	1.5	1920.82	0.487	21.43	23.18	9.7	7.38	6.45	6.72	7.55

SP spirulina powder, SF sorghum flour, GG: guar gum, H hardness, F fracturability, AA antioxidant activity, CI colour intensity, F flavour, S sweetness, G graininess, C crispiness

tray and baked in the oven at 170 °C for 25 min and cooled for 30 min at ambient temperature and packed in polyethylene packets using sealing machine (HP Impulse sealer, Sunray Industries, Bangalore, India) and stored at 25 °C before further analysis. Biscuits of each formulation were made three separate times. For chemical analysis, biscuits were ground using a mortar and pestle to a particle size of less than 1.0 mm before storage.

Rheological characteristics of dough

Texture profile analysis

First dough was sheeted and the sheeted dough was cut into a circular shape using cutters of 4.0 cm diameter, which was used to assess different rheological parameters at 25 °C, using a Texture Analyzer (Stable Micro Systems TA-XT, Exponent Lite, Surrey, UK) by the two-bite 'texture profile analysis' (TPA) method of Bourne (1978). The conditions for TPA: Probe: P/75; 75 mm Compression Platen; load cell, 5 kg; pre-test speed: 1 mm/s; test speed: 5 mm/s; post-test speed: 5 mm/s; target mode: strain; trigger force: 5 g; acquisition rate; 200 PPS (Points per second). Hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess, and chewiness were computed. The texture profile (force vs. time) curve for the treatments was used for the estimation of all the TPA parameters.

Extensograph analysis

Biscuit doughs were analysed for extensibility and resistance to extension using a Kieffer Extensibility Rig (A/KIE) in the Texture Analyser (Stable Micro Systems TA-XT, Exponent Lite, Surrey, UK). The test was performed at 25 °C, using mode: Probe: A/KIE; Kieffer Extensibility Rig; measured force in tension; option: return to start; pre-test speed: 2 mm/s; test speed: 3.3 mm/s; post test speed: 10 mm/s; target mode: distance; distance: 30 mm; trigger force: 5 g; data acquisition rate: 500 PPS. Three replications were conducted for these two parameters, following the AACC (2000).

Evaluation of biscuits

Biscuit hardness and fracturability

The biscuit hardness and fracturability were measured by the bend/snap (also called three-point break) technique of Gains (1991) using the Texture Analyzer. These were measured by Texture Analyser (Stable Micro Systems TA-XT, Exponent Lite, Surrey, UK). The compression strength of biscuits was measured using the following conditions: Test mode: compression; pre-test speed: 1 mm/s; test speed: 3 mm/s; post-test speed: 10 mm/s; target mode: distance; distance: 4 mm; trigger

force: 50 g; data acquisition rate: 500 PPS. Accessory: 3-Point Bending Rig (HDP/3PB) using 5 kg load cell, Heavy Duty Platform (HDP/90). The peak force (g) and the mean distance at break (mm) were recorded.

Antioxidant activity of biscuit by DPPH method

Determination of antioxidant activity of sample was done by 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) inhibition method as suggested by Nishino et al. (2000).

Measurement of antioxidant activity of biscuit by ABTS method

Determination of antioxidant activity of sample was done by 2, 2'-azinobis 3-ethylene benzothiazoline-6-sulphonic acid (ABTS) inhibition method as suggested by Re et al. (1999).

Sensory analysis

The sensory analysis of biscuits was carried out at 25 °C by a semi-trained panel of 10 judges drawn from staff and students of the Centre of Food Science and Technology at Banaras Hindu University, Varanasi (India). Panellists were asked to mark the scores according to the descriptive sensory score card (Table 2) by assigning a score for each quality attribute,

Table 2 Descriptive sensory score card used by panel to evaluate fortified biscuits

Sensory attributes and their definitions	Rating scales
Appearance	
Surface colour intensity: From light green to dark green	Not green = 0; Very green = 10
Flavour ^a	
Roasted cereal flavour: Intensity associated with cereals sufficiently heated to caramelize some starches and sugars	No roasted cereal flavour = 0; Intense roasted cereal flavour = 10
Baked biscuit flavour: Intensity of flavour associated with basic sugar biscuit	No baked flavour = 0; Intense baked flavour = 10
Sweetness	
Sweet: Fundamental taste sensation associated with sugars	No sweet taste = 0; Intense sweet taste = 10
Texture	
Graininess: Amount of small particles perceived by the tongue when the mass is gently compressed between the tongue and palate	Not grainy = 0; Very grainy = 10
Crispiness: Force and sound with which the sample ruptures	Not crispy = 0; Very crispy = 10

^a Flavour have been taken as average of roasted cereal flavour and baked biscuit flavour.

such as surface colour intensity, flavour, sweetness, graininess and crispiness. Descriptive sensory profiling of the biscuit samples was performed using the generic descriptive method of Einstein (1991).

