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Possible application of green algae as emulsifiers in foods and nutritional supplements

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The high protein content (43.4 %) and the ability of dietary fiber in *Spirulina (Arthrospira platensis*) to retain water or vegetable oil make the alga suitable for use as an emulsifier in colloidal or emulsion systems. This study aimed to investigate the influence of *Spirulina (Arthrospira platensis)* (4%, 8%, 12%) as emulsifier on the physical and thermodynamic stability of model emulsions by determining parameters such as Gibbs energy, enthalpy, and entropy. Thermodynamic stability was estimated spectrophotometrically, and physical stability was determined by the Kozin method. It was shown that increasing the *Spirulina* concentration leads to a decrease in Gibbs free energy and an increase in the physical stability of all emulsions (20%, 40%, 60% oil phases) that are finely dispersed and microscopically determined. The highest percentage of retained emulsion (100 %) was at 12 % *Spirulina* with an oil phase content of 40 % or 60 %. From a rheological point of view, emulsions with *Spirulina* at 20 % oil phase showed plastic body behavior, and those with 40 % and 60 % oil phase showed pseudo-plastic behavior.

Keywords: emulsions, *Spirulina*, microscopic determinations, thermodynamic parameters.

INTRODUCTION

Oil-in-water emulsions are often used in pharmaceutical and food technology. Sunflower oil is the most preferred ingredient for developing emulsions due to its high thermal stability, relatively long shelf life, and wide distribution in Europe [1]. The choice of an appropriate emulsifier plays a crucial role in the stability of the emulsion, ensuring a longer shelf life for the product. Vegetable proteins have a proven emulsifying capacity [2]. *Spirulina* is a source of proteins (40-60 %), carbohydrates (1-3 %), and lipids (25-40 %) [3]. Spirulina extracts are widely used in the cosmetic industry, and their inclusion in suitable pharmaceutical dosage forms for therapeutic purposes is being studied [4]. Due to their amphiphilic nature, proteins are quickly adsorbed and easily rearrange at the oil/water interface. The highly elastic protein film formed on the oil droplets maintains the repulsive forces between them and increases the emulsion stability [5].

The present study aims to investigate the influence of *Spirulina (Arthrospira platensis)* (4 %, 8 %, 12 %) on emulsions' physical and thermodynamic stability.

MATERIALS AND METHODS:

Materials

The model emulsions were prepared using *Spirulina* as an emulsifier and sunflower oil as an oil phase. *Spirulina (Arthrospira platensis)* was taken from a bioreactor in Varvara, Bulgaria, and sunflower oil "Kaliakra" was purchased from the supermarket. All other reagents were of analytical grade.

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Preparation of model emulsion

Nine model emulsions were made to assess the effect of oil concentrations (20 %, 40 %, 60 %) and *Spirulina* concentrations (4 %, 8 %, 12 %) on the stability of oil-in-water emulsions. The protein concentration was investigated for used microalgae; in our case, it is about 44 % [6]. The composition of model emulsions is presented in Table 1, and the scheme for their preparation is given in Figure 1. All emulsions were prepared as follows: *Spirulina* was added to distilled water (pH = 7.0), and the mixture was homogenized at 1000 rpm (ISOLAB Laborgeräte GmbH, Germany) for 3 minutes. Then, with continuous homogenization, the oil phase was added, and homogenization continued for another 5 minutes.

Water holding capacity

Water holding capacity (WHC) was determined using the method of Chau and Huang [7]. 1 g of the sample was homogenized with 10 mL of distilled water, and stored for 24 h at a temperature of 23-25 °C. It was centrifuged (2500G, 30 min), and the amount of the supernatant was measured. WHC was defined as mL of retained water from 1 g of sample.

Oil holding capacity

Oil-holding capacity (OHC) was determined according to the method of Chau and Huang [7]. The 1 g sample was homogenized with vegetable fat (1:10) and stored for 30 min at a temperature of 23-25 °C. It was centrifuged (2500G, 30 min), and the amount of the supernatant was measured. OHC was defined as mL of retained fat from one g of sample.

