



## Nutritional value, antioxidant and antibacterial activities of date syrup fortified with spirulina

Amira EL Arem<sup>1\*</sup>, Hayet Edziri<sup>2</sup>, Youssef Krichen<sup>3</sup> & Lotfi Achour<sup>1</sup>

<sup>1</sup> Laboratory of Bioresources, Biology Integrative and Valorization, Higher Institute of Biotechnology of Monastir, University of Monastir, Monastir, 5000, Tunisia.

<sup>2</sup> Laboratory of Transmissible Diseases and Biologically Active Substances, Faculty of Pharmacy, University of Monastir, Monastir, 5000, Tunisia.

<sup>3</sup> Tunisia Bio-Algae Company, Ksour Essef – Mahdia.

### Article info

Article history:

Received: 07 October 2024

Accepted: 05 September 2025

**Keywords:** Date syrup, spirulina, physicochemical composition, antioxidant activity, antibacterial activity.



Copyright©2025 JOASD

**\*Corresponding author**

amira.arem@yahoo.fr

**Conflict of Interest:** The authors declare no conflict of interest.

### Abstract

Dates and spirulina are two famous foods due to their richness in various nutrients and antioxidant compounds with very important therapeutic and nutritional effects. The aim of this study is to develop nutritious date syrup “Alig” fortified with spirulina. Firstly, a hedonic test of date syrups with spirulina at 2, 5 and 10% was carried out to evaluate the sensory properties of each sample and to choose the appropriate formulation. Then, the values of pH, titratable acidity (TA) and humidity, as well as the contents of total solids (TS), proteins, ash, lipids, sugars and carbohydrates of date syrup, spirulina and the chosen date syrup/spirulina formulation were determined. The total polyphenol (TPC) and condensed tannin (CTC) contents as well as the antioxidant and the antibacterial activities of these samples were also evaluated. Results showed that compared to the syrup without spirulina, the syrup enriched with 2% spirulina has the highest TS, ash, total lipids, proteins, TPC, and CTC, as well as pH and TA values, beside the most important antioxidant and antibacterial activities. In parallel, the present results indicate that this syrup is more interesting from the point of view energy value with 329.79 Kcal/day for 100 g of fresh syrup. We could conclude that date syrup fortified with 2% spirulina have very interesting nutritional value and could attribute to numerous health benefits.

### 1. INTRODUCTION

The world production of dates reached more than nine million tons in 2020, however only Deglet Nour (12% of world production) and “Medjoul” (1% of world production) varieties are particularly appreciated by consumers (Boughzala & Ben Mahmoud, 2022). In Tunisia, the “Deglet Nour” variety is largely cultivated in the southern regions and contributes to more than 60% of the total production due to its very good sensory quality and high commercial value. Whereas, the others varieties characterized by low commercial quality are less appreciated and represent more than 30% of Tunisian date production (Madi et al., 2024). To increase the value of these low-quality varieties, converting them into value-added products would benefit the local economy. Date syrup is the most prevalent product derived from dates. This thick,

dark liquid is obtained by heating date fruits and is free from fibers, sediments, impurities, and other foreign contaminants. It is obtained following two methods: 1) traditional domestic method by extraction and boiling down of the juice, and 2) industrial method by extraction, clarification and concentration of the juice. Similarly to date fruits, date syrups are very rich in various nutritional compounds such as organic acids, ash and mineral compounds, fatty acids, proteins with a wide selection of amino acids, pectin and vitamins (Abbès et al., 2013; Aleid & Haddadin, 2023; Bouhlali et al., 2020; Shahein et al., 2022). Besides bioactive natural compounds, including phenolic acids, flavonoids, tannins and carotenoids, which are well known to exhibit an extensive range of various biological activities such as antioxidants, antimicrobial, anti-inflammatory and

antiglycemic (Mimouni et al., 2015; Taleb et al., 2016a; Taleb et al., 2016b). In addition, the date syrup is used in traditional medicine to cure scurvy (Uba et al., 2015), prevent anemia and relieve rheumatic pains (Mony et al., 2022) and to facilitate and reduce labor pain in nulliparous women (Sohrabi et al., 2022).

Date syrup is a high energy food very rich in sugars ( $\geq 80\%$ ) consisting mainly of glucose (34.30%), fructose (32.60%), and a small amount of sucrose (0.20%) (Aleid & Haddadin, 2023). Consequently, it can serve as a great healthy alternative to refined sugar and other natural sweeteners. For this reason, date syrup is utilized extensively in the food industry as a natural sweetening agent in the formulation of bakery products, ice creams, chocolates, confectioneries, jams, beverages, and various desserts (Alqahtani et al., 2025; Faith et al., 2023; Gab-Allah et al., 2020; Mimouni et al., 2015). Furthermore, date syrup has been incorporated into functional dairy products such as probiotic milk and yogurt (Ali et al., 2023; Shahein et al., 2022) to enhance their organoleptic properties. In biotechnological applications, it acts as an effective carbon source in microbial fermentation processes, supporting the production of diverse metabolites including bioethanol and polyhydroxyalkanoates (Madi et al., 2024) and serves as a raw material for the production of date honey and highly concentrated fructose solutions (AlFaris et al., 2022).

Spirulina (*Arthrospira platensis*) is a cyanobacterium of 0.2 to 0.3 mm length. It is considered as the richest food of the world due to its veritable protein content (60-70%) beside essential fatty acids ( $\gamma$ -linolenic acid), vitamins, minerals (iron, potassium, calcium, zinc), pigments (chlorophylls A,  $\beta$ -carotene, phycocyanin and allophycocyanin), polysaccharides, and polyphenolic compounds (AlFadhly et al., 2022). These compounds attribute to spirulina potential health-promoting benefits include immunomodulation, antioxidant, antimicrobial and anti-inflammatory activities and positive effect against malnutrition, diabetes and anemia (AlFadhly et al., 2022; Wang et al., 2023). Spirulina algae can be manufactured in various forms including powder, liquid, oil, tablets, or capsules, and are utilized across numerous food industries. It was widely used as an ingredient in food bars, dairy products, snacks, cookies, pasta, functional beverages, ice cream and enriched flours frozen

desserts and condiments (AlFadhly et al., 2022; Podgórska-Kryszczuk, 2024).

