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Spirulina as a Functional Food Ingredient: Physical and Chemical Properties of Supplemented Cookies

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Abstract: In this study, the incorporation of microalgae Arthrospira platensis (Spirulina) in wheat-based snacks was investigated. Since cookies are one of the most popular baked food products, and are generally limited in vitamins, minerals, antioxidants and dietary fibre, a wheat-based cookie formulation was prepared and supplemented with 10% (w/w) dried Spirulina powder. The physical and chemical properties of the baked (at 200 ºC) cookies were then assessed via various physiochemical tests. The results of the tests were analysed through variance analysis (one-way ANOVA) and the Tukey's test (post hoc comparison) at a significance level of 95% ($p < 0.05$). The results showed a significant increase in the crude ash, crude fibre, crude protein, and antioxidant activity levels of the Spirulina-supplemented cookies (SC) compared to the control cookies (CC). The crude fat and crude carbohydrate levels significantly decreased in the SC. The moisture content and phenolic content remained unchanged in the comparative samples. These results suggest that Spirulina is a rich source of nutrients like protein, antioxidants, minerals, and fibre and can be used to enhance the nutritional properties of wheat-based snacks. At the same time, further work should be directed towards improving the phenolic content of Spirulina cookies by exploring the possibility of lower cooking temperatures.

Keywords: Algae, Alternate Ingredients, Arthrospira Plantensis, Edible Algae, Functional Foods, Spirulina, Supplementation, Sustainable Food Production.

Research Paper

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1. INTRODUCTION

Algae, including microalgae like Spirulina (*Arthrospira platensis*), offer a potential solution to the global food insecurity problem due to their ability to thrive in various water conditions and their rapid growth. Spirulina, in particular, is rich in valuable nutritional compounds and has been recognized for its health-promoting properties. With a growing interest in alternative foods and the projected market growth of algae products, there is a need to explore the viability of Spirulina as a sustainable and enriched food source. Spirulina is a cyanobacterium that thrives in warm alkaline waters. It is highly regarded for its nutritional composition, including proteins, vitamins (B12, A, and E), minerals (iron, calcium, and magnesium), and antioxidants. Spirulina's high protein content, including all essential amino acids, makes it an excellent protein source for various dietary preferences (AlFadhly *et al.*, 2022). The nutritional value of Spirulina contributes to several positive health effects. It has immune-boosting properties and exhibits anti-inflammatory characteristics (Angelina *et al.*, 2018), which may aid in preventing and managing chronic diseases like diabetes and cardiovascular disease (Bolanho *et al.*, 2014). Additionally, Spirulina has shown potential in reducing cholesterol levels and may have therapeutic applications in liver disease treatment (Burapan *et al.*, 2020).

1.1 Incorporation of Spirulina in Food Products

Spirulina has been successfully incorporated into various food products, including cookies, to enhance their nutritional value and health benefits. Previous studies have demonstrated an increase in protein content, antioxidant capacity, and phenolic content in Spirulina-fortified cookies. The acceptability of Spirulina-enriched shakes and pasta further supports the feasibility of incorporating Spirulina without compromising sensory quality.

Table 1: Different functional foods supplemented with Spirulina

Type of Supplemented Foods	Spirulina Biomass	References
Mixed Cookies	1% and 2%	Sahin (2020)
Composite Flour Cookies	2% and 5%	Bolanho *et al.*, (2014)
Traditional Egyptian Cookies	10-15%	Nakib *et al.*, (2019)
Wheat Biscuits	2% and 6%	Batista *et al.*, (2019)
Refined Wheat Bread	2%, 4%, 6% and 8%	Saharan & Jood (2017)
Bread	1% and 3%	Hafsa *et al.*, (2014)
Snack Bars	2% and 6%	Lucas *et al.*, (2020)
Dried Pasta	2-15%	Koli *et al.*, (2022)
Fresh Pasta	3%, 5%, 7%, and 10%	Raczyk *et al.*, (2022)
Corn Extrudates	2-8%	Tanska *et al.*, (2017)
Shakes	750 mg/100 g	Santos *et al.*, (2004)

The addition of Spirulina to cookie formulations can affect their physical and chemical properties. Studies have reported changes in color, hardness, moisture, and nutrient content in Spirulina-supplemented cookies. Texture stability, digestibility, and sensory preferences have also been evaluated, demonstrating the potential of Spirulina as a functional food ingredient. Spirulina's nutritional profile, minimal environmental impact, and potential to reduce food waste make it a sustainable alternative ingredient (Habib *et al.*, 2008). It has a lower carbon footprint compared to animal-derived protein sources, and its cultivation can utilize waste streams, improving resource efficiency (Hasler *et al.*, 2002).