Microscopic studies

The microscopic analysis performed to determine the distribution of oil globules in the model emulsions with the oil phase of vegetable sunflower oil (20, 40, and 60 %) and *Spirulina* (4, 8, and 12 %) as an emulsifier was carried out with a Levenhuk MED D30T digital microscope, using the Dino Capture 2.0 plugin, version 1.2 .0. and a PC sample Window Management Bar.

Determination of physical stability of emulsions

Emulsion stability was determined by Kozin [8] using a Nahita 2640 centrifuge. A five g emulsion sample was placed in a 10 cm^3 graduated centrifuge tube. Centrifugation was performed at angular velocity 314 $rad.s^{-1}$ for 20 min. The amount of retained emulsion was used to determine the stability of the emulsion *versus* separated water and oil phases.

Table 1. Composition of model emulsions with *Spirulina (Arthrospira platensis)* emulsifier

Sample	1	2	3	4	5	6	7	8	9
Emulsifier, % *(Spirulina (Arthrospira platensis))*	4	4	4	8	8	8	12	12	12
Oil phase (OP), % (sunflower oil)	20	40	60	20	40	60	20	40	60
Dispersion medium, % (water)	76	56	36	72	52	32	68	48	28

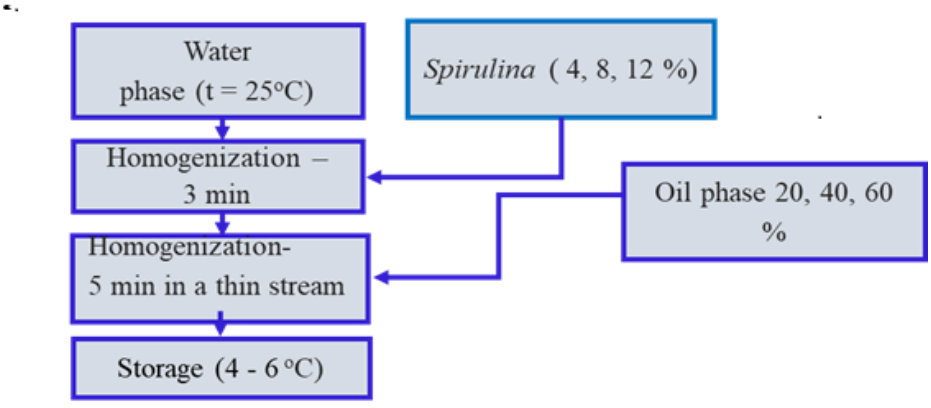

Figure 1. Technology for emulsion preparation.

Determination of the dispersity of emulsions

The method for determining dispersity was developed in [9]. Five g of the prepared emulsion was placed in a 50 cm^3 beaker and diluted with distilled water in a 100 cm^3 volumetric flask. Two cm^3 was taken from the prepared mixture with a pipette and transferred to a volumetric flask of 50 cm^3, and water was added to the mark and shaken, achieving a sample dilution of 1:500. A cuvette with a length path of 1.0 cm was used for measuring the transmission coefficient of the solution at a wavelength λ=540 nm relative to a control cuvette with distilled water. The amount of transmitted light determines the dispersion of the emulsion.

Thermodynamic stability of emulsions

The Gibbs free energy was calculated from equation 1.

$$\Delta G = -RTlnK \quad (1),$$

where R is the universal gas constant (R = 8.314 J/(K×mol)) and T is the absolute temperature (K). The experimental calculation of the equilibrium constant K was conducted by the "dilution method" described by Kendrow *et al*. [10]. Dilutions were made to each model sample with increasing volume concentrations from 0.2 to 1.0 % (v/v) in steps of 0.2. The optical density measurement's wavelength was determined from the absorption spectrum for the sample with the highest concentration. This was the wavelength at which the corresponding solution had maximum absorption. The equilibrium constant is the angular coefficient of dependence A=f(C).

The measurements were conducted by using a spectrophotometer (Genesys 10UV Thermo Scientific, Massachusetts, USA). The research was carried out with a cuvette of 1 cm path length at λ= 620 nm.

In addition to the Gibbs energy, the enthalpy and entropy were calculated from equations 2 and 3.

$$\frac{dlnK}{d(\frac{1}{T})} = \frac{-\Delta H}{R} \quad (2),$$

$$\Delta S = \frac{(\Delta H - \Delta G)}{T} \quad (3),$$

where ΔH is enthalpy ($kJ \times mol^{-1}$), ΔS is entropy ($kJ \times K^{-1} \times mol^{-1}$).

