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Full Length Research Paper

Vitamins, minerals, protein digestibility and antioxidant activity of bread enriched with *spirulina platensis* powder

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Bread is one of the most popular bakery products, generally made from refined wheat flour which is limiting in vitamins, minerals, antioxidants and dietary fibre. Therefore, in the present study, wheat flour was supplemented with *Spirulina* powder at 2, 4, 6 and 8% levels to improve its nutritional quality. Developed enriched breads were found organoleptically acceptable by the panelists up to 6% level. With regard to nutritional quality, among the fortified breads, 6% *Spirulina* fortified bread yielded higher contents of protein (15.43%), *in vitro* protein digestibility (69.17%), β-carotene (7.81 mg/100g), total carotenoids (28.97 mg/100g) and total lysine (2.24 g/16gN). Total and available calcium, phosphorus, magnesium, iron and zinc contents were also found higher in 6% *Spirulina* supplemented bread as compared to control bread. Antioxidant activity (16.61%) and total phenolic contents (1.89 mg GAE/g) were also found significantly higher in 6% *Spirulina* enriched bread.

Keywords: *Spirulina platensis* powder, bread, supplementation, organoleptic acceptability, nutritional quality.

INTRODUCTION

In recent years, with the increasing urbanization as well as the advancement in baking technology and changing food habits, the bakery food products are now becoming popular in urban and semi urban areas of the most developing countries (Mohammed *et al*., 2009; Vijayarani *et al*., 2012).

The consumers' preference for white bread resulted in the removal of 70-80% of the initial nutrient content of wheat due to grinding and sieving processes. The elimination of nutrients from the diet resulted in an increased incidence of diseases and disorders (Farcaș *et al*., 2014). In terms of nutrition, bread reflects largely the nutritional value of flour and other raw materials from which it is obtained, therefore, it is necessary to continuosly improve its nutritional and organoleptic attributes.

The objective of supplementing alternative ingredients in bread formulation is to improve the nutritional value of wheat flour particularly proteins, minerals, vitamins and dietary fibre (Hallen *et al*., 2004). Formulation of composite flour is vital for development of value added products with optimal functionality (Rehman *et al*., 2007). One of such composite flour may be wheat flour substituted with *Spirulina* powder, *Spirulina* is a blue-green algae, which is simple, one-celled form of algae that thrives in warm, alkaline fresh-water bodies. The name "*Spirulina*" is derived from the Latin word for "helix" or "spiral"; denoting the physical configuration of the organism when it forms swirling, microscopic strands (Vijayarani *et al*., 2012). *Spirulina* contains as much as 65-71% protein, chlorophyll, B-complex vitamins, vitamin E, iron, more beta-carotene than carrots and the essential fatty acid known as gamma-linolenic acid.

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Spirulina is one of the few plant sources of vitamin B_{12}, usually found only in animal tissues (Batista *et al*., 2013; Udayasree *et al*., 2013).
Therefore, in the prospect of valuation and exploitation of *Spirulina*, it seems judicious to undertake studies about the fortification of bakery products such as bread by a dry biomass of *Spirulina* with the aim of improving its nutritional profile.

MATERIALS AND METHODS

Procurement of raw materials

Spirulina platensis powder was purchased from HerboNutra, Wholesale Trader from New Delhi. Wheat variety (WH-1105) was procured from Wheat and Barley Section of the Department of Genetics and Plant Breeding, CCSHAU, Hisar. Other ingredients were purchased from local market. The sample of wheat grains was milled in grinding machine to obtain fine flour. The wheat flour was blended with *Spirulina platensis* powder at 2, 4, 6 and 8% levels for development of breads.

Preparation of breads

The breads formula included wheat flour and *Spirulina* powder at 98:2, 96:4, 94:6 and 92:8 (yeast 3g, sugar 10g, salt 1.75g, water ± 60 ml). The following baking schedule was adopted: mixing, optimum at room temperature; fermentation, 1 h 40 min at 30±1°C; remixing, 25 sec at room temperature; recovery (ferment), 25 min at 30±1°C; proofing, 55 min at 30±1°C; baking, 20 min at 240°C; cooling 25 min at room temperature.

