Journal of Nuts

Journal homepage: ijnrs.damghan.iau.ir

ORIGINAL ARTICLE

The Effect of Spirulina Algae Enrichment on the Quality of Pistachio Butter

Ahmad Shakerardekani[*1], Farzaneh Etemadi[2,3]

[1]*Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Iran*

[2]*MSc of Food Science & Technology field of Food Technology Agriculture Faculty, Kar Higher Education Institute, Rafsanjan, Iran*

[3]*Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

A R T I C L E I N F O

Keywords:

Enrichment;
Nutrition;
Oxidation;
Pistachios;
Sensory evaluation

A B S T R A C T

Pistachio butter is one of the pasty products made from pistachio kernels. This study investigated color characteristics, peroxide value, fat, protein, and carbohydrate content, and sensory evaluation of pistachio butter enriched with Spirulina algae (0, 5, and 10%) during three months of storage at 20°C. With increasing the percentage of the algae, the sample with 10% of the algae showed the highest protein content (around 25.4%). The highest and lowest fat content was observed in the control and the sample containing 10% algae, respectively. The lowest peroxide value (0.73 meq kg^{-1}) was observed in the sample containing 10% algae compared to others. The level of carbohydrates did not change significantly in all three specimens during storage. Sensory evaluation for taste, sweetness, color, spreadability, mouthfeel, and texture showed that the sample without algae and the specimen containing 5% algae did not differ much. With increasing the algae level to 10%, the sensory evaluation had a significant drop, and in some cases, there was a decrease in the score. The color evaluation of the samples showed that with increasing the algae level, the greenness of the specimen increased, *a value* decreased, and *b value* did not show a significant change. Overall, The amount of algae recommended in the pistachio butter formulation is 5%.

Introduction

Pistachio is one of the important families of *Anacardiaceae* and belongs to *Pistacia* species. Pistachio is a source of antioxidants and fatty acids that are useful for human health. Pistachio kernels are mainly used as dry nuts and to produce various types of products, including pistachio chocolate, pistachio halva, pistachio milk, pistachio breakfast cream, and pistachio butter. Pistachio butter is prepared by roasting and grinding raw pistachios with sugar (or other sweeteners) (Hosseini *et al.*, 2022, Nazoori *et al.*, 2022a, b, Shakerardekani and Shayegh, 2019, Shakerardekani *et al.*, 2020, Sharifkhah *et al.*, 2020, Roozban *et al.*, 2006). The quality of pistachio butter depends on its formula and production method. Usually, a mixture of 90% unsplit pistachio kernels, 10% sugar, and some flavoring, including vanilla and cardamom, are used to prepare pistachio butter. More than half of the pistachio butter formulation is a rich oil paste. The high amount

[*]**Corresponding author**: Email address: shaker@areeo.ac.ir
Received: 12 September 2022; Received in revised form: 5 December 2022; Accepted: 8 February 2023
DOI: 10.22034/jon.2023.1967656.1196

of fat in the formulation of pistachio butter makes it highly susceptible to spoilage, so it is necessary to control spoilage using various methods.

Spirulina platensis has a long history of use as food. The benefits of these microalgae are enormous compared to other plant food sources and algae. This algae has anti-viral and anti-cancer properties and strengthens the immune system, while no adverse effects have been observed on human cells (Asghari *et al.*, 2016). Spirulina not only has a strengthening effect on the immune system and stimulates the immune system but also increases the ability of the body to create a new generation of blood cells (Ravi *et al.*, 2010). *Spirulina platensis* and its metabolites have suggested a discernible trend in improving the value of healthy food products. Spirulina is rich in protein, essential fatty acids (γ-Linolenic acid), vitamins (especially vitamin B12 and vitamin A precursor), minerals (iron and calcium), pigments (phytocyanin, a rare colored protein), and sulfo-lipids. The green color of pistachio butter decreases during storage.

The biscuit and snack industry has been increasingly interested in incorporating spirulina algae into its products. The addition of spirulina up to 12.5% led to an increase in protein content (9.43–18.11%) (Haoujar *et al*., 2022). Algae, including spirulina, are used as a source of proteins, antioxidants, and biologically active molecules in the process of wheat cookies. The addition of 6% spirulina significantly increased protein content (up to 13.5%) (Batista *et al.*, 2019).

