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Original Article

The beneficial effect of fermented Spirulina platensis on reducing oxidative stress in patients with type 2 diabetes mellitus

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Abstract

Oxidative stress caused by hyperglycemia can lead to the intensification of hyperglycemia and an increased risk of diabetes complications. Spirulina platensis is a potent free-radical scavenger; it has the potential to be used as a substrate for fermentation by lactic acid bacteria. This study aimed to formulate two functional foods with antioxidant capacity (yogurt containing S. platensis powder / fermented S. platensis powder) for Type 2 Diabetes Mellitus (T2DM) patients and compare the antioxidant effects in diabetic subjects. In this article, for the first time, the antioxidant effect of fermented and non-fermented Spirulina was compared in a clinical study in 'T2DM' patients. By blood sampling, clinical parameters such as FBS, GSH, FRAP, MDA, and CRP before and after each treatment were measured and results were compared between two groups of intervention. Both products as functional foods have a positive effect on the health of diabetic patients by reducing FBS and increasing total antioxidant capacity, and this positive effect is more obvious when yogurt contains fermented lactic acid S. platensis is consumed by T2DM patients.

Keywords: Spirulina, Fermentation, Diabetes mellitus, Functional foods.

1. Introduction

Type 2 Diabetes Mellitus 'T2DM' is a major universal health problem closely linked to the obesity epidemic. In recent decades, the number of people with 'T2DM' has increased in the world. 'T2DM' is the third leading cause of death in the world, and the prevalence of diabetes in all age groups was 2.8% in 2000 and increased to 4.4% in 2020 [1]. 'T2DM' patients are at high risk for macrovascular complications such as cardiovascular disease and microvascular complications (including neuropathy, retinopathy, and nephropathy) due to hyperglycemia and insulin resistance syndrome. Available evidence confirms that one of the most important factors accelerating the development of 'T2DM' is oxidative stress [2-5]. Oxidative stress increases the number of free radicals in the body, accumulation of free radicals is the cause of various diseases. Free radicals are generally unstable and reactive chemical entities containing one or more unpaired electrons. The free radicals induce damage to cells by passing the unpaired electron resulting in oxidation of cell components and molecules. The formation of free radicals in diabetes due to increased lipid peroxidation and oxidation of non-enzymatic glycation of proteins leads to damage to cell organelles, and enzymes and increases insulin resistance due to oxidative stress [6-10]. Studies in rats suggest that *Spirulina* increases lipoprotein lipase activity and pancreatic insulin secretion. In animal studies, antioxidants have been shown to suppress oxidative stress-induced apoptosis of pancreatic β-cells. Therefore, dietary antioxidants such as *Spirulina* can help maintain the function of β-cells and prevent their damage [11, 12].

Some indicators of oxidative stress in the blood plasma of diabetic patients are changed. For example, people with diabetes have been reported to have inflammatory cytokines. In addition, diabetes is associated with low plasma iron-reducing capacity (FRAP). As a potent intracellular thiol, glutathione (GSH) plays a protective role against cellular oxidative stress and the erythrocytes of diabetics have significantly lower levels of glutathione. On the other hand, C-reactive protein (CRP) is associated with 'T2DM' as a general marker of systemic inflammation. Also, studies have shown that the consumption of *Spirulina* has reduced the level of blood sugar and 'malondialdehyde (MDA), a biomarker of oxidative stress, in the blood [13-16].

Many studies have been conducted on the therapeutic

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effect of blue-green algae on type 2 diabetes. *S. platensis* has anti-diabetic effects that stimulate the endocrine system, intermediate nutrient metabolism, and contribute to meeting nutrient needs. Scientists in Spain have shown that an extract of *Spirulina* containing phycocyanin is a potent free radical scavenger and inhibits microsomal lipid peroxidation [17, 18].

