Preparation of functional drinks using spirulina algae

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Abstract

Spirulina is gaining popularity as a food item due to its multiple health benefits for humans, which has led to increased commercial demand globally. It is high in proteins rich in essential amino acids and contains many important vitamins and minerals such as calcium and iron. Spirulina has been utilized to fortify drinks prepared from apples and bananas by adding at (zero "control samples" 5, 10, and 15%) of the total pulp added to the drink. The results revealed that increasing the content of spirulina enhanced the antioxidant properties and nutritional contribution of apple and banana drinks. The employment of pasteurization at (90°C/15sec) and pH (4) reduced the unpleasant taste as well as odor of spirulina. The application of 10% spirulina to apple and banana drinks produced the highest taste as well as odor score, followed by 5% addition, the control sample, and lastly the adding 15% spirulina. The supplemented drinks by 15% spirulina provided the most acceptable color and appearance but decreased the degree of texture, which tends to the grainy texture with a feel of roughness. The drinks of apple and banana containing 10 % of spirulina recorded the greatest overall acceptability followed by that containing 5 %, then the control sample, while 15% recorded the worst score. As a result, it is advised to use spirulina to fortify drinks since its high health value and antioxidant content. It must use a high degree of pasteurization for a short time as well as minimize pH drink for a suitable point to get rid of undesirable smell and taste, in addition, to use a higher degree of homogenization and thickening agents to avoid getting grainy texture and improve the degree of appearance and acceptance.

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1. Introduction

Food, particularly beverages, has an essential role in keeping one's life quality and promoting health, and prohibition of chronic illnesses. Increasing awareness of the value of high-quality beverages with health advantages, which causes increasing consumption of natural products, delaying the development of significant chronic illnesses stimulated by oxidative stress. Drink producers are being pressed to respond to the growing demand for functional natural drinks due to the therapeutic advantages these beverages provide. The unique combination of raw ingredients with various functional properties will lead to the creation of new products, which will eventually lead to fundamental inventions in the natural beverage sector that will benefit public health and prevent numerous diseases (Butu and Rodino, 2019).

Bananas represent the world's second largest fruit producer after citrus fruits (FAO, 2019), accounting for around 16% of worldwide fruit production. Bananas include vitamin C, which aids in healing and infection prevention, as well as vitamin B6, potassium, and dietary fiber. Pyridoxamine (V.B6) dosages reduced oxidative stress signs and ROS generation while maintaining normal blood glucose levels and assisting in the reduction of diabetes-related issues (Abdullah et al., 2019). Bananas have a high potassium content, substantially higher than other fruits. Potassium has many benefits, such as reducing stroke, preventing heart sickness, and lessening blood pressure. Potassium-wealthy foods may also help to prevent the development of chronic kidney disease and lower the risk of death in people with chronic kidney disease (Clegg et al., 2020). Banana has a high iron content, which promotes the synthesis of hemoglobin in the blood and red cells and is beneficial in instances of anemia. Iron also aids in protein metabolism and the correct functions of the centric nervous system. Because of their smooth texture and softness, bananas are used as a meal to cure digestive disorders. Dietary fiber in banana (resistant starch) has been attached to health advantages such as prevention of colonic diverticulosis, hypocholesterolemic, constipation, as well as digestive processes (Ciudad - Mulero and others, 2019).

Apples have high nutritional content and photochemistry, the fruit is well-known for its numerous health advantages and contains the highest amounts of antioxidant activity of any fruit. Apples stimulate various metabolic responses that promote human growth, development, and overall welfare. Apple is characterized by the least cholesterol-containing fruits as it contains many types of flavonoids, additionally its richness in dietary fiber. Apple consumption reverses oxidative damage to brain cells and lowers the hazard of diabetes. Apple polyphenols and macronutrients have powerful therapeutic benefits such as cholesterol reduction, hypoglycemic activity, chemopreventive impact, cardioprotective effect, and many other effects. Aside from being a low-calorie nourishment, apples aid in the treatment of depression, the prevention of obesity, the prevention of constipation, and the improvement of dental health (Hussain and others, 2021).

Humans have long consumed microalgae, dating back to the 16th century. Microalgae are integrated into many food formulations. Spirulina shows possible use as a constituent in the improvement of recently functional foods which the most important food industry developments (Lafarga et al., 2021). Because of the diversity and quantities of nutrients spirulina provides, NASA employed it as a dietary supplementation for spacemen. Many studies have proven the safety of using spirulina in food, and this has been approved by the administration of drug and food of US as a result of many confirmed toxicological studies (Tarantino, 2003). It is sold as a nutritional supplement or as a functional component for foods and beverages. Spirulina is an ideal food for people of all ages owing to its high nutritional content and suitability for a variety of lifestyles. Spirulina contains 55-70 % protein and a fat content of 5-6 % (on dry weight). Polyunsaturated fatty acids account for 1.5-2 % of the algae's total lipid content. Spirulina contains 36% of pufas on the form linolenic acid, different types of vitamins and minerals as well as pigments such as phycocyanin, carotenoids, chlorophyll, phycoerythrin, and xanthophylls (Henrikson, 2009; Falquet, 2012). This alga is utilized as a staple meal in humans because it contains both large and small components such as protein, linolenic acid, phenolic acids, provitamin A, VB12, VE, and iron without any discernible negative consequences. It is considered safe when cultivated correctly in pure environment and consumed in moderation (Tang and Sutter 2011). Several studies confirmed that spirulina applied as a functional food (Park et al., 2008) since it has more protein (60-70 %) than any plant (Wang and others, 2008). Spirulina is employed as both health food and food coloring (Shimamatsu, 2004). In terms of calcium, protein, carotene, and iron, spirulina outperforms milk, tofu, carrot, and spinach by 180 %, 670 %, 3100 %, and 5100 %, respectively. It contains five times the anti-inflammatory and antioxidant activity when compared to the same weight of fruits and vegetables (Moorehead et al., 2005). Spirulina produces phycocyanin, which gives the organism its blue color (Piñero-Estrada et al., 2001).