1.2 Conclusion

In conclusion, Spirulina offers a nutrient-rich microalga with potential health benefits. Incorporating Spirulina into food products, specifically cookies, has shown promise in enhancing their nutritional value without compromising sensory quality. This study aims to assess the physical and chemical properties of Spirulina-supplemented cookies and evaluate the functionality and sustainability of Spirulina as an alternative ingredient.

This research aims to assess the effectiveness of incorporating Spirulina into a wheat-based snack and explore its functionality as an alternative ingredient. The specific objectives include evaluating the impact of Spirulina on the physical and chemical properties of cookies, such as sensory properties, texture, nutritional value, and shelf-life.

2. MATERIALS AND METHODS

The experimental design of this research was divided into two phases; sample preparation and testing. Firstly, the preparation of wheat-based cookies was done by hand. This was followed by the analysis of the physical, chemical and nutritional properties of Spirulina enriched wheat cookies in a comparative study: whereby the control cookie, Spirulina cookie and Spirulina powder were subjected to physicochemical tests concurrently. The results obtained were analysed to determine whether or not Spirulina derived compounds can be functional in a baked, wheat product.

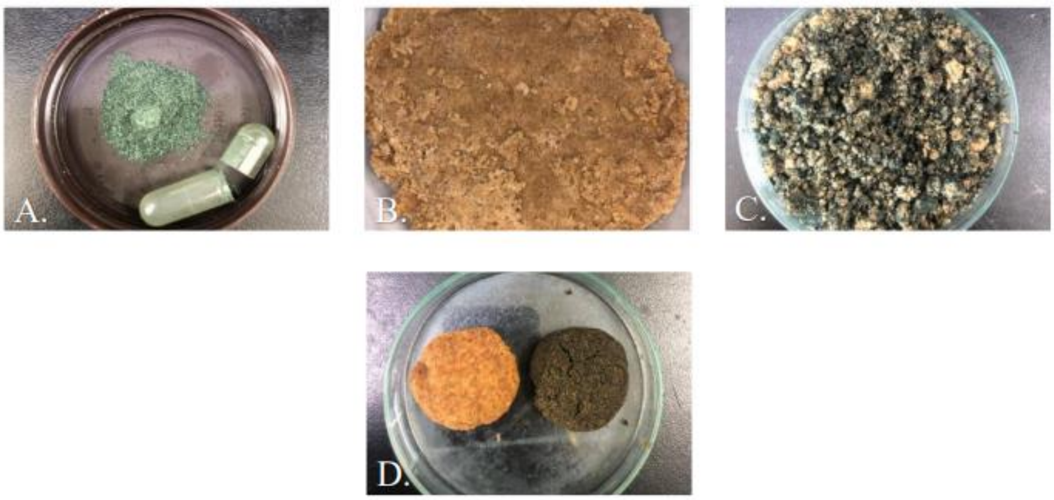

Figure 1: (A) GNC Spirulina Powder, (B) Prepared cookie mixture, (C) Cookie mixture with Spirulina and (D) The baked cookies

The testing to be carried out was broadly categorized into two types, each type measuring a different parameter of the food product, namely physical and chemical/nutritional testing. All test samples were taken in triplicates and were also subjected to duplicate testing in accordance to AOAC (Association of Official Agricultural Chemists) International's guidelines on accuracy and precision.

