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RESEARCH ARTICLE

Spirulina enriched gluten free quality protein maize (QPM) pasta as functional food

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ABSTRACT

The gluten-free Quality Protein Maize (QPM) and pasta made from such grains would have far-reaching implications for achieving nutritional stability. As a result, the aim of this study was to see whether QPM combined with legume flours could be used to make gluten-free pasta (pasta without gluten protein). Furthermore, the effect of adding legume flours and spirulina to QPM pasta (2, 4, 6, 8 and 10 %) on color, appearance, cooking, physical, nutritional and sensory quality was examined. The best-accepted combination of QPM (QPM flour 60 %, black gram flour 30 %, defatted soy flour 8 %, and guar gum 2 %-Q) and QPM pasta enriched with spirulina (QPM flour 60 %, black gram flour 30 %, Spirulina 6 %, defatted soy flour 2 % and guar gum 2 % -S) were compared to the control (100 % whole wheat flour-C). The functional and cooking parameters such as bulk density (0.54 kg/m^3), swelling index (1.3 g/g), and cooked weight (11.0 g) of the spirulina pasta were slightly higher with a reasonable cooking loss of 7.6 %. The nutrients such as protein (21.6 %), calcium (44 mg %), iron (8.6 mg %), lysine (3.1 g/100g protein), and tryptophan (0.81 g/100g protein) were reported in the dietary composition of QPM pasta enriched with 6 % spirulina (S). Further addition of spirulina resulted in 16-fold increase in the carotenoid content.

Keywords: Pasta; QPM; Spirulina; Microstructure; Carotenoids; Cooking loss

INTRODUCTION

Gluten is the main storage protein in wheat and is used in a variety of wheat products including flour, roti, noodles and pasta. Celiac patients who consume gluten-containing diets experience small intestinal villi atrophy, which results in a variety of symptoms including cramping, bloating, nausea, diarrhoea, anaemia, weakness, weight loss and vitamin and mineral deficiencies (Menon et al., 2016). As a result, gluten-free products that are high in nutritional content, appealing in appearance and have a long shelf life are more readily embraced and trusted by customers. To far, the most successful treatment for CD patients is a gluten-free diet, with rice, maize and sorghum being naturally gluten-free grains (Giuberti et al., 2015). The ELISA (enzyme-linked immunosorbent assay) was used by Moron et al. (2008) to detect gluten in cereal and discovered that gluten is not detectable in maize. As a result, maize is an excellent gluten-free substitute. Maize (Zea mays L.) is also another important cereal crop in the world providing basic nutrients like carbohydrates, protein, fat, vitamins and minerals, as well as phytochemicals like carotenoids, phenolic compounds and phytosterols. Despite its many advantages, maize suffers from a deficiency in two essential amino acids, lysine and tryptophan. The synthesis of quality protein maize (QPM) with twice as much lysine and tryptophan improved the quality of maize proteins. Many studies have proved that Algal supplementation can increase the protein content of cereals, since they are an excellent source of important amino acids. Because of the accompanying health claims, microalgal-based functional and nutraceutical products are becoming increasingly popular across the world. Various species of algae are utilized as therapeutic carriers for various physiological risks. Incorporating natural elements in the diet can improve the society's nutritional and immune state. For this reason, including microalgae into traditional diets might be a good way to acquire functional foods (Bashir et al., 2017).

Spirulina, also known as cyanobacteria or filamentous blue-green microalgae is well known as a good-quality protein source (60-70 g/100 g) since it is high in vitamins

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particularly vitamin B12 and pro-vitamin A, minerals, especially iron and essential fatty acids. Furthermore, phycocyanin, carotene and xanthophyll pigments, as well as tocopherols and phenolic compounds are responsible for the antioxidant activity of spirulina. The concentrated protein of spirulina (*Spirulina platensis*) makes it a perfect food supplement for people of all ages and lifestyles. Beta carotene, vitamin B12, iron, essential trace minerals and gamma-linolenic acid are all abundant in spirulina, because of which it will have a positive impact on health, particularly when it comes to immunity. During a 24-week study conducted by Bashir et al. (2017), the consistent spirulina ingestion (1–8 g/day) dramatically reduced malnutrition-related abnormalities in human volunteers. Many studies have demonstrated the hypolipidemic, anti-cancer, and diabetic and obesity-protective benefits of this microalgae, all of which might lead to important therapeutic applications. Spirulina is a fantastic raw material for nutritious cuisine because of these benefits (Marco et al., 2014). Further, spirulina has been called a "superfood" due to its unique composition (Morsy et al., 2014). Sauces Soups, snacks, candies, beverages, chocolates, bread, biscuits and cakes have all been supplemented with powdered *S. platensis* biomass so far. Previous workers used the spirulina in wheat pasta and noodle preparations at varying levels and reported positive health benefits (Mostolizadeh et al., 2020; Hussein et al., 2021; Özyurt et al., 2015). The invention of gluten-free pasta using QPM on the other hand is expected to expand the use of widely grown maize in human nutrition. As a result, the current research was designed to standardize the formulation of nutritionally enriched gluten-free QPM pasta enriched with spirulina powder and to assess its physical, nutritional and cooking properties.