Spirulina (Arthrospira) is a type of blue-green flagellate multicellular algae and a member of the Cyanobacteria family which grows in hot weather and alkaline conditions and contains essential fatty acids, essential amino acids, vitamins, minerals, and antioxidant components. It also contains high concentrations of β-carotene, vitamin B12, iron, trace elements, and the essential fatty acid g-linolenic acid. Therefore, *Spirulina* can compete as a functional food with many types of dietary supplements and has been designated as a health food by the World Health Organization (WHO) [19]. So, it can potentially become one of the best alternative treatments, according to the National Institute of Health (NIH). *S. platensis*, a subclass of *arthrospira*, contains about 60% protein consisting of 22 amino acids, including C-phycocyanin and allophycocyanin. Phycocyanin is a water-soluble blue pigment that gives *Spirulina* its bluish color. Phycocyanin is a powerful water-soluble antioxidant. The Japanese have found that phycocyanin protects the liver and kidneys during detoxification and activates the immune system. *Spirulina* is also a source of carotenoids. Carotenoids are vital antioxidants. The history of *Spirulina* consumption goes back many years. In recent decades, special attention has been paid to this food and it has been suggested as a beneficial food and also as a protein and vitamin supplement for humans [3, 5, 20, 21].

Numerous studies have been conducted on the safety of using these algae and its by-products. The general conclusion of the studies is that if the algae are grown correctly in a non-polluted environment and consumed in moderation, there are no adverse effects or side effects and it is completely safe. *Spirulina* is indicated as a non-toxic food supplement and has been declared a humanitarian tool in the fight against severe malnutrition by the WHO. *Spirulina* daily consumption has been attributed to hypolipidemic, hypoglycemic, antihypertensive, anti-inflammatory, neuroprotective and immunomodulatory properties (Anon n.d.) [22].

Fermentation is a process that helps break down large organic molecules via the action of microorganisms into simpler ones. Fermented foods can be considered great helpers in the complementary and alternative treatment of diabetes, improving mechanisms such as glycemic control, and antioxidant capacity [23-27].

In this study, we will investigate the effects of fermentation by a probiotic bacterium on *Spirulina*. Fermentation has long been used as a method to improve food properties and change the texture of food. Nowadays, the use of lactic acid bacteria (LAB) to improve the nutritional profile of food is a new area of research [23, 26, 28].

Lactobacillus plantarum, a lactic acid bacterium, has been suggested as a probiotic. Probiotic microorganisms are "live microbial food supplements that have beneficial effects on the host animal by improving its microbial balance". Lactic acid fermentation is known to enhance the nutritional properties and functional value of foods and their microbiological resistance to other pathogenic microorganisms, as well as to improve or modify their sensory properties. In recent years, several studies have been conducted in the field of different methods of *Spirulina* fermentation by lactic acid bacteria as a suitable technology to obtain new and innovative products with better nutritional and organoleptic properties [23-25].

This study aimed to enrich low-fat yogurt with fermented and non-fermented *Spirulina platensis* powder and to investigate and compare the effects in patients with 'T2DM' in Iran. FBS, FRAP as a Measure of "Antioxidant Power", GSH, MDA, and CRP were selected and studied as important indicators of blood serum in diabetic patients. In many articles, the property of reducing blood sugar by *Spirulina* has been mentioned. In this article, we investigated the changes in blood sugar with fermented *Spirulina* for the first time. the FRAP assay is presented as a novel method for assessing "antioxidant power." Ferric-to-ferrous ion reduction at low pH causes a colored ferrous-tripyridyl triazine complex to form. Since GSH is the most important intracellular antioxidant molecule and one of the indices of oxidative stress is the depletion of the antioxidant we can estimate the anti-oxidative stress effect of fermented *Spirulina* by measuring blood serum GSH. The amount of MDA is usually used as an indicator of oxidative stress in diabetic patients and many other diseases such as cancers. CRP is secreted by the liver in response to a variety of inflammatory cytokines. Levels of CRP increase very rapidly in response to inflammation. Thus, by measurement of CRP, we can estimate and monitor stress oxidative states before and after fermented and non-fermented *Spirulina* consumption.

