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

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#### **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

## ABSTRACT

Spirulina (Athrospira platensis) is very rich in protein, amino acids, fatty acids, vitamins and minerals and its incorporation into foods will enrich their nutritional values. The objectives of this study were to evaluate the acceptability of adding spirulina to traditional Egyptian cookies as a source of natural bioactive molecules and to assess the effect of the amount of added spiruling on their sensory properties (texture, shred, color, odor and taste) using a panel of 10 members. The results indicated that addition of spirulina to the cookies affected the texture, the mouth feel, the easiness with which breaking a cookie was made, the fragmentation and the appearance of the break line. The cookies that received no spirulina had smoother texture and moist-smooth mouth feel whereas those received spirulina had more sandycourse texture and heavy-chewy mouth feel. Increasing the spirulina content from 5 to 15% made the cookies more firm and harder to beak. Irregular large parts and continuous break lines were observed with the cookies that received no spirulina while more granules and smaller parts with irregular line were observed with all the cookies that received spirulina. The results showed that adding spirulina to cookies may help maintain their integrity and reduce breakage during packaging and distributions. The color of the control sample (0% spirulina) was yellow to yellow-orange and that of the samples that received 5 and 10% spirulina was tallow-green to green-yellow-green while that of the sample that received 15% spirulina was green-yellow-green to green-blue-green. All the baked cookies had a noticeable smell and the odor intensity ranged from faint to strong. The weighted average for the odor intensity was within the of weak odor. Increasing the amount of spirulina from 5 to 15% (3 fold) increased the odor intensity by 19.6 %. The nature of the smell of the cookies that received 0 and 5% spirulina was pleasant while that of the cookies that revived 10 and 15% spirulina was musty-seawater and fishy-seawater, respectively. Adding 5% spirulina did not affect the odor but higher concentrations of spirulina may require the addition of a strong aromatic compound to the cookies to musk the smell of spirulina. The addition of spirulina affected both the taste and the degree of acceptance. The taste of the cookies that received no spirulina was sweet-delicious with a high degree of acceptance while the taste of the cookies that received spirulina varied from sweet-sour to bitter-fishy with lower degree of acceptance. Adding 5% spirulina did not affect the taste. However, addition of a flavoring agent to cookies receiving higher concentrations of spirulina (10-15%) may be required to musk the taste of spirulina. The results showed that addition of spirulina enhanced the nutritional value of the cookies by increasing the protein content of the cookies and enriching them with vitamins, mineral, omega 3, 6, 7 and 9 fatty acids and amino acids, all of which have significant health benefits to school children. Therefore, a further work should be directed towards improving the smell and the taste.

Keywords: Anemia; Obesity; Stunting; Spirulina; Cookies; Nutrition; Amino Acids; Fatty Acids, Vitamins; Minerals; Sensory Evaluation; Texture; Shred; Color; Odor; Taste

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**Comment [HM1]:** 1. Present only important results in the abstract section. 500 words is too long for an abstract.

2. You have repeating sentence which can be merged.

3. You didn't assessed effect of spirulina on anemia, obesity, stunting...they can not appear as keywords. Again, Odor, color and taste...can be resume as sensorial properties No need to have too many keywords.

# 1. INTRODUCTION

Egypt has about 12.5 million school children in the age range of 5-18 [1]. A significant number of these children suffer from malnutrition specially those of low socioeconomic standard which resulted in several diseases including anemia, obesity and stunting.

Anemia is a condition in which the amount of red blood cells in the body is decreased below normal and it can make the child appear pale in color and feel cranky, tired and weak. Studies have indicated that anemia is a major public health problem among Egyptian school children [2-4]. Iron deficiency anemia was found to be the most common cause of anemia among Egyptian children affecting 30-43% of children under 6 years of age [5] whereas the prevalence of anemia among children in the age group of 6-12 years was found to be 12% [6-7].

Obesity is an excessive accumulation of fat that adversely affects well-being and health [8]. Obesity is a major public health problem that affects nearly 35 % of adults and 18% of teenagers in Egypt [9-11], the highest in the world. Obesity is more prevalent in children between 6 and 9 years of age and is more prevalent in girls than in boys [12-14]. Egypt is a country where most citizens receive enough food to silence their hunger but not enough to nourish their bodies, a phenomenon that would negatively affect the country's development. There is an urgent need to spread awareness about obesity, its consequences and find ways of prevention, especially among young children [15].

Stunting is the impaired growth and development (low height-for-age) that children experience from poor nutrition and inadequate psychosocial stimulation. Children are defined as stunted if their height-for-age is more than two standard deviations below the WHO Child Growth Standards Median [16]. Stunting remains a very important problem in Egypt, as one-third of children under 5 years of age are affected [16-17]. According to the United Nations Children's Fund (UNICEF), the largest number of stunted children (about 2.7 million) in the Middle East was in Egypt due to the socioeconomic conditions of a country [17]. Stunting in early life of child has adverse functional consequences on the child including: (a) poor cognition and poor school performance, (b) when stunting is accompanied by excessive weight gain later in childhood, it results in increased risk of nutrition-related chronic diseases in adult life such as diabetes, hypertension, and obesity and (c) lost productivity and reduced earnings later in life [18-19].

Household food security in Egypt is very fragile and fluctuating food prices can cause severe shocks resulting in malnutrition among the low-income families. Hunger and malnutrition can drive children away from schools. Malnutrition among Egyptian children results in: (a) 11% of children deaths, (b) 33% stunted children (age 6-18 years.) and their ability to comprehend and concentrate during class are impacted, (c) 2% of these children are likely to fail in education and (d) 6 % repetition rate in primary

governmental schools [19]. Therefore, the current Government of Egypt invests USD 110 million per year on the National School Feeding Programme which reaches 12.5 million pupils. The goals of this program are: (a) enhancing students' health by providing nutritious meals on a daily basis to increase their concentration in class, (b) educating the students and parents about the importance of the healthy nutritious meals and (c) motivating students to attend their classes and decrease school dropout rates and absences. However, the nutritional composition of theses meals must be enhanced [20].

Spirulina (Athrospira platensis) is a blue-green alga that has a great potential for use in food and food products because of its high nutritional composition (Table 1). The dark green color of Spirulina comes from the high amount of chlorophyll (plant blood) which is only one molecule different from the hemoglobin (human blood). No one fruit, vegetable or meat can provide all the nutrition elements the human body demands as Spirulina. It contains over 100 nutritional and bioactive compounds, is free of cholesterol, has only 2-4 cal/g, has a high digestibility (95%) and has an alkali pH which can protect the human body from the diseases resulting from acidic foods such as meat, sea food and cereals. The protein content in Spirulina is about 65-72% which is higher than that in the soybean and is easier to digest. Spirulina contain all the essential and non-essential amino acids (Table 2) which are 3-4 times those in fish and meat and 29 times those in soybeans. Spirulina contains more than 2000 enzymes that are beneficial for human health [25, 29-30]. The fatty acids (Table 3) contain omega 3 (alpha linolenic and docosahexaenoic) omega 6 (linolenic, gamma linolenic and dihomo-gamma linolenic), omega 7 (palmitoleic) and omega 9 (oleic and auric) oils [22, 28, 30]. Spirulina is very rich in mineral content (Table 4) including: calcium, phosphorus, iron, sodium, magnesium, potassium, manganese, zinc, boron, copper and molybdenum [23, 28]. The mineral contents in Spirulina are 28 and 58-fold of those in beef liver and spinach, respectively [31,32]. Spirulina contain several vitamins (Table 5) including: betacarotene (vitamin A), thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pyridoxine (vitamin B6), cyanocobalamin (vitamin B12), Da-tocopherol (vitamin E), biotin (vitamin H), folic acid, pantothenate and inositol [21-23,25-26]. The vitamin contents in spirulina are higher than those in liver, carrot, spinach and many vegetables [33].

In recent years, novel attractive healthy foods have been prepared from spirulina [30,33-35]. Traditional foods such as salad dressings, dips, puddings, gelled desserts, biscuits, cookies, bread, noodles, pasta, smoothies, ice cream and health drinks such as micro-algal sour milk and micro-algal green tea were supplemented with spirulina to add coloring and functional attributes, making the products more sensorial attractive with health benefits due to the high content of carotenoids, polyunsaturated fatty acids, antioxidant and anti-inflammatory compounds [22,30, 34-37].

Table 1. General composition of fresh dried spirulina [21-27].

Content	Value
Energy (Cal/g)	2.90
Moisture (%)	4 -5
Protein (%)	65 -72
Carbohydrate (%)	15 -25
Fibers (%)	3 -7
Lipids (%)	4 -7
Minerals (mg/g)	6 -12

Table 2. Amino acids in fresh dried spirulina [22, 24, 28].