MATERIALS AND METHODS

Raw materials

The primary raw material was Quality Protein Maize (QPM) grains which were obtained from the Regional Maize Research Station in Begusarai, Bihar and were treated with lime before being ground into flour as per Shobha et al. (2014). Wheat, black gram and defatted soy flour were purchased in a single lot from a local market and kept refrigerated until needed. Spirulina powder was procured from Shivani Organics, Vijayanagar, Bengaluru.

Formulation of gluten-free pasta

QPM pasta was standardized by combining 50, 60, 70 and 80 % QPM flour with sufficient amounts of black gram flour and defatted soy flour, as well as 2, 4, 6, 8 and 10 % spirulina. Guar gum (2 %) was used as a binding agent in both the combinations. Control pasta was prepared with 100 % whole wheat flour. Fig. 1 shows the results of trials to standardize the flour ratio.

Pasta making process

QPM, black gram, defatted soy flour, spirulina powder and guar gum flours were combined for two minutes before steaming for 1 hour at 100°C. After applying an optimal amount of lukewarm water (250 ml/500 g of mixed flour) and salt (1 g/100 g of mixed flour) to a laboratory pasta-making unit, the dough was kneaded for 30 minutes (Model: Dolly P3). The dough was chilled for 15 minutes at 4 °C. Preliminary trials were used to standardize the ideal steaming and refrigeration times. A ribbed tube (S shape) die was used to extrude the chilled dough. Depending on the form of the finished product, the cutter speed was adjusted to an optimal level (3 to 12 rpm). The extruded pasta was collected in trays and dried for three days at room temperature (27-28 °C and RH 65 %). Fig. 4 depicts photographs of the pasta-making process and best accepted pasta samples.

Organoleptic analysis

Twenty-one semi-trained judges conducted an organoleptic study of cooked pasta with different levels of QPM (50, 60, 70 and 80 %) and spirulina incorporation (2, 4, 6, 8 and 10 %) on a nine-point hedonic scale. The pasta was cooked for the optimum cooking time in water with a standardized amount of spice mixture.

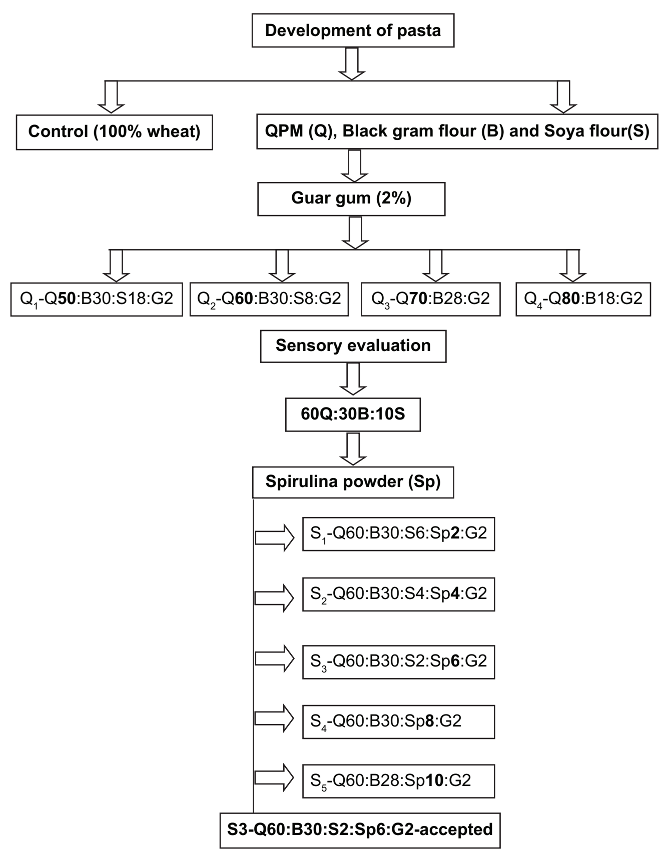

Fig 1. Different formulations of gluten free pasta.

Colour and texture analysis of pasta

The spectrophotometer was used to determine the colour of the cooked and uncooked pasta (Konica Minolta Instrument, Osaka, Japan; Model-CM 5). The CIE system was used to define colour coordinates (L*, a*, b*). A texture analyzer (Stable Microsystems Ltd, UK; model-HDi) was used to calculate the maximum force needed (hardness) to compress the uncooked and cooked pasta sample (single strand). A stainless steel cylindrical probe (2 mm) was used to compress pasta strands at pre-test speeds of 1.5 mm/s, test speeds of 2 mm/s and post-test speeds of 10 mm/s.

Cooking and physical properties of best-accepted pasta

Optimum cooking time (OCT), Swelling index (SI), cooking loss (CL), water holding capacity (WHC), cooked weight (CW) and cooked volume (CV) of the pasta were determined as per Jalgaonkar et al. (2019). The disparity between the initial oil used and the volume of the supernatant collected after centrifuging was used to quantify oil absorption capacity (OAC). The physical parameters such as expansion ratio (ER), water absorption index (WAI), water solubility index (WSI) and bulk density (BD) were assessed for the best-accepted pasta as per Benhur et al. (2015).