Statistical analysis

For optimization of high fibre and high protein biscuit, central composite rotatable design (CCRD) based on response surface methodology (RSM) was used. The experiments comprised of 20 trials as presented in Table 1. Optimization was done for textural, antioxidant and sensory properties of biscuit. Fitting of mathematical models and finally selecting variable levels by optimizing the response was employed as per the method given by Khuri and Cornell (1987).

A second-order polynomial model was fitted to study the relationship between the responses and the 3 factors (spirulina powder, sorghum flour, and guar gum). Design Expert 8.0.4 software was used to generate the design of the experiments, to fit model by multiple regression and to analyze the response surfaces. The response surfaces were drawn by plotting y as a function of two variables by keeping the third variable constant. The regression analysis of the responses was conducted by fitting linear and quadratic models as suitable in the case of the respective responses. Three replications were conducted for each trial of experiments.

Proximate analysis

Proximate composition of biscuits was determined by using standard methods. Moisture content was determined by the one-stage air oven procedure, fat were determined by soxhlet extraction, and dry ashing according to AACC (2000). Total dietary fibres and protein (by Kjeldahl method) was determined by AOAC (2008). Carbohydrate was determined by difference (Byrant et al. 1988). Vitamin B₁₂ was determined by the method given by Kumar et al. (2010). Total sugar was determined by using 3,4-Dimethylphenol as a reagent (Ramachandran and Gupta 1992). Cholesterol was determined by AOAC (1996) method. Minerals such as calcium, iron, potassium, magnesium, sodium and phosphorus were determined by microwave plasma atomic emission spectroscopy (MP-AES) method, given by AOAC (2003). The optimized biscuit was compared against the average nutrient composition of some common branded biscuits available in India.

Results and discussion

Shape and size

The biscuits obtained were circular in shape with an average radius of 0.9 to 1 cm and average height of 2.5 to 3 mm.

Rheological characteristics of dough

Hardness is the peak force measured during the first compression cycle (i.e., first bite) and is measured in g. Adhesiveness is the negative area of first cycle and is measured in g sec. Cohesiveness is the ratio between positive area of 2nd and 1st cycle and is dimensionless. Springiness is the ratio between time difference of 2nd and 1st cycle and is dimensionless. Gumminess is the product of hardness and cohesiveness and is measured in g. Chewiness is the product of hardness, cohesiveness and springiness and is measured in g. Resilience is how well a product “fights to regain its original position” and is measured as the ratio of area, covered under maximum force to base line and area, covered under initial point to maximum force and there is no unit for this parameter.

It was observed that the hardness increased when wheat flour content in the biscuit formulation was increased (Table 3). Increased gluten level through wheat flour, increased the cohesive property of the dough. The dough became more cohesive with higher level of wheat flour owing to the plasticity of gluten (Table 3). Variation in TPA profile might be due to lipid content as well as protein and starch quality. It has been reported that fat coats the surface of the flour particles inhibiting the development of the gluten proteins (Sudha et al. 2007b). Water uptake by the flour, in the absence of sufficient fat, would therefore result in dough hardness (O'Brien et al. 2003). Besides, other flour components such as starch and lipids along with sucrose can also affect the distribution of water in biscuit dough (Fustier et al. 2009).

Extensograph analysis gives information about the viscoelastic behaviour of dough. A combination of good resistance and good extensibility results in desirable dough properties (Rosell et al. 2001). It was observed that the biscuit dough is little resistant to extension and possessed low extensibility due to high percentage of fat (shortening) in the dough which restricts the formation gluten network responsible for dough extensibility but it also increased with increasing the level of wheat flour (Table 3). Similar results were also found by Karaoglu (2006). The dough tested was meant for preparation of biscuits and thus low extensibility is desired. In general, high extensibility of dough is required in bread making having high gluten content to make sure the entrapment of air during fermentation, while low extensibility of dough is required for biscuit dough and therefore it is known as ‘short dough’.

