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Integration of sprouting and extrusion for development of legume-cereal-based pasta

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Abstract

Legumes and whole grains are least preferred in processed food products owing to their lower digestibility, palatability, higher cooking time, and the presence of antinutritional factors. Sprouted flours of cereals and legumes provide nutritional advantages, and can get beyond these limitations. However, compositional differences also cause technical difficulties in processing. Consequently, research is necessary to determine the best combinations. The present work assessed the feasibility of substituting unique combinations of green gram, Bengal gram, pearl millet, and wheat with semolina in pasta. Two optimal formulations were established; one with a combination of sprouted wheat:sprouted Bengal gram:semolina (25:25:50); and the other with sprouted pearl millet:sprouted green gram:semolina (30:20:50). The consumer acceptability study yielded a good sensory score of 7.8 - 8.0 for sensory acceptability. These optimised formulations exhibited a notable increase in protein content (13.26 and 13.48%). The high *in vitro* protein digestibility (77.51 and 76.18%) confirmed higher bioaccessibility of protein. Meanwhile, tannins and phytates exhibited a significant decrease ($p < 0.05$) at 160.64 and 151.118 mg tannic acid equivalent/100 g, and 0.301 and 0.330 g/100 g, respectively. Starch and total sugars were 56.11 and 54.99%, and 8.83 and 10.50%, respectively. The DPPH inhibition activity for optimised combinations was 30.31 and 29.87%. The hardness decreased, and water absorption and cooking losses increased with higher levels of sprouted flour. The inclusion of sprouted flours improved the protein by 26 - 28%, total mineral content by 221 - 282%, and DPPH inhibition activity by 178 - 183%. The optimised combinations could effectively be utilised as nutritionally valuable pasta products based on sensory and quality parameters.

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Introduction

Cereal grains are principal components of diet, and a major contributor to human nutrition. However, they lack certain nutrients and health-promoting components, and need to be supplemented with legumes in processed food formulations for a better nutritional profile. The contemporary culture and the diversification of the grain processing facility shaped a necessity for simple and competent processing techniques for the development of newer products. The consumption of whole cereal grains is always encouraged owing to the associated positive health benefits. These benefits of whole grains are credited to the integrated effects of health-promoting components (micronutrients, dietary fibres, and phytochemicals, *etc*.), which are primarily confined to the outer bran layer and the germ. However, the lower palatability of whole grains, the presence of antinutritional factors, and lower digestibility limit their application in processed food products. Traditional food processing techniques are still being used regularly as they are simple, and can enhance the nutritional profile of processed food products. Germination or sprouting is one such technology that has been used traditionally for the preparation of fresh sprouts for salads. Also, its use at the industrial level is limited to malt preparation for the weaning food and brewery industry. However, the health benefits of such processing techniques are required to be applied in modern processed food products (Benincasa *et al*., 2019). Therefore, the integration of the nutritional benefits of traditional food processing techniques with the convenience of modern food processing

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technologies is a strategic approach towards the production of contemporary processed food products.

Germinated/sprouted grains are getting significant attention from food processors due to their positive health benefits. Sprouted grains are a substantial source of bioactive compounds such as phenolic acids, phytosterols, and tocopherols. Sprouting has been used for millennia to improve the nutritional status of cereals and pulses. It also enhances the digestibility of grains, especially legumes, by converting the complex nutrients into simpler forms, reduces the antinutritional factors, and improves the bioaccessibility of bioactive compounds (Lemmens *et al*., 2018). Germinated grains can be considered as one of the good functional foods that are used to moderate the likelihood of many ailments, and promote health. Sprouting has been known to enhance the levels of health-promoting nutrients such as phenolic compounds in addition to nutrients such as vitamins, minerals, and amino acids. It can also reduce the amount of antinutritional factors like phytic acid, trypsin inhibitors, oligosaccharides, and cyanogenic glycosides (Finnie *et al*., 2019). However, sprouting alters the functional, rheological, structural, and nutritional characteristics of grains. Therefore, it is important to elucidate the modifications occurring in pasta formulation enriched with sprouted grains through various analytical approaches, and to investigate such alterations in enriched pasta to establish optimal combinations. Researchers have recently been attracted to using sprouted grains in a variety of processed foods, including pastas, breads, and biscuits. Successful partial substitutions of sprouted oats and quinoa have produced biscuits and breads, respectively, which are palatable and high in nutrients. Although grains like sprouted quinoa and sorghum have been used to create pasta that is easily digested, and high in nutrients, the combination of cereals and legumes is not as well studied in this area. As a result, the present work extensively examined unique pairings of sprouted green and Bengal gram with pearl millet and wheat.

Pasta is a convenient product conventionally prepared from wheat semolina using cold extrusion technology. Among processed food products, pasta is extensively preferred by consumers due to its ease of preparation, variety of shapes, and longer storage stability. Milling or polishing whole grains to produce flour removes the outer bran layer which is naturally rich in minerals, dietary fibres, and bioactive compounds. Therefore, scientifically proven techniques with claimed technical and nutritional benefits can aid in the development of healthy pasta with legumes and whole grains to maximise the associated health benefits of whole grain consumption (Călinoiu and Vodnar, 2018). Conventional pasta products are prepared from wheat semolina; however, health concerns expanded their dimensions with the use of newer or non-conventional health-promoting ingredients in the pasta formulations (Giuberti *et al*., 2015). In the present work, to harness the nutritional benefits of sprouting and the convenience of cold extrusion, an attempt was done in order to produce pasta products using sprouted cereals and legumes.

Materials and methods

Raw materials and sprouting of grains

Wheat semolina, Bengal gram, green gram, wheat, and pearl millet were purchased from a local market. The grains were cleaned and graded in a cleaner-cum-grader machine, followed by washing. The washed grains were then soaked in water with a grain:water ratio of 1:20 for 6 h at 30 ± 2°C. Next, the grains were washed thrice to remove the froth. The grains were then spread on perforated trays lined with muslin cloth, and incubated for sprouting in a temperature-cum-humidity controlled chamber at 30 ± 2°C and 80% RH for an optimum time of sprouting depending on the type of grain. Sprouted grains were washed with disinfectant followed by rinsing with water. The sprouted grains were then placed in a tray dryer at 50°C until the moisture content reached 8%. The grains were then ground in a laboratory mill, passed through a sieve BSS 52, and stored in airtight containers at low temperatures until subsequent analyses.

