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Impact of Functional Stirred Low Fat Yoghurt Supplementation With *Spirulina platensis* Powder on Some Quality Characteristics and Therapeutic Effects *In Vivo*

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ABSTRACT

Yoghurt is one of the most consumed healthy and nutritious foods worldwide. Nutrition-related diseases are regularly found in developed or underdeveloped countries, wherein enriched dairy products can dramatically reduce the risk of these diseases. *Spirulina platensis* powder (SPP) is considered a good source of bioactive compounds exhibiting high antioxidant properties. In the present research, physicochemical and nutritional attributes of yoghurt supplemented with 0.5, 1, and 2% of SPP were investigated. The results showed that the addition of SPP decreased coagulation time, syneresis, and pH values. The supplementation with SPP increased yoghurt acidity as lactic acid, total solids content, total protein, fat, and total volatile free fatty acids. Stirred yoghurt treatments containing 0.5% of SPP displayed higher sensory acceptability than other treatments. Furthermore, *in vivo* therapeutic effects of the produced yoghurt were evaluated. The results revealed that SPP-supplemented yoghurt provided a novel technological approach to manufacture a functional food exhibiting prevention effects to cardiovascular diseases and improved liver, kidney, and heart functions.

Keywords: Stirred yoghurt, Spirulina, High-fat diet, Hypercholesterolemic

INTRODUCTION

Food with therapeutic benefits for human health has been identified and consumed in different cultures for over 2500 years (Shi *et al*., 2005). Considering the importance of quality and food safety, more attention was paid to consumers' health (Grunert, 2005). Nutrition scientists have mentioned that food products fortification by using natural resources is one of the best ways to improve food's overall nutrient intake with slight side effects (Nestle, 2013).

Yoghurt is the most popular functional dairy product worldwide (Ozer, 2010). Nowadays, numerous functional foods have been derived from algae (Zhao *et al*., 2018).

Cyanobacteria belonging to prokaryotic algae are more closely related to bacteria than other eukaryotic algae. Spirulina has been recognized as food and dietary supplement (Tomaselli, 1997). *Arthrospria platensis*, also characterized as spirulina, is a familiar microalgal species possessing higher protein content (65%) and nutritional value (Beheshtipour *et al.*, 2012). The Food and Drug Administration of the USA has categorized spirulina as "generally recognized as safe" for human consumption (FDA, 2003). In addition, the United Nations world food conference declared spirulina as "the best for tomorrow", and it gained a wide popularity in the last years as a food supplement (Kapoor and Mehta, 1993). NASA stated that the nutritional value for 1 kg of spirulina equals to that of 1000 kg of vegetables and fruits (Ravi *et al*., 2002). It was previously reported that spirulina has various potential health-promoting beneficial aspects in the treatment and prevention of several diseases, for instance cancers, male infertility, renal failure, and hypertension (Ghaeni and Roomiani, 2016 and Suliburska *et al*., 2016). Cardiovascular diseases are the main reason for diseases globally, causing approximately 17.7 million each year (WHO, 2019). Spirulina has gained a substantial research interest since it can be utilized as a functional food. This term refers to foods that have established to provide particular body functions, yielding health-stimulating properties and/or decrease the risk of diseases further than their nutritional roles (Ambrosi *et al*., 2008).

The present study aimed to evaluate the physicochemical characteristics and organoleptic properties of low-fat yoghurt supplemented with different concentrations (0.5, 1, and 2%) of *Spirulina platensis* powder (SPP). Furthermore, some therapeutic attributes of the obtained yoghurt were *in vivo* investigated.

MATERIALS AND METHODS

Materials

Fresh cow's milk (4.2% fat) was purchased from a private farm (Zagazig, Egypt) and standardized to 1.5% fat by partial cream separation. The average total solids content of the standardized cow milk was 12.98%. The contents of fat, protein, ash, and acidity represented 1.5, 3.53, 0.75, and 0.18%, respectively. SPP powder was acquired from the Department of Botany, Faculty of Science, Zagazig University (Egypt). In addition, ABT-3 starter culture comprising *Lactobacillus acidophilus*, *Streptococcus*

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thermophilus and *Bifidobacterium bifidum* was purchased from Christian Hansen Laboratory Copenhagen, Denmark. Powder crystalline cholesterol was obtained from El-Gomhoria Company for chemicals and medical equipments (Egypt).

Manufacture of stirred yoghurt

The manufacture of stirred yoghurt was carried out according to Tamime and Robinson (1999). Standardized cow milk was divided into four equal portions. The first portion was used to prepare the plain stirred yoghurt (control). The SPP was added to the other three portions at a concentration of 0.5, 1.0, and 2.0%, respectively. Every portion was heated to 90ºC for 15 min in order to dissolve SPP and pasteurize the mixture, and then rapidly cooled to 42ºC. According to the manufacturer's instructions, milk treatments with or without SPP were inoculated with ABT-3 starter culture, and then incubated at 42ºC until coagulation. The different batches were cold-stored overnight, stirred and bottled. Yoghurt treatments were stored in the refrigerator at 6±2ºC, and analyzed when fresh and after 5, 10, and 15 days.

Chemical composition, total phenolic content (TPC) and radical scavenging activity (RSA) of SPP:

The contents of moisture, crude protein, fat, crude fibers and ash in SPP were determined according to the AOAC (2012). The nutrition value of SPP was calculated using the formula of Nile and Khobragade (2009) as follow; [(9 x fat %) + (4 x protein %) + (4 x carbohydrate %)]. Besides, the total phenolic content was determined using Folin-Ciocalteu colorimetric method Škerget *et al*. (2005), and the results were reported as mg gallic acid equivalent (GAE)/100 g dry weight. Furthermore, the radical scavenging activity (RSA) of SPP was determined according to Batool *et al*. (2010) method using following equation;

$$RSA\ (\%) = [(A_0 - A_1)/A_0] \times 100$$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance of the extract.

Physicochemical properties of stirred yoghurt supplemented with SPP

The coagulation time (min) was determined Isanga and Zhang (2009), and the following formula was used to calculate the syneresis;

$$Syneresis\ (\%) = (V_1/V_2) \times 100$$

Where, V_1 refers to the volume of whey collected after drainage, and V_2 refers to the volume of the yoghurt sample.

The contents of total solids, fat, total protein, the titratable acidity and ash in stirred yoghurt treatments were determined according to the AOAC (2012) method. The variations in yoghurt sample's pH value throughout storage were determined by using a laboratory pH meter (HANNA, Instrument, Portugal). Finally, the total volatile fatty acids (TVFAs) in yoghurt samples were determined as described by Kosikowski (1978).

Sensory evaluation of stirred yoghurt supplemented with SPP:

Stirred yoghurt treatments were sensory evaluated by a taste panel of 10 experienced persons of the staff members of Food Science Department, Faculty of Agriculture, Zagazig University, according to Obi *et al*. (2010).

