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An assessment of the nutritional, physicochemical and sensory attributes of pasta enriched with fresh *Spirulina* wet biomass

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Abstract
World Health Organization conferred *Spirulina* (Microalgae) as a "super food" as it contains all amino acids, essential fatty acids, vitamins, minerals and pigments required to ensure a complete balanced diet. Regardless of the high nutritional content found in microalgae, only a few products containing microalgae are available into the market. Pasta is one of the best choices for enriching S*pirulina* because of its popularity among consumers, ease of preparation, handling and storage. Fresh biomass of *Spirulina* sp. (CCC 540) was used at 10%, 15% and 20% concentrations to enrich pasta prepared from semolina led to increase in protein, lipids, flavonoids, total phenols, iron, zinc, and calcium content of pasta by upto 38.73%, 19.42%, 86.12%, 10.95%, 180.86%, 5.58% and 17.80% respectively without causing any detrimental changes to the textural and sensory attributes. Pasta enriched with 20% *Spirulina* fresh biomass was reported “moderately like” had highest score of overall acceptability (7.52) after control and the purchase intention was evaluated as “probably would buy”. Therefore, this product can be used as functional food by consumers who look for a healthier diet.

Keywords: *Spirulina*, functional food, pasta, phenols, flavonoids, antioxidants

1. Introduction
Cyanobacteria (blue green algae) are prokaryotic organisms that contain chlorophyll-*a,* are photosynthetic microorganism which are small in size, unicellular, filamentous or colonial and can grow in all habitats under various environment conditions (Pagels *et al*., 2019; Rehakova *et al*., 2019) [31, 36]. Due to their high photosynthetic efficiencies, fast growth with low nutritional needs, microalgae are superior among terrestrial plants for biomass production (Costa and de Morais, 2011) [3]. Many of these microalgae and cyanobacteria have been widely cultivated due to their high added-value in human food and animal feed (Borowitzka. 2013) [11]. *Spirulina* sp. or *Arthrospira* sp. is an aquatic, filamentous blue green alga that is well known for use as a dietary supplement, pharmaceutical and animal feed owing to prophylactic and therapeutic nutrients that include protein, minerals, B-complex vitamins, γ-linolenic acid, β-carotene, vitamin E, trace elements and a number of unexplored bioactive compounds. *Spirulina* is consumed as a nourishing food concentrate as it contains 55–70% protein, 15–25% polysaccharides, 6–13% nucleic acids, 5–6% total lipids and 2.2–4.8% minerals (Wu *et al.,* 2016 and Gireesh, 2004) [47, 18] complex of 4% vitamins A, B, D, E, and K (Varga *et al*., 2002) [44] besides essential fatty acids (3,6 γ-linolenic acid, stearidonic acid ,α-linolenic acid, eicosapentaenoic acid, arachidonic acid, and docosahexaenoic acid (Andrica *et al*., 2015) [5]. Because of its apparent ability to stimulate whole human physiology, *Spirulina* exhibits plethora of beneficial functions therapeutic functions such as immunomodulation, anti-inflammatory, anti-allergic, anti-bacterial, antioxidant, antiviral, antidiabetic, anticancer (Wu *et al*., 2016) [47]. The cell wall of the *Spirulina* is composed of 86% digestible polysaccharide with high digestibility and lacks cellulose (Li & Qi, 1997) [25]. According to Santillan (1974) [38], the total nucleic acid content of *Spirulina* has been reported in the range of 4.2-6% on dry weight basis which is safe and does not pose any health risk upon consumption even upto 10g/day. Consisting all these properties without any adverse effect, cyanobacteria *Spirulina* has been accredited by the FDA (U.S.A.) and ANVISA (The Brazilian Health Regulatory Agency) with GRAS status. Despite of known health benefits and high nutritional content only a limited number of products of *Spirulina* are sold as tablets, dried powder and capsules are available. Nonetheless, currently, there is an increasing consumer demand for more new and sophisticated functional food products with added health benefits and that leads *Spirulina* biomass and its products as firmed position in the food market.

