



ISSN Print: 2664-844X  
 ISSN Online: 2664-8458  
 IJAFS 2024; 6(1): 208-211  
[www.agriculturaljournals.com](http://www.agriculturaljournals.com)  
 Received: 09-03-2024  
 Accepted: 13-04-2024

**Deepshikha Radhakrishnan**  
 Department of Quality Control  
 and Assurance, Krishna Delta  
 Institute of Agricultural  
 Sciences, Vijayawada, Andhra  
 Pradesh, India

**Rajyalakshmi Swaminathan**  
 Department of Quality Control  
 and Assurance, Krishna Delta  
 Institute of Agricultural  
 Sciences, Vijayawada, Andhra  
 Pradesh, India

## Physico-chemical and sensory evaluation of biscuits fortified with spirulina powder

**Deepshikha Radhakrishnan and Rajyalakshmi Swaminathan**

DOI: <https://doi.org/10.33545/2664844X.2024.v6.i1c.1373>

### Abstract

Spirulina (*Arthrospira platensis*) is among the richest microalgal sources of protein, iron, and beta-carotene, yet its incorporation into baked goods has received limited attention in South Asian food research. This research evaluated biscuits prepared by replacing refined wheat flour with spirulina powder at 0, 2, 4, and 6% (w/w) for physico-chemical properties, colour, texture, and sensory acceptability. Protein content rose from 7.3% in the control to 14.1% at the 6% level, and iron increased from 2.8 to 9.7 mg/100 g. Hardness decreased progressively from 42.6 N (control) to 31.4 N at 6% substitution. Sensory evaluation by a 25-member semi-trained panel showed that the 2% spirulina biscuit scored highest for overall acceptability (7.8/9), while the 6% variant was penalised for a strong algal aftertaste and dark green colour. Moisture uptake during 35-day ambient storage was higher in spirulina-containing samples. Results indicate that 2-4% spirulina fortification offers a practical route to improving the nutritional profile of wheat biscuits without major sensory compromise.

**Keywords:** Spirulina fortification, biscuits, physico-chemical analysis, sensory evaluation, functional bakery, protein enrichment, iron content, texture analysis, shelf stability, *Arthrospira platensis*

### Introduction

Biscuit-making traces its origins to ancient Roman army rations twice-baked bread strips designed for long shelf life. Centuries later, biscuits remain one of the most widely consumed bakery products on the planet, with annual global production exceeding 30 million tonnes [1]. Their popularity, low cost, and long shelf life make them attractive vehicles for nutrient fortification, particularly in regions where micronutrient deficiencies persist [2].

Spirulina (*Arthrospira platensis*) contains 55-70% protein on a dry-weight basis, along with high concentrations of iron (28.5 mg/100 g), beta-carotene (170 mg/100 g), and B-vitamins [3]. The United Nations World Food Programme has recommended spirulina as a dietary supplement for malnourished populations [4]. However, its intense green colour and distinctive marine-like odour present challenges when blended into cereal-based foods [5].

Previous work on spirulina-fortified bread [6], pasta [7], and cookies [8] indicated that replacement levels beyond 3-5% of flour weight tend to depress sensory scores, especially for colour and taste. For biscuits specifically, the low moisture and high sugar/fat matrix may mask off-flavours more effectively than bread dough. This research examined four replacement levels (0-6%) in a standard semi-sweet biscuit formulation, measuring proximate composition, physical properties, and consumer acceptability over a 35-day storage period. The work was carried out at the Krishna Delta Institute of Agricultural Sciences, Vijayawada, between August 2021 and February 2022.