Organoleptic acceptability

Organoleptic acceptability of developed breads were determined by a panel of 10 judges using a nine point Hedonic Rating Scale ranging from like moderately (9) to dislike extremely (1) for each organoleptic characteristics.

Nutritional evaluation of control and *Spirulina* supplemented breads

On the basis of organoleptic acceptability, *Spirulina* supplemented breads up to 6% were selected for further nutritional analysis. Total minerals i.e calcium, iron, magnesium and zinc in acid digested samples were determined by Atomic Absorption Spectrophotometer according to the method of Lindsey and Norwell (1969). Whereas, phosphorus was determined colorimetrically by using the method of Chen *et al*. (1956). In available minerals, iron in the samples were extracted according to the procedure of Rao and Prabhavathi (1978). Calcium and zinc were extracted by the method of Kim and Zemel (1986). *In vitro* protein digestability was carried out by using the modified method of Mertz *et al*. (1983). βeta-carotene was estimated by the method of AOAC (2000). Total carotenoids were determined by the method of Wellburn (1994) and total lysine was estimated as per the method described by Mertz *et al*. (1975).

Statistical analysis

The data were statistically analyzed in complete randomized design for analysis of variance according to the standard method Sheoran and Pannu (1999).

RESULTS AND DISCUSSION

Organoleptic acceptability

Mean scores of colour, appearance, aroma, texture and taste of breads were found ‘liked very much’ to ‘liked moderately’ by the panelists and found at par with their respective control samples up to 6 % level of incorporation but thereafter increasing the incorporation level i.e 8% resulted in significant change in sensory characteristics and found ‘liked slightly’ in sensory attributes by the panelists (Fig. 1). Similar results were also reported by the other workers in *Spirulina* powder incorporated bread and buns (Minh, 2014)

Total phenolic and antioxidant activity

DPPH free radical scavenging activity of wheat flour based bread was 11.90%. Whereas, on increasing the incorporation level of *Spirulina* powder i.e 2, 4 and 6% in wheat flour showed increasing trend i.e 13.47, 15.04 and 16.61%, respectively. Maximum antioxidant activity was observed in 6% *Spirulina*s upplemented bread and minimum was found in 2% *Spirulina* supplemented bread. Similar results were also reported by other workers in *Spirulina* incorporated products (Prabhasankar *et al*., 2009; Khan *et al*., 2013; Marco *et al*., 2014). Abd El Baky *et al*., (2015) reported that high quality of phycocyanin of *Spirulina platensis* may be responsible for strong antioxidant activity.
Total phenolic contents of control bread were 0.72 mg GAE/g, as these values were found to be significantly increased on increasing the level of incorporation of *Spirulina* powder. Maximum total phenolic content was observed in 6% *Spirulina* supplemented bread and minimum in 2% supplemented bread. These results are in agreement with those reported earlier by various workers in *Spirulina* supplemented bread, biscuits and pasta (Khan *et al*., 2013; Marco *et al*., 2014; Abd El Baky *et al*., 2015). Marco *et al*. (2014) and Abd El Baky *et al*. (2015) reported that algae are an important source of numerous bioactive metabolites such as phenolic compounds, phycocyanin and β-carotenoids.