Effect of ingredients on textural properties of high fibre and high protein biscuit

Hardness

Hardness refers to the force required to compress the material by a given amount. Hardness of the high fibre and high protein

Table 3 Effect of spirulina powder, sorghum flour, wheat flour and guar gum on the textural and rheological characteristics of biscuit dough

Composition				Textural							Extensograph	
SP (g/100 g)	SF (g/100 g)	WF (g/100 g)	GG (g/100 g)	HRD (g)	ADH (g s)	RES	COH	SPR	GUM (g)	CHW (g)	EXT (mm)	RTE (g)
5	22.5	77.5	1.5	14.36	−256.73	4.48	0.28	28.90	4.15	1.20	−1.26	5.84
7	30	70	2	13.87	−155.26	4.69	0.25	24.45	3.51	0.85	−0.06	5.53
3	30	70	2	14.22	−238.08	4.76	0.25	30.28	4.02	1.21	−1.13	5.56
5	9.88	90.11	1.5	16.27	−283.10	4.92	0.32	29.16	4.27	1.24	−7.98	13.03
5	22.5	77.5	0.65	14.29	−179.80	4.27	0.26	27.43	3.64	1.00	−0.03	5.73
5	22.5	77.5	2.34	14.65	−201.24	5.62	0.26	24.66	3.85	0.95	−0.88	5.84
1.63	22.5	77.5	1.5	14.50	−210.45	4.50	0.27	32.12	4.45	1.43	−1.90	5.68
3	15	85	2	16.13	−167.17	4.85	0.28	25.04	4.34	1.08	−2.61	7.86
7	15	85	1	15.28	−245.88	4.76	0.28	26.38	3.78	0.99	−3.44	7.73
5	35.11	64.9	1.5	12.73	−176.47	4.82	0.24	27.14	3.52	0.95	−1.28	4.83
3	15	85	1	15.07	−160.60	4.15	0.30	35.66	4.89	1.74	−2.32	7.37
3	30	70	1	13.23	−171.11	4.71	0.24	31.56	4.15	1.31	−0.95	5.01
7	30	70	1	13.58	−150.95	4.17	0.26	24.91	3.55	0.88	−1.70	5.65
7	15	85	2	16.13	−247.48	4.69	0.31	27.65	3.97	1.10	−2.66	9.64
8.36	22.5	77.5	1.5	14.36	−134.61	5.07	0.26	24.64	4.03	0.99	−5.45	7.25

SP spirulina powder, SF sorghum flour, WF wheat flour, GG guar gum, HRD Hardness, ADH adhesiveness, RES resilience, COH cohesiveness, SPR springiness, GUM gumminess, CHW chewiness, EXT extensibility, RTE resistance to extension

biscuit varied between 1545.14 and 2298.14 g (Table 1). The minimum hardness was obtained for experiment no. 6, and maximum was obtained for experiment no. 14 (Table 1). Table 4 depicts the coefficient estimates of hardness for high fibre and high protein biscuit.

Model F value of 22.92 (Table 5) implied that model was significant. The coefficient of estimation of biscuit hardness showed that the level of spirulina powder, sorghum flour and guar gum had positive effect on the hardness of biscuit with

effect of sorghum flour being most pronounced. It was observed that with increasing the level of sorghum flour and guar gum the hardness increases. These results are in conformity with the findings of Saha et al. (2011) who showed that hardness increases with the incorporation of 70 % finger millet flour. It has been reported that hardness increases with increasing the level of guar gum (Sudha et al. 2007b) and another study have been carried out with the incorporation of rice and barley bran (30 to 40 %) that increases the biscuit hardness (Sudha et al. 2007a).

Table 4 Coefficient estimate and model statistics for high fibre and high protein biscuit