Spirulina has garnered widespread attention for its health advantages due to its capability to change immunological and anti-inflammatory effects by decreasing mast cell histamine production (Karkos and others, 2008; Kumar and others, 2009). It also has anticancer, antiviral, anti-allergic properties, boosts immune device (Hirahashi et al., 2002; Cingi et al., 2008), lowers serum lipid levels and pressure of blood (Juarez-Oropeza et al., 2009), anti-obesity and diabetes effects (Kaur et al., 2009), defends versus radiation (Zhang and others, 2001), and depression (Frazer and others, 2005). Spirulina enhances the index of glycemic and patterns of lipid in the second type of diabetes and the reduction of cardiovascular risk factors (Gershwin and Belay, 2011). Spirulina contains antibacterial, anticancer, and anti-poisoning by heavy metal (Fe, Hg, Pb, and Cd) characteristics, besides immunostimulants and antioxidants (Hoseini et al, 2013). It stimulates the immune system by boosting resistance to virus infections, resulting in fewer colds and influenza (Henrikson, 1989), as well as the capacity to change hematopoiesis, enhancing the creation of antibodies and cytokines. It is also efficient against HIV, CMV, influenza, cancer, and herpes (Blinkova et al., 2001; Hernández-Corona et al., 2002). Phycocyanin, a selenium-rich spirulina extract, prevents atherosclerosis (Riss and others, 2007). Spirulina includes vitamin K, which is required for a range of biochemical functions including blood clotting, bone maintenance, atherosclerosis prevention, nerve protection, and inflammatory modulation (Vermeer, 2012; Willems and others, 2014). There is significant clinical data that suggests that increasing vitamin K dose may enhance bone health (Russell and Suter, 2015). Spirulina induces an immunomodulatory impact through improving resistance to infection, influencing hematopoiesis, and raising antibody as well as cytokine generation. Spirulina encourages the growth of healthy lactobacilli into the intestine allowing the formation VB6 which assists in energy emission (Baicus and Baicus, 2007). The main active ingredient in spirulina is phycocyanin, it had antioxidant and antibacterial properties. Phycocyanin is a cyclooxygenase inhibitor that induces apoptosis while also being anticancer and anti-inflammatory (Reddy et al., 2003).

It has been demonstrated that complete spirulina or a phycocyanin-wealthy fraction keep a good functional component of fruit juices (McCarty, 2007). To improve antioxidant status and vascular health, spirulina powder can be combined with other nutrients (McCarty et al., 2010). Spirulina is consumed as dietary supplementation as tablets, capsules, powder, or mixed with various types of healthy foods and beverages (Desai and Sivakami, 2004).

Combining spirulina with fruit drinks is a clever method to produce functional drinks since they include the same fiber, carbohydrates, and vitamins as fruit juice. Since the drink is made to meet nutritional, sensory, security, and technological requirements, the study aims to assess the drinks produced by mixing spirulina with apple and banana in terms of nutritional, sensory, and technological properties to determine the optimal spirulina addition ratios to achieve the highest quality of the functional drink produced.

2. Materials and method

2.1. Materials

2.1.1. Chemicals

All of the chemicals and reagents applied were of the analytical grade.

2.1.2. Spirulina

The dried spirulina was received from the Center of Aquaculture Research at Arab Academy for Science, Technology and Marine Transmit, Arab League.

2.1.3. Apples

Apples (Red Delicious) were purchased at the ripening stage from a market located in Ismailia, Egypt.

2.1.4. Banana

Fruits (Musa paradisiaca L.) obtained from market located in Ismailia, Egypt.

2.1.5. The starting materials

Sugar as well as citric acid that are utilized in the manufacture of spirulina drinks, are bought from local markets in Egypt's Ismailia Governorate.

2.1.6. Processing and preparation

2.1.6.1. Spirulina preparation

Spirulina has been grown, harvested, dried, ground and soft so it is ready to use.

2.1.6.2. Preparation of apple purees

The apples were washed, sliced into thin slices by hand with a knife, immersed in water twice their weight, and cooked at 90°C for 4 minutes. It is cooled after cooking, the pulp is obtained by squeezing it with hot water in (Moulinex blender, type: 741) and then running the liquid is passed through a cloth muslin to separate the coarse fibers, seeds, and stem cells. The resulting puree was utilized in the manufacturing process.

2.1.6.3. Banana puree preparation

Ripe bananas were bought, washed, and peeled by hand. The fruits were sever and dipped in twice their weight of water and boiled for 15 minutes, then cooled and juice extracted by (Moulinex blender, type: 741) after mashing with the boiling water, then filtered during the cloth muslin to obtain a clear banana juice.

2.1.6.4. Apple drink

Every 100 grams of the final drink has 50% ready-made pulp, which is equivalent to 16.5 grams of natural juice or puree devoid of fiber and seed, and this satisfies the Egyptian standards, which says that the drink must have less than 20% fruit pulp. The drink was made with 14 % TSS and a pH of 4.

2.1.6.5. Banana drink

The drink was prepared from 50% of the pre-prepared pulp to get 14% TSS and pH 4.

2.1.6.6. Apple and banana drink blends

Spirulina was added in the following rate: 0, 5, 10, and 15% depending on the fruit puree (juice without seeds and fibers before dilution), which accounts for 16.5 % of drink components. Sugar was used to modify the TSS to 14 %, and a 50 % (w/v) concentrated citric solution was used to adjust the pH to 4. The drinks were pasteurized for 15 s at 90 °C, then poured hot in clean, sterilized bottles with tight seals, cooled to 5 °C, and stored at room temperature (20-25°C) until they are examined.

2.2. Method

2.2.1. Proximate analysis

AOAC (2005) methodologies were applied to define moisture, crude protein, crude fat, total dietary fiber, and ash.

2.2.2. Brix (TSS)

At 20°C, the degree of Brix was determined using an Abbe refractometer (USA) C10.

