

---

***STUDIES ON THE NATURAL COLORES EXTRACTION FROM  
(MARINE ALGAE)***

*By*

***Prof .Dr .Sahar Osman***  
*Dept of Nutrition& Food Science,*  
*Faculty of Home Economics ,*  
*Minufiya University*

***Dr. Tarek Mohammed Afifi***  
*Dept of Nutrition& Food Science,*  
*Faculty of Home Economics ,*  
*Minufiya University*

***Khloud Mohammed Hamza***  
*Dept of Nutrition& Food Science,*  
*Faculty of Home Economics , Minufiya University*

**Research Journal Specific Education**  
**Faculty of Specific Education**  
**Mansoura University**

***ISSUE NO. 36, OCTOBER. 2014***

**مجلة بحوث التربية النوعية - جامعة المنصورة**  
**العدد السادس والثلاثون - أكتوبر ٢٠١٤**

---



## **STUDIES ON THE NATURAL COLORES EXTRACTION FROM (MARINE ALGAE)**

*Prof .Dr .Sahar Osman\* Dr. Tarek Afifi\* Khloud Hamza\**

### **Abstract**

The current trend for scientific research is moving towards the production of materials and natural food products to keep overall health of individuals so this research shows methods of extracting natural dyes from green algae (*Ulva lactuca* L) and red algae (*Plocamium cartilagineum*) & *Gelidium* sp, and used as colorants and preservatives in processed foods as an alternative to the industrial colors, and as a result they contain antioxidant materials at high is used as a preservative for foods. This study showed that pigments which extracted from green algae her best solvent (acetone aqueous 80%) at wavelength (450 nm) with an average (.6090), followed by (DMF 100% + Nitrogen liquid) degree (-196) under zero, when the wavelength (450 nm), average (.631) and be algae in the form of dried, the other side red algae best solvent for the extraction of pigment (Distilled water) to get the red pigment, but to get the green from red algae score the best solvent (DMF 100% + Nitrogen) at the wavelength (450 nm) average (1.335), followed by (M.A) at the same wavelength with an average (.988). The algae are processed before preparing for the extraction of the dye (soak and rinse grinding and sonication of the cells and tissues). The drying process for green was the best and freezing process red is the best Extracting the dye from algae. Been employed in the production of dyes pomegranate jelly from fruit kiwi. Because it is a treasure trove of natural dyes and carotenoids, vitamins and unsaturated fatty acids, protein and dietary fiber. That will affect positively on the overall health of individuals helps clearly to treat (obesity \_ diabetes \_ osteoporosis \_ increase immunity and prevention of cancer ..... etc. all from algae).

**Keywords:** Microalgae, Seaweeds, *Ulva lactuca*, *Plocamium*, Photopigments, solvents, extraction method, Chlorophyll, Carotene.

\* Faculty of Specific Education, Mansoura University, Egypt

## **Introduction**

Marine algae can be classified into brown, red, and green algae, which are referred to as Phaeophyceae, Rhodophyceae, and Chlorophyceae, (Khan et al., 2010). There are four kinds of chlorophyll found in marine algae (Holdt & Kraan, 2011). Algae and microalgae are referenced in the literature as sources of bioactive compounds for use as functional food ingredients (Plaza et al. 2009 ). Hence, the wide diversity of marine organisms is being recognized as rich sources of functional materials, including polyunsaturated fatty acids (PUFA), polysaccharides, natural pigments (NPs), essential minerals, vitamins, enzymes and bioactive peptides (Shahidi & Alasalvar, 2011). (NPs) have received particular attention. These NPs exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective activities. This contribution focuses on biological activities of marine algae-derived NPs and emphasizing their potential applications in foods as well as pharmaceuticals areas (Ratih and Se-Kwon ,2011). The study of seasonal variations showed that the best period to collect the seaweed for food use is between February and June ( Claire et al., 2010 ). Seaweeds, especially the red seaweeds appear to be an interesting potential source of food proteins (Denis et al., 2010).

Seaweeds contain up to 2% of dry weight of lipids and much of this lipid content is made up of PUFA with much of it occurring in the form of omega-3 and omega-6 lipids (Marsham et al ., 2007). The cell walls of marine algae are rich in sulfated polysaccharides (SPs) such as fucoidans in brown algae, carrageenans in red algae and ulvans in green algae. These SPs exhibit many beneficial biological activities such as anticoagulant, antiviral, antioxidative, anticancer and immunomodulating activities . Therefore, marine algae derived SPs have great potential for further development as products in nutraceutical, pharmaceutical and cosmeceutical areas ( Isuru et al ., 2011 ). In animal studies polysaccharides from various edible seaweed have been demonstrated to reduce plasma total cholesterol , LDL-cholesterol and TAG (Amano et al., 2005). several studies indicate a beneficial effect of oral administration of chondroitin sulphate on osteoarthritis ( Rainsford, 2009), through stimulation of extracellular matrix production by chondrocytes, anti-inflammatory effects and inhibition of cartilage degeneration (Kubo et al., 2009). These properties confer on the seaweed the potential to be used in food technology for the acquisition of

low-calorie food (Kadam et al., 2001), as well as in prevention of gastrointestinal (Lahaye & Kaeffer, 1997) and cardio-vascular (Bocanegra et al., 2009) diseases. seaweed represents one of the richest sources of natural antioxidants and antimicrobials (Cox et al., 2010). They are also an excellent source of vitamins such as A, B1, B12, C, D and E, riboflavin, niacin, pantothenic acid, folic acid as well as minerals such as Ca, P, Na, K and I (Dhargalkar & Pereira, 2005).