2.1 Sample Preparation

The three ingredient wheat cookies were made from whole wheat, coconut sugar and clarified butter in a self-devised recipe (Table 2) to keep the matrix as simple and reproducible as possible. Dried, pre-processed Spirulina powder was obtained in the form of pills (GNC, USA) to be added to the mixture to formulate the Spirulina cookies. 10 % w/w ratio was followed, where 1g of Spirulina powder was added to 9g of the cookie mixture (Figure 1). Finally, the cookies, weighing 10g each, were baked at a high temperature of 200°C and all the tests were carried out on these baked products.

Table 2: Cookie formulation

Ingredient	Quantity (g)
Whole Wheat Flour	128
Clarified Butter	64
Coconut Sugar	64
Spirulina Powder (in enriched cookies)	10% w/w in 10g cookie

2.2 Physical Testing

Physical tests are an integral part of food research, providing insights into the sensory, processing, and safety properties of food products. One important test is the measurement of moisture content, which plays a crucial role in determining the shelf life and processing characteristics of food. Moisture content can indicate the likelihood of spoilage and microbial growth, as well as impact the texture and consistency of the product. In this study, the moisture content test followed the guidelines outlined in AOAC 930.15. Representative samples were collected and thoroughly mixed to ensure homogeneity. These samples were then placed in clean, dry petri dishes and dried in an oven at 110°C for 3 hours or until a constant weight was achieved. After cooling, the samples were re-weighed, and the moisture content was calculated using the formula: Moisture content = [(W1 - W2) / W1] x 100%, where W1 represents the initial weight of the sample and W2 represents the final weight. By analyzing moisture content, manufacturers can optimize their processing conditions to achieve the desired product texture and consistency while ensuring adequate shelf life and microbial safety.

2.3 Chemical Testing

Food chemical tests are essential for evaluating the nutritional composition of food products, including the levels of vitamins, minerals, and macronutrients, to ensure compliance with specific nutritional requirements.

Ash Test: One such test is the ash content analysis, which provides valuable insights into the mineral content and overall nutritional quality of edible products. Following the guidelines of AOAC Official Method 942.05 and ISO 5984:2022, this study employed a protocol for ash content analysis. A dried sample (after moisture removal) weighing 2g was accurately weighed and homogenized (Ws). The sample was then placed in a pre-weighed and dry porcelain crucible (W1), which was subsequently subjected to a high temperature of 600°C in a muffle furnace for 2 hours. This process completely burned off the organic material, leaving behind only the mineral content as burnt ash. After cooling in a desiccator, the crucible with the ash was weighed altogether (W2). The percentage of ash content in the food sample was calculated using the formula: Ash Content = [(W2 - W1) / Ws] x 100%. This analysis enables the determination of essential minerals such as calcium, phosphorus, potassium, magnesium, iron, and zinc, which play crucial roles in various physiological functions within the human body.

Crude Fiber: The crude fiber content of the samples was determined using the AOAC 978.10 and ISO 6865:2000 methods. Briefly, the samples were ground to a fine powder and weighed accurately to 2 g. A 0.128 M solution of H_2SO_4 and a 0.313 M solution of NaOH were prepared. The 2 g sample was boiled for 30 minutes in 200 mL of acid first and filtered through a fine mesh. The filtrate was then digested for a second time for 30 minutes in the alkali, removing all the non-fibrous components of the food samples, leaving behind only crude fiber as the filtrate. The final filtrate was washed with hot water and dried in a hot air oven at 130 °C for 2 hours, until all residual moisture was removed. The dried residue was weighed and the crude fiber content was calculated using the following formula:
Crude Fiber Content = [W1 - W2/W1 x 100%]

Crude Fat: The crude fat content of the samples was determined using the AOAC Official Method 920.39 and ISO 1443:1973 methods. Briefly, up to 5 g of each sample was taken and weighed (Ws), and was then packed in a filter paper thimble while ensuring there was no leakage. The sample was extracted with an organic solvent of low boiling point using a Soxhlet apparatus. Petroleum ether was used and the extraction was carried

out at 60 °C for several hours until all the fat was extracted, which was indicated by the solvent turning completely clear. The solvent was removed by evaporating it from the extracted fat by placing the distillate in a hot air oven. The remaining extracted fat was weighed and recorded (W1). The crude fat content was calculated by using the following formula:
Crude Fat Content = [Ws – W1/ W1] x 100%