2.2.3. pH values

A pH meter Jenway 510 (UK) was used to determine the pH, which had previously been calibrated with two buffers at pH 4.0 and pH 7.0.

2.2.4. Titratable acidity:

Titration with 0.1N from NaOH to determine acidity as % citric acid, as given by AOAC (2000).

2.2.5. Ascorbic acid determination:

Ascorbic acid was calibrated against 2,6- Dichlorophenolindophenol to output a pink color and identified as mg/100g drink (Ranganna, 2009).

2.2.6. Sugars evaluation:

Shaffer and Hartman methodology, utilized to reducing and total sugars according to AOAC (2005).

2.2.7. Total pectic materials:

They determined using the (Carre & Hayness) technique, as described by Person (1976).

2.2.8. Total phenols (TPC)

TPC was estimated as mg equivalents from gallic acid for each 100 g sample using Folin-Ciocalteu according to Osorio-Esquivel et al. (2011). Methyl alcohol 80% containing 0.1% HCl was used to extract phenols from a 0.6 g sample at room temperature using a vibrating apparatus for 20 min. The mixing was centrifuged (1000×g /15 min) and the clear liquid was put into a 10 ml tube. With repeated extraction of the residue. For determination, 0.1 ml of methanol extract is taken, 0.9 ml of Folin-ciocalteu (1 Folin:10 water) is added to it, , left at room temperature for 5 minutes, then 0.75 ml of NaHCO3 7% was added, followed by strongly shaking for 30 s, followed by staying at room temperature for 90 min. With using gallic acid for making the standard curve at values ranging from 0 to 1 mg/ml, the absorbance achieved at 725 nm. All values were shown as mean (mg gallic equivalents/100 fresh weight) for 3 replications.

2.2.9. Total flavonoids

The flavonoids content of a methyl alcohol extract was determined using (Zhishen and others, 1999). 0.5 ml of the extract was mixed with 4.5 ml of distilled water, then 0.3 ml of 5% (w/v) of NaNO2 was added after 5 minutes, then 0.6 ml of AlCl3 10% was placed 6 min later, followed by 2 ml NaOH (1M) after 6 minutes, and finally 2.1 ml distilled water was added to the mixture. Absorbance at 350 nm was measured versus water (blank), and flavonoids were reported as mg quercetin equivalents per 100 g of drink.

2.2.10. Antioxidant activity

As stated by Brand-Williams et al. (1995), DPPH (Diphenylpicrylhydrazyl) is used to quantify antioxidant activity. Ten ml of methanol was added to 2.5 g sample, homogenized for 25 seconds at 20,500 rpm, and then centrifuged for 25 minutes at 4 C^o at 20,000 rpm. Methanol was used to dilute the supernatant up to 25 times, 1 ml of dilution is additional for 0.5 ml solution of 0.5 mmol DPPH (methanol-dissolved), and the mixture is stored in the dark for thirty minutes at room temperature. The measurement used 517 nm and was represented as AA%.

2.2.11. Color estimation

The determination was achieved by minolta reader CR-10 (Inc., Osaka, Japan). L* values of drink samples are expressed on transparency, a* values are expressed on a red to green scale, and b* values are displayed on a blue to green scale (yellow to blue). $H^* = \tan^{-1}(b^*/a^*)$ and $C^* = (a^{*2} + b^{*2})^{1/2}$ were used to compute the hue angle (H*) and chroma (C*).

2.2.12. Determination of vitamins:

All vitamins except vitamin C were determined by the HPLC system method according to AOAC (2005). The standard curve for each vitamin was made from five concentrations of the standard substance for each vitamin by HPLC. An Eelipse × BD-C18 column (4.6 × 250 mm 5 μm) with a linear gradient (95:5) methanol: water by flow rate 1 ml/min at 40 °C was applied to the column using a double pump. The curves were gotten at 325 nm using a multi-wavelength detector with a bandwidth of 8 nm.

2.2.13. Minerals analysis:-

Calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), chrome (Cr), selenium (Se), boron (B), and molybdenum (Mo) were determined using an atomic absorption spectroscopy technology as described by AOAC (2005). Phosphorus was determined by measuring the absorbance at 650 nm using the Ranganna (1978) technique.

2.2.14. Phytopigments determination

Chlorophylls, carotenoids, and phycocyanin are among the pigments investigated. Chlorophylls were extracted with dichloromethane for 12 hours at 4°C and evaluated using HPLC (Lee and Choe, 2009). After passing the solution through a hydrophobic membrane filter (20 μL), it was injected into an HPLC equipped with a C18 column (5.0 μm, 4.6×250 mm). The UV detector was used to measure absorption. The flow rate for mixed solution of ethylacetate, methanol, and water (50:37.5:12.5, v/v/v) was at 1.5 mL/min. The chlorophyll standard curve was used for measurement and identification. While AOAC method 970.64 (2005) employed to determine carotenoids by extraction with mixture formed of hexane, acetone, absolute ethanol, and toluene (10:7:6:7, v/v/v/v), and 20 µL was injected into HPLC. The column (10 µm, 3.9×300 mm) and a UV-Vis detector (436 nm) were used. The flow rate of the solvent prepared from hexane and isopropanol (97:3, v/v) was 1 ml/min. Phycocyanin was extracted by buffer phosphate 0.1 M at pH 6.8 and the spectrophotometer absorbance was estimated at the degrees illustrated in the equation according to Beer and Eshel (1985). Phycocyanin (mg/mL) =[(A618−A645)− (A592−A645) ×0.51]×0.15.

2.3. Sensory evaluation:
The sensory assessment was performed using the Awolu et al. (2018) (2018) with some alterations. For each variable of taste, odor, color, appearance, texture, and mouthfeel, ten panelists from the Food Technology Research Institute were requested to show their observations from 10 degrees. Five samples were used in the analysis. Overall acceptability was calculated using the average scores of the examined qualities.

2.4. Statistical analysis:
The SPSS program (Chicago, USA) version 17 was applied to determine the significance of treatment means at the (p 0.05) level.

