

LOSCHMIDT  
LABORATORIES



# Bi9540 Biotechnology and practical use of algae and fungi

## Lecture 6 – Algae in food industry



## History of algae as food source

- Ancient records show that algae were consumed also by populations of cavemen
- 500 BC macroalgae were collected in China
- 14<sup>th</sup> century report Aztecs harvesting *Arthrospira* using it for preparation of *tecuitlatl*
- Harvesting of *Arthrospira* in Chad dates back to Kanem Empire (9<sup>th</sup> century AD)

**Table 1** Algal biotechnology historical data

Alga	Year of first record		
	Collected	Cultivated	Processed
Macroalgae			
<i>Porphyra</i>	530	1640	–
<i>Chondrus/Gelidium/ Gracilaria</i>	∞	1950	1658
<i>Laminaria/Macrocytis/ Fucus</i>	∞	1731	1925
<i>Eucheuma</i>	∞	1971	1965
Microalgae			
Diatoms	∞	1863 (selective use)	1914
<i>Spirulina</i>	∞	1965	1985
<i>Chlorella</i>	–	1975	1994
<i>Dunaliella</i>	–	1982	1985
<i>Odontella</i>	–	2002	2003

## Traditional food

- Algae were traditionally consumed worldwide, however, during modern age the consumption vanished
- Strong tradition is in Asian countries (Japan, China, Korea, Philipines,...), Chad, Mexico
- Once traditional consuments, Ireland is now renewing interest in algae

## Global market

- 42 countries currently report commercial macroalgae activity
- China is the top producer of algae (*Laminaria* as prime)
- North Korea, South Korea, Japan, Philippines, Chile, Norway, Indonesia, USA, India – 10 countries contribute about 95 % to the global macroalgae market
- About 90 % of production is culture-based
- Asia covers 99 % of production (China 75 %)

## Global market

- *Porphyra*, *Kappaphycus*, *Undaria*, *Eucheuma*, *Gracilaria* and *Laminaria* make up 99 % of produced macroalgae
- World's total harvest increased from 3 million tons in 1981 to 13 million tons in 2002 (according to FAO)

### Total Macroalgae Harvest in All Fishing Areas of the World

All Fishing Areas of the World	2000	2001	2002
Red Macroalgae	2,275,141	2,472,253	2,791,006
Brown Macroalgae	5,608,074	5,453,534	5,782,535
Green Macroalgae	96,235	93,688	76,265

- *Porphyra* is the most valuable macroalgae

## Summary of Edible Algae and the Corresponding Food Item

Scientific Name	Common Name	Class
<i>Nostoc flagelliforme</i>	Facai	Cyanophyceae
<i>Arthrospira</i> sp.	Dihé/Tecuitlatl	Cyanophyceae
<i>Chondrus crispus</i>	Pioca/Irish moss	Floridophyceae
<i>Porphyra</i> spp.	Nori/Laber/Zicai	Bangiophyceae
<i>Palmaria (Rodimenia) palmata</i>	Dulse	Floridophyceae
<i>Callophyllis variegata</i>	Carola	Floridophyceae
<i>Asparagopsis taxiformis</i>	Limu kohu	Floridophyceae
<i>Gigartina</i> spp.	Botelhas	Floridophyceae
<i>Gracilaria coronopifolia</i>	Limu manauea	Floridophyceae
<i>Gracilaria parvisipora</i>	Ogo	Floridophyceae
<i>Gracilaria verucosa</i>	Ogo-nori/Sea moss	Floridophyceae
<i>Sargassum echinocarpum</i>	Limu kala	Pheophyceae
<i>Dictyopteris plagiogramma</i>	Limu lipoa	Pheophyceae
<i>Undaria pinnatifida</i>	Wakame	Pheophyceae
<i>Laminaria</i> spp.	Kombu	Pheophyceae
<i>Nereocystis</i> spp.	Black kelp	Pheophyceae
<i>Hizikia fusiforme</i>	Hiziki/Hijiki	Pheophyceae
<i>Alaria esculenta</i>	Oni-wakame	Pheophyceae
<i>Cladosiphon okamuranus</i>	Mozuku	Pheophyceae
<i>Codium edule</i>	Limu wawale'iole	Bryopsidophyceae
<i>Enteromorpha prolifera</i>	Limu 'ele'ele/green laver	Ulvophyceae
<i>Ulva fasciata</i>	Limu palahalaha	Ulvophyceae
<i>Caulerpa lentillifera</i>	Limu Eka	Charophyceae
<i>Monostroma nitidum</i>	Aonori	Ulvophyceae

## Major microalgae commercialized for human nutrition

<b>Alga</b>	<b>Annual production (t/year)</b>	<b>Producer country</b>	<b>Applications and products</b>
<i>Spirulina (Arthrospira)</i>	3000	China, India, USA, Myanmar, Japan	Human and animal nutrition, cosmetics (phycobiliproteins, powders, extracts, tablets, beverages, chips, pasta, liquid extract)
<i>Chlorella sp.</i>	2000	Taiwan, Germany, Japan	Human nutrition, aquaculture, cosmetics (tablets, powders, nectar, noodles)
<i>Dunaliella salina</i>	1200	Australia, Israel, USA, China	Human nutrition, cosmetics ( $\beta$ -carotene, powders)
<i>Aphanizomenon flos-aquae</i>	500	USA	Human nutrition (capsules, crystals, powder)
<i>Haematococcus pluvialis</i>	300	USA, India, Israel	Aquaculture, astaxanthin
<i>Cryptocodinium cohnii</i>	240t DHA oil	USA	DHA oil
<i>Shizochytrium sp.</i>	10t DHA oil	USA	DHA oil

Source: Adapted from Spolaore et al. (2006) and Gouveia et al. (2008b)



## Ge-Xian-Mi

- *Nostoc sphaeroides*
- Regional use as food or ingredient
- Traditionally in China where it can be found in rice fields
- Colonies can reach 2.5 cm in diameter
- Dried *Nostoc* can be sautéed with oysters, used in soups or as thickener for food



# Nostoc flagelliforme

- also known as faat choy, fa cai, black moss, hair moss or hair weed
- Chinese delicacy for about 2000 years
- Appreciated for herbal values and spiritual image



# Arthrospira

- Filamentous cyanobacterium
- Also called Spirulina
- Favorite in Mexico and Chad
- In 1940 Dangeard reported on dihé consumed in Chad and suggested it was Arthrospira
- Dihé is an important source of vitamin A



# Arthrospira

- Aztecs collected and consumed Arthrospira



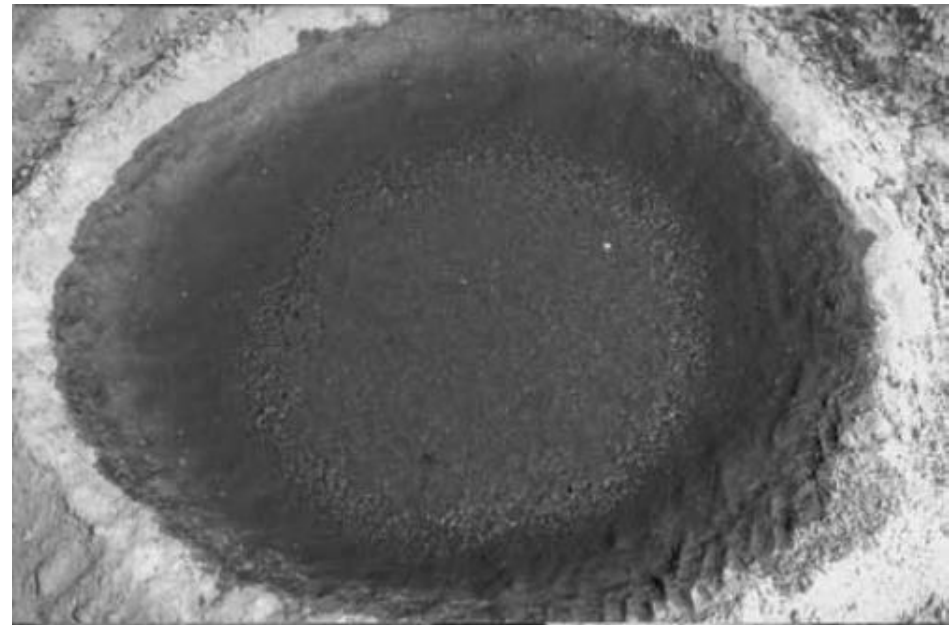
- *Tecuitlatl* (stone excrement) was consumed to boost energy. The food was some kind of dry cake



- Collected in Chad
- Consumed with maize, ...



Diet	Protein efficiency ratio
<i>Spirulina</i>	1.90
Maize	1.23
Rice	2.20
Wheat	1.15
Rice + <i>spirulina</i> (3:1)	2.35
Rice + <i>spirulina</i> (1:1)	2.40
Wheat + <i>spirulina</i> (3:1)	1.42
Wheat + <i>spirulina</i> (1:1)	1.90
Maize + <i>spirulina</i> (3:1)	1.80
Maize + <i>spirulina</i> (1:1)	1.72
Maize + oats + <i>spirulina</i> (3:2:5)	1.90
Maize + rice + <i>spirulina</i> (2:2:1)	1.95



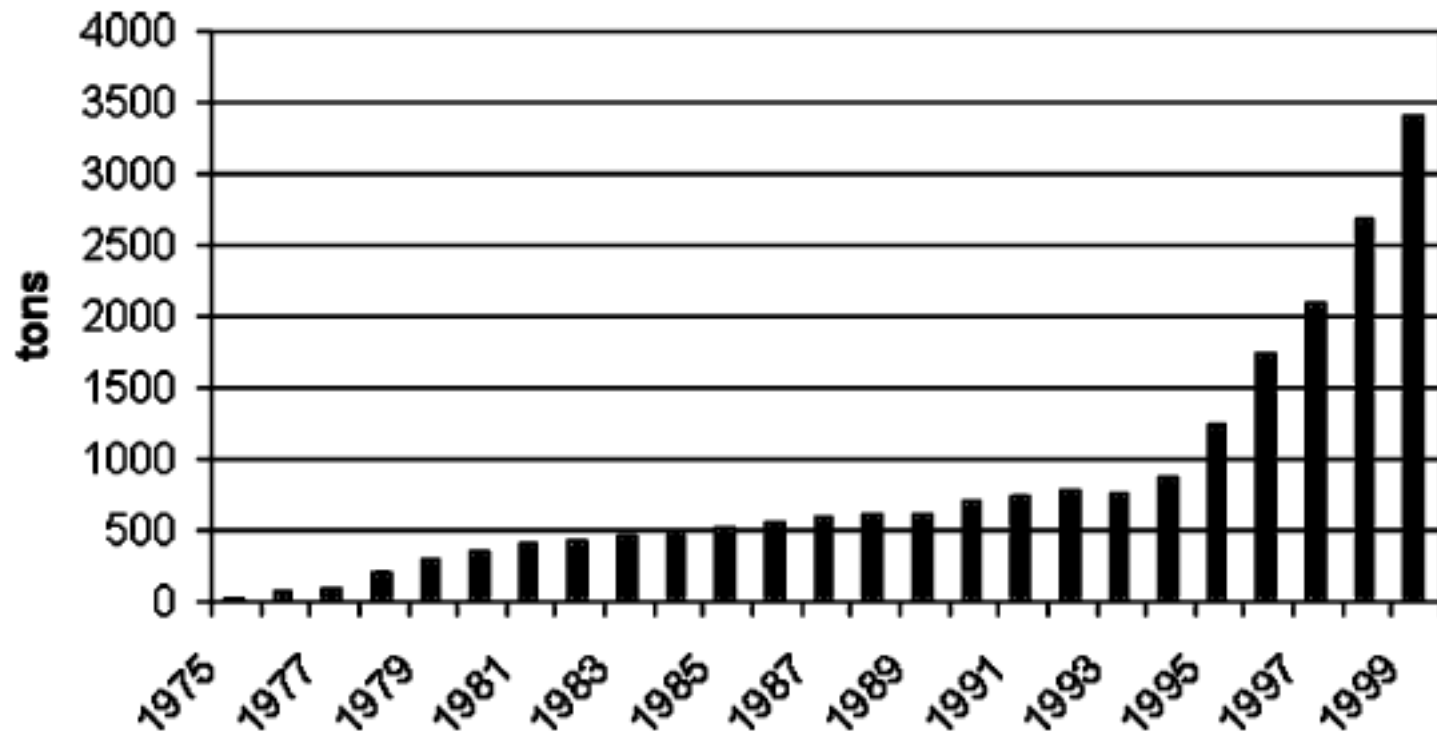


Fig. 2 World production of *Spirulina* biomass between 1975 and 1999 (1,000 tons = 1,016 t)