Polysaccharides from seaweeds have been reported to possess biological activities of potential medicinal values in addition to their current status as a source of dietary fibers and prebiotics (Smit, 2004). Phycobiliproteins are used as a natural dye in the food (C-phycoerythrin) and cosmetic industry (C-phycoerythrin and R-phycoerythrin). They are used as colorant in many other food products such as fermented milk products, ice creams (Sekar & Chandramohan, 2008). reported that the oral administration of several seaweeds can cause a significant decrease in the incidence of carcinogenesis in vivo. The SPs of *Porphyra* (Rhodophyceae) can induce cancer cell death via apoptosis in a dose-dependent manner in vitro without affecting the growth of normal cells (Kwon & Nam, 2006). Epidemiological evidence have shown h-carotene to prevent cancer of various organs like lungs, stomach, cervix, pancreas, colon, rectum, breast, prostate and ovary by means of antioxidant activity (Poppel and Goldbohm, 1995). Other than antioxidant property they can influence intracellular communication (Sies and Stahl, 1997), immune response (Hughes et al., 1997), neoplastic transformation and control of growth (Bertram and Bortkiewicz, 1995).

Most of the pigments have high nutritional value unlike their synthetic counterparts. Eco-friendliness: The process of production of natural dyes from algae does not involve the usage of harmful and/or polluting chemicals. The majority of these effluents are biodegradable and can also be reused as fodder, bioplastics etc. Non-Toxicity and Non Carcinogenicity: These reasons have contributed to the increase in the need for non-toxic eco-friendly colorants and dyes from Algae. Common Algal Pigments: (Phycocyanin, Phycoerythrin, B-car, Chl, Fucoxanthin). Algae contain a wide range of photosynthetic pigments. Three major classes of photosynthetic pigments are chlorophylls, carotenoids (carotenes and xanthophylls) and phycobilins. Phycocyanin and phycoerythrin belong to the major class of phycobilins photosynthetic pigment while fucoxanthin

and peridinin belong to carotenoid group of photosynthetic pigment (Deborah ,2013 ).

### ***Materials and methods***

#### ***Materials:***

##### ***Algal source :***

Two species of marine macroalgae ( green algae, *Ulva lactuca* L ) and red algae , (*Plocamium cartilagineum* & *Gelidium* sp ) ,the green of 5-8 cm in height but the red deeper 30 cm were obtained from an exposed rocky site near the edge of costal Miami Khalid ibn Walid , Alexandria , Mediterranean Sea, Egypt at April 2013 .The samples were washed thoroughly to remove adhering soil particles and immediately transferred to the laboratory in an ice box for further analysis .

***Reagents*** : Pure Acetone , Ethanol absolute , Methanol , N-N Dimethyl formamide (DMF), Petroleum ether , Magnesium Carbonate hydrated , Ascorbic acid and fructose ( laevulose ) were purchased from Gomhoureya Pharmaceuticals and chemicals ,co – Alexandria . Liquid nitrogen were from the Directorate of Veterinary Medicine – menufiya . Gelatin non- domestic were commercial grade and was purchased in a local market.

#### ***methods***

algae sample Preparation :The collected Algae samples and sent directly to the laboratory is immediately washed several times with water and under a strong spray of water to get rid of any plankton or impurities ( Sloth et al., 2006) . The clean samples were drained gently on filter paper ( Whatman 102 ) for a few minutes then placed the in plastic bottles and wrapped in aluminum foil according to (Buffan-Dubau & Carman, 2000 ) . Then kept in deep freezer at - 18 ° C . The samples were devided into three parts . The first was kept at deep freezer . the second were dried in an electric oven Drying Voltage 220V /50Hz – in Taiwan at 60 ° C for 70 h . The third was dried samples were grounded and kept in brown glass bottles for pigments extraction (Van Leeuwe et al ., 2006).

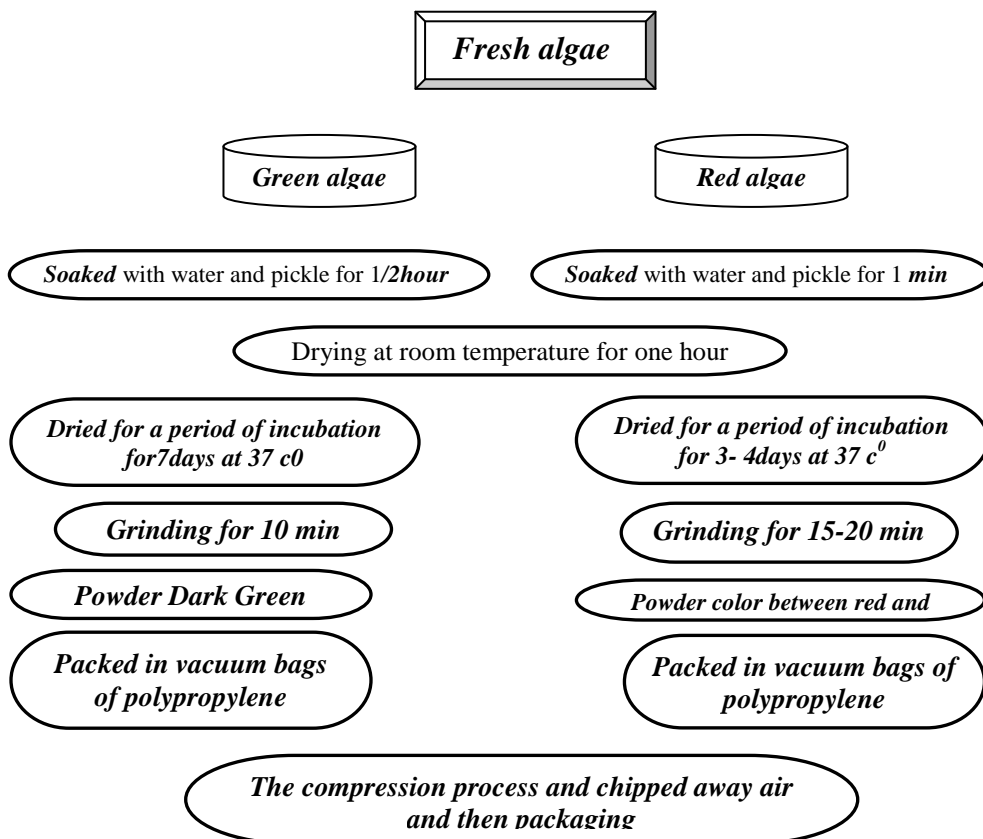


fig (1) : preparation of algae powder (spices).