***In vivo* characteristics of stirred yoghurt supplemented with SPP**

Animals and dietary treatments

Thirty western strain adult male healthy albino rats (90–120 g) purchased from the National Research Center (Egypt) were used. The animals were acclimatized for one week as an adaptation period, and then housed as groups (each dietary group consisting of 6 rats) in polypropylene cages under hygienic conditions in an air-conditioned animal house (Faculty of Pharmacy, Zagazig University). All experiments were carried out in accordance with relevant international regulations and guidelines.

Experimental design of diet and animal groups

Standard basic pellet diets were obtained from the central animal house of the National Research Center (Egypt). A standard diet was prepared from fine ingredients (100 g) according to AIN (1993). It mainly consisted of 60% carbohydrates, 22% protein, 12% fiber, 3.5% fat, and 2.40% ash. On the other hand, a high cholesterol diet was prepared according to Zulet *et al*. (1999), using the basil pellet diets described above, which was grounded and supplemented with 1% cholesterol, 15% buffalo fat, and 0.25% bile acid. The rats were divided into groups as shown in Figure 1. The diet was introduced in non-scattering feed cups to minimize food loss, and water was provided to the rats using a glass tube projecting through the cage wire.

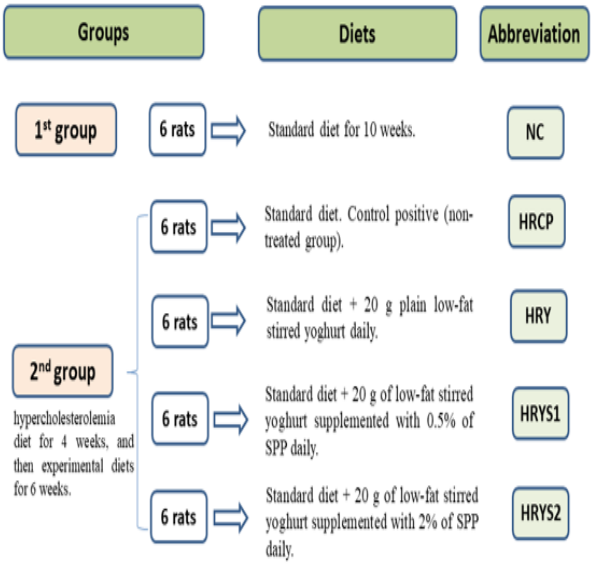


Figure 1. Experimental design of animal groups and diet.

Blood and organs collection

At the end of experiment period, the rats from different groups were fasted for 12 h, anesthetized, and the blood samples were collected from the portal vein into heparinized centrifuge tubes. The samples were centrifuged for 10 min at 3000 rpm to separate the plasma, and kept in plastic vials stored at –20°C until being analyzed. The kidney, liver, and heart were directly collected after animals' scarification, kept in neutral buffered formalin (10%), dehydrated in ascending concentrations of ethanol, cleared in xylol, and after that embedded in paraffin wax. The samples were stained by hematoxylin and eosin after being sectioned at 5 µm in thickness (Banchroft *et al*., 1996).

Histopathological investigation

Formalin-preserved organs were treated in an automated tissue processor. The processing comprised 2 preliminary steps of fixation and dehydration. The fixation

process included the immersion of tissues in buffered formalin (10%) for 48 h, subsequently using distilled water for 30 min in order to remove the fixative. Then, dehydration process was performed by running the tissues over graded alcohol series (70, 90, and 100%). Initially, the tissues were exposed to 70% alcohol for 2 h followed by 90% alcohol for 1.5 h, and then 2 cycles of absolute alcohol, each for 1 h. This process was followed by samples clearing in numerous changes of xylene. The tissues were immersed in a mixture composed of 50% xylene and 50% alcohol for 1 h, followed by pure xylene for 1.5 h. The different samples were saturated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4-5 um) were stained using hematoxylin and eosin (Sigma-Aldrich). The stained organ sections were observed and photographed by using a microscope (BX51; Olympus, Tokyo, Japan) equipped with a digital camera (DP71; Olympus). All images were captured at ×100 and ×400 magnification (Suvarna *et al*., 2013).

Biological evaluation Determination of rats growth parameters

The body weight gain (BWG) and feed efficiency ratio (FER) were determined as reported by Chapman *et al*. (1959) as follow:

BWG = (final weight - initial weight) / initial weight) × 100.

FER = body weight gain (g) / food intake (g).

Hematological analysis

Kidney functions; serum urea nitrogen was measured at 550 nm as described by Fawcett and Soctt (1960). While, the serum creatinine was measured at 510 nm according to Larsen (1972). Liver functions; the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was measured at 340 nm (Young, 2001).

Serum biochemical analysis (serum lipids profile)

Blood samples were allowed to stand in plastic tubes for 15 min at 37 °C, and then all specimens were centrifuged for 15 min at 3500 rpm (Zeng *et al*., 2013). After the centrifugation process, the serum was collected and lipids were analyzed immediately. Serum triglycerides were estimated according to the method reported by Triuder (1969). Furthermore, the total serum cholesterol was determined according to NIHP (1987). The high-density lipoprotein cholesterol (HDL-C) was estimated using the method reported by Grodon and Amer (1977). Low-density lipoprotein cholesterol (LDL-C) and the very-low-density lipoprotein cholesterol (VLDL-C) were determined as reported by Lee and Nieman (1996) as follow;

VLDL-C = Triglycerides/5.

LDL-C = Total cholesterol - (HDL-C + VLDL-C).

In addition, atherogenic index (AI) was determined as a logarithmic transformation of the ratio of triglycerides to HDL-C according to Zhu *et al*. (2018) as follow;

Atherogenic index = Log (TG/HDL-C).

The different parameters of serum lipid were analyzed in bio-diagnostic kits and investigated by using a spectrophotometer (model DU 4700).

Statistical analysis

The obtained data were statistically evaluated by the analysis of variance test using SPSS (Chicago, IL, USA, version 16) software. The results were expressed as mean ± SD and ± LSD. Two-tailed Student's t-test was applied to compare the different experimental groups. The significance level was considered for P values ≤ 0.05.

RESULTS AND DISCUSSION

Chemical composition, nutrition value, total phenolic contents and radical scavenging activity of SPP

The chemical composition of SPP is shown in Table 1. The results show that the total solids, protein/dry matter, fat/dry matter, carbohydrates, crude fibers, and ash contents were 96.14, 58.24, 7.15, 22.18, 3.49, and 3.12%, respectively. In a previous study, Salmeán *et al*. (2015) reported that the contents of protein, fat, carbohydrates, and dietary fibers of SPP accounted for 63, 4.30, 17.80, and 7.70%, respectively. In addition, the nutrition value of SPP represented 370.52 K cal/100 g powder. The obtained results showed that the total phenolic content of SPP was 256 mg GAE/100 g, and the radical scavenging activity represented 84.8%. The variations between the results obtained in this study and those reported in other literature may be attributed to the differences in SPP source and/or the analysis methods.

