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Compare the Chlorophyll Amount in Three Brown Algae Species of the Persian Gulf by Using Three Solvents and Applying Two Formulas

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Abstract: Chlorophyll is the green pigment in plants that helps to absorb sunlight and converts it into energy. It is believed that it is beneficial for the human body. Therefore in this study, the chlorophyll amount in three brown algae species (*Sirophysalis trinodis, Polycladia myrica* and *Colpomenia sinuosa*) of the Persian Gulf by using three solvents and applying two formulas was compared. Acetone, diethyl ether and methanol were used as solvents. Results showed that the chlorophyll amount in different species dependent on solvent type and cell wall structure of algae. The highest chlorophyll a and x+c contents were observed in *S. trinodis* species. Also, the highest content of chlorophyll c_1+c_2 was showed in *C. sinuosa* by the extraction with diethyl ether solvent. In both formulas was no significant difference between the same solvents in the amount of chlorophyll a. In general, the aim of this study due to the diversity and dispersion of algae and also their potential use in the food industry, cosmetics and pharmaceutical goods was to choose a suitable solvent for better extraction of chlorophyll as an active ingredient. Hence, in this study, the acetone was a better solvent.

Keywords: Chlorophyll, Brown Algae, Solvent, Formula, Persian Gulf

1. Introduction

Chlorophyll is a light-sensitive pigment with special electrical properties. All photosynthetic plants are containing chlorophyll a. But the existence of subsidiary chlorophylls such as b, c and d is depending on the type of plant. For example, higher plants have two chlorophyll a and b, which chlorophyll b has a structure similar to chlorophyll a. The difference between these two chlorophylls is in their R groups (if R is a CH_3 group, chlorophyll is the type of "a" and if R is a CHO group, chlorophyll is the type of "b"). Among the algae, the brown alga is lacking chlorophyll b [8]. The carotene level is also depending on the algae species and environmental conditions [2].

According to medical science, foods that contain chlorophyll can be wiped the blood flow, relieved the bad breath, deactivated the carcinogenic substance and prevented the tooth decay [3]. So, the presence of chlorophyll and its extraction is very important. But the human and animal bodies not have the ability to synthesize chlorophyll and must obtain it through diet. There are different techniques (the spectrophotometry, the fluorimetery, and the high-performance liquid chromatography) for the determination of the chlorophyll content [4]. These techniques can be used in various other fields of science and technology such as medicine, pharmaceuticals and food technology. For example, the spectrophotometric technique is a sample procedure which needs only general equipment and reagents, and can be applied in common laboratories [7]. Therefore in this study, the chlorophyll levels of three brown algae species

of the Persian Gulf were determined by using the spectrophotometric technique and three different solvents (acetone, diethyl ether and methanol) and also examined by applying two formulas.

2. Method

2.1. Sample Collection and Preparation

Three types of brown macroalgae called Sirophysalis trinodis, Polycladia myrica and Colpomenia sinuosa were collected from Qeshm Island (the southern coast of the Persian Gulf), washed with tap water to remove salt and epiphytes. Then they were sundried for 3 days and pulverized to obtain uniform particles (0.5 mm). The pulverized seaweed sample was stored in airtight plastic bags and was put up in a desiccator prior to design of experiments.

2.2. Extraction Process

The extraction process was done using the method of Jeffrey and Humphrey [6] and Dere et al. [2] with a slight modification. Briefly, 1 g sample with 50 ml of three solvents (acetone 100%, diethyl ether 95% and methanol 96%) was separately homogenized by a homogenizer (IKA T2 Digital, Ultraturrax) at 15000 rpm for 2 min at room temperature. Then, the mixture was filtered through two layer cheese cloths and centrifuged at 2500 rpm for 10 min. The supernatant was separated. The absorbance was measured at 400-700 nm using UV–Vis spectrophotometer (Biochrom Ltd, Cambridge CB4OFJ England). The experiments were performed in triplicate. Also in this study, two different formulas were used in the calculation of chlorophylls a, c_1 and c_2 in equal proportions and total carotene levels (Table 1).

