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THE THERAPEUTIC EFFECT OF ANTHROSPIRA PLATENSIS (SPIRULINA) BASED COOKIES ON HYPERLIPIDEMIA

Original Research

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ABSTRACT

Background: Hyperlipidemia, a major risk factor for cardiovascular diseases, is primarily associated with elevated lipid levels due to increased fat consumption and obesity. Conventional pharmacological treatments often come with adverse effects, necessitating the exploration of natural alternatives. *Arthrospira platensis* (Spirulina), a nutrient-dense cyanobacterium, has gained attention for its lipid-lowering, antioxidant, and cardioprotective properties. This study investigates the efficacy of spirulina-based cookies in improving lipid profiles in hyperlipidemic patients.

Objective: Objective of study are “to develop spirulina-based cookies and evaluate their sensory acceptability and to assess the impact of spirulina-based cookies on lipid profiles in hyperlipidemic patients.

Methods: A total of 60 hyperlipidemic patients (35-65 years) were recruited and randomized into three groups: control (T0), low-dose spirulina (T1, 7.5g/50g serving), and high-dose spirulina (T2, 15g/50g serving). Cookies were administered daily for 60 days, and blood samples were collected at baseline (Day 01), mid-intervention (Day 30), and post-intervention (Day 60) to assess total cholesterol, LDL, HDL, and triglyceride levels using enzymatic colorimetric analysis. Sensory evaluation was performed using a nine-point hedonic scale.

Results: Significant improvements were observed in lipid profiles among intervention groups compared to the control. At Day 60, cholesterol levels decreased from baseline (T0: 210.21±32.33 mg/dl; T1: 216.1±32.11 mg/dl; T2: 215.12±42.1 mg/dl) to (T0: 210.11±28.76 mg/dl; T1: 210±10.98 mg/dl; T2: 208±2.11 mg/dl) with p=0.031. LDL levels reduced significantly (T1: 135.05±21.06 mg/dl to 130.22±22.5 mg/dl, p=0.021; T2: 134.2±31.7 mg/dl to 129.43±6.75 mg/dl, p=0.011). Triglycerides also decreased (T1: 191.21±77.2 mg/dl to 186.54±76.43 mg/dl, p=0.04; T2: 190.33±69.4 mg/dl to 184.2±12.66 mg/dl, p=0.01). HDL levels improved significantly in intervention groups (T1: 41.66±1.77 mg/dl to 49.67±3.21 mg/dl, p=0.042; T2: 46.7±2.43 mg/dl to 51.32±3.21 mg/dl, p=0.032). Sensory evaluation showed high acceptability.

Conclusion: Spirulina-based cookies demonstrated a significant hypolipidemic effect, suggesting their potential as a functional dietary intervention for hyperlipidemia management. Their incorporation into daily nutrition may serve as an adjunctive strategy for cardiovascular risk reduction.

Keywords: Antioxidants, Cardiovascular Diseases, Cookies, Dyslipidemia, Functional Foods, Hyperlipidemia, *Spirulina platensis*.

INTRODUCTION

Hyperlipidemia, characterized by elevated levels of lipids and lipoproteins in the blood, is a major risk factor for cardiovascular diseases (CVDs), including atherosclerosis, ischemic heart disease, and stroke. Managing this condition requires a multifaceted approach, encompassing dietary modifications, lifestyle changes, and pharmacological interventions. Conventional lipid-lowering medications, such as statins and PCSK9 inhibitors, are widely used but may have adverse effects, including muscle pain, weakness, and high treatment costs, limiting their accessibility and long-term compliance. Consequently, there is growing interest in functional foods and natural alternatives with lipid-lowering properties, among which *Spirulina platensis*, a nutrient-rich cyanobacterium, has gained prominence for its therapeutic potential (1). Spirulina is a filamentous, blue-green microalga that thrives in both fresh and saltwater, particularly in tropical and subtropical regions. It is rich in bioactive compounds, including phycocyanin, polyunsaturated fatty acids, beta-carotene, tocopherols, and bioavailable phenolic compounds, which contribute to its antioxidant, anti-inflammatory, and lipid-lowering effects. Studies suggest that spirulina supplementation can modulate lipid metabolism by enhancing cholesterol excretion, increasing high-density lipoprotein (HDL) levels, and reducing low-density lipoprotein (LDL) and triglycerides. Additionally, spirulina contains niacin (vitamin B3), which plays a crucial role in lipid regulation and may help in dyslipidemia management (2).