### Material and Methods

#### Reagents and Chemicals

Spray-dried spirulina powder (protein 63.2%, moisture 4.8%) was procured from Parry Nutraceuticals Ltd, Chennai. Refined wheat flour (maida, 10.4% protein), hydrogenated vegetable fat (Vanaspati), powdered sugar, skim milk powder, ammonium bicarbonate, sodium bicarbonate, common salt, and vanilla essence were purchased from local suppliers in Vijayawada. All analytical-grade chemicals (H<sub>2</sub>SO<sub>4</sub>, NaOH, HCl, petroleum ether, boric acid) were obtained from Merck India. Kjeldahl catalyst tablets and Soxhlet extraction thimbles were from Borosil.

**Corresponding Author:**  
**Deepshikha Radhakrishnan**  
 Department of Quality Control  
 and Assurance, Krishna Delta  
 Institute of Agricultural  
 Sciences, Vijayawada, Andhra  
 Pradesh, India

## Material

Four biscuit formulations were prepared: control (100% wheat flour) and three spirulina-substituted variants at 2, 4, and 6% (w/w) of flour weight. The base recipe per 100 g flour comprised: fat 40 g, sugar 35 g, skim milk powder 3 g, ammonium bicarbonate 1.5 g, sodium bicarbonate 0.5 g, salt 0.8 g, vanilla essence 0.3 mL, and water to form a workable dough. Dough was sheeted to 4 mm thickness, cut into 50 mm rounds, and baked at 180°C for 12 minutes in a convection oven. Biscuits were cooled for 30 minutes and packed in metallised polyester-LDPE pouches for storage trials.

## Quality control and calibration

Analytical instruments were calibrated before each batch. The digital refractometer was checked against distilled water (0 °Brix). The Kjeldahl distillation unit was validated with ammonium sulphate standard (recovery  $99.3 \pm 0.4\%$ ). The texture analyser (TA.XT Plus, Stable Micro Systems) was calibrated with a 5 kg standard weight before compression tests. Colour measurements ( $L^*$ ,  $a^*$ ,  $b^*$ ) on a

HunterLab colorimeter were standardised against a white tile. All analyses were done in triplicate and means reported.

## Methods

Proximate analysis followed AOAC methods: moisture (925.10, oven at 105°C), crude protein (920.87, Kjeldahl,  $N \times 6.25$ ), crude fat (920.39, Soxhlet with petroleum ether), ash (923.03, muffle furnace at 550°C), and crude fibre (962.09). Carbohydrate was calculated by difference. Iron was determined by atomic absorption spectrophotometry after wet digestion. Biscuit hardness was measured as peak compression force (N) using a 36 mm cylindrical probe at 1 mm/s crosshead speed. Water activity was recorded with an AquaLab 4TE meter. Sensory evaluation used a 9-point hedonic scale with a 25-member semi-trained panel assessing colour, texture, taste, aroma, crispness, and overall acceptability. Storage trials ran for 35 days at  $30 \pm 2^\circ\text{C}$ ,  $65 \pm 5\%$  RH, with moisture and water activity measured at weekly intervals. Data were analysed by one-way ANOVA and Duncan's multiple range test at  $p \leq 0.05$ .

## Results

**Table 1:** Proximate composition and iron content of spirulina-fortified biscuits

| Parameter        | Control (0%) | Spirulina 2% | Spirulina 4% | Spirulina 6% |
|------------------|--------------|--------------|--------------|--------------|
| Moisture (%)     | 3.2          | 3.4          | 3.6          | 3.9          |
| Protein (%)      | 7.3          | 9.6          | 11.8         | 14.1         |
| Fat (%)          | 18.4         | 18.1         | 17.8         | 17.3         |
| Ash (%)          | 1.1          | 1.4          | 1.8          | 2.3          |
| Fibre (%)        | 0.6          | 0.8          | 1.1          | 1.4          |
| Carbohydrate (%) | 69.4         | 66.7         | 63.9         | 61.0         |
| Iron (mg/100 g)  | 2.8          | 5.1          | 7.4          | 9.7          |