However, incorporating spirulina into foods can reduce their overall acceptability due to its distinct taste, flavor, and color, which may not align with consumer preferences (Tiepo et al., 2021). To address this, date syrup can serve as a suitable medium to preserve the antioxidant properties of spirulina while masking its unfavorable taste. In other way, enriching date syrup made from low-quality dates with protein-rich food such spirulina could enhance its nutritional value.

Therefore, the aim of this study was to develop nutrient-rich syrup using "Alig" dates fortified with spirulina that are acceptable to consumers. Three formulations of date syrup/spirulina (2, 5 and 10%) were prepared and processed to sensorial evaluation and then the appropriated formula was evaluated for its physicochemical characteristics, antioxidant and antibacterial activities.

## 2. MATERIAL AND METHODS

### 2.1. Plant material and chemical

Fruits of a semi- dry date variety "Alig" were collected from Kebili region, southern Tunisia, during the 2018 harvest season at full maturity "Tamr stage". Five kilograms were directly divided into bags of 500 g and stored at - 20 °C until use. Spirulina used was in the form of dried powder, cultivated in Tunisia Bio-Algae Company, AL-Alia- Mahdia region. It has a blue-green color with an acceptable taste and odor.

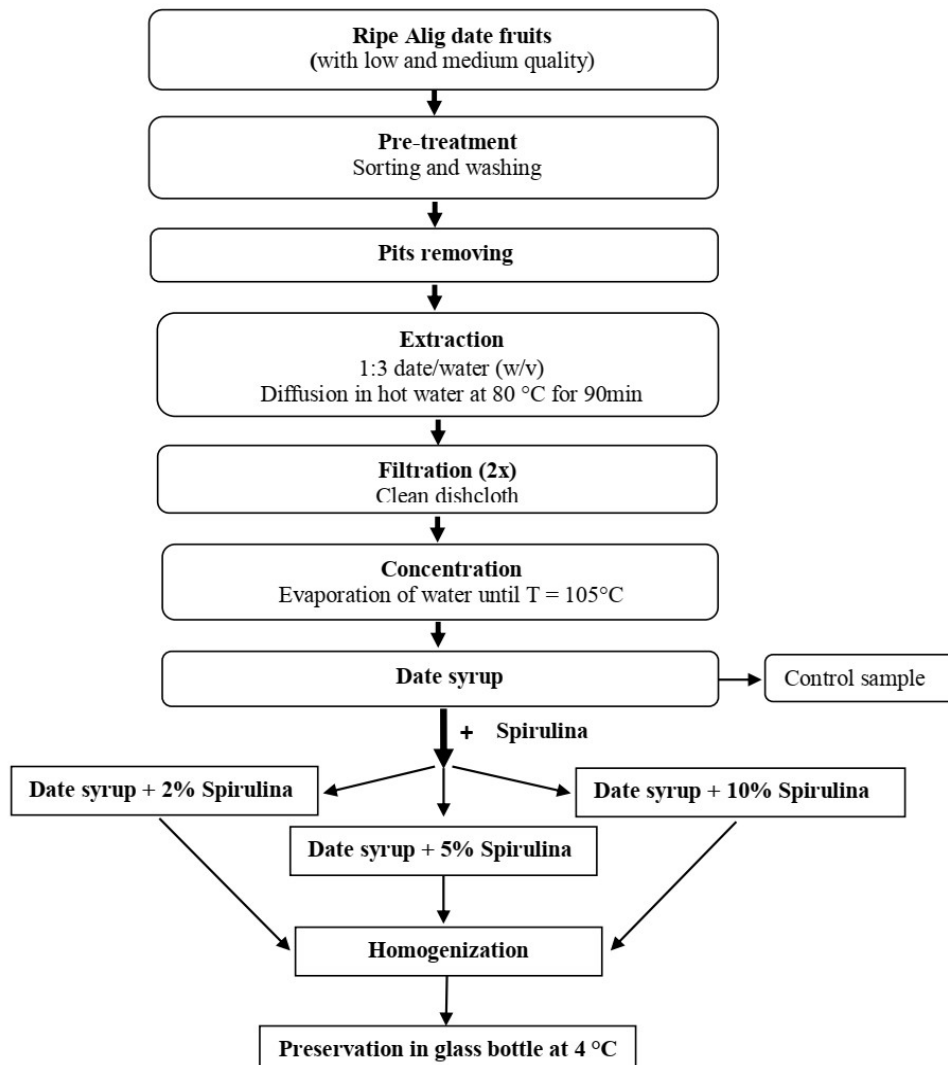
All chemical, standards and reagents used were purchased from Sigma-Aldrich Co. Ltd. (St. Louis, MO, U.S.).

### 2.2. Production of date syrup and its formulation with spirulina

Date syrup was prepared according to the traditional method. The extraction method adopted in this study is based on simple diffusion laws with proportion of 1:3 date/water (w/v). Fig. 1 outlining the different steps involved in the preparation of date syrup and its formulation with spirulina.

### 2.3. Sensory evaluation

Sixty healthy volunteers consisted of 33 men and 27 women were enrolled for this study with a middle age of 35 years to taste each formula and to answer to the hedonic test which was graduated from 1 to 9 (1 = dislike extremely and



**Fig. 1.** Organogram of the different steps involved in the preparation of date syrup and its formulation with 2, 5 and 10% spirulina.

9 =like extremely), taking into account the texture, color, taste and flavor of each formula. The volunteers used mineral water to rinse their mouths between the testing of each sample. The participants were blinded to avoid bias.

#### 2.4. Proximate analysis

Date syrup, spirulina, and developed date syrup/spirulina mixture were analyzed to determine their physicochemical properties according to methods described by EL Arem et al., (2011). Moisture content was determined by drying samples in an oven at 80°C until a constant weight was achieved. Meanwhile, ash content was determined using a muffle furnace at 530°C for 5 h. PH value was measured using a pH meter and the titratable acidity (TA) was determined by 0.1 N NaOH, expressed as citric acid equivalent. Total lipid content was measured using the Soxhlet method by

condensation for about 5–6 hours then the solvent was removed by evaporation. The protein content was determined according to the Bradford method (Bradford, 1976) using Bovine Serum Albumin (0-1.5 mg/mL) calibration curve. Total sugar content was determined according to Dubois method (Dubois et al., 1956) using calibration curve of glucose solution (0-1mg/mL). Total carbohydrates content was calculated according to the following equation:

$$\text{Total carbohydrates (\%)} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ total protein} + \% \text{ total lipids}).$$

Total solids content (TS) was calculated according to the following equation:

$$\text{TS (\%)} = 100 - \text{Moisture content}$$

Caloric value was calculated according to the following equation:

Caloric value (kcal/100 g) = [(% carbohydrates × 4) + (% protein × 4) + (% fat × 9)]

The date syrup yield was calculated according to the following equation:

Date syrup yield (%) =  $Q/q \times 100$

Where, Q is the quantity of date used (g) and q is the quantity of date syrup (g).