Amino Acids	Value
	(mg/g)
Alanine	7.7-46.6
Arginine	7.9-47.6
Aspartic Acid	12.1-72.8
Cysteine	0.9-5.6
Glutamic Acid	4.1-84.4
Glycine	5.3-31.9
Histidine	2.5-15.0
Isoleucine	5.4-32.6
Leucine	8.2-48.9
Lysine	4.4-26.2
Methionine	2.2-13.3
Phenylalanine	4.5-26.1
Praline	4.1-24.7
Serine	4.4-26.5
Threonine	4.7-28.1
Tryptophan	1.4-8.5
Tyrosine	4.0-23.8
Valine	6.2-37.4

Table 3. Fatty acids in fresh dried spirulina [22, 28, 30].

Fatty Acids	Value
Omega 3	
Alpha Linolenic	0.04 mg/g
Docosahexaenoic	0.04  mg/g
Omega 6	
Linolenic	33.0 mg/g
Gamma Linolenic	30.0 mg/g
Dihomo-gamma Linolenic	1.59 mg/g
Omega 7	
Palmitoleic	5.90 mg/g
Omega 9	
Oleic	0.50 mg/g
Erucic	0.07  mg/g

Table 4. Minerals in fresh dried spirulina [23,28].

Minerals	Value	
Calcium	168.00 mg/g	
Magnesium	2.55 mg/g	
Iron	0.52 mg/g	
	8.8	
Phosphorous	9.18 mg/g	
Potassium	18.30 mg/g	
Sodium	10.98 mg/g	
Manganese	19.00 μg/g	
Zinc	$2.00 \mu g/g$	
Boron	$30.00 \mu g/g$	
Copper	$3.00 \mu g/g$	
Molybdenum	$30.00  \mu \mathrm{g/g}$	
Selenium	5.00 μg/g	

Table 5. Vitamins in fresh dried spirulina [21-23,25-26] .

Vitamins	Value	
Water soluble vitamins		
B-complex vitamins		
Vitamin B1 (Thiamine)	238.00 mg/g	
Vitamin B2 (Riboflavin)	99.00 mg/g	
Vitamin B3 (Niacin)	3.67  mg/g	
Vitamin B5 (Pantothenic Acid)	3.4 mg/g	
Vitamin B6 (Pyridoxine)	13.20 mg/g	
Vitamin B9 (Folate)	94.00 μg/g	
Vitamin B12 (Cyanocobalamin)	6.60 μg/g	
Vitamin H (Biotin)	1.00 mg/g	
Choline	66.00 mg/g	
Vitamin C	58.80 mg/g	
Fat soluble vitamins		
Vitamin A (as Beta Carotene)	29.00 μg/g	
Vitamin E (Da-tocopherol)	5.0 mg/g	
Vitamin K	25.20 μg/g	
Alpha Carotene	$7.50 \mu\mathrm{g/g}$	
Beta Carotene	$1900.00  \mu g/g$	
Lutein and Zeaxanthin	126.00 µg/g	

Spirulina is a fast-growing microorganism and has high biomass growth and high protein yield (Table 6). For a given area, the harvest yield of spirulina is 10 times that of soybeans, 20 times that of corn and 200 times that of beef cattle [37]. It can be grown to produce proteins and functional bioactive nd function compounds.

The main aim of this study was to evaluate the acceptability of traditional Egyptian cookies containing spirulina as a source of natural bioactive molecules. The specific objectives were: (a) to determine the cookies characteristics (odor/smell, taste, color, texture and shred) using sensory evaluations, (b) to establish the most acceptable amount of spirulina that can be added to the cookies and (c) determine the nutritional value of the cookies.

## 2. MATERIALS AND METHODS

#### 2.1 Preparation of Cookies

The following ingredients were used: soft butter (100 g), sugar (100 g), wheat flower (280 g), 2 eggs (109 g), baking powder (10 g), salt (1 g), vanilla (1 g) and desired amount of spirulina (0, 5, 10 and 15% by weight of the wheat flower or 0, 14, 28 and 42 g of spirulina as replacements for wheat flower ). The butter, sugar, flower, eggs, baking powder, salt, vanilla were purchased from a supermarket in Cairo and spirulina was purchased from NP-Nutra, Gardena, California, USA

The butter and sugar were placed in the large bowl of a bowl-lift stand mixer (Model No. 4KV25HOXER, Kitchen Aid, Mississauga, Ontario, Canada) and beaten until became fluffy. The eggs and vanilla were added to the butter-sugar mixture. The wheat flower, baking powder, salt and the desired amount of spirulina were first mixed together and then added to the butter-sugar-eggs-vanilla mixture and mixed with continuous stirring. Four portions of 601g each were made. No spiruling was added to the first portion (control), 14 g spirulina were added to the second portion (5%), 28 g spirulina were added to the third portion (10%) and 42 g spirulina were added to the fourth portion (15%).

The oven was heated to 180°C (350°F). From each portion, cookies were made, each was about 5 cm in diameter and 0.5 cm in height. The cookies were placed on a cooking sheet placed in a baking tray. The baking trays were place in a convention countertop oven (Model No. TO4211SKT, Black & Dekker, Rayovac, Argentina) and the cookies were baked for 14 min. Each backed cookie weighed approximately 30 g.

Comment [HM2]: What do you mean by protein yield? did spirulina produced protein?

Comment [HM3]: Justify the choice of cookies as matrix for incorporation of spirulina

Comment [HM4]: Questions still pending without answers

- 1. Choice of proportions of spirulina?
- 2. Why use spirulina as wheat flower replacement?

Table 6. Biomass and protein yields and environmental growth conditions of spirulina [31-36].

Parameter	Value
Biomass yield (g/L)	4.30
Protein yield (g/L)	2.71
Temperature (C°)	30.00
pН	9.00

#### 2.2 Sensory Evaluation

Sensory evaluations were carried out on the baked cookies to determine some of the physical properties (texture and shredding/breaking) and to evaluate the acceptability of color, smell and taste of the cookies. The procedure described by Ghaly et al. [38] was followed. A panel of 10 evaluators was formed from among technicians, undergraduate and graduate students and professors in the Agricultural Engineering Department, Faculty of Agriculture, Cairo University. The panel included males and females who varied in age from 18 to 55 years. The sensory evaluation sheets used in this study (Figures 1-5) were those developed by Ghaly et al. [38].

#### 2.3 Nutritional Value

The nutritional contents of the cookies were analyzed. The analyses include the determination of energy, protein, amino acids, carbohydrate, fat, vitamins and mineral contents. These analyses were performed according to the procedures described in Official Methods of Analyses of the Association of the Official Chemists [39].

## 3. RESULTS AND DISCUSSION

## 2.1 Texture

The results of the texture appearance and mouth feel of the cookies are presented in Table 7. The addition of spirulina to the cookies affected both the texture and mouth feel compared to the control (0% spirulina). The cookies that received no spirulina (control samples) had smoother texture and moist-smooth mouth feel whereas those received spirulina had sandy-courses texture and heavy-chewy mouth feel. Increasing the percentage of spirulina made the surface of the cookies more sandy-courses and made the mouth feel more firmer and chewier.

Salehifar et al. [40] reported that the addition of 0.5-1.5% spirulina into traditional Iranan cookies did not alter the texture of the cookies. Lyer et al. [41] found that increasing spirulina content from 2 to 10% did not significantly alter the texture of biscuits. Sharma and Dunkwal [42] reported that the incorporation of 10% spirulina into biscuits did not have any significant effect on the biscuits texture. Lemes et al. [43] noted no statistical differences in the textures of pasta samples containing spirulina at concentrations of 0, 5 and 10%. However, Morsy et al. [44] reported that the addition of 2.5-7.5% spirulina to several extruded products did not significantly alter the texture of products but concentrations above 7.5 % had a significant effect on the texture of these products. Vijayarani et al. [44] noticed slight

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differences in the texture of extruded products when the s	spirulina content was increased from 5% to 15%.
trey. Ghaly et al. [38] reported	
Name:	Date:

The texture is the appearance, finish or consistency of a surface of a substance. It is the characteristic of the physical structure of an object given by the size, shape, arrangement and proportions of its parts. It could also be defined as the way that a food feels in the mouth.

A-Please describe the surface appearance and mouth feel of the samples using the following list

Appearance	Mouth Feel
Coarse	Heavy
Clumpy	Rough
Grating	Dry
Gritty	Firm
Grainy	Chewy
Granular	Sticky
Sandy	Sandy
Smooth	Grainy
Fuzzy	Smooth
Slimy	Moist
Other (Specify)	Other (Specify)

## **RATING**

Sample	Appearance	Mouth Feel
1		
2		
3		
4		

Thank you for your time

Fig. 1. Texture evaluation sheet (adopted from Ghaly et al. [38]).

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Name:	Date:
Shredding/breaking is a method of cutting or b	reaking food into small pieces.