#### **pigments Extraction : -**

Three extraction solvents were investigated: methanol/ ammonium acetate, ethanol, acetone/water (90:10) . Methanol/ammonium acetate extractions were initiated by adding 5 ml methanol to cell pellets in 13×100 mm glass tubes with polypropylene caps. The resulting mixtures were homogenized by vortex mixing with adding of one gram of sterilized sand . Tubes were then placed in crushed ice in the dark for 2 h, mixed at 30 min intervals and, after 2 h, 0.5 mL of 0.5 M ammonium acetate, pH 7.2, was added to each tube. Samples were mixed again and then stored overnight at -20 °C before .

Ethanol and acetone/water (80:10) extractions followed a similar pattern. Cell suspensions were homogenized by vortex mixing with was added one gram of sterilized sand , and then placed in crushed ice for 2 h in the dark. Samples ( powder or frozen ) were vortex mixed at 30 min intervals. After two hours, all samples were placed in a  $-20\text{ }^{\circ}\text{C}$  freezer overnight. Ammonium acetate (0.5 M) was not added to the acetone , ethanol based extractions. The following morning all samples were mixed and centrifuged to pellet residual cell material (Wright et al., 1991) . After the above described sample preparation part samples was treated with acetone was frozen for 1 day at  $-18\text{ }^{\circ}\text{C}$  prior to liquid nitrogen freezing ( $-196\text{ }^{\circ}\text{C}$ ) , and then treated three times at 24h intervals with acetone ( Henriques1 et al., 2007 ).

much stronger solvent was used extract pigments dimethyl formamide (DMF 100% ) (Porra et al., 1989). Each samples ( powder or frozen ) was incubated in 3ml solvent in a 15-ml polyethylene tube for 24 h under dark conditions in a refrigerator with occasional vortex mixing of the contents. Since no debris or samples particles were in suspension, the samples were not centrifuged. Chlorophyll a was then photometrically quantified using the equation of ( Porra et al. 1989). samples were shock-frozen with liquid nitrogen prior to lyophilisation by liquid nitrogen . Frozen filters were stored for 1 day and freezing samples were processed immediately after drying. Besides mechanical grinding homogenisation of lyophilised and frozen samples using a sterilized sand homogenizer for 3 \_ 6 min, a stepwise extraction protocol was adopted to evaluate the chlorophyll extraction efficiency. Lyophilised and frozen samples were homogenized with or without DMF and compared with non-homogenised samples. All homogenised and non-homogenised samples were kept in 3–5 ml DMF for 24 h in the refrigerator. After centrifugation for 3 min at 6700g , chlorophyll a concentration of the supernatants was photometrically determined. The pellets were resuspended in 3–5 ml DMF and kept for another 24 h before another chlorophyll quantification was carried out. This procedure was repeated a third time. The lyophilised and frozen samples were also compared with samples which were first frozen for 1 day at  $-18\text{ }^{\circ}\text{C}$  prior to liquid nitrogen freezing ( $-196\text{ }^{\circ}\text{C}$ ) and lyophilisation, and then treated three times at 24-h intervals with DMF.



The acetone / Magnesium Carbonate extract was prepared by ground the thallus was dried gently with absorbent filter papers (Whatman 102 )for a few seconds and weighed. The thallus was ground manually in a porcelain mortar in a cold and darkened fume cupboard to prevent photo-oxidative breakdown of labile pigments. 100 % acetone and small amount of magnesium carbonate were added in order to prevent the accidental formation of chlorophyll metabolites. This procedure was repeated until the algal thallus became colorless, 5 ml of ethyl ether were added and the extract was filtered. The extract was concentrated by a low pressure rotary evaporator ( Czech republic , 220V – 95 VA 50 Hz ) at 25 °C ( Jeffrey et al ., 1997 ) .

each sample was repeat treatment three times at 24-h intervals to carried out the pigments with all solvent's used, and then were determined Spectrophotometer at wavelength ( 450nm , 550 nm ,650 nm , 650 nm ) nm according to (Wright et al., 1996 ; Hegazi et al ., 1998 ) method.

#### ***Pigment Determination : -***

Determination of total chlorophylls (T-Chl ) : Three grams of green and red algae was homogenized in acetone (30 ml, 80%) , ethanol ( 30 ml, 98 % ) , methanol (30ml, 99% ) , DMF ( 25 ml ,100% ) and incubated overnight in dark at 4°C for complete extraction followed by centrifugation at 6700g for 3-5 min. Total chlorophyll (T- Chl ) , chlorophyll a ( Chl a) and chlorophyll b (Chl b) in the supernatant were determined spectrophotometrically (6105 UV / Vis , JENWAY , UK ) at wavelength 664 nm and 400 \_ 700 nm according to Lichtenthaler, H.K., 1987 ; Hegazi et al ., 1998 method.

Determination of total carotenoids (T- CAR): The total carotenoids were determined spectrophotometrically at 450 - 630 nm according to AOAC ,1995 ; wright et al ., 1991 ( b-carotene was used as a standard ) .

#### ***Drying the fruit \_ pigment mixture :***

kiwi and pomegranate fruits were homogenized in a mixer( brouns ,Germany ) then mixed to pigment green and to pigment with red pigment and mixed separately with the pigment extract ( 2 : 1 v/v ) . The mixture was dried at 60 ° C for 3 days in an electric draught oven (dual purpose - incubator 220V /50Hz – in Taiwan ) and then grinded for 3 min a mixer . And called as fruits - pigment powder .

**Preparation of jellies : -**

The jellies were prepared according to ( CODEX , 2009 ; Oakenfull, D et al ., 1984) , with some modification , 5 gram White powder, gelatin non-domestic were commercial grade, 5 gram fructose , 0.25 gram ascorbic acid, 10 gram kiwi and pomegranate Powder .

**Procedure: -**

The jellies were prepared by mixing the water,( pomegranate , kiwi ) Powder , fructose , ascorbic acid and peel extract. This combination was maintained with continuous stirring, and the gelatin were then poured slowly to avoid the formation of lumps. The final mixture was covered and boiled for 1 min. Then, the mixture was poured immediately into cylindrical glass containers and capped. The jars were completely submerged in boiling water for 5 min, left at room temperature and, finally, were refrigerated at 4 \_C. sensory evaluation was performed with fresh samples .