Table 1. Chemical composition, nutrition value, total phenolic contents, and radical scavenging activity of SPP

Parameter	Unit	Means ± S.D
Nutrition value	K cal/100 g	370.52
Moisture	%	3.84±0.12
Total solids	%	96.14±1.13
Protein/dry matter	%	58.24±1.41
Fat/dry matter	%	7.15±0.23
Carbohydrates	%	22.81±1.00
Crude fibers	%	3.49±0.28
Ash	%	3.12±0.06
Total phenolic contents	mg GAE/100 g	256±5.72
Radical scavenging activity	%	84.80±0.70

Physicochemical properties of stirred yoghurt supplemented with SPP

Table 2 indicated that the control sample scored the longest coagulation time as compared to other treatments containing SPP (147, 139, 132, and 126 min, respectively), which might attributed to that the addition of SPP accelerated the coagulation process caused by the influences of SPP nutritional attributes on the activity of the starter culture strains. These results are in line with Beheshtipour *et al*. (2012), who reported that *S. platensis* promoted lactic acid bacteria growth in milk and dairy products. Furthermore, the control sample had a higher syneresis value as compared to stirred yoghurt treatments supplemented with SPP. The syneresis decreased with the increase in SPP supplementation level, which might attributed to the higher protein and dietary fiber contents of SPP (Barkallah *et al*. 2017). It was also shown that the syneresis increased with the progress in the storage period up to 15 days, owing to the impact of the starter culture bacteria on the curd. There is a direct relationship between syneresis and the total solids content in yoghurt since syneresis is reduced with the increase in total solid contents (Alirezalu *et al*., 2019). It is well recognized that the increase in syneresis throughout the storage period is regularly related to whey expulsion promoted by the severe

rearrangements in casein network (Ramirez-Santiago *et al*., 2010).

Table 2 shows that the acidity of stirred yoghurt treatments was significantly ($P \leq 0.05$) affected by SPP supplementation. In the meantime, the supplementation with 1 and 2% increased yoghurt's acidity. Additionally, the acidity increased with the progress in the storage period up to 15 days. On the other hand, yoghurt treatments supplemented with SPP exhibited lower pH values than the control with significant differences ($P \leq 0.05$) throughout the storage period. (Barkallah *et al.*, 2017) reported that by using different concentrations of spirulina (0.25, 0.5, 0.75, and 1%), no significant variations in the pH of yoghurt samples were detected. Similar results were obtained by Varga *et al.* (1999) who pointed out that the pH values of milk inoculated with a mixed culture of *L. bulgaricus* and *S. thermophilus* in addition to spirulina were lower than the control samples throughout fermentation.

Also, Table 2 shows that the total solids content significantly increased with the increase in SPP supplementation. There were significant differences in the fat contents of stirred yoghurt from different treatments when fresh and throughout the storage period. The content of the total protein significantly ($P \leq 0.05$) increased with the increase of SPP supplementation, and that may be due to its higher content (60-70 g/100 g) of protein (Belay, 2013). The same Table explains the changes in the ash content of stirred yoghurt from different treatments during storage. There were significant ($P \leq 0.05$) differences in the ash content with the increase in SPP supplementation as compared to the control sample. It was also shown that there were significant ($P \leq 0.05$) changes in TVFAs content of stirred yoghurt from different treatments thoughout storage period. Samples supplemented with 2% SPP scored higher TVFAs value, while the control sample was the lowest.

Table 2. Physicochemical properties and chemical composition of low-fat stirred yoghurt supplemented with different concentrations of SPP

Parameter	Storage period (days)	Treatment			
		Control	0.5% SPP-yoghurt	1% SPP-yoghurt	2% SPP-yoghurt
Coagulation time (min)		147±0.65^{a}	139±0.41^{b}	132±0.70^{c}	126±0.36^{d}
Syneresis (g/100 g)	Fresh	18.20±0.47^{a}	17.04±0.12^{b}	16.88±0.43^{b}	14.88±0.22^{c}
	5	20.12±0.64^{a}	18.67±0.32^{b}	17.44±0.71^{c}	16.32±0.51^{d}
	10	22.55±0.36^{a}	21.02±0.56^{b}	19.79±0.26^{c}	18.44±0.33^{d}
	15	24.00±0.37^{a}	22.84±0.26^{b}	21.65±0.21^{c}	20.54±0.21^{d}
Acidity (lactic acid %)	Fresh	1.05±0.30^{c}	1.19±0.03^{b}	1.21±0.04^{b}	1.35±0.30^{a}
	5	1.08±0.12^{c}	1.21±0.10^{bc}	1.29±0.01^{b}	1.38±0.12^{a}
	10	1.09±0.02^{c}	1.29±0.02^{b}	1.30±0.02^{b}	1.39±0.02^{a}
	15	1.10±0.11^{c}	1.31±0.01^{b}	1.35±0.01^{ab}	1.40±0.11^{a}
pH value	Fresh	4.57±0.32^{a}	4.41±0.12^{b}	4.33±0.02^{a}	4.24±0.11^{c}
	5	4.52±0.22^{a}	4.39±0.06^{b}	4.30±0.04^{bc}	4.21±0.05^{c}
	10	4.50±0.23^{a}	4.37±0.02^{b}	4.28±0.01^{c}	4.29±0.01^{c}
	15	4.48±0.04^{a}	4.36±0.05^{b}	4.25±0.09^{c}	4.26±0.05^{c}
Total solids (%)	Fresh	12.24±0.17^{c}	12.45±0.13^{b}	13.12±0.17^{a}	14.12±0.32^{a}
	5	12.71±0.14^{c}	12.53±0.20^{b}	13.40±0.21^{a}	14.25±0.41^{a}
	10	12.94±0.11^{c}	12.60±0.32^{b}	13.68±0.24^{b}	14.57±0.33^{a}
	15	13.98±0.18^{bc}	12.72±0.23^{b}	13.90±0.11^{b}	14.80±0.23^{a}
Fat (%)	Fresh	1.50±0.14^{b}	1.63±0.02^{b}	1.77±0.01^{ab}	1.85±0.02^{a}
	5	1.50±0.31^{b}	1.70±0.04^{b}	1.87±0.01^{a}	1.90±0.01^{a}
	10	1.60±0.36^{b}	1.75±0.01^{a}	1.95±0.06^{a}	2.07±0.04^{a}
	15	1.65±0.12^{b}	1.80±0.05^{a}	2.00±0.03^{a}	2.10±0.01^{a}
Total protein (%)	Fresh	3.62±0.21^{c}	3.81±0.10^{b}	4.04±0.02^{ab}	4.20±0.03^{a}
	5	3.66±0.23^{c}	3.88±0.05^{b}	4.11±0.01^{a}	4.25±0.01^{a}
	10	3.72±0.26^{c}	3.90±0.02^{b}	4.16±0.05^{a}	4.32±0.02^{a}
	15	3.72±0.25^{bc}	3.93±0.01^{b}	4.24±0.11^{a}	4.38±0.05^{a}
Ash (%)	Fresh	0.75±0.54^{c}	0.78±0.03^{bc}	0.80±0.05^{b}	0.82±0.01^{a}
	5	0.77±0.09^{c}	0.81±0.02^{b}	0.84±0.10^{ab}	0.86±0.03^{a}
	10	0.80±0.04^{bc}	0.82±0.01^{b}	0.85±0.02^{b}	0.88±0.01^{a}
	15	0.82±0.01^{c}	0.85±0.01^{b}	0.90±0.04^{ab}	0.91±0.03^{a}
Total volatile fatty acids (0.1 N NaOH/100g)	Fresh	7.67±0.21^{d}	9.88±0.23^{c}	11.15±0.20^{b}	13.20±0.11^{a}
	5	8.76±0.45^{d}	11.29±0.10^{c}	12.30±0.14^{b}	14.35±0.23^{a}
	10	9.96±0.52^{d}	12.00±0.20^{b}	13.10±0.10^{b}	15.50±0.41^{a}
	15	11.03±0.34^{d}	13.10±0.15^{c}	14.25±0.22^{b}	16.44±0.26^{a}

Means with dissimilar superscript letters are significantly different ($P \leq 0.05$).