Table 1. *Two formula used in the calculation of brown algae chlorophyll (µg / g of fresh weight).*

	The first formula	**The second formula**
Acetone	$C_a = 11.75\ A_{662} - 2.350\ A_{645}$ $C_b = 18.61\ A_{645} - 3.960\ A_{662}$ $C_{x+c} = 1000\ A_{470} - 2.270\ C_a - 81.4\ C_b / 227$	$C_a = 11.47\ A_{664} - 0.40\ A_{630}$ $C_{c1+c2} = 24.36\ A_{630} - 3.73\ A_{664}$
Diethyl ether	$C_a = 10.05\ A_{662} - 0.766\ A_{644}$ $C_b = 16.37\ A_{644} - 3.140\ A_{662}$ $C_{x+c} = 1000\ A_{470} - 1.280\ C_a - 56.7\ C_b / 230$	$C_a = 11.47\ A_{664} - 0.40\ A_{630}$ $C_{c1+c2} = 24.36\ A_{630} - 3.73\ A_{664}$
Methanol	$C_a = 15.65\ A_{666} - 7.340\ A_{653}$ $C_b = 27.05\ A_{653} - 11.21\ A_{666}$ $C_{x+c} = 1000\ A_{470} - 2.860\ C_a - 129.2\ C_b / 245$	$C_a = 11.47\ A_{664} - 0.40\ A_{630}$ $C_{c1+c2} = 24.36\ A_{630} - 3.73\ A_{664}$

C_a = Chlorophyll a, C_b = Chlorophyll b, C_{x+c} = Total carotene, C_{c1+c2} = Chlorophylls c_1 and c_2 in equal proportions

2.3. Statistical Analysis

Statistical analysis was performed using Analysis of Variance (ANOVA). The LSD and Duncan tests (p = 0.05) was used to determine any significance of differences between specific means (Sigma Stat, 21 version, 2012, USA). All determinations were performed in triplicate, and the data are expressed in terms of mean ± standard deviation (SD).

Table 2. *Chlorophyll contents of three brown algae species in different solvents (µg / g fresh weight) and applying two formulas.*

		Formula (1)			**Formula (2)**		
		Acetone	**Diethyl ether**	**Methanol**	**Acetone**	**Diethyl ether**	**Methanol**
S. trinodis	C_a	5.41 ± 0.94^{ab}	3.35 ± 0.93^{cd}	4.25 ± 1.71^{bc}	5.80 ± 0.94^{a}	4.16 ± 1.15^{bcd}	4.14 ± 1.64^{bcd}
	C_b	-	-	-	-	-	-
	C_{x+c}	2.40 ± 0.26^{a}	2.11 ± 0.55^{ab}	1.46 ± 0.54^{bcd}			
	C_{c1+c2}				1.28 ± 0.46^{b}	0.94 ± 0.22^{b}	1.11 ± 0.79^{b}
P. myrica	C_a	4.46 ± 0.65^{abc}	2.69 ± 0.36^{de}	3.42 ± 0.41^{cd}	5.06 ± 0.79^{ab}	3.32 ± 0.55^{cd}	3.41 ± 0.61^{cd}
	C_b	-	-	-	-	-	-
	C_{x+c}	1.93 ± 0.13^{abc}	1.46 ± 0.26^{bcd}	1.01 ± 0.22^{de}			
	C_{c1+c2}				2.09 ± 1.60^{b}	2.36 ± 0.22^{b}	2.01 ± 0.79^{b}
C. sinuosa	C_a	0.63 ± 0.14^{f}	1.52 ± 0.19^{ef}	1.09 ± 0.24^{f}	1.22 ± 0.24^{ef}	1.70 ± 0.38^{ef}	1.21 ± 0.19^{ef}
	C_b	-	-	-	-	-	-
	C_{x+c}	0.35 ± 0.05^{ef}	1.29 ± 0.20^{cd}	0.07 ± 0.13^{f}			
	C_{c1+c2}				1.52 ± 0.40^{b}	5.75 ± 1.09^{a}	1.44 ± 0.29^{b}

C_a = Chlorophyll a, C_b = Chlorophyll b, C_{x+c} = Total carotene, C_{c1+c2} = Chlorophylls c_1 and c_2 in equal proportions.
Results are expressed as Mean±SD (n = 3).