Please describe the easiness with which breaking is made, the appearance of the broken parts and the breaking line of the samples using the following list.

Toughness/Easiness	Appearance of Fragments	Breaking Line
Firm and hard to break	Beaks into granules	Uniform
Soft and easy to break	Breaks into large parts	Smooth
Sticky and hard to separate	Breaks into irregular parts	Irregular
Sticky and separate to clumps	Breaks into sticky parts/clumps	Continuous
Other (Specify)	Other (Specify)	Other (Specify)

# RATING

Sample	Toughness	Fragmentation	Break Line
1			
2			
3			
4			

Thank you for your time

Fig. 2. Shred/break evaluation sheet (adopted from Ghaly et al. [38]).

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3. T			
Name:			

Date:		
Date.		

The color is the property of reflecting light of a particular

wavelength. The distinct colors of the spectrum are red, orange, yellow, green, blue, indigo, and violet. Each of these colors is shading into the next as shown in the diagram.



Please identify the color of the samples according to the above diagram and rate the saturation of the color using scale of 1:10 (dull=1 and vivid=10).

**RATING** 

Color	Saturation
	Color

Thank you for your time

Fig. 3. Color evaluation sheet (adopted from Ghaly et al. [38]).

Name	Date:
1 tallic	Dutc.

Oder/Smell is the property of substance that activates the sensory smell. The intensity is the perceived strength of odor/smell sensation. Hedonic assessment is the process of rating on a scale ranging from extremely unpleasant to extremely pleasant. The characters of the odor are the ability to distinguish the nature of odor/smell.

A-Please rate the samples as to the presence of odor/smell (Intensity) and the odor/smell Hedonic Tone using the following scale

Intensity		Hedonic Tone	Hedonic Tone		
No odor	0	No Smell	0		
Very Faint	1-2	Extremely Pleasant	1-2		
Faint	3-4	Pleasant	3-4		
Weak	5-7	Neutral	5-7		
Strong	8-9	Un Pleasant	8-9		
Very Strong	10	Intolerable	10		

B- Please describe the character of the odor/smell of each sample by giving an appropriate descriptive term using the list below. You may use a term of your choice which you feel properly describes the odor/smell.

Mold Yeast
Musty Ammonia
Fish Animal feed
Stagnant water Sour
Sea water Rotten cabbage
Earthy Other (Please specify)

### **RATING**

Sample	Presence	Hedonic Tone	Odor
	Rating	Rating	Description

1	
2	
3	
4	
	Thank you for your time
Fig. 4. Odor evaluation sheet (adopted from Ghaly et al. [38]).	
Name:	Date:

Taste is the sensation of a flavor perceived in the mouth and throat on contact with a substance. The characters of the taste are the ability to distinguish flavors. The Degree of acceptability is the process of rating the taste on a scale ranging from nasty to delicious

A-Please rate the samples as to the characters and the hedonic tone of flavor.

Characters	Degree of Acceptance	
Sweet	Nasty	1
Vinegar	Bad	2-3
Sour	Unpleasant	4-5
Bitter	Tasteless	6-7
Salty	Pleasant	8-9
Coffee	Delicious	10
Pumpkin		
Others (Specially)		

## **RATING**

Sample	Taste	Hedonic Tone
1		
2		
3		
4		

Thank you for your time

Fig. 5. Taste evaluation sheet (adopted from Ghaly et al. [38]).

Table 7. Effect of spirulina on the texture and mouth feel of cookies.

Spirulina	Texture	Panelists	Mouth Feel	Panelists
(%)	Appearance			
0	Smooth	6	Moist and Smooth	10
	Sandy	2		
	Course	2		
5	Smooth	4	Moist and Smooth	6
	Sandy	2	Heavy and Chewy	4
	Course	4	, ,	
10	Smooth	3	Heavy and Chewy	7
	Sandy	3	Firm & Chewy	3
	Course	4		
15	Smooth	2	Firm & Chewy	8
	Sandy	3	Rough and Chewy	2
	Course	5	<i>G</i>	<del>-</del>

Texture is appearance, finish or consistency of the surface of a cookie

Mouth fell is the feeling of moistness and dryness or chewiness and smoothness of a substance in the mouth.

that the addition of spirulina to chocolate chip oatmeal cookies affected their texture and mouth feel as compared to the original cookies (no spirulina added) but increasing the percentage of spirulina from 3 to 9% did not show any significant differences.

Answer to this question still pending

Line 212 to 223, authors just listed reported studies, where is the link with authors works? excessive citation of published work is not discussion or interpretation of data. Authors should say here what could explain the modification of texture while spirulina is added? what is the biochemical mechanism of that texture modification? Is it lack of wheat flour which explain what is observed? And base on texture which spiking percentage get good score?

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## 3.2 Shred/Fragmentation/Beak Line

The toughness (the degree of easiness with which breaking cookies is made), fragmentation (appearance of the broken parts) and the appearance of the break line were evaluated for the cookies receiving different amounts of spirulina. The results are presented in Table 8. The addition of spirulina affected the easiness with which breaking a cookie was made, the fragmentation and the appearance of the break line. Increasing the spirulina content made the cookies more firm and harder to beak. However, the addition of 5% spirulina did not affect the toughness of the cookies. The panel reported a toughness rating of soft-easy to beak for both 0 and 5% spirulina and firm-easy to beak to firm-hard to break for the cookies that received 10 and 15% spirulina, respectively

Also, higher content of spirulina affected the fragmentation and the appearance of the break line. However, irregular large parts and continuous beak lines were observed with the cookies that received no spirulina while more granules and smaller parts with irregular line were observed with all the cookies that received spirulina. Increasing the percentage of spirulina made the parts firmer and stickier. The results showed that adding spirulina to cookies may help maintain their integrity and reduce breakage during packaging and distributions.

Salehifar et al. [40] reported that the addition of 0.5-1.5% *spirulina* into cookies did not alter their brittleness. Morsy et al. [44] reported that the addition of 2.5-7.5 *spirulina* did not significantly alter the brittleness and the firmness of the extruded products but concentrations above 7.5% significantly altered the firmness and the brittleness of these products. Ghaly et al. [38] reported that the addition of 3-9% spirulina to chocolate chip oatmeal cookies affected the easiness with which the cookies were broken but did not affect their fragmentation nor the appearance of the break line.

## 3.3 Color

The color of the baked cookies is shown in Figure 6 and the color rating results are presented in Table 9. The sensory panel members described the color of the control sample (0% spirulina) as yellow and yellow-orange and the samples that received the 5 and 10% spirulina as green, tallow-green and green-yellow-green while the sample that received 15% spirulina as green, tallow-green, green-yellow-green and Green-blue-green. The color shifted from dark green to bluish green with the increase of spirulina content.

The intensity or saturation of the color was rated in scale of 1:10 with 1 considered dull color and 10 considered vivid color. The intensity rating of the color by the sensory panel varied from 6.88±0.89 for the

Table 8. Effect of spirulina on the toughness, fragmentations and breaking line of cookies.

Spirulina Toughness		ness	Fragm	ent	Breaking Line	
Content (%)	Description	Panelists	Type	Panelists	Description	Panelists
0	Soft and easy	10	Irregular parts	4	Continuous	6
	to break		Large parts	6	Irregular	4
5	Soft and easy to break	10	Granules	5	Continuous	3
			Large parts	5	Irregular	7
10	Firm and easy to break	8	Granules	4	Continuous	3
			Large parts	4	Irregular	7
	Firm and hard to break	2	Sticky parts	2		
15	Firm and easy to break	7	Granules	4	Continuous	3
			Large parts	3	Irregular	7
	Firm and hard to break	3	Sticky parts	3	C	
T 1			1			

Toughness is the easiness with which breaking of a cookie is made Fragmentation is the appearance of the broken parts of a cookie Break line is the appearance of the breaking line of a cookie



Fig. 6. Samples of baked cookies with varying amounts of spirulina.

Table 9. Effect of spirulina on the color of cookies.

Spirulina Content	Saturation	Color	Panelists
(%)	Rating		
0	7.00±0.00	Yellow	2
	$6.88 \pm 0.89$	Yellow-Orange	8
5	$7.00\pm0.00$	Green	1
	$7.66 \pm 0.67$	Yellow-Green	2
	8.37±0.38	Green-Yellow-Green	7
10	7.86±0.87	Green	4
	$7.33 \pm 0.33$	Yellow-Green	3
	$7.83 \pm 0.87$	Green-Yellow-Green	3
15	9.50±0.21	Green	4
	$8.00\pm0.00$	Yellow-Green	1
	$10.00\pm0.0$	Green-Yellow-Green	1
	$9.10\pm0.29$	Green-Blue-Green	4

The saturation of the color is the intensity of the color in a scale of 1 (dull): 10 (vivid).

yellow-orange color to  $10.00\pm0.00$  for the green-yellow-green color. The results obtained from the sensory panel showed that increasing the amount of spirulina in the cookies increased the vividness of their color.