Sensory evaluation of stirred yoghurt supplemented with SPP

The sensory evaluation scores of yoghurt samples are presented in Table 3. The results revealed significant ($P \leq 0.05$) differences between the control sample and treatments containing 0.5% of SPP. As shown, samples with higher SPP concentrations (1 and 2 %) exhibited lower sensory acceptability scores as compared to the control and the samples containing 0.5% of SPP.

The addition of SPP slightly changed the yoghurt colour to blue-green because of the microalgae used, which directed the panelists to consider it as an undesirable appearance. Furthermore, insoluble SPP particles' graininess was mostly observed in the treatments containing 2% of SPP. It was also shown that the flavour of yoghurt samples decreased by increasing SPP addition. The undesirable flavour resulted from SPP supplementation might attributed to the compounds produced from lipids oxidation in addition to the metallic off-flavors produced by minerals (Shimamatsu, 2004). The study of Barkallah *et al.* (2017) concluded that using 0.25% of SPP did not cause substantial differences in the organleptic properties of yoghurt.

Table 3. Sensory evaluation of low-fat stirred yoghurt supplemented with different concentrations of SPP

Parameter	Storage period (days)	Treatment			
		Control	0.5% SPP-yoghurt	1% SPP-yoghurt	2% SPP-yoghurt
Taste	Fresh	4.39±0.32^{a}	4.41±0.21^{a}	4.24±0.34^{b}	3.98±0.28^{b}
	5	4.24±0.43^{a}	4.33±0.25^{a}	4.13±0.22^{b}	3.85±0.23^{c}
	10	4.13±0.24^{a}	4.27±0.22^{a}	4.03±0.14^{b}	3.83±0.43^{c}
	15	4.01±0.54^{a}	4.15±0.34^{a}	4.00±0.21^{b}	3.65±0.54^{c}
Flavour	Fresh	4.24±0.50^{a}	4.14±0.43^{b}	3.84±0.57^{b}	3.38±0.23^{c}
	5	4.33±0.20^{a}	4.04±0.23^{b}	3.81±0.34^{c}	3.56±0.52^{c}
	10	4.41±0.32^{a}	4.08±0.38^{b}	3.75±0.31^{c}	3.32±0.20^{d}
	15	4.50±0.43^{a}	4.07±0.32^{b}	3.60±0.26^{c}	3.27±0.28^{d}
Colour	Fresh	4.21±0.21^{a}	4.23±0.32^{a}	4.15±0.54^{b}	3.17±0.23^{c}
	5	4.28±0.32^{b}	4.32±0.23^{a}	4.11±0.36^{c}	3.69±0.27^{d}
	10	4.32±0.38^{b}	4.45±0.52^{a}	4.05±0.37^{c}	3.65±0.21^{d}
	15	4.26±0.43^{b}	4.31±0.21^{a}	4.01±0.46^{c}	3.09±0.28^{d}
Consistency	Fresh	4.34±0.21^{a}	4.31±0.28^{a}	4.17±0.32^{b}	3.84±0.43^{c}
	5	4.29±0.65^{a}	4.19±0.34^{b}	4.01±0.27^{c}	3.78±0.32^{d}
	10	4.26±0.34^{a}	4.24±0.35^{a}	4.15±0.18^{b}	3.66±0.36^{c}
	15	4.19±0.53^{a}	4.14±0.24^{a}	4.03±0.15^{b}	3.51±0.17^{c}

Means with dissimilar superscript letters are significantly different ($P \leq 0.05$).

Growth parameters of rats fed on low-fat stirred yoghurt supplemented with SPP

The body weight gain of rats fed on plain stirred yoghurt and low-fat stirred yoghurt supplemented with SPP is shown in Table 4. SPP-supplemented yoghurt showed a tendency to increase the body weight of rats. It was shown that the body weights of hypercholesterolemic rats group increased through the feeding period. The data revealed that the final body weights of hypercholesterolemic rats were significantly higher than other rats fed on the standard diet and standard diet plus (plain stirred yoghurt and/or stirred yoghurt supplemented with SPP), which might be due to that the dietary fat is associated with an increased risk of obesity (Steijns, 2008). These results are in line with the findings of Ngongang *et al*. (2016), who reported that rats fed only on a hypercholesterolemic diet recorded higher body weights.

The positive control group (non-treated group) rats fed on a high-fat diet scored the highest score in food intake **(**g/day), and there were no significant differences in food intake **(**g/day) between the other groups. Also, the HRY group (rats fed on standard diet and plain stirred yoghurt) gained higher FER scores followed by HRYS2 group (rats fed on standard diet and 20 g of stirred yoghurt containing 2.00 % SPP), with non-significant differences in FER among other groups. Different results were obtained by Zommara *et al*. (2013), who pointed out that feeding rats on Zabady drink containing different forms of organic and inorganic selenium enhanced the antioxidant properities without affecting the growth parameters and lipids metabolism of rats.

Serum lipids profile of rats fed on low-fat stirred yoghurt supplemented with SPP

It was reported that spirulina is useful for improving blood lipids profile (Torres *et al*., 1998). The existence of high cholesterol levels in the blood is named hypercholesterolemia. The utilization of spirulina in the treatment of several diseases, including cholesterol degradation was reported. Iwata *et al*. (1990) revealed that the increase of triglycerides level in liver and serum is inhibited with lipase activity. Ramamoorthy and Premakumari (1996) illustrated that HDL-C is good cholesterol. The blood serum lipids profile of rats fed on diverse experimental diets is presented in Table 4. Hyperlipidemia induces abnormal higher blood and liver index concentrations, including total cholesterol, triglycerides, LDL-C, and low levels of HDL-C. Consumption of 1% of cholesterol in the diet resulted in a substantial increase in the total cholesterol (246.3 mg/dL) of blood. Elevated cholesterol level is 240 mg/dL or above is considered to be a hypercholesterolemia. It indicates that HDL decreased and LDL increased (Kim and Kim, 2005). In the positive control group, hypercholesterolemia was effectively persuaded as compared to the other groups. However, a reduction in the total cholesterol was observed in hypercholesterolemia rats fed on yoghurt supplemented with SPP; the reduction was higher in yoghurt supplemented with SPP than plain yoghurt. LDL-C is sometimes called bad cholesterol. Its particles directly influence atherogenic process (Zeng *et al*., 2013). These results are in harmony with Vasiljevic and Shah (2007), who revealed that regular consumption of yoghurt containing live cultures and probiotic strains caused an obvious decline in the serum cholesterol levels. Similar results were also reported by Park and Lee (2016), who stated that the addition of spirulina in the diet had shown a significant reduction in the concentrations of both total cholesterol and LDL-C in the plasma. With regarde to the influence of low-fat stirred yoghurt supplemented with SPP on the AI of rats, it was shown that the control positive group fed on a high-fat diet scored the highest AI score, while it was decreased by feeding the rats on the different experimental diets (Table 4).