3. Results

Chlorophyll a, x+c and c_1+c_2 contents in three brown macroalgae species of *S. trinodis* and *P. myrica* and *C. sinuosa* were determined (Table 2). The highest chlorophyll a content was observed in *S. trinodis* species. In terms of chlorophyll x+c (the total carotene) contents, the significant difference was observed between the species (P < 0.05). The highest content of the total carotene was showed in *S. trinodis* and the lowest in *C. sinuosa.* Also, results indicated that chlorophylls c_1 and c_2 contents in equal proportions were significant between three

algae species ($P < 0.05$). The highest content of chlorophylls c_1+c_2 was observed in *C. sinuosa* by the extraction with diethyl ether solvent. In relation to chlorophyll b, there was no absorption because of the absence of chlorophyll b in brown algae, so it was neglected. On the other hand, results indicated that there was a significant relationship between solvents used in the chlorophyll extraction (Table 2), especially between the acetone and others. In both formulas, the best solvent in the chlorophyll extraction from *S. trinodis*, *P. myrica* and *C. sinuosa* species was acetone, acetone and diethyl ether, respectively. Generally, it has been found that the use of different solvents in order to extract chlorophyll, show different results. Its reason can be because of the various structures of the cell wall and the plant type. These results were consistent with Wellburn [11] studies. However, there are very little results in relation to the brown algae chlorophyll.

4. Discussion

Chlorophyll is an important source of nitrogen storage and a predominant constituent in green plants and algae with positive effects on oxidation, inflammation and wound healing [5]. Also, chlorophyll can act as a powerful antioxidant and reducers of free radicals, prevents the destruction of lymphocytes [3] against carcinogens and protects the body from the lipid peroxidation of low density lipoprotein [9]. Although there is the chlorophyll a in all algae groups, in the present study it was observed that the amount of chlorophyll a in different species depends on the type of solvent to extract them. For example, other researchers also found that the solvents used in the pigment extraction have an important effect, so that in the studies on Scenedesmus quadricauda and Selenastrum capricornutum microalgae and solvents such as ethanol, methanol and acetone, the best solvent was ethanol [10]. Overall in addition to the solvent type, other findings from many researches indicate that factors such as environment conditions, the light, the water depth and the variety of the cell wall structures can affect the amount of chlorophyll. Therefore, the use of suitable methods and materials for breaking up cell walls will be useful for chlorophyll extraction. The total carotene content is various in algae groups and it was proved that more carotene pigments belonging to Phaeophyta group. In these samples studied, the carotene content in S. trinodis was observed higher in comparison with the other two brown algae. The chlorophyll c_1+c_2 contents were high in P. myrica and C. sinuosa species. There was an inverse relationship between chlorophyll a and c_1+c_2, x+c. In fact, results indicated that when chlorophyll a decreases, the carotene increases and the algae turns to yellow. In some studies it has been said that the best solvent for extraction of chlorophyll, is methanol. But it should be noted that the selection of the method and solvent in connection with pigments extraction is useful. For example, although methanol is a good solvent in some extraction methods but it is a toxic solvent. In this project, the acetone and diethyl ether in both formulas were respectively the best solvent for S. trinodis, P. myrica and C. sinuosa species in the field of chlorophyll extraction. With all the above, these tests still need to be explored further in the future, because there is high demand for use of natural pigments of algae in food products, particularly dairy and beverages [1] as food additives and also in cosmetics.

5. Conclusion

As a result, this study shows that macroalgae can be a promising natural resource for chlorophyll and also, chlorophyll extraction with different solvents can help to better use in food-drug industry as potential functional ingredients.

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