The amount of adding spirulina to biscuits during the fortification process varies 5-15% (Sonam *et al*., 2017). The findings of the sensory evaluation revealed that the control sample had a lower score for most of the sensory characteristics. It was found that the incorporation of spirulina into biscuits resulted in an increase in the percentage of protein (9.09 %). Nutritional biscuit content might be improved by adding certain spirulina percentages to the foods that school-children eat for snacks (El Nakib *et al.*, 2019).

The production of a wide variety of dairy products includes using spirulina algae. Spirulina algae are used to improve the nutritional content of fermented dairy products. *Spirulina platensis* (1.2%) was used in the production process to make a functional ice cream with a high nutritional value (AlFadhly *et al.*, 2022).

Because *Spirulina platensis* is known to be rich in protein and has a green color, it has the potential to be used in the formulation of pistachio butter to improve the green color and protein content. Therefore, the purpose of this study was to improve color characteristics, fat, protein, and carbohydrate content, sensory evaluation, and oxidation stability of pistachio butter enriched with Spirulina algae at the beginning and three months after storage**.**

Materials and Methods

Materials and devices

Solvents, reagents, and chemicals were purchased from Merck and Folica companies. A spectrophotometer (Biowave, WPA S2100, and UK) was used to measure the absorbance of compounds.

Sample preparation method

Pistachio butter was prepared according to its standard formulation and method suggested by Shakerardekani *et al.* (2020). For this purpose, pistachio kernels were roasted at 110°C. The roasted kernels were grinded with 10% sugar using a stone mill. First, the control sample (without Spirulina algae) was prepared. In Other specimens, five or ten percent of Spirulina algae power were added to the roasted kernels and sugar before grinding in a stone mill. The pistachio butter samples were packed in poly ethylene container (250 g). The prepared samples were evaluated in terms of physicochemical, sensory, and fat, protein, and

carbohydrates changes of pistachio butter at the beginning and after three months of storage at ambient temperature (20±2°C).

Protein measurement

Kjeldahl's method was used to measure protein content. In the digestion stage, first, 0.7 to 5.3 grams of food was weighed and poured into Kjeldahl's balloon. After digestion and distillation, the volume of acid used in the titration stage was recorded, and nitrogen content was calculated. The percentage of protein was also obtained from the product of nitrogen percentage in pistachio protein factor (5.3) (ISIRI, 2001).

Fat measurement

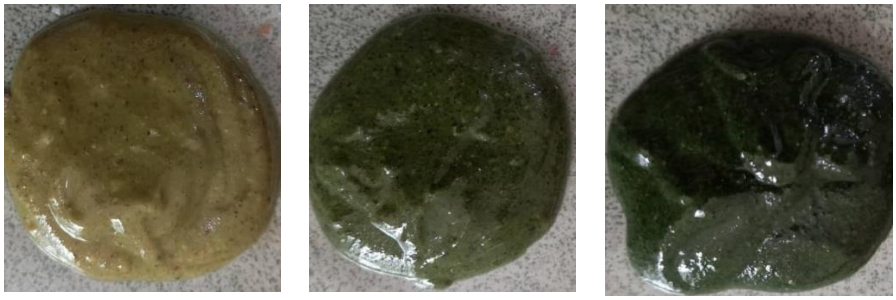
The Soxhlet method was used to measure the amount of fat (ISIRI, 2001).

Carbohydrate measurement

The Fehling method mentioned was used to measure carbohydrates (ISIRI, 2001).

Color measurement

The samples were photographed from a distance of 20 cm above (Fig. 1) and analyzed with the Image J software. In the end, after photographing, the values of b, a, and L were calculated. The *L value* represents the brightness of the sample, and its range varies from zero (pure black) to 100 (pure white). The *a value* shows the degree of redness (red to green), and the *b value* shows the degree of yellowness (yellow to blue).