Kidney functions of rats fed on low-fat stirred yoghurt supplemented with SPP

The kidney functions of rats fed on experimental diets is tabulated in Table 4. Feeding hypercholesterolemia rats on stirred yoghurt supplemented with SPP prevented the rise of mean serum creatinine and urea concentrations. The prevention rate increased with the increase in SPP addition. The control positive group scored 0.98 and 93.67 mg/dL for creatinine and urea, respectively. These parameters had decreased to 0.73 and 71.67 mg/dL for creatinine and urea, respectively by feeding the hypercholesterolemia rats on stirred yoghurt containing 2% of SPP.

Liver functions of rats fed on low-fat stirred yoghurt supplemented with SPP

The liver functions of rats fed on experimental diets are shown in Table 4. The feeding of hypercholesterolemia rats on stirred yoghurt supplemented with SPP prohibited the rise of serum ALT and AST activities. The reduction in liver enzymatic activities for ALT was recorded at 84.00 u/L in the control positive group to 53.00 u/L in hypercholesterolemia rats fed on stirred yoghurt containing 2% of SPP. In the case of AST activities, the liver enzymatic activities was 74.00 u/L in control positive group, while the hypercholesterolemia rats fed on stirred yoghurt containing 2% of SPP recorded 50.00 u/L.

Histopathological examination of rat organs

Several animal studies have investigated the potential protective effect of spirulina against toxicity-related various cytotoxic agents, such as hepatotoxicity, nephrotoxicity, and cardiotoxicity (Khan *et al*., 2005). Many publications have reviewed the health aspects of spirulina during the last years. Experimental approaches by using spirulina preparations in potential health influences including antibacterial, antioxidant, immunomodulation, anticancer, antiviral, and positive belongings to obesity, malnutrition, hyperlipidemia, anemias, inflammatory allergic reactions, and diabetes were described Falquet and Hurni (1997). The histological variations in the liver, kidney and heart of each group are shown in Table 5.

Table 4. Effect of feeding hypercholesterolemia rats on low-fat stirred yoghurt supplemented with SPP

Parameter	Animal groups					LSD (0.05)
	NC	HRCP	HRY	HRYS1	HRYS2	
Body weight gain %	120.50±16.29 ^{a}	172.45±33.61 ^{a}	145.08±26.19 ^{a}	153.11±36.85 ^{a}	158.51±14.99 ^{a}	49.25
Food intake (g/day)	16.18±0.74 ^{b}	22.00±2.00^{a}	16.08±1.36 ^{b}	15.47±1.41 ^{b}	17.13±1.22 ^{b}	2.55
Food efficiency ratio (g)	0.139±0.139 ^{a}	0.152±0.004 ^{a}	0.193±0.025 ^{a}	0.164±0.085 ^{a}	0.191±0.017 ^{a}	0.074
Serum lipids profile						
Triglycerides	118.67±7.37 ^{d}	158.00±6.00 ^{a}	137.33±1.53 ^{b}	129.00±1.00^{c}	124.00±1.00^{cd}	7.92
Total cholesterol (mg/dL)	141.00±3.61 ^{d}	246.33±5.51 ^{a}	185±5.00 ^{b}	169.67±5.03 ^{c}	157.33±2.08 ^{d}	8.05
HDL-C (mg/dL)	51.00±1.00 ^{a}	37.00±3.46 ^{d}	42.67±1.15 ^{c}	50.67±1.15 ^{b}	51.33±0.58 ^{a}	3.25
LDL-C (mg/dL)	66.27±3.87 ^{e}	178.40±6.26 ^{a}	114.87±5.28 ^{b}	93.20±5.80 ^{c}	81.20±2.20 ^{d}	8.93
VLDL-C (mg/dL)	23.73±1.47 ^{d}	31.60±1.20 ^{a}	27.47±0.31 ^{b}	25.80±0.2 ^{c}	24.80±0.20 ^{cd}	1.58
Atherogenic index (mg/dL)	0.007 ^{d}	0.270 ^{a}	0.148 ^{b}	0.046 ^{c}	0.023 ^{c}	0.06
Kidney functions						
Creatinine (mg/dL)	0.68±0.01 ^{e}	0.98±0.01 ^{a}	0.86±0.01 ^{b}	0.80± 0.02 ^{c}	0.73±0.02 ^{d}	0.03
Urea (mg/dL)	68.00±2.65 ^{d}	93.67±4.73 ^{a}	82.33±3.06 ^{b}	76.33±1.53 ^{c}	71.67±1.53 ^{cd}	5.36
Liver functions						
ALT (u/L)	46.67±5.51 ^{e}	84.00±2.00 ^{a}	72.33±1.53 ^{b}	63.00±4.36 ^{c}	53.00±2.08 ^{d}	6.30
AST (u/L)	45.00±2.00 ^{d}	74.00±8.00 ^{a}	62.33±1.53 ^{b}	55.67±2.08 ^{bc}	50.00±2.65 ^{cd}	7.35

Groups abbreviation, see Figure 1.

Means with dissimilar superscript letters are significantly different ($P \leq 0.05$).

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 5: Lesion score of the many histopathological lesions among different groups

Organ	Lesions	Animal groups				
		NC	HRCP	HRY	HRYS1	HRYS2
Liver	Fatty change	-	+++	++	+	+
	Hydropic degeneration	-	+++	++	+	++
	Dilated sinusoids	-	++	++	-	+
	Inflammatory cells	-	++	++	+	+
Kidney	Fatty change	-	+++	++	+	+
	Hydropic degeneration	-	+++	++	++	++
	Renal casts	-	++	++	+	++
	Hemorrhage	-	+	++	+	+
	Inflammatory cells	-	++	++	+	++
Heart	Fatty change	-	+++	++	+	+
	Hyaline degeneration	-	+	++	+	++
	Congestion of intermuscular B.vs	-	++	++	+	+

Groups abbreviation, see Figure 1.

- : No alterations; +: Mild (25-35% alterations); ++: Moderate (40-65% alterations); +++: Severe (up to 65% alterations).