The therapeutic efficacy of spirulina has been demonstrated in both preclinical and clinical studies. Its hypolipidemic effects are attributed to its ability to influence gut microbiota composition, particularly by increasing *Prevotella* and *Firmicutes*, which facilitate bile acid metabolism and cholesterol reduction. Additionally, spirulina exhibits hepatoprotective properties, mitigating fatty liver disease, a condition closely associated with hyperlipidemia (3). Beyond its role in lipid modulation, spirulina is recognized for its immunostimulatory, anti-cancer, and neuroprotective properties, making it a versatile candidate for therapeutic applications (4). Given its nutritional profile and safety, spirulina has been identified by the United Nations as a sustainable food source with significant health benefits (5). Despite extensive research on spirulina's lipid-lowering potential, there remains a lack of product development focusing on its integration into functional foods, particularly in Pakistan. Functional food-based interventions, such as spirulina-enriched products, offer a promising alternative for individuals with hyperlipidemia who may not tolerate conventional medications. Spirulina-based cookies present an innovative approach to incorporating this microalga into the daily diet while ensuring palatability and compliance. The bioavailability of spirulina's bioactive components through food-based delivery systems requires further exploration to assess its effectiveness in hyperlipidemic individuals (6-8). This study aims to develop spirulina-based cookies and evaluate their sensory attributes and therapeutic potential in hyperlipidemic patients. By assessing lipid profile changes over a 60-day intervention period, the study seeks to determine the efficacy of spirulina-based cookies in improving lipid metabolism, thereby contributing to the development of functional foods with clinical significance.

METHODS

The study employed a controlled experimental design to evaluate the therapeutic effects of spirulina-based cookies on hyperlipidemic patients. A total of 60 participants, aged between 35 and 65 years, were initially recruited from both public and private hospitals in Layyah and Lahore. After accounting for participant dropouts, the final sample included 20 participants in each group. The participants were randomly assigned into three groups: a control group (T0) receiving standard cookies without spirulina, a low-dose experimental group (T1) consuming cookies containing 7.5 g of spirulina per 50 g serving, and a high-dose experimental group (T2) consuming cookies containing 15 g of spirulina per 50 g serving (9,10). The inclusion criteria required participants to have a total cholesterol level above 200 mg/dL, LDL cholesterol above 100 mg/dL, triglycerides above 150 mg/dL, and HDL cholesterol below 60 mg/dL. Individuals with normal lipid profiles, those younger than 35 or older than 65 years, pregnant or lactating women, and those on chronic illness medications were excluded from the study. A rigorous pre-screening process ensured that all enrolled participants met the inclusion criteria, with lipid profiles assessed before intervention to confirm eligibility (11,12).

Spirulina was procured from Ayub Research, Faisalabad, and incorporated into cookie formulations. The cookies were prepared using whole wheat flour (ranging from 35–42.5 g, depending on the spirulina dose), spirulina powder, vanilla essence, salt, and stevia as a low-calorie sweetener. The dough was rolled into shapes and baked at 180°C for 12–15 minutes or until the edges turned golden brown, ensuring proper texture and consistency (13,14). Physical characteristics of the cookies, including thickness, diameter, and spreading ratio, were assessed using standardized methods. Thickness was measured using a vernier caliper, while diameter was determined using a ruler. Sensory evaluation was conducted using a nine-point hedonic scale, where participants rated attributes such as taste, texture, color, appearance, and overall acceptability, with 1 representing “like extremely” and 9 representing “dislike tremendously.” Each participant received an evaluation sheet to record their responses (15-18).

