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Extended Abstract

Comparison of Different Cell-wall Disruption and Fatty Acid Extraction from *Dunaliella Salina* Microalgae

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Abstract

As extracted oil from microalgae is highly affected by selected cell-wall breaking method and type of the solvent used, thus an appropriate choice matters in which it might affect the quantity. This study was conducted to determine the most effective method on *Dunaliella salina* microalga cell disruption and solvent by comparing several methods. According to the results, the most efficient technique for oil extraction from *Dunaliella salina* microalgae was recorded as combination of enzymatic and homogenization methods (2.26±0.02 g.L-1), followed by enzymatic method with 3% cellulose and 1.5% flavourzyme (2.04±0.02 g.L-1), and finally ultrasonication (1.61±0.00 g.L-1). Based on the fatty acid profile, C16:0, C18:1 and C18:2 fatty acids were recorded as the main constituents ethanol was the most effective solvent by extraction of 8.22%, 1.07% and 5.18% of above mentioned fatty acids. Furthermore, present results demonstrated that in order to efficiently extract lipid from *Dunaliella salina*, enzymatic and homogenization methods exhibited the most efficient technique for cell disruption.

Keywords: Cell Destruction, *Dunaliella Salina*, Homogenization, Lipid Extraction

Introductıon

Microalgae have a lot of commercial and nvironmental importance as the food chain base and oxygen producer, and also a natural source of valuable compounds such as fatty acids, steroids and carotenoids. Many protocols used to break the tiny algae cell include mechanical (Hoekman *et al*., 2012; Santos *et al*., 2012) chemical and thermal methods (Gordillo et al., 1998; Peterson et al., 1983) microviums (Meier, 1955), and enzymatic hydrolysis (Choi *et al*., 2014). In choosing a preferred method to destroy cells, it should be considered factors such as the cell wall nature and quality of the targeted methbolites and the dimentions of the work (commercial or experimental) (McMillan *et al*., 2013; Nascimento *et al*., 2013). In the present study, the test was tested if the enzyme hydrolysis was combined with the use of cellulase and proteinase enzymes, it can be combined with homogenization or ultrasound, so as to

determine how much it would break the microscopic cells and thereby increase the efficiency of oil extraction. On the other hand, regarding to the fact that acid profile the extracted fatty by each solvent differes form each other, the fatty acid profiles were compare to each other.

Material and methods

Microalgae *Dunaliella salina* from the collection of alga culture was prepared at Uromia University. For cultivation of algae, a modified BM medium with sodium bicarbonate was used at 1.25 g / l. The destruction of the cell wall with ultrasound, homogenizer, enzyme and combination of enzymatic methods and synthesis of enzymatic method with mechanical mechanics was performed. The identification and measurement of the fatty acids available in the extracted lipid samples were carried out by gas chromatography method.

Results and discussion

Three methods for the most effective method of destruction and digestion of the cell wall and lipid extraction of *D. Salina* was examined. The effectiveness of the cell wall destruction was measured by the total amount of extracted lipid. The destruction of the cell wall causes better access to components within the cell (Gordillo *et al*., 1998). All techniques of cell wall destruction used in this study indicated the effective break down of the cell wall of *D. Salina* even though the quantity of oil extraction is varied by each method (Table 1 and 2, Figure 1 and 2).

Zhang *et al*. (2016) They investigated the amount of cell wall destruction and lipid extraction from *D. Salina* microalgae in different ways and reported that: the amount of extraction of lipids was 26% by ultrasound method, 22% biological method, 17% homogenizer and 10% self-confidence. The ultrasound and biological methods for degrading the Donatella cells are better than other methods and in terms of energy consumption, the biological method has the potential to produce large-scale lipid production(Zhang *et al*., 2016.). The extracted oil fatty acid profiles with different solvents (water, ethanol, methanol) identified by the GC device are shown in Table (3). The dominant fatty acids are palmitic acid (C16:0) oleic acid (C18:1) linoleic acid (C18:2) linolenic acid (C18:3), which contains 80% of total fatty acids. Other fatty acids include palmitoleic acid (C16: 1) and arachidic (C20:0) percentages. The fatty acids identified in this study were similar to those reported by Hana *et al*. (2004). Based on the results of this study, the highest amount of extracted oil with 3% cellulose and 1.5% flourzyme was 2.04±0.0264 g/L, based on the dry weight of algae and the lowest level was related to 1% cellulose and 1% flourzyme 0.733±0.0737 g/L in terms of dry weight of algae and in ultrasound method, the maximum extract extraction value of 1.613±0.007 g/L was related to breaking the cell wall in a period of three minutes and the lowest level was 0.493±0.025 g/L for 13 min and among different methods of breaking the cell wall, the combination of enzymatic and homogenizer methods with a rate of extraction of oil was recorded 2.266±0.0251 g/L.