2. Material and Methods

2.1. Bacterial strains preparation

Lyophilized *Lactobacillus plantarum* PTCC 1058 (LAB 1058) was obtained from the Persian Type Culture Collection- Iranian Research Organization for Research and Technology (Tehran, Iran). Before use, the Lactobacillus strains were activated by adding 10 ml of MRS broth at 37 °C.

2.2. Fermentation of *Spirulina* with bacteria

S. platensis powder was produced by spray drying technology at Berke Sabz Mad Asia (Delijan, Iran). The nutritional value of the algae used was evaluated in the laboratory by using standard laboratory methods (US Pharmacopoeia) and equipment for chemical analysis of materials such as High-Performance Liquid Chromatography (HPLC), and the results are summarized in Table 1.

The powder was dissolved in distilled water (4% w/v) and frozen and thawed for 3 cycles. *Spirulina* medium was prepared by adding the same volume of autoclaved glucose solution (2% w/v). The cultured LAB 1058 was inoculated in the *Spirulina* medium (5% v/v) in the fermenter at 37 °C for 36 h. The effects of fermentation were studied by pH changes and sensory properties and reports are presented in results. The color and odor of the fermented *Spirulina* differed from the non-fermented solution.

According to Niccolai et al., (2019) to ensure that no bacteria remain active in fermented *Spirulina platensis* biomass, 5 grams of *S. platensis* powder were poured into 100 mL Erlenmeyer flasks with 50 mL deionized water

without LAB 1058 inoculation. The flasks were incubated at 37 ° C for 72 h. Samples were taken at 0, 24, 48, and 72 hours and the growth of bacteria was determined by the plate counting method. No growth of microorganisms was observed. The fermented *Spirulina* was directly freeze-dried at -40 ° C and processed into a powder.

2.3. Fortified yogurt preparation

Low-fat yogurt prepared according to Iranian food standard no 5562. 4 grams of fermented and non-fermented *Spirulina* powder was added to 100 grams of low-fat yogurt; which diabetics were asked to consume twice a day for three weeks. The yogurts were prepared weekly and provided to consumers each week.

2.4. Clinical trial

Before the study began, all participants were given a written informed consent form to complete. Anthropometric parameters such as height, weight, age, gender, waist circumference, and type of diet were collected as baseline information. Body Mass Index (kg/m2) was calculated. In addition, subjects were interviewed individually for general characteristics, lifestyle habits, and food consumption. Blood pressure was measured with an automatic blood pressure monitor (Breuer Company, Germany) after 15 min of rest. During the intervention period of 21 days, participants were asked to continue their usual diet and not to consume any supplements, functional foods, or changes in the type of unusual foods. After completing the above steps and announcing that the participants were fully prepared for the intervention, participants were asked to return to the center the next day fasting to perform the pre-test. 10 mL of blood was drawn from the subjects and the blood samples were immediately sent to the laboratory to separate the serum by centrifuge. FBS, FRAP, HS-CRP, and GSH were measured for each patient. Participants were randomly assigned to either a *Spirulina* or fermented *spirulina* group. When subjects entered the study, they selected a numbered sealed envelope containing their random allocation to the *Spirulina* group (intervention-1) or the fermented *Spirulina* group (no intervention-2). Thus, at random 50 patients received fermented *Spirulina* powder, and 50 patients received non-fermented *Spirulina* powder.

On the 22nd day, 10 mL of blood was taken from each patient again for follow-up tests.

2.5. Assay for FBS content

FBS was measured using the glucose oxidase method (Pars Azmoon kit, Iran).