Several studies using *Spirulina* in different form of foods have been performed such as noodles, mayonnaises, doughnuts, pasta, salad dressing and gelled-desserts. Lucas *et al*., (2018) [26] prepared snacks with the use of rice and organic corn flours with 2.6% *Spirulina* enrichment and found increase of 28.1% in lipids, 22.6% in protein and 46.4% in minerals with sensory acceptance and without significantly affecting its physical parameters (expansion index and hardness). Addition of *Spirulina* sp. *Tetraselmis* sp. or *Chlorella* sp. at different concentration (0.5-2.0% w/v) to prepare broccoli soups, revealed higher antioxidant activities and phenolic content as compared to the control soups (Lafarga *et al*., 2019) [23]. Similarly dried noodles were formulated with addition of 9% *Spirulina* paste and which projected 38.6% and 8.88% protein and 17.51 mg/100g and 0.06 mg/100g (β-carotene) in *Spirulina* enriched noodle and control noodle respectively. The noodles were in accordance to Indonesian National Standard in term of nutritional, physical and sensorial aspects (Agustini *et al*., 2015) [4]. Among the other developed foods, there are doughnuts (Rabelo *et al*., 2013) [35], nutritive shake for old people (Santos *et al*., 2016) [39] and extruded snacks (Joshi *et al*., 2014) [20]. Pasta is preferred among the various foods prepared with different microalgal enrichment due to well-acceptability by all age groups, ease of preparation, low cost and wide consumption due to nutritive values and sensory values. Muresan (2016) [29] incorporated *Spirulina* biomass (5%) in fresh pasta, resulting in increased nutritional compositions without significantly affecting sensory, cooking and textural quality. Although many studies have investigated the use of dry *Spirulina* powder in the concentration ranges of 2-10% in pasta, there are limited studies for the use of fresh wet *Spirulina* biomass to enrich different foods including pasta. In consequences of these, the objectives of this research work were to: (i) evaluate the effect of fresh wet *Spirulina* biomass (SWB) incorporation on the nutritive value, cooking attributes of pasta and, (ii) determine the consumer preferences for fresh SWB supplemented pasta.

2. Material and Methods

2.1 *Spirulina* strain and biomass Production

The *Spirulina* sp. CCC 540 was procured from Centre for Conservation and Utilisation of Blue Green Algae (CCUBGA), Indian Agricultural Research Institute, New Delhi. India. The *Spirulina* strain was grown and maintained in 100 mL autoclaved Zarrouk's medium (Zarrouk, 1966) [48] contained in 250 mL Erlenmeyer flasks at 28±2° C and light intensity of 55-60 μmol photon m^{-2} s^{-1} with photoperiod of 16h:8h light and dark rhythm. Further, it was cultivated in raceway ponds using medium containing commercial fertilizers (NaCl-1g/L, Na_2CO_3-9g/L, $MgSO_4$-0.2g/L, single super phosphate-0.1g/L, Na_2CO_3-9g/L and Sufala: N:P:K (19:19:19)-1g/L). The biomass was harvested after 21-25 days of *Spirulina* growth. It was filtered, washed properly and used directly to prepare pasta.

2.2 Pasta Processing

Fresh pasta elaborated into fusilli shape (Fig 1) was prepared using commercial *durum* wheat semolina, carboxymethyl cellulose (1%), water and fresh SWB at 10, 15, and 20% (w/w, *n* = 3) and control one prepared without *Spirulina* biomass addition. Laboratory scale equipment (Dolly La Montiferrina, Italy) were used for the preparation. The pasta were dehydrated in hot air oven at 60±2 ºC under high humidity until final moisture of 7-8% (w/w).

Fig 1: Fusilli control pasta and *Spirulina* wet biomass (SWB) enriched pasta with increasing order of *Spirulina* biomass (10%, 15% and 20%).

2.3 Nutritional evaluation

Percentage nutrient composition (protein, fat and ash) analysis was done according to AOAC (Association of official Analytical Chemists) methods. The protein content was estimated by micro Kjeldahl method (AACC, 2000) [1]. The total lipids content was determined by Folch method (Folch, 1957) [16] using chloroform: methanol extraction process. Anthrone method (Ludwig & Goldberg, 1956) [27] was used for carbohydrates content estimation. Atwater conversion factors were used to calculate calorific value of pasta (Merril & Watt, 1973) [28]. Moisture content in pasta was estimated by difference in weight method upon drying in the oven at 100 ºC (AOAC, 1984) [6] and muffle furnace (method 08-01, AACC, 2000) [1] was used to determine ash content. Phenolic content as Gallic acid equivalent (mg GAE 100 g^{-1}) and Flavonoids content as pimol Quarcet equivalent per gram (QE/g) in pasta samples were analysed spectrophotometrically according to Singleton *et al*., 1999 [41] and Barnum, 1977 [8] respectively. *In vitro* antioxidant activity was determined as 2, 2-diphenyl- 1-picryl hydrazyl (DPPH) assay by Apak, Güclü, Özyürek & Celik, (2008) [7] and ferric reducing antioxidant activity (FRAP) expressed as μmol Trolox equivalents (TE) 100 g^{-1} respectively according to Benzie & Strain (1996) [10]. Minerals estimation in pasta samples was done after digestion of pasta samples with di-acid (HNO_3: $HClO_4$ 4:1). Zinc and iron content estimation in extract prepared was done using atomic absorption spectrophotometer (Z-Xpress 8000, Spectrum) while calcium content was estimated using flame photometer (Watanabe & Olsen, 1965). Cooking quality parameters of pasta samples were determined by AACC method 16-50 (AACC, 1983).

