PROCEEDINGS OF SCIENCE

Physical parameters of chocolate with the addition of *Arthrospira platensis*

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The physicochemical properties of chocolate mass with 85% cocoa content and 3%, 5%, and 10% of *Arthrospira platensis* have been studied. The algae were grown into a bioreactor in Varvara, Bulgaria. The present work aims to create an innovative chocolate product with appropriate physicochemical properties and organoleptic characteristics, contributing to the healthy nutrition of consumers. To achieve this goal, a technological scheme was developed for obtaining chocolate mass with *Arthrospira platensis*. Rheological, X-ray structural, and sensory analyses were performed. Data from reflective microscopy and fluorescence spectroscopy were also obtained. X-ray analysis shows peaks of crystalline sucrose and cocoa. In addition to these peaks in the X-ray diffraction pattern of the sample with 10% *Arthrospira platensis* content, some peaks may be due to complex crystals of the protein structure of freshwater algae. The rheological curves of the samples show that the studied systems are non-Newtonian fluids. Increasing the concentration of *Arthrospira platensis* does not affect the stickiness, softness, and hardness. According to sensory analysis, the samples with 5% *Arthrospira platensis* have a pronounced sweet taste, and those with 3% - a cocoa taste. Reflective microscope images were taken to examine the fracture section.

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1. Introduction

Chocolate - amazing, but its journey starts from a tree called Theobroma cacao and its seeds. These seeds go through two important processes fermentation and roasting–two processes without which the taste of chocolate would not be what it is. People like chocolate for the energy it gives and its action as an aphrodisiac, but mostly for the effect it has on the mood. In the scientific literature, there are many studies on its health benefits - those related to heart health [1, 2], others with the regulation of blood pressure [3], an increase of good cholesterol [4, 5], has a beneficial effect on cerebrovascular diseases [6, 7], peripheral vascular diseases [8] and others [2].

Arthrospira platensis is an excellent source of essential amino acids, vitamins, and several macro- and micronutrients, and is also an excellent non-animal source of protein. However, it also contains several other, more specific compounds beneficial to human health, such as phycocyanobilin, which has antioxidant and anti-inflammatory effects on the body [9-11]. There is plenty of research on *Arthrospira platensis* supporting its health benefits, making it an easy dietary supplement to recommend. For example, it reduces the risk of cardiovascular problems [12] because of its beneficial property of regulating blood pressure [13, 14] and blood cholesterol [12, 14]. It has proven antioxidant [15-17] and anti-inflammatory properties [17], improves the metabolism of various lipids [12, 18, 19] and glucose [12, 19], and all these beneficial properties improve the defenses of the human body.

Population growth leads today to the development and inclusion of various food additives, such as those that are traditional for the given region [20, 21] and/or are products of high biological value, with beneficial effects on health [22, 23].

The present work aims to create an innovative chocolate product with appropriate physicochemical properties and organoleptic characteristics contributing to the healthy eating of consumers.

2. Materials and methods

2.1. Sample

Three samples of chocolate bonbons made according to the technological scheme given in Figure 1 were examined. They were prepared from:

- Ghirardelli dark chocolate couverture with 85% cocoa content, purchased from the commercial network; and

- *Arthrospira platensis* biomass grown in a bioreactor /Varvara, Bulgaria/ purchased from a manufacturer after conventional drying;

The *Arthrospira platensis* content in them is:

Sample 1- 3% *Arthrospira platensis*

Sample 2- 5% *Arthrospira platensis*

Sample 3- 10% *Arthrospira platensis*

2.2. Preparation of the samples

The technological scheme is presented in fig. 1. The chocolate couverture was individually warmed to 40 °C. Conventional dried *Arthrospira platensis* was added and then homogenized with couverture. The resulting mass was cooled to 30 °C, then tempered, formed, and cooled to 18-20 °C.