Salehifar et al. [40] reported that the addition of 0.5-1.5% spirulina into traditional Iranian cookies did not alter their color compared to that of the original cookies. Morsy et al. [44] reported that the addition of 2.5% spirulina to several extruded products did not change their color, but the addition of 5-12.5% spirulina to these products significantly altered their color. Lemes et al. [43] noted that the addition of 5% spirulina to pasta did not change its color from that of the original pasta, but the addition of 10% altered the color of the pasta. Vijayarani et al. [45] noticed slight change of the color of extruded products when the spirulina content was increased from 5 to 15%. Sharma and Dunkwal [42] found change in the color of biscuits due to the addition of 10% spirulina. Lyer et al. [41] found that increasing the content of spirulina from 2 to 10% in Indian biscuits decreased the color appearance acceptance and concluded that addition of up to 5% of spirulina may be acceptable. Ghaly et al. [38] reported a change of the color of chocolate chip oatmeal cookies when spirulina was added to the cookies and increasing the spirulina content from 3 to 9% increased the vividness of the color. They stated that the color of the cookies was acceptable as reported by the members of the sensory panel.

## 3.4 Odor

All the baked samples had a noticeable smell. The odor intensity was measured on a scale of 0: 10 (0= no odor and 10= very strong odor) and the results are shown in Table 10. The results indicated that the odor intensity ranged from faint (4.06-4.89) to strong (8.19-8.69) for the all cookies. However, the number of panelists who reported strong odor increased with increasing the spirulina content. The weighted average for the odor intensity was 6.11, 5.53, 6.02 and 6.63 for the cookies receiving 0, 5, 10 and 15% spirulina, all of which are rated weak odors. Increasing the amount of spirulina from 5 to 15% (3 fold) only increased the odor intensity by 19.6 % (from 5.33 to 6.63).

The nature of the smell (Hedonic Tone) was also rated on a scale of 1:10 with a score of 1-2 considered as extremely pleasant odor and a score of 10 considered as intolerable odor. The sensory panel rating for the Hedonic Tone was 4.06, 4.63, 5.78 and 6.33 for the cookies that received 0, 5, 10 and 15 % *spirulina*, respectively. The nature of the smell of the cookies that received 0 and 5% spirulina was pleasant (cookies smell and sweat-yeast smell, respectively) while that of the cookies that revived 10 and 15% spirulina was must-seawater and fishy-seawater, respectively. The weighted average for the Hedonic Tone was 2.97, 3.88, 4.69 and 4.74 for the cookies receiving 0, 5, 10 and 15% spirulina, all of which are within the pleasant odor range. Increasing the amount of spirulina from 5 to 15% (3 fold) increased the Hedonic Tone by 18.14 % (from 3.88 to 4.74). The results showed that adding 5% spirulina did not affect the odor

Table 10. Effect of spirulina on the odor of cookies.

Spirulina Content	Odor Intensity	Panelists	Hedonic Tone	Panellists	Odor Description	
(%)	4.00+0.16 (E.1.)		2.66+0.22 (F. )		0.1:	
0	4.89±0.16 (Faint)	4	2.66±0.32 (Extremely Pleasant)	8	Cookie	Formatted: Highlight
	$6.57\pm0.76$ (Weak)	5	4.06±0.12 (Pleasant)	2		<u> </u>
	8,69±0.00 Strong)	1				
5	4.06±0.24 (Faint)	4	2.43±0.11 (Extremely Pleasant)	6	Sweet-Yeast	
	6.17±0.56 (Weak)	5	4.05±0.22 (Pleasant)	2		
	8,19±0.00 Strong)	1	8.06±0.18 (Unpleasant)	2		
10	4.28±0.22 (Faint)	3	2.18±0.15 (Extremely Pleasant)	4	Musty-Seawater	
	6.32±0.56 (Weak)	5	4.06±0.19 (Pleasant)	3	-	
	8,29±0.32 Strong)	2	8.66±0.14 (Unpleasant)	3		
15	4.38±0.19 (Faint)	2	2.33±0.09 (Extremely Pleasant)	3	Fishy-Seawater	Formatted: Highlight
•	6.47±0.56 (Weak)	5	4.41±0.12 (Pleasant)	3		Tarinastea: Highlight
	8,41±0.32 (Strong)	3	9.06±0.32 (Unpleasant)	4	1 )	

Odor intensity is the perceived strength of odor sensation on a scale of 0 (no odor) :10 (very strong odor) Hedonic Tone is the rating odor on a scale of 1 (no smell): 10 (intolerable smell.)

and the addition of a strong aromatic compound to musk the smell of *spirulina* may be required with higher concentrations (10-15%) of spirulina.

Sharma and Dunkwal [42] found that the addition of 10% spirulina into biscuits did not significantly alter the smell of the biscuit as compared with the biscuits without *spirulina*. Lemes et al. [43] noted no difference in the odor of pasta containing 5- 10 % spirulina. Vijayarani et al. [44] found no significant differences in the odor of extruded products containing 5- 15% spirulina. Ghaly et al. [38] reported no change of the smell of chocolate chip oatmeal cookies when spirulina was added to the cookies at 3% and increasing the spirulina content from 3 to 9% increased the smell of spirulina and stated that a strong aromatic compound may be require to musk the smell of spirulina.

# 3.5 Taste

The sensation of flavor perceived in the mouth and throat on contact with pieces of cookies was evaluated by the sensory panel. The degree of acceptance of the taste was also rated on a scale of 1 (nasty): 10 (delicious). The results are presented in Table 11. The addition and/or increasing the amount

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of spirulina affected both the taste and the degree of acceptance. The taste of the cookies that received no spirulina was rated sweet/delicious with a degree of acceptance between 8.33 and 10.00 (sweet and delicious). The taste of the cookies that received spirulina varied from sweet to sour or sour-fishy or bitter-fishy and the degree of acceptance also varied from 8.06 (Sweet) to 4.16 (unpleasant) for the cookies receiving 5% spirulina to from 8.09 (Sweet) to 2.38 (bad) for the cookies receiving 15% spirulina. The results showed that adding 5% spirulina did not affect the taste but addition of a flavoring agent to the cookies to musk the taste of spirulina may be required with higher concentrations of spirulina (10-15%).

Lyer et al. [41] reported that increasing spirulina content in Parathas bread and biscuits from 2 to 10%) changed the taste but remained acceptable. Sharma and Dunkwal [42] found that the addition of 10% spirulina into biscuits did not result in any significant change in the taste. Lemes et al. [43] noted differences in the taste of pasta containing 5% spirulina compared to the pasta containing no *spirulina*. Morsy et al. [44] reported that the addition of 2.5% spirulina did not significantly alter the taste of the extruded products, but higher concentrations of 5-12.5% resulted in an undesirable taste. Ghaly et al. [38] reported the addition of 3% spirulina to chocolate chip oatmeal cookies did not change their taste but increasing the spirulina content in the cookies from 6 to 9% may require a strong aromatic compound to musk the smell of spirulina.

Table 11. Effect of Spirulina on the taste of the cookies.

Spirulina Content (%)	Taste	Degree of Acceptance	Panellists
	Sweet	8.33±0.35 (Pleasant)	6
	Delicious	10.00±0.00 Delicious	4
5	Sweet	8.06±0.34 (pleasant)	6
	Sour	6.66±0.14 (Tasteless)	2
	Bitter	4.16±0.38 (Unpleasant)	2
10	Sweet	8.11±0.26 (Pleasant)	5
	Sour and Fishy	4±0.34 (Unpleasant)	2
	Bitter and Fishy	2±0.44 (Bad)	3
15	Sweet	8.09±0.18 (Pleasant)	4
	Sour and Fishy	4±0.54 (Unpleasant)	3
	Bitter and Fishy	2±0.38 (Bad)	3

Taste is the sensation of flavor perceived in the mouth and throat on contact with a substance. The degree of acceptance is the rating of taste on a scale of 1 (nasty): 10 (delicious)

#### Provide answer to this

<u>Line 367 to 369 what can explain the taste which decreases when spirulina concentration increases? Are</u> there bitter compounds in spirulina which could justify the lost in sweet taste?

In table 12, where are standard deviation? Authors must perform ANOVA to compare the nutrient content of the different spiked sample. Also in methodology part spiking was carried out at 0,5, 10, 15 and 20%, why in table 12 the level is different?

In table 12, add a column of the composition of spirulina,

When I look line protein in table 12, spirulina at 0% have 5.44% of protein and fortification at 20% (6.91%), something is wrong the protein content should be more than that because spirulina is mostly made of protein.

Same with carbohydrates, why replacing wheat flour which is mainly made of carbohydrates by spirulina which is mainly made of protein, normally, carbohydrates content should decrease.