Crude Carbohydrate: The crude carbohydrate content of the samples was determined using the anthrone reagent method. Briefly, a 0.2% anthrone reagent was prepared by dissolving 0.2 g of anthrone powder in 100 mL of concentrated sulfuric acid. A stock solution of glucose with the concentration 200 µg/mL was prepared to run a standard curve. The stock was serially diluted to prepare 8 dilutions ranging from 10-200 µg/mL. 1 mL of ethanolic extracts of each sample with a concentration of 25 µg/mL were used to run the test. All the glucose dilutions were made up to 1 mL using distilled water, followed by 5 mL of the anthrone reagent being added to both these dilutions and the sample extracts. The prepared mixtures were incubated at 100 °C in a water bath by submerging the test tube rack in the boiling water completely for 10 minutes. After cooling down the test tubes to room temperature, the absorbance of the greenish-blue colour that developed was measured using a spectrophotometer at a wavelength of 620 nm, and the standard curve was plotted. The crude carbohydrate content was calculated using the glucose standard curve and was expressed as a percentage of the original sample weight.

Crude Protein: The crude protein content of the samples was determined using the Folin-Lowry method. Briefly, a stock solution of bovine serum albumin (BSA) was prepared and diluted to create a standard curve. The ethanolic extracts of the samples were then diluted and reacted with the Folin-Lowry reagents, forming a colored complex whose absorbance was measured at 660 nm using a spectrophotometer. The crude protein content was calculated using the BSA standard curve and was expressed as a percentage of the original sample weight.

Total Phenolic Content: The total phenolic content of the samples was determined using the Folin-Ciocalteu (FC) assay. Briefly, a stock solution of gallic acid was prepared and diluted to create a standard curve. The ethanolic extracts of the samples were then diluted and reacted with the FC reagents, forming a colored complex whose absorbance was measured at 760 nm using a spectrophotometer. The total phenolic content was calculated using the gallic acid standard curve and was expressed as a percentage of the original sample weight.

Antioxidant Activity: The antioxidant activity of the samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams *et al.*, 1995). Briefly, a stock solution of DPPH was prepared and diluted to create a standard curve. The ethanolic extracts of the samples were then diluted and reacted with the DPPH solution, forming a yellow-colored complex. The absorbance of the complex was measured at 517 nm using a spectrophotometer. The antioxidant activity was calculated as the percentage inhibition of DPPH by the samples.

Statistical Analysis

Statistical analysis of the experimental data was performed using STATISTIX from Analytical Software (version 8.1), through variance analysis (one way ANOVA), by the Tukey's test – Post Hoc Comparison at a significance level of 95% ($p < 0.05$). All results are presented as average ± standard deviation.

3. RESULTS AND DISCUSSION

Table 3: Comparative results based on different nutritional parameters

Tests	Spirulina Powder (SP)	Spirulina Cookie (SC)	Control Cookie (CC)
Moisture Content (%)	4.40 ± 0.19^a	3.23 ± 1.15^a	3.05 ± 0.52^a
Ash Content (%)	6.53 ± 1.25^a	3.78 ± 0.17^b	1.58 ± 0.49^c
Fibre Content (%)	4.95 ± 0.49^a	5.49 ± 0.53^a	3.77 ± 0.23^b
Crude Fat (%)	6.21 ± 0.98^a	23.61 ± 1.97^b	27.46 ± 1.38^c
Crude Carbohydrate (µg/g)	92.17 ± 1.90^a	299.32 ± 9.19^b	438.61 ± 50.43^c
Crude Protein (µg/g)	211.69 ± 18.34^a	88.59 ± 7.25^b	22.29 ± 1.75^c
Phenolic Content (mg GAE/g)	13.08 ± 1.32^a	9.560 ± 0.56^b	9.460 ± 0.62^b
Antioxidant Activity (%)	52.25 ± 0.28^a	21.37 ± 0.09^b	14.98 ± 0.47^c

All values are expressed as mean ± S.D for analysis in triplicates. Superscripts along the rows indicate significant difference ($p < 0.05$). Values in this table pertain to the composition of raw spirulina powder and baked cookies.