Chemical analysis of pasta

The proximate analysis of the pasta samples, as well as raw ingredients was determined as per Raghuramulu et al. (2003). An atomic absorption spectrophotometer (AAS: Agilent Technologies Series AA) was used to assess the mineral content of the samples, which included calcium, copper, iron, manganese and zinc. Phosphorus was measured spectophotometrically at 430 nm to determine its concentration (Raghuramulu et al., 2003). A Flame photometer (CL378: version 1.0) was used to estimate sodium and potassium content. Spectrophotometric method was used to examine the limiting amino acids in QPM such as lysine and tryptophan. The total carotenoids in pasta were measured with a spectrophotometer using the process of Ranganna, (1986).

Microstructure of pasta

The microstructure of the uncooked pasta samples was analysed by following the procedure of Giuberti et al. (2015) using Scanning Electron Microscope (SEM, TM303OPlus, HITACHI Co., Japan), at×1500, ×2000 and ×4000 magnifications.

Statistical analysis

The Duncan test was used to determine the importance of variations in the findings (P\0.01).

The R software (R studio) was used to do the statistical interpretation of the data.

RESULTS AND DISCUSSION

Organoleptic analysis of pasta

The organoleptic analysis revealed that there was no substantial variation (p>0.01) in appearance, colour and flavour between treatments of QPM pasta (Fig. 2A). Before and after cooking, the Q2 (Q60:B30:S8:G2) pasta had a uniform yellow colour and thickness, smoothness and a polished surface without roughness. The sample Q2 was found to be very close to control in texture, appearance and flavour according to the panellists. The pasta developed a starchy mouth coating as the amount of QPM flour increased (Q1- Q4) along with distinct maize taste. Even Q2 had a mild starchy mouth coating in this study but it was tolerable. Prior to extrusion, steaming and refrigeration techniques helped to hydrate starch resulting into decrease of the distinctive starchy taste of maize and the stickiness of the pasta. These organoleptic properties were previously observed by other researchers (Maity et al., 2012; Alam et al., 2013; Raja et al., 2014) when protein and fibre sources were added to improve the sensory properties of pasta. Since, maize flour contains no gluten the addition of gum and black gram flour resulted in the manufacture of rupture-free pasta with good sensory quality. Earlier studies (Romero et al., 2017; Mirhosseini et al., 2015) found that adding gums like xanthan gum and guar gum

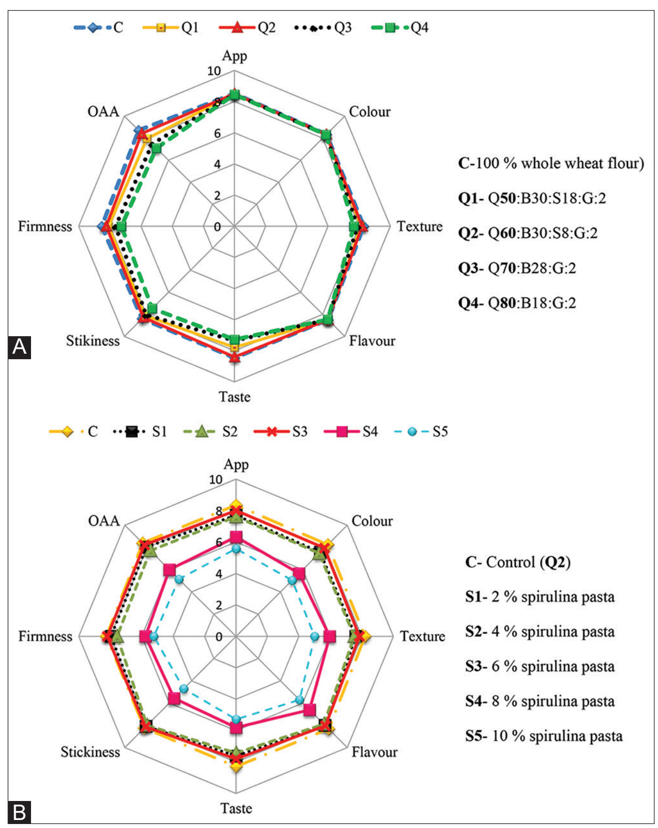

Fig 2. A) Organoleptic evaluation of QPM and B) Organoleptic evaluation of QPM pasta with different levels of spirulina incorporation.