2.4 Textural analysis

The textural analyses (stickiness, firmness and cutting force) of cooked pasta was conducted by Texture Analyzer (TA-XT2; Stable Micro Systems Ltd., Surrey, UK) using software Exponent (6.1).

2.5 Sensory evaluation

Fifty seven semi-trained panelists (40 males and 17 females), aged between 21 to 45 years, evaluated the freshly cooked pasta for sensory attributes served in random order two hours post meal. The evaluation was done using 9-point hedonic scale (0=not acceptable; 9=highly acceptable).

2.6 Statistical analysis

Test of significance when the p-value was <0.01, was calculated with Tukey's HSD test for comparison of treatment effects for each of the parameters by SPSS-16.0 statistical package.

3. Results and Discussion

3.1 Nutritional composition

The biomass of *Spirulina* sp. CCC 540 was observed to contain 65.71%, 6.94%, 21.87% and 8.34% protein, total lipids, carbohydrates and ash respectively and minerals contents as Zn, Ca and Fe recorded were 1.87, 107.83 and 26.59 mg/100g respectively. Similarly, Tańska, Konopka & Ruszkowska (2017) [43] used *Spirulina* powder to prepare corn extrudates that also contained 60.7%, 7.2%, 15.2% and 7.9% protein, fat, carbohydrates and ash respectively. Upon *Spirulina* enrichment in pasta, there was significant variation observed in nutritional values such as protein, carbohydrates and total lipids (Table 1). The control pasta prepared from semolina contained 11.52% protein and fresh *Spirulina* wet biomass (SWB) (concentration of 10%, 15% and 20% w/v) enriched pasta reported an increase in protein upto 38.73%. The enhancement in protein content with fresh wet *Spirulina* biomass addition is in conformity with several studies conducted by researchers (Lemes *et al*., 2012; Selmo & Salas-Mellado, 2014) [24, 40] and is mainly due to high protein content of fresh wet *Spirulina* biomass. An increase in lipids content ranged from 9.41 to 19.42% in pasta enriched with various levels (10%, 15% and 20% w/v) of SWB against the control semolina pasta (2.99% total lipids) (Table 1). Tańska *et al*., (2017) [43] prepared corn extrudates with 8% *Spirulina* addition and found increase of 4% in protein, 0.4% in total lipids, 0.4% in ash and 0.7% in fibers. Similar increase in nutrient content were reported by Agustini *et al* (2017) [3] in noodles and Lucas *et al*., (2018) [26] in snacks during enrichment with *Spirulina*. Semolina pasta (control), containing 71.68% carbohydrates content, also reported a proportional decrease in carbohydrates' content upon enrichment with SWB at various levels (10%, 15% and 20% w/w) on dry weight basis and the carbohydrates content was 64.40%, 62.47% and 60.90% respectively (Table 1). The corn extrudates prepared by Tańska *et al*., 2017 [43] also observed a decrease in carbohydrates content from 79.4% to 74.3% in control and enriched with *Spirulina* respectively. The decrease in carbohydrates content (10-12%) was also reported in pasta prepared with 2% *Spirulina* as compared to control (Fradique *et al*., 2010) [17].

The control pasta samples were found to contain 1.13, 21.57, 1.28 mg/100g zinc, calcium and iron content on dry weight basis and their content in SWB enriched pasta increased up to 5.58%, 17.80% and 180.86% respectively (Table 1). The results are in agreement with the study on bread prepared with 10% *S. platensis* (Burcu *et al*., 2016) [12] that reported 14.0, 45.95 and 3.24 mg/100g magnesium, calcium and iron content, respectively over control. Similar findings were also reported by several researchers (Özyurt *et al*., 2015; Pagnussatt *et al*., 2014) [30, 32] for *Spirulina* enriched pasta, biscuits or cookies.