## 2.5. Measurement of total polyphenolic and condensed tannin contents

The extraction and the quantification of total polyphenolic contents (TPC) in date syrup, spirulina and date syrup fortified with 2% spirulina were carried out according to Al-Farsi et al. (2005) method with some modifications: 1 g of each sample was stirred with 25 mL of ethanol/water (70:30) for 30 min. Then, the solution was filtered using Whatman No. 1 paper and centrifuged (Hettich universal 320R centrifuge, Tuttlingen, Germany) at 1000g for 15 min, and each supernatant was collected and used for total polyphenolic assay. 200 µL of each extract were mixed with 1.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min. Then 1.5 mL of NaHCO<sub>3</sub> solution (60 g/L) was added to the mixture and incubated for 90 min at 22 °C. Absorbance was measured at 725 nm using a UV spectrophotometer (Jenway visible spectrophotometer 115 VAC model, Cole-Parmer, Chicago, IL, USA). TPC was calculated according to calibration curve of gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per 100 g of dry weight (DW).

Condensed tannin content (CTC) was determined with the vanillin in acidic medium according to the method of Julkunen-Titto (1985) using catechin as standard. The results were expressed as mg catechin equivalent (CE)/100g DW.

## 2.6. Determination of the antioxidant activity

Free radical scavenging activities of date syrup, spirulina and date syrup mixed with 2% spirulina were evaluated using the DPPH free radical assay according to the method described by Hassanzadeh et al. (2022). The radical scavenging activity was expressed as the inhibition percentage of the DPPH radical.

% Inhibition = [(Absorbance of control - Absorbance of the test sample) / Absorbance of control] × 100.

## 2.7. Evaluation of the antibacterial activity

### 2.7.1. Microbial Strains

Four reference strains were chosen for antibacterial investigation: one Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, and three Gram negative bacteria's: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 13883. These species were provided by the Laboratory of Microbiology, Hospital of Fattouma Bourguiba, Monastir.

### 2.7.2. Microdilution Method

The minimum inhibitory concentration (MIC) preventing visible bacterial growth was measured by the broth microdilution method. All extracts stock solutions of date syrup, spirulina and date syrup enriched with 2% spirulina were prepared by dissolution in 10% dimethyl sulfoxide (DMSO). Tested extracts were diluted to the highest concentration (50 mg/mL) and serial dilutions were prepared. A volume of 100 µL of brain-heart broth (BHI Broth, Sigma Aldrich, Steinheim, Germany) was added into the 96-well plates with the bacteria. Then, 20 µL of the overnight culture was adjusted to 0.5 McFarland turbidity. The plates were incubated for 24 h at 37 °C. Ciprofloxacin was used as antibacterial positive control (Hamdi et al., 2023).

## 2.8. Statistical analysis

All parameters were determined in triplicate for each sample. Statistical analysis was performed with SPSS (11.0). Duncan's test ( $p \leq 0.05$ ) was used to determine significant differences between means.

## 3. RESULTS & DISCUSSION

### 3.1. Sensory evaluation of the different formulas of date syrup and spirulina

To compare the consumer overall acceptability for the different formulas of date syrup/spirulina, the 9-point hedonic scale was used in this study (Fig. 2). Results indicated that adding spirulina to date syrup at concentrations of 2, 5, and 10% influenced significantly its sensory characteristics. Likewise, earlier research has demonstrated that the inclusion of spirulina in food products alters sensory properties, varying with the concentration levels used (Lucas et al., 2020; Mimouni et al., 2015). Specifically, the addition of a lower concentration of spirulina (2%) to date syrup resulted in higher overall acceptability scores compared to the higher concentrations (5 -

10%). This preference for lower concentration of spirulina may be linked to the lesser amount of nutrients added, which enhances the sensory qualities of the syrup. Substituting date syrup with 2% spirulina yielded the highest scores and was favored by consumers as the best and most acceptable option. As a result, this ratio was utilized for the preparation of functional syrup made from Alig dates and spirulina.

105°C. Moreover, this yield is in line to that reported by Ganbi (2012) when have used the water bath extraction method (38.94 %), but it is very lower compared to those obtained by ultrasonic (83.10 %), enzymatic (83.96 %) and microwave (77.52 %) extraction methods. These differences could be attributed mainly to the extraction method used.

The present study showed that Alig syrup was very riche in TS (83.98%), total sugars (66.17%)