Rheology

The rheological measurements were performed at (20 ± 1) °C using Thermo Scientific HAAKE Viscotester 550 (Germany). The analyses were conducted in an SV DIN coaxial cylinder sensor at shear rates ranging from 0.0123 s^{-1} to 1000 s^{-1}. The data for the shear stress as a function of the share rate for each model were investigated. The instrument software application performed the mathematical modeling.

Statistical analysis

The data on the three parallel measurements of investigated parameters were processed to obtain the mean value and the standard deviation (SD). Dispersion analysis was used to compare the mean values with a significance level of $p < 0.05$. A nonlinear model was obtained by the IBM SPSS Statistic 26 computer program, USA.

RESULTS AND DISCUSSION

The type of dietary fiber (soluble and insoluble) consumed affects human physiology. Soluble and insoluble dietary fiber content determines water and vegetable oil holding capacity.

Our study showed that *Spirulina* has a WHC and OHC of 1.1 g/g and 1 g/g, respectively. This fact confirms the thesis by Suzuki *et al*. [11] that algae are suitable stabilizers of colloidal and emulsion systems.

The main characteristics (emulsion stability and dispersity) of emulsions with oil phase (OP) from vegetable sunflower oil 20, 40, and 60 % and emulsifier *Spirulina* 4, 8, and 12 % were investigated.

Table 2 presents the results of the Kozin test for physical stability of model emulsions with OP of vegetable sunflower oil (20 %, 40 %, and 60 %) and *Spirulina* content of 4, 8, and 12 % as an emulsifier.

Table 2. Physical stability of emulsions.

	Separated phase, ml			Retained emulsion, %
	O	W	E	
1	-	0.5	3.5	46.70
2	-	0.8	3.7	40.00
3	-	0.5	5.0	26.67
4	-	1.0	3.0	57.13
5	-	0.5	4.0	43.81
6	-	-	6.2	27.09
7	-	0.3	0.2	93.30
8	-	-	-	100.00
9	-	-	-	100.00

Increasing the amount of *Spirulina* increased the percentage of retained emulsion, regardless of the OP content. At 12 % *Spirulina* as emulsifier and 40 and 60 % OP, emulsion stability was 100 %. At 20 % OP and 12 % emulsifier, emulsion stability is high – about 93 %. Therefore, when developing emulsions with 20%, 40%, and 60% OP vegetable sunflower oil, it is appropriate to use 12 % *Spirulina* as an emulsifier.

The dispersity of O/W emulsions with the emulsifier freshwater algae *Spirulina (Arthrospira platensis)* with 20 %, 40 %, and 60 % OP of vegetable sunflower oil was determined, and the extinction values (E, %) were measured. The results are presented in Table 3.

Table 3. Dispersion of model emulsions with emulsifier *Spirulina (Arthrospira platensis*) (SP) and Oil Phase (OP) from vegetable sunflower oil.

Emulsifier/oil phase ratio			
Sample (SP/OP)	1	4	7
E,%	0.075	0.085	0,087
Sample (SP/ OP)	2	5	8
E,%	0,106	0.124	0.129
Sample (SP/ OP)	3	6	9
E,%	0.168	0.228	0.640

The optical density (extinction) values increased with an increase in the amount of *Spirulina (Arthrospira platensis)* as an emulsifier (Table 3) and the OP from 20 % to 40 % or 60 %. This trend was most pronounced in the 60 % emulsions with 4 %, 8 %, and 12 % *Spirulina (Arthrospira platensis).* The optical density of emulsions with 12% *Spirulina (Arthrospira platensis)* as an emulsifier and 60 % OP were 3.8 times higher than those with 4 % emulsifier. Model emulsions 3, 6, and 9 with 12 % emulsifier and 20 %, 40 %, and 60 % OP are the most dispersed.

