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Characterization of phenolic compounds and antioxidant activity in spirulina-enriched cookies

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Abstract

This study explores the phenolic profile and antioxidant potential of cookies enriched with *Spirulina platensis*, a microalga known for its functional bioactivity. The growing demand for health-enhancing bakery products has driven interest in incorporating natural antioxidants into widely consumed formats like cookies. Using spectrophotometric and chromatographic techniques, we quantified total phenolic content (TPC), identified individual phenolic compounds, and evaluated antioxidant activity via DPPH and ABTS assays in control and spirulina-fortified samples. Our findings demonstrate a marked increase in both phenolic concentration and radical scavenging capacity following spirulina enrichment, supporting its role in functional bakery product development.

Keywords: Spirulina, phenolic compounds, antioxidant activity, functional cookies, total phenolic content

1. Introduction

Cookies are among the most widely consumed baked products worldwide due to their taste, texture, and convenience. However, conventional cookies are often considered nutritionally poor, typically high in saturated fat and simple carbohydrates, and low in functional compounds such as antioxidants. In response to rising public health concerns, particularly those related to oxidative stress and chronic disease, there is a growing emphasis on the development of functional bakery products. These aim to deliver both sensory appeal and physiological benefits.

Among the natural bioactive ingredients under investigation, *Spirulina platensis*, a filamentous cyanobacterium, has gained significant attention. It is rich in protein, polyunsaturated fatty acids, vitamins, minerals, and phenolic compounds—bioactives known to mitigate oxidative damage and support metabolic health. Spirulina's antioxidant potential is largely attributed to its phenolic acids, flavonoids, and unique pigments such as phycocyanin. However, limited data exist on how spirulina's functional compounds behave when incorporated into thermally processed foods like cookies.

Phenolic compounds in food matrices have been extensively studied for their ability to neutralize free radicals, reduce inflammation, and provide a protective effect against non-communicable diseases. Nevertheless, the stability of phenolics during baking and their impact on final antioxidant activity require careful evaluation. Previous studies have reported promising results when incorporating spirulina into bakery formulations, but comprehensive characterization of its phenolic profile post-baking remains insufficient.

The aim of this study is to assess the phenolic content and antioxidant activity of cookies enriched with *Spirulina platensis*. Using both spectrophotometric and chromatographic methods, we provide quantitative and qualitative insights into the retention and behavior of phenolic compounds during cookie production. Furthermore, we evaluate the antioxidant potential of the final product to determine the efficacy of spirulina fortification in enhancing health-promoting properties in a widely consumed food format

2. Materials and Methods

2.1 Cookie Preparation

Two types of cookies were formulated: a control (without spirulina) and a test group enriched with *Spirulina platensis* powder. All ingredients were of analytical grade and food

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safe. The standard shortbread formulation included wheat flour (100 g), butter (50 g), granulated sugar (30 g), and water (15 mL). For the test cookies, spirulina powder was added at 3% (w/w) relative to the total flour content. The dough was homogenized using a planetary mixer for 5 minutes, rolled to a uniform thickness of 5 mm, cut into 5 cm diameter discs, and baked at 180 °C for 12 minutes. After cooling, cookies were sealed in airtight containers and stored at room temperature until analysis.

2.2 Extraction of Phenolic Compounds

After freeze-drying, cookie samples were ground into a fine powder using a laboratory grinder. An aliquot of 2 g from each sample was mixed with 20 mL of 80% methanol (v/v) and vortexed for 5 minutes. The mixture was then sonicated at 40 kHz for 30 minutes at room temperature and shaken on an orbital shaker at 200 rpm for 1 hour. The supernatant was collected by centrifugation (4000 rpm, 15 min), filtered through a 0.45 µm membrane, and stored at 4 °C in amber vials until further analysis.

2.3 Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu colorimetric method. Briefly, 0.5 mL of each methanolic extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and incubated for 5 minutes. Next, 2.0 mL of 7.5% sodium carbonate solution was added. The reaction mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was read at 765 nm using a UV-Vis spectrophotometer. Gallic acid was used to prepare the calibration curve (10-100 mg/L), and results were expressed as mg gallic acid equivalents (GAE) per 100 g dry weight.

