BIOCHEMICAL COMPOSITION AND FATTY ACID PROFILE OF THE MARINE MICROALGA *Isochrysis galbana* DRIED WITH DIFFERENT METHODS

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**MATERIALS AND METHODS**

**Algae culture**

The initial purified seed of *I. galbana* was obtained from the Persian Gulf Biotechnology Park (Gheshm, Iran) and transported to Zakarya-e-Razi Laboratory Complex, IAU University (Tehran, Iran). The algae was grown in f2 medium (Guillard and Ryther, 1962) under culture conditions: 3511 ± 3511 lux, temperature (°C) 20 ± 2°C, and 2.5% salinity. After 14 days of culture, the algae was harvested by using a Sigma 3-30KS centrifuge (Osterode, Germany) at 7100 × g for 15 min at 4°C.

**Drying methods**

The fresh microalgal biomass (120 L) was dehydrated using four different drying techniques: sun dried (SD) at 22–36 °C for 2 days, freeze dried (FD) at -84°C under high vacuum conditions (0.04 mbar) for 12 h by a Christ Alpha 1–4 freeze dryer (Christ, Germany), oven dried (OD) at 60 °C for 12 h, and spray dried (SPD) (liquid suspension of algae) through a Buchi B-191 spray dryer (Buchi Laboratoriums-Technik AG, Switzerland) at 140–150 °C and 80–85 °C as the inlet and outlet temperatures, respectively for 6–8 seconds.

**Chlorophyll a, b and carotenoid contents**

Chlorophyll (Chl) contents of each treatment was determined using method described by Yang et al. (1998). Briefly, the dried samples (10 mg) were mixed with 5 mL of 80% acetone in a vortex for 5 min, centredug at 1500 × g for 5 min, and the supernatant was collected. Chl a (µg/mL) = 12.25 × A663 - 2.25 × A645 and Chl b (µg/mL) = 20.31 × A645 - 2.25 × A663.

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In this paper, the effects of different drying methods on proximate composition, pigment contents, and fatty acids profile of *Isochrysis galbana* were evaluated. The microalgal biomass was dried by freeze drying (FD), oven drying (OD), spray drying (SPD), and sun drying (SD) methods to identify the best procedure with the lowest negative impacts on the algae nutritional values including pigments, proximate composition, and fatty acid profile. The highest protein content was obtained in FD (62.20±0.15 %). Also, the lipid content of the dried microalgae was significantly influenced by the drying process, while among all treatments, the highest and lowest values were measured in FD (13.77±0.42 %) and SD (11.68±0.16 %) samples, respectively (P<0.05). The highest chlorophyll a (0.90±0.028 µg/mg), b (0.605±0.007 µg/mg), and total carotenoids (1.057±0.056 µg/mg) were observed in FD samples. However, the lowest levels of chlorophylls and total carotenoid pigments were obtained in SD and SPD, respectively. The lipid profiling analysis showed the highest level of polyunsaturated fatty acids (PUFAs) in FD method (41.51%), while the maximum saturated fatty acids were observed in SD (54.89 %) followed by OD (51.41%). Also, the highest docosahexaenoic acid (12.41 %) were measured in FD compared to others (P<0.05). In conclusion, freeze drying method would be an efficient dewatering post-harvesting technique for the marine microalga *I. galbana* with the lowest impact on the nutritional quality in particular PUFAs content compared to the other studied drying methods.

**Keywords:** Carotenoids, Drying methods, Fatty acids, *Isochrysis galbana*
Total carotenoids ($\text{mg g}^{-1}$)

\[
\text{Total carotenoids} = \frac{\text{Chl a} + \text{Chl b}}{1000 \times A_{470}} - (1.90 \times \text{Chl a} - 0.14 \times \text{Chl b})
\]

Where, A is the optical density (OD) by the spectrophotometer.