Histopathological examination of rats liver

Spirulina has hepatoprotective properties by decreasing the liver lipids profile. The histopathological analysis indicated that spirulina mixed active substance supplementation decreased liver lesions and improved hepatocyte abnormality (Chen *et al*., 2019). This lipid-lowering property was attributed to the C-phycocyanin molecule in spirulina (Nagaoka *et al*., 2005). In addition, Gorban *et al*. (2000) pointed out that the administration of spirulina prohibited the transformation of chronic hepatitis into the hepatic cirrhosis. The histological variations in the liver and liver biopsy of each group are shown in Table 5 and Figure 2.

The first slide corresponds to a photomicrograph (1×100, 2×400) of the negative control group fed on a standard diet for 10 weeks. The liver shows hepatic cords (arrow head) and normal central veins (arrow). The second slide is a photomicrograph (3×100, 4×400) of the control positive hypercholesterolemia rats fed on a standard diet for 6 weeks.

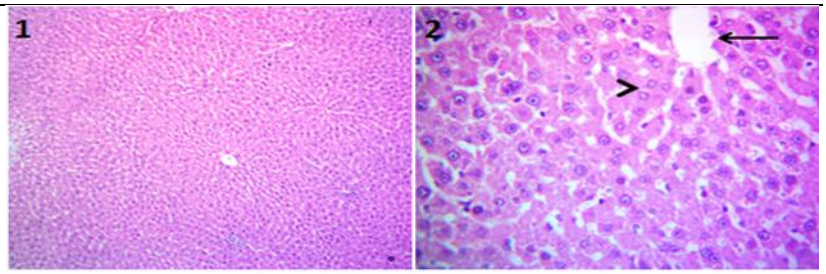
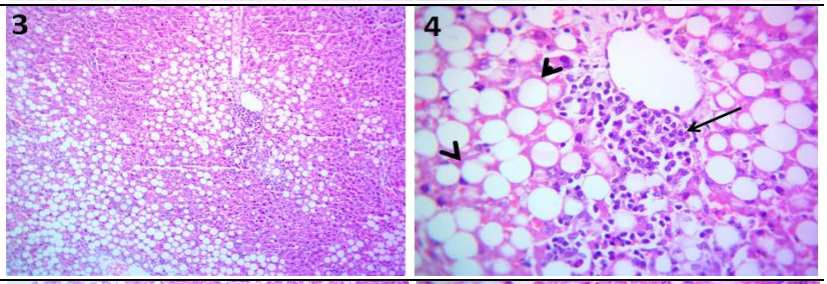
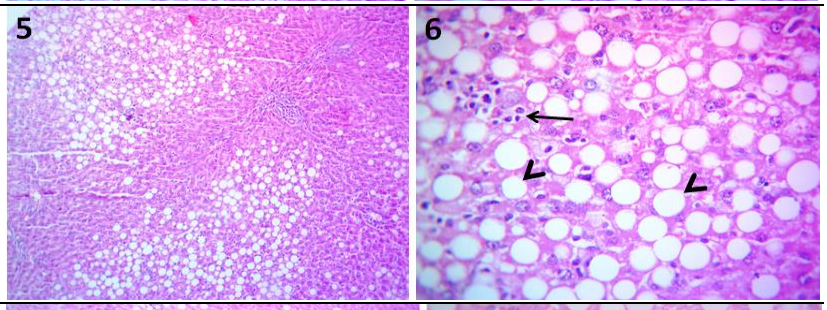
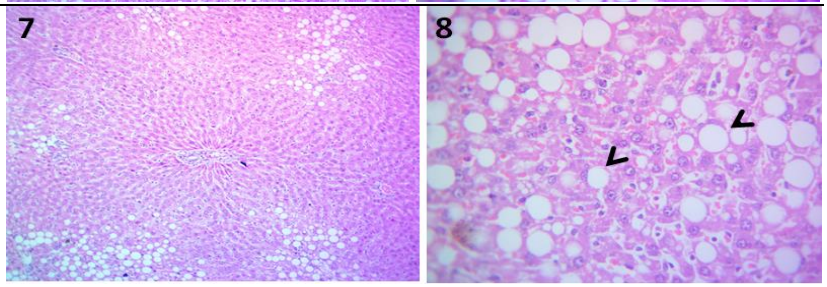
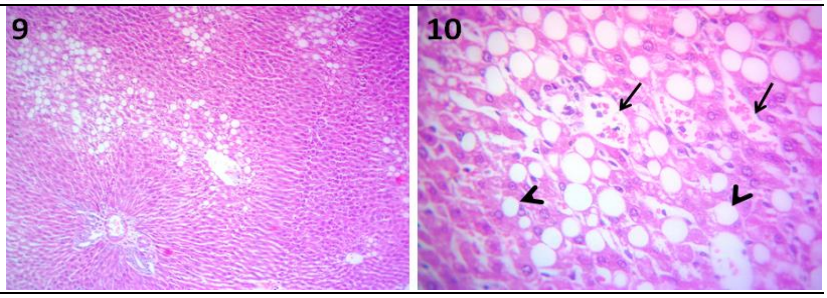
Animal groups	Liver	
	H & E ×100	H & E ×400
NC		
HRCP		
HRY		
HRYS1		
HRYS2		

Figure 2. Liver histopathological examination of rats fed on different experimental diets. Groups abbreviation is shown in Figure 1; H & E, sections stained with hematoxylin and eosin; x, magnification power

The liver sections showed widely distributed fatty change which represented by replacement of hepatocytes by large clear and sharp vacuole with peripherally located nuclei. Round cells infiltration around the central vein and degenerative variations in few hepatocytes were observed. The third slide represents a photomicrograph (5×100, 6×400) of hypercholesterolemia rats fed on standard diet and plain low fat stirred yoghurt for 6 weeks. The liver displays round cells aggregations within hepatic sinusoids (arrow) and fat change within a large number of hepatic parenchyma (arrow heads). The fourth slide is a photomicrograph (7×100, 8×400) of hypercholesterolemia rats fed on the standard diet and stirred yoghurt supplemented with 0.5% of SPP for 6 weeks. The liver shows fatty change within few numbers of hepatic parenchyma (arrow heads). The fifth slide is the photomicrograph (9×100, 10×400) of hypercholesterolemia rats fed on standard diet and stirred yoghurt containing 2% of SPP for 6 weeks. The liver displays dilated sinusoids

(arrows) and fatty change within a few number of hepatocytes parenchyma (arrow heads).

Histopathological examination of rats kidney

The histological changes in the kidney and kidney biopsy of each group are shown in Table 5 and Figure 3. The first slide is a photomicrograph (11×100, 12×400) of the negative control group fed on a standard diet for 10 weeks. The kidney displays glomerular structures and renal tubular epithelium (arrow head). The second slide corresponds to a photomicrograph (13×100, 14×400) hypercholesterolemia rats fed on the standard diet for 6 weeks. The kidney shows tunica media of renal blood vessels (arrow) and fatty changes within renal tubular epithelium (arrow heads), degenerative variations in a moderate number of renal tubules (curved arrow), dilated tubular lumen (black star) and perivascular edema (red star).