2.4 HPLC Analysis of Phenolic Profile

Phenolic compound profiling was conducted using high-performance liquid chromatography (HPLC) equipped with a diode array detector (DAD). Separation was achieved on a C18 reversed-phase column (250 mm × 4.6 mm, 5 µm) using a gradient mobile phase: solvent A (0.1% formic acid in water) and solvent B (acetonitrile). The flow rate was maintained at 1.0 mL/min, and the injection volume was 20 µL. Detection was performed at 280 nm and 320 nm. Phenolic standards included caffeic acid, ferulic acid, chlorogenic acid, and quercetin. Identification was based on retention time comparison and spectral matching.

2.5 Antioxidant Assays

Two *in vitro* assays were used to evaluate antioxidant capacity: DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). For the DPPH assay, 100 µL of extract was added to 3.9 mL of DPPH solution (0.1 mM in methanol). The mixture was incubated in the dark for 30 minutes, and absorbance was read at 517 nm. For the ABTS assay, ABTS radicals were generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating the solution in the dark for 12-16 hours. The radical solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm before use. Then, 100 µL of extract was added to 3.9 mL of ABTS solution and measured at 734 nm after 6 minutes. Results from both assays were expressed as µmol Trolox equivalents (TE) per gram of dry weight.

3. Results

3.1 Total Phenolic Content

The TPC of spirulina-enriched cookies was significantly higher than that of the control group. Control cookies

yielded 42.5 ± 1.3 mg GAE/100g DW, while spirulina cookies reached 89.4 ± 2.1 mg GAE/100g DW, more than doubling the phenolic content.

3.2 HPLC Phenolic Profiling

HPLC-DAD revealed enhanced concentrations of key phenolic acids in the spirulina group, particularly ferulic acid (6.2 µg/g vs. 2.1 µg/g in control) and chlorogenic acid (4.7 µg/g vs. 1.5 µg/g). Minor increases were also noted in quercetin and caffeic acid levels.

3.3 Antioxidant Activity

As summarized in Table 1, both antioxidant assays demonstrated superior radical scavenging capacity in spirulina-fortified cookies.

Table 1: Antioxidant activity of control and spirulina-enriched cookies as measured by DPPH and ABTS assays.

Sample	DPPH (µmol TE/g)	ABTS (µmol TE/g)
Control Cookies	5.8 ± 0.2	6.3 ± 0.3
Spirulina-Enriched	12.6 ± 0.4	13.8 ± 0.6

4. Discussion

The results confirm that spirulina is a valuable source of phenolic compounds capable of enhancing the antioxidant potential of cookies. The increase in TPC aligns with previous studies emphasizing the contribution of spirulina's phycocyanin and flavonoid content to oxidative stress mitigation.

The HPLC data suggest that specific phenolic acids in spirulina remain stable during the baking process, although some degradation may occur. Importantly, the preservation of functional compounds post-baking reinforces the feasibility of spirulina fortification in commercial bakery production.

The antioxidant activity measured by DPPH and ABTS confirms that spirulina inclusion yields not only higher phenolic content but also bioactive functionality, validating its role as a natural antioxidant enhancer.

5. Conclusion

This study presents clear evidence that spirulina enrichment significantly enhances the phenolic content and antioxidant potential of cookies. The integration of *Spirulina platensis* into a conventional shortbread recipe led to a marked increase in total phenolic concentration and radical scavenging activity, as measured by DPPH and ABTS assays. High-performance liquid chromatography confirmed the presence of several key phenolic compounds—most notably ferulic acid, chlorogenic acid, and caffeic acid—in appreciable concentrations, demonstrating the compound-specific contribution of spirulina to the overall phytochemical profile.

Importantly, the retention of phenolic activity post-baking underscores the thermal stability of these compounds when incorporated into food matrices such as cookies. These findings are significant not only for food scientists but also for the functional food industry, where spirulina offers a natural and sustainable means of improving the nutritional profile of widely consumed snack items.

Nevertheless, this study also highlights the need for further investigation. Future work should focus on the bioavailability of spirulina-derived phenolics following ingestion, sensory acceptance among different consumer groups, and the optimization of formulation parameters for

industrial-scale applications. Moreover, clinical trials are necessary to substantiate the health claims associated with regular consumption of spirulina-enriched baked goods.

In conclusion, spirulina holds considerable promise as a fortifying agent in functional cookie development, offering both nutritional enhancement and potential antioxidant protection. Its inclusion aligns with current trends in health-oriented food innovation, representing a valuable step toward the creation of bakery products with tangible health benefits.

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