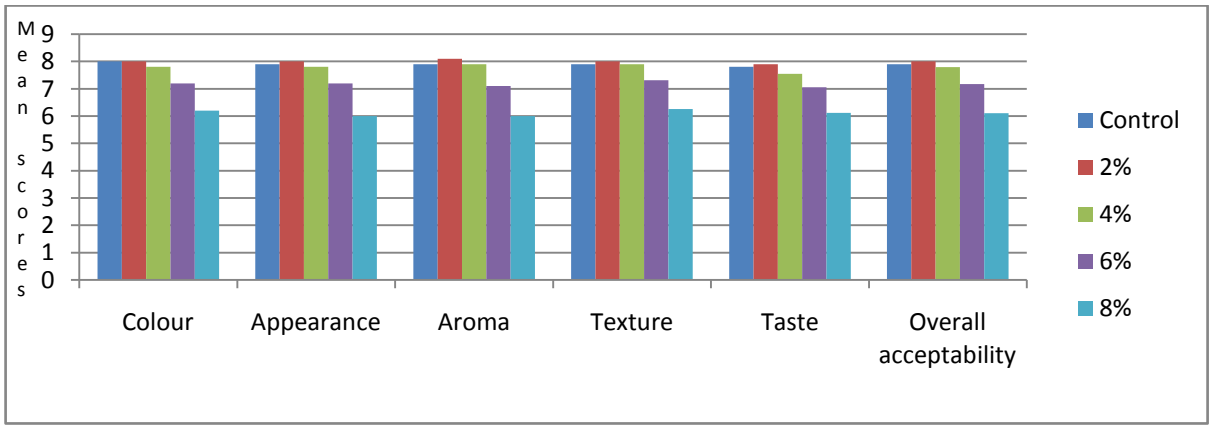

Fig 1. Mean score of organoleptic characteristics of *Spirulina platensis* powder supplemented breads.

Table 1. Total phenolic content and antioxidant activity of breads supplemented with *Spirulina platensis* powder (dry matter basis).

Types of Breads	Total phenolic contents (mg GAE/g)	DPPH free radical scavenging activity (%)
Control (100% WF)	0.72±0.03	11.90±0.74
Supplementation level (%) WF : SP		
98 : 2	0.95±0.04	13.47±0.99
96 : 4	1.29±0.06	15.04±1.00
94 : 6	1.89±0.13	16.61±0.85
CD (P≤0.05)	0.13	0.99

Values are means ± SE of three independent determinations
WF = Wheat flour SP = *Spirulina* powder

βeta-carotene and total carotenoids

β-carotene and total carotenoids were not detected in control bread, while in *Spirulina* supplemented breads, β-carotene and total carotenoids increased gradually and significantly on increasing the level of *Spirulina* powder in wheat flour (Table 2). The values were found in the range of 2.31 to 7.81 and 9.34 to 28.97 mg/100g, respectively in 2, 4 and 6% *Spirulina* supplemented breads. This increase in β-carotene and total carotenoids in supplemented breads might be due to higher content of β-carotene and total carotenoids in *Spirulina* powder as reported by Dominguez (2013). Other researchers also reported the similar results in *Spirulina* enriched products (Abd El Baky *et al*., 2014).

Total and available minerals

Spirulina fortified bread exhibited significantly higher amount of total calcium, phosphorus, magnesium and iron as compared to control bread (Table 3). Zinc content was differed non-significantly. As on increasing the level of *Spirulina* supplementation resulted in significant improvement in mineral contents. Among the supplemented bread, 6% *Spirulina* supplemented bread had significant higher amount than 4 and 2 per cent supplemented breads. It might be due to addition of *Spirulina* powder which contained many folds higher mineral contents than wheat flour (Vijayarani *et al*., 2012). These results are also in agreement with those reported by other workers in *Spirulina* supplemented bread, muffins and biscuits (Minh, 2014; Shahbazizadeh *et al*., 2015; Ghaly *et al*., 2015).
In vitro availability of calcium, iron and zinc were also found higher in all the three types of supplemented breads as compared to control bread (Table 4). It might be due to absence of phytic acid in *Spirulina* powder which is known to bind the divalent cations and hence reduce their bioavailability (Chaudhary, 2011).