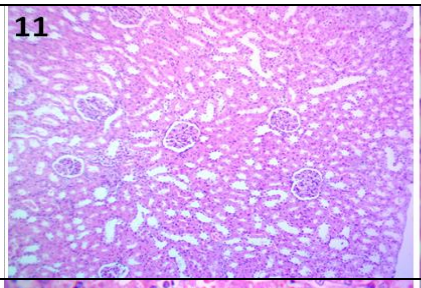
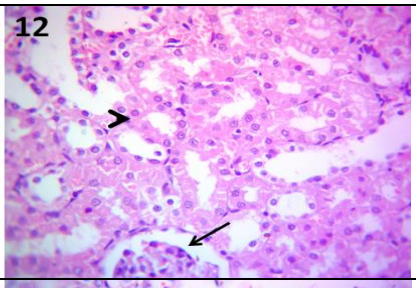
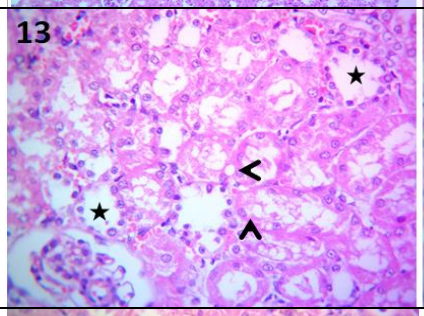
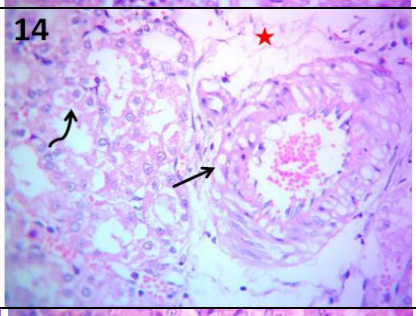
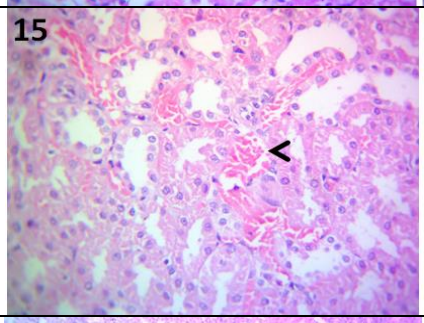
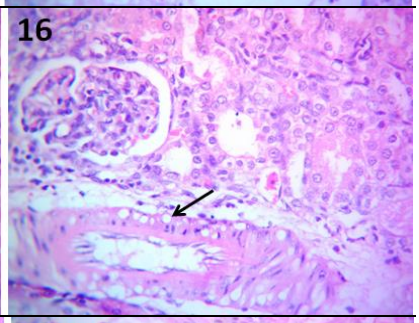
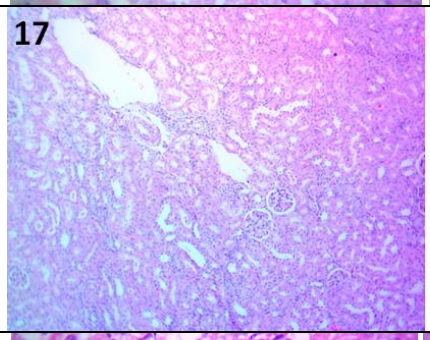
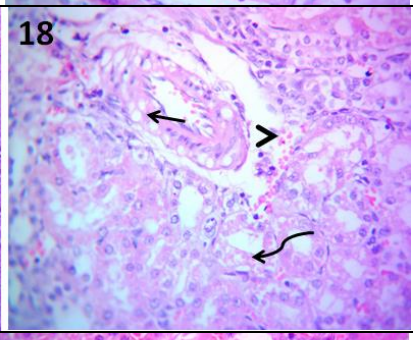
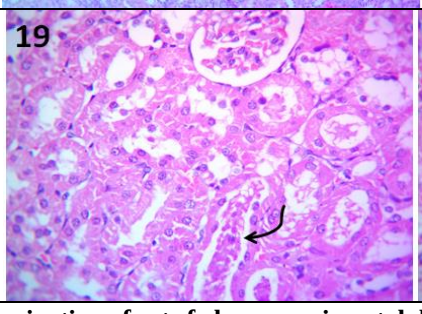
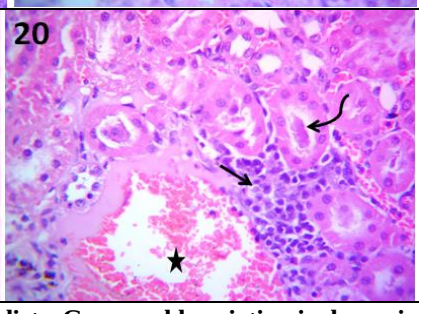
Animal groups	Kidney	
	H & E ×100	H & E ×400
NC		
HRCP		
HRY		
HRYS1		
HRYS2		

Figure 3. Kidney histopathological examination of rats fed on experimental diets. Groups abbreviation is shown in Figure 1; H & E, sections stained with hematoxylin and eosin; x, magnification power.

The third slide is a photomicrograph (15×100, 16×400) of hypercholesterolemia rats fed on the standard diet and plain stirred yoghurt for 6 weeks. The kidney exhibits vacuolated tunica media of renal blood vessels (arrow) and hemorrhage between renal tubules (arrow head). The fourth slide is the photomicrograph (17×100, 18×400) of hypercholesterolemia rats fed on the standard diet and stirred yoghurt supplemented with 0.5% of SPP for 6 weeks. Kidney shows necrotic changes within renal tubular epithelium (curved arrow), vacuolated tunica media of renal blood vessels (arrow) and perivascular hemorrhage (arrow head). The fifth slide is a photomicrograph (19×100, 20×400) of hypercholesterolemia rats fed on the standard diet and stirred yoghurt containing 2% of SPP for 6 weeks. The kidney shows intratubular hyaline casts (curved arrows), round cells infiltration between renal tubules (arrow), and congestion of renal blood vessel (star).

Histopathological examination of rats heart

Several reports have suggested that spirulina might have a beneficial effect on cardiovascular diseases prevention (Chamorro *et al.*, 2002). In addition, Ramamoorthy and Premakumari (1996) administered spirulina supplements in patients with ischemic heart disease, and observed a substantial reduction in the blood cholesterol, LDL-C, triglycerides, and an increase in HDL-C. The histological changes in the heart and heart biopsy of results of each group are shown in Table 5 and Figure 4. The first slide is the photomicrograph (21×100, 22×400) the negative control group fed on the standard diet for 10 weeks. The heart shows normal histomorphology of cardiomyocytes (arrow head). The second slide corresponds to the photomicrograph (23×100, 24×400) of the hypercholesterolemia rats fed on standard diet for 6 weeks. The heart displays tunica intima of some cardiac blood vessels (arrow) in addition to congestion of some cardiac blood vessel (star) and fatty change within some cardiomyocytes (arrow heads). The third slide is a photomicrograph (25×100, 26×400) of hypercholesterolemia