Biochemical analysis involved measuring participants' lipid profiles at three time points: baseline (pre-intervention), day 30, and day 60 post-intervention. Blood samples (5 mL) were collected from participants following overnight fasting and analyzed for total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol. Lipid profile analysis was performed using an enzymatic colorimetric method with an automated biochemical analyzer. This method involved the use of enzymatic reactions to quantify lipid concentrations, ensuring precision and reliability in data collection (19,20). The treatment groups received their respective doses of spirulina-based cookies daily for two months. The T1 group consumed two to three biscuits per day, amounting to a daily intake of 7.5 g spirulina, while the T2 group received the same number of biscuits with 15 g spirulina per day. The control group received non-spirulina cookies. This intervention aimed to determine dose-dependent effects of spirulina on lipid metabolism and overall acceptability (21,22).

Ethical considerations were meticulously followed throughout the study. Approval was obtained from the Institutional Review Board (IRB). Written informed consent was obtained from all participants, who were briefed about the study objectives, risks, and their right to withdraw at any stage. Confidentiality was maintained by assigning unique participant IDs, and no personal identifiers were disclosed in the study findings. The study adhered to the principles outlined in the Declaration of Helsinki for ethical medical research (23). Statistical analysis was conducted to determine the significance of changes in lipid profiles before and after the intervention. The results were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) test was performed to compare means between groups, and a p-value of less than 0.05 was considered statistically significant (24).

RESULTS

The study analyzed the effects of spirulina-based cookies on lipid profiles in hyperlipidemic patients across three groups: a control group (T0), a low-dose spirulina group (T1), and a high-dose spirulina group (T2). A total of 60 participants were initially enrolled, with 20 participants retained in each group after accounting for dropouts. Demographic data revealed that male participants constituted the majority, with the highest proportion observed in T2 (80%), followed by T0 and T1 (both at 70%). Female representation was highest in T0 and T1 (30%) and lowest in T2 (20%). Age distribution showed that the majority of participants in T2 (55%) were aged 35-39 years, whereas the distribution was more evenly spread in T0 and T1. The chi-square test yielded a p-value greater than 0.05, indicating no significant differences in gender and age distribution across the groups.

Cholesterol levels were assessed at baseline (Day 01), mid-intervention (Day 30), and post-intervention (Day 60). In the control group (T0), mean cholesterol levels remained nearly unchanged (210.21 mg/dl at baseline, 210.20 mg/dl on Day 30, and 210.11 mg/dl on Day 60), with an F-value of 2.113 and a non-significant p-value of 0.057. In contrast, the T1 group exhibited a decrease from 216.1 mg/dl at baseline to 210 mg/dl on Day 60, with an F-value of 7.213 and a p-value of 0.041, indicating statistical significance. The most pronounced reduction was observed in the T2 group, where cholesterol levels decreased from 215.12 mg/dl to 208 mg/dl by Day 60, with an F-value of 8.431 and a p-value of 0.031, confirming statistical significance. LDL levels followed a similar trend. The control group (T0) showed no significant change over time (133.4 mg/dl on Day 01, 133.1 mg/dl on Day 30, and 133.1 mg/dl on Day 60), with an F-value of 0.12 and a p-value of 0.763, indicating non-significance. In T1, LDL levels declined from 135.05 mg/dl at baseline to 130.22 mg/dl by Day 60, with an F-value of 22.01 and a p-value of 0.021, confirming statistical significance. T2 exhibited the greatest reduction, from 134.2 mg/dl to 129.43 mg/dl, with an F-value of 101.56 and a p-value of 0.011, demonstrating a highly significant effect.