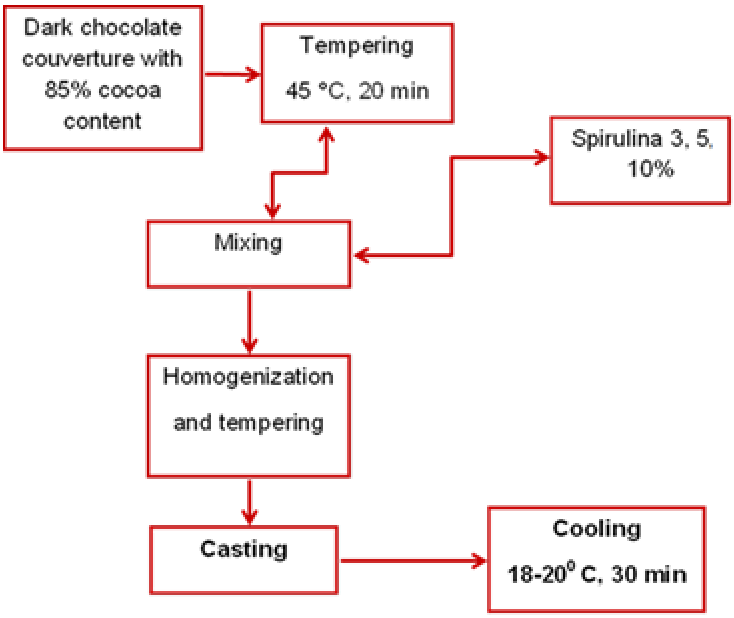


Figure 1: Technological scheme for chocolate product with *Arthrospira platensis*

2.3. Used methods:

2.3.1. Rheological studies

Rheological measurements were performed at 40±1 °C using a Thermo Scientific HAAKE Viscotester 550 (Germany). This temperature is used because all the solid glycerides in the oil fraction of the chocolate mass are in a molten state. 100 g of chocolate mass is grated, placed in a glass cup with a volume of 250 cm^2, and melted in a water bath at a temperature of 55°C. The chocolate mass in the glass is stirred periodically so that it does not absorb air. It was then cooled at room temperature to 43°C. 62 g of chocolate mass was analyzed in an SV DIN coaxial cylindrical sensor at shear rates ranging from 0.0123 s^{-1} to 1000 s^{-1}. The shear stress data as a function of partition percentage for each sample was examined.

2.3.2. Fluorescence spectroscopy

Fluorescence spectra of whole and cross-cut chocolate samples with *Arthrospira platensis* were obtained with a portable spectrometer AvaSpec-ULS2048CL-EVO, Avantes-Apeldoorn (Apeldoorn, The Netherlands). Measurements were performed for light excitation wavelengths of 285 nm, 385 nm, 470 nm, and 530 nm. The measurement scheme is shown in fig. 2, and the measurement methodology is the same as Ropelewska et al [24].

2.3.3. Microscopy of chocolate samples

The middle part of investigated samples was observed with an Olympus SZ61 stereomicroscope, Olympus ltd., Tokyo, Japan, operating in the bright field with reflected light at 70× objective and 8× eyepiece magnification. The photos were taken with a 5Mp×Wi-fi camera.

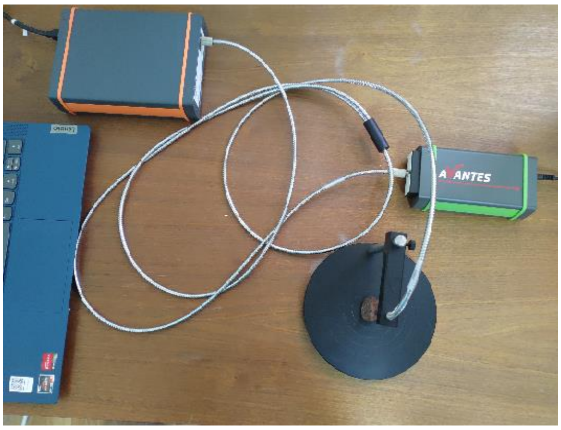


Figure 2: Experimental set up for fluorescence measurements

2.3.4. X-ray structural analysis

The evaluation of crystalline and amorphous phases presented in the chocolate was provided by X-ray analysis. Powder XRD patterns of the chocolate samples were collected in the range from 5.3 to 80°2θ. The step of the scan was constant and equal to 0.02°2θ. The counting time was 52.5 sec./step. The equipment uses was Bruker D8 Advance diffractometer (Germany) with Cu tube and LynxEye detector. Phase identification was performed with the Diffracplus EVA using ICDD-PDF2 (2021) Database.

2.3.5. Sensory analysis

A sensory analysis of the developed compositions of chocolate products was carried out. The indicators "Taste intensity” and “Structural profile" of samples with 85% cocoa mass and concentrations of *Arthrospira platensis* (3. 5, and 10%) were made. For this reason, a panel of trained sensory experts evaluated the products. Each participant tested the product and gave "descriptive" indicators to fill out the system of questions accurately.