2.6. Assay for FRAP content

Total antioxidant capacity (TAC) was determined using the Kiazist TAC kit (Kiazist, Iran), according to the FRAP method. The principle of the FRAP method is based on the reduction of the ferric tripyridyl triazine complex to the ferrous tripyridyl triazine with an intensive color, detectable at 450 nm. The FRAP reagent consisted of 300 mM acetate buffer (pH=3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl3-6H2O in the ratio of 10:1:1. An increase in the absorbance of the reaction corresponded to the increased reducing power in the sample. Briefly, the cells were washed with PBS and the cell lysates were prepared and centrifuged at 15,000 rpm, after which the supernatants were collected. Then, 150 µl of Kiazist TAC reagent was added to 100 µl of cell lysate (collected supernatant) and incubated at room temperature for 45 min. Absorbance was read using a Rayto RT-2100C microplate reader. Standard solutions of Fe2+ were prepared from ferrous sulfate (FeSO4-7H2O) in distilled water and the concentrations of samples were calculated based on the standard curve and expressed as Nano mole per milligram of protein.

2.7. Assay for GSH content

The GSH content was measured using a GSH kit (Kiazist, Life Sciences, Iran) based on its manufacturer's instructions. GSH evaluation was performed by ELISA reader (Biotek, USA) against blank at 405 nm.

2.8. Assay for MDA content

The MDA content was measured using an MDA kit (Kiazist, Life Sciences, Iran) based on its manufacturer's instructions. MDA calculation was performed by absorbance reader (Biotek, USA) against blank at 540 nm.

2.9. Assay for CRP test

Hs-CRP levels were measured with ultra-sensitive latex-enhanced immunoassay in Valiasr Hospital laboratory- Arak- Iran.

2.10. Statistical analysis

Mean and standard deviation were used to describe quantitative variables. Percentage and frequency were used for qualitative variables. To compare the quantitative variables and conduct the analysis by the objectives of the project, the Colmogorov-Smirnof one-sample test was used to check the normality of the data. According to this test, a p-value of less than 0.05 means that the data are not normally distributed and we used non-parametric tests.

3. Results

The Chemical and nutritional composition of *Spirulina* cultivated in different parts of the world is different due to growing conditions. The nutritional composition of dried powdered *Spirulina* grown in freshwater (Delijan- Iran) is summarized in Table 1.

The effects of fermentation were studied by pH changes and sensory properties. Untreated *Spirulina* was found to have the highest pH value (6.2 ± 0), increasing to a pH of 4.1 by the end of the fermentation treatment (36 h). Also, the color of the *Spirulina* solution was blue-green before fermentation, which changed to dark green color after fermentation (Figure 1).

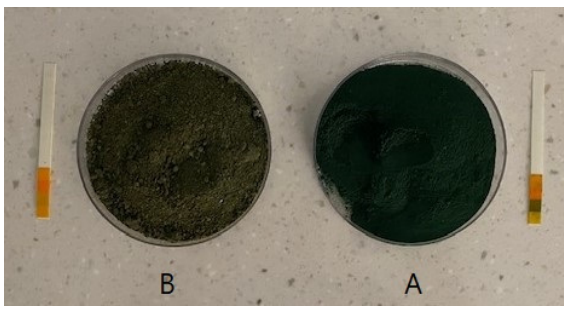

Fig. 1. Comparison of color differences, before and after intervention. A: *S. platenesis* powder before fermentation. B: *S. platenesis* powder after fermentation.

Table 1. Chemical investigation of ingredients in *S. platensis* used in the research.

Content	Unit	Results
Protein	gr/100	60
Total Fat	gr/100	10
Carbohydrate	gr/100	26.5
Vitamin A (Retinol)	IU/100 gr	217666.6
Vitamin B_1 (Thiamine)	mg/100	14.5
Vitamin B_2 (Riboflavin)	mg/100	4.96
Vitamin B_3 (Niacin)	mg/100	15.63
Vitamin B_5 (Pantothenic acid)	mg/100	12.6
Vitamin B_6 (Pyridoxine)	mg/100	10.2
Vitamin B9 (Folic acid)	mg/100	15
Vitamin B_{12} (Cyanocobalamin)	mg/100	1.24
Vitamin C (Ascorbic acid)	IU/100 gr	10.3
Vitamin D_3 (Calciferols)	IU/100 gr	23144
Vitamin E (Alpha-tocopherol)	mg/100	2.50

Table 2. Comparison of clinical parameters before and after consumption of yogurt containing *S. platensis* extract and yogurt containing fermented *S. platensis* extract.