HDL levels were also evaluated to determine the beneficial impact of spirulina-based cookies on lipid metabolism. The control group (T0) showed minimal variation, with HDL levels at 40.34 mg/dl at baseline and 40.54 mg/dl at Day 60, yielding an F-value of 2.43 and a p-value of 0.055, indicating a non-significant change. In T1, HDL increased from 41.66 mg/dl to 49.67 mg/dl, with an F-value of 5.88 and a p-value of 0.042, demonstrating statistical significance. The T2 group displayed the highest increase, from 46.7 mg/dl at baseline to 51.32 mg/dl at Day 60, with an F-value of 3.45 and a p-value of 0.032, confirming significance. Triglyceride levels were assessed in a similar manner. The control group (T0) exhibited no significant change over time (190.21 mg/dl on Day 01, 190.1 mg/dl on Day 30, and 190.1 mg/dl on Day 60), with an F-value of 3.12 and a p-value of 0.214. In contrast, the T1 group showed a reduction from 191.21 mg/dl at baseline to 186.54 mg/dl by Day 60, with an F-value of 3.41 and a p-value of 0.04, demonstrating statistical significance. The

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most significant decrease was observed in the T2 group, where triglyceride levels dropped from 190.33 mg/dl to 184.2 mg/dl, with an F-value of 2.54 and a p-value of 0.01.

Sensory evaluation results indicated that participants rated the cookies based on texture, taste, color, aroma, and overall acceptability using a nine-point hedonic scale. The majority of participants (85%) favored sweetness, while 15% found the cookies slightly bitter. Regarding color, 75% preferred the darker shade of spirulina-based cookies, while 25% favored lighter tones. For texture, 60% of participants favored crunchiness, while 20% preferred breakability and 20% hardness. The aroma evaluation showed that 87% of participants favored a slightly rancid scent, while 15% preferred a toasted aroma. The overall acceptability rating was 100%, indicating high consumer approval of the spirulina-based cookies. Physical characteristics of the cookies were also measured, revealing an average thickness of ½ inch, a diameter of 4-4.5 inches, and an optimal spread ratio ensuring balance in taste and texture. These parameters confirmed the suitability of spirulina incorporation in baked products without compromising structural integrity or palatability.

Pairwise comparison using the Tukey test for cholesterol levels demonstrated significant differences between the control and treatment groups. On Day 30, the mean difference between T0 and T1 was 40.87 mg/dl ($p=0.002$), and between T0 and T2 was 39.87 mg/dl ($p=0.007$), indicating significant reductions in cholesterol levels in the intervention groups. However, no significant difference was observed between T1 and T2. By Day 60, cholesterol reduction was more pronounced, with T0 versus T1 showing a mean difference of 73.987 mg/dl ($p<0.001$) and T0 versus T2 showing 69.654 mg/dl ($p<0.001$). No significant difference was found between T1 and T2 at this stage, suggesting similar effectiveness in both intervention groups. For triglycerides, Tukey pairwise comparisons on Day 30 showed no significant difference between T0 and T1 ($p=0.765$), while T0 versus T2 demonstrated a significant reduction ($p=0.002$). On Day 60, significant reductions were observed in both treatment groups compared to the control, with T0 versus T1 showing $p<0.001$ and T0 versus T2 also showing $p<0.001$.

The Tukey test for LDL levels on Day 30 indicated significant reductions in both intervention groups compared to the control ($p=0.000$ for both T0 vs. T1 and T0 vs. T2). However, no significant difference was noted between T1 and T2 ($p=0.453$). By Day 60, the reductions in LDL levels remained highly significant ($p=0.000$ for T0 vs. T1 and T0 vs. T2), with no strong evidence for a difference between T1 and T2 ($p=0.654$). For HDL levels, Tukey pairwise comparisons on Day 30 showed a significant increase in T1 compared to T0 ($p=0.001$), while T2 also exhibited a significant increase compared to T0 ($p=0.006$). However, no significant difference was found between T1 and T2 ($p=0.870$). By Day 60, HDL increases remained significant, with T0 versus T1 showing a mean difference of -4.430 mg/dl ($p=0.001$) and T0 versus T2 showing -4.200 mg/dl ($p=0.001$), while T1 and T2 did not differ significantly ($p=0.560$).