The calorific value of pasta (per 100g) was calculated based on its nutrient' composition that was in the range of 339.65-359.71 calories. It was found that the calorific values were reduced in SWB enriched pasta due to less carbohydrates content of SWB enriched pasta (Table 1). The ash content in semolina pasta was estimated to be 0.57% (Table 1) and upon enriching pasta with SWB at different levels, a proportional increase in ash content which was found to be 1.06%, 1.11% and 1.17% in 10%, 15% and 20% SWB enriched pasta respectively. Agustini *et al*., (2017) [3] prepared *Spirulina* enriched noodles that were also found to increase in ash content upto 9%. It was concluded that increase in ash content of different foods upon *Spirulina* enrichment was due to higher content of minerals (Sudarmadji *et al*., 1997) [42].

Table 1: Nutritional composition, phenolic, flavonoids and antioxidant content of *Spirulina* wet biomass (SWB) enriched pasta.

Component	Control	10 % SWB	15% SWB	20% SWB
Moisture (%)	6.21 ± 0.182^{b}	5.54 ± 0.051^{a}	6.03 ± 0.022^{a}	6.01 ± 0.130^{a}
Calories (in 100g)	359.71 ± 2.74^{b}	343.24 ± 2.78^{b}	341.64 ± 1.98^{b}	339.65 ± 2.16^{a}
Carbohydrates (%)	71.68 ± 1.48^{c}	64.39 ± 1.38^{bc}	62.47 ± 0.83^{b}	60.90 ± 0.93^{a}
Protein (%)	11.52 ± 0.38 ^{d}	14.06 ± 0.36^{c}	15.19 ± 0.13^{b}	15.98 ± 0.25^{a}
Fat (%)	2.99 ± 0.14^{c}	3.27 ± 0.24^{ab}	3.44 ± 0.16^{a}	3.57 ± 0.10^{a}
Ash (%)	0.57 ± 0.002^{b}	1.06 ± 0.045^{a}	1.11 ± 0.027^{a}	1.17 ± 0.071^{a}
Iron (mg/100g)	1.28 ± 0.020^{c}	2.23 ± 0.003^{ab}	2.67 ± 0.020^{ab}	3.60 ± 0.010^{a}
Calcium (mg/100g)	21.57 ± 0.66^{ab}	23.24 ± 0.03^{ab}	24.36 ± 0.23^{ab}	25.41 ± 0.13^{a}
Zinc (mg/100g)	1.13 ± 0.002^{a}	1.16 ± 0.001^{a}	1.17 ± 0.004^{a}	1.19 ± 0.007^{ab}
Phenol content (mg GAE/100g)	50.25 ± 1.72^{c}	52.50 ± 1.45^{ab}	53.00 ± 1.22^{ab}	55.75 ± 1.66^{a}
Flavonoids content (pimol QE gram)	63.65 ± 0.30^{c}	95.63 ± 2.00^{ab}	105.21 ± 3.52^{b}	118.46 ± 4.72^{a}
DPPH (% inhibition)	42.80 ± 1.76^{c}	44.18 ± 1.56^{ab}	45.33 ± 0.78^{ab}	46.08 ± 0.42^{a}
FRAP (µmol TE/g)	2.52 ± 0.08^{c}	3.04 ± 0.06^{b}	3.34 ± 0.14^{ab}	3.58 ± 0.04^{a}

All values are calculated on dry weight basis and are average values for analysis in triplicates.

3.2 Total phenolics, Flavonoids and *In vitro* antioxidant activity

The total phenolics, flavonoids content and *in vitro* antioxidant activity were recorded in increasing order with SWB enrichment. The total phenolics content (TPC) of pasta were found 52.50, 53.00 and 55.75 mg GAE $100g^{-1}$ in (10%, 15% and 20% w/v) of SWB enriched pasta respectively over the control (50.25 mg GAE $100g^{-1}$) (Table 1). Kumar *et al*., (2018) [22] prepared bars with *Spirulina* addition and reported TPC in the range from 5.56-7.90 mg GAE/g while in control it was 0.98±0.03mg GAE/g of TPC. These findings are in accordance with several studies related to development of *Spirulina* enriched different food products (Vatsala & Sudesh., 2017; Khan *et al*., 2013; Abd El Baky *et al*., 2015) [45, 21, 22]. SWB enriched pasta also recorded a significant increment in flavonoids content from 50.25% to 86.12% as compared to control pasta (63.65 pimolQE/g). Agustini *et al*., (2015) [4] reported 6.92 GAE/g TPC, 110.13 quercetin equivalent/g total flavonoids and 33.07 antioxidant activity IC50 in dried extract of *Spirulina* biomass.