0% 5% 10%

Fig. 1. Pistachio butter with 0, 5 and 10 % *Spirulina platensis*

Measurement of peroxide value

Titration method was used to extract the oil and calculate the peroxide value. Forty grams of pistachio butter was poured into the filter paper and weighed to extract the oil. Then, 120 ml n-hexane was poured on each sample, and after 48 hours, the filter paper was taken out with the specimen inside and placed inside a desiccator for 48 hours to prevent moisture absorption and evaporation of n-hexane and then weighed. Then a combination of oil and n-hexane was poured into the rotary device, and the solvent was evaporated under vacuum conditions. The obtained oil was collected and stored at -18°C. The oil content (%) was calculated through the following formula (ISIRI, 2001):

$$\text{Oil content (\%)} = (\text{Initial weight} - \text{Secondary weight})/ \text{Initial weight} \times 100$$

Sensory test

To perform the sensory test, ten trained evaluators who have the necessary skills to perform this test and the QDA method (15 cm linear scale) were used (Shakerardekani and Kavoosi). Finally, the quality, the

result of the evaluators' opinion about the product-specific features, was converted into quantity. This test measured the characteristics of taste, mouth feel, texture, sweetness, spreadability, and color (Table 1). A code was assigned to all treatments, and in addition to an evaluation form, it was provided the evaluators with a glass of water and other equipment, such as a spoon.

Table 1. Glossary for sensory evaluation.

Sensory property	Ranking	Description
Spreadability	Good-bad	Ability to spread on bread
Mouthfeel	Good-bad	Creamy mouthfeel
Taste	Good-bad	Delicious
Texture	Soft-rigid	Rigid to soft
Color	Low green-high green	Amount of pistachio butter green color
Sweetness	suitable-unsuitable	Amount of sweetness

Data analysis

The statistical design used in this research was a factorial experiment in a completely randomized design. A comparison of mean parameters (3 replications) was made at a 5% level using Tukey's test (Minitab software version 20).

Results

Percentage of fat, protein, and carbohydrates

The results indicated that the samples did not show significant change during the three months. However, with increasing the level of algae, the fat content decreased (Table 2).

Table 2. Fat, protein, and carbohydrate content of pistachio butter with different Spirulina algae (%).

Treatment	Fat (%)	Protein (%)	Carbohydrate (%)
0% Spirulina algae, Month 0	43.5 ± 0.4a*	20.3± 0.1c	20.3± 0.1c
0% Spirulina algae, Month 3	43.2± 0.4ab	20.2± 0.1c	20.2± 0.1c
5% Spirulina algae, Month 0	42.7 ± 0.3ab	22.6± 0.1b	22.6± 0.1b
5% Spirulina algae, Month 3	42.0 ± 0.3bc	22.4± 0.1b	22.4± 0.1b
10% Spirulina algae, Month 0	40.9± 0.8c	25.3± 0.1a	25.3± 0.1a
10% Spirulina algae, Month 3	40.8± 0.4c	25.2± 0.1a	25.2± 0.1a

The values with different letters show significant differences at 5% level.

The results (Table 2) showed that with increasing the percentage of algae, the protein content in pistachio butter increased and changed little with time. The sample containing 10% algae had the highest protein content. The highest and lowest fat content was observed in the control and the specimen containing 10% algae, respectively. Treatments containing Spirulina algae had more carbohydrates than samples without algae. Moreover, with increasing the percentage of Spirulina algae, the carbohydrate content also increased, and with time, no significant difference in the carbohydrate content was observed.

Color

There was a significant difference between the three examined samples at a 5% level. According to Table 3, with an increase in the level of algae, the brightness (*L value*) decreased. The results showed that by increasing the Spirulina algae to 10%, the *a value* decreased significantly, the amount of greening also increased, and the samples showed a significant difference. Moreover,

the *b value* was examined for all three samples, and the three samples did not show a significant difference.

Table 3. Effect of Spirulina algae on pistachio butter color during storage.

Treatment	L*	a*	b*
0% Spirulina algae, Month 0	59.87± 10.60b	1.35± 0.021a	15.83± 0.063a
0% Spirulina algae, Month 3	96.86± 2.12a	1.41± 0.014a	15.89± 0.028a
5% Spirulina algae, Month 0	60.36± 2.82b	0.11± 0.007b	15.72± 0.042a
5% Spirulina algae, Month 3	49.36 ± 1.41b	0.13± 0.007b	15.62± 0.035a
10% Spirulina algae, Month 0	32.25± 0.21c	-1.16± 0.063d	14.70± 0.282b
10% Spirulina algae, Month 3	31.65± 0.21c	-0.77± 0.063c	14.60± 0.141b

The values with different letters show significant differences at a 5% level.