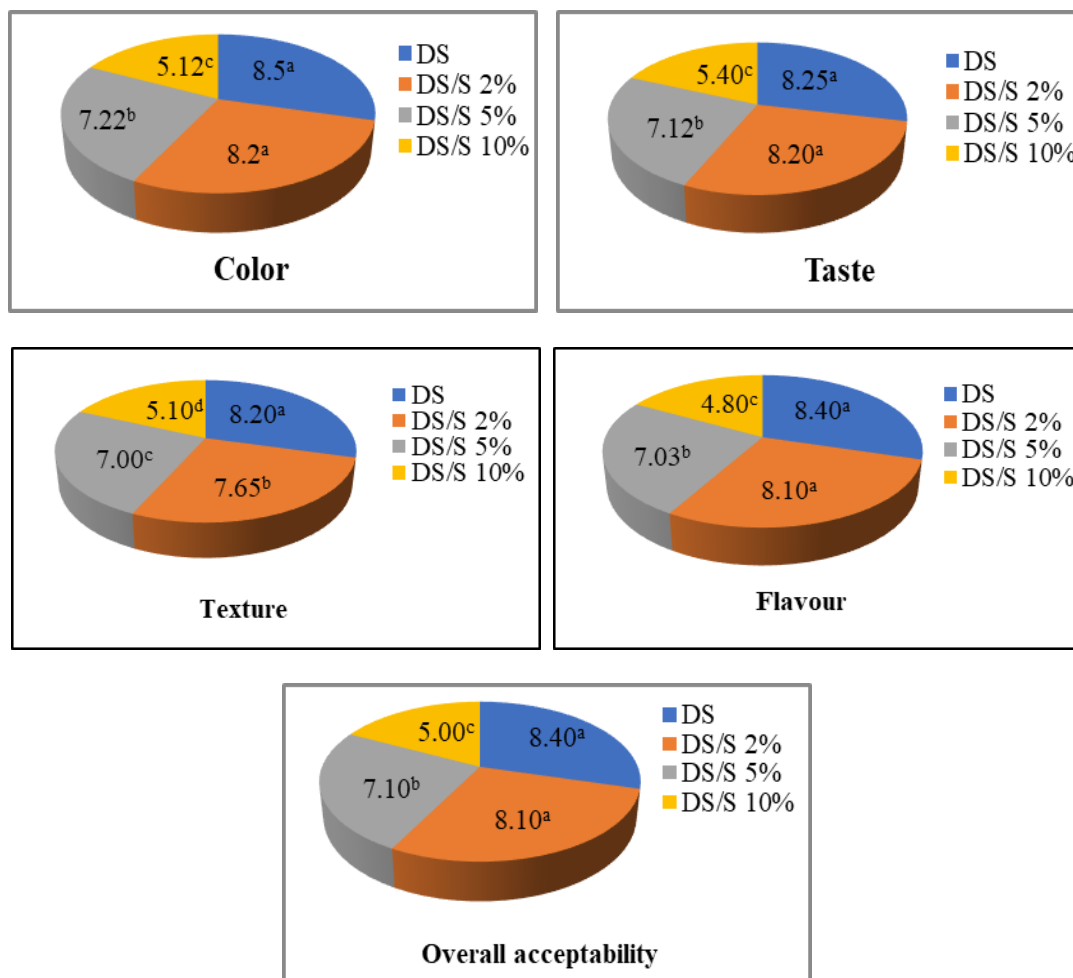


Fig. 2. Sensory properties of different formulas of date syrup with spirulina

DS: Date syrup; S: spirulina.

<sup>a, b, c</sup>: Means ± standard deviation (n = 60) followed by different letters indicate significant difference between samples according to Duncan’s test (p ≤ 0.05).

### 3.2. Physicochemical properties

The physicochemical composition of date syrup, spirulina and date syrup fortified with 2% spirulina are shown in Table 1.

Using the diffusion method followed by a concentration of date syrup to 72° Brix give a yield of 37%. This yield is similar to that found by Mimouni et al. (2023) for Algerian date syrups when concentrated at 65°C but is higher as compared to the same syrups concentrated at

and carbohydrates (78.57%). The amount of TS is related mainly to the sugar content due to the high sugar concentration in the syrup. Previous studies proved that glucose and fructose are the major sugar fractions of date syrup (Aleid & Haddadin, 2023; Ganbi, 2012; Mahdi et al., 2020). Comparing the total sugars content of Alig syrup to that of the original date fruit (EL Arem et al., 2011), we concluded that there was an augmentation in the total sugars level. This rise can be explained by the highest quantity of

**Table 1.** Physicochemical composition of tested samples

|                           | Date syrup                | Spirulina                 | Date syrup + 2% Spirulina |
|---------------------------|---------------------------|---------------------------|---------------------------|
| Extraction yield (%)      | 37                        | -                         | -                         |
| Moisture (%)              | 16.02 ± 0.2 <sup>a</sup>  | 9.14 ± 0.02 <sup>c</sup>  | 15.27 ± 0.3 <sup>b</sup>  |
| Total solids (%)          | 83.98 ± 0.2 <sup>c</sup>  | 90.85 ± 0.02 <sup>a</sup> | 86.91 ± 0.4 <sup>b</sup>  |
| Ash (%)                   | 1.97 ± 0.02 <sup>c</sup>  | 10.34 ± 0.01 <sup>a</sup> | 2.67 ± 0.03 <sup>b</sup>  |
| Total protein (%)         | 3.25 ± 0.24 <sup>c</sup>  | 66.49 ± 0.06 <sup>a</sup> | 4.56 ± 0.02 <sup>b</sup>  |
| Total Sugars (%)          | 66.17 ± 0.16 <sup>a</sup> | 21.11 ± 0.06 <sup>c</sup> | 64.49 ± 0.40 <sup>b</sup> |
| Total Carbohydrates (%)   | 78.57 ± 0.07 <sup>a</sup> | 12.69 ± 0.02 <sup>c</sup> | 77.25 ± 0.02 <sup>b</sup> |
| Total lipids (%)          | 0.19 ± 0.04 <sup>c</sup>  | 1.34 ± 0.02 <sup>a</sup>  | 0.25 ± 0.03 <sup>b</sup>  |
| Titrateable acidity (%)   | 0.81 ± 0.02 <sup>c</sup>  | 2.10 ± 0.02 <sup>a</sup>  | 0.93 ± 0.03 <sup>b</sup>  |
| pH                        | 5.05 ± 0.01 <sup>c</sup>  | 6.61 ± 0.03 <sup>a</sup>  | 5.51 ± 0.01 <sup>b</sup>  |
| Caloric value (kcal/100g) | 328.99                    | 328.78                    | 329.49                    |

<sup>a, b, c</sup>: Means ± standard deviation (n = 3) followed by different letters in the same line represent significant difference between samples according to Duncan's test (p ≤ 0.05).

date fruit used to produce a given quantity of syrup, because during syrup preparation we eliminated the presscake especially fibers including cellulose, pectocellulose and we kept the extractable part of date fruit which consists mostly of sugars syrup.

The moisture content of date syrup is an important factor that determines the syrup's ability to stay fresh and avoid spoilage by yeast fermentation. Moisture was the thirst predominant component in Alig syrup (16%). This value is higher than that found by Aleid and Haddadin (2023) for the Saudi Arabia date syrup (12%) but lower than those found by Bouhlali et al. (2020) (ranging from 30.41 to 45.93%) for Algerian and Marocain date fruit syrups respectively. These differences in moisture content may depend on the manufacturing process, the volume of water used, the temperature and the cooking time.

The protein, ash and lipid contents and the TA of the Alig syrup presented in Table 1 are in line to those previously found by Abbès et al. (2011) and Mahdi et al. (2020). The lowest quantity of protein observed in date fruit syrup compared to the original date fruit reported in our previous work (EL Arem et al., 2011) might be due to the degree of protein transformation during heat process through Maillard reactions. The protein quantity of the studied syrup was in accord to those found by (Bouhlali et al., 2020) for Moroccan date syrups ranging from 2.34 to 4.62%, but very high compared to those reported by Abbès et al. (2011) (0.97 – 1.50%) for Tunisian date syrups and Ganbi (2012) for Arabie Saudia date syrups (1.85-2.72%). These differences may be attributed to the extraction

and concentration methods applied for syrups production.