Preparation of pasta

To determine the minimum and maximum levels of sprouted flour replacement in pasta formulation, preliminary trials were carried out based on quality factors such as sensory, integrity of structure after cooking, and nutritional value. These findings led to an experimental investigation of a 50% substitution using various combinations of sprouted flour. Wheat semolina was supplemented with sprouted flours of wheat (SW) and Bengal gram (SBG), and pearl millet (SPM) and green gram (SGG), in different proportions (25:25:50, 20:30:50,

30:20:50, 10:40:50, 40:10:50, and 50:50:0) for each set of experiments. Semolina and sprouted grains flour blended at different levels were uniformly combined and sieved together to ensure an even mixing of dry fractions. The homogenously mixed flour formulation was added with distilled water to attain a moisture content of 32% with a combination of hydroxypropyl methyl cellulose and guar gum at a level of 0.5% each. The wet flour formulation was again mixed in a pasta extruder (La Monferrina, Italy) for 15 min. The hydrated pasta formulation was passed through the pasta extruder attached to a spiral die. Extruded pasta samples were subjected to drying in a tray dryer at 50°C to attain a final moisture content below 10%. The ready pasta samples were filled in polyethylene terephthalate packages of 250 g capacity for subsequent quality assessment. Pasta with 100% wheat semolina was treated as a control or reference sample for comparison purposes.

Nutritional analysis

Moisture, fat, protein, and ash contents were estimated by AOAC (2001) method nos. 925.09, 969.24, 950.48, and 923.03, respectively. The free fatty acids were analysed following AOAC (2001) method. *In vitro* protein digestibility was determined according to Akeson and Stachman (1964). The starch content and total sugars were determined according to Clegg (1956).

Colour index

Colour index coordinates L^*, a^*, and b^* of sprouted pasta samples were measured using a Hunter Lab colorimeter (45/0L, USA), which was previously standardised using white and black control tiles. The L^* indicates the lightness of the sample on a scale of 100 to 0, where 100 signifies absolute white and 0 signifies black. The a^* indicates redness (positive values) and greenness (negative values), while the b^* indicates yellowness (positive values) and blueness (negative values). The difference in the L^*, a^*, and b^* values between the test and reference samples was calculated as total colour difference (ΔE) using the Minolta equation (Sethi *et al*., 2020), Eq. 1:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad \text{(Eq. 1)}$$

where, $\Delta L = L\text{-}L_0$, $\Delta a = a\text{-}a_0$, $\Delta b = b\text{-}b_0$; L, a, and b = values of sprouted pasta samples; and L_0, a_0, and b_0 = values of control pasta sample.

Antioxidant activities

The DPPH scavenging activity (Yamaguchi *et al*., 1998), total phenolics (Singleton *et al*., 1999), ferric-reducing ability (Benzie and Strain, 1996), and total flavonoids (Lees and Francis, 1972) were assessed to evaluate the antioxidant potentials of sprouted grains and pasta supplemented with sprouted grains.

Antinutritional compounds

The tannins were estimated by the Folin-Ciocalteu method according to Makkar *et al*. (1993), with slight modifications. The sample extract was added with distilled water, Folin-Ciocalteu phenol reagent, and 35% sodium carbonate solution, and further diluted with distilled water. The mixture was mixed in a vortex mixer, and held for 30 min at room temperature. For the estimation of tannins, standard solutions of tannic acid at a concentration ranging from 20 - 100 µg/mL were prepared. The absorbance of the samples and standards were measured using a UV-visible spectrophotometer (Shimadzu UV-2600i) at 700 nm against the distilled water as blank. The concentration of tannins in a sample was determined in terms of mg/mL of tannic acid. The phytates were estimated according to Davis and Reid (1979). For this, sample extract diluted with distilled water was added with ammonium ferric sulphate. The contents were thoroughly mixed and held in boiling water for 20 min, followed by immediate cooling. Afterward, the mix was added with amyl alcohol and ammonium thiocyanate, followed by centrifugation at 1,000 rpm for 5 min. Standard solutions of sodium phytate at concentrations varying from 10 - 200 µg/mL were prepared. The absorbance of the samples and standards were recorded at 465 nm against the amyl alcohol as blank.

Cooking quality

The optimal cooking time was estimated using AACC (2000). Dehydrated pasta samples were boiled in distilled water. Optimal cooking time was measured as a time required where the inner hard core disappeared when the sample was pressed between two flat surfaces. This indicated the gelatinisation of starch present in the pasta sample.

The water absorption upon cooking was assessed according to Petitot *et al*. (2010). The amount of water absorbed by the samples was calculated by evaluating the change in weight of the

cooked sample in comparison to raw (uncooked) pasta. The cooking losses were determined by dehydrating the cooking water in an oven at 100°C, and estimating the weight of residual solids on a percentage basis of the initial sample.

Textural attributes

The cooked pasta samples were measured for textural properties in terms of texture profile analysis (TPA) using Stable Micro Systems, TA-XT2i texture analyser integrated with a 50 kg load cell. The test samples were cooked for an optimal cooking time in water (1:25). To avoid the effect of time on the textural parameters of cooked pasta, the measurements were conducted 5 min after cooking (Sethi *et al*., 2020). A compression plate (P 75 mm) was performed with the following test settings: pre-test, test, and post-test speed of 2.0, 1.0, and 5.0 mm/s, respectively; strain 50% in compression mode; and a trigger force of 5 g. A total of eight replications were performed for every sample, and each set of measurements for a sample was completed within 10 min of cooking the sample.

Sensory evaluation and consumer acceptability studies

The sensory assessment and consumer acceptability studies were accomplished to obtain the buyer response toward the sensory satisfaction of the sprouted pasta samples in comparison to the reference pasta sample. For sensory analysis, 25 semi-trained evaluators from the age group 20 - 40 years with no food allergies were selected. The sprouted grain pasta samples were reviewed for their sensory acceptability concerning colour/appearance, flavour, texture, taste, and overall acceptability as sensorial attributes. The samples were cooked in water for an optimal time as assessed previously, and served in warm conditions to the evaluators. The samples in random coding were assessed on a typical nine-point hedonic scale, with a score of 9 as an indicator of extreme likeness, and 1 being extreme dislikeness. Optimised pasta samples were evaluated for consumer acceptability during two consecutive Farmer's fairs at Punjab Agricultural University, Ludhiana, India. Seventy-five participants of varied age groups ranging from 12 to 60 years participated in the study. The pasta samples were cooked and added with mild condiments for consumer acceptability studies in the way pasta products are consumed in general. The samples were served at a warm temperature for accurate assessment.