Values are means of triplicate analyses. Carbohydrate by difference

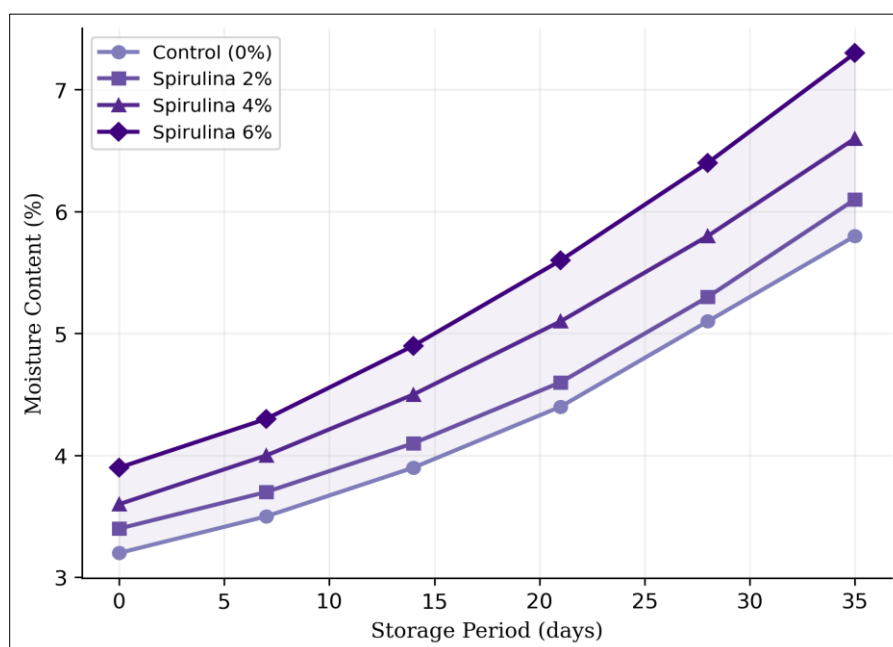


**Fig 1:** Moisture content of spirulina-fortified biscuits during 35-day ambient storage ( $30 \pm 2^\circ\text{C}$ ,  $65 \pm 5\%$  RH)

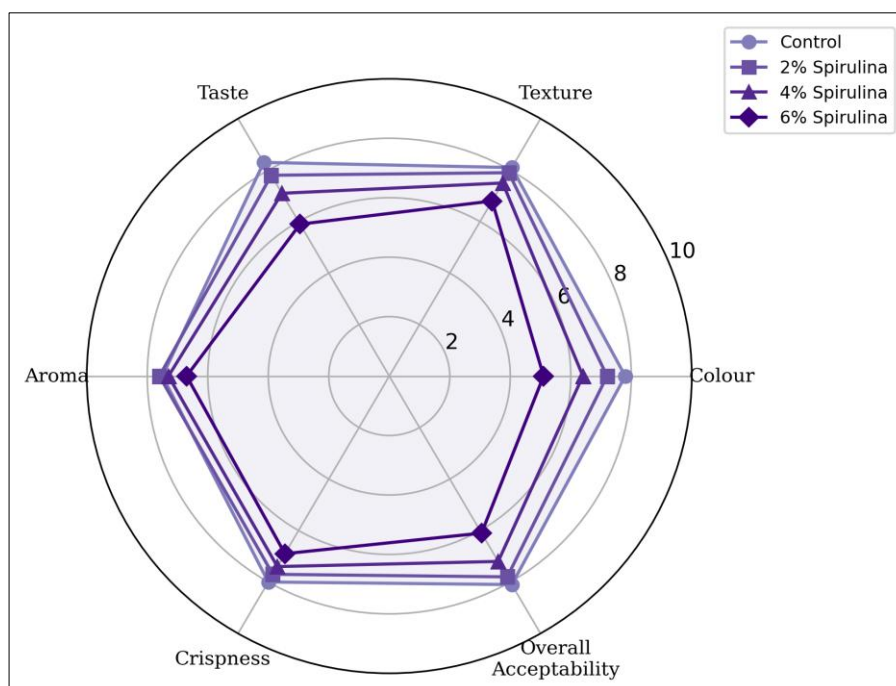
Protein increased linearly with spirulina level, rising from 7.3% to 14.1% at 6% substitution nearly doubling. Iron content showed a similar trend, reaching 9.7 mg/100 g at 6%, which represents about 35% of the recommended daily

intake for adult women. Moisture uptake during storage was faster in spirulina samples, reaching 7.3% at day 35 in the 6% variant versus 5.8% in the control.