Variables	Intervention 1- *S. platensis* yogurt consumers' group N=50	Intervention 2- fermented *S. platensis* yogurt consumers group N=50	
	mean± SD	mean± SD	P. value**
FBS			
Before intervention	150.44± 27.85	156.24± 46.09	0.74
After intervention	140.44± 22.82	140.56± 33.95	0.76
P. value*	0.001	0.001	
FRAP			
Before intervention	0.80± 0.19	0.89± 0.32	0.76
After intervention	0.91± 0.30	1.32± 0.65	0.01
P. value*	0.015	0.001	
GSH			
Before intervention	509.63± 193.09	523± 161.50	0.57
After intervention	574.20± 181.13	695.70± 136.12	0.01
P. value*	0.001	0.001	
CRP			
Before intervention	2.80± 1.83	3.14± 1.76	0.25
After intervention	2.68± 1.77	1.97± 1.26	0.68
P. value*	0.291	0.001	
MDA			
Before intervention	1.34± 0.42	1.42± 0.43	0.032
After intervention	1.14± 0.34	0.34± 0.42	0.010
P. value*	0.76	0.001	

p. value*: Comparison of results before and after treatment within each intervention group. *p*. value**: Comparison of results before and after treatment between intervention groups.

A total of 110 patients with 'T2DM' participated in the plan, 100 patients remained in the plan and 10 people withdrew from the intervention for personal reasons. We have summarized the results in Tables 2 and 3, which are used to interpret the results.

The average FBS, FRAP, GSH, MDA, and CPR values before and after supplementation with yogurt enriched with *S. platensis* extract and fermented *S. platensis* are summarized in Table 2. From the results, there is a significant difference in the intervention group No. 1 (*S. platensis* yogurt consumer group) in terms of "FBS (P=0.001), FRAP (P=0.015), and GSH (P=0.001)". However, no significant difference was found in CRP (P=0.291) and MDA(P=0.76). In intervention, no. 2 (group of fermented *S. platensis* yogurt consumers), all five factors studied, FBS(P=0.001), FRAP (P=0.015), GSH(P=0.001), MDA(P=0.001), and CRP(P=0.001) showed significant differences. Table 2 shows the mean value of each clinical parameter in the two treatment groups compared. Results show that there is a significant difference in GSH, MDA, and FRAP after the treatments comparing the two treatment groups. consumption of fermented products

Table 3. Comparison of differences between before and after intervention.

groups		FBS	GSH	FRAP	CRP	MDA
Intervention 1- *S. platensis* yogurt consumers' group	Differences between before and after intervention	-10.00	64.56	0.11	-10.00	-0.1938
	Std.D	14.29	132.86	0.27	14.29	0.342
	P.Value	0.008	0.53	0.20	0.002	0.200
Intervention 2- fermented *S. platensis* yogurt consumers group	Difference between before and after intervention	-15.68	171.81	0.42	-1.17	-0.22
	Std.D	22.6	141.65	0.53	1.15	0.37
	P.Value	0.01	0.20	0.01	0.01	0.001

resulted in a significant increase in GSH and FRAP levels AND a significant decrease in MDA level after treatment compared to the non-fermented *S.platensis* treatment.