Sample with 0% spirulina presents 5.44% of protein but no amino acid, is it normal?

When we see which ingredient were used "soft butter (100 g), sugar (100 g), wheat flower (280 g), 2 eggs (109 g), baking powder (10 g), salt (1 g), vanilla (1 g)" Authors are saying that even egg only did not contain any vitamin or mineral? Otherwise why there are no results?

<u>Line 397 to 412, the way of presenting and interpreting results is not good.</u> What is important here is to explain the increase or decrease in nutrient of each sample when the level of spirulina is increased.

Authors must perform principal component analysis of the chemical composition of spirulina, the spiking level and their sensorial parameter assessed; in other to visualize which nutrient are link to a specific sensorial parameter. This will enable author to choose the sample with good sensorial score and nutritional value.

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## 3.6 Nutritional Content

The nutritional values of 30 g cookies containing different amounts of spirulina are shown in Table 12. The addition of spirulina has enhanced the nutritional value of the cookies by increasing the protein and adding vitamins, mineral, omega 3. 6, 7 and 9 fatty acids and amino acids. *Spirulina* is much better source of protein (65%) than milk (4.3%), eggs (13.3%), pulses (24%) and soybean (43.2%). The beta carotene in *spirulina* (1900  $\mu$ g/g) is much higher than that in carrots (18.9  $\mu$ g/g), spinach (55.8  $\mu$ g/g) and mango (27.4  $\mu$ g/g). The iron content in *spirulina* (0.522 mg/g) is also higher than spinach (0.109 mg/g) and soybean (0.115 mg/g). Table 13-17 show the health benefits of amino acids, water soluble and fat-soluble fatty acids, minerals and vitamins found in spirulina.

Because of its high contents of highly valuable bioactive compounds, spirulina has been used to stimulate the immune system by enhancing the production of antibodies and cytokines and, thus, improving the resistance to infections in humans. Spirulina preparations have proved to be effective against HIV, herpes virus, cytomegalovirus and influenza virus as well as preservation of the resident intestinal micro flora (especially lactic acid *bacilli* and *bifid* bacteria) and decreasing of *Candida albicans* level. Many of the phytonutrients in spirulina function as antioxidants and anti-inflammatory nutrients (Table 18) working together in synergistic fashion to provide cardiovascular benefits. Because of this unique combination of antioxidant and anti-inflammatory nutrients, Spirulina has several health benefits including: cardiovascular support and prevention of cardiovascular diseases, heart, kidney and liver disease, obesity, neurodegenerative disease, arthritis, allergies, prevention of breast, cervical, colon and esophageal cancers, cholesterol control and improved regulation of blood sugar [21-25,28-30,34,46,61-63].

## 4. CONCLUION

The incorporation of spirulina into cookies will enrich their nutritional values by increasing the protein content and adding vitamins, minerals, omega 3 and omega 6 oils and amino acids. *Spirulina* is a good source of protein, beta carotene and iron. The protein content in *spirulina* (65%) is much higher than that in milk (4.3%), eggs (13.3%), pulses (24%) and soybean (43.2%). The beta carotene in *spirulina* (1900  $\mu$ g/g) is much higher than that in carrots (18.9  $\mu$ g/g), spinach (55.8  $\mu$ g/g) and mango (27.4  $\mu$ g/g). The iron content in *spirulina* (0.522 mg/g) is also higher than spinach (0.109 mg/g) and soy bean (0.115 mg/g). However, adding spirulina to cookies affected their smell, color, appearance, texture and taste.

The addition of spirulina to the cookies affected both the texture and mouth feel compared to the control (0% spirulina). The cookies that received no spirulina had smoother

texture and moist-smooth mouth feel whereas those received spirulina had sandy-courses texture and heavy-

Table 12. Nutritional facts for cookies with different spirulina contents.

A		Spirulina C	Content (%)	
Amount per Cooke	0	3	6	9
Calories (Cal)	145.000	147.100	149.200	151`.300
Carbohydrate (g)	19.400	19.540	19.680	19.820
Fat (g)	4.990	5.039	5.088	5.137
Omega 3 (mg)		6.550	13.100	19.650
Omega 6 (mg)		45.213	90.426	135.639
Protein (g)	5.440	5.930	6.420	6.910
Alanine (mg)		21.700	43.400	68.100
Arginine (mg)		21.000	42.000	63.000
Aspartic Acid (mg)		29.400	58.800	88.200
Cysteine (mg)		2.100	4.200	6.300
Glutamic Acid (mg)		30.100	60.200	90.300
Glycine (mg)		13.300	26.600	39.900
Histidine (mg)		14.000	28.000	42.000
Isoleucine (mg)		15.400	30.800	46.200
Leucine (mg)		25.000	50.000	57.000
Lysine (mg)		11.200	22.400	33.600
Methionine (mg)		3.600	7.200	10.800
Phenylalanine (mg)		7.000	14.000	21.000
Praline (mg)		10.500	21.000	31.500
Serine (mg)		11.900	23.800	35.700
Threonine (mg)		12.600	25.200	37.800
Tryptophan (mg)		3.500	7.000	10.500
Tyrosine (mg)		8.400	16.800	25.200
Valine (mg)		16.100	32.200	48.300
Vitamin				
A (μg)		20.300	40.600	60.900
B1 (mg)		166.600	333.200	499.800
B2 (mg)		69.300	138.600	207.900
B3 (mg)		2.569	5.138	7.707
B5 (mg)		2.380	4.760	7.140
B6 (mg)		9.240	18.480	27.720
Β9 (μg)		65.800	131.600	197.400
B12 (μg)		4.620	9.240	13.860
C (mg)		41.160	82.320	123.480
E (mg)		3.500	7.000	10.500
H (mg)		0.700	1.400	2.100
K (μg)		17.640	35.280	0.198
Alpha Carotene (µg)		5.250	10.500	15.750
Beta Carotene (mg)		1.330	2.660	3.990
Lutein and Zeaxanthin (µg)		88.200	176.400	264.600
Choline (mg)		46.200	92.400	138.600
Folic Acid (µg)		0.027	0.054	0.081
Pantothenic Acid (µg)		0.004	0.007	0.0108
Minerals				
Calcium (mg)		117.600	235.200	352.800
Magnesium (mg)		1.785	3.570	5355
Iron (mg)		0.364	0.728	1.092
Phosphorous (mg)		6.426	12.852	19.278
Potassium (mg)		12.810	25.620	38.430
Sodium (mg)	157.363	165.049	172.735	180.421
Manganese (μg)		1.400	2.800	4.200
Zinc (µg)		13.300	26.600	39.900
Boron (µg)		27.300	54.600	81.900
Copper (µg)		2.100	4.200	6.300
Molybdenum (μg)		21.000	42.000	63.000
Selenium (µg)		3.500	7.000	10.500

Cookie Weight = 30 g Cookie Size = 5 cm diameter x 0,5 cm height

Table 13. Health benefits of amino acids in spirulina [46-49].