**Proximate composition**

Residual moisture in each sample (1 g) was determined through an automatic moisture analyzer (Sartorius MA30, Germany) at 105°C for up to 60 min until reached a constant weight of the sample. Total protein was calculated (N x 6.25) by the Kjeldahl method (AOAC, 2000). The extraction of total lipids was carried out in accordance with Bligh and Dyer (1959) method. Total ash content was calculated by a muffle furnace at 550 °C for 8 h.

**Fatty acids profile**

Fatty acids (FA) composition of different dried samples were measured according to Coenen et al. (1993) method and the results were expressed as % of total fatty acids (TFAs). FAs were trans esterified in 2% H2SO4-methanol solution at 80°C for 1 h, followed by adding n-heptane to the mixture prior to stirring and centrifugation at 2150 g for 10 min. Fatty acid methyl esters (FAMEs) were analyzed by a Younglin ACME 6100 Gas Chromatograph (Anyang, Korea) equipped with a mass selective detector (Dikmacap-2330, a capillary column DB-2560 (30 m x 0.25 mm x 0.25 μm), and helium used as the carrier gas with a flow rate of 2.6 mL/min. The injection temperature, oven temperature, and interface temperature were set at 250, 200 and 260 °C, respectively, and the split ratio was 1:100.

**Statistical analysis**

All measurements were carried out in triplicates and the mean data and their standard errors were obtained. All variances were checked for normality and homogeneity, and one-way analysis of variance (ANOVA) was used to determine the significance of differences in the dependent variables. Post-hoc Tukey-test at reliability level of 5% was used to identify differences between each level of treatment.

**RESULTS**

**Changes in the biochemical composition**

Results of the proximate biochemical composition are summarized in Table 1. The highest protein (62.20±0.15 %) and moisture (6.46±0.134 %) contents were determined in FD and SD treatments, respectively. The lipid content was significantly elevated in SPD samples (P<0.05). Also, minimum level of lipid content (17.89±0.12%) was observed in FD samples. The highest and lowest ash content were obtained in FD (13.77±0.42 %) and SD (11.68±0.16%), respectively.

### Table 1 Proximate composition (% dry weight) of dehydrated * Isochrysis galbana* by different drying methods. OD: oven dried, FD: freeze dried, SD: sun dried, and SPD: spray dried.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OD %</th>
<th>FD %</th>
<th>SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>3.94±0.29 b</td>
<td>4.03±0.14 a</td>
<td>4.10±0.22 b</td>
</tr>
<tr>
<td>Protein</td>
<td>60.08±0.53 a</td>
<td>62.20±0.15 a</td>
<td>60.53±0.38 a</td>
</tr>
<tr>
<td>Lipid</td>
<td>19.13±0.09 c</td>
<td>17.89±0.12 c</td>
<td>20.72±0.22 a</td>
</tr>
<tr>
<td>Ash</td>
<td>12.83±0.52 a</td>
<td>13.77±0.42 a</td>
<td>12.38±0.37 a</td>
</tr>
</tbody>
</table>

Changes in the pigment contents

As shown in Table 2, the highest content of Chl a was obtained in FD (0.902±0.028 µg/mg) compared to other treatments (P<0.05). Also, the highest Chl b was measured in FD (0.605±0.007 µg/mg), while the minimum value was obtained in SD (0.490±0.004 µg/mg). Further, the highest total carotenoid was obtained in FD (1.057±0.056 µg/mg), while SPD samples showed the minimum value (0.735±0.028 µg/mg) (P<0.05).

### Table 2 Pigment contents (µg/mg dry weight) of dehydrated * Isochrysis galbana* by different drying methods. OD: oven dried, FD: freeze dried, SD: sun dried, and SPD: spray dried.

<table>
<thead>
<tr>
<th>Pigments</th>
<th>OD µg/mg</th>
<th>FD µg/mg</th>
<th>SD µg/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>0.738±0.004 a</td>
<td>0.902±0.028 b</td>
<td>0.776±0.014 a</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>0.510±0.002 b</td>
<td>0.605±0.007 a</td>
<td>0.507±0.003 b</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>0.844±0.049 b</td>
<td>1.057±0.056 a</td>
<td>0.735±0.028 b</td>
</tr>
</tbody>
</table>

| Mean values (±standard error) followed by different letters in the same row indicate a statistical difference (n=3, P<0.05).