Table (1) : preparation technological the jell kiwi and jell pomegranate of supplement extracts green and red color algae (2 :1).

Pigment	Natural jell pomegranate	Natural jell Kiwi
Pomegranate Juice	6.7 g	-----
Kiwi juice	-----	6.7 g
Gelatin	5g	-----
fructose	15g	5g
Water	150 ml	80 ml
Red pigment from algae	3.3 g	-----
Green pigment from algae	-----	3.3g
Ascorbic acid	.25g	.25g
Mixture fruit and pigment	2:1	2:1

**Sensory evaluation**

Level agreement analysis was carried out by a panel of 10 panelist, In the Faculty of Economics Laboratory Home. taste and Appearance , color , Size and shape , appearance, consistency, Flavor , Taste, Transparent and general agreement of Product evaluation jelly . using 9 point hedonic scale ( 4 = acceptable, 6 = average, 8 = good ( J .Ventura et al ., 2013 ) .

### Statistical analysis :-

Data were expressed as mean  $\pm$  SD Statistical significance of differences between groups was assessed using one – way analysis of variance ( ANOVA ). A value of  $P < 0.05$  was considered for significance Statistical analysis was performed using spss 17 for windows software ( spss Inc , Chicago , IL , USA ) .

### Result are reported as :

significant differences :

.  $P < 0.05$  \* = Statistically significant

.  $P < 0.01$  \*\* = Statistically high significant

.  $P < 0.001$  \*\*\* = Statistically very high significant

### Result :

Table (2) : specific absorption coefficients for green pigments at wavelength by solvents applied for analysis .

solvent	sample	Wavelengths		
		630	550	450
( A+D) acetone + drying	extracted (1)	0.191	0.055	1.368
	extracted (2)	0.056	0.387	0.459
	extracted (3)	0	0	0
Mean $\pm$ Std		0.0823 $\pm$ 0.0982	0.1473 $\pm$ 0.209	0.6090 $\pm$ 0.696
DMF + N dimethyl formamide + nitrogen	extracted (1)	0.022	0.06	0.155
	extracted (2)	0.332	0.032	0.655
	extracted (3)	0.046	0.246	1.082
Mean $\pm$ Std		0.133 $\pm$ 0.172	0.113 $\pm$ 0.116	0.631 $\pm$ 0.464
( A+ N ) acetone + nitrogen	extracted (1)	0.046	0.041	0.397
	extracted (2)	0.101	0.121	0.325
	extracted (3)	0	0	0
Mean $\pm$ Std		0.049 $\pm$ 0.051	0.054 $\pm$ 0.062	0.241 $\pm$ 0.212
A + Mgco3 acetone + Magnesium carbonate	extracted (1)	0.055	0.06	0.279
	extracted (2)	0.075	0.06	0.279
	extracted (3)	0.116	0.131	0.364
Mean $\pm$ Std		0.082 $\pm$ 0.031	0.083 $\pm$ 0.041	0.307 $\pm$ 0.049

extracted (1) : the first extraction from sample .

extracted (2) : the second extraction from the same sample.

extracted (3) : the third extraction from the same sample.

Table (3) :- Mean and standard Deviation for all solvent to green algae :-

Wavelengths	630	550	450
Mean ± Std (A+ D !)	0.0823 ±0.0982	0.1473 ± 0.209	0.6090 ± 0.696
Mean ± Std (DMF + N!)	0.133 ± 0.172	0.113 ± 0.116	0.631 ±0 .464
Mean ± Std (A + N !)	0.049 ± 0 .051	0.054 ± 0.062	0.241 ± 0.212
Mean ± Std (A + Mgco3!)	0.082 ±0 .031	0.083 ± 0.041	0.307 ± 0.049

! solvent , ( A+D) acetone + drying , ( A+ N ) acetone + nitrogen ,(DMF) ) dimethyl formamide (Mgco3 Magnesium carbonate .

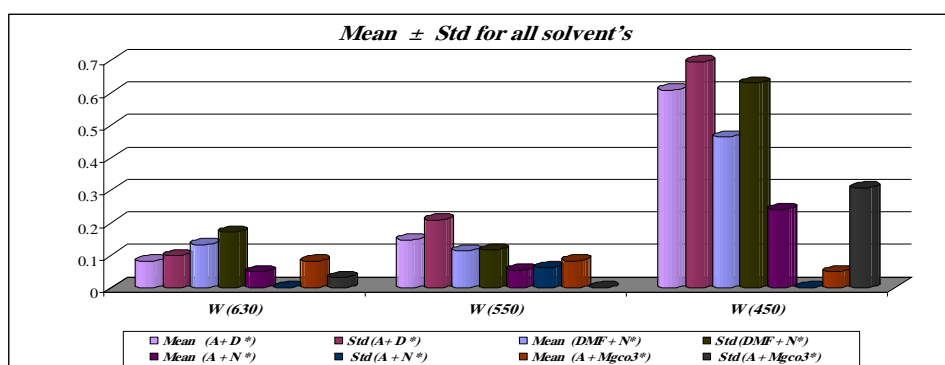


Fig (2):- Mean and standard Deviation for all solvent to green algae :-

Table (2) ,Table (3) & Fig (2) explains the specific absorption coefficients for when the waves pigments and solvents used for the analysis highlights the percentages of degrees of absorption of the pigment with every type of solvent at different wavelengths ( 450 , 550 , 630 nm ) respectively . the same wavelengths mean and standard deviation to Acetone + Drying ( .6090 ± .696 , .1473 ± .209 , .0823 ± .0982 ) . DMF 100% + N the average and standard deviation ( .6090 ± .696 , . 113 ± .116 , .133 ± .172 ) . With Acetone freeze + N the mean and std ( .241 ± .212 , 054 ± .062 , .049 ± .051 ) . Acetone + Mgco3 mean and std ( .307 ± .049 , .083 ± .041 , .082 ± .031 ) . The best average degree of absorption at the wavelength (450 nm ) Acetone to drying . In (Fig (1) found that the higher extraction for the greater proportion of the pigment , to supply only found the third stage of the highest in the extraction degree of absorption of the pigment and special solvents acetone drying and (DMF + N ) , especially at wavelength 450 nm of (Jeffrey and Humphrey ,1975)

Table (4) : specific absorption coefficients for red pigments at wavelength by solvents,s applied for analysis .