The moisture content of the spirulina powder (SP) was 4.4%, while the moisture contents of the control cookie (CC) and the spirulina-supplemented cookie (SC) were 3.23% and 3.05%, respectively. These results indicate that the addition of SP to the cookie mix did not significantly increase the moisture content of the cookies.

The ash content of the SP was 6.53%, while the ash contents of the CC and SC were 1.58% and 3.78%, respectively. These results indicate that the addition of SP to the cookie mix significantly increased the ash content of the cookies.

The crude fibre content of the SP was 4.95%, while the crude fibre contents of the CC and SC were 3.77% and 5.49%, respectively. These results indicate that the addition of SP to the cookie mix significantly increased the crude fibre content of the cookies.

The results of this study suggest that the addition of SP to wheat cookies can be an effective way to increase the nutritional value of the cookies. The SP is a good source of minerals and dietary fibre, and the addition of SP to the cookies significantly increased the levels of these nutrients in the cookies.

The results of this study are consistent with the results of other studies that have investigated the effects of spirulina supplementation on the nutritional value of food products. For example, a study by Tańska *et al.*, (2017) found that the addition of spirulina to corn extrudates significantly increased the ash and crude fibre contents of the extrudates. Similarly, a study by Raczyk *et al.*, (2022) found that the addition of spirulina to pasta significantly increased the insoluble fibre content of the pasta.

The results of this study suggest that the addition of SP to wheat cookies is a promising way to improve the nutritional value of these cookies. The SP is a good source of minerals and dietary fibre, and the addition of SP to the cookies significantly increased the levels of these nutrients in the cookies. These results suggest that spirulina-supplemented cookies could be a healthy and nutritious snack option.