Peroxide value

The peroxide value in pistachio butter in three samples during three treatments (Table 4) showed that the peroxide value in the treatments increased with time. The peroxide value for the treatments without Spirulina algae was higher than the ones containing Spirulina. In other words, the treatment with 10% spirulina had a lower peroxide value than the other two treatments. The sample without algae showed a significant difference from the specimen containing 10% algae, but no significant difference was observed compared to the 5% sample.

Table 4. Effect of Spirulina algae on the peroxide value of pistachio butter during storage.

Treatment	Peroxide value (meq kg^{-1})
0% Spirulina algae, Month 0	0.87± 0.028abc
0% Spirulina algae, Month 3	0.92± 0.042ab
5% Spirulina algae, Month 0	0.80± 0.021bc
5% Spirulina algae, Month 3	0.95± 0.021a
10% Spirulina algae, Month 0	0.76± 0.042c
10% Spirulina algae, Month 3	0.84± 0.056abc

The values with different letters show significant differences at a 5% level.

Sensory evaluation

The sensory evaluation showed that the sample containing 5% algae got a better score while increasing the algae level to 10% showed a noticeable drop in the taste score. As a result, the sample containing 5% algae got the highest score after three months. Furthermore, the passage of time had no adverse effect on the taste of pistachio butter with Spirulina algae (Table 5).

Table 5. Sensory evaluation of pistachio butter with Spirulina algae after 3 months' storage.

Treatment	Texture	Mouthfeel	Spreadability	Color	Sweetness	Taste
0% Spirulina algae, Month 0	12.7 ± 0.3a	11.9 ± 0.1a	12.2 ± 1.1a	11.6 ± 1.7ab	11.4 ± 1.5a	12.1 ± 0.8a
0% Spirulina algae, Month 3	11.7 ± 0.3a	11.6 ± 1.6a	11.9 ± 0.8a	11.3 ± 1.0ab	8.9 ± 0.8a	10.3 ± 1.9a
5% Spirulina algae, Month 0	11.3 ± 1.7b	9.3 ± 1.3a	10.9 ± 0.8a	12.4 ± 0.5a	10.3 ± 0.1a	8.2 ± 3.4a
5% Spirulina algae, Month 3	12.7 ± 0.4a	12.4 ± 0.6a	11.4 ± 0.1a	12.6 ± 0.4a	10.1 ± 0.8a	9.1 ± 3.7a
10% Spirulina algae, Month 0	8.8 ± 0.8b	6.3 ± 2.9ab	9.6 ± 1.3a	6.9 ± 2.3bc	7.9 ± 0.6a	7.9 ± 1.1a
10% Spirulina algae, Month 3	12.2 ± 0.3a	5.9 ± 1.5b	9.6 ± 2.6a	5.5 ± 1.1c	9.9 ± 3.7a	9.5 ± 3.0a

The values with different letters show significant differences at a 5% level.

The examination of the color score for the samples showed that the amount of green color increased with increasing the level of the algae, and during the past three months, the specimens containing the algae were able to maintain the quality of the color better. Examining the sweetness score of pistachio butter enriched with different amounts of the algae showed that increasing the level of the algae decreased the score by the evaluators, although this decrease was not significant. As a result, it can be said that increasing the algae level over time had no adverse effect on sweetness. The mouthfeel evaluation showed that the sample containing 5% algae got a better score, while increasing the algae level to 10% showed a noticeable drop in the mouthfeel score. As a result, the sample containing 5% algae got the highest score after three months. Examining the pistachio butter texture of treatments containing Spirulina algae and pistachio butter without algae (Table 5) did not show a significant difference. In addition, the passage of time had no adverse effect on the texture of pistachio butter with Spirulina algae. The results showed that by increasing the algae level up to 10%, a decrease in some sensory characteristics was observed. Therefore, the sample containing 5% algae scored higher than the 10% algae sample.