## Porphyra sp.

- Popularly known as Nori (Japan, Gim (Korea) or Zacai (China))
- One of the most nutritious macroalgae
- Protein content 25-50 % (75 % digestible)
- Source of trace elements and vitamins
- Excellent source of iodine
- Low in sugars (only 0.1 %)
- Taste of nori caused by content of alanine, glycine and glutamic acid





# Porphyra

- First cultivations in Japan and Korea date back to 17<sup>th</sup> cent.
- Common names are found in 16 languages, which proves widespread usage of the alga
- Nearly 133 species are known (28 from Japan)
- In Japan, the annual production of *Porphyra* species is valued at 100 billion yen (USD 1.5 billion)
- Sushi (Japan), Gimbap (Korea) are most important foods using nori

# Laminaria

- One of the biggest algae
- Native to Japan and Korea (cultivation since 1730)
- Millions of tons of *kombu* produced annually
- 10 % proteins, 2 % fat
- Source of minerals
- Served with salmon



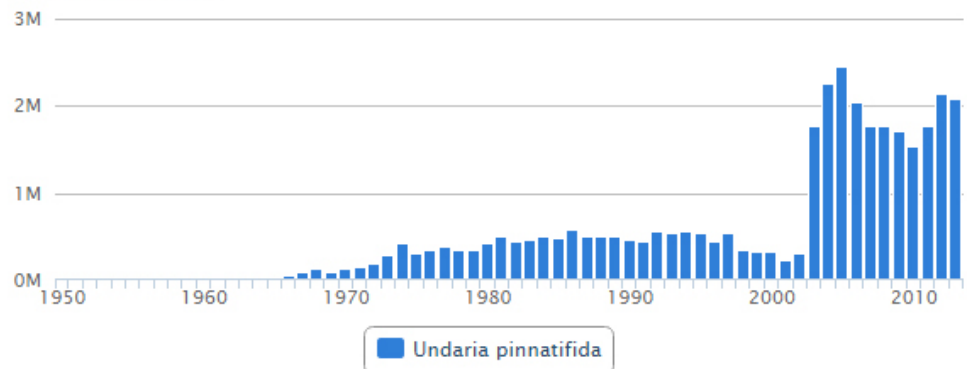
# Undaria

- Second most valuable edible macroalga
- Millions of tons of *wakame* produced annually
- Processed as variety of food products
  - Salted *wakame*
  - *Haiboshi wakame*
  - Cut *wakame*
- 16.3 g protein / 100 g



Global Aquaculture Production for species (tonnes)

Source: [FAO FishStat](#)



# Ulva

- Known as Sea lettuce
- Abundant around British Isles, China, Japan
- *Ulva lactuca* consumed in Scotland in soups and sallads
- Ulvas consumed by manatees 😊
- High in protein and minerals
- Rotting algae expel hydrogen sulfide



# Chlorella

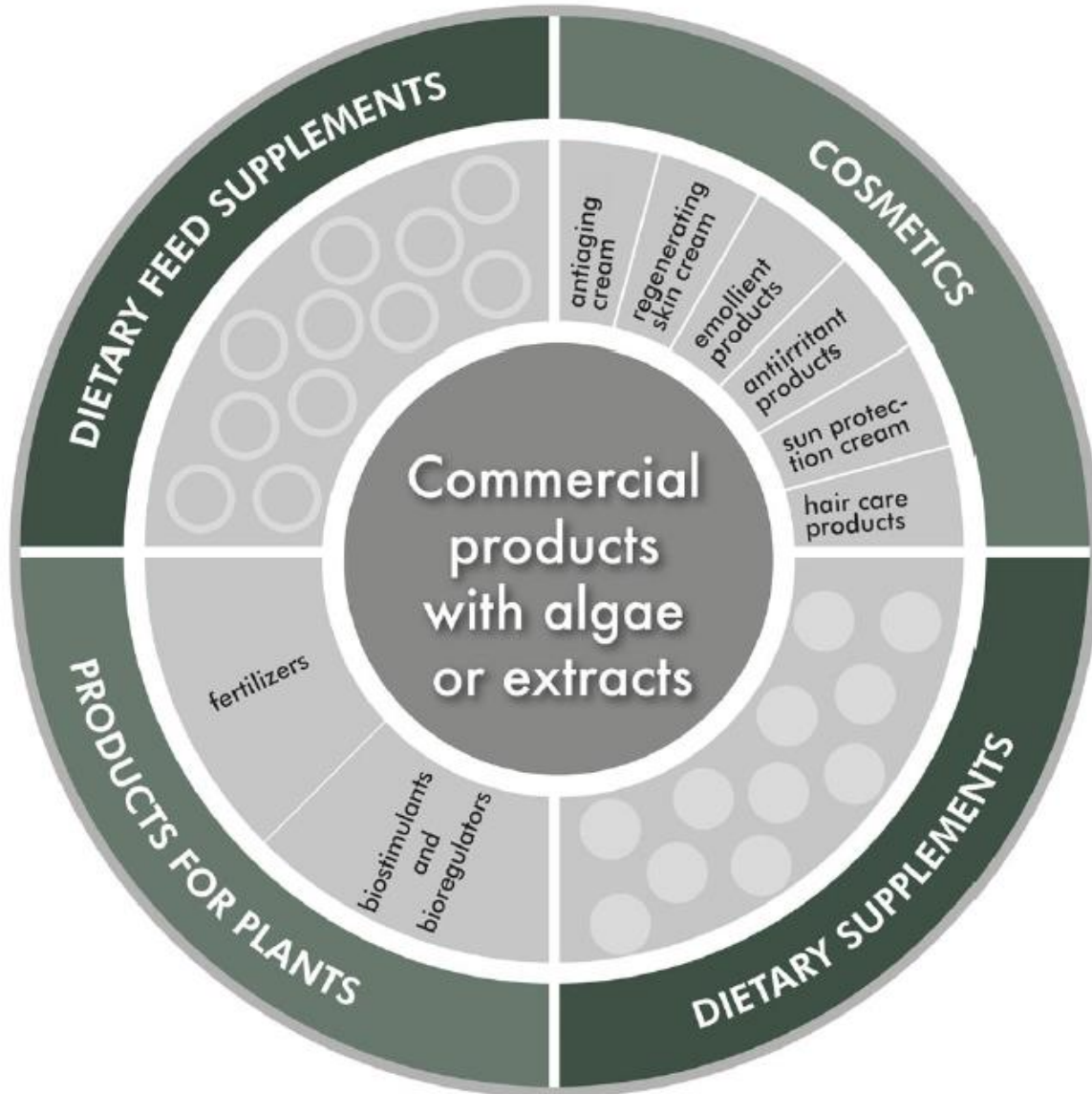
- Unicellular nonmotile green algae
- Although it is the most promising taxon concerning health issues, the production doesn't reach Arthrospira and others
- Widely used for processing of human dietary supplements and animal feed
- Rich in protein, PUFAs, and minerals
- Study from 2002 suggesting endotoxin presence in Chlorella was never proved
- Favorite for detoxification of organism



## Interkosmos program

- Chlorella was tested in microgravity conditions by soviet space program





Commercial products with algae or extracts

DIETARY FEED SUPPLEMENTS

COSMETICS

DIETARY SUPPLEMENTS

PRODUCTS FOR PLANTS

antitaging cream

regenerating skin cream

emollient products

antirritant products

sun protection cream

hair care products

fertilizers

biostimulants and bioregulators

# Algal extracts

## POLYSACCHARIDES



### **Brown algae**

(*Phaeophyta*):

- alginate
- cellulose
- fucoidan
- laminarin



### **Red algae**

(*Rhodophyta*):

- agar
- carrageenan
- cellulose
- furcellaran
- mannan
- porphyran
- xylan



### **Green algae**

(*Chlorophyta*):

- amylose, amylopectin
- cellulose
- inulin
- mannan
- pectin
- xylan
- ulvan

## PIGMENTS



### **Phycobilins**

- **phycocyanin**
  - cyanobacteria (Blue-green algae)
- **phycoerythrin**
  - Red algae (*Rhodopyta*)



### **Carotenoids**

- **carotene:**
  - $\alpha$ -carotene
  - $\beta$ -carotene
  - lycopene
- **xanthophyll:**
  - astaxanthin
  - fucoxanthin
  - zeaxanthin
  - lutein



### **Chlorophylls**




- chlorophyll a
- chlorophyll b
- chlorophyll c



## COMPOUNDS WITH ANTI-OXIDANT ACTIVITY

glutathione (GSH)		
vitamins	ascorbate (vitamin C)	
	tocopherol (vitamin E)	• $\alpha$ -, $\gamma$ -, $\delta$ - tocopherol
carotenoids	$\alpha$ -carotene and $\beta$ -carotene	
	fucoxanthin and astaxanthin	
polyphenols	phlorotannin – brown algal polyphenol	<ul style="list-style-type: none"> <li>• fucol</li> <li>• phlorethol</li> <li>• fucophlorethol</li> <li>• fuhalol</li> <li>• isofuhalol</li> <li>• eckol</li> </ul>
	catechin	<ul style="list-style-type: none"> <li>• catechin (3-hydroxyflavan)/catechin gallate</li> <li>• epicatechin/epicatechin gallate</li> <li>• epigallocatechin/epigallocatechin gallate</li> </ul>
	phenolic acid	
	flavonoids	<ul style="list-style-type: none"> <li>• anthocyanins</li> <li>• flavonols</li> <li>• flavanols</li> <li>• flavanones</li> <li>• flavones</li> <li>• isoflavones</li> </ul>
	tannins	
	lignans	
mycosporine-like amino acids	mycosporine-glycine	

## PLANT GROWTH-PROMOTING SUBSTANCES/HORMONES

plant hormones (phytohormones)	cytokinins	
	auxins	
	gibberellins	
	abscisic acid (ABA)	
	ethylene	
betaines		
polyamines		
sterols	fucosterol	 Brown algae (Phaeophyta)
	cholesterol	
	cholesterol	 Red algae (Rhodophyta)
	ergosterol	
	24-methylenecholesterol	
	cholesterol	 Green algae (Chlorophyta)

## OTHER COMPOUNDS

vitamins	B <sub>12</sub> , K, C, E, A, D		
minerals	K, Ca, Mg, Na, Zn, Cu, Co, I, B		
peptides and proteins			
lectins			
lipids	fatty acids	polyunsaturated fatty acids (PUFAs)	<ul style="list-style-type: none"> <li>• <math>\gamma</math>-linolenic acid (GLA)</li> <li>• arachidonic acid (AA)</li> <li>• eicosapentaenoic acid (EPA)</li> <li>• docosahexaenoic acid (DHA)</li> </ul>
	sterols		
diterpenes	dolabellanes		
	hydroazulenoids		
	xenicanes		
	extended sesquiterpenoids		

# Polysacharides

- Agar, alginate, carragenan

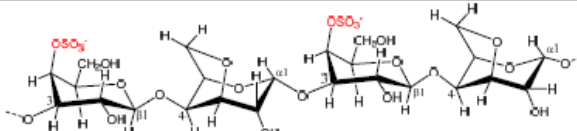
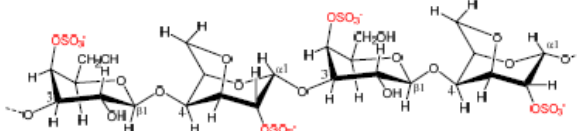
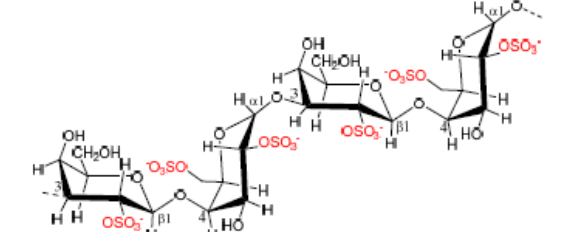
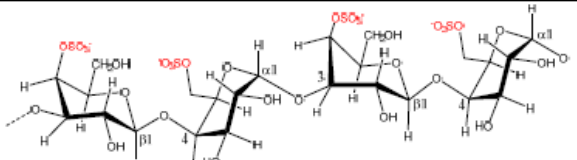
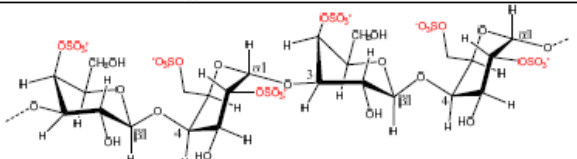
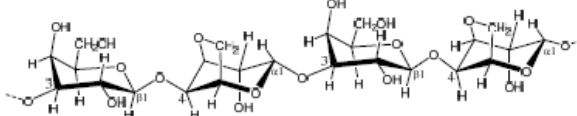
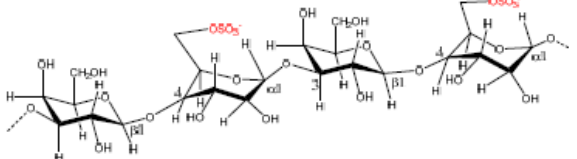
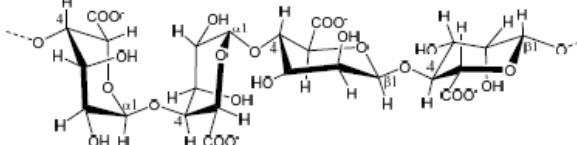
The market for seaweed-derived hydrocolloids, agars, alginates, and carrageenans [1].