The pH is one of the most important measurements in food quality that may influence the length of storage time of food products. The pH value of the studied syrup was of 5.05 ± 0.01. This funding was in line with those reported in previous studies ranging from 4.10 to 6.15 (Abbès et al., 2011; Mahdi et al., 2022). This acidic pH value in Alig syrup may be related to either the removal of dispersed or insoluble components of an amphoteric type or structural changes of a few of the syrup's acidic molecules during processing.

As shown in Table 1, spirulina is a rich source of nutrients with an important amount of protein (66.49%) and total solids (90.85%), an appreciate amounts of total sugars (21.11%), total carbohydrates (12.69%) and ash (10.34%) and a suitable amount of lipids (1.34%) beside a TA and a pH value of 2.10% and 6.61, respectively. These amounts are in line to those found in previous studies (Bchir et al., 2019; Mesbah et al., 2022; Seghiri et al., 2019). The addition of spirulina at 2% to date syrup leads to a significant (p ≤ 0.05) decrease in the moisture, total sugars and carbohydrates contents. However, the amounts of TS, ash, protein and lipids and in the TA and the pH values were significantly (p ≤ 0.05) increased in comparison to unsupplemented syrup (Table 1). In accord to these results, an increase in TS and a decrease in moisture levels have been observed in date nectar, in yogurt, in ricotta cheese and in ice cream fortified with spirulina (Aljobair et al., 2021; Bchir et al., 2019; Ismail et al., 2023; Tiepo et al., 2021). The increase in the TS percentage may be attributed to the rise in the levels of

protein, fiber, and carbohydrates found in spirulina.

Conversely, fortifying date syrup with 2% spirulina resulted in a slight reduction in total sugars and carbohydrates content compared to unfortified syrup. Nonetheless, spirulina can enrich date syrup with various sugars such as glucose, mannose, galactose, rhamnose, and xylose, along with glycogen (AlFadhly et al., 2022). Consequently, the carbohydrates included in spirulina are simple to digest, as well as nutrient-dense, and may be consumed by elderly individuals and those who have intestinal malabsorption (AlFadhly et al., 2022). Our results are in accord to those found in various products fortified with spirulina like shakes for the elderly (Santos et al., 2016), yoghurt (Mesbah et al., 2022) and snack bars for schoolchildren nutrition (Lucas et al., 2020).

As reported in Table 1, the ash, total protein, and total lipids contents in date syrup supplemented with 2% spirulina were much higher than those of unsupplemented sample. In agreement with these findings, previous studies showed that the fortification of products with spirulina such as date nectar, whey based dairy drink, snack bars, ricotta cheese and yoghurt, enhanced the level of protein, ash and lipids in comparison to the control (Aljobair et al., 2021; Bchir et al., 2019; Ismail et al., 2023; Lucas et al., 2020). The rise of ash content in the final product is a result of the presence of significant amounts of essential minerals such as phosphorus, sodium, magnesium, calcium and potassium in spirulina (Paula da Silva et al., 2021). According to a study conducted by Janda-Milczarek et al. (2023), the inclusion of powdered spirulina in the daily diet led to a marked increase in the concentrations of essential elements, including iron (673 mg/kg), magnesium (4151 mg/kg) and potassium (16,686 mg/kg), while simultaneously resulting in significantly lower sodium content (9868

mg/kg). Proteins are essential nutrients for the correct functioning of the body, as they are its main building components. Spirulina is very rich of proteins and is a good source of essential amino acids such as histidine, threonine, phenylalanine, tyrosine, valine, leucine, isoleucine, methionine and cysteine beside to non-essential amino acids (AlFadhly et al., 2022; Aljobair et al., 2021). Consequently, the elevation of protein level in date syrup fortified with 2% spirulina may be followed by an elevation in its amino acid profile. Correspondingly, Aljobair et al. (2021) attributed the augmentation of the amino acid composition of date nectar fortified with spirulina to the high protein level and the high quality of the amino acid profile of spirulina.

Therefore, the present study demonstrated that *spirulina platensis* contributes to increasing the nutrients and caloric values of date syrup and may therefore increase the demand as functional foods at all stages of life.

### 3.3. Phytochemical composition and the antioxidant activity

The data presented in Table 2 shows that date syrup extract was characterized by important contents of TP ( $492.87 \pm 1.19$  mg GAE/100g DW) and CT ( $123.27 \pm 1.04$  mg CE/100g DW), and had a great free radical scavenging activity ( $91.22 \pm 0.21\%$ ).

A significant ( $p \leq 0.05$ ) linear correlation was obtained ( $R^2 = 0.904$ ) between DPPH radical scavenging activity of date syrup and the TPC. This high correlation coefficient indicates that polyphenolics are one of the main compounds responsible for the scavenging effect of date syrup. In fact, Abbès et al. (2013) evaluated the polyphenolic and tannin contents of two Tunisian date syrups extracted at 60 °C and 100 °C and showed that the hydrolysable tannins were degraded under high temperature (100 °C),

**Table 2.** Phytochemical contents and the antioxidant activity of tested samples

|  | Date syrup               | Spirulina                | Date syrup + 2% Spirulina |
|--|--------------------------|--------------------------|---------------------------|
| <b>TPC (mg GAE/100g DW)</b>                | 492.87±1.19 <sup>b</sup> | 141.42±1.08 <sup>c</sup> | 552.76±1.48 <sup>a</sup>  |
| <b>CTC (mg CE/100 DW)</b>                  | 123.27±1.04 <sup>b</sup> | 61.22±1.74 <sup>c</sup>  | 133.23±1.68 <sup>a</sup>  |
| <b>Antioxidant activity (% Inhibition)</b> | 91.22±0.21 <sup>a</sup>  | 64.03±0.12 <sup>b</sup>  | 92.67±0.38 <sup>a</sup>   |

<sup>a, b, c</sup>: Means ± standard deviation (n = 3) followed by different letters in the same line represent significant difference between samples according to Duncan's test ( $p \leq 0.05$ ). TPC: Total polyphenolic content. CTC: Condensed tannin content. DW: Dry weight.