Factor	Coefficient estimate								
	H (g)	F (mm)	AA (% DPPH inhibition)	AA (% ABTS inhibition)	CI	F	S	G	C
Intercept	1923.18	0.49	13.98	15.52	7.11	8.86	7.73	6.79	7.65
A-SP	17.50 ^a	0.01 ^b	5.07 ^c	4.19 ^d	1.46 ^c	−0.49 ^f	−0.63 ^g	0.03 ^h	−0.00 ⁱ
B-SF	319.39 ^a	0.13 ^b	0.04 ^c	0.05 ^d	−0.07 ^c	−0.01 ^f	−0.01 ^g	1.27 ^h	0.66 ⁱ
C-GG	30.66 ^b	0.01 ^c	−0.40 ^a	−0.05 ^a	0.08 ^d	0.00	0.00	0.00	−0.00
AB	−26.94 ^a	−0.01 ^b	—	0.20 ^c	—	0.01 ^d	0.01 ^e	0.01 ^f	0.05 ^g
AC	17.19 ^a	0.00	—	−0.11 ^b	—	−0.01 ^c	−0.01 ^d	−0.01 ^e	−0.04 ^f
BC	−22.35 ^a	−0.01 ^b	—	0.19 ^c	—	0.02 ^d	0.02 ^e	0.02 ^f	0.05 ^g
A ²	−16.29 ^a	−0.01 ^b	—	0.24 ^c	—	−0.33 ^d	−0.19 ^e	−0.09 ^f	−0.06 ^g
B ²	−25.93 ^a	−0.01 ^b	—	0.09 ^c	—	−0.07 ^d	−0.08 ^e	−0.73 ^f	−0.23 ^g
C ²	24.86 ^a	0.01 ^b	—	0.03 ^c	—	−0.03 ^d	−0.04 ^e	−0.04 ^f	−0.03 ^g

SP spirulina powder, SF sorghum flour, GG guar gum, H hardness, F fracturability, AA antioxidant activity, CI colour intensity, F flavour, S: sweetness, G graininess, C crispiness. Values represented with different superscripts in a row differ significantly ($p < 0.01$)

Table 5 ANOVA and model statistics for high fibre and high protein biscuit

Term	Responses								
	H (g)	F (mm)	AA (% DPPH inhibition)	AA (% ABTS inhibition)	CI	F	S	G	C
Model	Quadratic	Quadratic	Linear	Quadratic	Linear	Quadratic	Quadratic	Quadratic	Quadratic
F Value	22.92	27.48	38.03	59.29	46.03	92.99	54.00	179.71	18.54
P > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Mean	1942	0.49	15	16.53	7.4	8.56	7.46	6.58	7.57
Standard deviation	51.75	0.01	1.41	0.55	0.36	0.068	0.0979	0.074	0.11
CV%	2.66	3.83	9.41	3.33	4.96	0.80	1.31	1.136	1.58
R ²	0.95	0.96	0.87	0.98	0.89	0.98	0.97	0.99	0.94
Adjusted R ²	0.91	0.92	0.85	0.96	0.87	0.97	0.96	0.98	0.89
Predicted R ²	0.73	0.76	0.82	0.91	0.84	0.95	0.87	0.96	0.82
Lack of fit	0.20	0.12	0.80	0.52	0.77	0.58	0.09	0.16	0.88

H hardness, F fracturability, AA antioxidant activity, CI colour intensity, F flavour, S sweetness, G graininess, C crispiness

Fracturability

Fracturability refers to the ease with which the material will break. Fracturability of the high fibre and high protein biscuit ranged between 0.341 and 0.644 mm (Table 1). The minimum fracturability was obtained for experiment no. 6, whereas maximum was obtained for experiment no. 14 (Table 1). Table 4 depicts the coefficient estimates of fracturability for high fibre and high protein biscuit.

Model F value of 27.48 (Table 5) implied that model was significant. The coefficient of estimation of biscuit fracturability showed that the level of spirulina powder, sorghum flour and guar gum had positive effect on the fracturability of biscuit with effect of sorghum flour being most pronounced. It was observed that with increasing the level of sorghum flour and guar gum the fracturability increases. Sudha et al. (2007b) reported that fracturability of biscuit was increased with the level of guar gum due to its emulsification property. Brennan and Samyue (2004) found that dietary fibre enriched biscuits had higher fracturability due to reduced resistance for snapping during texture analysis. Similarly, the high content of fibre in the product under study also enhanced its fracturability.

Effect of ingredients on antioxidant properties of high fibre and high protein biscuit

Antioxidant activity as DPPH inhibition

Antioxidant activity of the high fibre and high protein biscuit varied from 7.78 % to 21.43 % DPPH inhibition (Table 1). The minimum value was obtained for experiment no. 11, and maximum was obtained for experiment no. 20 (Table 1). Table 4 depicts the coefficient estimates of antioxidant

activity as DPPH inhibition for high fibre and high protein biscuit.