Protein, in vitro protein digestibility and total lysine

Protein content in the fortified breads found to be increased significantly with increase in the percentage of *Spirulina* powder (Table 5). The increase in protein content could obviously be due to the significant quality of protein (65-71%) in *Spirulina* as reported by Udayasree *et al*. (2013). Other workers also reported significantly

Table 3. Total mineral contents of *Spirulina platensis* powder supplemented breads (mg/100g, dry matter basis).

Types of Breads	Calcium	Phosphorus	Magnesium	Iron	Zinc
Control (100% WF)	45.58±0.12	342.04±0.57	122.37±0.59	3.53±0.38	2.07±0.57
Supplementation level (%) WF : SP					
98 : 2	56.58±0.32	350.29±0.44	125.44±0.63	4.44±0.36	2.13±0.24
96 : 4	68.43±0.64	359.61±0.38	128.51±0.42	5.53±0.46	2.14±0.56
94 : 6	79.76±0.60	368.43±0.52	131.71±0.33	6.33±0.54	2.19±0.59
CD (P≤0.05)	1.56	1.61	1.68	1.47	NS

Values are means ± SE of three independent determinations
WF = Wheat flour SP = *Spirulina* powder NS = Non-significant

Table 4. Available minerals of *Spirulina platensis* powder supplemented breads (%, dry matter basis).

Types of Breads	Iron	Calcium	Zinc
Control (100% WF)	47.24±1.16	53.13±3.99	54.68±5.76
Supplementation level (%) WF : SP			
98 : 2	48.44±3.94	54.69±2.78	56.21±2.13
96 : 4	50.05±5.99	56.25±4.24	57.74±3.59
94 : 6	52.65±3.22	58.82±5.70	59.27±4.85
CD (P≤0.05)	1.00	0.93	1.06

Values are means ± SE of three independent determinations
WF = Wheat flour SP = *Spirulina* powder

Table 5. *In vitro* protein digestibility (%) and total lysine (g/16gN) content of breads supplemented with *Spirulina platensis* powder (dry matter basis).

Types of Breads	Protein	*In vitro* protein digestibility	Total lysine
Control (100% WF)	11.80±0.60	63.62±1.67	1.90±0.03
Supplementation level (%) WF : SP			
98 : 2	13.00±0.76	65.47±1.73	2.01±0.02
96 : 4	14.22±0.37	67.32±5.43	2.12±0.17
94 : 6	15.43±0.32	69.17±0.91	2.24±0.16
CD (P≤0.05)	1.80	1.17	0.10

Values are means ± SE of three independent determinations
WF = Wheat flour SP = *Spirulina* powder

higher protein content in *Spirulina* supplemented biscuits and breads (Fradique *et al*., 2010; Figueira *et al*., 2011; Marco *et al*., 2014).

In vitro protein digestibility and total lysine were also found significantly higher in *Spirulina* supplemented breads, 6% *Spirulina* supplemented bread had significantly higher amount followed by 4 and 2% supplemented breads. *In vitro* digestibility i.e69.17, 67.32 and 65.47%, respectively were found significantly higher than their control bread. Similarly, total lysine content was also found significantly higher in all the supplemented breads than control. It might be due to higher *in vitro* protein digestibility (92.59%) and total lysine (5.72g/16gN) contents in *Spirulina* powder (Ghaly *et al*., 2015). They also reported that *Spirulina* incorporated products had significantly higher content of *in vitro* digestibility and total lysine as compared to control bread. Our results are also in agreement with those reported by other workers in *Spirulina* fortified products (Tomaselli, 2004; Marco *et al*., 2014; Ghaly *et al*., 2015).

CONCLUSION

It may be concluded from the present study that 6% *Spirulina* incorporated bread found organoleptically acceptable by the panelists. In terms of nutritional quality, antioxidant activity, β-carotene, total carotenoids, protein,

digestibility, total lysine and minerals were found significantly higher. Hence, due to unique nutritional profile and presence of bioactive compounds in *Spirulina* powder, its use in food products may be encouraged to improve the nutritional status of general population and to overcome the nutritional deficiencies.

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