Microstructural examination

The optimised sprouted grains pasta samples and control pasta were evaluated for microstructural analysis with a JSM -6610 LV, JEOL, scanning electron microscope. The images of dried pasta samples were attained at magnifications ranging from 100× to 1,500×. For this, the samples were dried to eliminate the probable moisture at 45°C for 24 h. The pasta samples were fixed with tape to a circular specimen stub, and coated vertically with gold nanoparticles. The images were obtained at 5.0 KV acceleration voltage.

Statistical analysis

Results are expressed as mean ± standard error of three replications in each set of experiments. ANOVA test was used for the numerical evaluation of the variations at 5% level of significance. Means were segregated for analogous sets using Tukey's *t*-test with SPSS 16.0.

Results and discussion

Nutritional and quality characteristics of sprouted flour

The sprouting parameters were optimised based on quality parameters such as length of sprouts, nutritional parameters (protein, fat, and ash contents), *in vitro* protein digestibility, water absorption capacity, free fatty acids, viscosity, and colour index. The optimum time for SGG, SBG, SPM, and SW was 24, 36, 36, and 36 h, respectively, at 30°C and 80% RH. The quality parameters of sprouted flours are represented in Table 1. Different sprouted grains exhibited distinct physico-chemical profiles. Under optimum conditions, the length of different grain sprouts was measured between 20.00 and 22.45 mm. The flour of SGG, SBG, SPM, and SW expressed water absorption capacities of 213.67, 237.03, 150.55, and 132.74%, respectively. SGG showed the maximum protein (27.66%) and total mineral (4.03%) content, while SPM expressed the maximum fat content (4.29%). The *in vitro* protein digestibility of SGG, SBG, SPM, and SW was measured at 74.04, 74.98, 68.56, and 76.24, respectively. Proteolysis is known to increase the digestibility of proteins after the sprouting process. These changes are the results

Table 1. Quality parameters of different sprouted flours.

Sprouted flour	Length of sprout (mm)	Protein (%)	Fat (%)	Total mineral (%)	*In vitro* protein digestibility (%)	Water absorption (%)	*L**	*a**	*b**	FFA (%)
Green gram	20.7 ± 3.58	27.66 ± 0.82	2.09 ± 0.27	4.03 ± 0.06	74.04 ± 1.44	213.67 ± 6.04	39.58 ± 2.50	3.73 ± 0.72	29.42 ± 2.08	0.286 ± 0.00
Bengal gram	22.45 ± 4.56	21.56 ± 0.96	2.74 ± 0.30	3.09 ± 0.09	74.98 ± 1.02	237.03 ± 4.86	40.31 ± 2.23	11.01 ± 0.83	23.5 ± 1.96	0.371 ± 0.00
Pearl millet	20.00 ± 3.91	9.82 ± 0.86	4.29 ± 0.11	1.13 ± 0.04	68.56 ± 0.86	150.55 ± 4.78	50.88 ± 0.87	5.15 ± 0.45	24.23 ± 1.12	0.394 ± 0.00
Wheat	22.06 ± 4.25	10.46 ± 1.04	1.52 ± 0.08	1.52 ± 0.10	76.24 ± 0.82	132.74 ± 5.26	51.50 ± 1.20	9.48 ± 0.71	28.53 ± 0.54	0.344 ± 0.06

Values are mean ± standard deviation.

of the activation of proteases, reduction in the protease inhibitors, and breakdown of complex proteins. The free fatty acid content of different sprouted flours varied from 0.286 to 0.394%.

Different antinutrients are present in cereals and legumes, which reduce the absorption of nutrients in the body by making them unable for absorption. Sprouting is a technique that reduces the antinutrients in legumes and cereals. It changes the molecular arrangements to the macroscopic level. Sprouting reactivates the biochemical reactions leading to the breakdown of various constituents, and hence reduction in antinutrients. Tannins were decreased in all the grains after sprouting, as evident from Table 2. Similarly, a considerable decrease in phytates was observed in SGG, SBG, SW, and SPM after sprouting. Similar degradation of phytates in wheat was reported by Faltermaier *et al*. (2015).

Amylases catalyse the breakdown of starch (amylopectin and amylose) into simple sugars (glucose and maltose) in sprouted grains. The starch content in legumes green gram and Bengal gram decreased after sprouting from 44.16 to 35.35% and 54.61 to 42.20%, respectively, while the sugar content in these two legumes increased from 12.62 to 15.56% and 11.25 to 14.69%, respectively (Table 2). The cereals, pearl millet, and wheat also expressed a change in starch and sugar contents after sprouting. In pearl millet and wheat, the starch content decreased from 64.17 to 51.14% and 68.39 to 50.86%, respectively, while the sugar content increased from 3.69 to 4.35% and 5.68 to 7.20%, respectively. A comparative trend was also observed, where sprouted Bengal gram showed an increase in sugar content upon sprouting (Sibian *et al*., 2016).

The antioxidant activities were measured in terms of DPPH scavenging activity (%), total phenolics, Ferric-reducing activity, and total flavonoids. Legumes and cereals expressed increased antioxidant activities after sprouting (Table 2). The DPPH scavenging activity (%), total phenolics, FRAP activity, and total flavonoids expressed a significant improvement after sprouting in SGG, SBG, SW, and SPM. This could have been due to the hydrolysis of conjugated phenolic components in the food grains. Sprouting has been stated as a mode of enhancing the total phenolic content and DPPH inhibition activity of wheat (Alvarez-Jubete *et al*., 2010). Ramesh *et al*. (2011) also observed increased antioxidant activities (reducing power, DPPH scavenging activity, total phenolics, and total antioxidants) in green gram after sprouting.