Model F value of 38.03 (Table 5) implied that the model was significant. The coefficient of estimation of biscuit antioxidant activity (% DPPH inhibition) showed that the level of spirulina powder and sorghum flour had positive effect. The level of guar gum had a non-significant negative effect on the antioxidant activity (% DPPH inhibition).

Figure 1a shows the response surface plot for antioxidant activity (% DPPH inhibition) as influenced by the level of spirulina powder and sorghum flour. It can be observed that the major effect was of spirulina powder as with increase in its amount, antioxidant activity of biscuit increased remarkably. This antioxidant potential has been attributed mainly due to phycocyanin and phycocyanobilin prepared from spirulina and can function as a potent inhibitor of NADPH oxidase which acts as the chief source of pathological oxidant stress in a wide range of health disorders (Dartsch 2008; Anbarasan et al. 2011).

Antioxidant activity as ABTS inhibition

Antioxidant activity of the high fibre and high protein biscuit varied from 10.34 % to 23.18 % ABTS inhibition (Table 1). The minimum value was obtained for experiment no. 11, and maximum was obtained for experiment no. 20 (Table 1). Table 4 depicts the coefficient estimates of the antioxidant activity as ABTS inhibition for high fibre and high protein biscuit.

Model F value of 59.29 (Table 5) implied that model was significant. The coefficient of estimation of biscuit antioxidant activity (% ABTS inhibition) showed that the level of spirulina powder and sorghum flour had positive effect. The level of guar gum had a non-significant negative effect on the antioxidant activity (% ABTS inhibition).

Fig. 1 Response surface plots showing the effect of level of spirulina powder and sorghum flour on **a** antioxidant activity as % DPPH inhibition and **b** antioxidant activity as % ABTS inhibition

Figure 1b shows the response surface plot for antioxidant activity (% ABTS inhibition) as influenced by the level of sorghum flour and spirulina powder. It can be observed that the major effect was of spirulina powder as with increase in its amount, antioxidant activity of biscuit increased remarkably. Zouari et al. (2011) also observed that the free radical scavenging activity of spirulina enriched pasta increased with the increasing level of spirulina.

Effect of ingredients on sensory properties of high fibre and high protein biscuit

Colour intensity

Colour intensity refers to the surface colour of the biscuit i.e. from light green to dark green due to spirulina powder. Sensory score for colour intensity ranged from 5.0 to 9.7 (Table 1). The minimum score was obtained for experiment no. 11, and maximum was obtained for experiment no. 20 (Table 1). Table 4 depicts the coefficient estimates of the colour intensity for high fibre and high protein biscuit.

Model F value of 46.03 (Table 5) implied that model was significant. The coefficient of estimation of biscuit colour

intensity showed that the level of spirulina powder and guar gum had positive effect and only the level of sorghum flour had negative effect on the colour intensity of biscuit with effect of spirulina powder being most pronounced. It was observed that addition of higher amount of spirulina powder gave a higher score for colour intensity. It has been reported that some natural pigments are found in *Spirulina platensis*. These pigments are chlorophyll a, chlorophyll b, carotenoids and phycocyanin which are responsible for the characteristic colors (Albert et al. 2012). These results are in conformity with the findings of Dubey and Kumari (2011) who showed that the frozen yoghurt prepared with 6 % spirulina was found the best on the basis of sensory attributes.

Flavour

Flavour refers to the intensity associated with spirulina powder, cereals sufficiently heated to caramelize some starches and sugars. Sensory score for flavour ranged from 7.38 to 9.02 (Table 1). The minimum score was obtained for experiment no. 20, and maximum was obtained for experiment no. 11 (Table 1). Table 4 depicts the coefficient estimates of flavour for high fibre and high protein biscuit.

Model F value of 92.99 (Table 5) implied that model was significant. The coefficient of estimation of biscuit flavour showed that the level of spirulina powder and sorghum flour had negative effect and only the level of guar gum had positive effect on the flavour. It was observed that as level of guar gum increased, sensory score for flavour also increased but has minor effect and similar results were found when biscuit was fortified with guar gum (Sudha et al. 2007b) and the major effect was of spirulina powder as with increase in its amount, sensory score for flavour of biscuit decreased remarkably. Flavour score decreases with increase in level of spirulina powder as it has a foul odour, and a flavour that most find unappealing and so its addition to biscuit counteracts its original flavour (McCarty et al. 2010). Similarly, Dubey and Kumari (2011) reported that flavour score of spirulina enriched yoghurt decreased with the increasing level of spirulina.