Table 1: Cooking and physical parameters of developed pasta

Variations	L*±SD		a*±SD		b*±SD		Chroma±SD		Hue angle±SD		Hardness±SD (N)	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
QPM pasta												
Control (100:W)	54.4^{a}±1***	46.3^{d}±0.07	6.2^{d}±0.2	5.7^{c}±0.07	21.9^{e}±0.8	17.3^{e}±0.1	22.8^{d}±0.8	18.2^{c}±0.1	74^{b}±0.2	15.1^{c}±0.2	40.54^{a}±0.3***	0.32^{c}±0.1
Q_1(Q50:B30:S18:G: 2)	48^{b}±0.5	56.9^{a}±0.03***	9.4^{a}±0.3***	8.2^{a}±0.1***	28^{d}±0.2	20.8^{d}±0.1	29.5^{c}±0.2	22.3^{b}±0.2	71.3^{d}±0.5	10.6^{e}±0.2	16.4^{c}±0.6	0.54^{ab}±0.07
Q_2(Q60:B30:S8:G: 2)	47.5^{b}±0.3	53.9^{b}±0.05	8.8^{b}±0.3	6^{b}±0.01	29.3^{c}±0.6	21.5^{c}±0.2	30.6^{b}±0.7	22.3^{b}±0.2	73.3^{c}±0.3	14.5^{d}±0.03	24.2^{b}±0.07	0.61^{a}±0.2**
Q_3(Q70:B28:G: 2)	47.2^{bc}±0.5	53.2^{c}±0.06	8.2^{c}±0.2	5.2^{d}±0.07	31.4^{a}±0.3***	21.8^{b}±0.07	32.5^{a}±0.3	22.4^{b}±0.08	75^{a}±0.1	16.9^{b}±0.2	15.3^{d}±0.15	0.35^{bc}±0.1
Q_4(Q80:B18:G: 2)	46.1^{c}±1.4	52.8^{c}±0.7	8.1^{c}±0.07	4.7^{e}±0.1	30.8^{b}±0.3	22.6^{a}±0.1***	31.8^{a}±0.3***	23.1^{a}±0.1***	75.2^{a}±0.2***	18.7^{a}±0.6***	13.2^{e}±0.2	0.29^{c}±0.1
F-value	72.5***	745.8***	115.8***	899.1***	295***	1101.7***	275.5***	849.5***	112.4***	514.5***	8810.4***	4.7**
S.Em±	0.38	0.14	0.11	0.045	0.22	0.063	0.23	0.068	0.14	0.13	0.11	0.06
CD at 5%	1.13	0.42	0.33	0.13	0.65	0.18	0.69	0.2	0.43	0.39	0.35	0.19
Spirulina pasta												
Control (Q2)	47.2^{a}±0.5***	53.9^{a}±0.05***	8.8^{a}±0.3***	6^{a}±0.01***	29.3^{a}±0.6	21.5^{a}±0.2***	30.6^{a}±0.7***	22.3^{a}±0.2***	73.3^{d}±0.3	14.5^{b}±0.03	24.2^{a}±0.07***	0.61^{a}±0.2***
S_1 (2%)	38.2^{b}±0.9	36.3^{b}±0.03	2.5^{b}±0.05	1.5^{b}±0.08	16^{b}±0.4	15.6^{b}±0.2	16.2^{b}±0.4	15.7^{b}±0.3	81^{b}±0.2	56.9^{b}±3.05	16.3f±0.1	0.32^{b}±0.06
S_2 (4%)	29.9^{c}±0.4	35.87^{b}±1.6	2.3^{c}±0.04	1.4^{bc}±0.1	12.3^{c}±0.2	11.8^{c}±0.3	12.5^{c}±0.2	11.8^{c}±0.4	79.5^{c}±0.1	61.8^{b}±6.7	16.6^{e}±0.1	0.29^{b}±0.08
S_3 (6%)	28.3^{d}±0.2	33.2^{c}±0.7	2^{d}±0.06	1.2^{c}±0.07	11^{d}±0.07	7.4^{d}±0.2	11.2^{d}±0.07	7.5^{d}±0.2	79.6^{c}±0.3	67.1^{b}±4.1	21.6^{b}±5.9	0.35^{b}±0.04
S_4 (8%)	27.4^{e}±0.8	30.6^{d}±2.8	1.8^{d}±0.08	0.75^{d}±0.1	10.2^{d}±0.6	6.75^{e}±0.04	10.4^{e}±0.6	6.8^{e}±0.05	79.9^{c}±0.8	112.1^{b}±22.7	18^{c}±0.22	0.29^{b}±0.09
S_5 (10%)	25.2f±0.8	27.8^{e}±1.4	1^{e}±0.2	0.44^{e}±0.2	8.6^{e}±0.7	6.7^{e}±0.2	8.6f±0.7	6.7^{e}±0.2	83.2^{a}±0.5***	280.3^{a}±59.3**	17.1^{d}±0.1	0.26^{b}±0.04
F-value	805.7***	197.4***	1323.6***	1145.7***	1207.8***	3369.3***	1298.1***	3471.3***	250.4***	3.92**	1619.8***	7.15***
S.Em±	0.295	0.66	0.077	0.06	0.219	0.1	0.22	0.1	0.21	47.56	0.07	0.048
CD at 5%	0.862	1.92	0.229	0.17	0.641	0.3	0.655	0.31	0.614	138.82	0.23	0.14

W: whole wheat flour, Q_1-Q_4: QPM pasta, S_1-S_5: spirulina pasta, Q: QPM flour, B: blackgram flour, S: defatted soy flour, G: guargum, S_1:Q60:B30:S6:Sp2:G: 2, S_2:Q60:B30:S4:Sp4:G: 2, S_3:Q60:B30:S2:Sp6:G: 2, S_4:Q60:B30:Sp8:G: 2, S_5:Q60:B28:Sp10:G: 2L*: brightness, a*: redness, b*: yellowness, Chroma=, $\sqrt{a^2+b^2}$ Hue angle (^{O}) = $\tan^{-1}$(b/a). Where, 'a' +ve=redness, 'a' –ve=greenness, 'b' +ve=yellowness, 'b' -ve=blueness, N: Newton, SD standard deviation for five determinations, ** significant at 5% (p<0.05), *** significant at 1% (p<0.01) and data followed by different letters (a-f) are significantly different at 1% (p<0.001), 5% (p<0.05) and the same letter were not significantly different (p=0.001, *P*=0.05).