3. Results and Discussion
The approximate analysis (moisture, protein, fat, ash, carbohydrates, and crude fiber) of apple puree, banana puree, and spirulina powder used in this study was shown in Tables (1). According to the tabulated data in Table (1), the measured moisture value for dry Spirulina was 4.74 %. Spirulina is often recognized as one of the world's richest whole food sources, especially when compared to plant foods. It is commonly used as a dietary supplement. As seen in the table, dried spirulina has a high concentration of proteins, lipids, and carbohydrates, with the value of 62.84 %, 6.93 %, and 10%, Abd El Baky (2015) and others stated the same results. When compared to typical dietary proteins, spirulina proteins comprise all of the necessary amino acids, notably leucine, valine, and isoleucine, but are lacking in methionine, cysteine, and lysine (meat, eggs, or milk). It does, however, surpass all vegetable proteins and so serves as an alternate protein source (Gershwin and Belay, 2008; Henrikson, 2009). Spirulina carbohydrates are a branching polysaccharide polymer that is structurally similar to high molecular weight glycogen and possesses antiviral and immunomodulatory properties Parages et al., (2012).

Table 1: Approximate analysis of apple puree, banana puree, and spirulina powder.

Analysis / Treatments	Moisture	Protein	Fat	Ash	Carbohydrates	Crude fiber
Spirulina	4.74 ± 0.21	62.84 ± 0.22	6.93 ± 0.39	7.47 ± 0.09	10 ± 0.33	10 ± 0.38
Apple Puree	86.11 ± 0.23	0.19 ± 0.17	0.23 ± 0.18	0.63± 0.15	11.15 ± 0.38	1.952 ± 0.14
Banana puree	74.2± 0.33	1.41 ± 0.18	0.43 ± 0.19	0.75 ± 0.14	20.29 ± 0.55	2.22 ± 0.35

Each assessment is the mean of three replicates ± S.E.

In addition to, high ash and crude fiber levels of 7.47 and 10, respectively. It should be noted that the high spirulina concentration in raw fibers results in a granular or coarse texture in large quantities, as well as a look that resembles a foamy or buttery texture after the fibers are impregnated with water during manufacture. Accordingly, the pulp will easily separate from the water in the final drinks, so strong homogenization with the homogenizer with narrow holes and high pressure is required. The moisture level in the prepared fruits puree varied from 74.2 % in banana puree to 86.11 % in apple puree. Protein contents were from 0.19 to 1.41 % in apple and banana puree, respectively. According to the findings, apple puree has a low amount of fat (0.23 %), but banana puree has a significant proportion of fat (0.43 %). In terms of ash content, the results revealed that each of the fruits had less than 1%. Banana puree had a higher ash content than apple puree. The high ash content of the fruits puree stimulates the use of the fruits as the primary component in the creation of drink. Carbohydrates are widely acknowledged as the most important fruit quality parameters influencing palatability, quality and discoloration formation, processability, and viscosity. The banana puree has the most carbohydrates (20.29 %), followed by apple puree (11.15 %). As indicated in Table 1, banana puree included a large quantity of crude fiber compared with apple puree with values 2.22 %, and 1.95, respectively.
Physicochemical properties of fresh apple puree and banana puree were showed in Table 2.

Table (2): The physicochemical parameters of apple and banana purees.

Analysed item	Apple Puree	Banana puree
Total Solids%	13.89%	25.8%
Total Soluble Solids o Brix	12.9±0.2	21.9±0.2
pH values	5.83±0.05	4.82±0.03
Titratable acidity%	0.28±0.02	0.46±0.06
Ascorbic acid mg/100g	44.34±0.55	9.17±0.22
Total Sugars%	10.77±0.43	19.44±0.63
Reducing Sugars%	7.35±0.25	5.58±0.19
Non-Reducing Sugars%	3.42±0. 38	13.86±0.63
Total Pectic substance%	2.33±0.18	1.92±0.15
Total phenols (mg/100g)	345.25+10	24.35±2.24
Color index (O.D. at 420 nm)	0.29±0.08	0.274±0.09
Antioxidant activity%	59.58±0.47	51.13±0.39
Carotenoids (µg/100g)	0.42±0.08	0.52±0.09
Total flavonoids (mg/100g)	175.91±0.72	18.72±0.65

Each assessment is the mean of three replicates ± S.E. (on a wet weight basis)

The higher total solids content elevated the final product quality. It should be noted that banana puree is superior to apple puree because the proportion of solids in bananas (25.8 %) is nearly double that of apples (13.89 %). The results revealed that apple puree had lower acidity (0.28%) than banana puree (0.46%). The nutritional and vital value of a drink grows as the vitamin C of the fruit derived from juice increases. In this aspect, apple puree has a greater vitamin C concentration (44.34 mg/100g) than banana puree (9.17 mg/100g). Banana puree contains more total sugars (19.44 %) than apple puree (10.77 %). Apples, on the other hand, have a higher concentration of reducing sugars than bananas, which encourages browning more. Apple puree has more pectic components (2.33 %) than banana puree (1.92 %), indicating a better possibility for turbidity stability after processing and throughout storage. It is observed that apple puree contains more total phenols than banana puree, which explains why apple fruit enzymatically browns faster than banana fruit. It also explains why enzymatic browning develops in higher amounts and at a faster rate in apples that are constantly concentrated at temperatures below 70°C and, in certain situations, below 65°C. When apples are not available, the stored concentrated apples are used to make drinks. The high quantity of phenolic antioxidants in drinks after preparation and pasteurization inhibits the oxidation process. The color index relates to non-enzymatic browning as evaluated by the absorption of an alcoholic extract of puree at 420nm. It is noticed that the non-enzymatic browning for apple puree is greater than that of banana puree. Perhaps this is due to apples having a larger quantity of reducing sugars than bananas. Finally, it was noticed that apple puree had a higher antioxidant activity (59.58 %) and total flavonoids (175.91 mg/100g) than banana puree in terms of antioxidant activity (51.13 %) and flavonoids (18.72 mg/100g). This will reflect on the entire physicochemical and sensorial properties for both drinks, especially when the percentages of spirulina added to each of them are equal, making apple juice superior in these parameters as can be seen in Tables (5 and 6).