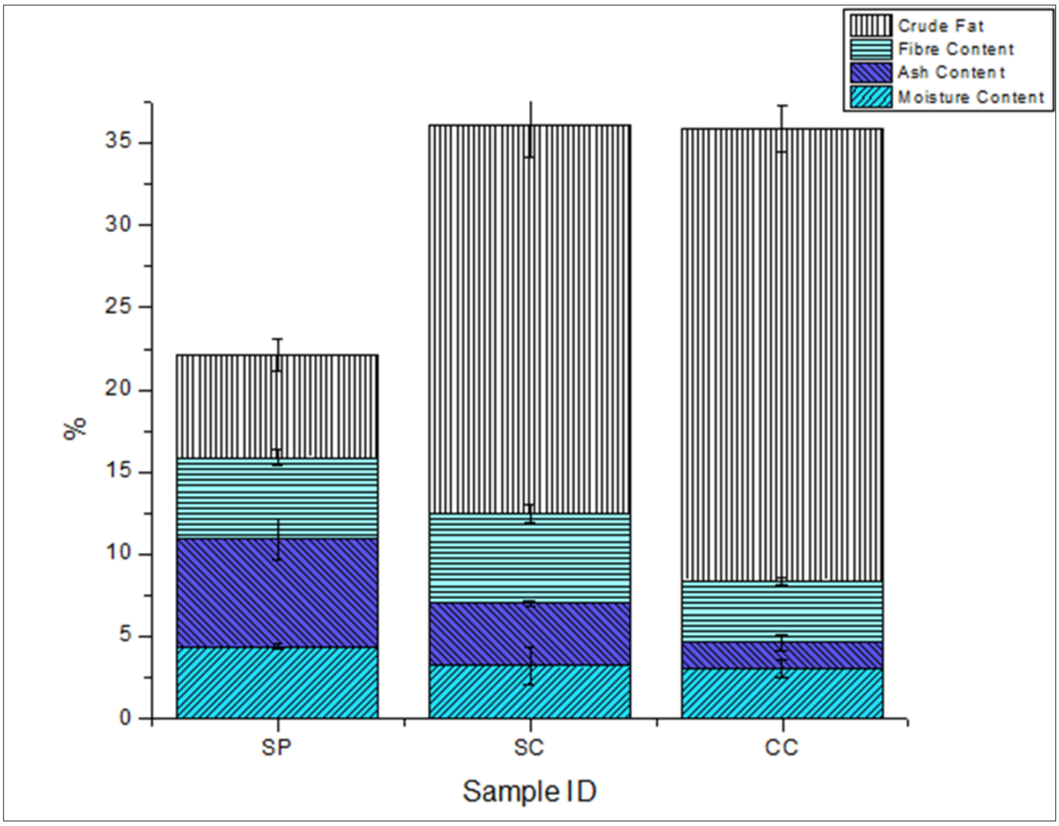

Figure 2: Crude Fat, Fibre, Ash and Moisture contents of SP, SC and CC expressed in percentages

The crude fat content of the spirulina powder (SP) was 6.21%, while the crude fat contents of the control cookie (CC) and the spirulina-supplemented cookie (SC) were 23.61% and 27.46%, respectively. The decrease in crude fat content in the SC as compared to the CC was significant ($p < 0.05$). This is due to the fact that the wheat, which formed the main constituent of these cookies, is low in fat and has approximately 1.3% fat content. However, the other major component in this cookie which also served as a binding agent as no moisture was used, was clarified butter and like most cooking fats, it is close to 100% fat content (Garone, 2022). This is why the CC contains quite an increased amount of fat content, and removing 10% of the mixture and replacing it with spirulina that is not rich in fats as detailed earlier, decreased the total fat content in the supplemented cookies by about 16.3%.

The decrease in crude fat content upon the addition of spirulina is a well-documented phenomenon. Several researchers have reported significant decreases in lipid content upon the addition of spirulina to various food products, including cookies, bread, yogurt, and chocolate. This is because spirulina is not rich in fats, and the addition of spirulina to a food product results in a dilution of the fat content.

The crude carbohydrate content of the SP was 92.17 µg/g, which is very low. This is because carbohydrates only make up around 15-25% of the dry weight of spirulina and most of this content is made up of complex carbohydrates, such as glucosamine and rhamnosamine. The crude carbohydrate content of the CC was 438.61 µg/g, which is significantly higher ($p < 0.05$) than the SC (299.32 µg/g). This is because the CC is wheat-based, and wheat is a high-carbohydrate food. The addition of spirulina to the SC resulted in a dilution of the carbohydrate content, as the carbohydrate content of spirulina is very low.

The decrease in crude carbohydrate content upon the addition of spirulina is also a well-documented phenomenon. Several researchers have reported that the addition of spirulina to food products results in a decrease in the carbohydrate content. This is because spirulina is not rich in carbohydrates, and the addition of spirulina to a food product results in a dilution of the carbohydrate content.