Table 3 shows a comparison of the differences in clinical parameters before and after each treatment. Based on the differences between the values of clinical parameters before and after the consumption of functional yogurts by 'T2DM' patients, it can be seen that the FBS value has a significant difference in both groups ($p = 0.008$). Similar results are also observed for CRP ($p= 0.002$). GSH, MDA, and FRAP have no significant difference when supplemented with yogurt enriched with *S. platensis* extract. The value of GSH in consumers of fermented *S. platensis* yogurt showed no significant difference, but there was a significant difference in the case of FBS, MDA, CRP, and FRAP ($p\leq 0.05$). It can be concluded that both products, as functional foods, have a positive effect on the health of diabetics by lowering FBS and increasing total antioxidant capacity, although this positive effect is more pronounced in the fermented product.

4. Discussion

In this study, the effect of *Spirulina* on diabetic patients was investigated. Since previous studies had confirmed the positive effect of *Spirulina* on hematological factors in diabetic individuals, researchers aimed to examine its effects in processed forms and assess its impact on the hematological factors of diabetic individuals.

To this end, two groups consisting of men and women with different diabetic histories were subjected to experimentation. One group received unprocessed *Spirulina*, while the other group received fermented *Spirulina* along with yogurt as a dietary supplement. The results from both groups were examined in several different scenarios.

S. platensis antioxidant effect was reported before Dartsch, 2008. While the effects of *Spirulina* fermented with a variety of bacteria, including probiotics, have been less discussed, conducting clinical research on humans seems necessary and interesting [29].

In the present study, two types of functional foods consisting of low-fat yogurt mixed with non-fermented and fermented *S. platensis* powder were prepared. Type 2 diabetic patients were selected as a vulnerable group to high oxidative stress caused by the high glucose in the blood, and the effects of two types of formulated functional foods were investigated and compared. The results showed that oral administration of both functional foods can reduce blood glucose levels and modulate oxidative stress in diabetic patients, and this positive effect was stronger in the consumer group of fermented products.

According to Lee et al., *Spirulina* is supposed to improve immune capacity, but no significant changes in fasting blood sugar (FBS) plasma levels were observed. In contrast, in the current study, the administration of 8 g/day *Spirulina* and its fermented products for three weeks resulted in a reduction in plasma FBS levels in patients with 'T2DM'. On the other hand, blood glucose levels in mice induced by alloxan DM were reported to be reduced after oral administration of 20 mg/kg body weight *S. platensis*[30].

Based on the results of this study, positive effects of *Spirulina* consumption on increasing GSH and FRAP levels were observed. It was also found that consumption of fermented *Spirulina* had a greater effect on increasing GSH and FRAP and decreasing MDA. So, fermentation can be an effective method to improve the properties of *Spirulina*. A similar observation has been reported that GSH activity is increased in selected tissues in diabetic patients treated with *Spirulina*. Comparison of FRAP before and after fermentation of *Spirulina* has been reported that the bioactive profile of *Spirulina* improved in vitro by 12 to 72 h fermentation with the lactic acid bacterium *Lactobacillus plantarum*.

According to Castro et al., *Spirulina* has been confirmed to be a suitable substrate for *L. plantarum*, thus, holding a potential for the production of probiotic-based products. Fermentation increased the nutraceutical value of *Spirulina*, significantly ($P \leq 0.05$). According to the results of this study, fermentation increased the anti-oxidative value of *spirulina*. There are a limited number of studies that have examined the properties of fermented spirulina and it is suggested that more studies be done in this field [15, 31,32].

Mixed probiotic fermentation of *Spirulina* has also been carried out and the results showed that probiotic fermentation has a good effect on *Spirulina* and can serve as a new procedure for developing new *Spirulina*-containing food items. In this study, the *Spirulina* fermentation process was used and its desirable effects were observed in moderating the oxidative stress status of T2DM patients.

In a study, Moghadam et al., investigated the combined effect of exercise and *Spirulina* consumption on inflammatory factors in diabetic men and concluded that consumption of *S. platensis* with exercise and even without exercise reduced inflammatory factors [33].