<b>Product</b>	<b>Global Production (ton/year)</b>	<b>Retail Price (US\$/kg)</b>	<b>Approximate Gross Market Value (US\$ million/year)</b>
Agars	10,600	18	191
Alginates	30,000	12	339
Carrageenans	60,000	10.4	626

Physico-chemical properties for agar and carrageenans. The numbers are estimates.

Viscosity values are given as (centipoise, cP) that is equivalent to  $\text{N}\cdot\text{s}\cdot\text{m}^{-2}$  [56].

<b>Properties</b>	<b>Agar</b>	<b>Carrageenan</b>
Solubility	Boiling water	Boiling water
Gel Strength (1.5% at 20 °C)	700–1000 g/cm <sup>3</sup>	100–350 g/cm <sup>3</sup>
Viscosity (1.5% at 60 °C)	10–100 centipoise	30–300 centipoise
Melting point	85–95 °C	50–70 °C
Gelling point	32–45 °C	30–50 °C

Seaweed Source	Products	Main Chemical Structures	Applications
<i>Kappaphycus alvarezii</i>	$\kappa$ -Carrageenan		Gelling agent (stiff and brittle gel)
<i>Eucheuma spinosum</i>	$\iota$ -Carrageenan		Gelling agent (flexible soft gel)
<i>Gigartina</i> spp. <i>Chondrus</i> spp.	$\lambda$ -Carrageenan		Thickener
<i>Kappaphycus alvarezii</i>	$\mu$ -Carrageenan		$\kappa$ -Carrageenan precursor
<i>Eucheuma spinosum</i>	$\nu$ -Carrageenan		$\iota$ -Carrageenan precursor
<i>Gelidiella</i> spp. <i>Gelidium</i> spp.	Agar/Agarose		Microbiology Gelling agent (strong and rigid)
<i>Porphyra umbilicalis</i>	Porphyran		Agar precursor
<i>Laminaria</i> spp. <i>Sargassum</i> spp.	Alginate		Gelling agent

# Summary of Commercially Exploited Algae and the Corresponding Extracts

Scientific Name	Class	Extracts
<i>Gracilaria chilensis</i>	Floridophyceae	Agar
<i>Ahnfeltia plicata</i>	Floridophyceae	Agar/Carrageenan
<i>Gelidium lingulatum</i>	Floridophyceae	Agar
<i>Pterocladia</i> spp.	Floridophyceae	Agar
<i>Hypnea</i> spp.	Floridophyceae	Agar
<i>Chondrus crispus</i>	Floridophyceae	Carrageenan
<i>Gigartina skottsbergii</i>	Floridophyceae	Carrageenan
<i>Gigartina canaliculata</i>	Floridophyceae	Carrageenan
<i>Mazzaella laminaroides</i>	Floridophyceae	Carrageenan
<i>Sarcothalia crispata</i>	Floridophyceae	Carrageenan
<i>Kappaphycus alvarezii</i>	Floridophyceae	Carrageenan
<i>Eucheuma denticulatum</i>	Floridophyceae	Carrageenan
<i>Iridaea</i> spp.	Floridophyceae	Carrageenan
<i>Laminaria hyperborea</i>	Phaeophyceae	Alginate
<i>Laminaria digitata</i>	Phaeophyceae	Alginate
<i>Laminaria japonica</i>	Phaeophyceae	Alginate
<i>Laminaria saccharina</i>	Phaeophyceae	Alginate
<i>Macrocystis pyrifera</i>	Phaeophyceae	Alginate
<i>Ascophyllum nodosum</i>	Phaeophyceae	Alginate
<i>Durvillea potatorum</i>	Phaeophyceae	Alginate
<i>Ecklonia</i> spp.	Phaeophyceae	Alginate
<i>Lessonia nigrescens</i>	Phaeophyceae	Alginate
<i>Lessonia trabiculata</i>	Phaeophyceae	Alginate

# Agar

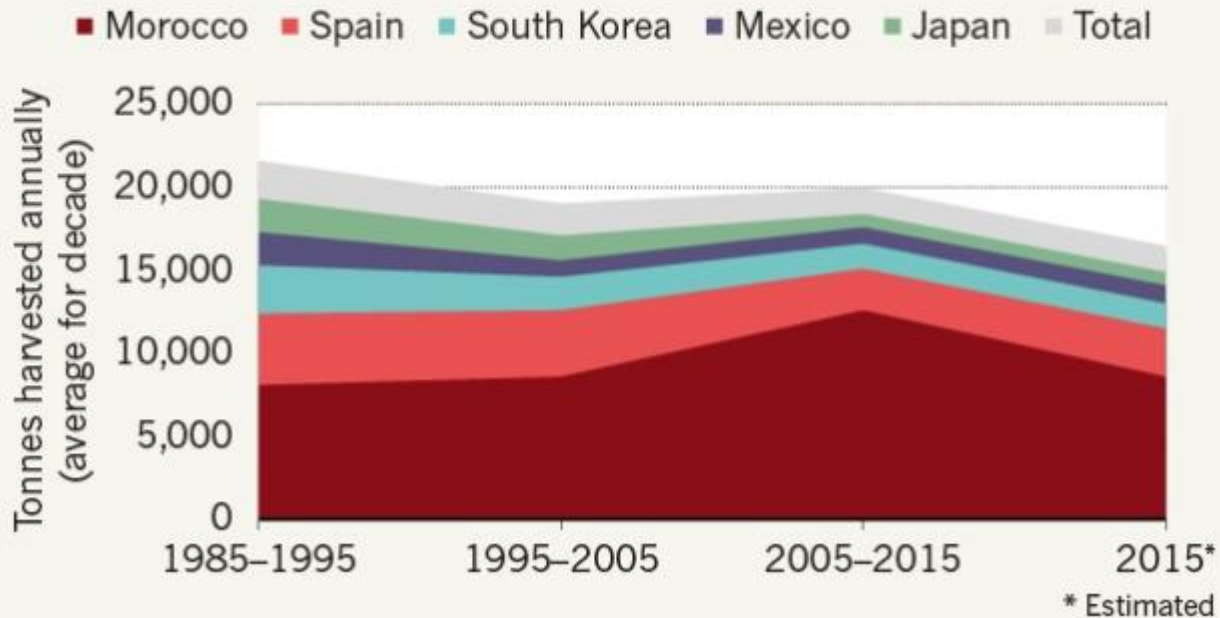
- D- and L-galactopyranose
- Name derived from Malaysian word 'agar-agar,' which literally means 'macroalgae'.
- As gelling agent kanten it is known from Japan since 17<sup>th</sup> cent.
- Most importantly is agar used as solidifier for media in microbiology
- Non-ionic purified fraction is known as agarose

# Agar

- High-grade (bacteriological) agar is extracted from *Pterocladia* and *Gelidium*
  - Spain, France, Portugal, Morocco, California, Mexico, New Zealand, South Korea, India, Chile, Japan, ...
- Food-grade is extracted from *Pterocladia*
- Low grade agars can be isolated from *Gracilaria* and *Hypnea*
- Agar production is valued at approx. \$200 million annually

## SEAWEED SHORTAGE

Harvests of *Gelidium* seaweed, from which the agar used in labs is made, are shrinking — particularly in Morocco, which is the world's major supplier.



# Alginate

- Constituents of Phaeophyceae cell wall
- Mannuronic and guluronic acid
- Composition of heteropolysacharide blocks depends on species and extraction procedure
- Most suitable for alginate extraction are brown algae (Laminariales and Fucales) grown in cold water (<20°C)
- Approx. 50,000 tons are produced annually
  - Scotland, Norway, China, USA
  - Production is valued approx. USD 215 mil annually



# Alginate

- Alginates have variety of applications
  - Thickening of food (E401) – sodium alginate
  - Stabilizers of ice cream
  - Bandager, fabrics
  - Thickening paste for printing

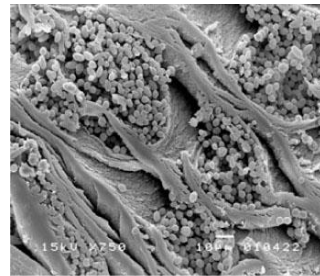
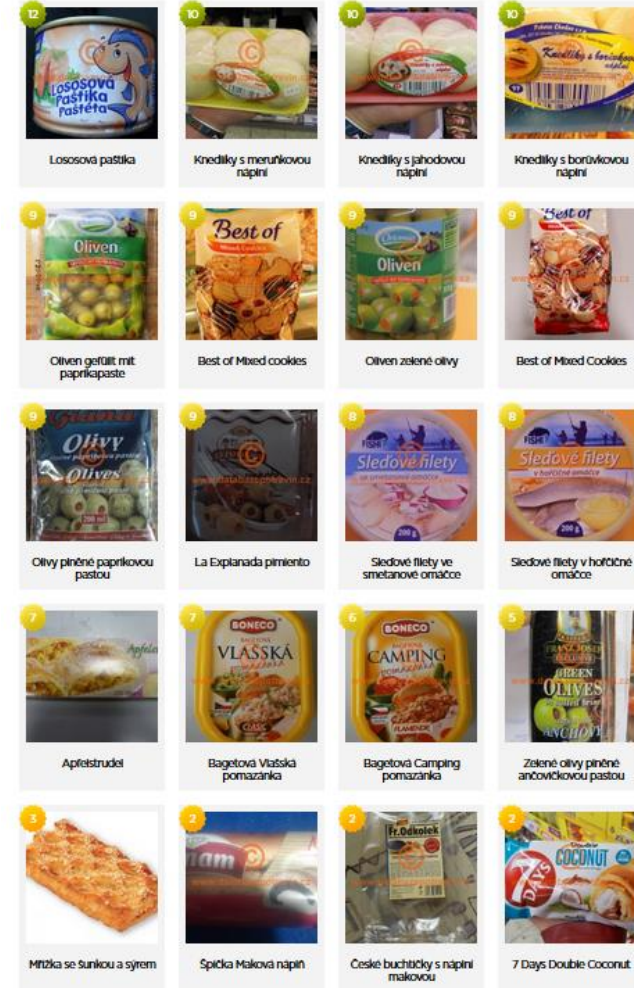


Figure 3. Yeast cells in the alginate gel layer of the ALM after 72 h of ethanol fermentation

# Carrageenan

- D-galactopyranose units
- *Carrageenan* is colloquial Irish word for macroalgae
- Known from Ireland since 1810
- *Chondrus crispus* used to be sole source, nowadays  
*Eucheuma*, *Ahnfeltia* and *Gigartina* are also exploited
- 30,000 tons of carrageenan produced annually
- As of 2011, global sales of carrageenan were estimated at  
\$640 million

# Carrageenan

- Most raw material now comes from the Phillipines
- There are three main commercial classes of carrageenan:
  - **Kappa** forms strong, rigid gels in the presence of potassium ions; it reacts with dairy proteins. It is sourced mainly from *Kappaphycus alvarezii*
  - **Iota** forms soft gels in the presence of calcium ions. It is produced mainly from *Eucheuma denticulatum*
  - **Lambda** does not gel, and is used to thicken dairy products.