Amino Acid	Health Benefits
Alanine	Is a critical player in the body's <i>protein biosynthesis</i> and has certain regulatory functionality, the liver absorbs alanine from the blood and converts it into pyruvate to enables a faster and more effective supply of energy to the body. I it supports the immune system and prevents kidney stones which are produced by the body as insoluble toxic compounds, stimulates the production of glucagon when blood sugar is too low and supports the generation of glucose from other amino acids, protects the prostate gland from an irregular enlargement which cause severe pain during urination, reacts with glucose which leads to an increased production and excretion of insulin, increases physical fitness when combined with exercise and protects from cardiovascular illnesses.
Arginine	Reduces blood pressure and improves blood flow, reduces risk of heart disease and type 2 diabetes, helps increase insulin levels and decrease blood glucose levels in diabetes, stimulates the immune system to act on and destroy pathogens, reduces anxiety, increases stamina, improves wound healing, maintains memory during aging, increases fertility and fights Aging.
Aspartic acid	Improves muscle strength, increases lean body mass and boosts energy levels, increases natural testosterone production which is needed for building muscles, increases endurance and improves performance, regulates hormone production, boosts growth hormones and positively affects insulin-like growth factors, enhances the level of nitric oxide which helps increase muscular pumps and vascularity and plays a major role in enhancing memory enhancers and preventing depressants.
Cysteine	Neutralizes free radicals that can damage cells and tissues, prevents kidney and liver damage, prevents side effects of drugs and environmental toxins, improves psychiatric disorders and decreases withdrawal symptoms, prevents relapse in cocaine addicts, relieves symptoms of respiratory conditions and reduces inflammation in bronchial tubes and lung tissues, improves cystic fibrosis, asthma, pulmonary fibrosis and nasal and sinus congestion, boosts brain health and slows the loss of cognitive ability in people with Alzheimer's, improves fertility in men and women, stabilizes blood sugar, reduces heart disease and improves immune function.
Glutamic acid	Improves gastrointestinal health such as irritable bowel syndrome, ulcerative colitis, diverticulosis, diverticulitis, leaky gut, joint pain, autoimmune response, Hashimoto's disease, arthritis and skin issues like psoriasis, boosts brain health, improves diarrhea by balancing mucus production, promotes muscle growth and decreases muscle wasting, cleanses the body from high levels of ammonia, burns fat, helps suppress insulin levels and stabilizes blood glucose.
Histidine	Protects heart, reduces blood pressure, reduces oxidative stress, reduces inflammation, decreases insulin resistance, prevents obesity, protects skin from UV radiation, improves brain function, prevents blood clots, suppresses seizures, protects eyes from inflammation and prevents oxidative stress.
Isoleucine	Is antiaging and anti-inflammation and is important for synthesis of hemoglobin and other proteins. It increases muscle mass, prevents essential amino acid deficiency in individuals on protein-restricted diets, treats hot flushes in postmenopausal women, improves vision disturbance, dermatitis and diarrhea, detoxifies nitrogen wastes, heals wound, treats erectile dysfunction, diabetes, hair losses, inflammation, osteoarthritis, rectal diseases, insomnia, weight loss and cancer, improves blood circulation, reduces cholesterol, boosts the immune system and muscle growth and improves fertility.
Lucien	Helps in weight loss, protects against liver and colorectal cancer, manages blood glucose levels and prevents complications of diabetes, keeps blood pressure and cholesterol within target ranges, prevents heart disease and stroke, helps maintain strong bones and teeth, improves mood and memory, improves gut health and helps getting a good night's sleep.
Lysine	Is important for normal growth and muscle turnover. It protects against and treats cold sores by blocking arginine, reduces anxiety by blocking stress response receptors, improves calcium, iron, and zinc absorption and retention, reduces blood pressure, promotes wound healing by helping create collagen, helps produce enzymes, antibodies and hormones, supports the immune

system, treats herpes virus, treats poor concentration, irritability, nausea and red eyes problems, treats hair losses and supports hair growth, treats anorexia, prevents bone loss, promotes healthy growth of skin, prevents plaque buildup in arteries and treats shingles.

Table 13. Continued (Health benefits of amino acids in spirulina).

Amino Acid	Health Benefits
Methionine	Provides an important role relating to the growth of new blood vessels, heals wounds, treats drug withdrawal, schizophrenia, radiation, copper poisoning, asthma, allergies, alcoholism and depression, supports the proper function of the immune system, reduces risk of colorectal cancer, lowers tremors in Parkinson's patients, builds bone strength and helps treat the effects of liver disease.
Phenylalanine	Is direct precursor of tyrosine in the human body and is used as a marker for Parkinson's Disease. It treats depression and pain, treats vitiligo which is a skin condition where the skin loses its colorist, increases natural opioids in the body by inhibiting the enzyme carboxypeptidase which degrades endogenous opioids in the body, alleviates alcohol withdrawal symptoms, enhances the effectiveness of acupuncture anesthesia and improves ADHD symptoms in the short term.
Praline	Is vital for nerve conduction and brain function, improves digestion, helps with weight loss, reduces the risk of certain cancer, provides anti-Inflammatory and anti-aging benefits, prevents skin problems, stimulates hair growth and prevents hair loss, reduces the risk of coronary heart disease and minimizes the risk of stroke, reduces the risk of breast cancer, promotes colon health and facilitates regular bowel movements, improves bone and teeth health, reduces blood pressure, strengthens the immune system, plays an important role in maintaining good skin, aids in the elimination of toxins and waste from the body and prevents the occurrence of wrinkles and pigmentation
Serine	Is a precursor to other amino acids like glycine and cysteine and is important in cell communication within the brain. It assists in production of immunoglobulins and antibodies for a healthy immune system, helps in the absorption of creatine that helps build and maintain the muscles, treats brain diseases such as amyotrophic lateral sclerosis (ALS), chronic fatigue syndrome and Alzheimer disease, plays a role in forming of all four bases of DNA and RNA (adenine, guanine, cytosine, thymine, and uracil, assists in production of antibodies (immunoglobulins), plays a central role in information processing, assists in stimulating the nervous system, produces serotonin which ultimately affects mood, digestion and sleep, increases levels of creatine which promotes muscle mass in the body, reduces symptoms of the brain disease (HSAN1) that causes loss of sensation in the legs and feet, treats seizures, increases blood flow to the brain, improves Huntington's disease, slows the appearance of wrinkles and decreased the presence of pre-existing wrinkles, combats depression and schizophrenia and helps relieve anxiety.
Threonine	Treats various nervous system disorders including spinal spasticity, multiple sclerosis, familial spastic paraparesis and amyotrophic lateral sclerosis, treats different types of depression, makes up elastin, collagen and enamel protein, promotes the proper fat metabolism in the liver, aids the digestive and intestinal tracts to function more smoothly and helps in metabolism in the upper reaches of the small intestine (ileum), produces the mucus gel layer that covers the digestive tract which is a barrier to digestive enzymes that can damage the intestines, produces antibodies to boost the immune system, supports the liver and treats amyotrophic lateral sclerosis (Lou Gherigs Disease),
Tryptophan	Is a precursor to the brain neurotransmitter serotonin (low serotonin production in the brain leads to depression, anxiety, mood disorders, insomnia, poor cognition). It improve sleep quality and lifts mood, reduces depression and anxiety, helps with recovery from addictions, reduces headaches and migraines, maintain a healthy weight, contributes to the therapy of autism, cardiovascular disease, cognitive function, chronic kidney disease, depression, inflammatory bowel disease, multiple sclerosis, sleep, social function and microbial infections, facilitates the diagnosis of certain conditions such as human cataracts, colon neoplasms, renal cell carcinoma and prognosis of diabetic nephropathy, brings on natural calming effects, induces sleep, fights

anxiety, helps burn more body fat, stimulates the release of growth hormones and reduces food cravings for carbohydrates and works in the brain and central nervous system to boost feelings of well-being, connection and safety

Table 13. Continued (Health benefits of amino acids in spirulina).

#### Amino Acid

#### Health Benefits

#### Tyrosine

Is a precursor for three important neurotransmitters (dopamine regulates reward and pleasure centers and memory and motor skills, norepinephrine and adrenaline which are responsible for the fight-or-flight response to stressful situations as they prepare the body to "fight" or "flee" from a perceived attack or harm, thyroid hormones which are produced by the thyroid gland and primarily responsible for regulating metabolism, melanin which is the pigment that gives skin, hair and eyes their color, improves mood and addresses mental health disorders like depression, bipolar or obsessive-compulsive disorder. It improves brain function, mood and stress response, helps with regulating mood swings caused by premenstrual syndrome (PMS) or premenstrual dysphoric disorder (PMDD), increases good cholesterol levels and reduces bad cholesterol levels, maintains optimal health of the digestive system, skin, hair and eyes, treats sleep-related conditions like insomnia, sleep apnea and bruxism (teeth grinding), helps relieve facial pain, assists in quitting smoking, aids with attention deficit-hyperactivity disorder (ADHD) and Tourette's syndrome, aids with brain maturation of the infant and plays key roles in the neurobehavioral regulations of food intake, satiation and sleep-wake rhythm.

## Valine

Is needed for proper mental functioning and is vital for the muscle metabolism and the growth of muscle tissues as it assists in maintaining the proper amount of nitrogen in the body, plays an important role in building muscle and helps to decrease muscle soreness, helps in stress management, boosts immune system and healthy growth, improves mental focus during exercise which is thought to result from the fatigue, prevents muscle wasting by supplying the muscles with extra glucose for energy production during intense physical activity, helps with liver and gallbladder diseases as well as damage to these organs caused by alcoholism and drug abuse, treats or even reverses hepatic encephalopathy or alcohol-related brain damage, helps remove potentially toxic excess nitrogen from the liver by transporting nitrogen to other tissues throughout the body as required, promotes normal growth, repairs tissues, regulates blood sugar and provide the body with energy and helps stimulate the central nervous system.

Table 14. Health benefits of fatty acids in spirulina [25-26,50-52].