Colour profile of pasta

The effect on the colour profile of pasta enriched with sprouted legumes and cereals is depicted in Figure 1a. A prominent difference was observed in the colour profile of the pasta samples prepared with the addition of sprouted grains with reference to the control sample. The L^* values decreased significantly ($p < 0.05$) with the addition of sprouted legumes and cereal flours. The L^*, a^*, and b^* of the pasta added with different combinations of SGG and SPM varied from 34.18 to 40.86, 2.49 to 3.28, and 16.14 to 18.25, respectively. However, in comparison, an increased degree of lightness was observed in pasta samples prepared with SBG and SW. The L^*, a^*, and b^* values of the pasta prepared with different combinations of SBG and SW varied between 41.23 to 48.18, 5.87 to 7.54, and 20.00 to 22.39, respectively. The addition of SBG significantly increased the redness in the pasta as depicted by an increase in the a^* value, while the addition of SGG and SPM augmented the greenness and significantly lowered the yellowness in the pasta samples. These results indicated significant deviation in the colour profile of pasta samples enriched with the sprouted legume and cereal flours in comparison to control pasta, as depicted by total colour difference (ΔE). The $\Delta E\%$ value ranged from 16.57 to 22.26% in the case of SBG and SW, and from 22.36 to 28.86% in the case of pasta prepared with SGG and SPM. Variations in the colour coordinates of pasta samples may be credited to the pigments present in different sprouted flours. Gull *et al*. (2015) also observed a decrease in L^* values, and an increase in a^* and b^* values in pasta supplemented with pearl millet, finger millet, and carrot pomace. A significant increase in the a^* value was observed in pasta samples supplemented with sprouted sorghum flour (Marengo *et al*., 2015). Since the conventional pasta is creamish white, the light colour was preferred by the consumers. However, with increasing awareness towards health foods, especially using traditional grains has taken a front seat, the pasta with darker colour hues were also equally liked and accepted by the consumers. Also, the darker tones are even indicative of the usage of higher levels of non-conventional grains and the natural appearance of the formulation.

Table 2. Antinutritional factors, antioxidant activities, and starch and sugar contents in raw, sprouted flour, and optimised products.

		Antinutrient		Carbohydrate		Antioxidant activity			
	Sample	**Tannin (mg tannic acid equivalent/100 g)**	**Phytate (g/100 g)**	**Starch (%)**	**Total sugar (%)**	**DPPH inhibition activity (%)**	**Total phenolic (mg gallic acid equivalent/100 g)**	**FRAP (mg ascorbic acid equivalent/100 g)**	**Flavonoid (mg quercetin/100 g)**
Raw flour	Green gram	545.404 ± 30.27	0.724 ± 0.06	44.16 ± 1.48	12.62 ± 0.19	48.17 ± 0.26	316.48 ± 24.07	317.09 ± 23.22	146.13 ± 7.41
	Bengal gram	631.516 ± 21.90	1.121 ± 0.03	54.61 ± 1.31	11.25 ± 0.15	50.25 ± 0.69	372.39 ± 21.95	348.63 ± 8.28	233.69 ± 6.57
	Wheat	434.656 ± 24.53	1.186 ± 0.01	68.39 ± 1.27	5.68 ± 0.06	38.99 ± 0.57	171.94 ± 24.00	286.93 ± 11.84	98.06 ± 4.79
	Pearl millet	529.132 ± 23.85	0.791 ± 0.15	64.17 ± 0.58	3.69 ± 0.05	47.39 ± 0.80	237.02 ± 11.94	362.18 ± 14.56	221.80 ± 10.04
Sprouted flour	Green gram	354.239 ± 15.22	0.547 ± 0.23	35.35 ± 1.53	15.56 ± 0.23	55.19 ± 1.41	408.92 ± 15.91	601.26 ± 19.26	229.01 ± 13.19
	Bengal gram	384.179 ± 17.22	0.712 ± 0.04	42.20 ± 0.88	14.69 ± 0.23	58.42 ± 1.97	406.54 ± 28.23	727.38 ± 11.28	316.96 ± 13.20
	Wheat	185.076 ± 10.01	0.682 ± 0.02	50.86 ± 1.55	7.20 ± 0.16	48.42 ± 2.16	198.03 ± 16.50	330.02 ± 17.16	132.84 ± 4.80
	Pearl millet	297.486 ± 16.24	0.530 ± 0.13	51.14 ± 0.45	4.35 ± 0.04	53.14 ± 1.77	367.75 ± 19.08	546.39 ± 23.11	311.70 ± 16.02
SW:SBG:S (25:25:50)	Pasta 1	160.643 ± 12.22	0.301 ± 0.07	56.11 ± 0.35	8.83 ± 0.15	30.31 ± 1.20	112.06 ± 7.49	200.02 ± 7.31	40.00 ± 2.99
SPM:SGG:S (30:20:50)	Pasta 2	151.118 ± 4.41	0.330 ± 0.10	54.99 ± 1.05	10.50 ± 0.16	29.87 ± 1.70	126.08 ± 5.21	158.56 ± 13.45	73.85 ± 5.85
Control (100% semolina)	Pasta	144.26 ± 3.68	0.325 ± 0.22	74.52 ± 1.34	2.98 ± 0.56				

Values are mean ± standard deviation.

Figure 1a. Colour index of pasta supplemented with sprouted flours. C: control; 1A: 25:25:50, 2A: 20:30:50, 3A: 30:20:50, 4A: 10:40:50, 5A: 40:10:50, and 6A: 50:50:0 (Sprouted pearl millet:Sprouted green gram:Semolina). 1B: 25:25:50, 2B: 20:30:50, 3B: 30:20:50, 4B: 10:40:50, 5B: 40:10:50, and 6B: 50:50:0 (Sprouted wheat:Sprouted Bengal gram:Semolina). Measurements were made in quadruplicate at different positions of sample surface, and averaged.