# Algal nutritional values

Commodity	Protein	Carbohydrates	Lipids
Egg	47	4	41
Meat	43	1	34
Milk	26	38	28
Rice	8	77	2
Soybean	37	30	20
Anabaena cylindrica	43 – 56	25 – 30	4-7
Chlorella vulgaris	51 – 58	12 – 17	14 – 22
Dunaliella bioculata	49	4	8
Haematococcus	48	27	15
Spirulina platensis	64	25	7

Soy vs. Algae	Soy	Algae*
Protein	44%	55%
Lipids	2%	18%
Carbohydrates	39%	15%
Ash	15%	12%

## Chemical composition of different algae (w/w)

Alga	Proteins	Carbohydrates	Lipids
<i>Aphanizomenon flos-aquae</i>	62	23	3
<i>Chlorella pyrenoidosa</i>	57	26	2
<i>Chlorella vulgaris</i>	51–58	12–17	14–22
<i>Porphyridium cruentum</i>	28–39	40–57	9–14
<i>Schizochytrium</i> sp.	-	-	50–77
<i>Arthrospira maxima</i>	60–71	13–16	6–7

Source: Adapted from Becker (2007) and Chisti (2007)

# Kombu and Wakame nutritional values

## Vitamin Contents of Marine Algae *Wakame* (*U. pinnatifida*) and *Kombu* (*L. japonica*) (in mg [100 g d.w.]<sup>-1</sup>)

Vitamins	Kombu	Wakame
β-carotene	2.99 ± 0.09	1.30 ± 0.12
Retinol equivalent	0.481 ± 0.015	0.217 ± 0.006
Vitamin B <sub>1</sub>	0.24 ± 0.02	0.30 ± 0.04
Vitamin B <sub>2</sub>	0.85 ± 0.08	1.35 ± 0.09
Vitamin B <sub>6</sub>	0.09 ± 0.01	0.18 ± 0.02
Niacin	1.58 ± 0.14	2.56 ± 0.11

## Dietary Fiber Content of *Wakame* (*U. pinnatifida*) and *Kombu* (*L. japonica*) (% d.w.)

	Soluble	Insoluble	Total
Kombu	32.6	4.7	37.3
Wakame	30	5.3	35.3

## Mineral Composition of *Wakame* (*U. pinnatifida*) and *Kombu* (*L. japonica*) (in mg [100 g d.w.]<sup>-1</sup>)

Minerals	Kombu	Wakame
Ca	880 ± 20	950 ± 30
Mg	550 ± 15	405 ± 10
P	300 ± 10	450 ± 12
I	170 ± 5.5	26 ± 2.4
Na	2532 ± 120	6494 ± 254
K	5951 ± 305	5691 ± 215
Ni	0.325 ± 0.020	0.265 ± 0.015
Cr	0.227 ± 0.073	0.072 ± 0.026
Se	<0.05	<0.05
Fe	1.19 ± 0.03	1.54 ± 0.07
Zn	0.886 ± 0.330	0.944 ± 0.038
Mg	0.294 ± 0.017	0.332 ± 0.039
Cu	0.247 ± 0.076	0.185 ± 0.016
Pb	0.087 ± 0.021	0.079 ± 0.015
Cd	0.017 ± 0.007	0.028 ± 0.006
Hg	0.054 ± 0.005	0.022 ± 0.003
As	0.087 ± 0.006	0.055 ± 0.008

**Table 1** Amino acid composition in mg g<sup>-1</sup> protein of wakame (*U. pinnatifida*) and nori (*P. purpurea*)

Amino acid	<i>U. pinnatifida</i>	<i>P. purpurea</i>
Aspartic acid	75.60±12.12	66.58±3.63
Serine	33.96±3.04	46.25±2.47
Glutamic acid	120.85±20.26	83.04±6.13
Glycine	65.75±7.8	75.39±6.26
Histidine	17.11±1.17	22.04±1.00
Arginine	88.19±8.49	89.98±8.14
Threonine	29.22±1.24	50.10±3.98
Alanine	97.57±8.20	80.54±7.29
Proline	44.26±3.89	37.97±0.19
Cystine	3.26±0.30	4.58±0.55
Tyrosine	20.99±0.64	29.38±0.92
Valine	58.48±4.77	47.98±3.61
Methionine	1.41±0.21	13.73±0.43
Lysine	39.96±3.40	29.91±1.01
Isoleucine	50.82±4.36	34.44±1.03
Leucine	86.14±7.39	53.23±1.45
Phenylalanine	48.46±3.93	78.15±2.50

**Table 2** Mineral content (mg 100 g<sup>-1</sup> dry weight) of wakame (*U. pinnatifida*) and nori (*P. purpurea*)

Mineral	<i>U. pinnatifida</i>	<i>P. purpurea</i>
Calcium	693.2±7.6	359.2±4.1
Phosphorus	1070.0±7.0	720.2±6.1
Iron	7.94±0.80	10.5±0.11
Magnesium	630.2±8.2	233.9±7.2
Zinc	3.86±0.27	3.29±0.24
Iodine	9.6±0.73	0.54±0.05
Sodium	3,511.0±26.0	728.2±4.04
Potassium	5,679.0±22.3	1,602.0±4.03
Manganese	0.69±0.02	2.53±0.05
Copper	0.19±0.01	0.57±0.02

**Table 3** Vitamin content of wakame (*U. pinnatifida*) and nori (*P. purpurea*). Results are expressed in dry weight of sample

Vitamin	<i>U. pinnatifida</i>	<i>P. purpurea</i>
Vitamin A (UI kg <sup>-1</sup> )	4,729±23.3	23,830±17.2
Vitamin B <sub>1</sub> (mg kg <sup>-1</sup> )	0.30±0.03	0.40±0.02
Vitamin B <sub>2</sub> (mg kg <sup>-1</sup> )	0.68±0.03	1.89±0.09
Vitamin B <sub>5</sub> (mg kg <sup>-1</sup> )	2.0±0.11	2.7±0.12
Vitamin B <sub>8</sub> (µg g <sup>-1</sup> )	0.22±0.01	0.10±0.01
Vitamin B <sub>12</sub> (µg 100 g <sup>-1</sup> )	0.16±0.01	2.90±2.7
Vitamin B <sub>6</sub> (mg kg <sup>-1</sup> )	1.5±0.02	0.9±0.08
Vitamin B <sub>3</sub> (mg kg <sup>-1</sup> )	<5	<5
Folic acid (µg g <sup>-1</sup> )	0.79±0.08	<0.02
Vitamin C (mg 100 g <sup>-1</sup> )	3.10±0.11	9.73±0.31
Vitamin E (mg kg <sup>-1</sup> )	6.3±0.12	9.3±0.27

**Table 2.** Chemical composition (g 100 g<sup>-1</sup> dry weight) of *Gracilaria salicornia* and *Ulva lactuca*

Seaweed	Crude lipid	Crude protein	Crude fibre	Dry weight	Ash content
<i>G. salicornia</i>	2.00 ± 0.92 <sup>a</sup>	9.58 ± 0.15 <sup>a</sup>	10.4 ± 0.89 <sup>a</sup>	9.98 ± 0.15 <sup>a</sup>	38.91 ± 1.62 <sup>a</sup>
<i>U. lactuca</i>	0.99 ± 0.00 <sup>a</sup>	10.69 ± 0.67 <sup>a</sup>	5.6 ± 1.69 <sup>b</sup>	5.96 ± 0.33 <sup>b</sup>	18.03 ± 2.37 <sup>b</sup>

Results are the means of triplicate determinations ± SD.

Values in columns with different superscripts are significantly different ( $P < 0.05$ ).

<sup>a,b</sup> Means in columns with different letters are significantly different ( $P < 0.05$ ).

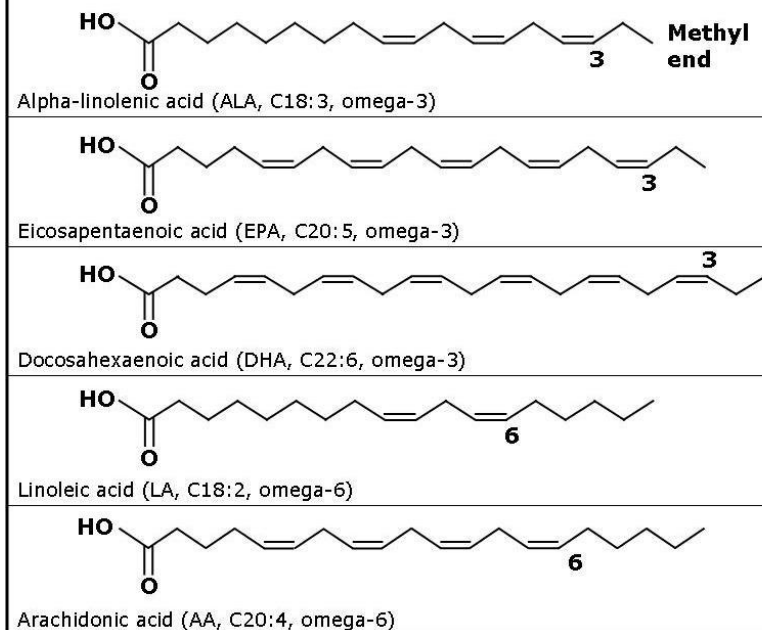
**Table 3.** Amino acid concentrations in *Gracilaria salicornia* and *Ulva lactuca*

Amino acid	<i>G. salicornia</i> (mg g <sup>-1</sup> protein) <sup>†</sup>	<i>U. lactuca</i> (mg g <sup>-1</sup> protein) <sup>†</sup>	Cereal (Pellett <sup>22</sup> )	Legume (Pellett <sup>22</sup> )	FAO/WHO/UNU <sup>11</sup> requirement pattern
Aspartic acid	53.9 ± 8.4 <sup>a</sup>	49.7 ± 5.6 <sup>b</sup>	–	–	–
Glutamic acid	75.9 ± 6.3 <sup>a</sup>	70.7 ± 6.6 <sup>a</sup>	–	–	–
Serine	34.6 ± 2.1 <sup>a</sup>	28.7 ± 2.4 <sup>b</sup>	–	–	–
Glycine	75.6 ± 3.8 <sup>a</sup>	39.7 ± 2.1 <sup>b</sup>	–	–	–
Histidine	14.3 ± 2.7 <sup>a</sup>	15.2 ± 1.5 <sup>a</sup>	–	–	–
Arginine*	75.78 ± 3.5 <sup>a</sup>	37.9 ± 1.4 <sup>b</sup>	–	–	–
Threonine*	32.9 ± 2.2 <sup>a</sup> (0.96)	31.1 ± 2.5 <sup>b</sup> (0.91)	33.6	40	34
Alanine	75.5 ± 7.3 <sup>a</sup>	43.3 ± 2.4 <sup>b</sup>	–	–	–
Proline	39.8 ± 5.6 <sup>a</sup>	37.9 ± 2.1 <sup>b</sup>	–	–	–
Tyrosine*	75.9 ± 5.6 <sup>a</sup>	23.4 ± 4.2 <sup>b</sup>	–	–	–
Valine*	41.4 ± 4.9 <sup>a</sup> (1.18)	39.2 ± 3.5 <sup>b</sup> (1.12)	51.1	50.5	35
Methionine*	77.5 ± 2.8 <sup>a</sup> (3.1)	5.9 ± 1.4 <sup>b</sup> (0.23)	41.1	25.3	25 <sup>5</sup>
Isoleucine*	30.3 ± 3.4 <sup>a</sup> (1.08)	21.7 ± 2.6 <sup>b</sup> (0.77)	39.8	45.3	28
Leucine*	76.6 ± 6.1 <sup>a</sup> (1.16)	45.1 ± 4.8 <sup>b</sup> (0.68)	86.5	78.9	66
Phenylalanine*	32.7 ± 5.7 <sup>a</sup> (1.72)	28.4 ± 3.6 <sup>b</sup> (0.82)	83.0 <sup>‡</sup>	84.9 <sup>‡</sup>	63
Lysine*	77.1 ± 5.8 <sup>a</sup> (1.32)	25.4 ± 0.5 <sup>b</sup> (0.43)	30.5	67.1	58
Tryptophan	ND	ND	–	–	–
Essential amino acids	520.18 ± 22.47 <sup>a</sup>	258.1 ± 11.6 <sup>b</sup>	–	–	328
Non-essential amino acids <sup>A</sup>	369.62 ± 33.0 <sup>a</sup>	285.2 ± 17.27 <sup>b</sup>	–	–	661
Total amino acids	889.78 ± 22.64 <sup>a</sup>	543.3 ± 15.14 <sup>b</sup>	–	–	–