causing increase of non-tannin content. Comparing our results with those reported in the literature we noticed that there are higher than those found in date syrups reported by Shahein et al. (2022) (472.14 mg/100 g and 72.84%) and in Alig syrup reported by Abbès et al. (2013) (409.85 mg GAE/100g FW and 43.17%) for TP and antioxidant activity, respectively. Difference between studies may be attributed to variety origin, soil fertilization, storage conditions, sampling, method and condition of extraction process. In other way, it could be noticed that the incorporation of spirulina to date syrup enhanced its TP and CT contents to  $552.76 \pm 1.48$  mg GAE/100g FW and  $133.23 \pm 1.68$  mg CE/100g FW, respectively, and improved its antioxidant activity to  $92.67 \pm 0.38\%$ . Similar behaviors in raising the total polyphenolic value and improvement in the antioxidant activity were obtained by the addition of spirulina powder to various food products (AlFadhly et al., 2022; Hassanzadeh et al., 2022; Mesbah et al., 2022). In fact, date syrup and spirulina both contain various polyphenolic compounds with a great capacity to scavenge free radicals like salicylic, chlorogenic, sinapic, caffeic, and cinnamic acids, beside various classes of flavonoids like isoflavones, flavonols, flavanones, and dihydrochalcones (Abbès et al., 2013; Guldás et al., 2022). These natural phenolic compounds likely possess a wide range of chemical and biological properties, including antioxidant and free radical-scavenging activities and can act as antioxidants synergistically or individually. These compounds are variable, and their amounts in the extract depend on the type and condition of extraction. Guldás et al. (2022) examined the phenolic profile of honey enriched with spirulina in comparison to a control one. They observed a strong antioxidant composition characterized by a blend of phenolic compounds present in both the control honey and the spirulina supplement. Furthermore, spirulina is a rich source of several natural bioactive

compounds, including phycocyanin, carotenoids (especially  $\beta$ -carotene), polyunsaturated fatty acids, xanthophyll, polysaccharides, vitamins and minerals that are associated with its antioxidant properties (AlFadhly et al., 2022).

### 3.4. Antibacterial activity

Date syrup, spirulina and date syrup fortified with spirulina were investigated for their antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* and represented as the minimum inhibitory concentration (MIC) (Table 3). Results showed that all sample extracts are more effective in inhibiting the growth of Gram-positive bacteria (MIC of 2.5-5 mg/mL) than Gram-negative bacteria (MIC of 5 - 30 mg/mL). The difference in sensitivity between Gram-positive and Gram-negative bacteria can be attributed to the morphological variations among these microorganisms, particularly in terms of cell wall permeability (Bereksi et al., 2018). In other way, no significant ( $p \geq 0.05$ ) difference was found in the MIC (20 - 30 mg/mL) for all sample extracts against *E. Coli* and *P. aeruginosa* suggesting that date syrup, spirulina and date syrup fortified with spirulina exert the same effect in retarding bacterial growth. However, *spirulina platensis* showed an interesting inhibitory activity against *S. aureus* and *K. pneumoniae* with MIC of 2.5 mg/mL and 5 mg/mL, respectively.

In comparison to the standard antibiotic ciprofloxacin used, we found that date syrup, spirulina and date syrup/spirulina extracts are less active against all tested microorganisms. This result may be due to the composition of extracts used consisting of complex mixtures of bioactive compounds. It has been shown that the antimicrobial activity of plant extracts was influenced by their chemical composition, the diffusion characteristics of their components, and the inherent resistance of the target microorganisms (Bereksi et al., 2018).

**Table 3.** Antibacterial activity of tested samples (MIC: mg/mL)

|                               | Date syrup      | Spirulina        | DS + 2% S        | Ciprofloxacin      |
|-------------------------------|-----------------|------------------|------------------|--------------------|
| <i>Staphylococcus aureus</i>  | $5 \pm 0.25^c$  | $2.5 \pm 0.11^b$ | $2.5 \pm 0.18^b$ | $0.031 \pm 0.22^a$ |
| <i>Escherichia coli</i>       | $20 \pm 0.15^b$ | $20 \pm 0.18^b$  | $20 \pm 0.20^b$  | $0.062 \pm 0.18^a$ |
| <i>Pseudomonas aeruginosa</i> | $30 \pm 0.10^b$ | $30 \pm 0.14^b$  | $30 \pm 0.18^b$  | $0.125 \pm 0.14^a$ |
| <i>Klebsiella pneumoniae</i>  | $20 \pm 0.14^d$ | $5 \pm 0.20^b$   | $10 \pm 0.20^c$  | $0.125 \pm 0.16^a$ |

<sup>a, b, c</sup>: Means  $\pm$  standard deviation ( $n = 3$ ) followed by different letters in the same line represent significant differences between sample according to Duncan's test ( $p \leq 0.05$ ). DS: Date syrup; S: Spirulina; MIC: Minimum inhibitory concentration

In other way, comparing the results of the present study to those reported in the literature, we note that our findings are more significant than those obtained by Taleb et al. (2016a) that have reported a MIC of 30 mg/mL against the growth of *E. coli* and *S. aureus*. Similarly, Mimouni et al. (2023) proved that date syrup has interesting inhibitory activities against *E. coli* and *S. aureus*. On the other hand, Usharani et al. (2015) evaluated the antibacterial activity of spirulina ethanolic extract against pathogenic bacteria and fungi and found MIC values higher than those found in the present study, varying from 5 to 10 mg/mL for *E. coli*, *P. aeruginosa* and *K. pneumoniae*. However, for *S. aureus*, we have revealed a MIC value in the spirulina ethanolic extract of 2.5 mg/mL, which are greater. These differences may be attributed to sample origin, extraction method and solvent used. The fortification of date syrup with spirulina improved its antibacterial activity against *S. aureus* (MIC: 2.5 mg/mL) and *K. pneumoniae* (MIC: 10 mg/mL). These results may be related to a synergic effect of different compounds present in spirulina and date syrup like phenolic and flavonoids compounds (catechin, quercetin, gallic acid, apigenin, kaempferol) which were known for their high antibacterial potency. In fact, Taleb et al. (2016a) evaluated the antibacterial activity of date syrup polyphenols and have demonstrated their capacity to inhibit the growth of *E. coli* and *S. aureus* by generating H<sub>2</sub>O<sub>2</sub>, and assumed that date syrup polyphenols are active intermediates directly involved in inducing oxidative stress in bacteria as a result of hydrogen peroxide generation. In another study, Alshuniaber et al. (2021) isolated polyphenolic compounds from spirulina and assessed their antibacterial activity against drug-resistant foodborne bacterial pathogens. They discovered that fraction B of the methanolic extract exhibited greater efficacy against both Gram-positive and Gram-negative pathogens compared to the control antibiotic, attributed to the presence of potential secondary metabolites such as benzophenone and dihydro-methylphenylacridine and carbanilic acid. Moreover, previous study showed that the C-phycoerythrin, lipids and fatty acids mainly  $\gamma$ -linolenic acid, terpenols, sterols, polysaccharides, peptides, and protein metabolites secreted by spirulina exhibit a potent antibacterial activity against some pathogenic bacteria and fungus (Seghiri et al., 2019).