to gluten-free pasta improved the colour and overall quality of the product. From control to Q4 (Q 80:B18:G2) decrease in firmness and stickiness scores (Figs. 2A and 2B) showed a low to high degree of firmness and stickiness, respectively. The panelists gave a slightly higher sensory score (7.85±0.65) for the QPM pasta supplemented with 6 % spirulina. In case of QPM pasta with 8 and 10 % spirulina powder, due to high concentration of spirulina powder resulted into more stickiness and were disliked by the majority of the panelists due to loss of shape and high stickiness after cooking (Fig. 2B). Apart from the good taste and flavour, the 6 % spirulina incorporated pasta (S3) held its shape during cooking without cracks, breaks or disintegration; the product also had a solid texture, nonsticky surface and less cooking loss (Table 1). In a study, use of spirulina powder combined with maize flour in the preparation of extruded crisps increased the final product's market acceptability (Joshi et al., 2014). The rice soy crisp supplemented with 6 % spirulina (RSC-S6) received the highest approval score (8.50) from the judges in another review (Bashir et al., 2017).

Color and texture analysis of pasta

The colour is affected by a number of reactions that occur during the extrusion process. The non-enzymatic browning reaction (Maillard reaction, sugar caramelization) and pigment degradation are the most common. On both uncooked and cooked pasta tests, lightness L* decreased marginally as the amount of QPM flour in the recipe was increased (Table 1). The higher the L* rating, the lighter the pasta samples were. QPM pasta without spirulina (Q2) had the highest L* value (47.5±0.3). With the incremental rise of spirulina supplementation from 2 to 10 %, a reduction in lightness was found significantly (Table 2). QPM flour improved the yellowness of the pasta, as shown by a highly important positive impact in terms of QPM pasta's b* (blue-yellow) value. As the amount of spirulina in the basic formulation increased, the colour of the formulation darkened significantly. The inclusion of spirulina reduced the a* and b* values as well. The dark blue-green colour of spirulina, as compared to QPM and legume flours (black gram and defatted soy flour) is responsible for this pattern.

In both uncooked and cooked pasta, the hardness of the pasta decreased dramatically ($p<0.05$) as the percentage of QPM flour increased (Table 1). Since the proportion of ingredients blended for Q2 (Q60:B30:S8:G:2) helped in the formulation of rupture-free structures and improved the hardness of pasta, QPM pasta with 60 % QPM flour (Q2) was harder (24.2±0.07 N and 0.61±0.2 N) compared to others (Q1, Q3 and Q4). The study of Wojtowicz and Moscicki, (2014) found similar patterns in precooked wheat pasta fortified with legume flours. The hardness of the QPM pasta enriched with spirulina powder was significantly

Table 2: Color and texture analysis of pasta

Pasta samples	Cooking properties							Physical parameters			
	OCT±SD (min)	SI±SD (g/g)	CL±SD (%)	CV±SD (%)	CW±SD (g)	WHC±SD (g/g)	OAC±SD (g/g)	WAI±SD (g/g)	WSI±SD (%)	BD±SD (Kg/m^3)	ER±SD
Control pasta (C)	6.2^c±0.03	1.1^b±0.003	5.5^c±0.02	43.3^c±0.02	10.2^c±0.02	0.74^c±0.01	0.3^a±0.03***	1.6^c±0.13	3.7^c±0.1	0.47^b±0.003	8.9^a±0.02***
QPM pasta (Q)	7.5^a±0.01***	1^c±0.002	7.4^b±0.02	64.5^a±0.01***	11.3^a±0.01***	1^b±0.001	0.2^b±0.01	2.7^b±0.2	6.6^b±0.3	0.48^b±0.0015	8.8^b±0.03
Spirulina pasta (S)	7.3^b±0.03	1.3^a±0.003***	7.6^a±0.01***	55.3^b±0.01	11^b±0.01	1.1^a±0.03***	0.17^c±0.002	3^a±0.04***	7.4^a±0.2***	0.54^a±0.02***	8.6^c±0.03
F-value	3220.9***	121.3***	6986.8***	2355538***	4832.8***	389.8***	229.9***	183.6***	297.8***	32.08***	117***
S.Em±	0.012	0.013	0.014	0.0069	0.0081	0.010	0.0036	0.055	0.112	0.006	0.014
CD at 5%	0.038	0.042	0.043	0.021	0.025	0.031	0.011	0.17	0.346	0.019	0.044

C: 100% whole wheat flour, Q: Q60:B30:S8:G: 2, S: Q60:B30:S2:Sp6:G: 2, OCT: optimum cooking time, SI: swelling index, CL: cooking loss, CV: cooked volume, CW: cooked weight, WHC: water holding capacity, OAC: oil absorption capacity, WAI: water absorption index, WSI: water solubility index, BD: bulk density, ER: expansion ratio, ***significant at 1% ($p<0.01$) and SD standard deviation for five determinations and data followed by different letters (a-c) within the same column indicate statistically significant difference at 1% ($p<0.01$) between the results and the same letter were not significantly different ($p=0.01$).

($p<0.05$) increased (Table 1). The softness of uncooked and cooked spirulina pasta improved, as the amount of spirulina powder was gradually increased. Similarly, Morsy et al. (2014) observed a related phenomenon in which replacing corn flour with 10 % spirulina increased texture parameters in all produced extrudates. In comparison, Joshi et al. (2014) found that corn extrudates containing 7.5 % spirulina were harder than pure corn extrudates.