The amount of phytopigments, minerals, and vitamins in spirulina is given in Table 3. Spirulina is a high-quality source of vitamins B, C, D, E, as well as K. Spirulina is a high-quality source of thiamin, often known as vitamin B1, with 4.1 mg/100g. The daily intake of VB1 in the US is 0.2 - 1.4 mg/day as established by the Academy of Sciences and this depending on age group or specific instances. 25 g from spirulina per day will roughly fulfill the body's daily requirements. Thiamine is essential for many crucial activities in the body, including the neurological system, muscular performance, nerve transmission, several enzyme processes, glucose metabolism, and hydrochloric acid generation (necessary for digestion).

Spirulina is one of the greatest sources of vitamin B2 or riboflavin, including 4.17 mg/100g of vitamin B2, which is higher than beef liver, which is regarded as the highest source (3.46 mg/100g). An adult person requires between 1.1 and 1.4 mg/day, with exceptions requiring greater or lower quantities. A lack of vitamin B2 causes a variety of health problems, including skin and mucous membrane inflammation. VB2 is necessary for the body's growth and health, and also for the production of red cells blood. It participates in all metabolic activities involving lipids, amino acids, and carbohydrates as well as generates the energy required by the human body. It is important to enhance the functions of enzymes and contribute to the formation of amino acids and fatty acids, as well glutathione, cellular respiration, and it maintains the eye mucous membranes and skin. Only 20g of these algae provides the body with VB1 (thiamine), VB2 (riboflavin), and VB3 (niacin) (Gershwin and Belay, 2008; Sharma et al., 2011).

Table (3) Phytopigments, minerals, and vitamins in spirulina.

Vitamins		**Minerals mg/100g**		**Phytopigments**	
Vitamin B1 (thiamine) mg/100g	4.1	**Ca**	1115.52	**phycocyanin**	12.55 %
Vitamin B2 riboflavin mg/100g	4.17	**K**	1806.12	**Chlorophyll**	1.64 %
Vitamin B3 (Niacin) mg/100g	14.05	**Mg**	389.80	**Carotenoids**	630 mg/100g
Vitamin B5 (Pantothenic acid) mg/100g	127	**Na**	713.76	**β-caroten**	285 mg/100g
Vitamin B6 (pyridoxine) mg/100g	0.67	**P**	1011.32	**Xanthophylls**	0.304 mg/100g
Vitamin B7 (Biotin) ug/100g	11	**Fe**	115.20	**Zeaxanthin**	153 mg/100g
Vitamin B9 (Folate) ug/100g	152	**Cu**	1.23		
Vitamin B12 (cobalamin) ug/100g	210	**Zn**	5.92		
Vitamin C mg/100g	13.51	**Mn**	2.41		
Vitamin E (alpha-tocopherol) mg/100g	7.34	**Cr**	0.50		
Vitamin K phylloquinone) ug/100g	1167	**Se**	22.02		
inositol mg/100g	80.34	**B**	2.70		
		Mo	0.33		

Vitamin B6 (active pyridoxine) is a pyridoxal phosphate that is essential for amino acid metabolism, muscle glycogen breakdown, neurotransmitter synthesis, histamine synthesis, haemoglobin formation and functionality, and gene expression are all affected. This vitamin's lack and excess both induce peripheral nerve dysfunction, often known as peripheral neuropathy. The recommended daily dose varies depending on the age group and the condition, and it ranges from 1-2 mg per day. This vitamin is present in spirulina by 6.7 g per kg.

VB7 (biotin) is an imidazole derivative produced by intestinal bacteria, and biotin deficiency is uncommon because a large portion of it is reused several times before being excreted in the urine or feces. It is necessary to consume 30 to 60 µg of it per day. Spirulina contains 11 micrograms per 100 grams. Biotin is responsible for metabolic processes as well as the health of the skin, nails, and hair, and a lack of it causes hair loss, skin infections, a lack of appetite, dizziness, and even depression marks.

Spirulina has 152 µg of folate/100 g. Folate, also known as VB9, is essential for the creation of DNA, RNA, as well as the production of red blood cells and the metabolization of amino acids required for cell reproduction. The daily folic acid intake required for adults is 400 µg from meals or supplements. This vitamin is required for the appropriate metabolization of protein and lipids, as well as the maintenance of the gastrointestinal tract, skin, hair, neurological system, muscles, and other tissues in the body. It is required during times of fast development, such as pregnancy, puberty, and childhood. Folate Helps in anemia treatment.

Vitamin B12, commonly known as cobalamin, helps in DNA synthesis as well as fatty acid and amino acid metabolism. It is thought to be essential for the correct functioning of the neurological system and brain, as well as the development of red blood cells in the bone marrow. The daily-recommended dietary intake for vitamin B12 is 2.4 µg. Spirulina is high in vitamin B12, each 100 g containing 210 µg.

Spirulina has 13.51mg/100g of vitamin C, which is a little quantity when compared to the fruit. Vitamin E's antioxidant function is most effective at high oxygen concentrations, thus it is concentrated in red blood cells, respiratory membranes, and the retina. It also preserves the phospholipids of cellular and subcellular membranes by inhibiting unsaturated fatty acid oxidation. As the intake of pufa grows, so does the demand for vitamin E. Adults should take 15 mg vitamin E each day.

The fat absorption depends on vitamin K, which is produced in the human body by bacteria in the intestine. Daily recommendations range from 90 to 120 µg. Blood coagulation, bone building as well as blood vessel creation are all facilitated by vitamin K. Spirulina is amongst the greatest sources of vitamin K, with 1.2 mg /100g.