Table 1. *D. Salina* cell wall destruction with ultrasound

Time (min)	Amplitude (%)	Extraction oil (g/L)
3	100	0.613±0.007[a]
8	100	0.578±0.0042[b]
13	100	0.493±0.025[c]

Table 2. *D. Salina* cell wall destruction with homogenizer

Time (min)	Speed (rpm)	Extraction oil (g/L)
3	7200	1.21±0.037[a]
3	11200	1.162±0.04[b]
3	15600	0.973±0.039[c]

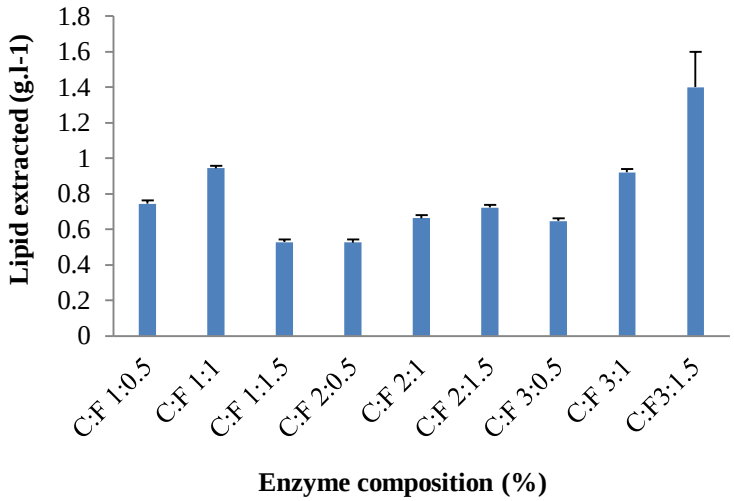

Figure 1. Comparison of the application of different levels of cellulase and protease enzymes and the amount of extracted oil from *D. Salina* (C: Cellulase, F: Florzyme)

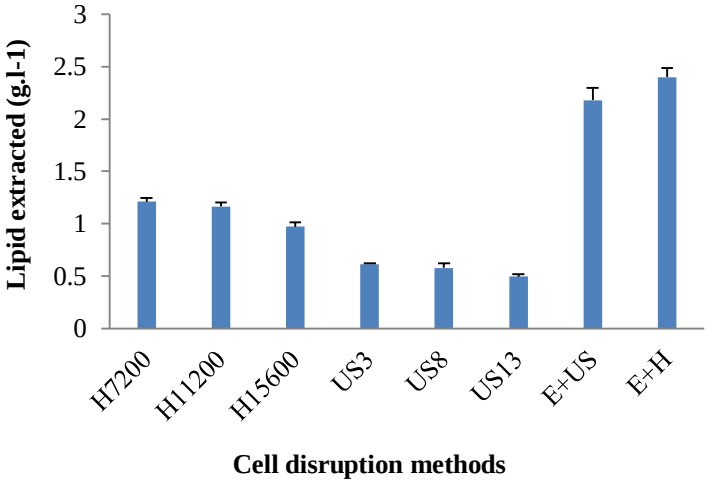

Figure 2. Different methods of degradation of *D. Salina* fine peppermint cell wall and fat extraction (H: Homogenizer, US: Ultrasound, E: Enzyme)

Table 3. Profile of extracted fatty acid oils with different solvents in *D. Salina*

Fatty Acid	Solvents		
	Ethanol	Methanol	Water
Saturation			
C16:0	1.079	1.954	3.434
C18:0	2.096	0.492	0.00
C20:0	4.379	2.694	12.917
UnSaturation			
C16:1	11.672	0.666	1.832
C18:1	8.220	0.145	12.300
C18:2	5.183	0.126	3.186
C18:3	20.276	0.155	1.962
C20:1	8.107	0.542	0.00
C20:2	3.885	2.037	22.088
C20:3	0.00	0.409	0.842
C20:4	2.290	0.843	5.443
C20:5	1.079	0.334	0.00
C22:6	2.096	6.085	0.00
C24:1	4.379	64.949	0.00

Conclusion

Based on the results of this study, the combination of enzymatic and homogenization methods for cell wall destruction and lipid extraction with ethanol solvent represents the best method for extracting the highest lipid from *D. Salina* microalgae.

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