Cooking and physical properties of pasta

The optimum cooking time (OCT) for control pasta was 6.2±0.03 minutes while gluten-free pasta (Q and S) had slightly longer cooking times (Table 2). Since, the spirulina protein network restricts water diffusion through the central zone of pasta during preparation, spirulina pasta had a high OCT. The degradation of the gluten protein network in control pasta allows water to diffuse through it more easily thus decreasing cooking time. The swelling index of spirulina pasta was slightly ($p<0.01$) higher (1.3±0.003 ml/g) (Table 2). Our findings are due to the microalgae's ability to absorb water and hold it in the protein-starch network resulting into a higher swelling index. Cooking loss of wheat-based pasta has been proposed to be 8 % by Indian standard [BIS: 1485(2010)], so all three pasta samples in this study is within the range of approval (5.5±0.02, 7.4±0.02, and 7.6±0.01 %, respectively for control, QPM and spirulina pasta). The results of gluten-free pasta (QPM and spirulina pasta) cooking loss is somewhat higher than wheat pasta, owing to the solubilization of poorly attached gelatinized starch from the product's surface (Table 2).

The overall water absorption index (WAI) value of 3±0.04 g/g was found in spirulina pasta, while the minimum WAI value of 1.6±0.13 g/g was found in control pasta (Table 2). The differences in WAI of extrudates may be due to protein denaturation, starch gelatinization and coarse fibre swelling during extrusion. The WSI ranged from 3.7±0.19 % (control pasta) to 7.4±0.26 % (spirulina pasta), meaning that adding legume flour to QPM and spirulina pasta increased WSI (Table 2). Spirulina pasta had a slightly higher bulk density (BD) value (0.54±0.02 Kg/m^3), which may be attributed to spirulina's higher protein content which has been shown to cause water diffusion in the matrix. The observations (Tanska et al., 2017, Bashir et al., 2017 and Joshi et al., 2014) influence of spirulina inclusion is highly variable and can be related to the processing parameters used in the production of pasta such as extruder type, raw materials, moisture, temperature and so on.

Nutritional composition of pasta samples

Spirulina powder had a crude protein content of 59.2±0.01 g, according to Table 3. Also our protein content findings are consistent with Tanska et al. (2017), who found that spirulina contained 60.7 % protein. The moisture content of control, QPM and spirulina pasta was 8.5±0.03, 8.4±0.03 and 8.4±0.03 g/100 g, respectively, meaning that the moisture level was <14 % in all pasta varieties which was ideal for longer shelf life (Table 3). In terms of protein content, spirulina pasta had a slightly higher protein content (21.6±0.03 g/100 g) than control pasta (10.2±0.03 g/100 g) showing a favorable association between substituting defatted soy flour, black gram flour and spirulina powder in the formulation. The results of Table 4 shows that spirulina pasta contains slightly more calcium (44.6±0.3 mg/100 g), iron (8.6±0.04 mg/100 g), phosphorous (398±3.1 mg/100 g),

Table 3: Proximate composition of raw ingredients and best accepted pasta samples

Raw ingredients	Moisture±SD (g)	Protein±SD (g)	Fat±SD (g)	Crude fiber±SD (g)	Ash±SD (g)	CHO±SD (g)	Energy±SD (Kcal)
Wheat flour	10.7^{c}±0.09	10.1^{e}±0.02	1.3^{e}±0.02	1.2^{e}±0.07	1.1^{e}±0.02	75.8^{a}±0.6***	355^{ab}±2.5
QPM flour	10.8^{b}±0.04	10.2^{d}±0.02	3.8^{b}±0.02	2.3^{d}±0.02	1.4^{d}±0.07	71.4^{b}±0.2	360.6^{a}±0.6***
Black gram flour	11.2^{a}±0.07***	24.8^{c}±0.03	1.4^{d}±0.03	3.3^{c}±0.03	3.2^{c}±0.03	56^{c}±0.2	339.7^{d}±15.6
Soy flour	6.2^{d}±0.02	49.8^{b}±0.03	1.9^{c}±0.07	3.4^{b}±0.02	6.8^{b}±0.02	31.9^{d}±0.1	343.8^{cd}±0.3
Spirulina powder	5.8^{e}±0.05	59.2^{a}±0.01***	6.9^{a}±0.02***	7.8^{a}±0.07***	7.4^{a}±0.02***	12.9^{e}±0.2	350.5^{bc}±0.5
F-value	9291.3***	35754***	18060***	12558***	26612***	36862***	7.01***
S.Em±	0.027	0.011	0.017	0.022	0.018	0.139	3.17
CD at 5%	0.082	0.035	0.052	0.066	0.053	0.41	9.37
Pasta samples							
Control pasta (C-100:W)	8.5^{a}±0.03***	10.2^{c}±0.03	1.3^{c}±0.03	1^{c}±0.02	1.5^{c}±0.03	77.4^{a}±0.1***	362.3^{c}±0.2
QPM pasta (Q - Q60:B30:S8:G: 2)	8.4^{b}±0.03	17.1^{b}±0.03	3.3^{a}±0.02***	1.1^{b}±0.02	2.8^{b}±0.02	67.1^{b}±0.1	367.3^{a}±0.2***
Spirulina pasta (S -Q60:B30:S2:Sp6:G: 2)	8.4^{b}±0.03	21.6^{a}±0.03***	3.1^{b}±0.02	1.3^{a}±0.07***	2.9^{a}±0.02***	62.7^{c}±0.1	365.3^{b}±0.2
F-value	13.07***	147255***	7496.4***	41.93***	3833.4***	16385***	623.18***
S.Em±	0.015	0.014	0.012	0.021	0.012	0.058	0.10
CD at 5%	0.046	0.046	0.039	0.065	0.037	0.18	0.31