Sweetness

Sweetness refers to the fundamental taste sensation associated with sugars. Sensory score for sweetness ranged from 6.45 to 8.12 (Table 1). The minimum score was obtained for experiment no. 20, and maximum was obtained for experiment no. 11 (Table 1). Table 4 depicts the coefficient estimates of sweetness for high fibre and high protein biscuit.

Model F value of 54.006 (Table 5) implied that model was significant. The coefficient of estimation of biscuit sweetness showed that the level of spirulina powder and sorghum flour had negative effect and only the level of guar gum had positive effect on the sweetness. It was observed that as level of guar

Table 6 Constraints and criteria for optimization of high fibre and high protein biscuit

Name	Goal	Lower Limit	Upper Limit
Spirulina powder	Maximize	3	7
Sorghum flour	Maximize	15	30
Guar gum	Minimize	1	2
Colour intensity	Is in range	5	9.7
Flavour	Is in range	7.38	9.02
Sweetness	Is in range	6.45	8.12
Graininess	Is in range	4.8	7.4
Crispiness	Maximize	6.65	8.15
Hardness	Is in range	1545.14	2298.14
Fracturability	Is in range	0.34	0.64
Antioxidant activity (% DPPH inhibition)	Maximize	7.78	21.43
Antioxidant activity (% ABTS inhibition)	Maximize	10.34	23.18

where, lower weight = 1, upper weight = 1, importance = 3

gum increased, sensory score for sweetness also increased but has minor effect and the major effect was of sorghum flour and spirulina powder as with increase their amounts, sensory score for sweetness of biscuit decreased remarkably. Serrem et al. (2011) reported that protein from the soy flour reacts with the sugar in the biscuits to form maillard reaction products. Thus, soy flour is negatively related with the sweetness score. Similarly, Mohsen et al. (2009) reported that sweetness decreased with increasing level of soy-protein isolate in wheat cookies. Thus, results are in conformity with the above findings.

Graininess

Graininess refers to the amount of small particles perceived by the tongue when the mass is gently compressed between the tongue and palate. Sensory score for graininess ranged from

Table 8 Proximate composition of high fibre and high protein biscuit

Nutrient	Market biscuits* Amount per 100 g	Optimized biscuit Amount per 100 g
Moisture	2.90 ^a g	3.80 ^b g
Total sugar	23.8 ^a g	22.10 ^a g
Dietary fibres	2.20 ^b g	4.50 ^c g
Protein	5.60 ^a g	13.72 ^b g
Fat	20.9 ^a g	20.00 ^a g
Ash	1.50 ^b g	2.50 ^c g
Carbohydrates	59.15 ^a g	55.48 ^a g
Cholesterol	10.00 ^b mg	25.70 ^c mg
Vitamin B 12	0.07 ^a µg	24.60 ^b µg
Calcium	74.12 ^b mg	48.06 ^c mg
Iron	1.62 ^a mg	4.05 ^b mg
Potassium	155.26 ^b mg	267.78 ^c mg
Magnesium	20.02 ^a mg	86.95 ^b mg
Sodium	403.22 ^b mg	572.42 ^c mg
Phosphorus	106.25 ^a mg	160.04 ^b mg

*Market survey of some common branded biscuits available in India

4.8 to 7.4 (Table 1). The minimum score was obtained for experiment no. 6 and maximum was obtained for experiment no. 14 (Table 1). Table 4 depicts the coefficient estimates of graininess for high fibre and high protein biscuit.

Model F value of 179.71 (Table 5) implied that model was significant. The coefficient of estimation of biscuit graininess showed that the level of sorghum flour and guar gum had positive effect and only the level of spirulina powder had negative effect on the graininess of biscuit with effect of sorghum flour being most pronounced. It was observed that as level of sorghum flour increased, sensory score for graininess also increased. These results are in conformity with findings of Ezeogu et al. (2008) who showed that graininess increased with the level of sorghum flour due to hard vitreous endosperm cells of sorghum grain that remain intact during

Table 7 Predicted optimization solutions for high fibre and high protein biscuits optimized using design expert software 8.0.4