### Table 3 Fatty acids profile (% total fatty acid) of dehydrated *Isochrysis galbana* by different drying methods. OD: oven dried, FD: freeze dried, SD: sun dried, and SPD: spray dried.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>OD %</th>
<th>FD %</th>
<th>SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprylic acid</td>
<td>0.18±0.01 a</td>
<td>0.51±0.02 b</td>
<td>1.00±0.03 a</td>
</tr>
<tr>
<td>Capric acid</td>
<td>2.59±0.09 a</td>
<td>3.88±0.01 a</td>
<td>3.87±0.32 a</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>1.47±0.08 a</td>
<td>0.9±0.01 d</td>
<td>2.82±0.04 a</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>16.91±0.14 a</td>
<td>16.03±0.12 a</td>
<td>16.88±0.18 a</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>1.93±0.15 a</td>
<td>18.33±0.12 a</td>
<td>21.99±0.03 a</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>1.06±0.01 a</td>
<td>0.91±0.04 a</td>
<td>2.48±0.33 a</td>
</tr>
<tr>
<td>Arachidic acid</td>
<td>0.06±0.02 a</td>
<td>0.03±0.01 a</td>
<td>0.49±0.01 a</td>
</tr>
<tr>
<td>Heneicosylic acid</td>
<td>1.19±0.05 a</td>
<td>0.08±0.01 a</td>
<td>0.07±0.02 a</td>
</tr>
<tr>
<td>Behenic acid</td>
<td>0.05±0.00 a</td>
<td>0.04±0.00 a</td>
<td>0.08±0.00 a</td>
</tr>
</tbody>
</table>

### Table 4 Pigments (µg/mg dry weight) of dehydrated *Isochrysis galbana* by different drying methods. OD: oven dried, FD: freeze dried, SD: sun dried, and SPD: spray dried.

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</table>

| Mean values (±standard error) followed by different letters in the same row indicate a statistical difference (n=3, P<0.05).
Changes in fatty acids compositions

FAs composition of dewatered *I. galbana* by different drying procedures is given in Table 3. Myristic acid (14:0) and palmitic acid (C16:0) were the most abundant saturated fatty acids (SFA) which were the highest values in all treatments except for FD samples. Palmitoleic acid (C16:1) and oleic acid (C18:1) were primarily proportions of MUFA, and the highest values was measured in FD treatment (7.76±0.05 % of TFAs and 16.50±0.99 % of TFAs, respectively). Linoleic acid (C18:2), stearidonic acid (C18:4) and docosahexaenoic acid (C22:6) were the major fatty acids in PUFAs component. Also, the highest linoleic acid (11.94±0.20 % of TFAs), stearidonic acid (14.41±0.30 % of TFAs), and DHA (12.41±0.08 % of TFAs) contents were obtained in FD samples compared to other treatments (P<0.05).

**DISCUSSION**

The results indicated that the highest protein content was obtained in FD method compared to other methods. The reason why a higher protein level was seen in FD samples compared to hot air-drying methods might be in part due to the loss of protein by thermal breakdown and with some volatile nitrogen based compounds which can lead to reducing of crude proteins content. However, FD probably preserve the protein content of microorganisms against direct heating.

**REFERENCES**


CONCLUSION

We found that different drying methods had significant impact on some chemical and biochemical compositions, particularly fatty acids profile of *I. galbana*. The freeze-dried samples showed the highest level of protein, lipid, Chl a, b, and carotenoids, whereas the lowest levels were measured in sun drying method. Our findings highlights that different drying methods not only could cause different fat yield, but also could affect the total fatty acid production. The sun-dried biomass showed the highest content of SFAs, including C14:0 and C16:0, and the maximum level of MUFA and PUFAs were obtained in the freeze-dried samples. The results confirmed that freeze drying method can provide a better preservation method compared to other dehydration methods with the lowest opposition in *I. galbana*.