3. Results and discussion

Figure 3 presents the results of the rheology of samples with 85% cocoa mass and concentrations of *Arthrospira platensis* (3, 5, and 10%). The resulting curves show that the samples have the character of non-Newtonian fluids depending on the applied mechanical impact. For all analyzed samples, it is found that with increasing velocity gradient (D, s^{-1}), viscosity values (η, Pa.s) decrease. Therefore, at a temperature of 40°C, all the solid glycerides in the oil fraction of the chocolate mass are in a molten state. The sample with a concentration of 10% of *Arthrospira platensis* has a similar viscosity at 1 s^{-1} for the indicated temperature with cocoa butter extracted from raw beam kernel [25].

It can be noted that decreasing the concentration of *Arthrospira platensis* (3, 5, 10 %) does not affect the character of the rheological curves. Increasing the concentration of *Arthrospira platensis* at all velocity gradients resulted in an increase in the viscosity of the samples. A similar trend was observed when inulin was added to chocolate milk [26].

The results of the microscopic analysis are presented in fig. 4. At 3% and 10% *Arthrospira platensis*, a uniform distribution of *Arthrospira platensis* in the cocoa mass volume was observed, while at 5% *Arthrospira platensis* there was no uniform distribution, and cracks (fractures) appeared.

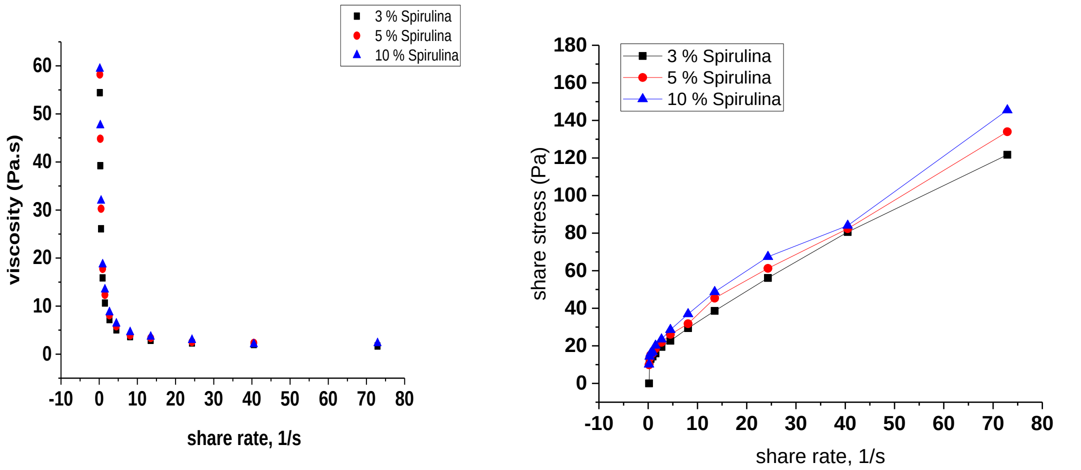
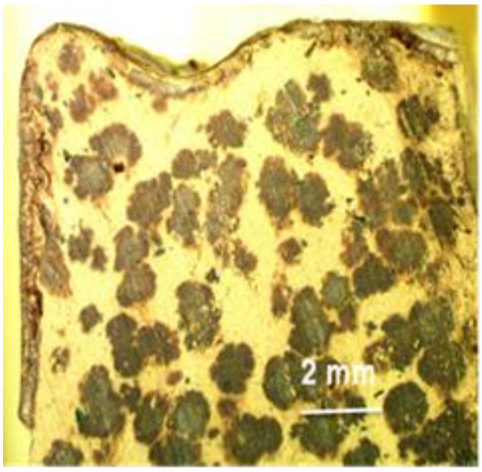
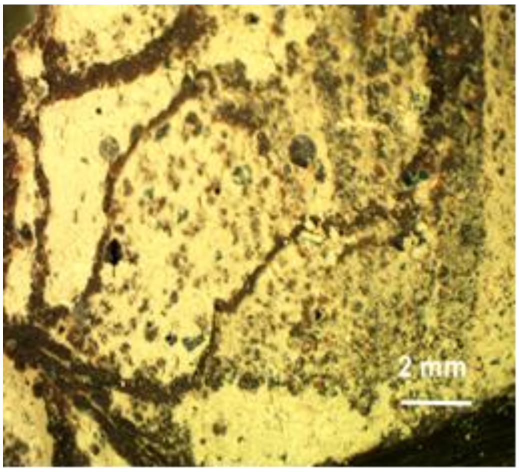