Factors			Responses								Desirability		
SP (g/100 g)	SF (g/100 g)	GG (g/100 g)	H (g)	F (mm)	AA (% DPPH inhibition)	AA (% ABTS inhibition)	CI	F	S	G	C		
7.00	30.00	1.00	2100.05	0.565	19.48	20.22	8.43	7.97	6.81	7.21	7.94	0.910	Selected
7.00	30.00	1.01	2099.7	0.564	19.47	20.22	8.44	7.97	6.82	7.21	7.94	0.908	
6.98	30.00	1.00	2100.49	0.565	19.43	20.18	8.42	7.98	6.82	7.21	7.94	0.908	
7.00	29.81	1.00	2095.69	0.563	19.48	20.22	8.44	7.97	6.82	7.20	7.93	0.907	
6.96	30.00	1.00	2100.85	0.565	19.40	20.14	8.41	7.98	6.83	7.21	7.94	0.906	
7.00	29.73	1.00	2093.74	0.562	19.48	20.22	8.44	7.97	6.82	7.20	7.93	0.906	
7.00	29.66	1.00	2092.01	0.561	19.48	20.21	8.44	7.97	6.82	7.20	7.93	0.905	

SP spirulina powder, SF sorghum flour, GG guar gum, H hardness, F fracturability, AA antioxidant activity, CI colour intensity, F flavour, S sweetness, G graininess, C crispiness

milling, which is related to the fact that the sorghum starch granules are encapsulated by hydrophobic cross-linked kafirins. Serrem et al. (2011) reported that graininess in sorghum biscuits was increased with the increasing level of sorghum flour.

Crispiness

Crispiness refers to the force and sound with which the sample ruptures. Sensory score for crispiness ranged from 6.65 to 8.15 (Table 1). The minimum score was obtained for experiment no. 6 and maximum was obtained for experiment no. 14 (Table 1). Table 4 depicts the coefficient estimates of crispiness for high fibre and high protein biscuit.

Model F value of 18.54 (Table 5) implied that model was significant. The coefficient of estimation of biscuit crispiness showed that the level of spirulina powder and guar gum had negative effect and only the level of sorghum flour had positive effect on the crispiness. It was observed that as level of sorghum flour increased, sensory score for crispiness also increased. Serrem et al. (2011) reported that crispiness in sorghum biscuits was probably because of the absence of gluten and increased with the level of sorghum flour. Similarly, crispiness in millet enriched biscuits have been reported to be influenced by the level of incorporation of the millet (Chakraborty et al. 2011).

Optimization

The numerical optimization technique of the Design-Expert software (8.0.5) was used for simultaneous optimization of the multiple responses. ANOVA, constraints and criteria for optimization of high fibre and high protein biscuit were selected as shown in Tables 5 and 6. The desired goals for each factor and response were chosen. Responses obtained after each trial were analyzed to visualize the interactive effect of various parameters on sensory, textural and antioxidant attributes of biscuits. RSM gave 7 optimized solutions for the composition of ingredients with maximum desirability (0.910) for solution no. 1 (Table 7).

Proximate composition of biscuit

The proximate composition of the optimized biscuit compared with average composition of market biscuits was determined and the results are presented in Table 8. It can be seen that the optimized biscuits had high level of dietary fibres (4.50 g) and protein content (13.72 g), as compared to the average composition of the market samples. Also the average composition of micronutrients such as vitamin B₁₂, iron, potassium, magnesium, sodium and phosphorus was higher as compared to the market samples.

Conclusions

Addition of spirulina powder in biscuit increased its protein as well as antioxidant potential in proportion to the level of spirulina powder added. The level of sorghum flour along with wheat flour in biscuit increased its fibre as well as changed the rheological characteristics of biscuit dough and quality of biscuits. However, at high levels of fortification (>7 % spirulina powder and >30 % sorghum flour), it adversely affected the textural and sensory attributes of flavour and graininess. Therefore the limiting level of fortification is needed. Composite flour (30:70) was found best in terms rheological characteristics as well as biscuit quality and found to be in optimized range. Optimized high fibre and high protein biscuit had hardness 2100.05 g, fracturability 0.565 mm, antioxidant activity as 19.48 % DPPH inhibition and 20.22 % ABTS inhibition, colour intensity score 8.43, flavour score 7.97, sweetness score 6.81, graininess score 7.21 and crispiness score 7.94. The results obtained in the present investigation suggest that the high fibre and high protein biscuit could be made with composition of spirulina powder 7 g/100 g, sorghum flour 30 g/100 g and guar gum 1 g/100 g.

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