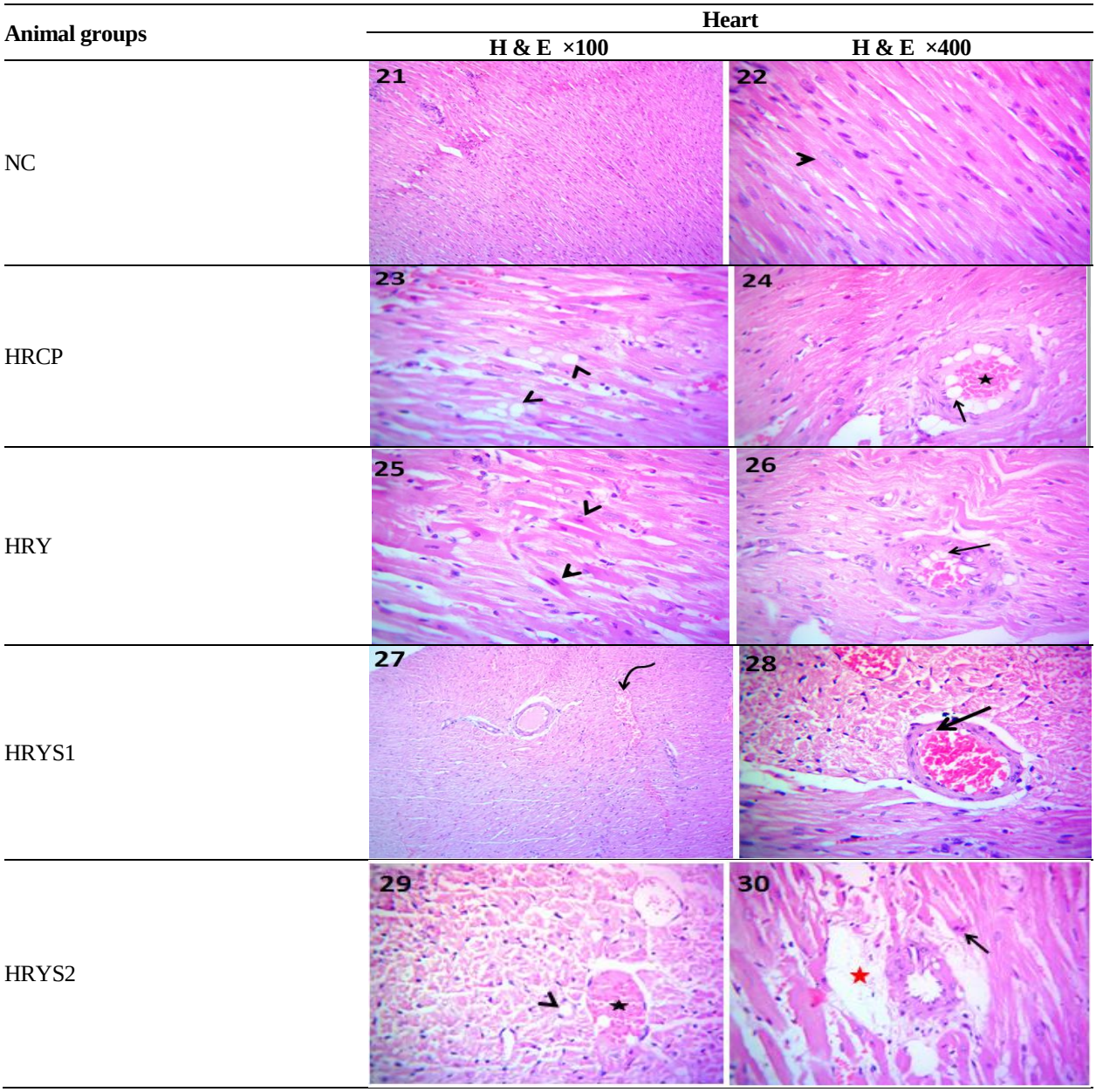


Figure 4. Heart histopathological examination of rats fed on experimental diets. Groups abbreviation is shown in Figure 1; H & E, sections stained with hematoxylin and eosin; x, magnification power.

rats fed on the standard diet and plain stirred yoghurt for 6 weeks. The heart shows vacuolated tunica intima of some cardiac blood vessels (arrow) and hyaline degenerations (arrow head) in few myocardial bundles.

The fourth slide refers to the photomicrograph (27×100, 28×400) of hypercholesterolemia rats fed on the standard diet and stirred yoghurt containing 0.5% of SPP for 6 weeks. The heart exhibits congested intramuscular blood vessels (curved arrow) and vacuolated tunica intima (arrow). The fifth slide is a photomicrograph (29×100, 30×400) of hypercholesterolemia rats fed on standard diet and stirred yoghurt containing 2% of SPP for 6 weeks. The heart shows fat globules within a little number of cardiomyocytes (arrow head), congested intermuscular blood vessels (black star), perivascular edema (red star), and hyaline degeneration of some cardio-myocytes (arrow).

CONCLUSION

In this study, anovel functional low-fat stirred yoghurt supplemented with different levels of SPP were prepared and evaluated. The obtained results revealed that SPP-supplemented stirred yoghurt treatments improved the biological and histopathological parameters of hypercholesterolemia rats. The findings of this work could pave the way for the implementation of SPP in other novel functional dairy products.

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تأثير تدعيم يوغورت مقلب وظيفي منخفض الدهن بمسحوق *Spirulina platensis* على بعض خصائص الجودة والتأثيرات العلاجية في كائن حي

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يعتبر اليوغورت أحد أكثر الأطعمة الصحية والمغذية إستهلاكًا على مستوى العالم. عادةً ما توجد الأمراض المتعلقة بالتغذية في البلدان النامية أو تحت النامية، حيث يمكن لمنتجات الألبان المدعمة أن تقلل بشكل كبير من مخاطر هذه الأمراض. يعتبر مسحوق الإسبريولينا (SPP) مصدرًا جيدًا للمركبات النشطة حيوياً والتي تتميز بنشاطها العالي المضاد للاكسدة. في الدراسة الحالية، تم تقييم الخصائص الفيزوكيميائية والتغذوية لليوغورت المقلب المدعم بـــ 0.5 ، 1 و 2 ٪ من SPP. أدت إضافة SPP إلى تقليل وقت التجبن وكمية الشرش المنفصل من الخثرة وقيم الـــ pH. أدى التدعيم بـــ SPP إلى زيادة محتوى المواد الصلبة الكلية والحموضة مقدرة كحامض لاكتيك والبروتين والدهن والأحماض الدهنية الطيارة الحرة الكلية. أظهرت عينات اليوغورت المدعم بنسبة 0.5٪ قبولاً حسياً أعلى مقارنة بالمعاملات الأخرى. بالإضافة إلى ذلك، فقد صممت الدراسة الحالية لدراسة بعض التأثيرات العلاجية لليوغور ت منخفض الدهن والمدعم بمسحوق الإسبريولينا داخل الجسم الحي. وأشارت النتائج إلى أن تدعيم اليوغورت بـــ SPP قد أتاح نهجًا تكنولوجيًا جديدًا لإنتاج غذاء وظيفي ذو تأثيرات إيجابية مضادة لأمراض تصلب الشرايين ويحسن وظائف الكبد والكلى والقلب.