W: whole wheat flour, Q: QPM flour, B: blackgram flour, S: defatted soy flour, Sp: spirulina powder, G: guar gum, CHO: carbohydrate, *** significant at 1% ($p<0.01$), SD standard deviation for five determinations and data followed by different letters (a-e) within the same column indicate statistically significant difference at 1% ($p<0.01$) between the results and the same letter were not significantly different ($p=0.01$).

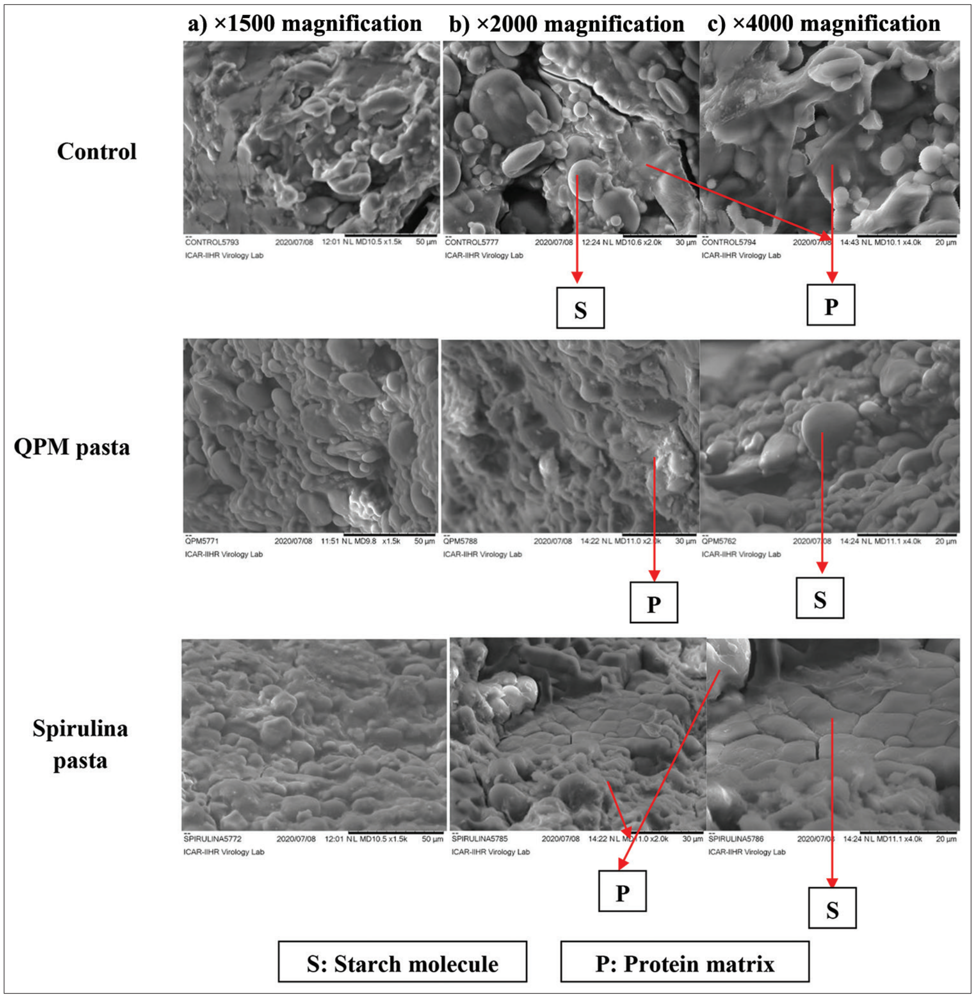

Fig 3. SEM images of pasta.

Table 4: Minerals composition of developed pasta

Pasta samples	Minerals							
	Ca±SD (mg)	Cu±SD (mg)	Zn±SD (mg)	Fe±SD (mg)	P±SD (mg)	Mn±SD (mg)	K±SD (mg)	Na±SD (mg)
Control pasta (C)	29^{c}±0.3	0.39^{b}±0.015	1.9^{c}±0.04	4.7^{c}±0.3	308^{c}±4.5	1.5^{a}±0.03***	81.3^{c}±0.3	16.2^{c}±0.1
QPM pasta (Q)	40.2^{b}±0.6	0.56^{a}±0.5***	2.9^{a}±0.03***	5.4^{b}±0.04	386^{b}±3.9	0.87^{b}±0.04	172.1^{b}±0.3	20.2^{b}±0.1
Spirulina pasta (S)	44.6^{a}±0.3***	0.57^{a}±0.03***	2.3b±0.04	8.6^{a}±0.04***	398^{a}±3.1***	0.79^{c}±0.03	177.9^{a}±0.6***	28.9^{a}±0.1***
F-value	1717.5***	21.34***	1181.6***	558.2***	787.2***	361.7***	69151***	13701***
S.Em±	0.194	0.021	0.015	0.087	1.74	0.023	0.20	0.055
CD at 5%	0.598	0.075	0.048	0.271	5.36	0.079	0.63	0.17