Nutritional composition of pasta enriched with sprouted flour

The nutritional composition of pasta samples formulated with combinations of SBG and SW, and SGG and SPM, is depicted in Table 3. The protein, fat, mineral, and *in vitro* protein digestibility of pasta with SBG and SW content ranged between 11.46 - 15.98, 2.40 - 3.96, 1.26 - 2.50, and 66.09 - 67.54, respectively. The protein, fat, mineral, and *in vitro* protein digestibility content of pasta with SGG and SPM ranged between 11.32 - 16.98, 3.03 - 4.54, 1.72 - 2.40, and 61.65 - 67.73, respectively. A significant increment in all three components *i.e*. protein, fat, and mineral contents of pasta enriched with sprouted flours was detected. The optimised pasta samples with SBG and SW observed a 221.15 and 26.52% increase in ash and protein contents with respect to the reference pasta sample. The pasta samples with optimised levels of SGG and SPM observed a 282.69 and 28.62% increase in ash and protein contents with respect to the reference pasta sample. The addition of legumes and whole grains improved the nutritional profile (protein and total mineral contents) of pasta. Also, sprouting is known to enhance the nutritional profile of the grains, and additionally contribute to the increased values. Comparable results of high protein, fat, and mineral contents in cookies have been reported by Sibian and Riar (2020). The *in vitro* protein digestibility of pasta with sprouted grains was higher than the control pasta, as sprouting improved the *in vitro* protein digestibility due to the breakdown of peptides into smaller fractions. Protein digestibility can be improved by sprouting, and can also alter the protein content and amino acid composition (Ikram *et al*., 2021). The effect of sprouting on protein content can also depend on the equilibrium between enzymatic degradation of protein and protein synthesis (Benincasa *et al*., 2019). Sprouting displayed an increment of 5 - 49% in digestible protein, and demonstrated a positive association with sprouting duration. Additionally, there was a positive correlation between phytate decrease and protein digestibility, suggesting that phytate inhibits digestible protein (Sharma and Gujral, 2020). Similar findings on increased *in vitro* protein digestibility were reported in cookies formulated from germinated triticale, kidney beans, and chickpeas (Sibian and Riar, 2020).

Cooking quality of pasta

Data illustrating the effect of the addition of SGG-SPM and SBG-SW on pasta samples are presented in Table 3. The cooking quality of pasta substituted with non-gluten flours was typically lower than that of semolina pasta, exhibiting low firmness and considerable cooking loss. Since these formulations lack a suitable protein network, starch polymers are less effectively entrapped in the matrix (Marti and Pagani, 2013). The water absorption (%) upon cooking pasta samples supplemented with sprouted legume and cereal flours showed an increment in the water absorption capacity. The samples with an optimum formulation of SGG-SPM and SBG-SW yielded water absorption capacities of

Table 3. Nutritional composition, *in vitro* protein digestibility, and cooking quality of pasta supplemented with sprouted flours.

Sample	Formulation	Protein (%)	Fat (%)	Total mineral (%)	*In vitro* protein digestibility (%)	Water absorption (%)	Cooking time (min)	Cooking loss (%)
Semolina	100%	10.48 ± 0.34^{a}	0.633 ± 0.02^{a}	0.518 ± 0.02^{a}	71.33 ± 1.43^{b}	96.42 ± 3.06^{a}	4.0 ± 0.00^{a}	3.75 ± 0.10^{a}
Sprouted wheat:Sprouted Bengal gram:Semolina	25:25:50	13.26 ± 0.74^{bc}	3.96 ± 0.11^{efg}	1.334 ± 0.05^{b}	77.51 ± 1.24^{ab}	121.07 ± 5.23^{d}	4.5 ± 0.00^{b}	7.10 ± 0.21^{bc}
	20:30:50	13.64 ± 0.74^{bcd}	3.50 ± 0.23^{de}	1.804 ± 0.01^{cd}	76.66 ± 1.89^{ab}	115.18 ± 3.23^{cd}	4.5 ± 0.00^{b}	6.99 ± 0.27^{b}
	30:20:50	12.72 ± 0.37^{abc}	2.40 ± 0.34^{b}	1.670 ± 0.16^{c}	77.34 ± 1.62^{ab}	102.36 ± 4.93^{abc}	4.5 ± 0.00^{b}	7.16 ± 0.16^{bc}
	10:40:50	14.20 ± 0.88^{ef}	2.77 ± 0.30^{bc}	1.669 ± 0.06^{c}	76.09 ± 1.03^{ab}	122.13 ± 3.47^{d}	4.5 ± 0.00^{b}	6.91 ± 0.06^{b}
	40:10:50	11.46 ± 0.62^{ab}	3.24 ± 0.12^{cd}	1.257 ± 0.18^{b}	77.54 ± 1.44^{ab}	110.16 ± 4.61^{abcd}	4.5 ± 0.00^{b}	7.66 ± 0.18^{cd}
	50:50:0	15.98 ± 0.85^{def}	3.63 ± 0.13^{def}	2.498 ± 0.09^{h}	71.19 ± 1.04^{a}	97.73 ± 3.57^{ab}	5.0 ± 0.00^{c}	9.22 ± 0.09^{g}
Sprouted pearl millet:Sprouted green gram:Semolina	25:25:50	14.26 ± 0.74^{cde}	3.27 ± 0.08^{cd}	1.993 ± 0.021^{def}	77.73 ± 1.39^{ab}	117.34 ± 3.91^{cd}	4.0 ± 0.00^{a}	7.83 ± 0.11^{de}
	20:30:50	14.44 ± 0.59^{cde}	3.18 ± 0.10^{cd}	2.114 ± 0.006^{efg}	73.08 ± 1.82^{a}	113.13 ± 3.33^{bcd}	4.5 ± 0.00^{b}	8.21 ± 0.13^{def}
	30:20:50	13.48 ± 0.54^{bc}	3.03 ± 0.07^{bcd}	1.868 ± 0.005^{cde}	76.18 ± 1.98^{ab}	118.56 ± 3.25^{d}	4.5 ± 0.00^{b}	8.42 ± 0.11^{ef}
	10:40:50	16.20 ± 0.52^{bcd}	4.13 ± 0.03^{efg}	2.285 ± 0.010^{fgh}	72.84 ± 3.37^{a}	114.07 ± 4.96^{cd}	4.5 ± 0.00^{b}	8.49 ± 0.16f
	40:10:50	11.32 ± 0.34^{ab}	4.54 ± 0.03^{g}	1.721 ± 0.004^{cd}	72.84 ± 3.37^{ab}	112.15 ± 3.99^{abcd}	4.5 ± 0.00^{b}	7.08 ± 0.18^{bc}
	50:50:0	16.98 ± 0.41^{f}	4.27 ± 0.03^{fg}	2.402 ± 0.074^{gh}	71.65 ± 1.39^{a}	102.11 ± 3.23^{abc}	5.0 ± 0.00^{c}	9.26 ± 0.21^{g}

Values are mean ± standard deviation. Means followed by different superscripts are significantly different ($p < 0.05$).