In vitro antioxidant activity measured as FRAP activity and percentage inhibition of DPPH ranged from 2.52 to 3.58 µmol TE g^{-1} and 42.80-46.08% respectively. 20% SWB enriched pasta showed significant improvement in FRAP activity and percentage inhibition of DPPH which was 42.24% and 7.67% respectively (Table 1). Similarly, Muresan *et al*. (2016) [29] reported increase in DPPH scavenging activity with addition of 5% *Spirulina* biomass. Bread wheat pasta prepared with 5, 10 and 20% dry *Spirulina* biomass enrichment reported FRAP activity in the range from 1.93-4.24 µmol TE g^{-1} over control pasta (1.16 µmol TE g^{-1}) (Marco., 2014) [37].

3.3 Cooking quality
Optimum cooking time (OCT) was reported to be 5.20-5.35 minutes showing a marginal change. No change in the OCT and texture attributes was reported in fresh spaghetti prepared with *Chlorella vulgaris* and *Spirulina maxima* (Fradique *et al*., 2010) [17]. The presence of *Spirulina* protein hinders the development of gluten network and for that reason OCT reduced with increase in concentration of *Spirulina* biomass after first increase in cooking time. After cooking, the increase in true volume, weight and cooking loss are also important indicators for determining physical changes and the quality of pasta. Pasta enriched with 15% SWB recorded 24.17% and 58.13% increase in length and breadth of pasta respectively. Volume displacement of toluene that represents true volume of pasta as presented in table 2 recorded 87.43% to 92.48% volume displacement when enriched with SWB as compared to control pasta (100%). SWB enriched pasta after cooking also showed higher increment in weight (154.43% to 182.77%) over control (137.82%). Prabhasankar *et al*. (2009) [34] reported similar observations for microalgae enriched pasta. The cooking loss of SWB enriched pasta were found in the range 6.26 to 6.86% as compared to control (4.13%) (Table 2). According to Dick and Young, (1998) the technological acceptable limit of cooking loss was ≤8% and all the pasta samples were found in this acceptable limit.

Table 2: Cooking quality of Spirulina wet biomass (SWB) enriched pasta.

Parameter	Control	10 % SWB	15% SWB	20% SWB
Cooking Time (Min.)	5.35 ± 0.06^{a}	5.25 ± 0.05^{ab}	5.25 ± 0.08^{b}	5.20 ± 0.02^{b}
Change in Length (%)	30.97 ± 7.90^{a}	25.97 ± 7.39^{a}	24.26 ± 4.43^{a}	24.17 ± 7.24^{a}
Change in Breadth (%)	47.11 ± 6.88^{ab}	56.00± 5.40^{a}	50.24 ± 7.07^{b}	58.13 ± 4.47^{a}
Gruel loss (%)	4.13 ± 0.15^{c}	6.86 ± 036^{a}	6.71 ± 0.36^{a}	6.26 ± 0.31^{bc}
Increase in wt after cooking (%)	137.82 ± 3.41^{c}	154.43 ± 1.62^{b}	174.57 ± 1.98^{ab}	182.77 ± 2.37^{a}
Volume displacement (%)	10.00 ± 0.00^{a}	9.25 ± 0.13^{ab}	8.88 ± 0.21^{ab}	8.74 ± 0.29^{bc}

3.4 Textural analysis
The textual attributes of cooked pasta are also important parameters that govern the acceptability of the pasta. The significant variations were observed for different textural attributes *viz*. stickiness, firmness, cut force, cohesiveness and consistency of SWB enriched cooked pasta. The mean cut force and firmness varied from 542.11 to 637.28g and 3062.90 to 4912.19g respectively (Table 3). *Spirulina* (8%) enriched rice-soy crisp was found lower in hardness (61.0kg) than the control (96.3kg) without *Spirulina* addition (Bashir *et al*., 2017) [9]. The *al dante* feeling of pasta is measured by its stickiness. The stickiness recorded was higher in SWB enriched pasta (32.74-38.53 g.sec) than control (23.68 g.sec) and the consistency of the 20% SWB enriched and control cooked pasta was recorded at 13.46 kg.s and 23.98 kg.s respectively (Table 3). The smooth texture and pasta stickiness are also related to consistency of cooked pasta.