Inositol is a substance being used to treat a number of ailments, including diabetic nerve aches, neurological problems, cancer, and attention deficit and hyperactivity disorder. Lithium-based medical therapy has several side effects. It is also used to treat diseases related to polycystic ovarian syndromes, such as hypertension, excessive triglycerides, and high testosterone levels. It can be used to treat a wide range of neurological diseases. Per 100 grams of spirulina, there are 1.1 grams of calcium, 1.8 grams of potassium, 1.3 grams of sodium, and 2 grams of phosphorous. Similar results have been documented by (Henrikson, 2009; Falquet, 2012) who found a higher quantity of various micronutrients in Spirulina's particularly (iron, calcium, phosphorus, and potassium), making it an excellent dietary supplement for vegetarians. They also mentioned that Spirulina's mineral concentration varies depending on the source and production circumstances. Furthermore, they discovered that phosphorus, calcium, and magnesium are present in comparable amounts to those found in milk. Finally, they discovered that Spirulina was the richest iron source, with absorption rates exceeding 60% when compared to ferrous sulfate (present in iron supplements). Calcium is an element that the human need in large quantities. Calcium is the largest component of the human body, making up 90% of the components of bones and teeth. It is necessary for the health of the muscular, circulatory, and digestive systems, as well as the creation of bones and the synthesis and function of blood cells. Moreover, it is associated with growth and development properties and aiding in hematopoiesis and clotting, nerve signaling, muscle relaxation, and contraction (Narvaez Andino and Reyes Toval, 2013). The average estimated requirement (EAR) of calcium an expectant woman takes a minimum of 1 grams of calcium. A diurnal dosage (1.3 g) of calcium is regarded extremely acceptable for children aged 9 to 18, and it is lowered in the elderly until it reaches 1 g per day to avoid osteomalacia, while women over 50 require 1,2 g/day (Gonzalez Sanchez et al., 2012). The amount of calcium that an individual obtains through food does not exceed the required amount per day (Aballay, 2012); as a result, poor calcium consumption may result in osteoporosis and a high risk of fractures, particularly in postmenopausal women (Zamudio et al., 2015). Therefore, it should increase the consumption of foods rich in calcium or fortified with nutritional supplements such as spirulina. In addition to the above minerals, magnesium, iron, copper, chromium, selenium, zinc, manganese, boron, and molybdenum are present in lower amounts: 14.80, 315.20, 1.23, 5.92, 6.11, 0.50, 0.02, 2.70, and 0.33 mg/100 g, respectively.

Spirulina contains a high concentration of phytopigments with high antioxidant activity, such as phycocyanin, chlorophyll, carotenoids, β-carotenes, xanthophylls, and zeaxanthin, which explains the high protection power against oxidative free radicals in spirulina-containing products, as well as disease prevention and increased lifespan in consuming countries for this type of algae. Due to the high biological value of the carotenoids and β-carotene present in spirulina, this alga can be used to relieve vitamin A deficiency in some parts of the world Tang and Suter, 2011. Table 4 presents the approximate analysis of apple and banana drinks mixed with spirulina in different proportions. The percentage of moisture in the drinks was close to some, the range was between 85.57 to 85.94% for all samples. As for the apple drinks, the humidity gradually decreased with the increase in the percentage of added spirulina. The moisture percentage in the control sample was 85.94, and the percentage of spirulina decreased the moisture to 85.65% by increasing spirulina addition to 15%. The same decrease was observed in the banana drinks when spirulina powder was gradually increased to reach the lowest moisture content in sample B15 to 85.57%. As the amount of spirulina powder supplementation increases, so does the crude protein of the beverages. The treatments that had 15% Spirulina added to the pulp, whether apples or bananas, had the highest protein content. The protein percentages in apple and banana drink samples containing 15% spirulina were 1.8576 and 2.0595 %, respectively. This could be owing to spirulina's high protein content, which ranges from 50 to 70%, and the results are regarded to be identical (Habib and others, 2008; Henrikson, 2009; Vijayarani et al., 2012; Bahlol et al., 2018).

Table 4: Approximate analysis of apple and banana drinks blended with or without spirulina.

Analysis / Treatments	Moisture	Protein	Fat	Ash	Carbohydrates	Crude fiber
Apple drinks (CA)	85.94^{a}	0.0055^{d}	0.0066^{d}	0.0182^{d}	13.8534^{a}	0.0563^{d}
Apple and Spirulina at 5% (A5)	85.85^{a}	0.6205^{c}	0.0745^{c}	0.0913^{c}	12.9095^{b}	0.1542^{c}
Apple and Spirulina at 10% (A10)	85.75^{a}	1.2378^{b}	0.1426^{b}	0.1647^{b}	11.9523^{c}	0.2526^{b}
Apple and Spirulina at 15% (A15)	85.65^{a}	1.8576^{a}	0.2109^{a}	0.2384^{a}	10.9918^{d}	0.3513^{a}
Banana drinks (CB)	85.89^{a}	0.0695^{d}	0.0212^{d}	0.0370^{d}	13.6529^{a}	0.1094^{d}
Banana and Spirulina at 5% (B5)	85.79^{a}	0.7301^{c}	0.0941^{c}	0.1155^{c}	12.6356^{b}	0.2147^{c}
Banana and Spirulina at 10% (B10)	85.68^{a}	1.3934^{b}	0.1673^{b}	0.1945^{b}	11.6043^{c}	0.3205^{b}
Banana and Spirulina at 15% (B15)	85.57^{a}	2.0595^{a}	0.2407^{a}	0.2737^{a}	10.5693^{d}	0.4267^{a}

The diverse letters within the same pillar for the same variety of fruit indicate considerable differences between the mean of various treatments