117.34 and 102.36%, respectively, whereas the control sample showed 96.42% water absorption upon cooking. A similar trend of higher water absorption upon cooking was reported in the pasta enriched with fermented sorghum flour by Marengo *et al*. (2015). The cooking time of supplemented pasta ranged between 4.5 and 5.0 min in comparison to 4 min of cooking time of 100% semolina pasta. The loss of solids in water during cooking is an important quality parameter of pasta products. The cooking losses (%) expressed a significant increase with the increased proportion of sprouted flours in the pasta formulation. The pasta samples prepared with the SGG-SPM combination showed losses between 7.08 and 9.26%, while the pasta prepared with different levels of SBG-SW expressed losses between 7.10 and 9.22%. The control sample showed 3.75% loss upon cooking. Cooking loss of up to 17% was reported in pasta samples enriched with sprouted pearl millet and finger millet flour by Gull *et al*. (2015). The increased cooking loss can be credited to the addition of non-gluten protein in flour formulation. Because gluten forms a structural association that is accountable for maintaining the structural integrity of pasta while cooking, non-gluten flours form a weak network that discharges more solids into the water (Laleg *et al*., 2017). Relatable observations on escalating cooking losses in pasta products have been reported with non-conventional formulations such as split pea and faba bean (Petitot *et al*., 2010).

Textural properties of cooked pasta

The textural properties of cooked pasta supplemented with sprouted grain flours were evaluated as textural profile analysis (TPA). The test measured hardness, springiness, cohesiveness, gumminess, chewiness, and resilience as presented in Table 4. The hardness of 100% semolina pasta (3.23 N) was significantly higher than the pasta supplemented with sprouted flours (varied from 0.94 to 2.96 N). Similar observations were reported by Bouasla *et al*. (2017) in gluten-free rice pasta enriched with legume flours, and by Wójtowicz and Mościcki (2014) in precooked wheat pasta supplemented with legume flours. The type of grain and degree of substitution may affect how sprouted grain affects pasta texture. The structural breakdown of protein and starch brought on by sprouting may have contributed to the observed loss in hardness by forming a less cohesive matrix (Sergiacomo *et al*., 2024). This change might be attributed to the creation of cracks and discontinuities inside the structure due to the fibre fractions and weakened structure caused by the addition of non-gluten components. A significant decrease in gumminess and chewiness upon the addition of sprouted flours was observed in different pasta samples. Wójtowicz and Mościcki (2014) also observed a decrease in firmness and chewiness in pasta samples upon the addition of legume flours such as white beans and yellow peas. The optimised pasta samples with formulations SW:SBG:SM of 25:25:50 and SPM:SGG:SM of 30:20:50 were close to the control sample with 100% semolina, in terms of all the measured parameters such as hardness, springiness, cohesiveness, gumminess, chewiness, and resilience.

Sensory evaluation and consumer acceptability of pasta

The pasta samples supplemented with SGG-SPM and SBG-SW were evaluated for colour/appearance, aroma/flavour, taste, texture, and overall acceptability by a semi-trained sensory panel. The results are depicted in the form of a radar chart in Figure 1b. The sensory evaluation was the important parameter in the standardisation of the pasta formulation with sprouted flour. The sensory scores for colour/appearance, aroma/flavour, taste, texture, and overall acceptability of SGG-SPM pasta ranged between 7.0 - 7.4, 6.5 - 7.3, 6.65 - 7.65, 6.7 - 7.4, and 7.05 - 7.6, respectively, and for SBG-SW pasta ranged between 7.0 - 7.7, 6.9 - 7.4, 6.9 - 7.5, 6.8 - 7.6, and 7.1 - 7.5, respectively. The optimised samples of pasta supplemented with sprouted grain flour were also evaluated for consumer acceptability during an agricultural fare by 75 consumers of different age groups. The average overall acceptability scores on a 9-point hedonic scale for SGG-SPM and SBG-SW were found to be 7.8 and 8.0, respectively. The optimised pasta samples with formulations SW SBG:SM of 25:25:50 and SPM:SGG:SM of 30:20:50 expressed sensory scores for different parameters, and were at par with the control sample. Sprouting of food grains has been reported to improve sensory characteristics due to the conversion of complex carbohydrates into simple sugars (Heiniö *et al*., 2001).

Table 4. Textural properties of cooked pasta supplemented with sprouted flours.

Sample	Formulation	Hardness (N)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Semolina	100%	3.23 ± 0.18^{g}	0.36 ± 0.01^{ef}	0.34 ± 0.02^{c}	111.54 ± 5.14^{f}	39.85 ± 3.30^{e}	0.13 ± 0.01^{c}
Sprouted pearl millet:Sprouted green gram:Semolina	25:25:50	1.41 ± 0.12^{b}	0.28 ± 0.02^{cd}	0.12 ± 0.03^{a}	17.02 ± 5.48^{ab}	4.77 ± 1.74^{a}	0.03 ± 0.01^{a}
	20:30:50	2.04 ± 0.13^{def}	0.17 ± 0.06^{ab}	0.09 ± 0.01^{a}	18.87 ± 4.25^{ab}	3.37 ± 1.93^{a}	0.02 ± 0.00^{a}
	30:20:50	2.94 ± 0.17^{g}	0.36 ± 0.02^{ef}	0.29 ± 0.09^{bc}	86.07 ± 28.25^{ef}	31.13 ± 11.67^{cde}	0.12 ± 0.04^{c}
	10:40:50	1.47 ± 0.10^{b}	0.30 ± 0.02^{de}	0.15 ± 0.02^{a}	22.90 ± 2.80^{ab}	6.90 ± 1.13^{ab}	0.05 ± 0.00^{ab}
	40:10:50	1.63 ± 0.08^{bc}	0.22 ± 0.01^{bc}	0.10 ± 0.01^{a}	16.25 ± 1.73^{ab}	3.59 ± 0.60^{a}	0.02 ± 0.00^{a}
	50:50:0	0.96 ± 0.06^{a}	0.14 ± 0.02^{a}	0.08 ± 0.01^{a}	8.12 ± 1.38^{a}	1.15 ± 0.31^{a}	0.02 ± 0.00^{a}
Sprouted wheat:Sprouted Bengal gram:Semolina	25:25:50	2.36 ± 0.16^{f}	0.40 ± 0.02^{f}	0.31 ± 0.02^{c}	75.75 ± 8.63^{dc}	30.72 ± 4.58^{cde}	0.13 ± 0.01^{c}
	20:30:50	2.22 ± 0.15^{ef}	0.40 ± 0.02^{f}	0.37 ± 0.02^{c}	83.72 ± 7.02^{ef}	33.69 ± 0.93^{de}	0.15 ± 0.00^{c}
	30:20:50	1.93 ± 0.04^{cde}	0.39 ± 0.02^{f}	0.31 ± 0.09^{c}	60.36 ± 19.19^{cde}	23.65 ± 8.59^{cd}	0.11 ± 0.04^{c}
	10:40:50	1.68 ± 0.09^{bcd}	0.39 ± 0.01^{f}	0.28 ± 0.03^{bc}	47.59 ± 8.21^{bcd}	18.58 ± 3.77^{bc}	0.10 ± 0.02^{bc}
	40:10:50	1.01 ± 0.08a	0.30 ± 0.02^{de}	0.14 ± 0.01^{a}	14.37 ± 1.88^{a}	4.38 ± 0.70^{a}	0.03 ± 0.00^{a}
	50:50:0	1.61 ± 0.09^{bc}	0.32 ± 0.01^{de}	0.19 ± 0.01^{ab}	30.50 ± 3.39^{abc}	9.74 ± 1.49^{ab}	0.05 ± 0.01^{ab}