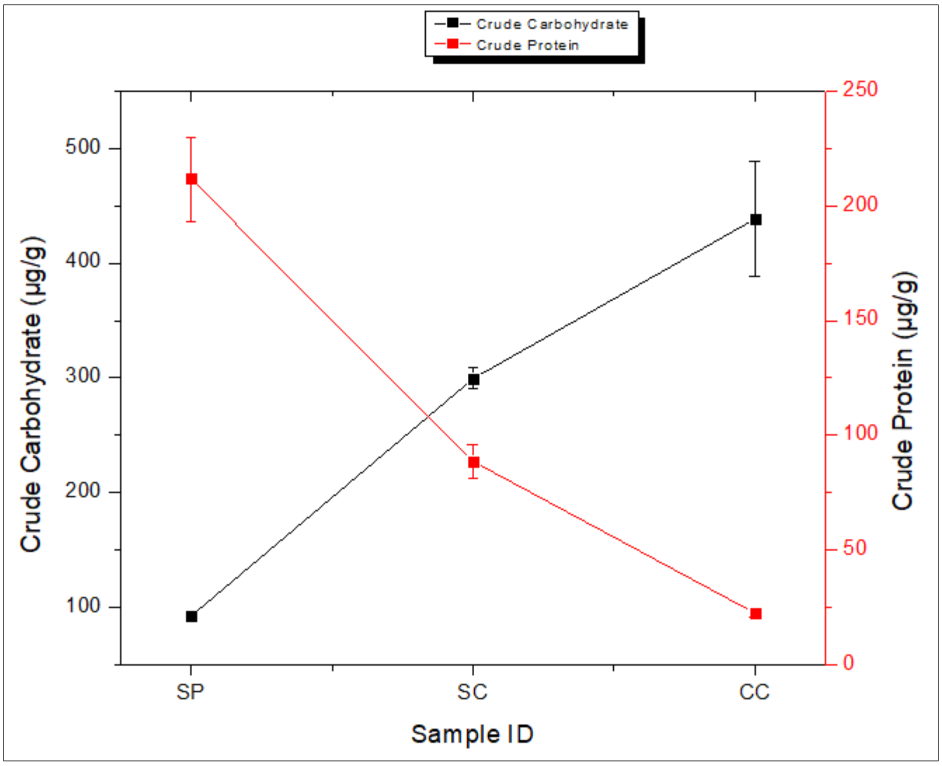

Figure 3: Comparison of Crude Carbohydrate and Crude Protein of the samples expressed in µg/g, indicating inverse values

The addition of spirulina to the cookies resulted in a significant increase in protein content. The control cookie (CC) had a protein content of 22.29 mg/g, while the spirulina-supplemented cookie (SC) had a protein content of 88.59 mg/g. This is due to the high protein content of spirulina, which can range from 60-70%.

The addition of spirulina to the cookies did not result in a significant change in total phenolic content. The CC had a total phenolic content of 13.08 mg/g, while the SC had a total phenolic content of 13.58 mg/g. This is likely due to the degradation of labile phenolics during baking.

The addition of spirulina to the cookies resulted in a significant increase in antioxidant activity. The CC had an antioxidant activity (AOA) of 14.98%, while the SC had an AOA of 21.37%. This is due to the antioxidant properties of spirulina, which contain a variety of compounds with antioxidant activity, such as carotenoids, flavonoids, and phycocyanin.

The results of this study are consistent with the findings of other studies that have investigated the effects of spirulina supplementation on the nutritional profile of food products. For example, a study by Koli *et al.*, (2022) found that the addition of spirulina to pasta resulted in a significant increase in protein content. Similarly, a study by Saharan and Jood (2021) found that the addition of spirulina to bread resulted in a significant increase in antioxidant activity.

The results of this study also suggest that the addition of spirulina to wheat cookies can be a way to improve the antioxidant activity of the cookies. This is important because antioxidant activity can help to protect the body against damage caused by free radicals. Free radicals are unstable molecules that can damage cells and tissues, and they have been linked to a number of chronic diseases, such as cancer and heart disease.