The control apple drink had a fat level of 0.0066 %. Spirulina-supplemented samples steadily increase their fat content as the percentage of spirulina rises. A15 recorded the highest fat percentage (0.2109 %). Despite adding the same quantity of spirulina to the comparable samples, the percentage of fat in all banana drink samples was greater than that of apple drink, which might be attributed to the higher percentage of fat in banana fruit than apple fruit. The ash content of the control sample of apple and banana drinks was the lowest, followed by the other mixtures, and this was similar to what Bahlol et al., (2018) found. The highest ash levels were recorded by A15 (0.2384%) and B15 (0.2737%), respectively. The ash level of the drinks rose as the spirulina amount increased. The carbohydrate content of the control sample in apple and banana drinks was 13.8534 and 13.6529 %, respectively, and decreased with increased spirulina powder supplementation. It achieved the lowest rate of carbohydrate in the samples with 15% spirulina in both apples and bananas, and the carbohydrate content in which reached 10.9918 and 10.5693 percent, respectively. Perhaps this is due to spirulina powder's lower carbohydrate content when compared to banana and apple fruit pulp. When the spirulina powder dose was increased in comparison to the control samples, the fiber proportion of both the apple and banana beverages increased somewhat. Its because spirulina has more fiber than apple or banana pulp. When apple drink samples are compared to banana drink samples, it is discovered that the fiber level in the banana drink samples is higher than in the apple drink samples, which is owing to the higher raw fiber content in the banana fruit compared to the apple fruit.

Table 5: Physicochemical parameters of apple and banana drinks blende with or without spirulina

Treatments / Analysis	**(CA)**	**(A5)**	**(A10)**	**(A15)**	**(CB)**	**(C5)**	**(C10)**	**(C15)**
Total soluble solids (Brix)	14.0^{a}	14.0^{a}	14.0^{a}	14.0^{a}	14.0^{a}	14.0^{a}	14.0^{a}	14.0^{a}
pH values	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}
Titratable acidity%	0.32^{b}	0.36^{b}	0.38^{ab}	0.42^{a}	0.49^{b}	0.51^{b}	0.54^{b}	0.59^{a}
Ascorbic acid mg/100g	1.10^{c}	1.48c	2.22^{b}	3.34^{a}	0.36^{c}	0.73^{c}	1.48^{b}	2.60^{a}
Total Sugars%	10.96^{a}	11.04^{a}	11.12^{a}	11.19^{a}	10.72^{a}	10.80^{a}	10.88^{a}	10.96^{a}
Reducing Sugars%	7.46^{a}	7.51^{a}	7.56^{a}	7.61^{a}	3.00^{a}	3.02^{a}	3.05^{a}	3.07^{a}
Non Reducing Sugars%	3.40^{a}	3.42^{a}	3.45^{a}	3.47^{a}	7.61^{a}	7.67^{a}	7.73^{a}	7.78^{a}
Color index(O.D. at 420 nm)	0.412^{a}	0.293^{b}	0.214^{c}	0.180^{c}	0.433^{a}	0.387^{b}	0.315^{c}	0.188^{d}
Antioxidant activity%	23.51^{d}	42.01^{c}	59.81^{b}	73.41^{a}	17.62^{d}	33.46^{c}	56.37^{b}	65.94^{a}
Total phenols (mg/100g)	8.46^{d}	19.34^{c}	38.68^{b}	58.02^{a}	0.95^{d}	14.88^{c}	28.83^{b}	42.77^{a}
Total flavonoids%	4.31^{d}	81.50^{c}	158.69^{b}	235.88^{a}	0.73^{d}	65.45^{c}	130.29^{b}	195.13^{a}
Chlorophyll (mg/100g)	0.07^{d}	0.90^{c}	1.73^{b}	2.56^{a}	0.05^{d}	0.69^{c}	1.34^{b}	1.98^{a}
L*	69.4^{d}	72.5^{c}	74.2^{b}	78.2^{a}	54.6^{d}	58.1^{c}	61.8^{b}	63.6^{a}
a*	0.8^{a}	-0.5^{b}	-1.0^{b}	-1.1^{b}	0.5^{a}	-0.3^{b}	-0.4^{b}	-0.5^{b}
b*	1.1^{a}	-2.3^{b}	-5.5^{c}	-6.5^{d}	1.0^{a}	-2.6^{b}	-3.8^{c}	-9.0^{d}
Hue	53.97^{d}	77.74^{c}	79.70^{b}	80.39^{a}	63.43^{d}	83.42^{c}	83.99^{b}	86.82^{a}
Chroma	1.36^{d}	2.35^{c}	5.59^{b}	6.59^{a}	1.12^{d}	2.62^{c}	3.82^{b}	9.01^{a}

The diverse letters within the same pillar for the same variety of fruit indicate considerable differences between the mean of various treatments

Table 5 shows the physicochemical parameters of apple and banana drinks, mixed with or without spirulina. The drinks were made at a Brix concentration 14% and a pH of 4. In general, the titrated acidity of the banana drinks was greater than that of the apple drinks. The measured acidity rose as the quantity of spirulina added to the drink increased. When we compare the apple drink control sample (0.32 %) to the apple drink samples with spirulina added, we find a gradual increase in acidity as the amount of spirulina added increases. The acidity of the banana drink alone or fortified with increasing amounts of spirulina followed the same pattern as the acidity of the apple drink alone or fortified with spirulina. In terms of vitamin C results, it is worth noting that the vitamin content in the sample raised as the proportion of spirulina added to the drink increased. When a considerable amount of spirulina is put in various drinks, it increases their biofunctional and nutritional quality, as well as their stability. By increasing the concentration of spirulina added to drinks, all indices such as total sugars, reducing sugars, non-reducing sugars, total phenols, antioxidant activity carotenoids, total flavonoids, chlorophyll, L*, hue, and chroma were raised. Antioxidant activity in drinks is increased by increasing the amount of spirulina added. This is attributed to an increase in the compounds responsible for higher antioxidant activity in spirulina, such as phenols, flavonoids, and chlorophyll, which resulted in a decrease in a color index and increased transparency in the drinks. Abd El Baky et al. (2015) discovered that adding Spirulina to various foods improves the biofunctional and nutritive quality of the meal as well as its durability against auto-oxidation. The higher antioxidant activity might also be due to the phycocyanin protein found in spirulina, which is a potent antioxidant with high radical scavenging capabilities since it is a cyclooxygenase inhibitor or due to the higher content of phenols, flavonoids, chlorophylls, and carotenoids (Reddy et al., 2003). The decrease in a* value as the concentration of spirulina added to the drink relates to an increase in the green color, which results in the green-blue color of spirulina, whilst the decrease in b* value refers to an increase in the blue color. It should be mentioned that the hue color values of spirulina-supplemented drinks range from dark green (86.2) to yellow-green (77.74). Chroma reflected the purity of the hue, which improved as the quantity of spirulina was raised. This is responsible for the decrease in browning color as a result of the antioxidant compounds provided by spirulina.