Table 1: Demographics Data

Group	Male (%)	Female (%)	Age 35-39 (%)	Age 40-44 (%)	Age 45-49 (%)	Age 50-54 (%)	Age 55-59 (%)	Age 60-65 (%)
T0	70	30	25	25	25	25	25	25
T1	70	30	40	15	20	25	15	25
T2	80	20	55	15	20	10	15	15

Table 2: Cholesterol Levels Over Time

Group	Day 01 (mg/dl)	Day 30 (mg/dl)	Day 60 (mg/dl)	F-value	P-value
T0	210.21	210.2	210.11	2.113	0.057
T1	216.1	213.81	210	7.213	0.041
T2	215.12	211	208	8.431	0.031

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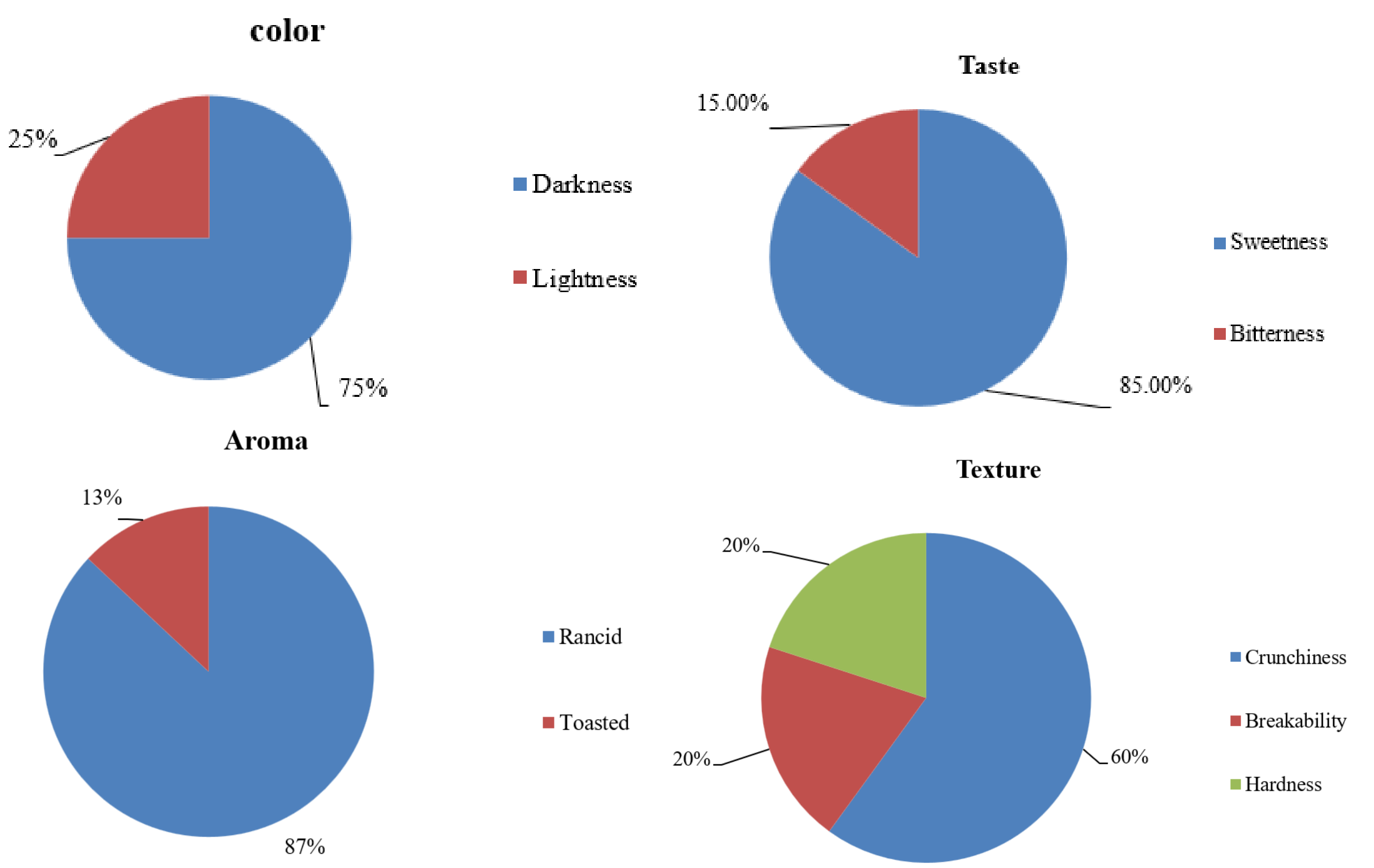
Table 3: LDL Levels Over Time