Fatty Acid	Health Benefits
Alpha Linolenic	Is an extremely important nutrient for muscles and body growth and is responsible for the production of red blood cells. It improves cognitive function, treats anemia, circulates oxygen, prevents chronic diseases, boosts immune system, treats fatigue and helps reverse insomnia.
Docosahexaenoic	Important for bone formation, maintenance of bone health and prevention of osteoporosis. It prevents diabetes, maintains heart health, prevents migraine headaches, relieves anxiety and helps with premenstrual syndrome.
Linolenic	Builds and maintains strong bones, helps alleviate back pain, keeps bones in their proper shape, helps muscles function properly, protects against cancer and diabetes, prevents arthritis and osteoporosis, helps in maintaining optimal body weight in both males and females, helps the nervous system maintain a proper pressure in arteries, suppresses the growth of polyps which has the potential to lead to cancer, protects against premenstrual depression, prevents kidney stones, controls alkaline pH level, regulates blood pressure and protects teeth by keeping the jaw bone strong and sturdy throughout your life
Gamma Linolenic	Improves brain function, reduces osteoarthritis symptoms, prevents and treats yeast infections, helps metabolize insulin, helps with kidney stones, supports metabolic processes, protects against oxidative stress and prevents vitamin D deficiency.
Dihomo-gamma Linolenic	Enables the body to form red blood cells, helps maintain healthy bones, blood vessels, nerves and immune function, contributes to iron absorption, prevents cardiovascular disease and osteoporosis, lowers cholesterol and high blood pressure, maintains proper level of white blood cells or neutrophils which fight off infection, maintains proper level of bone mineral density and avoids risk of osteoporosis, plays an important role in maintaining collagen, helps prevent or delay arthritis and helps reduce the production of free radicals.
Palmitoleic	Promotes good bone health and is good for thyroid health, regulates blood sugar level, kick-starts metabolism, protects against diseases, relieves inflammation, combats PMS Syndrome., prevents epileptic seizures, boosts vitamin absorption, supports digestion, improvs cognitive function and serves as a co-enzyme to assist metabolic activities in the human body.
Oleic	Is a good antimicrobial agent and trading allergy. It prevents cardiovascular diseases, ischemic stroke and osteoporosis, maintains good bone density and prevents cancer.
Erucic	Prevents cardiovascular diseases and prostate cancer, maintains normal blood pressure, prevents and treats dermatitis and photosensitivity, lung swelling and airway narrowing, allergies, asthma and common cold.

**Table 15.** Health benefits of minerals in spirulina [21,28,53-55].

Mineral	Health Benefits
Boron	Improves brain function, reduces osteoarthritis symptoms, prevents and treats yeast infections, helps metabolize insulin, helps with kidney stones, supports metabolic processes, protects against oxidative stress and prevents vitamin D deficiency.
Calcium	Is beneficial to postmenopausal women with an increased risk of low bone density and is responsible for building the bone structure, strong teeth and fetal development, strengthens the jawbones that keep the teeth in position, ensures teeth are strong and less exposed to damage from bacteria and tartar, promotes calcium bone levels in children and adolescents, promotes the maintenance of bone mass in adults, decreases the risk of bone fracture in elderly adults, slows the rate of bone loss, keeps muscles strong, maintains the circulatory system, manages the digestive process, encourages bone growth., treats sarcoidosis, treats kidney failure and lactose intolerance.
Copper	Helps maintain healthy bones, blood vessels, nerves and immune function, contributes to iron absorption, prevents cardiovascular disease and osteoporosis, lowers cholesterol and high blood pressure, maintains proper level of white blood cells or neutrophils which fight off infection, maintains proper level of bone mineral density and avoids risk of osteoporosis, plays an important role in maintaining collagen, helps prevent or delay arthritis and helps reduce the production of free radicals.
Iron	Is an extremely important nutrient for muscles and body growth and is responsible for the production of red blood cells, treats anemia, circulates oxygen, prevents chronic diseases, improves cognitive function, boosts immune system, treats fatigue and helps reverse insomnia.
Magnesium	Is important for bone formation, maintenance of bone health and prevention of osteoporosis. It prevents diabetes, maintains heart health, prevents migraine headaches, relieves anxiety and helps with premenstrual syndrome.
Manganese	Is good for thyroid health and is crucial for promoting good bone health, regulates blood sugar level, kick-starts metabolism, protects against diseases, relieves inflammation, combats PMS Syndrome., prevents epileptic seizures, boosts vitamin absorption, supports digestion, improvs cognitive function and serves as a co-enzyme to assist metabolic activities in the human body.
Molybdenum	Is a good antimicrobial and trading allergy. It prevents cardiovascular diseases, ischemic stroke and osteoporosis, maintains good bone density and prevents cancer.
Phosphorus	Is important for preventing cardiovascular diseases and prostate cancer, maintains normal blood pressure, prevents and treats dermatitis and photosensitivity, lung swelling and airway narrowing, allergies, asthma and common cold.
Potassium	Is good for improving cognitive function and is a gastro protective. It prevents and treats Cohn's disease, heart disease, prostate and colon cancer, leukemia, respiratory disease, oxidative stress, helps in cancer cell prevention and glucose metabolism, modulates antiaging, lowers cholesterol and treats obesity.
Zinc	Protects against breast and colon cancer, leukemia, neural degradation, heart disease, liver fibrosis, obesity, diabetes, antiaging and skin sun damage

Table 16. Health benefits of water-soluble vitamins in spirulina [21,23,28,53-60].

Vitamin	Health Benefits
Thiamine (B1)	Boosts energy production, safeguards the nerves system, slows the body aging process, stimulates digestion and enhances memory, helps to prevent Alzheimer's disease, improves appetite, boosts body immunity, treats alcoholism and improves mood
Riboflavin (B2)	Helps body growth and overall good health, helps the body break down carbohydrates, proteins and fats to produce energy, allows oxygen to be used by the body.
Niacin (B3)	Reduces risk of heart diseases, improves mental health, treats diabetes, alleviates symptoms of arthritis, lowers levels of triglycerides, improvs skin function and treats impotency, lowers bad cholesterol and regulates digestion.
Pantothenic acid (B5)	Stimulates hormone production, relieves stress, keeps the heart healthy, reduces fatigue and provides stamina to the body, assists in skin and hair care, helps the body generate more hemoglobin, heals wounds and prevents rheumatoid arthritis
Pyridoxine (B6)	Supports healthy skin, detoxifies the liver, enhances the health of blood vessels, improves cognitive function, assists in relieving mood swings, curies anemia, supports eye health, assist in relieving the symptoms of rheumatoid arthritis, prevents diabetes, assist in relieving carpal tunnel syndrome, treats pregnancy related nausea and treats hair loss.
Folate (B9)	Prevents birth defects, premature aging and heart attacks, improvs the working of the human heart by removing homocysteine which is one of the major causes of heart attacks at early ages, controls the cholesterol level in the heart and ensures that the cardiovascular system is saved from various disorders, curs mental disorder, helps the body produce more red blood cells, combats depression, acts as a coenzyme, supports muscle build-up, combats free radicals, prevents cancer and aids fertility.
Cobalamin (B12)	Supports the normal function of nerve cells, assists in red blood cell formation and DNA synthesis, benefits the body by boosting energy, improves memory, helps prevent heart disease, improves heart health by decreasing homocysteine, prevents major birth defects, supports bone health and prevents osteoporosis, reduces risk of macular degeneration, improves mood and symptoms of depression, prevents the loss of neurons and supports healthy hair, skin and nails
Ascorbic acid (C)	Reduces the risk of chronic diseases, helps battle high blood pressure, fights heart disease risk factors, reduces blood uric acid levels, prevents gout attacks, prevents iron deficiencies by improving iron absorption and boosts immunity by helping white blood cells function better
Biotin (H)	Helps maintain <b>p</b> roper metabolic function, controls the level of sugar in the bloodstream, enhances the condition of skin, maintains strong and beautiful nails and healthy hair, treats multiple sclerosis and diabetics, relieves muscle cramps, balances cholesterol levels and assists in obesity, antiaging and ulcer healing
Choline	Prevents breast and colon cancer, leukemia, neural degradation, heart disease, liver fibrosis, obesity, diabetes and skin sun damage.

Table 17. Health benefits of fat-soluble vitamins in spirulina [21,23,28,53-60].