Solvent	Wavelengths	Wavelengths		
		630	550	450
methanol , ammonium acetate	extracted (1)	0.235	0.405	0.537
	extracted (2)	0.415	0.344	1.399
	extracted (3)	0.278	0.165	1.027
Mean ± Std		0.309 ± 0.094	0.305 ± 0.125	0.988 ± 0.432
dimethyl formamide + nitrogen	extracted (1)	0.525	0.935	1.313
	extracted (2)	0.433	0.363	1.427
	extracted (3)	0.42	0.34	1.264
Mean ± Std		0.459 ± 0.057	0.546 ± 0.337	1.335 ± 0.084
Acetone+ nitrogen	extracted (1)	0.3	1.141	0.1027
	extracted (2)	0.671	0.924	1.425
	extracted (3)	0.148	0.362	0.417
Mean ± Std		0.321 ± 0.244	0.279 ± 0.249	0.842 ± 0.765
Acetone + Magnesium carbonate	extracted (1)	0.057	0.362	1.0295
	extracted (2)	0.367	0.476	1.495
	extracted (3)	0.539	0	0
Mean ± Std		0.321 ± 0.244	0.279 ± 0.249	0.842 ± 0.765
Water	extracted (1)	0.257	0.983	0.965

extracted (1) : the first extraction from sample .

extracted (2) : the second extraction from the same sample.

extracted (3) : the third extraction from the same sample.

Table (5) :- Mean and standar Deviation for all solvent to red algae

Wavelengths	630	550	450	Appearance
Mean ± Std (M +Acetate!)	0.309 ± 0.094	0.305 ± 0.125	0.988 ± 0.432	Red greenish
Mean ± Std (DMF + N!)	0.459 ± 0.057	0.546 ± 0.337	1.335 ± 0.084	Green reddish reddish reddish
Mean ± Std (A + N !)	0.321 ± 0.244	0.279 ± 0.249	0.842 ± 0.765	Red + tissues
Mean ± Std (A + Mgco3!)	0.321 ± 0.244	0.279 ± 0.249	0.842 ± 0.765	Green + tissues

NOTE : ( M.A) methanol , ammonium acetate,(DMF) ) dimethyl formamide, (N) nitrogen , (Mgco3 ) Magnesium carbonate , ! solvent .

Mean ± Std for all solvent's

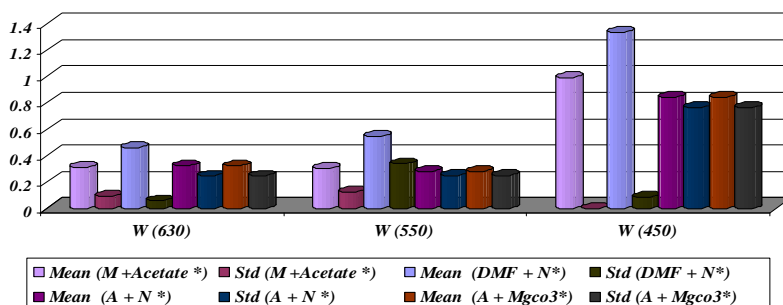


Fig (3) :- Mean and standard Deviation for all solvent to reed algae

Data in table (4) , Table (5) & Fig (3) According to the table of coefficients specific absorption of pigments red with different types of solvents and different wavelengths (630 , 550 , 450 nm ) for all solvents . We found that mean and standard deviation with Methanol 95% + ammonium acetate .5M ( .309 ± .094 , .305 ± .125 , .988 ± .432 ) . Was found average and standard deviation with DMF 100% + N (.459 ± .057 , .546 ± .337 , 1.335 ± .084 ) . in same table found that mean and standard deviation with Acetone 80% + (N) ( .321 ± .244 , .842 ± .765 , .279 ± .249) . It found that mean and std with acetone 80% + Mgco3 (.321 ± .244 , .279 ± .249 , .842 ± .765 ) . When water extraction degrees of absorption was (0.965 , 0.983 , 0.257) . The best degree of absorption at the wavelength ( 450 nm ) for DMF + N Followed by methanol + ammonium acetate , the more length and extraction stages (Wright et al., 1996). And emerged stronger solvent for the pigment ( Methanol 95% + ammonium acetate .5M ) followed by ( DMF + N ) citing by ( Jeffery & humphery 1975 ) .

Table (6) : Degrees of characteristics for the new, products as jelly (pomegranate and kiwi) .

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	AppearanceA	6.400	11	1.200	0.362
	AppearanceB	5.100	11	0.700	0.211
Pair 2	ColourA	7.600	11	1.114	0.336
	ColourB	4.700	11	0.900	0.271
Pair 3	SizeA	7.000	11	1.414	0.426
	SizeB	7.700	11	0.900	0.271
Pair 4	TextureA	7.400	11	0.917	0.276
	TextureB	8.200	11	0.748	0.226
Pair 5	ConsistencyA	6.900	11	0.700	0.211
	ConsistencyB	8.400	11	0.663	0.200
Pair 6	FlavorA	6.900	11	0.700	0.211
	FlavorB	4.400	11	0.663	0.200
Pair 7	TasteA	6.200	11	0.600	0.181
	TasteB	8.000	11	0.632	0.191
Pair 8	TransparentA	7.100	11	1.446	0.436
	TransparentB	5.400	11	0.663	0.200
Pair 9	acceptanceA	6.700	11	0.900	0.271
	acceptanceB	5.300	11	1.100	0.332