#### 4. CONCLUSION

The research has focused in the development of novel date syrup fortified with 2% spirulina. This innovative syrup possesses a distinguished composition characterized by elevated levels of protein, ash, and polyphenolic compounds, as well as potent antioxidant and antibacterial activities, all of which enhance its nutritional and caloric value. Given spirulina's rich nutritional composition, its inclusion in the date syrup formulation resulted in a functionally enriched product with improved health benefits. These findings open the possibility to use the date syrup fortified with spirulina as natural ingredients in the formulation of novel nutraceutical foods contributing to the increasing demand of consumers by products elaborated with natural additives. Future researches are necessary to elucidate their bioavailability when they are ingested as a part of a food matrix.

#### Acknowledgment

The authors extend their appreciation to European Union for funding this work through the EMORI program and managed by the ANPR.

#### REFERENCES

- Abbès, F., Bouaziz, M.A., Blecker, C., Masmoudi, M., Attia, H. & Besbes, S. (2011). Date syrup: Effect of hydrolytic enzymes (pectinase/cellulase) on physicochemical characteristics, sensory and functional properties. *LWT - Food Science and Technology*, 44, 1827-1834.
- Abbès, F., Kchaou, W., Blecker, C., Ongena, M., Lognay, G., Attia, H. & Besbes, S. (2013). Effect of processing conditions on phenolic compounds and antioxidant properties of date syrup. *Industrial Crops and Products*, 44, 634-642.
- Aleid, S.M. & Haddadin, J.S. (2023). Valorization and Chemical Constituents Assessments of Khalas dates fruit, syrup and pits. *Current Research in Nutrition and Food Science*, 11, 77-87.
- AlFadhly, N.K.Z., Alhelfi, N., Altemimi, A.B., Verma, D.K., Cacciola, F. & Narayanankutty, A. (2022). Trends and technological advancements in the possible food applications of spirulina and their health benefits: A Review. *Molecules*, 27, 5584.
- AlFaris, N.A., AlTamimi, J.Z., AlMousa, L.A., AlGhamidi, F.A. & Albaridi, N.A. (2022). Date-derived industries: A review of common products, manufacturing methods, and leading

- countries. Emirates Journal of Food and Agriculture, 34, 86-97.
- Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M. & Shahidi, F. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. Journal of Agricultural and Food Chemistry, 53, 7592-7599.
- Ali, A., Khalid, S., Irfan, A. & Din, A. (2023). Formulation and evaluation of date syrup flavored soy milk yogurt, a novel plant based alternative of dairy products. Biology and Life Sciences Forum, 26, 1-7.
- Aljobair, M.O., Albaridi, N.A., Alkuraieef, A.N. & AlKehayez, N.M. (2021). Physicochemical properties, nutritional value, and sensory attributes of a nectar developed using date palm puree and spirulina. International Journal of Food Properties, 24, 845-858.
- Alqahtani, N.K., Alnemr, T.M., Makki, H.M.M., Ali, D.O.M., Mohamed, H.A., Saleh, F.A., Tami, S.H., Darrag, H.M., Taha, A.A., Salih, Z.A. & Aboufarrag, H.T. (2025). Date syrup (dibs) as healthy natural sweetener ingredient in peanut butter processing: Impact on physico-chemical, sensory, textural profile and microstructure properties. LWT - Food Science and Technology, 221, 117590.
- Alshuniaber, M.A., Krishnamoorthy, R. & AlQhtani, W.H. (2021). Antimicrobial activity of polyphenolic compounds from spirulina against food-borne bacterial pathogens. Saudi Journal of Biological Sciences, 28, 459-464.
- Bchir, B., Felfoul, I., Bouaziz, M.A., Gharred, T., Yaich H., Noumi, E., Snoussi, M., Bejaoui, H., Kenzali, Y., Blecker, C. & Attia, H. (2019). Investigation of physicochemical, nutritional, textural, and sensory properties of yoghurt fortified with fresh and dried spirulina (*Arthrospira platensis*). International Food Research Journal, 26, 1565-1576.
- Bereksi, M.S., Hassaine, H., Bekhechi, C. & Abdelouahid, D.E. (2018). Evaluation of antibacterial activity of some medicinal plants extracts commonly used in Algerian traditional medicine against some pathogenic bacteria. Pharmacognosy Journal, 10, 507-512.
- Bouhlali, E.T., Derouich, M., Meziani, R., Bourkhis, B., Filali-Zegzouti, Y. & Alem, C. (2020). Nutritional, mineral and organic acid composition of syrups produced from six Moroccan date fruit (*Phoenix dactylifera* L.) varieties. Journal of Food Composition and Analysis, 93, 103591.
- Boughzala, Y. & Ben Mahmoud, N. (2022). Valorization of the date industry in Tunisia by combining "modern" and "traditional" knowledge and techniques: difficulties, successes and prospects. ISTE Ltd. London, UK, 1-20.
- Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 7, 248-54.
- Dubois, M., Gilles, K., Hamilton, K., Rebers, A. & Smith, F. (1956). Colorimetric method for determination of sugars and related Substances. Analytical Biochemistry, 28, 350-356.
- EL Arem, A., Guido, F., Saafi, E.B., Issaoui, M., Zayene, N., Ferchichi, A., Hammami, M., Helal, A.N. & Achour, L. (2011). Chemical and aroma volatile compositions of date palm (*Phoenix dactylifera* L.) fruits at three maturation stages. Food Chemistry, 127, 1744-1754.
- Faith, E.O., Majiyabo, E.M., Eunice, A.A., Jasper, O.E. & Smah, A.C. (2023). Rheological properties and the evaluation of the effects of date syrup (*Phoenix dactylifera* L.) sugar substitute on food composition of Bambara flour (*Vigna subterranea*) composite bread. Journal of Primary Care and General Practice, 6, 1-7.
- Gab-Allah, R.H. & Shehta, H., 2020. A new functional whey beverage, containing calcium and date syrup (Dibs). Egyptian Journal of Nutrition, 35, 53-75.
- Ganbi, H.H.A. (2012). Production of nutritious high quality date (*phoenix dactylifera*) fruits syrup (dibs) by using some novel technological approaches. Journal of Applied Sciences Research, 8, 1524-1538.
- Guldas, M., Gurbuz, O., Cakmak, I., Yildiz, E.& Sen, H. (2022). Effects of honey enrichment with *spirulina platensis* on phenolics, bioaccessibility, antioxidant capacity and fatty acids. LWT- Food Science and Technology, 153, 112461.
- Hamdi, A., Horchani, M., Ben Jannet, H., Snoussi, M., Noumi, E., Bouali, N., Kadri, A., Polito, F., de Feo, V. & Edziri, H. (2023). *In vitro* screening of antimicrobial and anti-coagulant activities, ADME profiling, and molecular docking study of citrus *limon* L. and citrus *paradisi* L. cold-pressed volatile oils. Pharmaceuticals, 16, 669.
- Hassanzadeh, H., Ghanbarzadeh, B., Galali, Y. & Bagheri, H. (2022). The physicochemical properties of the spirulina-wheat germ-enriched high-protein functional beverage