C: 100% whole wheat flour, Q: Q60:B30:S8:G: 2, S: Q60:B30:S2:Sp6:G: 2, Ca: calcium, Cu: cupper, Zn: zinc, Fe: iron, P: phosphorous, Mn: manganese, K: potassium and Na: sodium, *** significant at 1% (p<0.01), SD standard deviation for five determinations and data followed by different letters (a-c) within the same column indicate statistically significant difference at 1% (p<0.01) between the results and the same letter were not significantly different (p=0.01).

potassium (177.9±0.6 mg/100 g), and sodium (28.9±0.1 mg/100 g) than control. Spirulina pasta has roughly two times more iron than regular pasta (Pastor-Cavada et al., 2011) due to the high concentrations of iron in dry spirulina powder. The lysine content of control pasta (3.1±0.03 g/100 g of protein) was greater than that of QPM pasta and the addition of spirulina powder significantly (p<0.01) improved the lysine content (Table 5). The tryptophan content of control pasta (0.83±0.02 g/100 g) is considerably higher (p<0.01) than spirulina pasta (0.81±0.01 g/100 g) due to the addition of spirulina powder and even the tryptophan content of protein was significantly higher (p<0.01). According to FAO guidelines, normal tryptophan intake should be 1.1 g/day (Pastor-Cavada et al., 2011) and it is found that both QPM pasta and spirulina pasta will fulfill this minimum requirement. Since, spirulina powder was added at the rate of 6 %, the carotenoid content in pasta samples increased from 0.24±0.05 to 3.9±0.08 mg/100 g (Table 5). Enrichment of spirulina powder resulted into 16-fold rise

Table 5: Limiting amino acid and total carotenoids content of developed pasta

	Limiting amino acid (g/100g of protein)		Total carotenoids±SD (mg/100g)
Pasta samples	**Lysine±SD**	**Tryptophan±SD**	
Control pasta (C)	3.1^{a}±0.03***	0.83^{a}±0.02***	0.24^{c}±0.05
QPM pasta (Q)	2.7^{b}±0.2	0.71^{b}±0.01	0.61^{b}±0.06
Spirulina pasta (S)	3.1^{a}±0.03***	0.81^{a}±0.01***	3.9^{a}±0.08***
F-value	35.01***	55.77***	458910***
S.Em±	0.047	0.0087	0.0029
CD at 5%	0.14	0.026	0.0091

C: 100% whole wheat flour, Q: Q60:B30:S8:G: 2, S: Q60:B30:S2:Sp6:G: 2, SD standard deviation for three determinations, *** significant at 1% (p<0.01), SD standard deviation for five determinations and data followed by different letters (a-c) within the same column indicate statistically significant difference at 1% (p<0.01) between the results and the same letter were not significantly different (p=0.01).

Fig 4. Images from experiment of pasta.

in carotenoid levels in spirulina pasta, as dried spirulina powder absorbs 360-500 mg of total carotenoids per 100 g (Siva Kiran et al., 2015).

Microstructure of pasta (SEM)

The control pasta's microstructure picture (SEM) revealed a thick and compact gelatinized starch framework (2000×). Gluten encapsulates the starch granules as a protective coat in control pasta (Fig. 3) and thereby reduces the subsequent degradation of starch and overall leaching of starch during cooking (4000×). The protein matrix was not formed in the case of gluten-free pasta (Q and S) resulting in detachment of part of the protein from the network and starch granules exposed outside (4000×). There was a thin layer of protein matrix (1500×) on the spirulina pasta. The addition of spirulina to gluten-free QPM pasta helped in the formation of a protein matrix in which starch granules are dispersed (Fig. 3). Many cracks were visible on the surface of spirulina pasta, suggesting that water could penetrate the interior of the pasta during cooking (4000×). This may be due to shrinkage the sample during preparation process or strain within the pasta dough during the drying process. As a result, when compared to control pasta, gluten-free pasta (Q and S) had a higher cooking loss (Table 2). Our observations were consistent with those of Detchewa et al. (2016), Li et al. (2019) and Sung et al. (2005).

CONCLUSION

The study demonstrated that adding spirulina powder to QPM pasta improved nutritional content in terms of protein, crude fibre, calcium, zinc, iron, lysine, tryptophan, and carotenoids. The bulk density (0.54 kg/m3), swelling index (1.3 g/g) and cooked weight (11.0 g) of the spirulina pasta were slightly higher with a reasonable cooking loss of 7.6 %. In spirulina-infused gluten-free pasta where starch granules were dispersed among them, the formation of a protein network was observed. In our study, the formulated gluten-free pasta from QPM enriched with 6 % spirulina powder was found to be suitable for the development of nutritious and appropriate pasta that serves as a gluten-free breakfast item suitable for people of all ages who are gluten-intolerant as well as providing dietary versatility to regular consumers.

Authors' contributions

Veena U. K: Conducted research work and data analysis; Shobha D: Guidance, correction of the manuscript, paper writing; Neena Joshi: Research design and guidance; Darshan M.B: Color and texture analysis; Benherlal P.S: Assisted in biochemical analysis.

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