Microscopic analysis established the distribution of oil globules in model emulsions with OP of vegetable sunflower oil 20 %, 40 %, and 60 % and *Spirulina (Arthrospira platensis)* (4 %, 8 %, and 12 %). Microscopic observation was carried out 10 minutes after the preparation of the emulsions. The addition of *Spirulina* reduces the size of the oil globules in the emulsions, and as its concentration increases, the fine dispersion of the emulsions increases. The results are presented in Figure 2.

Fig. 2 shows that the prepared emulsions are polydisperse systems – their particles have different sizes.

As the *Spirulina* content increased to 12 % and the OP increased to 40 % and 60 %, small-sized oil globules predominated. The uniform distribution of tiny oil droplets facilitates the adsorption of the protein molecules of the microalgae by the lipid globules, which prevents their coalescence and, therefore, leads to the destabilization of the model emulsion. A similar fact was described by Hebishy *et al.* (2017) [12].

Samples with 4 % *Spirulina* and OP 20 % and 40 % sunflower oil have larger sizes and, therefore, are unstable as the oil globules interact and coalescence is observed.

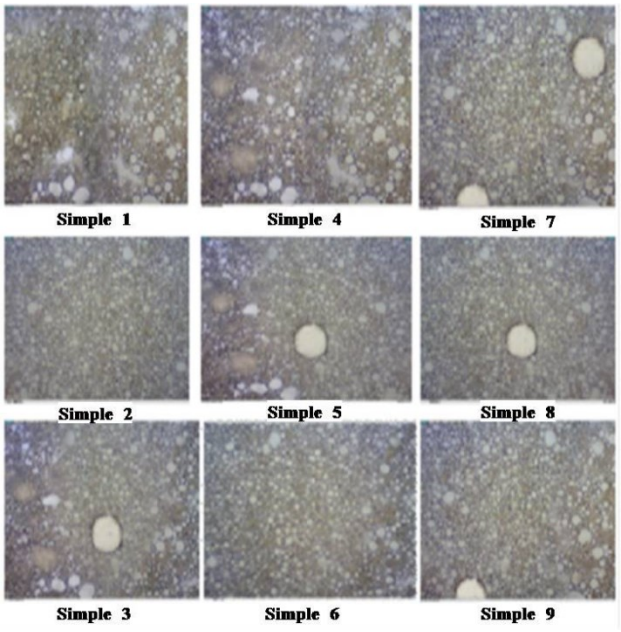

Figure 2. Microscopic analysis of model emulsions.

Sunflower oil was chosen for the OP of the developed emulsions, as it contains medium-chain fatty acids (C16:0 to C18:0). Taha and others (2018) [13] noted that the lower molecular weight of medium-chain triglycerides reduced surface tension more effectively contributing to forming a stable emulsion. The choice of vegetable oil is essential for the characteristics of the emulsion and its stability since the above properties depend on the physicochemical characteristics (viscosity, solubility, surface tension) of the oil [14].

The model emulsions' thermodynamic parameters, such as Gibbs free energy, enthalpy, and entropy, were calculated using equations 1-3. The results are presented in Table 4. The content of *Spirulina* increases, and the Gibbs free energy decreases. This parameter is used as a criterion for evaluating the thermodynamic stability of emulsions. At a negative value of the G parameter, spontaneous emulsification occurs. The more negative the free energy value, the more thermodynamically stable is the emulsion, according to Mehta and Kaur (2011) [16]. Increasing the concentration of sunflower oil increases the stability of the emulsions and leads to more negative Gibbs free energy values. Therefore, the emulsion system with the highest content of *Spirulina* (12 %) possessed the highest Gibbs modulus-free energy. Thus, the most incredible thermodynamic samples are also physically stable. Comparable results have been observed for other food emulsions [12, 16].

The conclusion made above is also confirmed by Figure 3, representing the dependence of Gibbs free energy on the concentration of *Spirulina* as an emulsifier and the oil content of the emulsion.

Table 4. Thermodynamic parameters - free Gibbs energy, enthalpy, and entropy. Samples with 4 % *Spirulina* and OP 20 % and 40 % sunflower oil have larger sizes and, therefore, are unstable as the oil globules interact and coalescence is observed.