- based on pearcantaloupe juice. Food Science and Nutrition, 10, 3651-3666.
- Ismail, H.A., El-Sawah, T.H., Ayyash, M., Adhikarid, B. & Elkot, W.F. (2023). Functionalization of Ricotta cheese with powder of *spirulina platensis*: physicochemical, sensory, and microbiological properties. International Journal of Food Properties, 26, 1, 1968-1983.
- Janda-Milczarek K., Szymczykowska K., Jakubczyk K., Kupnicka P., Skonieczna- Zydecka K., Pilarczyk B., Tomza-Marciniak A., Ligenza A., Stachowska E. & Dalewski B. (2023). Spirulina Supplements as a Source of Mineral Nutrients in the Daily Diet. Applied Sciences, 13, 1011.
- Julkunen-Titto, R. (1985). Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. Journal of Agricultural and Food Chemistry, 33, 213-217.
- Lucas, B.F., da Rosa, A.P.C., de Carvalho, L.F., de Morais, M.G., Santos, T.D. & Costa, J.A.V. (2020). Snack bars enriched with spirulina for schoolchildren nutrition. Food Science and Technology, 40, 146-152.
- Madi, F., Hachicha, R., Gamero, J.E.R., Gupte, A.P., Gronchi, N., Haddad, M., Favaro, L., Casella, S. & Basaglia, M. (2024). Exploitation of spoilage dates as biomass for the production of bioethanol and polyhydroxyalkanoates. Renewable Energy, 220, 119655.
- Mahdi, Z.I., El-Sharnouby, G.A. & Sharoba, A.M. (2022). Physicochemical properties and microbiological quality of dates syrup prepared from some Egyptian and Iraqi dates palm (*Phoenix dactylifera* L.) Fruits. Egyptian Journal of Chemistry, 65, 175-184.
- Mesbah, E.E., Matar, A.A. & Karam-Allah, A.A. (2022). Functional properties of yoghurt fortified with *spirulina platensis* and milk protein concentrate. Journal of Food and Dairy Sciences, 13, 1-7.
- Mimouni, M., Atika, B. & Hafsia, B. (2023). Bioactive metabolite composition and biological properties of date syrups of four cultivars. Journal of Food and Nutrition Research, 11, 158-167.
- Mimouni, Y., Siboukeur, O. & Merabet, I. (2015). Effect of adding the spirulina (*Arthrospira platensis*), to date syrup on glycemic response and its effectiveness to reduce post prandial blood glucose. International Journal of Science and Research, 4, 1-7.
- Podgórska-Kryszczuk, I. (2024). *Spirulina* - An invaluable source of macro- and micro-nutrients with broad biological activity and application potential. Molecules, 29, 5387.
- Santos, T.D., Freitas, B.C.B., Moreira, J.B., Zanfonato, K. & Costa, J.A.V. (2016). Development of powdered food with the addition of spirulina for food supplementation of the elderly population. Innovative Food Science and Emerging, 37, 216-220.
- Seghiri, R., Kharbach, M. & Essamri, A. (2019). Functional composition, nutritional properties, and biological activities of Moroccan spirulina microalga. Journal of Food Quality, 1-11
- Shahein, M.R., Atwaa, E.S.H., Elkot, W.F., Hijazy, H.H.A., Kassab, R.B., Alblihed, M.A. & Elmahallawy, E.K. (2022). The impact of date syrup on the physicochemical, microbiological, and sensory properties, and antioxidant activity of bio-fermented camel milk. Fermentation, 192, 2-13.
- Paula da Silva S., Ferreira do Valle A. & Perrone D. (2021). Microencapsulated *Spirulina maxima* biomass as an ingredient for the production of nutritionally enriched and sensorially well-accepted vegan biscuits. LWT - Food Science and Technology, 142, 110997.
- Taleb, H., Maddocks, S.E., Morris, R.K. & Kanekanian, A.D. (2016a). The antibacterial activity of date syrup polyphenols against *S. aureus* and *E. coli*. Frontiers in Microbiology, 7, 198.
- Taleb, H., Morris, R.K., Withycombe, C.E., Maddocks, S.E. & Kanekanian, A.D. (2016b). Date syrup derived polyphenols attenuate angiogenic responses and exhibits anti-inflammatory activity mediated by VEGF and COX-2 expression in endothelial cells. Nutrition Research, 36, 636-647.
- Tiepo, C.B.V., Gottardo, F.M., Mortari, L.M., Bertol, C.D., Reinehr, C.O. & Colla, L.M. (2021). Addition of *spirulina platensis* in handmade ice cream: Physicochemical and sensory effects. Brazilian Journal of Development, 7, 88106-88123.
- Usharani, G., Srinivasan, G., Sivasakthi, S. & Saranraj, P. (2015). Antimicrobial activity of *spirulina platensis* solvent extracts against pathogenic bacteria and fungi. Advances in Biological Research, 9, 292-298.
- Wang, Y.Y., Xu, B.L., Dong, C.M. & Yan-Yan, S. (2023). The nutritional value of spirulina and utilization research. Life Research, 6, 1-12.