Discussion

Percentages of fat, protein, and carbohydrates

The results obtained from the fat percentage test for the treatments showed that by increasing the algae level, the amount of fat decreased, which was to be expected. According to the results, with increasing the percentage of the algae, the protein content in pistachio butter increased, which could be due to the protein in Spirulina algae because these algae are rich in all kinds of amino acids and have high absorbability, which can play a vital role in human nutrition. This algae contains balanced amounts of proteins, which are easily digestible and quickly relieve hunger feelings (Gershwin and Belay, 2007, Khazaei *et al.*, 2016). Moreover, treatments containing Spirulina algae had more carbohydrates than samples without algae, which was expected considering the compounds of Spirulina.

Color

According to the results, with increased Spirulina algae, the amount (a) decreased significantly, and the amount of greening also increased. Morshedi *et al.* (2014) reported a similar case of pistachio butter containing green pumpkin seed paste (without algae).

Peroxide value

The peroxide value for the treatments without Spirulina algae was higher than for treatments containing spirulina in this research. Souzankar *et al.* (2018) reported that enriching wafers with *Arthrospira platensis* microalgae showed lower peroxide values. Ratrinia and Komala (2022) indicated that adding R. apiculata leaf powder could slow down an increase in peroxide value in chocolate bars during their shelf life.

Sensory evaluation

Among the sensory characteristics of pistachio butter, color is one of the salient qualitative characteristics effective in choosing and attracting the consumer's attention, and it indicates things such as enzymatic and chemical reactions and microbial growth (Hedayati and Niakousari, 2015). Spirulina is one of the blue-green filamentous multicellular microalgae (Jung *et al.*, 2019). In this research, with increasing the algae level, the amount of green color increased, and during the past three months, samples containing the algae have maintained the color quality better. Ekantari *et al.* (2019) investigated how to make one type of spirulina that people noticed its flavor and texture. The results

showed that the texture, aroma, and taste of the chocolate did not change even when the spirulina was added.

Conclusions

This study investigated some compositions, physicochemical, and sensory properties of pistachio butter enriched with Spirulina algae. The sample with 10% algae content was better than the other two samples in terms of protein content. Moreover, checking the fat content showed that the sample with 10% algae had the lowest fat content. Furthermore, the peroxide value showed a significant decrease with increasing the algae level, and the sample with 10% algae in the first treatment had the lowest peroxide value (0.73) compared to the two other samples. In addition, checking the carbohydrate content in three samples did not change significantly. Sensory evaluation for taste, sweetness, color, spreadability, mouthfeel, and texture showed that the samples without algae and 5% algae were not much different. However, by increasing the algae level to 10%, sensory evaluation in some characteristics, such as taste and mouthfeel, decreased significantly. Evaluating the color of the samples showed that with increasing the algae level, the darkness of the product increased. On the other hand, increasing the level of green algae in the sample, i.e., *a value*, decreased. Generally, we concluded that 5% Spirulina algae had a more favorable effect on pistachio butter samples.

Acknowledgements

This work was supported by the Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, under grant 31/20/1/97384. The author s also acknowledges the cooperation of the Iran Pistachio Research Center.

Conflict of interest

The authors report that there are no competing interests to declare.

References

AlFadhly NK, Alhelfi N, Altemimi AB, Verma DK, Cacciola F & Narayanankutty A (2022). Trends and technological advancements in the possible food applications of Spirulina and their health benefits: A Review. Molecules. 27(17), 5584.

Asghari A, Fazilati M, Latifi AM, Salavati H & Choopani A (2016). A review on antioxidant properties of Spirulina. Journal of Applied Biotechnology Reports. 3(1), 345-351.

Batista AP, Niccolai A, Bursic I, Sousa I, Raymundo A, Rodolfi L, Tredici MR (2019). Microalgae as functional ingredients in savory food products: application to wheat crackers. Foods. 8(12), 611.

Ekantari N, Budhiyanti SA, Fitriya W, Hamdan AB, Riaty C (2019) Stability of chocolate bars fortified with nanocapsules carotenoid of *Spirulina platensis*. In IOP Conference Series: Earth and Environmental Science, p. 012079. IOP Publishing.

El Nakib DM, Ibrahim MM, Mahmoud NS, Abd El Rahman EN, Ghaly AE (2019) Incorporation of Spirulina (*Athrospira platensis*) in traditional Egyptian cookies as a source of natural bioactive molecules and functional ingredients: Preparation and sensory evaluation of nutrition snack for school children. European Journal of Nutrition & Food Safety. 9(4), 372-397.