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	AppearanceA & AppearanceB	11	0.310	0.354
Pair 2	ColourA & ColourB	11	0.279	0.405
Pair 3	SizeA & SizeB	11	-0.550	0.080
Pair 4	TextureA & TextureB	11	-0.408	0.213
Pair 5	ConsistencyA & ConsistencyB	11	0.517	0.104
Pair 6	FlavorA & FlavorB	11	-0.129	0.705
Pair 7	TasteA & TasteB	11	0.264	0.434
Pair 8	TransparentA & TransparentB	11	0.063	0.855
Pair 9	acceptanceA & acceptanceB	11	-0.212	0.531

Table (6), Fig (4) : Degrees of sensory characteristics for the New products as jelly ( pomegranate and kiwi). Of the General assessment of the product jelly component of algae pigments and dried fruit tissue was observed as follows : Appearance of red algae gets 6.4 but Jelly gets green algae 5.1 , color degree evaluate the color of the red 7.6 and much more than them degrees green evaluate 4.7 , the Size and shape of the observed of convergence in size degrees, exceeded slightly to give the red and green 7: 7.7respectively , And a supplement to assess the textures of the red over the other is to score 7.4 degree in green while the score 8.2 degree , Consistency and texture it was agreed that it is worth the number 7.4 and number 6.8 green , flavored red tops in green flavor for the following degrees respectively 6.9 : 8.4 , As for the taste even both two classes and closed at 6.2 : 8 Degree , Transparent great for both for two varieties and degree of public acceptance of the two varieties , 7.1: 5.4 and green was the best characteristic of the red .

### ***Discussion***

#### ***Priorities for the extraction is based on***

- 1 - Stability of the pigment extract.
- 2 - Optimization of the extraction efficiency of pigments.
- 3 - Good resolution of peak shape and separation.
- 4- over the stability of the dye .

Extract the pigment stability during storage in the auto sampler is of paramount importance , so the choice of solvent extraction requires special attention . Has been applied to various solvent extraction accurately, but so far have not been discussed in the context of automation. In this paper will reconsider the use of two of the most commonly used solvent extraction , methanol , acetone and other types of solvents (ethanol and methanol with ammonium acetate and water ), but the best is acetone Wa And, we will give here the way highly reproducible based on the extraction of 90% in acetone, in combination with freeze drying , and packaging of water generally higher extraction efficiency is achieved by the use of methanol as solvent extraction in combination with a mechanical disorder ( Wright et al , 1997). The main criterion for choosing the most suitable solvent for the method based on automation , however, must be based on solvent stability . Low stability of the pigments in methanol . Several papers have already been described how methanol encourages to configure allomers of chlorophyll (Brereton et al ., 1994 ) .



Acetone, on the other hand , provides a stable environment . This has been shown in fact in our study. Although pigment extracts can be safely stored for up to 18 hours , the 12-hour limit and can be pursued . In theory, the acetone is known to have extractability less( Jeffery & humphery 1975 ).

For other solvents are less in the degree of separation and efficiency as well as the presence of remnants of the algae cells and tissues with the dye in the solvent . It also found that the drying of algae on the degree of 50 ° C for green better than red because it can maintain color degree on the red reverse (Porra et al., 1989).

Discussed in this research is clear resulting application for automation in the analysis of dyes depends on the extraction of algae suitable for methanol as well as storage for long periods of the sample in automatic (e.g. Claustre et al., 2004) can lead to a rapid deterioration in the pigment . The Master Standard to choose solvent be based on the stability of the solvent but stability of the pigment in the methanol is low (Brereton et al., 1994) , It is known that acetone least have a lower extractability of chlorophylls from the protein matrix (Nakamura and Watanabe, 2001) , showed the efficiency of acetone top of methanol . DMF ( 100% ) the easiest way to enhance the efficiency of extraction is to prolong the time of extraction, however the length of time lead to the destruction of chlorophyll with the passage of time and the cells frozen in liquid nitrogen lypophilised highest level of chlorophyll a and extraction is carried out under the dark to prevent light photooxidation pigments ( Kowalewska and Szymczak, 2001) , And are compared of solvents the different of the extract (Wright et al., 1997) DMF more Efficient but a because of toxicity to can not be the recommendation by the followed by acetone and is based the promotion allomers from the chlorophyll ( Brereton et al., 1994) .

Color dye extracted degrees Citing with all Mercandente et al., 1999) , (Zapata et al. ,2000), (Van Heukelem andThomas,2001) ( Wright et al , 1997) , (SIMON W ,2005 ) .

**For green algae**

Treatment solvent	degree Color
A+ Mgco3	Lime-green light by 50%
A+ N + F	Transparent to the color yellow Sloped by 35%
A + D	One of the best ways is given Olive Green 90%
E + F	Less than color tone drying Green average by 50%
E+ D	Green the average score of 60%
DMF +N	Green very verdant green maximum rate of 90%

N : Liquid nitrogen / (DMF) dimethyl formamide , ( A+D) acetone + drying , (A+ N) acetone + nitrogen ,Mgco3 Magnesium carbonate , F ( frozen ) , E ( ethanol ) .

**For reed algae**

Treatment solvent	degree Color
Water	Dark red watermelon somewhat.
A + F	Bloody red color by 80%
A+ Mgco3	Dark lime-green 70%
M + A .5	Red and pink in the first conclusion and lime-green in the second conclusion
DMF + N	Red given me for the conclusion first, followed by the second conclusion Olive Green 90%

N : Liquid nitrogen / (DMF) dimethyl formamide , ( A+ F) acetone + frozen , (A+ N) acetone + nitrogen , Mgco3 Magnesium carbonate , F ( frozen ) , E ( ethanol ) , ( M.A) methanol , ammonium acetate .

**REFERENCE**

- Amano H, Kakinuma M, Coury D, Ohno H, Hara T. Effect of a seaweed mixture on serum lipid level and platelet aggregation in rats. *Fish Sci* 2005;71:1160–6.
- Bertram, J.S., Bortkiewicz, H., 1995. Dietary carotenoids in habit neoplastic transforma -tion and modulate gene expression in mouse and tumor cells. *American Journal of Clinical Nutrition* 62, 13275–13365
- Bocanegra, A., Bastida, S., Benedí, J., Ródenas, S., & Sánchez-Muniz, F. J. (2009). Characteristics and nutritional and cardiovascular-health properties of seaweeds. *Journal of Medicinal Food*, 12(2), 236–258.