Values are mean ± standard deviation. Means followed by different superscripts are significantly different ($p < 0.05$).

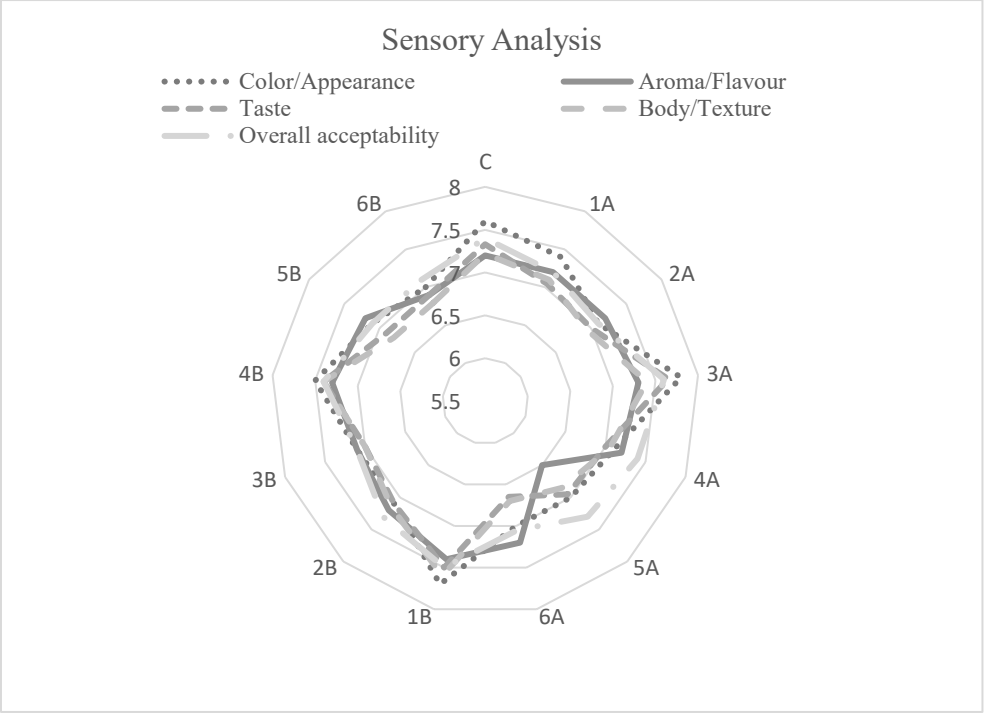

Figure 1b. Radar chart representing sensory characteristics of pasta supplemented with sprouted flours. C: control; 1A: 25:25:50, 2A: 20:30:50, 3A: 30:20:50, 4A: 10:40:50, 5A: 40:10:50, and 6A: 50:50:0 (Sprouted pearl millet:Sprouted green gram:Semolina). 1B: 25:25:50, 2B: 20:30:50, 3B: 30:20:50, 4B: 10:40:50, 5B: 40:10:50, and 6B: 50:50:0 (Sprouted wheat:Sprouted Bengal gram:Semolina).

Antinutritional factors, antioxidant activity, and sugar-starch content of pasta

The optimised pasta with SGG and SPM showed tannins at 160.64 mg tannic acid equivalent/100 g, and phytates at 0.3 g/100 g, while the optimised pasta with SBG and SW showed tannins and phytates at 151.12 mg tannic acid equivalent/100 g, and 0.43 g/100 g, respectively (Table 2). Through the activation of phytase, *de novo* production of phytase, and the leaching of water-soluble phytate during soaking, sprouting can lower phytate concentrations (Gibson *et al*., 2018). Phytate reduction is also significantly influenced by temperature and the length of soaking and sprouting time. Phytate reduction of 6 - 46% has been reported by various researchers in different grains (Elliott *et al*., 2022). Demir and Bilgiçli (2020) also observed a reduction in phytate content (antinutrient) in quinoa upon germination. Also, pasta enriched with sprouted quinoa flour expressed reduced phytate content.

The optimised pasta with SGG and SPM showed DPPH scavenging activity, total phenolics, FRAP, and total flavonoids at 30.31%, 112.06 mg gallic acid equivalent/100 g, 200.03 mg ascorbic acid equivalent/100 g, and 40.00 mg quercetin/100 g, respectively, while the optimised pasta with SBG and SW showed DPPH scavenging activity, total phenolics, FRAP, and total flavonoids at 29.87%, 126.08 mg gallic acid equivalent/100 g, 158.57 mg ascorbic acid equivalent/100 g, and 73.85 mg quercetin/100 g, respectively (Table 2). Since different enzymes hydrolyse reserve macronutrients and cell walls, germination enhances total phenolics and related antioxidant activities through either *de novo* production of phenolics or the release of bound phenolic compounds (Xu *et al*., 2009). Analogous observations in total phenolic content and antioxidant activity were observed by Demir and Bilgiçli (2020) in pasta enriched with sprouted quinoa flour.

The sugar and starch contents of optimised pasta with SGG and SPM were 56.11 and 8.83%, respectively, whereas these were measured at 54.99 and 10.50%, respectively (Table 2), in pasta supplemented with SBG and SW. Sprouting-induced activation of α-amylase degrades starch into sugars. As the germination process progresses, the number of low molecular weight oligosaccharides increases, and the starch content decreases, hence decreasing the swelling capacity (Singh *et al*., 2015). Montemurro *et al*. (2019) reported a decrease of 3.3 times in starch and an increase of 7.8 times in sugars in sprouted wheat, barley, chickpeas, lentils, and quinoa.