Vitamin	Health Benefits
A	Maintains healthy vision, protects eyes from night blindness and age-related decline, ensures normal function of immune system and organs, aids the proper growth and development of babies in the womb, lowers risk of Hodgkin's lymphoma, cervical, lung and bladder cancers, supports a healthy immune system, reduces the risk of acne, supports bone health and reduces the risk of fractures.
E	Balances cholesterol, ffights free radicals, prevents disease development, repairs damaged skin, protects against skin cancer from ultraviolet rays, treats scars, acne and wrinkles, helps scalp from becoming dry and flakey, makes hair look healthier and fresher, balances hormones, maintains a healthy weight, keeps a regular menstrual cycle and reduces the cramping, anxiety and cravings, reduces menstrual blood loss, decreases the risk of age-related macular degeneration, protects against Alzheimer's disease, lowers cancer risk and improve growth and development of infants and children.
K	Prevents the calcification of the arteries, maintains the integrity of the bone structure, promotes absorption and utilization of calcium, slows down the bone density loss, regulates menstrual cycle and bleeding, reduces inflammation related to cancer, helps stabilize patients suffering from liver cancer, helps brain function fully, improve cognitive function, prevents tooth decay, prevents arthritis and osteoporosis, heals wounds and keeps digestion and cardiovascular systems up and running.
Alpha carotene	Removes destructive free radicals from the body before they cause the tissue damage that can lead to chronic diseases like heart disease and cancer, prevents cancer by stimulating cell-to-cell communication and promotes a strong component of a longevity and healthy mined.
Beta carotene	Assist in maintaining longevity or healthy mined, helps immune systems, protects against free radicals, lowers the risk of developing cancer and heart diseases, prevents the deterioration of cognition and compensates for some of the damage to the lungs caused by oxygen free radicals.
Lutein	Prevents eye diseases including age-related macular degeneration (AMD), cataracts and retinitis, prevents colon cancer, breast cancer, type 2 diabetes and heart disease, reduces inflammation, preserves skin health and filters high-energy wavelengths of visible light which slows down the rate of oxidative stress.
Zeaxanthin	Filters harmful high-energy blue wavelengths of light resulting in the protection and maintaining healthy cells in the eyes, improves the contrast in the eyes and stimulates a faster recovery process from light damage, increases macular pigment density resulting in significant improvements in visual processing speed, supports aging eyes, maintains healthy skin growth, improves cognitive functioning and supports brain health in older adults,

Table 18. Health benefits of spirulina antioxidant/anti-inflammatory compounds [21-25,28-30,34,46,61-63].

Group	Compound	Health Benefits
	Cyanidins	Help in treating and preventing liver disease, various types of cancer, diabetes and arthritis, assists in cholesterol modulation and cartilage protection
Anthocyanins	Pelargonidins	Help in treating and preventing colon and liver cancer, neurodegenerative disorder, parkinsonism, meal induced postprandial inflammation, hepatitis B and gastrointestinal digestion
Flavonols	Procyanidins	Help in treating and preventing cardiovascular disease, coronary heart disease, assists in stabilization of collagen in joints, blood vessels and muscles inflammation, provides nutritional support to reduce capillary permeability and antiplatelet aggregation
	Catechins	Prevents and assists in treating obesity, cardiovascular disease, various types of cancer, myocardial infection, assist in cholesterol modulation,, atherosclerosis and antiplatelet aggregation
	Gallo-catechins	Prevents skin cancer and treats HIV, helps bone metabolism, protect against neurodegenerative disease, UV-B damage, diabetes, melanoma, acts as antimicrobial and antimetastatic
	Epicatechins	Prevents cardiovascular diseases, periodontal diseases, various types of cancer, hepatitis C and blastocyst. It modulates testosterone secretion, improves insulin resistance and glucose tolerance.
	Kaempferol	Prevents cardiovascular diseases, ischemic stroke, cancer and osteoporosis. Helps maintain good bone density, acts as an antimicrobial and assists with allergy.
	Quercetin	Prevents cardiovascular diseases and prostate cancer. It modulates blood pressure, treats dermatitis, lung swelling and airway narrowing, assists and improves allergies and asthma and treats common cold.
	Ellagic acid	Prevents Cahn's disease, heart disease, prostate and colon cancer, leukemia, respiratory disease and oxidative stress. Improves glucose metabolism, antiaging, modulates cholesterol, prevent and treats obesity, is a gastroprotective and assists in ulcer healing
Hydroxy- benzoic acids	Gallic acid	Prevents and treats breast and colon cancer, leukemia, neural degradation, heart disease, liver fibrosis and obesity. Treats diabetes and skin sun damage and modulates aging.
	Vanillic acid	Protects against ulcerative colitis, oxidative brain damage, colorectal cancer and HIV. Assists with immune system regulation, malaria and ant sickling, and is ai effective antimicrobial.
	Salicylic acid	Protects and treats colorectal cancer and blood thinning, reduces pain, hep with skin cleansing, removal of warts and corns, curs acne, calluses and dandruff.
Hydroxy- cinnamic acids	Cinnamic acid	Protects against lung adenocarcinoma and breast cancer, improves diabetes, assist with obesity, gastrointestinal hormone secretion and mycobacterium tuberculosis, improves mood and cognition, assists with allergy and antimalarial activity, is an effective antifungal.
	Coumaric acid	Prevents heart disease, liver disease, stomach cancer and renal toxicity. Improves diabetes, assists with immune system regulation and cholesterol modulation.
	Caffeic acid	Prevents liver cancer, HIV, AIDS, neurodegenerative disease, chlamydia pneumonia infection. Assists with hypertension, male fertility and immune system regulation.
	Ferulic acid	Prevents kidney disease, bone degenerative disease, breast and liver cancer, colon and

		prostate cancer, tongue and lung cancer. Protects skin from photo damage, prevents and treats diabetes, slows aging, assists with cholesterol modulation and menopausal symptoms
Tannins	Ellagitannins	Prevents heart disease, prostate cancer, inhibits cancer growth, reduces gastric inflammation, lowers blood sugar, assists with obesity and modulates aging.
Tannins	Gallo-tannins	Prevents colorectal cancer, eye disease, diabetes, assists with abdominal pain, diarrhea, antimicrobial activities
Stilbenes	Resveratrol	Prevents cardiovascular disease, Alzheimer, inhibits cancer growth, helps with obesity and diabetes, lowers high blood pressure and lowers cholesterol.

chewy mouth feel. Increasing the percentage of spirulina made the surface of the cookies more sandy-courses and made the mouth feel firmer and chewier.

The addition of spirulina affected the easiness with which breaking a cookie was made, the fragmentation and the appearance of the break line. Increasing the spirulina content made the cookies more firm and harder to beak. However, the addition of 5% spirulina did not affect the toughness of the cookies. The panel reported a toughness rating of soft-easy to beak for the cookies that received 0 and 5% spirulina and firm-easy to beak to firm-hard to break for the cookies that received 10 and 15% spirulina, respectively. Also, higher content of spirulina affected the fragmentation and the appearance of the break line. Irregular large parts and continuous lines were observed with the cookies that received no spirulina while more granules and smaller parts with irregular line were observed with all the cookies that received spirulina. Increasing the percentage of *spirulina* made the parts firmer and stickier. The results showed that adding *spirulina* to cookies may help maintain their integrity and reduce breakage during packaging and distributions.

The sensory panel members described the color of the control sample (0% spirulina) as yellow and yellow-orange and the samples that received the 5 and 10% spirulina green as yellow-green and green-yellow-green while the sample that received 15% spirulina as green, yellow-green, green-yellow-green and Green-blue-green. The color shifted from dark green to bluish green with the increase of spirulina content. The intensity rating of the color by the sensory panel varied from 6.88±0.89 for the yellow-orange color to 10.00±0.00 for the green-yellow-green color. Increasing the amount of spirulina increased the vividness of the color

All the baked samples had a noticeable smell. The odor intensity ranged from faint (4.06-4.89) to strong (8.19-8.69). The weighted average for the odor intensity was 6.11, 5.53, 6.02 and 6.63 for the cookies receiving 0, 5, 10 and 15% spirulina, all of which are within the weak odor range. Increasing the amount of spirulina from 5 to 15% (3 fold) only increased the odor intensity by 19.6 % (from 5.33 to 6.63). The sensory panel rating for the Hedonic Tone was 4.06, 4.63, 5.78 and 6.33 for the cookies that received 0, 5, 10 and 15 % spirulina, respectively. The nature of the smell of the cookies that received 0 and 5% spirulina was pleasant while that of the cookies that revived 10 and 15% spirulina was must-

seawater and fishy-seawater, respectively. The weighted average for the Hedonic Tone was 2.97, 3.88, 4.69 and 4.74 for the cookies receiving 0, 5, 10 and 15% spirulina, all of which are within the pleasant odor range. Increasing the amount of spirulina from 5 to 15% (3 fold) increased the Hedonic Tone by 18.14% (from 3.88 to 4.74). The results showed that adding 5% spirulina did not affect the odor and the addition of a strong aromatic compound to the cookies to musk the smell of spirulina may be required with higher concentrations (10-15%) of *spirulina*.

The addition and/or increasing the amount of spirulina affected both the taste and the degree of acceptance. The taste of the cookies that received no spirulina was rated sweet/delicious with a degree of acceptance between 8.33 and 10 while the taste of the cookies that received spirulina varied from sweet-sour to bitter-fishy and the degree of acceptance also varied from 8.06 (Sweet) to 4.16 (unpleasant)a for the cookies receiving 5% spirulina to from 8.09 (Sweet) to 2.38 (bad) for the cookies receiving 15% spirulina. The results showed that adding 5% spirulina did not affect the taste and the addition of a flavoring agent to musk the taste of spirulina may be required with higher concentrations of spirulina (10-15%).

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## **COMPETING INTERESTS**

The authors have declared that no competing interests exist.

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