- Buffan-Dubau, E. & Carmen, K. R. 2000. Extraction of benthic microalgal pigments for HPLC analyses. Mar. Ecol. Prog. Ser. 204:293-7.
- Claire Denis a, Michele Morançais a, Min Li a, Estelle Deniaud a, Pierre Gaudin a, Gaetane Wielgosz-Collin a, Gilles Barnathan a, Pascal Jaouen b, Joel Florence a,\* ( 2010) . Study of the chemical composition of edible red macroalgae Grateloupia turuturu from Brittany (France) . Food Chemistry 119 , 913-917.
- Deborah Robertson. (2013) . Dyes and Colorants from Algae. <http://www.clarku.edu/faculty/robertson/Laboratory%20Methods/Pigments.html>.
- Denis, C., Morançais, M., Li, M., Deniaud, E., Gaudin, P., Wielgosz-Collin, G., Barnathan, G., Jaouen, P., & Florence, J. (2010). Study of the chemical composition of edible red macroalgae Grateloupia turuturu from Brittany (France). Food Chemistry, 119(3), 913-917.
- Dhargalkar, V.K. and N. Pereira. 2005. Seaweed: promising plant of the millennium. Sci. Culture 71: 60-66.
- Heyward, A.J., 1991. Chlorophyll extraction from marine microalgae: verification and extension of an improved technique. Proceedings of the Australian Society of Phycology and Aquatic Botany, p. 9
- Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: Functional food applications and legislation. Journal of Applied Phycology, 23, 543-597.
- Hughes, D.A., Wright, A.J.A., Finglas, P.M., Peerless, A.C.J., Bailey, A.L., Astley, S.B., Pindar, A.C., Southon, S., 1997. The effect of h-carotene supplementation on the immune function of blood monocytes from healthy male non smokers. Journal of Laboratory and Clinical Medicine 129, 309-317.
- Isuru Wijesekaraa, Ratih Pangestutia, Se-Kwon Kima,b, . (2011) . Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae . Carbohydrate Polymers 84 , 14 - 21 .
- Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemie und Physiologie der Pflanzen 167, 191-194.
- Khan, S., Kong, C., Kim, J., & Kim, S. (2010). Protective effect of Amphiroa dilatata on ROS induced oxidative damage and MMP expressions in HT1080 cells. Biotechnology and Bioprocess Engineering, 15, 191-198 .

- Kubo M, Ando K, Mimura T, Matsusue Y, Mori K. Chondroitin sulfate for the treatment of hip and knee osteoarthritis: current status and future trends. *Life Sci* 2009;85:477–83.
- Kowalewska, G., Szymczak, M., 2001. Influence of selected abiotic factors on the decomposition of chlorophylls. *Oceanologia* 43 (3), 315–328.
- Kwon, M. J., & Nam, T. J. (2006). Porphyran induces apoptosis related signal pathway in AGS gastric cancer cell lines. *Life Sciences*, 79, 1956–1962.
- Lahaye, M., & Kaeffer, B. (1997). Seaweed dietary fibres: Structure, physico-chemical and biological properties relevant to intestinal physiology. *Science des Aliments*, 17(6), 563–584.
- Lawrenz, E., Fedewa, E.J., Richardson, T.L., 2011. Extraction protocols for the quantification of phycobilins in aqueous phytoplankton extracts. *J. Appl. Phycol.* 23, 865–871 .
- Lewitus, A.J., White, D.L., Tymowski, R.G., Geesey, M.E., Hymel, S.M., Noble, P.A., 2005. Adapting the CHEMTAX method for assessing phytoplankton taxonomic composition in southeastern U.S. estuaries. *Estuaries* 28, 158–170.
- Marsham S, Scott GW, Tobin ML. Comparison of nutritive chemistry of a range of temperate seaweeds. *Food Chem.* 2007;100:1331–1336.
- Mantoura, R. F. C. & Llewellyn, C. A. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase highperformance liquid chromatography. *Anal. Chim. Acta* 151:297–314.
- Mercandente AZ (1999) *Pure Appl Chem* 71:2263.
- Plaza, M., M. Herrero, A. Cifuentes, and E. Ibañez. 2009. Innovative natural functional ingredients from Microalgae. *Journal of Agricultural and Food Chemistry* 57: 7159–7170.
- Porra, R.J., Thompson, W.A., Kriedmann, P.E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta* 975, 384–394.
- Porra, R.J., 1991. Recent advances and re-assessments in chlorophyll extraction and assay procedures for terrestrial, aquatic, and marine organisms, including

- recalcitrant algae. In: Scheer, H. (Ed.), Chlorophylls. CRC Press, Boca Raton, pp. 31–57.
- Poppel, G.V., Goldbohm, R.A., 1995. Epidemiological evidences for h-carotene and cancer prevention. American Journal of Clinical Nutrition 62, 1393S–1402S.
  - Ratih Pangestutia, Se-Kwon Kima,b,\* . ( 2011 ) . Biological activities and health benefit effects of natural pigments derived from marine algae. J O U R N A L O F F U N C T I O N A L F O O D S 3 , 2 5 5 - 2 6 6 .
  - Rainsford KD. Importance of pharmaceutical composition and evidence from clinical trials and pharmacological studies in determining effectiveness of chondroitin sulphate and other glycosaminoglycans: a critique. J Pharm Pharmacol 2009;61: 1263–70.
  - Schumann, R., Rentsch, D., Go ¨ rrs, S., Schiewer, U., 2001. Composition and quantities of seston particles along a salinity and eutrophication gradient in coastal waters of the Southern Baltic Sea: significance of detritus and transparent mucoid material. Marine Ecology Progress Series 218, 17–31.
  - Sekar, S., & Chandramohan, M. (2008). Phycobiliproteins as a commodity: Trends in applied research, patents and commercialization. Journal of Applied Phycology, 20, 113–136.
  - Shahidi, F., & Alasalvar, C. (2011). Marine oils and other marine nutraceuticals. In Handbook of seafood quality, safety and health applications (pp. 444–454). Wiley Online Library.
  - Sies, H., Stahl, W., 1997. Carotenoid and intracellular communications via gap junction. International Journal of Vitamin and Nutrition Research 67, 364–367.
  - SIMON W. WRIGHT .( 2005 ) . Analysis of phytoplankton populations using pigment markers . June 29 – July 1 .
  - Sloth, J.K., Weibe, M.G., Eriksen, N.T., 2006. Accumulation of phycocyanin in heterotrophic and mixotrophic cultures of the acidophilic red alga Galdieria sulphuraria. Enzymes Microb. Technol. 38, 168–175
  - Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. Journal of Applied Phycology, 16, 245–262.
  - Suzuki, R., Ishimaru, T., 1990. An improved method for the determination of phytoplankton chlorophyll using N,N-dimethylformamide. Journal of the Oceanographic Society of Japan 46, 190–194.
  - Van Heukelem L, Thomas C (2001) J Chromatogr 910:31.