In this experimental study, we did not investigate the changes that may occur in the mixture of *Spirulina* algae with yogurt over time. Therefore, it is suggested to study the physical, chemical, and antioxidant properties of this functional food (fermented and non-fermented *Spirulina*-enriched yogurt) over time and estimate its shelf life.

S. platensis has the potential to be a 'miracle food sup-

plement' for diabetics. It can be concluded that functional products containing *S. platensis* such as yogurt should be designed and formulated, not only to have positive health effects and improve the state of oxidative stress in diabetics but also to provide a varied diet for consumers. The results of this research show that fermentation of *S. platensis* with probiotic bacteria increases the bioavailability of the algae's contents and can improve the nutritional properties of foods fortified with *S. platensis*.

With a comparison conducted between the male and female populations in both groups, the results were presented in Figures 2-5. A comparative classification was employed such that in Figure 2, the group consuming unprocessed *Spirulina* was analyzed for hematological reduction factors, namely FBS and CRP. In both male and female groups, a reduction in the levels of FBS and CRP was observed. However, while men exhibited logical trends in changes, women displayed sinusoidal patterns. A similar pattern occurred in Figure 3 for factors GSH, MDA, and FRAP. As previously mentioned, these factors increase with Spirulina consumption.

In the second group, which consumed fermented Spirulina, due to its stronger antioxidant effect, remarkable changes occurred. As evident in Figures 4 and 5, the graphs became sharper, indicating the positive effect and influential impact of the fermentation process on increasing the antioxidant effect of *Spirulina*. The difference between men and women groups may stem from their different physiological characteristics. For this reason, it is suggested that this factor (sex)can be investigated in future studies.

5. Conclusion

Mixed probiotic fermentation of *Spirulina* has also been shown to have a positive effect on its properties 1, making it a potential ingredient for developing new *Spirulina*-containing food items. In our study, the fermentation process of *Spirulina* was used, and its desirable effects were observed in moderating the oxidative stress status of type 2 diabetes patients.

It is worth noting that the physical, chemical, and antioxidant properties of *Spirulina*-enriched yogurt, both fermented and non-fermented, should be studied over time to estimate its shelf life.

Overall, *S. platensis* has the potential to be a "miracle food supplement" for diabetics. Functional products containing *S. platensis*, such as yogurt, can have positive health effects and improve the state of oxidative stress in diabetics while providing a varied diet for consumers.

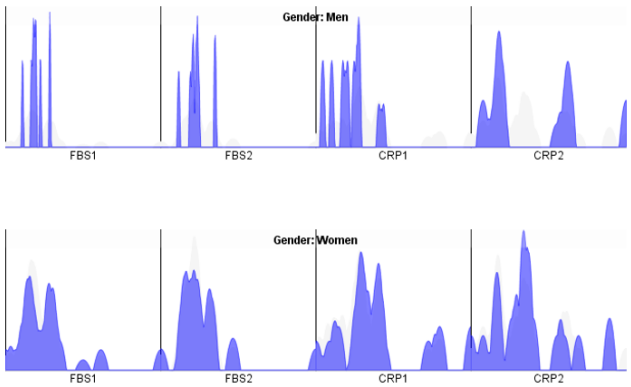

Fig. 2. Comparison between different genders in the first group: Decreased blood factors (*Spirulina* without fermentation).

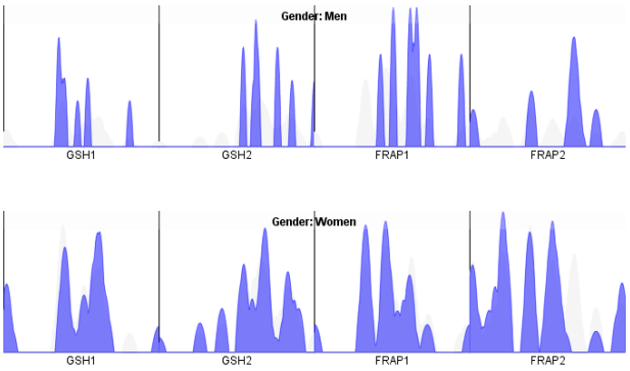

Fig. 3. Comparison between different genders in the first group: Increased blood factors (*Spirulina* without fermentation).