**Table 2:** Physical and sensory properties of spirulina-fortified biscuits

| Parameter             | Control | 2%   | 4%    | 6%    |
|-----------------------|---------|------|-------|-------|
| Hardness (N)          | 42.6    | 38.7 | 35.2  | 31.4  |
| Spread ratio          | 7.1     | 6.8  | 6.5   | 6.1   |
| L* (lightness)        | 68.4    | 54.2 | 43.1  | 34.8  |
| a* (green-red)        | -1.2    | -6.8 | -10.4 | -13.7 |
| Colour score (9 pt)   | 8.1     | 7.2  | 6.4   | 5.1   |
| Overall acceptability | 8.1     | 7.8  | 7.2   | 6.1   |

Sensory scores are means from a 25-member semi-trained panel

**Fig 2:** Comparing sensory scores of spirulina-fortified biscuits at four substitution levels

### Comprehensive interpretation

The 2% spirulina biscuit retained sensory acceptability close to the control (7.8 vs 8.1) while delivering 31.5% more protein and 82.1% more iron. At 4%, overall acceptability fell to 7.2 still within the acceptable range ( $\geq 7.0$ ) but colour and taste scores dropped noticeably. The 6% variant crossed below acceptability thresholds for colour (5.1) and showed visible grittiness noted by 60% of panellists. Hardness decreased because spirulina's high protein content competes with gluten for water, weakening the biscuit matrix.

### Discussion

The protein enrichment observed here is consistent with earlier findings by Batista *et al.* [6], who reported a 1.8× protein increase in wheat cookies at 6% *Chlorella*. Spirulina's protein content (63.2%) is substantially higher than that of wheat flour (10.4%), so even a small substitution produces a large nutrient shift. The near-doubling of protein at 6% replacement agrees with the mass-balance expectation:  $0.06 \times 63.2 + 0.94 \times 10.4 \approx 13.6\%$ , close to the observed 14.1% [9].

Colour deterioration at higher spirulina levels is the primary barrier to consumer acceptance. The intense green due to chlorophyll and phycocyanin pigments pushed L\* values from 68.4 (pale golden) to 34.8 (dark olive-green) at 6%. Masking strategies such as cocoa powder addition or encapsulated spirulina may mitigate this, though they add cost [10]. The drop in hardness from 42.6 to 31.4 N actually improved mouthfeel for some panellists, who described the

2% variant as 'pleasantly crisp' a reminder that texture changes are not always negative [11].

Moisture uptake was higher in spirulina biscuits, likely because of the hygroscopic nature of algal proteins and polysaccharides [12]. This has implications for shelf life: at 7.3% moisture after 35 days, the 6% variant was approaching the 8% threshold typically associated with loss of crispness in semi-sweet biscuits [13]. Moisture-barrier packaging or desiccant sachets would be advisable for commercial-scale production.

### Conclusion

Spirulina powder at 2% (w/w) replacement of wheat flour produced biscuits with significantly higher protein and iron content while maintaining sensory acceptability comparable to the control. The 4% level also fell within acceptable sensory ranges and delivered even greater nutritional gains, though colour and taste scores began to decline. At 6%, sensory penalties particularly for colour and aftertaste outweighed nutritional benefits for the tested panel. Moisture uptake during storage was faster in spirulina-fortified samples, suggesting that improved packaging is needed. A 2-4% spirulina fortification window appears practical for commercial biscuit production aimed at addressing protein and iron deficiencies.

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