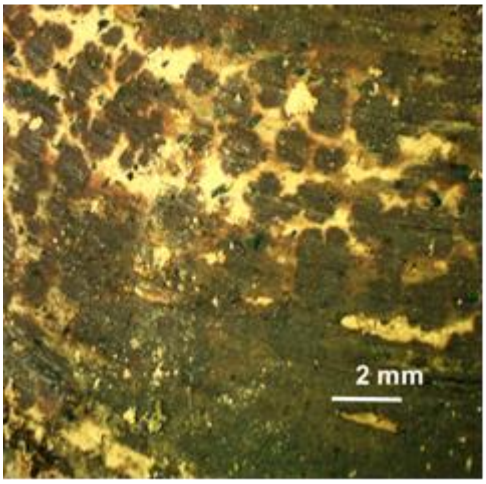
Figure 3: Rheological behavior of chocolate samples, containing *Arthrospira platensis* at temperature 40 °C



a) 85% cocoa + 3% *Arthrospira platensis*



b) 85% cocoa + 5% *Arthrospira platensis*



c) 85% cocoa + 10% *Arthrospira platensis*

Figure 4: Microscopy analysis of chocolate samples

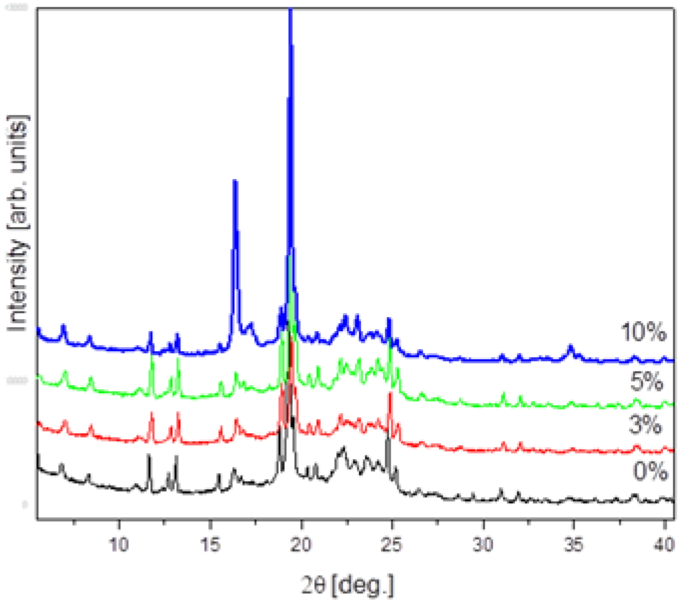


Figure 5: X-ray structural analysis of chocolate with *Arthrospira platensis*

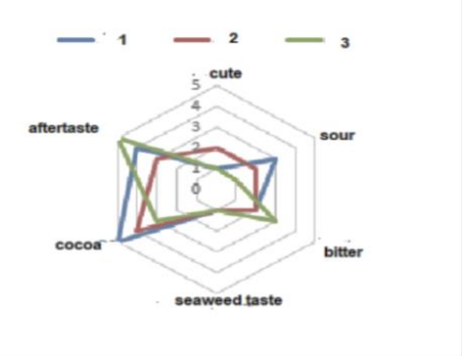
X-ray analysis of the pure chocolate sample (fig. 5) shows peaks corresponding to crystalline sucrose identical with ICD-PDF2 file #00-024-1977 and cocoa butter – the most intensive peak at 4.6Å (19.2°) and some others [27]. In addition to these peaks, in the X-ray diffraction pattern of the sample with 10% *Arthrospira platensis* content, some new peaks at 5.43 Å (16.3°), 5.17Å (17.1°) and 2.58Å (34.8°) can be seen. They may originate from the complex crystals or the protein structure of fresh water algae [28].

We conducted research on 'Flavor Intensity', 'Structure Profile' and 'Sample Surface'. The products were evaluated by a panel of trained sensory experts. Each participant tests the product and gives "descriptive" indicators. Specific indicators have been developed based on the organoleptic indicators and corresponding to the chocolate product descriptions. Samples were presented in a different order to each trained participant. The data are presented in fig. 6, and the results of the analysis can be summarized:

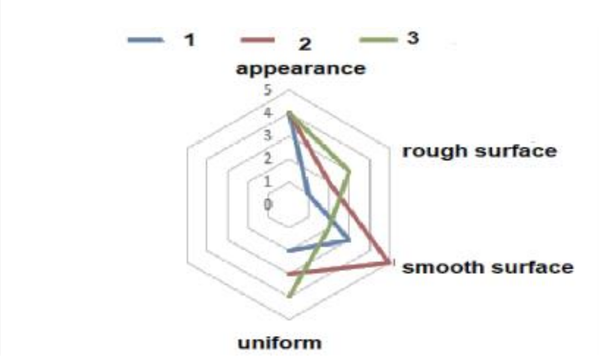
- In sample 1, tasters reported a strong cocoa flavor and almost no aftertaste. The most pronounced aftertaste is sample 3, with a strongly pronounced bitter taste. At 5% content, *Arthrospira platensis* , according to tasters, has a markedly sweet taste.