- Van Leeuwe M, Villerius L, Roggeveld J, Visser R, Stefels J, (2006) An optimized method for automated analysis of algal pigments by HPLC. *Mar. Chem.* 102, 267-275.
- Volk, S.L., Bishop, N.E., 1968. Photosynthetic efficiency of a phycocyanin-less mutant of *Cyanidium*. *Photochemistry and Photobiology* 8, 213–221.
- Wright, S.W., Jeffrey, S.W., Mantoura, R.R.C., Llewellyn, C.A., Bjomland, T., Repeta, D., Welschmeyer, N., 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.* 77, 183–196.
- Wright SW, Thomas DP, Marchant HJ, Higgins HW, Mackey MD, Mackey DJ (1996) *Mar Ecol Prog Ser* 144:285
- Wright, S. W., Jeffrey, S. W. & Mantoura, R. F. C. 1997. Evaluation of methods and solvents for pigment extraction. In Jeffrey, S. W., Mantoura, R. F. C. & Wright, S. W S. W. [Eds.] *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*. UNESCO Publishing, Paris, pp. 261–82.
- Zapata M, Rodríguez F, Garrido JL (2000) *Mar Ecol Prog Ser* 195:29.
- Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthesis. *Biomembrane. Methods Enzymol.*, 147: 350-382.
- AOAC, 1995. *Official Methods Of Analysis*. Association of Official Analytical Chemists, 16th Ed., K Hlrich. Arlington, Virginia
- Janeth Ventura a, Francisco Alarcon-Aguilar a, Ruben Roman-Ramos a, Efrain Campos- Sepulveda c, Maria L. Reyes-Vega b, V. Daniel Boone-Villa b, Edgar Ivan Jasso-Villagomez a, Cristobal N. Aguilar b, ( 2013 ) . Quality and antioxidant properties of a reduced-sugar pomegranate juice jelly with an aqueous extract of pomegranate peels . *Food Chemistry* 136 , 109 - 115 .
- M. Henriques<sup>1</sup>, A. Silva<sup>2</sup> and J. Rocha\*, 2 , (2007) . Extraction and quantification of pigments from a marine microalga: a simple and reproducible method . ©FORMATEX 2007.

## دراسات على إستخلاص الألوان الطبيعية من الطحالب البحرية

أ.د سحر عثمان الشافعي، ا.م.د طارق عبد الرحمن، خلود محمد حمزة\*

### الملخص العربي

يتجة البحث العلمي نحو الخضراء (خس البحر)، الطحالب الحمراء (بلوكامبيوم كارتيلاجينيوم) & جليديوم SP لإنتاج المواد ومنتجات غذائية الطبيعية للحفاظ على الصحة العامة للأفراد حتى يظهر هذا البحث أساليب استخراج الصبغات الطبيعية لنوع من الطحالب، وتستخدم الأطعمة الملونة والمواد الحافظة المصنعة كبديل لألوان صناعية، ونتيجة أنها تحتوي على مواد مضادة للأكسدة بنسبة عالية جدا لذلك تستخدم كمادة حافظة للأطعمة. وأظهرت هذه الدراسة أن الأصباغ التي تستخرج من الطحالب الخضراء أفضل مذيبي لها (الأسيتون مائي ٨٠٪) عند الطول الموجي (٤٥٠ نانومتر)، بمتوسط (٠.٦٠٩٠)، يليه (الداي ميثيل فورماميد بنسبة 100 ٪ + النيتروجين السائل) درجة (- ١٩٦) تحت الصفر، عندما الطول الموجي (٤٥٠ نانومتر)، متوسط (٠.٦٣١) وتكون الطحالب في صورة مجففة، ومن الجانب الاخر وجدت الطحالب الحمراء أفضل المذيبات لاستخراج الصباغ (الماء المقطر) للحصول على الصبغة الحمراء، ولكن للحصول الصبغة الخضراء من الطحالب الحمراء يسجل أفضل مذيب (الداي ميثيل الفورماميد 100 ٪ + النيتروجين السائل) عند الطول الموجي (٤٥٠ نانومتر)، المتوسط (١.٣٣٥)، تليها (الميثانول + اسيتات الامونيوم ٠٥، مولر) في نفس الطول الموجي بمتوسط (٠.٩٨٨). وتتم معالجة الطحالب قبل إعداد لاستخراج الصبغة (نقع وشطف طحن و صوتنة من الخلايا والأنسجة). وعملية التجفيف للخضراء كان أفضل لاستخراج الصبغة ووعملية التجميد الأحمر هو الأفضل لاستخراج الصبغة. استخدمت في إنتاج الأصباغ جيلي من فاكهة الرمان الكيوي. ولأن الأعشاب البحرية كنز من الصبغات الطبيعية والكاروتينات والفيتامينات والأحماض الدهنية غير المشبعة، والبروتين والألياف الغذائية من شأنها أن تؤثر إيجابا على الصحة العامة للأفراد، يساعد بشكل واضح لعلاج (السمنة - مرض السكري - هشاشة العظام - وزيادة مناعة والوقاية من السرطان. الخ .... كل من الطحالب).

\* قسم التغذية وعلوم الاطعمة كلية الاقتصاد المنزلى - جامعة المنوفية- مصر