**Table 4.** Relative fatty acid content of *Gracilaria salicornia* and *Ulva lactuca* (% of total fatty acid content)

Fatty acids	<i>G. salicornia</i>	<i>U. lactuca</i>
C 12:0	6.98 ± 0.50 <sup>a</sup>	6.03 ± 0.85 <sup>a</sup>
C 14:0	5.5 ± 0.86 <sup>a</sup>	5.53 ± 0.13 <sup>a</sup>
C16:0	33.39 ± 8.86 <sup>a</sup>	34.33 ± 2.65 <sup>a</sup>
C 16:1	2.46 ± 0.12 <sup>a</sup>	2.48 ± 0.08 <sup>a</sup>
C 18:0	3.04 ± 0.66 <sup>a</sup>	2.44 ± 0.29 <sup>a</sup>
C 18:1	11.72 ± 2.01 <sup>a</sup>	2.63 ± 0.41 <sup>b</sup>
C 18:2 ω6	1.45 ± 0.38 <sup>b</sup>	4.89 ± 0.78 <sup>a</sup>
C 18:3 ω3	1.65 ± 0.04 <sup>b</sup>	2.77 ± 0.06 <sup>a</sup>
C 20:4 ω6	8.05 ± 1.98 <sup>a</sup>	8.53 ± 0.27 <sup>a</sup>
C 20:5 ω3	1.53 ± 0.27 <sup>b</sup>	3.65 ± 0.31 <sup>a</sup>
C 22:5 ω3	4.7 ± 0.19 <sup>a</sup>	4.98 ± 0.89 <sup>a</sup>
Saturated fatty acids	48.92 ± 6.83 <sup>a</sup>	48.34 ± 3.67 <sup>a</sup>
Monounsaturated	16.36 ± 1.54 <sup>a</sup>	5.11 ± 0.5 <sup>b</sup>
PUFAs	17.30 ± 1.18 <sup>b</sup>	24.84 ± 1.03 <sup>a</sup>
PUFAs ω6	10.14 ± 0.7 <sup>b</sup>	13.43 ± 0.5 <sup>a</sup>
PUFAs ω3	7.89 ± 0.43 <sup>b</sup>	11.41 ± 0.52 <sup>a</sup>
Ratio ω6/ω3	1.2	1.17

**FIG. 1 OMEGA-3 AND OMEGA-6 FATTY ACIDS**



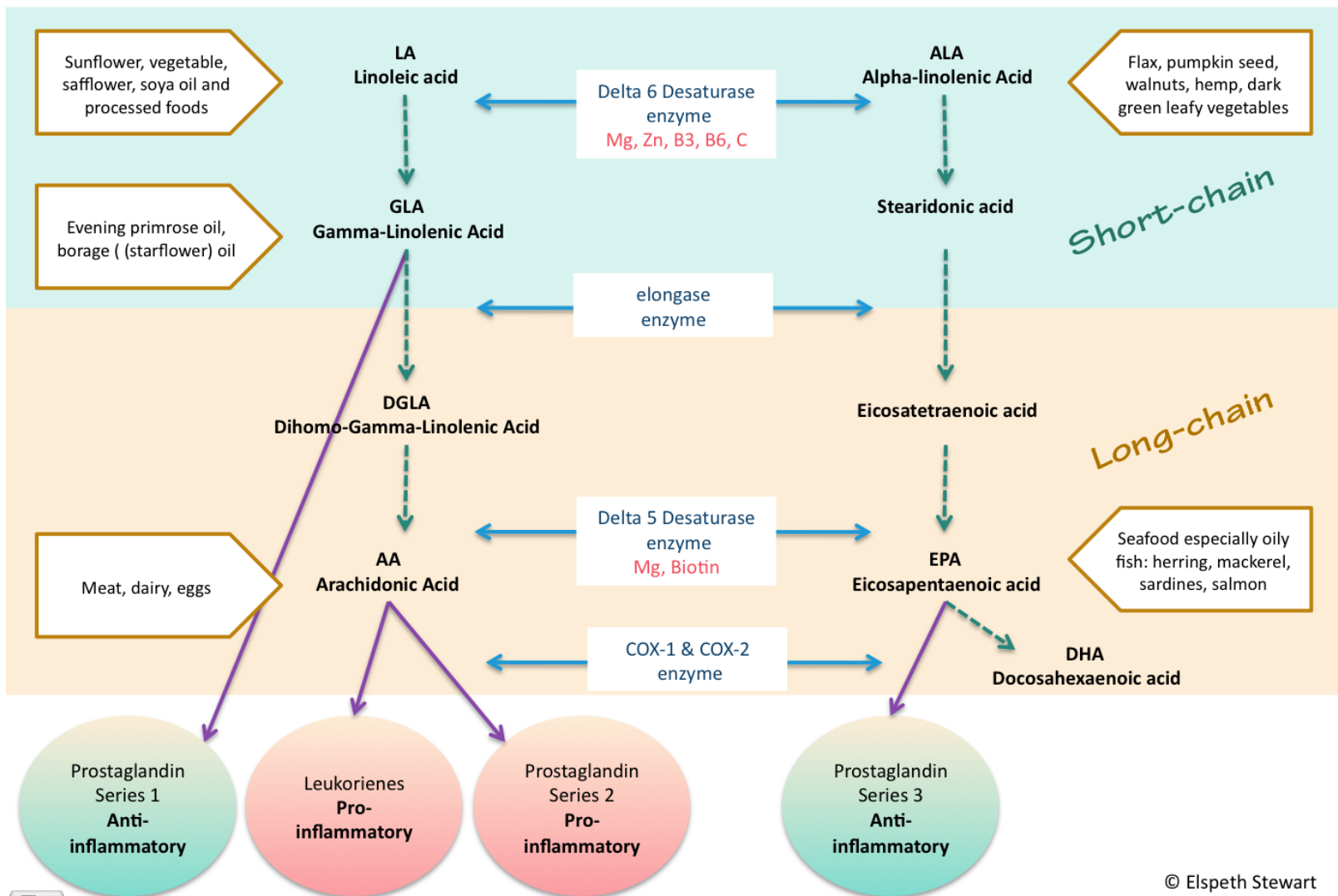
**Table 5.** Elemental composition in *Gracilaria salicornia* and *Ulva lactuca* (mg 100 g<sup>-1</sup> dry weight)

Mineral	<i>G. salicornia</i>	<i>U. lactuca</i>	Foodstuff (USDA <sup>29</sup> )				
			Lettuce	Cabbage	Carrots	Broccoli	Spinach
Potassium	11 380.06 ± 73.45 <sup>a</sup>	2414.02 ± 26.89 <sup>b</sup>	956.42	1931.1	3747.2	3381.2	4798.8
Calcium	948.45 ± 7.7 <sup>b</sup>	2782.13 ± 11.12 <sup>a</sup>	177.48	368.95	386.43	502.9	851.4
Sodium	1035.92 ± 61.48 <sup>b</sup>	1805.44 ± 58.6 <sup>a</sup>	138.04	141.3	807.99	353.1	679.4
Iron	67.35 ± 7.77 <sup>b</sup>	199.45 ± 5.86 <sup>a</sup>	4.23	4.63	3.51	7.81	23.3
Manganese	4.16 ± 0.05 <sup>a</sup>	2.11 ± 0.22 <sup>b</sup>	1.23	1.24	1.64	2.184	7.71
Nickel	0.92 ± 0.03 <sup>a</sup>	0.76 ± 0.01 <sup>a</sup>	-	-	-	-	-
Copper	0.57 ± 0.07 <sup>b</sup>	1.45 ± 0.21 <sup>a</sup>	0.14	0.18	0.52	0.52	1.11
Cobalt	0.24 ± 0.05 <sup>a</sup>	0.15 ± 0.03 <sup>b</sup>	-	-	-	-	-
Total cations	13 438 ± 143 <sup>a</sup>	7205.51 ± 102 <sup>b</sup>	-	-	-	-	-



## Omega 6 pathway

## Omega 3 pathway



# Dietary supplements, pharmaceuticals

**Table 2** Microalgal species with high relevance for biotechnological applications

Species/group	Product	Application areas	Basins/reactors
<i>Spirulina platensis</i> /Cyanobacteria	Phycocyanin, biomass	Health food, cosmetics	Open ponds, natural lakes
<i>Chlorella vulgaris</i> /Chlorophyta	Biomass	Health food, food supplement, feed surrogates	Open ponds, basins, glass-tube PBR
<i>Dunaliella salina</i> /Chlorophyta	Carotenoids, $\beta$ -carotene	Health food, food supplement, feed	Open ponds, lagoons
<i>Haematococcus pluvialis</i> /Chlorophyta	Carotenoids, astaxanthin	Health food, pharmaceuticals, feed additives	Open ponds, PBR
<i>Odontella aurita</i> /Bacillariophyta	Fatty acids	Pharmaceuticals, cosmetics, baby food	Open ponds
<i>Porphyridium cruentum</i> /Rhodophyta	Polysaccharides	Pharmaceuticals, cosmetics, nutrition	Tubular PBR
<i>Isochrysis galbana</i> /Chlorophyta	Fatty acids	Animal nutrition	Open ponds
<i>Phaedactylum tricorutum</i> /Bacillariophyta	Lipids, fatty acids	Nutrition, fuel production	Open ponds, basins
<i>Lyngbya majuscula</i> /Cyanobacteria	Immune modulators	Pharmaceuticals, nutrition	

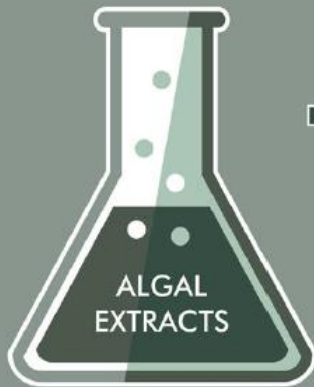
- Supplements rich in natural active compounds
- Healthy nutrition, immunomodulation, etc.
- Studies have proven positive effects on human health

## Biologically active compounds

- Mostly microalgae, but also macroalgae are used as source of biologically active compounds with variety of activities:
  - Antibacterial
  - Antiviral
  - Antifungal
  - Antioxidative
  - Antiinflammatory
  - Antitumor
  - ...



# PROPERTIES OF COMPOUNDS IN ALGAL EXTRACTS



<p>➔ <b>antibacterial</b></p>	• proteins
	• polyphenols
	• polysaccharides
	• pigments: chlorophyll and carotenoids
	• PUFAs
	• PUFAs
<p>➔ <b>antifungal</b></p>	• pigments: chlorophyll and carotenoids
	• terpens
	• phenols
<p>➔ <b>antioxidative</b></p>	• proteins
	• mycosporine-like amino acids
	• glutathione
	• polyphenols
	• polysaccharides
	• PUFAs
	• carotenoids
• tocopherol	
<p>➔ <b>antiinflammatory</b></p>	• ascorbate
	• proteins
	• carotenoids
	• polysaccharides
	• sterols - fucosterol
	• polyphenols - phlorotannins
<p>➔ <b>antitumor</b></p>	• porphyrin derivatives: pheophorbide a and pheophytin a
	• polyphenols
	• carotenoids
<p>➔ <b>antiviral</b></p>	• polysaccharides
	• proteins
	• diterpens
	• polyphenols
<p>➔ <b>antiviral</b></p>	• polysaccharides
	• carotenoids
	• carotenoids