Table 3: Textural attributes of *Spirulina* wet biomass (SWB) enriched pasta

Level of incorporation of *Spirulina*	Firmness (g)	Cut force (g)	Stickiness (g.sce)	Consistency (g.sec)	Cohesiveness (g)
Control	4912.19 ± 201.08^{a}	637.28 ± 27.56^{a}	23.68 ± 1.25^{c}	23.98 ± 2.77^{a}	522.87 ± 16.92^{b}
10%	3456.21 ± 176.06^{ab}	575.34 ± 31.64^{ab}	38.53 ± 0.54^{bc}	15.32 ± 1.64^{ab}	502.76 ± 16.74^{ab}
15%	3335.72 ± 124.18^{b}	572.74 ± 22.53^{ab}	35.32 ± 1.62^{a}	16.42 ± 1.43^{ab}	476 ± 13.74^{b}
20%	3062.90 ± 118.25^{bc}	542.11 ± 33.74^{c}	32.74 ± 1.34^{ab}	13.46 ± 1.63^{c}	432.64 ± 11.74^{c}

3.5 Sensory attributes
The evaluation of the average findings on sensory attributes by the 57 selected panelists are represented in Figure 2. The 20% SWB enriched pasta and control pasta were reported to have average overall acceptability score of 7.52 and 7.91 respectively. The value of color, aroma and appearance of pasta enriched with 20% SWB (7.31, 7.29 and 7.55) were found close to the value of control pasta (7.36, 7.42 and 7.64 respectively) (Fig 2). The enrichment of pasta with *Spirulina* helped to provide bright green color that refined its appearance and improve the acceptability. Pasta enriched with 20% SWB was evaluated as "like moderately" with highest score among all SWB enriched pasta preparations after control. Similarly, addition of *Spirulina* (1-4%) in rice flour bread reported no negative affect on the choice of evaluators for sensory analysis (Selmo & Salas-Mellado, 2014) [40].

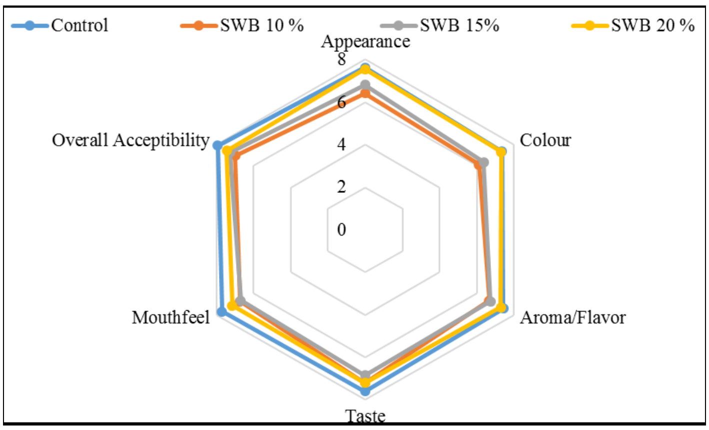

Fig 2: Acceptability scores of *Spirulina* wet biomass (SWB) enriched pasta

4. Conclusion
The observed results reveal that pasta enriched with fresh *Spirulina* wet biomass recorded significantly higher nutrient and chemical composition in terms of proteins, total lipids, minerals and ash content when compared to the control pasta. SWB enriched pasta also reported higher flavonoids, antioxidants activity and total phenolics content than the control pasta without any supplementation. The cooking quality of the pasta did not have any adverse effect of the *Spirulina* wet biomass addition, particularly the cooking losses, that is generally used to check pasta cooking performance by consumers and industry. The textural attributes were also in acceptable technological limits that favours enrichment of *Spirulina* biomass. The panelists favorably evaluated the pasta samples for its sensory attributes and found pasta enriched with 20% *Spirulina* wet biomass as most acceptable with high purchase intention. The pasta enriched with 20% SWB recorded an increase of 38.73% in protein, 19.42% in lipids, 86.12% in flavonoids, 10.95 in total phenols, 180.86 in iron, 5.58% in zinc and 17.80 in calcium content. The use of fresh wet *Spirulina* biomass directly for supplementation also reduced the time and energy required for drying of Spirulina. *Therefore*, pasta enriched with nutritious *Spirulina* can be used as functional food and providing an option to overcome nutrients deficiency and feasible for improving the nutritional health.

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