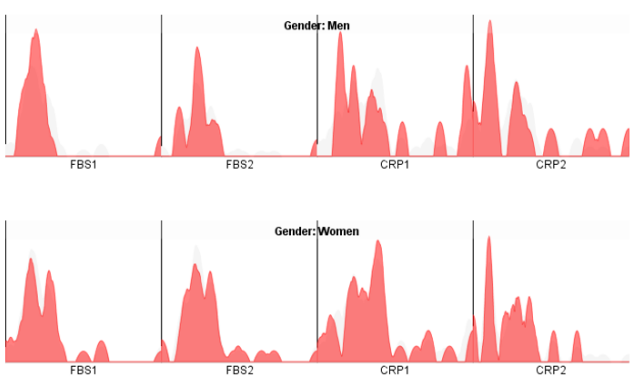

Fig. 4. Comparison between different genders in the second group: Decreased blood factors (Fermented *Spirulina*).

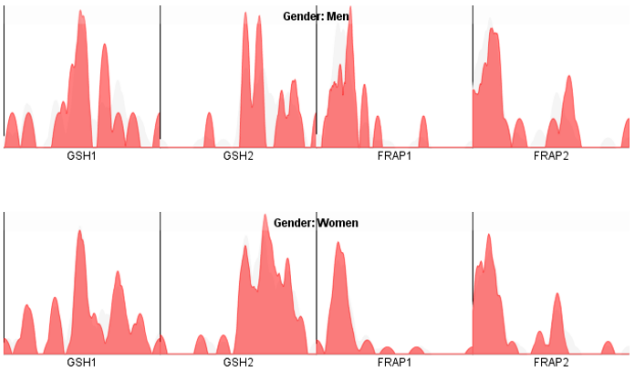

Fig. 5. Comparison between different genders in the second group: Increased blood factors (Fermented *Spirulina*).

The effects of *Spirulina* fermented with a variety of bacteria, including probiotics, have been less discussed, and conducting more clinical research on humans seems necessary and interesting.

Abbreviations

T2DM: Type 2 Diabetes Mellitus; FBS: Fasting Blood Sugar; GSH: Glutathione; MDA: Malondialdehyde; FRAP: Ferric-Reducing Antioxidant Power; CRP: C-reactive protein.

Conflict of interests

The authors declare that they have no conflict of interest.

Consent for publications

All authors read and approved the final manuscript for publication.

Ethics approval and consent to participate

Approval of the research protocol: This study has been approved by the Ethics Committee of the Islamic Azad University; Science and Research Branch, Tehran, Iran. The ethics code related to this survey is IR.IAU.SRB.REC.1401.094.

This study was approved by the Iranian Registry of Clinical Trials (IRCT). study/trial number: IRCT 65709.

Informed consent

All patients and participants in the research were informed about the aims of the study and any possible side effects of the drugs and interventions.

Availability of data and material

Data supporting this study can be available upon request from the first author (Aida Ghafari Ashtiani).

Authors' contributions

Study concept and design: Ayda Ghaffari Ashtiani, Morteza Gharibi,

Analysis and interpretation of data: Rahmatollah Moradzadeh

Drafting of the manuscript: Ayda Ghaffari Ashtiani

Critical revision of the manuscript for important intellectual content: Anousheh Sharifan

Statistical analysis: Rahmatollah Moradzadeh

Acquisition of data: Ayda Ghaffari Ashtiani- Anousheh Sharifan

Administrative, technical, and material support: Morteza Gharibi- Ayda Ghaffari Ashtiani

Study supervision: Anousheh Sharifan- Morteza Gharibi

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