Extracted compound	Algal species	Extraction method	Target
<b>(a) Antibacterial activity</b>			
PUFAs	<i>Gracilaria corticata</i> <i>Ulva fasciata</i> <i>Enteromorpha compressa</i>	Solvent extraction with hexane, chloroform, ethyl acetate, chloroform:alcohol (1:1), methanol by soaking the material in the solvents thrice overnight at room temperature (1:3v/v)	Bacteria pathogenic to fish: <i>Edwardsiella tarda</i> , <i>Vibrio alginolyticus</i> , <i>Pseudomonas fluorescens</i> , <i>P. aeruginosa</i> , <i>Aeromonas hydrophila</i>
Fats (palmitic acid)	<i>Ulva reticulata</i>	Extraction of powdered algal samples with ethanol, chloroform, petroleum ether, water. Samples were soaked in the solvents for 24 h and homogenized in a blender with the solvents at room temperature	<i>Escherichia coli</i> ,
Proteins (amino acids)	<i>Caulerpa occidentalis</i>		<i>P. aeruginosa</i> ,
Bioflavonoids (rutin, quercetin, and kaempferol)	<i>Cladophora socialis</i> <i>Dictyota ciliolata</i> <i>Gracilaria dendroides</i>		<i>Staphylococcus aureus</i> ,
Sulfated polysaccharide	<i>Sargassum swartzii</i>		<i>Enterococcus faecalis</i>
		Dried seaweed powder was extracted with water at 90–95°C for 16 h. The syrup was filtered through filter paper, cooled, and precipitated with ethanol	<i>S. aureus</i> , <i>Proteus vulgaris</i> , <i>E. coli</i> , <i>Bacillus subtilis</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhi</i> , <i>Shigella flexineri</i> , <i>Klebsiella pneumoniae</i> , <i>E. faecalis</i> , <i>Aeromonas hydrophilla</i>
Carotenoids, alkaloids, favanoids, fatty acids, saponins, amino acids, carbohydrates	<i>Chlorococcum humicola</i>	The algae were centrifuged to remove the water content. Fresh biomass was extracted for 15 min with organic solvents: acetone, benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane, methanol	Effect of pigments: $\beta$ -carotene and chlorophyll on: <i>E. coli</i> , <i>P. aeruginosa</i> , <i>B. subtilis</i> , <i>Salmonella typhimurium</i> , <i>K. pneumoniae</i> , <i>Vibrio cholerae</i> , <i>S. aureus</i>
Fatty acids, phytol, fucosterol, neophytadiene, palmitic, palmitoleic, and oleic acids	<i>Himantalia elongate</i> , <i>Synechocystis</i> sp.	PLE in accelerated solvent extractor equipped with a solvent controller. Three different solvents hexane, ethanol, water were used	<i>S. aureus</i> , <i>E. coli</i>
PUFAs, indolic derivative, $\beta$ -ionone, neophytadiene	<i>Dunaliella salina</i>	Sub- and supercritical CO <sub>2</sub> extraction	<i>E. coli</i> , <i>S. aureus</i>
Short-chain fatty acids	<i>Haematococcus pluvialis</i>	PLEs were performed with hexane and ethanol at different temperatures: 50, 100, 150, 200°C for 20 min	<i>E. coli</i> , <i>S. aureus</i>

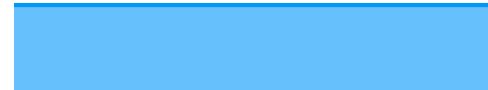
**(b) Antiviral activity**

Polysaccharide	<i>Constantinea simplex</i> <i>Farlowia mollis</i>	Frozen samples were combined with citrate-phosphate buffer at pH 7.0, homogenized in a blender, and incubated at 4°C overnight	Mice: Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), vaccinia virus, vesicular stomatitis virus, encephalomyocarditis virus, Semliki Forest virus, murine cytomegalovirus
Sulfated polysaccharide	<i>Sargassum patens</i>	Seaweed was washed and extracted with boiling water for 2 h. After centrifugation, the supernatant was concentrated and precipitated with ethanol	Vero cells (African green monkey kidney cell line): HSV-2
Sulfated galactofucan, fucan, galactan; depolymerized galactofucan sulfate, galactofucan sulfate	<i>Undaria pinnatifida</i> <i>Splachnidium rugosum</i> <i>Gigartina atropurpurea</i> <i>Plocamium cartilagineum</i>	For <i>U. pinnatifida</i> and <i>S. rugosum</i> : dry, ground samples were extracted for 6 h with 1% (w/v) H <sub>2</sub> SO <sub>4</sub> at 20°C, 0.2 M HCl at 20°C, or 2% CaCl <sub>2</sub> at 75°C. For <i>G. atropurpurea</i> and <i>P. cartilagineum</i> : dry, ground algae were placed in NaHCO <sub>3</sub> solution (0.05 M), left to swell (20min) then heated (90°C, 2 h)	Human foreskin fibroblast: HSV-1 and 2
Sulfated polysaccharide	<i>Padina pavonia</i>	Algal biomass was extracted with water at 80°C for 2 h (twice)	Vero cell culture: HSV, Hepatitis A (HAV, Hep A)
Bromophenols	<i>Polysiphonia morrowii</i>	Freeze-dried alga was extracted with 80% (v/v) methanol (in water, 80% MeOH) at 80°C eight times, each of which took 1 h for a total of 10 h	Fish pathogenic viruses: infectious hematopoietic necrosis virus and infectious pancreatic necrosis virus
Sulfated polysaccharide	<i>Sphaerococcus coronopifolius</i> <i>Boergeseniella thuyoides</i>	Polysaccharides from seaweed powder were extracted in hot distilled water at 80°C for 4 h with magnetic stirring	Vero cells culture: human immunodeficiency virus (HIV) and HSV-1
Polysaccharide	<i>Acrosiphonia orientalis</i>	Polysaccharides were extracted from the dried fronds using 0.1 N HCl at 95°C for 12 h and the extract was precipitated by adding ethanol	Shrimp pathogen—white spot syndrome virus
Diterpenes	<i>Dictyota pfaffii</i> <i>D. menstrualis</i>	Air-dried specimens were extracted with CH <sub>2</sub> Cl <sub>2</sub> /MeOH (7:3) and MeOH	HSV-1
Diterpenes	<i>D. menstrualis</i>	Extraction with CH <sub>2</sub> Cl <sub>2</sub> /MeOH	HIV type 1 (HIV-1)

(c) Antifungal activity

Carotenoids, alkaloids, flavonoids, fatty acids, saponins, amino acids, carbohydrates	<i>C. humicola</i>	The algal samples were centrifuged to remove the water content. Fresh algae were extracted for 15 min with organic solvents: acetone, benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane, methanol	Effect of pigments: $\beta$ -carotene and chlorophyllon: <i>Candida albicans</i> , <i>Aspergillus niger</i> , <i>A. flavus</i>
Terpenes and phenols (terpenes were present in all algal extracts, phenols for *)	<i>Styopodium zonale</i> <i>Laurencia dendroidea</i> , <i>Ascophyllum nodosum</i> (*) <i>Sargassum muticum</i> (*) <i>S. filipendula</i> , <i>S. stenophyllum</i> , <i>Pelvetia canaliculata</i> (*) <i>Fucus spiralis</i> <i>Laminaria hyperborea</i> <i>Gracilaria edulis</i>	The algae were washed, air-dried, powdered, and extracted with ethanol (95%)	<i>Colletotrichum lagenarium</i> , <i>A. flavus</i>
Phenols	<i>Padina pavonica</i> <i>Sargassum vulgare</i>	First method—marine alga was macerated for 3 days in methanol at room temperature in an orbital shaker; the second extraction was in methanol using a Soxhlet extractor for 6 h	<i>Candida</i>
Fatty acids, phytol, fucosterol, neophytadiene, palmitic, palmitoleic and oleic acids	<i>H. elongate</i> , <i>Synechocystis</i> sp.	PLE in accelerated solvent extractor equipped with a solvent controller. Three solvents hexane, ethanol, water were used	<i>C. albicans</i> , <i>A. niger</i>
PUFAs, indolic derivative, $\beta$ -ionone, neophytadiene	<i>D. salina</i>	Sub- and supercritical CO <sub>2</sub> extraction	<i>C. albicans</i> , <i>A. niger</i>
Short-chain fatty acids	<i>H. pluvialis</i>	PLEs were performed with hexane and ethanol at different temperatures: 50, 100, 150, 200°C for 20 min	<i>C. albicans</i> , <i>A. niger</i>

(d) Antioxidative activity			
Sulfated polysaccharide	<i>S. swartzii</i>	Dried seaweed powder was extracted with water at 90–95°C for 16 h. The syrup was then filtered through filter paper, cooled, and precipitated with ethanol	Total antioxidant activity of the extract Reducing power of the extract 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay Hydrogen peroxide scavenging assay 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) inhibition assay
Sulfated polysaccharides: (1) iota, kappa, and lambda carrageenans, fucoidan, (2) fucans	(1) <i>Fucus vesiculosus</i> (2) <i>Padina gymnospora</i>	Powdered algae were suspended with NaCl. pH was adjusted to 8.0. For proteolytic digestion protease from <i>Esporobacillus</i> was added. Incubation at 60°C under shaking lasted for 24 h	Superoxide anion scavenging activity Hydroxyl radical scavenging activity Liver microsomal (from Wistar rats) lipid peroxidation
Phenols	<i>Bifurcaria bifurcata</i> <i>Cystoseira tamariscifolia</i> <i>Fucus ceranoides</i> <i>Halidrys siliquosa</i> <i>Porphyra yezoensis</i>	Accelerated solvent extraction system. The biomass was extracted with a mixture of dichloromethane methanol (1:1, v:v) at 75°C and 1500 psi	DPPH Reducing activity $\beta$ -carotene–linoleic acid system
Usujilene—kind of mycosporine-glycine like amino acid		Ground freeze-dried material was extracted with <i>n</i> -hexane, ethyl acetate, acetone, chloroform/methanol (2:1), methanol, and hot water (90°C) under stirring	Ferric thiocyanate method Thiobarbituric acid method
Phenols	<i>P. pavonica</i> <i>S. vulgare</i>	First method—marine alga was macerated for 3 days in methanol at room temperature in an orbital shaker; the second extraction was in methanol using a Soxhlet extractor for 6 h	DPPH
Phenols	<i>Caulerpa racemosa</i>	Seaweed powder was placed into an extraction vessel and was extracted with solvent under different MAE conditions	Hydroxyl radical scavenging assay DPPH determination of reducing power
Phenols	<i>A. nodosum</i> <i>P. canaliculata</i> <i>F. spiralis</i> <i>Ulva intestinalis</i>	SLE and PLE was employed to extract algae with 100% water, ethanol/water (80:20, v:v), and acetone/water (80:20, v:v)	DPPH Ferric reducing antioxidant power Ferrous ion chelating capability assay
Fatty acids, phytol, fucosterol, neophytadiene, palmitic, palmitoleic, and oleic acids	<i>H. elongate</i> <i>Synechocystis sp.</i>	PLE in accelerated solvent extractor equipped with a solvent controller. Three solvents hexane, ethanol, water were used	Trolox equivalent antioxidant capacity assay
Carotenoid	<i>D. salina</i>	PLEs were performed using an accelerated solvent equipped with a solvent controller. Three solvents hexane, ethanol, water were used	Trolox equivalent antioxidant capacity assay
Antioxidants—carotenoids	<i>Spirulina platensis</i>	Extractions were performed in accelerated solvent extractor equipped with a solvent controller. Three solvents hexane, petroleum ether, ethanol were used. Extractions were performed at temperatures (60, 115, 170°C) and extraction times (3, 9, and 15 min)	DPPH
Polyphenol, flavonoid	<i>Chlorella vulgaris C-C</i>	Supercritical fluid equipment and ultrasonic extraction	DPPH Ferric reducing antioxidant power Metal chelating activity Superoxide anion radical scavenging capacity