- Tasters reported that sample 2 had the smoothest surface compared to the other samples, while sample 3 had the roughest surface.

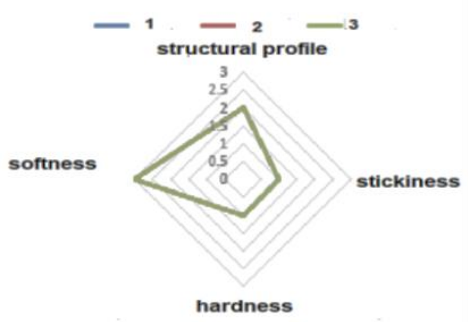
- Regardless of the concentration of added *Arthrospira platensis* (3, 5, and 10%), the samples had the same values for the indicators "stickiness," "softness“, and "hardness“.



According to the sample taste



According the sample surface



According to structural profile

Figure 6: Results from sensory analysis

According to sensory evaluation, "fracture" indicator is highest in the presence of 5% *Arthrospira platensis.* The latter result correlates with images taken with a reflection microscope. There are clearly defined cracks inside the product only in sample 2

A dependence is observed **-** as the content of *Arthrospira platensis* increases, the shine of the chocolate products increases. The consistency scores for all samples were very close. The values were between 5 and 6 points. The latter fact shows that the content of *Arthrospira platensis* does not significantly affect the consistency of the product. The porosity of all products is the same.

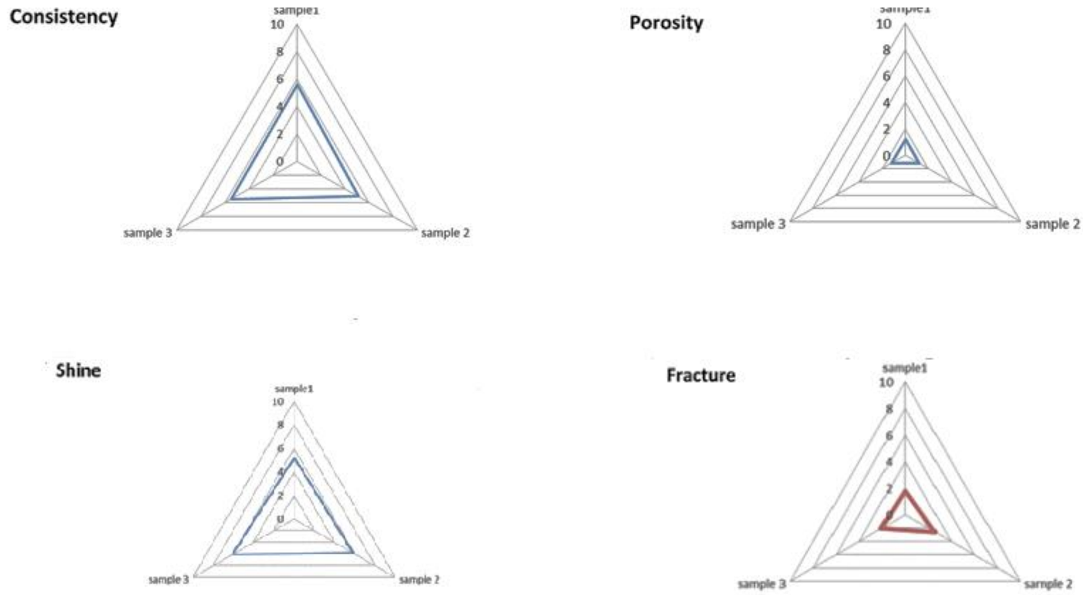


Figure 7: Results from sensory analysis for consistency, fracture, porosity and shine

4. Conclusion:

The addition of *Arthrospira platensis* (3 and 5%) to 85% cocoa content in chocolate products does not negatively affect taste perceptions and are suitable additions to create a new product. Some deviations in the physicochemical parameters for the 5% supplement may be due to technological errors. A 10% addition of *Arthrospira platensis* is definitely not suitable due to the observed change in taste which is leading among consumers.

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