Group	Day 01 (mg/dl)	Day 30 (mg/dl)	Day 60 (mg/dl)	F-value	P-value
T0	133.4	133.1	133.1	0.12	0.763
T1	135.05	133.12	130.22	22.01	0.021
T2	134.2	131.7	129.43	101.56	0.011

Table 4: HDL Levels Over Time

Group	Day 01 (mg/dl)	Day 30 (mg/dl)	Day 60 (mg/dl)	F-value	P-value
T0	40.34	40.54	40.54	2.43	0.055
T1	41.66	45.32	49.67	5.88	0.042
T2	46.7	48.44	51.32	3.45	0.032



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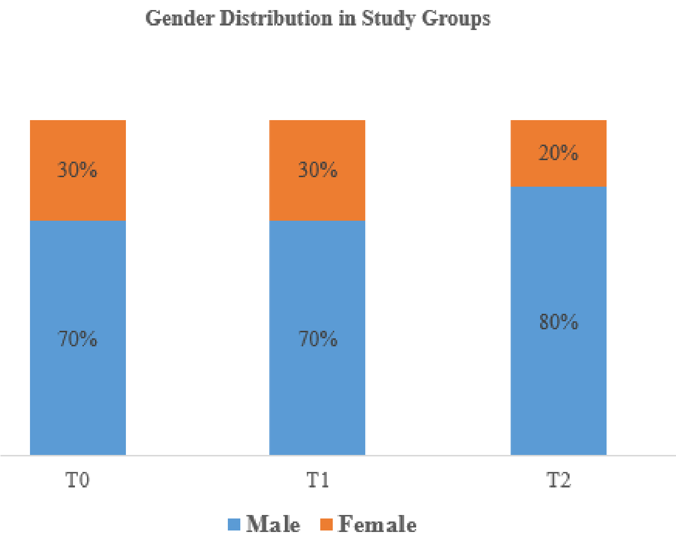