Sample	ΔG ± SD [kJ mol^{-1}]	ΔH ± SD [kJ mol^{-1}]	ΔS [kJ mol^{-1} K^{-1}]
1	-5.28±0.05	-19.64±1.05	-0.048±0.002
2	-4.97±0.14	-19.50±1.11	-0.049±0.002
3	-6.48±0.54	-20.16±1.24	-0.046±0.002
4	-5.19±0.94	-19.60±0.76	-0.048±0002
5	-5.23±0.74	-19.62±0.49	-0.048±0.001
6	-5.63±0.64	-19.79±0.89	-0.048±0.001
7	-7.17±2.04	-20.46±1.32	-0.045±0.002
8	-7.03±1.24	-20.40±1.34	-0.045±0.001
9	-6.00±1.34	-19.95±1.07	-0.046±0.001

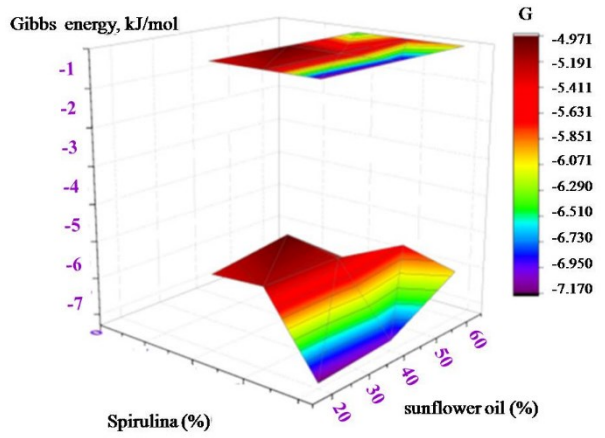

Figure 3. Graphical dependence between the Gibbs free energy and mass % O/W of emulsion prepared with *Spirulina.*

Enthalpy determines the thermal effect of emulsion systems. The negative value of enthalpy indicates that the processes are defined as exothermic. Entropy is related to the phase separation of the emulsion system and is a measure of its disorder. In the studied samples, the entropy shows shallow negative values.

Except for the amount of emulsifier, the results show that the amount of sunflower oil also affects the emulsion stability. Emulsions prepared with lower *Spirulina* concentrations and more significant amounts of the oil phase were characterized by higher Gibbs energy values. When the emulsifier amount increased, the emulsions' stability according to the resulting Gibbs energies was better, in addition to a smaller amount of oil phase. This is associated with the emulsifier nature that leads to forming of a particular emulsion. The free water binds to the emulsifier and leads to gelling processes. Because of the connection of water with the emulsifier, the system's viscosity probably increases, which is another criterion for stability and explains the more excellent stability of the respective emulsions. To confirm the statement, rheological tests were carried out at 200 °C. From a rheological point of view, emulsions with 20 % oil phase had plastic behavior, and those with 40 % and 60 % oil phase had pseudo-plastic behavior. Results are presented in Figure 4.

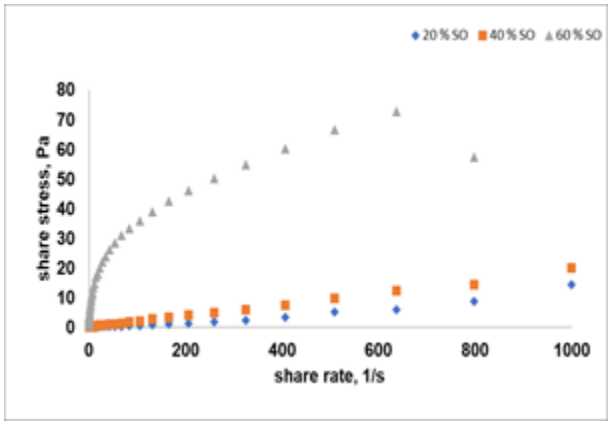

Figure 4. Dependence between share stress and share rate for model emulsions with *Spirulina* as an emulsifier.

CONCLUSIONS

Spirulina platensis has excellent potential for use in the food industry as a stabilizer and in the pharmaceutical industry as an emulsifier to enrich food products and replace synthetic substances in supplements. In developing emulsion systems in food and pharmaceutical technologies, concentrations of 12 % *Spirulina* as an emulsifier with 20, 40, and 60 % oil phase of vegetable sunflower oil can be recommended for high emulsion stability.

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