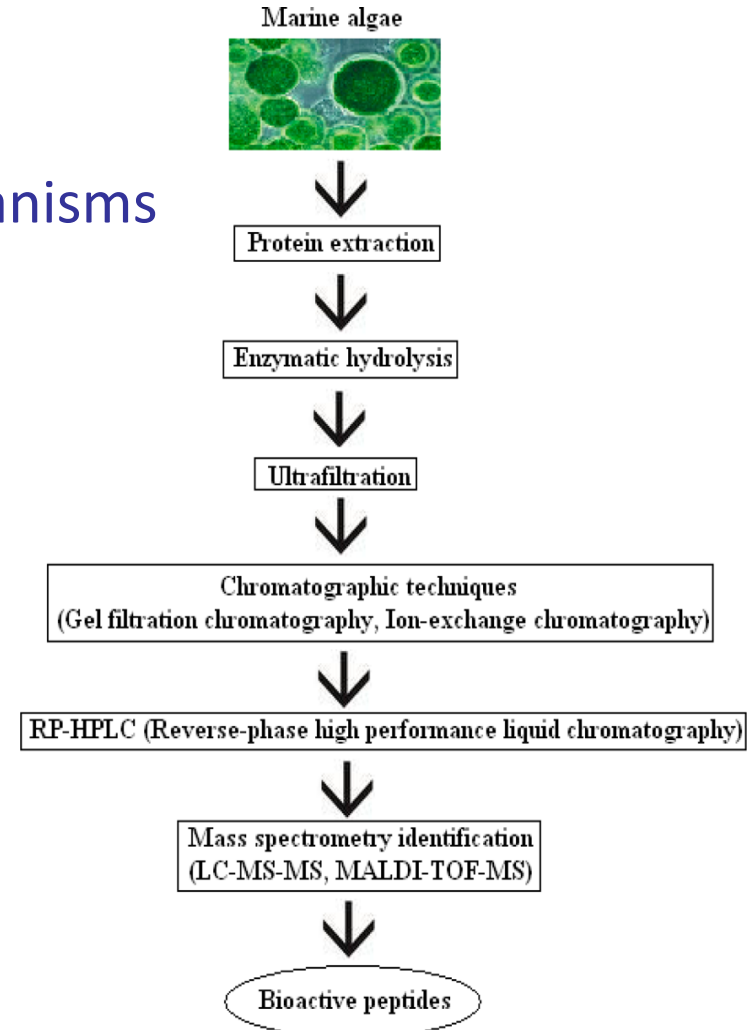
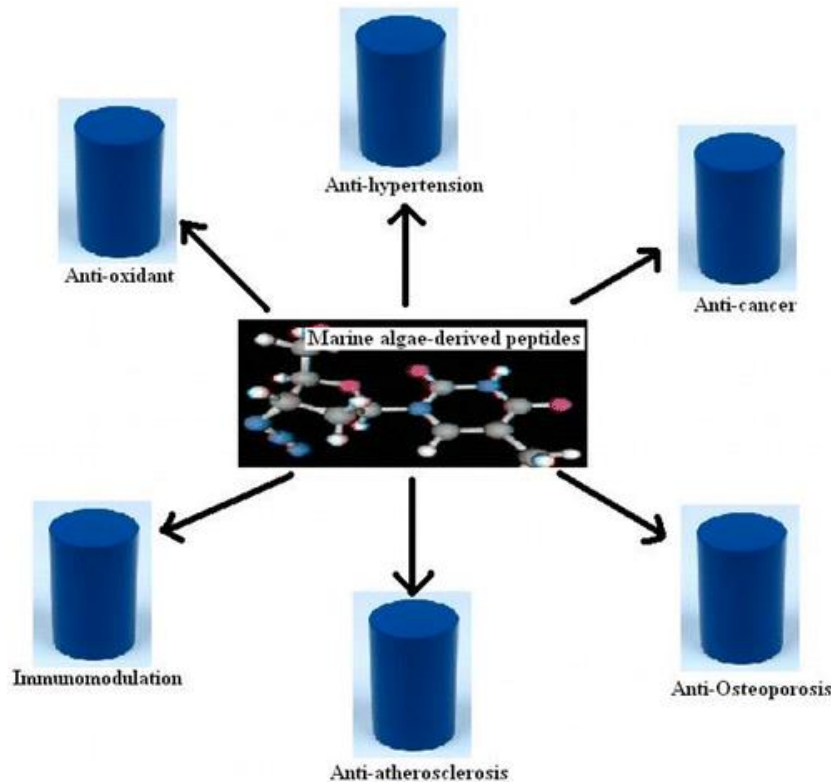
(e) Anti-inflammatory activity

Fucosterol, phlorotannins (phloroglucinol, eckol, dieckol, 7-phloroeckol, phlorofucofuroeckol A, dioxinodehydroeckol)	<i>Eisenia bicyclis</i>	The powdered leafy thallus of alga was refluxed with methanol for 3 h	Inhibition against production of lipopolysaccharide (LPS) induced nitric oxide (NO) and tert-butylhydroperoxide induced reactive oxygen species, suppression against expression of inducible NO synthase, and cyclooxygenase-2 in LPS-stimulated RAW 264.7 macrophages
Porphyrin derivatives: pheophorbide a, pheophytin a	<i>Saccharina japonica</i>	The powder of the whole plant of alga was refluxed with methanol for 3 h	Inhibitory activities against LPS-induced NO production, inducible NO synthase, and cyclooxygenase-2 expression in RAW 264.7 murine macrophage cells
Lactones, phenols, triterpenes, steroids, reduced carbohydrates	<i>Dichotomaria obtusata</i>	Distilled water was added to algal powder and vortexed in a shaker for 24 h at room temperature	Tests in mice: ear edema induced by 12-O-tetradecanoylphorbol acetate and writhing induced by acetic acid
<b>(f) Antitumor activity</b>			
Fucoidan	<i>Fucus evanescens</i>	Hot extraction	Mice with transplanted Lewis lung adenocarcinoma
Polyphenol: phlorotannin—dioxinodehydroeckol	<i>Ecklonia cava</i>	The lyophilized powder of alga was percolated in hot EtOH. The crude extract was partitioned with organic solvents to yield <i>n</i> -hexane, CH <sub>2</sub> Cl <sub>2</sub> , EtOAc, and <i>n</i> -BuOH fractions, as well as an H <sub>2</sub> O residue	Inhibition of the proliferation of human breast cancer cells
Phenols	<i>B. bifurcata</i> <i>C. tamariscifolia</i> <i>F. ceranoides</i> <i>H. siliquosa</i>	Accelerated solvent extraction system. The biomass was extracted with a mixture of dichloromethane methanol (1:1, v:v) at 75°C and 1500 psi	Cytotoxic assay with three different tumoral cells lines (Daudi, Jurkat, and K562)
Crude polysaccharide	<i>Sargassum coreanum</i>	Biomass was pulverized into powder with a grinder. Buffer solution was added to the dried sample and then Neutrase. The reaction was performed for 12 h	HL-60 (human promyelocytic leukemia cell line); >30-kDa fraction of crude polysaccharides exhibited a marked anticancer activity in HL-60 cells
Polyphenol, flavonoid	<i>C. vulgaris C-C</i>	Supercritical fluid equipment and ultrasonic extraction	Extract of <i>C. vulgaris C-C</i> inhibits human lung cancer H1299, A549, and H1437 cells in a dose-dependent manner



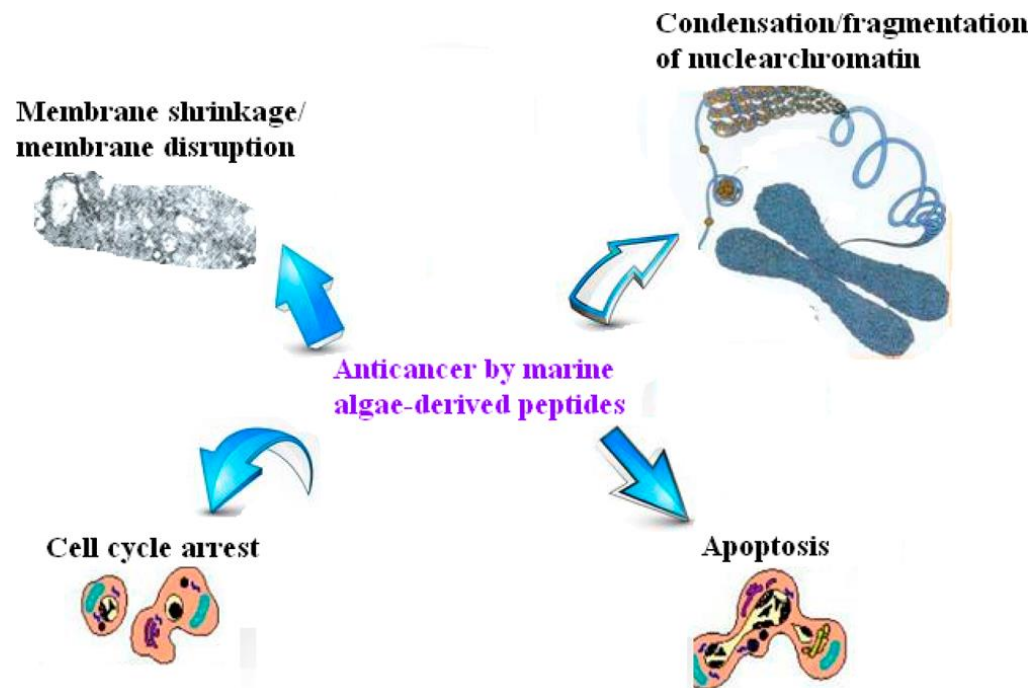
# Bioactive peptides from algae

- Small peptides (2-20 AA)
- Variety of activities on cells and organisms



# Anticancer peptides

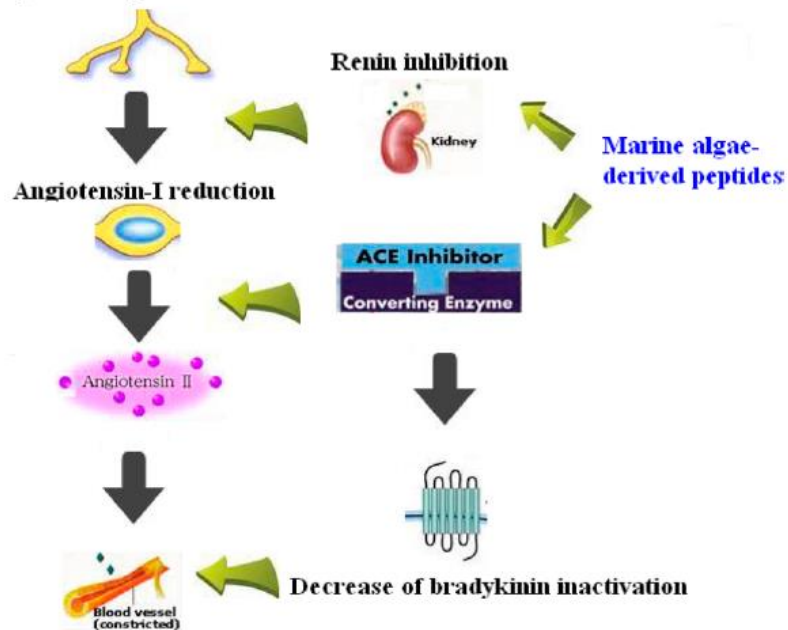
peptide name or sequence	source	enzyme	IC <sub>50</sub>	in vitro/in vivo	mechanism of action
VECYGPNRPQF	<i>Chlorella vulgaris</i>	pepsin	70 µg/mL	in vitro (gastric cancer AGS cells)	antiproliferation and post-G1 cell cycle arrest
polypeptide CPAP	<i>Chlorella pyrenoidosa</i>	papain, trypsin, and alcalase	426 µg/mL	in vitro (HepG2 cells)	apoptosis
polypeptide Y2	<i>Spirulina platensis</i>	trypsin, alcalase, pepsin, and papain	61 µg/mL	in vitro (MCF-7 and HepG-2 cells)	



# Antihypertensive biopeptides

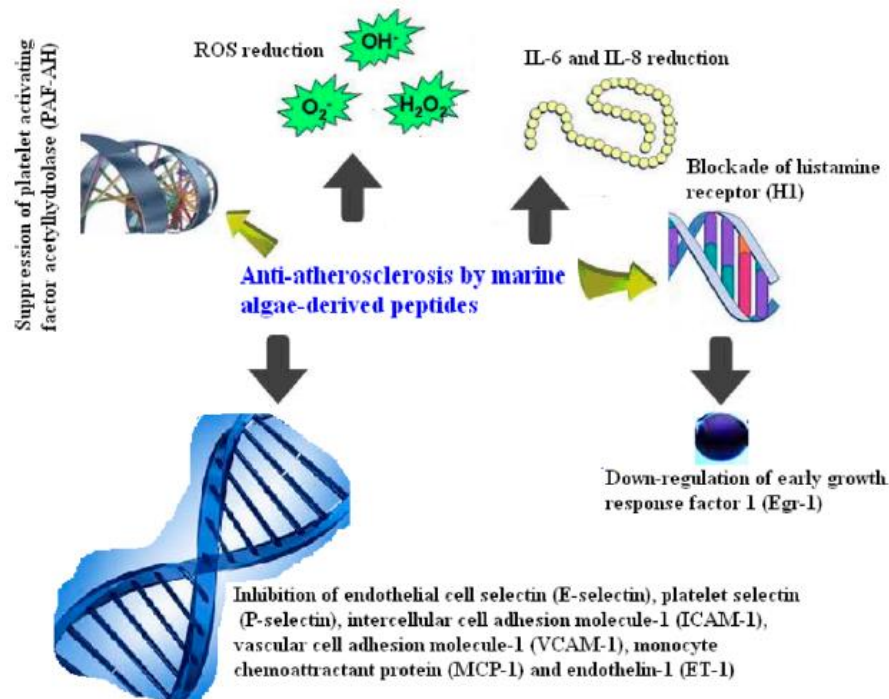
peptide name or sequence	source	enzyme	IC <sub>50</sub>	in vitro/in vivo	mechanism of action
YH, KY, FY, IY	<i>Undaria pinnatifida</i>	no enzyme used	2.7–43.7 $\mu\text{mol/L}$	in vitro and in vivo (rats)	ACE inhibition
enzymatic digests	<i>Ecklonia cava</i>	Kojizyme, Flavourzyme, Neutrase, Alcalase, and Protamex	2.33–3.56 $\mu\text{g/mL}$	in vitro	ACE inhibition
VECYGPNRPQF	<i>Chlorella vulgaris</i>	pepsin	29.6 $\mu\text{M}$	in vitro	ACE inhibition
VEGY	<i>Chlorella ellipsoidea</i>	Protamex, Kojizyme, Neutrase, Flavourzyme, Alcalase, trypsin, $\alpha$ -chymotrypsin, pepsin, and papain	128.4 $\mu\text{M}$	in vitro and in vivo (rats)	ACE inhibition
GMNNLTP, LEQ	<i>Nannochloropsis oculata</i>	pepsin, trypsin, $\alpha$ -chymotrypsin, and papain	123–173 $\mu\text{M}$	in vitro	ACE inhibition
IRLIIVLMPILMA	<i>Palmaria palmata</i>	papain	3.3 mM	in vitro	renin inhibition