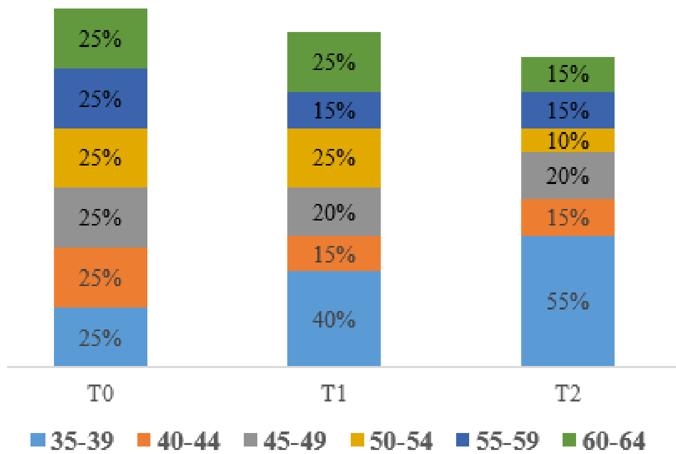

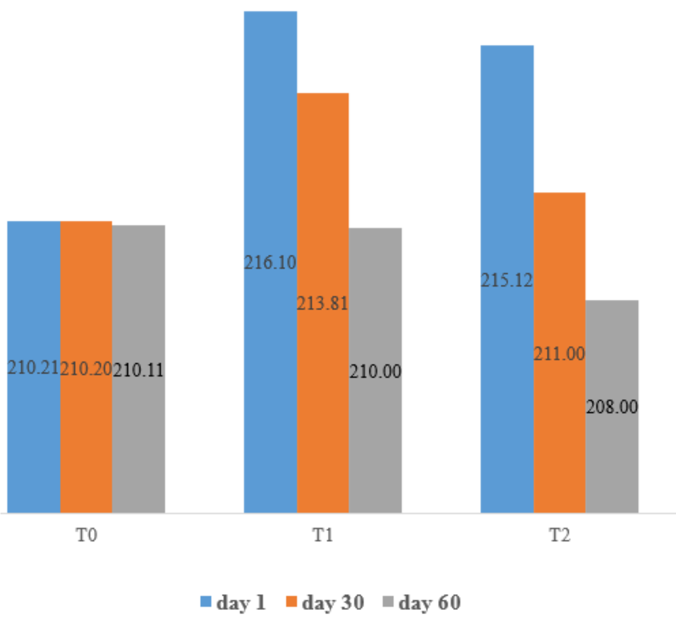

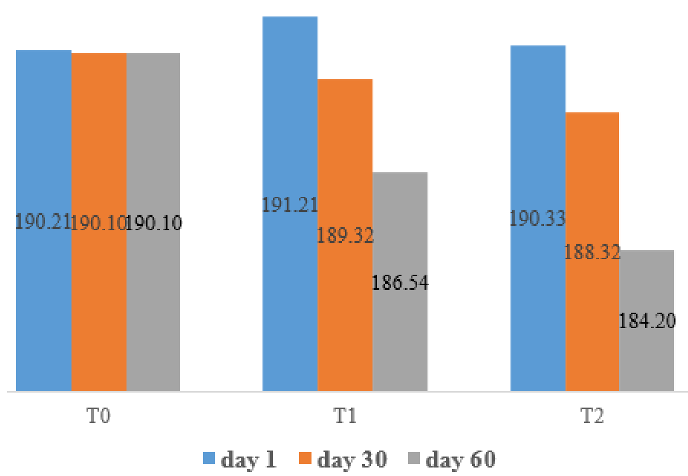

DISCUSSION

The findings of this study indicate a significant reduction in cholesterol, LDL, and triglyceride levels in hyperlipidemic patients who consumed spirulina-based cookies, while HDL levels showed an increase. These results align with previous research demonstrating the lipid-lowering potential of *Spirulina platensis*, attributed to its bioactive compounds such as phycocyanin, gamma-linolenic acid, and niacin, which enhance cholesterol excretion and improve lipid metabolism. The reduction in LDL levels suggests that spirulina may play a role in preventing atherosclerosis, a major risk factor for cardiovascular diseases. The increase in HDL levels further supports its protective effect, given that HDL facilitates reverse cholesterol transport and reduces the risk of plaque formation in arteries (25,26). The intervention groups exhibited a dose-dependent response, with the high-dose spirulina group (T2) demonstrating slightly greater reductions in total cholesterol, LDL, and triglycerides compared to the low-dose group (T1). However, the statistical comparison between T1 and T2 revealed no significant differences, suggesting that the lipid-lowering effects of spirulina may plateau beyond a certain dosage. This finding supports existing evidence that the hypolipidemic effects of spirulina are dose-dependent up to an optimal level, after which no additional benefit is observed. The control group (T0), which received cookies without spirulina, showed no significant changes in lipid profiles, confirming that dietary intervention with spirulina was responsible for the observed improvements (27.28).