Table 6: Organoleptic scores of apple and banana drinks mixed with or without spirulina.

Treatments / Analysis	(CA)	(A5)	(A10)	(A15)	(CB)	(C5)	(C10)	(C15)
Taste	8.48±0.59^b	8.52±0.52^b	9.07±0.63^a	5.73±0.69^c	7.73±0.70^c	8.39±1.02^b	9.28±0.79^a	6.60±0.44^d
Odor	7.12±0.39^c	8.28±0.59^b	9.38±0.42^a	7.03±0.78^c	6.92±0.46^c	8.24±0.74^b	8.74±0.66^a	5.67±0.89^d
Color	8.26±0.26^d	8.74±0.70^c	9.36±0.34^b	10.0±0.75^a	6.82±0.79^d	7.31±0.51^c	7.89±0.92^b	9.43±0.48^a
Appearance	6.69±0.67^d	7.89±0.43^c	8.18±0.51^b	9.33±0.46^a	7.06±0.17^d	7.67±0.28^c	8.22±0.63^b	8.74±0.75^a
Texture	9.22±0.67^a	7.72±0.62^b	7.33±0.62^c	5.42±0.82^d	8.18±0.65^a	7.36±0.37^b	6.35±0.18^c	5.74±0.53^d
Overall Acceptability	7.95±0.52^c	8.23±0.57^b	8.66±0.50^a	7.51±0.70^d	7.35±0.55^c	7.80±0.58^b	8.10±0.64^a	7.24±0.62^d

The diverse letters within the same pillar for the same variety of fruit indicate considerable differences between the mean of various treatments

Results of sensory assessments of color, taste, odor, appearance, texture, and overall acceptability are presented in Table 6. Sensory evaluation, in general, is the ultimate guide to quality in the eyes of the consumer. Where the results reflect the best results that were recorded by the addition percentage (10 % spirulina), this drink achieved the highest score when compared to other drinks. The sensory test results agreed with those of Fradique et al. (2010), Liu et al. (2012), Bahlol et al., (2018) in different fruit juices enhanced with spirulina. According to the committee members' average ratings of apple drinks and their mixtures with spirulina, A10 drinks taste as well as odor better than CA, A5, and A15 drinks. Because of the increased spirulina powder composition, A15 is less acceptable. In terms of the banana drink alone and the drink blended with spirulina, the taste as well as odor of sample B10 were the best, followed by sample B5, then CB and finally B15. Because of the supremacy of the taste as well as odor of the spirulina on the sample and the poor taste as well as odor of bananas, sample B15 was the least tasty. The average rating for color or appearance of apple drinks and their mixing was highest for A15 and lowest for CA. An increase in spirulina supplementation develops and raises the level of green, making apple drinks more appealing. According to panelists' mean scores, B15 had the most acceptable color and appearance of banana drinks when compared to other samples, whereas CB had the lowest. The texture is character that defines surface properties, mechanical properties, and structure that we feel with our senses.

The texture of food describes how it feels in your mouth, such as how firm, soft, crunchy, or smooth it is. Control sample regarded apple and banana have a superior texture than all samples fortified by spirulina and the texture worsens with the increase in the concentration of spirulina, as the grainy texture increases and the feeling of roughness increases. A10 had the greatest mean sensory score apple drinks for overall acceptability followed by A5 then control sample, whereas the lowest score was A15. The banana drink and the mixed drink with spirulina followed the same pattern of overall acceptability. Treatment B10 had the greatest overall acceptability, whereas treatment B15 had the lowest. The review concluded that the A10 and B10 were the most acceptable because they did not degrade the product's quality while also improving its taste, appearance, and color. Finally, the findings of apple and banana drinks mixed with spirulina show that drinks blended containing 10% spirulina achieved higher in all attributes and were more acceptable to the panelists due to improved nutritional and health value, as well as improved acceptance rates for taste, smell, and color, in addition to general acceptance. This suggests that spirulina, which has higher levels of all physical and chemical properties, improves the nutritional value of apple and banana drinks. According to McCarty (2007), McCarty et al (2010), and Bahlol et al. (2018), spirulina improved fruit juice quality, chemical and nutritional composition, and sensory appeal; the outcomes of this study confirm these conclusions.

4. Conclusions

According to the findings of immediate research, the powder of spirulina includes necessary nutrients. Since it boosts antioxidant and nutritional qualities. For its high nutritious content, it may be utilized to fortify several products. In this study, it was utilized to fortify drinks such as apple and banana. The results revealed that pasteurizing drinks at 90˚C moreover, lowering pH to 4 led to reduction in the unpleasant taste and smell of spirulina. The study revealed that fortifying apple and banana drinks, with up to 10% from the pulp used to manufacture the drink, enhanced nutritional and health value, likewise taste, smell, and color, in addition to overall acceptability. Adding 10% spirulina had greater quality features than the addition of 5% spirulina, and then the CA was compared to additionally 15% spirulina. The last treatment (15%) was achieved the highest color and appearance only but decreased the rest quality parameters such as texture which became more grainy with a roughness. To create a commercial product with high-quality characteristics, three points must be considered during the industry of functional drinks made by mixing spirulina with apple or banana pulp. The first is to use high pasteurization heat for a short time as well as lower the pH to the greatest extent possible to remove the unpleasant taste and smell of spirulina. The second step to obtain soft texture must use a higher degree of harmonization as possible to avoid a grainy and roughness texture. Finally, suitable thickening agent should use to maintain the texture stable and provide a marketable appearance.

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