Microstructure of pasta

Scanning electron images depicting microstructural changes in dried pasta samples at magnifications ranging from 100 – 1,500× are represented in Figure 2. Microstructural images of the dried control pasta indicated undamaged round to oblong starch granules with smooth surfaces and compact arrangements with an apparent attachment of proteins. Starch granules in the control sample were more regular in size and shape, and appeared slightly swollen. A higher amount of intact starch granules was observed in control pasta. The inclusion

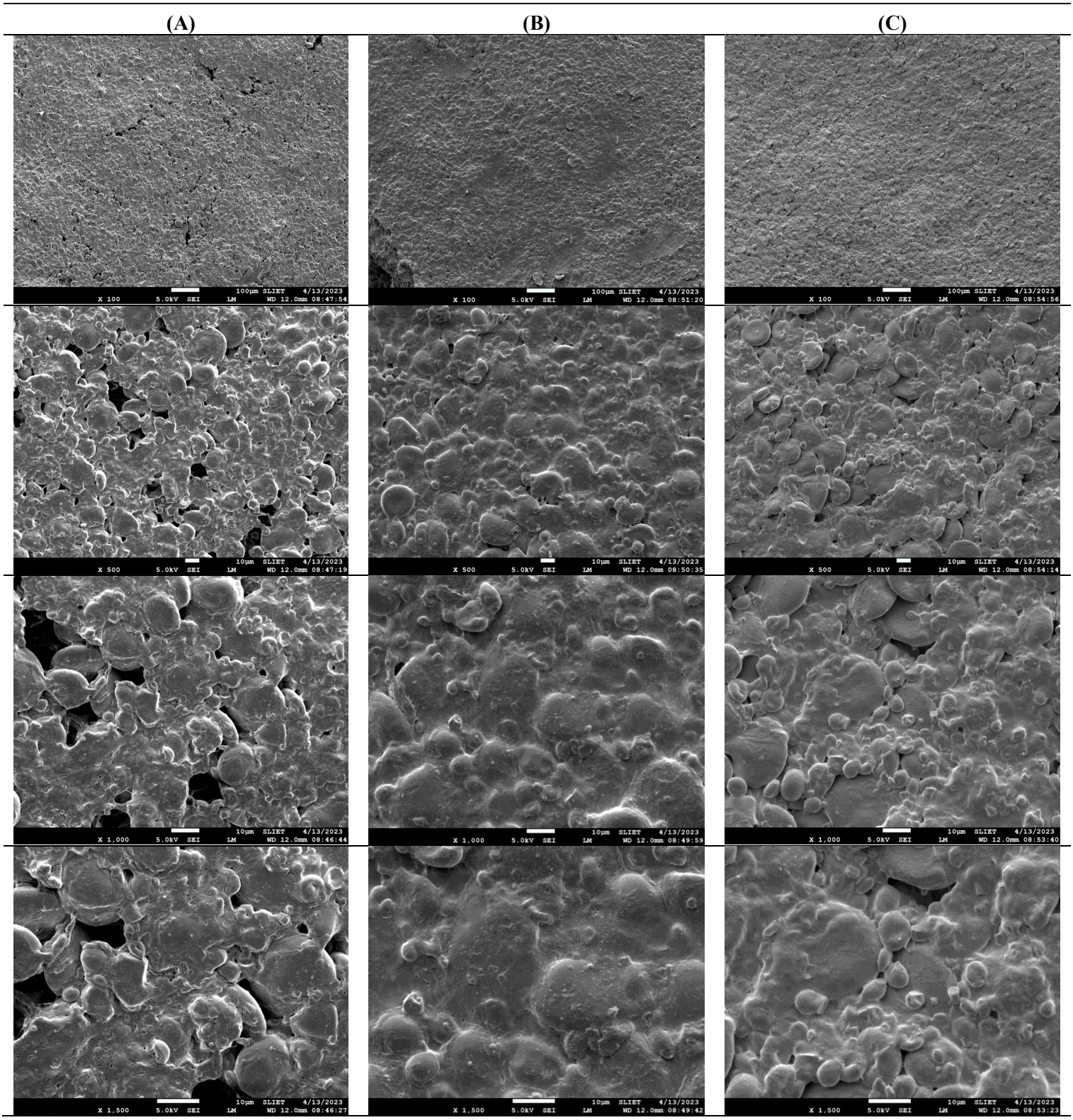

Figure 2. Microstructure of pasta samples at 100×, 500×, 1,000× and 1,500× magnifications of **(A)** optimised pasta with SGG and SPM, **(B)** optimised pasta with SBG and SW, and **(C)** control pasta.

of non-gluten flours in the pasta caused disordered starch-protein complexes making the arrangement less dense as they dilute the starch molecules. More irregularities in the shape and size of starch granules were observed in pasta samples enriched with sprouted flour. A distinct rough and corrugated surface was seen in pasta supplemented with SBG and SW. However, more irregularities in the structure and more corrugations were observed in pasta supplemented with SGG and SPM. Similar observations were reported by Bouasla *et al*. (2017) with interrupted protein-starch arrangement and rough and corrugated surfaces, with the addition of yellow peas in rice pasta. Pasta enriched with legume flours such as white pea, yellow pea, and lentil also indicated structural irregularities, and rough or corrugated surfaces upon addition of these flours (Wójtowicz and Mościcki, 2014). Also, small holes and cracks on the surface with irregular shapes and sizes in pasta enriched with sprouted finger millet and green banana flour were reported (Krishnan and Prabhasankar, 2010).

Conclusion

Pasta products are widely consumed around the world, demonstrating it as a preferred matrix for nutrients delivery. Nutritious formulation along with health-promoting bioactives can improve its nutritional value, and provide several health benefits to the intended consumers. Efforts to improve the nutritional value of pasta by the inclusion of sprouted food grains have resulted in pasta with better nutritional profile, high antioxidant activity, reduced antinutrients, and high protein digestibility. The developed pasta has good sensorial properties, making it more attractive to consumers. A good technological quality in pasta supplemented with a better nutritional profile and consumer acceptability along with scientific innovation can turn the business into a profitable enterprise. This development can aid in expanding innovative pasta products to health-conscious consumers, and provide newer opportunities to the processors for value-addition.

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