The sensory evaluation results indicated high consumer acceptability, with the majority of participants favoring the taste, texture, and appearance of spirulina-based cookies. Despite spirulina's strong pigment and distinct taste, the formulation appeared to balance these properties effectively, making it a feasible alternative for integrating spirulina into functional foods. The high overall acceptability suggests that spirulina-based cookies can be a sustainable dietary intervention for individuals managing hyperlipidemia without reliance on pharmacological treatment (29,30). The lipid-lowering effects of spirulina observed in this study align with previous findings that identified its role in reducing oxidative stress, inflammation, and hepatic lipid accumulation, all of which contribute to dyslipidemia. The potential mechanism underlying these effects involves the modulation of gut microbiota, particularly the increase in *Prevotella* and *Firmicutes*, which enhance bile metabolism and cholesterol clearance. The hepatoprotective effects of spirulina may also contribute to lipid regulation by improving liver function and reducing fat accumulation, which is often associated with hyperlipidemia (31,32).

Although this study provides compelling evidence for the effectiveness of spirulina in lipid management, several limitations must be acknowledged. The study duration of 60 days may not fully capture the long-term effects of spirulina on lipid metabolism. A longer follow-up period could provide insights into the sustainability of these effects over time. Additionally, while lipid profile parameters were measured, other metabolic markers such as liver enzymes, inflammatory cytokines, and insulin sensitivity were not assessed, which could have provided a more comprehensive understanding of spirulina's impact on metabolic health. Compliance with the intervention was not explicitly monitored, and dietary variations among participants were not controlled, which may have influenced the outcomes (33,34). Future research should explore the long-term effects of spirulina-based dietary interventions, particularly in diverse populations with varying dietary habits and metabolic conditions. Investigating the synergistic effects of spirulina with other functional ingredients, such as fiber or probiotics, could enhance its lipid-lowering potential. Further studies should also assess the bioavailability of spirulina's active compounds in different food matrices to optimize its therapeutic efficacy. Large-scale clinical trials with extended follow-up periods and a broader range of biochemical assessments are warranted to establish standardized dietary recommendations for spirulina in the management of hyperlipidemia (35). Despite its limitations, this study highlights the potential of spirulina-based cookies as an effective, palatable, and non-pharmacological intervention for improving lipid profiles in hyperlipidemic individuals. The findings contribute to the growing body of evidence supporting the use of functional foods in the prevention and management of metabolic disorders, reinforcing the need for further research and development in this area.

CONCLUSION

The findings of this study highlight the potential of spirulina-based cookies as an effective dietary intervention for managing hyperlipidemia. The incorporation of *Anthrospira platensis* into functional foods demonstrated beneficial effects on lipid metabolism, contributing to reductions in harmful cholesterol levels while enhancing protective lipid mechanisms. The bioactive compounds in spirulina, particularly phycocyanin and omega-3 fatty acids, may play a key role in these improvements, offering a natural alternative for cardiovascular risk management. Given the promising results, spirulina-based cookies could serve as a valuable addition to comprehensive hyperlipidemia treatment strategies. While this research underscores their potential, further studies are necessary to deepen the understanding of their long-term effects and mechanisms, paving the way for broader clinical applications in metabolic health management.

Author Contribution

Author	Contribution
Zartasha Gull*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Kanwal Fatima	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Ahmad Ibn e Yousaf	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Azzah Khadim Hussain	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Qaswara Affaf	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Anza Ahmad	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Faziyya Latif	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Saif Ullah Khan	Writing - Review & Editing, Assistance with Data Curation

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