## Angiotensinogen reduction



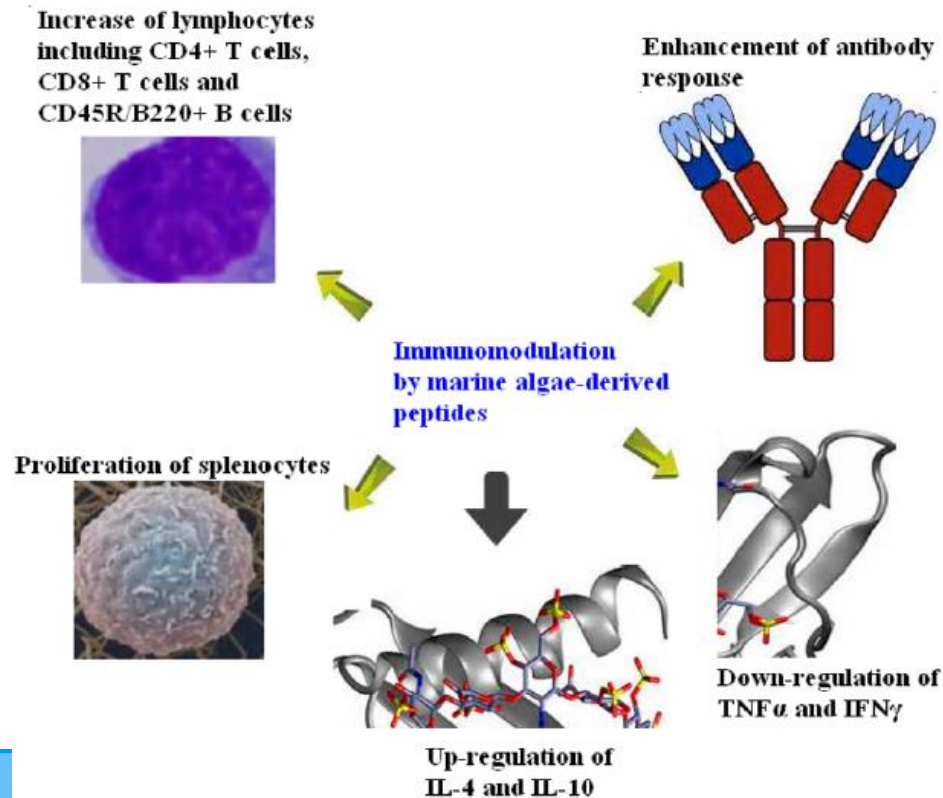
# Antiatherosclerotic peptides

peptide name or sequence	source	enzyme	IC <sub>50</sub>	in vitro/in vivo	mechanism of action
VECYGPNRPQF	<i>Chlorella</i> sp.	pepsin, Flavourzyme, Alcalase, and Papain		in vitro (endothelial cells Svec4-10 and macrophage RAW 264.7 cells)	inhibition of vascular adhesion molecules (E-selectin, ICAM, VCAM, MCP-1 and ET-1) gene expression
NIGK	<i>Palmaria palmata</i>	papain	2.32 mM	in vitro	platelet activating factor acetylhydrolase (PAG-AH) inhibition
LDAVNR, MMLDF	<i>Spirulina maxima</i>	trypsin, $\alpha$ -chymotrypsin, and pepsin		in vitro (EA hy926 cells and U937 cells)	adhesion inhibition/anti-inflammatory (inhibition of IL-6, IL-8, MCP-1, P-selectin, ROS, and Egr-1)



# Immunomodulatory biopeptides

peptide name or sequence	source	enzyme	IC <sub>50</sub>	in vitro/ in vivo	mechanism of action
protein hydrolysates	<i>Chlorella vulgaris</i>	pancreatin		in vivo (mice)	stimulation of both humoral and cell-mediated immune functions (T-dependent antibody response and the reconstitution of delayed-type hypersensitivity response)
protein hydrolysates	<i>Ecklonia cava</i>	Kojizyme		in vivo (mice)	increases in lymphocytes, monocytes, and granulocytes; down-regulation of TNF- $\alpha$ and IFN- $\gamma$ , up-regulation of IL-4 and IL-10
protein hydrolysates	<i>Porphyra columbina</i>	trypsin, alcalase	2.1–5.6 g/L	in vivo (rats)	cytokine modulations (inhibition of TNF- $\alpha$ and IFN- $\gamma$ , increase of IL-10)



## Summary of Commercially Exploited Algae and the Corresponding Products or Applications

### Other areas of algae biotechnological applications

Scientific Name	Class	Products/Applications
<i>Lyngbya lagerheimii</i>	Cyanophyceae	Sulpholipids/spirulan
<i>Nostoc</i> spp.	Cyanophyceae	Cryptophycin 1
<i>Arthrospira</i> spp.	Cyanophyceae	Health food
<i>Palmaria mollis</i>	Floridophyceae	Abalone feed
<i>Phymatolithon calcareum</i>	Floridophyceae	Fertilizers
<i>Lithothamnion coralloides</i>	Floridophyceae	Fertilizers
<i>Nannochloropsis</i> spp.	Eustigmatophyceae	EPA/fish fry feed
<i>Monodus subterraneus</i>	Eustigmatophyceae	EPA
<i>Skeletonema</i> spp.	Bacillariophyceae	Fish fry feed
<i>Chaetoceros</i> spp.	Bacillariophyceae	Fish fry feed
<i>Nitzschia alba</i>	Bacillariophyceae	EPA
<i>Nitzschia laevis</i>	Bacillariophyceae	EPA
<i>Petalonia binghamiae</i>	Phaeophyceae	fucoxanthin
<i>Scytosiphon lomentaria</i>	Phaeophyceae	fucoxanthin
<i>Ascophyllum nodosum</i>	Phaeophyceae	Fertilizers
<i>Sargassum</i> spp.	Phaeophyceae	Fertilizers
<i>Laminaria digitata</i>	Phaeophyceae	Animal feed
<i>Macrocystis pyrifera</i>	Phaeophyceae	Abalone feed
<i>Isochrysis</i> spp.	Haptophyceae	DHA/fish fry feed
<i>Tetraselmis</i> spp.	Haptophyceae	Fish fry feed
<i>Pavlova</i> spp.	Haptophyceae	Fish fry feed
<i>Cryptocodinium cohni</i>	Dinophyceae	DHA
<i>Euglena gracilis</i>	Euglenophyceae	$\beta$ -1,3-glucan
<i>Haematococcus pluvialis</i>	Chlorophyceae	astaxanthin
<i>Dunaliella salina</i>	Chlorophyceae	$\beta$ -carotene
<i>Chlorella</i> spp.	Chlorophyceae	Health food/fish fry feed

## Livestock feed

- Feeding of algae (mainly *Chlorella*) to cattle or pigs leads to better growth and improved meat quality
- Even small addition has positive effect



**Table 5** Results of *Chlorella* feeding trials with sows and piglets during farrowing at the Regional Research Center (LVA; Iden, Germany; Weber and Grimmer 2001)

Parameter	Trial 1		Trial 2		Trial 3		Total	
	Control	Alga	Control	Alga	Control	Alga	Control	Alga
Sow daily weight gain								
Lactating time (g/day)	290	305	319	318	303	300	304	308
Weight after lactating (kg)	7.5	7.9	8.5	8.5	7.2	7.18	7.8	7.8
End weight (kg)	23.8	24.9	26.9 <sup>a</sup>	29.8 <sup>b</sup>	24.5	25.7	25.1 <sup>a</sup>	26.8 <sup>b</sup>
Husbandry (days)	42	42	46.2	45.8	47	46.1	45	44.6
Piglet daily weight gain								
Growth (g/day)	388	404	396 <sup>a</sup>	466 <sup>b</sup>	369	403	386 <sup>a</sup>	424 <sup>b</sup>
Feed conversion (kg/kg)	1.67	1.66	1.74	1.66	1.73	1.57	1.71	1.63
Dead animals	0	0	1	0	3	0	4	0

<sup>a,b</sup>Level of significance  $P > 0.05$



# Aquacultures

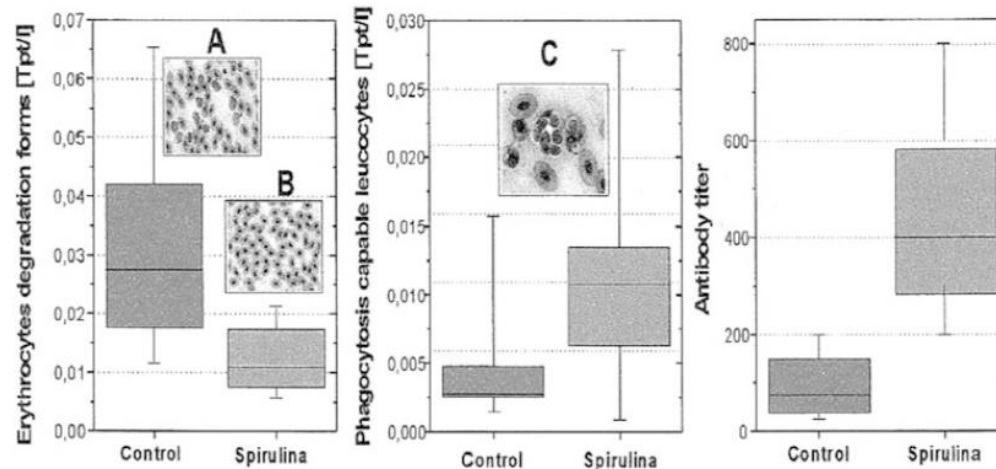
- Enhancement and improvement of fish production

**Table 6** Important genera of microalgae used in aquaculture

Taxon	Genera
Bacillariophyta	<i>Skeletonema</i> , <i>Chaetoceros</i> , <i>Phaeodactylum</i> , <i>Nitzschia</i> , <i>Thalassiosira</i>
Prymnesiophyta	<i>Isochrysis</i> , <i>Pavlova</i>
Prasinophyceae	<i>Tetraselmis</i>
Chlorophyceae	<i>Chlorella</i> , <i>Scenedesmus</i> , <i>Dunaliella</i>
Cyanobacteria	<i>Spirulina</i>



**Fig. 4** Influence of adding microalgae to feed for carp. *A-C* Views of cells mentioned in y-axis (Schreckenbach et al. 2001)



	Products currently on market	Producers of current products	Micro-algae / product from micro-algae
Cosmetics	Personal care skin products	Solazyme (US) + Unilever Fuji Chemicals [77]	(NA)
		Soliance (France) [78]	<i>Spirulina</i>
		LVMH (France) [87]	<i>Chlorella</i>
		Daniel Jouvance (France) [87]	(NA)
		Algenist /Solazyme (USA, California) [79]	'Alguronic acid' (trade name for a undetermined mix of polysaccharides produced by micro-algae clogging filters in algae cultures)
	Anti aging skin product (lipid)	Soliance (FR) [78]	<i>Skeletonema costatum</i>
		Exsyrmol S.A.M. (Monaco) [87]	<i>Arthropira (Spirulina)</i>
		Pentapharm (Switzerland) [87]	<i>Nannochloropsis</i> <i>Dunaliella Salina</i>
	Hydrating skin product	Soliance [78]	<i>Porphyridium cruentum</i>
		Codif (France) [87]	<i>Chlorella</i>
Anti – inflammation (peptide)	Soliance [78]	<i>Phaeodactylum tricornutum</i>	
Slimming products	Soliance [78]	<i>Dysmorphococcus globosus</i>	
Other products	Fluorescent protein markers	Martek/DSM	(NA)
	Stable isotope biochemicals	Spectra Gases/Martek/DSM [87]	(NA)