Gershwin ME, Belay A (2007) Spirulina in human nutrition and health. CRC press. 328 Pages.

Haoujar I, Haoujar M, Altemimi B, Essafi A, Cacciola F (2022) Nutritional, sustainable source of aqua

feed and food from microalgae: A mini review. International Aquatic Research. 14(3), 157-167.

Hedayati S, Niakousari M (2015) Effect of coatings of silver nanoparticles and gum arabic on physicochemical and microbial properties of green bell pepper (C *apsicum annuum*). Journal of Food Processing and Preservation. 39 (6), 2001-2007.

Hosseini N, Rezanejad F, ZamaniBahramabadi E (2022) Effects of soil texture, irrigation intervals, and cultivar on some nut qualities and different types of fruit blankness in pistachio (*Pistacia vera* L.). International Journal of Horticultural Science and Technology. 9(1), 41-53.

ISIRI (2001) Pistachio butter: specification and test methods (NO. 5691). 1ST edition. Karaj: Institute of Standards and Industrial Research of Iran.

Jung F, Krüger-Genge A, Waldeck P, Küpper JH (2019) *Spirulina platensis*, a super food? Journal of Cellular Biotechnology. 5(1), 43-54.

Khazaei E, Shahidi F, Mortazavi SA, Mohebbi M (2016). Study of different levels of micro-algae *Spirulina platensis* on the Microstructure and Psychochemical and Sensory Characteristics of Kiwi pastille. Iranian Food Science and Technology Research Journal. 12(1), 21-33.

Morshedi A, Razavi SMA (2014) Investigating the rheological and sensory characteristics of the mixture of pistachio paste and pumpkin seed paste. In The First Iranian Pistachio National Conference.

Nazoori F, ZamaniBahramabadi E, Mirdehghan H (2022) Effect of sulfur pesticide on the quality of fresh pistachios in cold storage. International Journal of Horticultural Science and Technology. 9(4), 453-462.

Nazoori F, ZamaniBahramabadi E, Mirdehghan H, Yousefi M (2022) Preharvest application of sulfur as pesticide on fresh hull and kernel of pistachio (*Pistacia vera* L.). International Journal of Horticultural Science and Technology. 9(1), 117-129.

Ratrinia P, Komala U (2022) The effect of addition mangrove leaves powder to lipid oxidation of chocolate bar during the shelf life. In IOP Conference Series: Earth and Environmental Science, p. 012023. IOP Publishing.

Ravi M, De SL, Azharuddin S, Paul SF (2010) The beneficial effects of Spirulina focusing on its immunomodulatory and antioxidant properties. Nutrition and Dietary Supplements. 2, 73-83.

Roozban MR, Mohamadi N, Vahdati K (2006) Fat content and fatty acid composition of four Iranian pistachio varieties grown in Iran. Acta Horticulturae. 726, 573-577.

Saltmarsh M (2020) Saltmarsh's Essential Guide to Food Additives. Royal Society of Chemistry.

Shakerardekani A, Behmaram K, Bostan A (2020) The effect of replacing honey with sugar on oxidative stability properties of pistachio butter. Pistachio and Health Journal. 3(4), 33-49.

Shakerardekani A, Kavoosi M, Use of pistachio meal and mono-and diglyceride in the production of low-fat pistachio butter. Journal of Nutrition and Food Security. Accepted.

Shakerardekani A, Shayegh R (2019) Exploring the possibility of replacing date powder with sugar in pistachio butter formulation. Pistachio and Health Journal. 2(4), 73-82.

Sharifkhah M, Bakhshi D, Pourghayoumi M, Abdi S, Hokmabadi H (2020) Effect of pollination time on yield and antioxidant properties of some pistachio cultivars. International Journal of

Horticultural Science and Technology. 7(1), 51-58.

Sonam K, Neetu S (2017) A comparative study on nutritional profile of spirulina cookies. International Journal of Food Science and Nutrition. (3), 100-102.

Souzankar R, Chaichi- Nosrati S, Movahhed A (2018) Enrichment of coated wafers by addition of micro algae arthrospira (Spirulina) platensis powder. Iranian Journal of Nutrition Sciences & Food Technology. 13(2), 51-60.