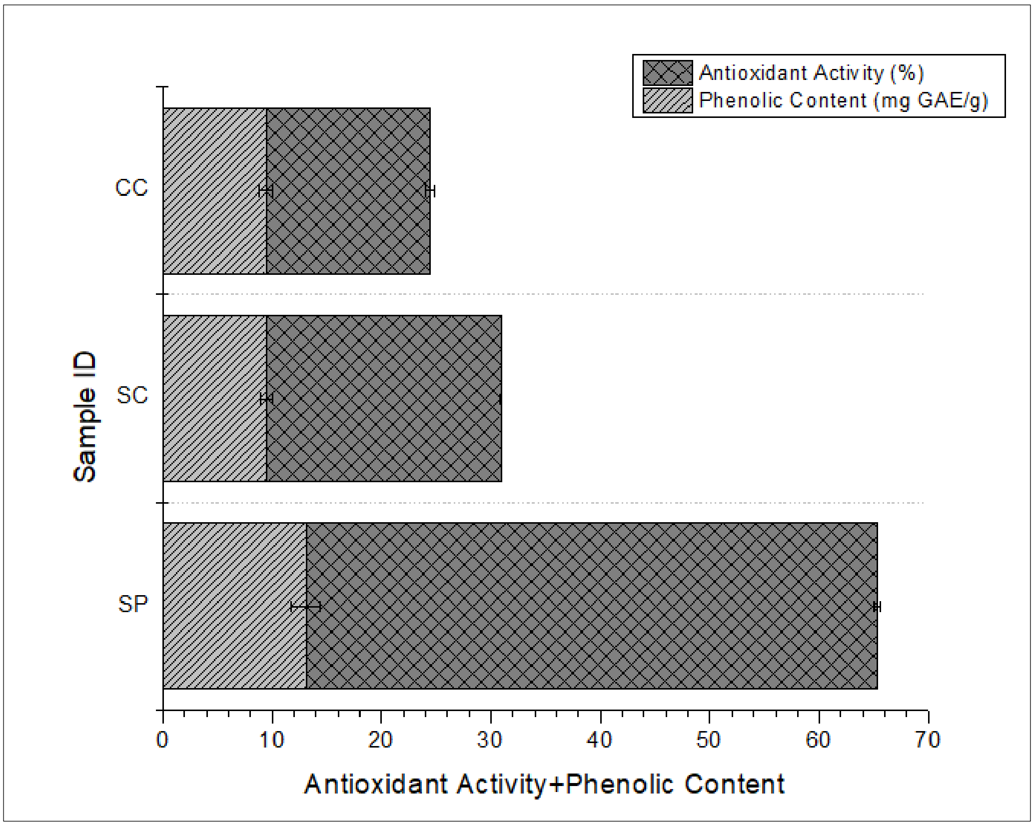

Figure 4: TPC and AOA of SP, SC and CC represented side by side, showing a far greater increase in AOA values than TPC upon SP supplementation

Regardless, the comparison between the SC and CC confirms the antioxidative nature of Spirulina. Even considering the high temperature denaturing effects, adding a small amount of spirulina biomass to a food product still has a significant effect in the antioxidative nature of the modified food product. This suggests that microalgae like Spirulina can successfully be incorporated in both raw and cooked forms of foods to obtain the antioxidative advantages of the added ingredient.

4. CONCLUSION

This study aimed to evaluate the effect of the incorporation of microalgae Arthrospira platensis (Spirulina) in a wheat-based cookie, while also exploring the functionality of Spirulina derived compounds in foods cooked at a high temperature. A wheat-based cookie formulation was prepared and supplemented with 10% w/w dry Spirulina powder, followed by the assessment of various physical and chemical parameters. The three samples; SP, SC and CC were then compared with each other to ascertain the level of change in each nutritional component. The results obtained showed a significant increase ($p < 0.05$) in the crude ash, crude fiber, crude protein and antioxidant activity levels in the SC as compared to the CC, owing to the high values of these compounds in the SP used for enrichment. The ash content, which signifies the levels of minerals, increased to 3.78% in the SC from the 1.58% in the CC. Similarly for crude fiber, a value of 5.49% was calculated for the 10% SC, meanwhile the CC gave a value of 3.77%, amounting to a 45.6% increase in the SC as compared to the CC. The SP used proved to be a rich source of protein (211.69 µg/g), the addition of which increased the protein content in the SC from 22.29 µg/g (in the CC) to 88.59 µg/g. The supplementation also resulted in a large jump in antioxidant activity, because of the raw SP giving a DPPH inhibition value of 52.25% - causing the AOA to jump to 21.37% in the SC from 14.98% in the CC. Almost a doubling of the values was observed as a result of Spirulina addition. For the rest of the nutrients (crude fat, crude carbohydrate) a significant decrease in the SC was observed owing to the reduction of the fatty-wheat matrix in the modified cookies. Moreover, moisture content and phenolic content remained more or less unchanged in the comparative samples. The former does not affect the nutritive value of the cookies however, the latter can definitely be improved. This study can be expanded by working on maintaining and increasing phenolic content by altering either the cooking temperature or extraction methods. Additionally, it is important to mention that a sensory evaluation was not carried out in this research, so the acceptability of this snack in consumers is unknown. In conclusion, the overall results indicated that Spirulina is an excellent source of protein, minerals, fiber and antioxidants and can successfully be used to enhance the nutritional properties of an otherwise nutritionally poor snack. It can make for a cheap alternative to traditional protein sources, given that it is appropriately commercialized.

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