

# EXTRACTION OF PROTEIN FROM MACROALGAE

INGRID UNDELAND, PROFESSOR  
CHALMERS –FOOD AND NUTRITION SCIENCE





# Food and Nutrition Science-Chalmers *-Marine research group*





# We need a protein shift

The climate cannot handle the amount of animals we eat. There needs to be a protein shift, according to researcher Karolina Östbring who is involved in the Sustainability Week. Her vision is to create a platform for research on vegetable proteins at LU.

The protein shift: will Europeans change their diet?



## The protein shift: From fauna to flora

•Published on August 25, 2016

### The Protein Shift: Plant-Based Options



by Sanna Delmonico, MS, RDN, CHE on Oct 14, 2016

**CEC Feature:** How we're getting more protein in our diets without eating more meat.



Livsmedel  
ifokus.se LivsmedelsFöreningen

Livsmedelsdagarna LivsmedelsFöreningen Om Livsmedel i Fokus Branschguiden

11 MAJ 2016

### Proteinskifte på gång

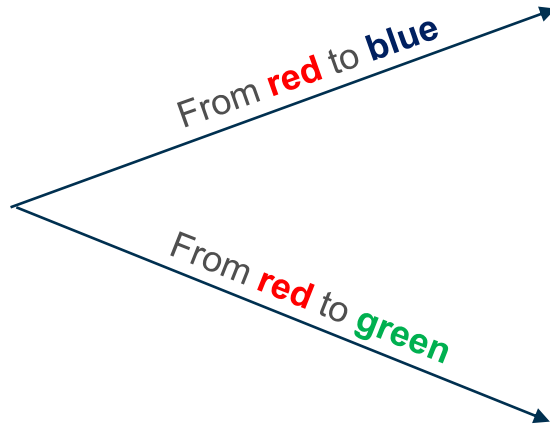
Livsmedelsindustrin står inför ett proteinskifte. Vilka utmaningar och möjligheter finns? Det tänker konsultföretaget Macklean belysa i nästa nummer av Insikter som presenteras på Livsmedelsdagarna den 7 september. För att få input till rapporten, bjöd Macklean in studenter till en workshop.



en av studenterna som deltog i Mackleans workshop om proteinskiftet. Längat till Ulf Berglund.



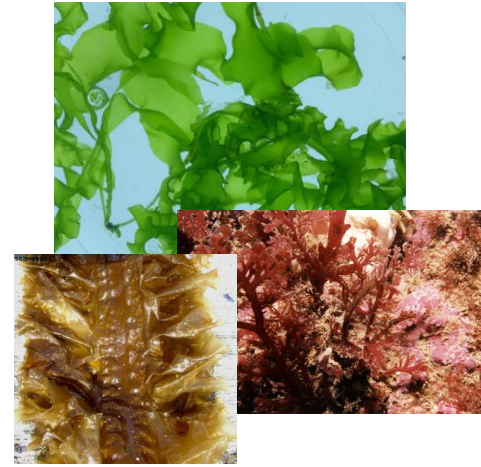
# The protein shift!



# The protein shift!



From **red** to **blue**



- No need for watering/fresh water
- No need for pesticides and insecticides
- Remediates N, P
- Uptake of CO<sub>2</sub>
- Effective growth rate per area
- No competition for arable land



# Potential to cultivate large amounts!

*Photo from Göran Nylund, GU*

<90 ton algae biomass/ha/year



**4-15 tons protein/ha/year**

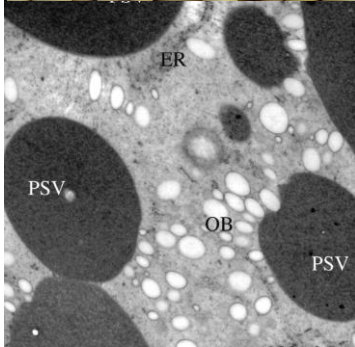
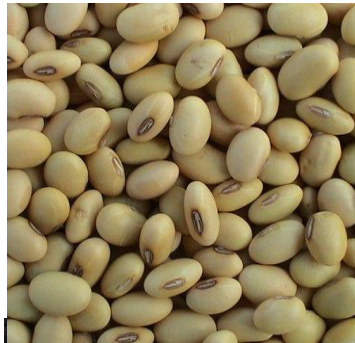
*Photo from Wikimedia Commons*

2.5 ton soy beans/ha/year



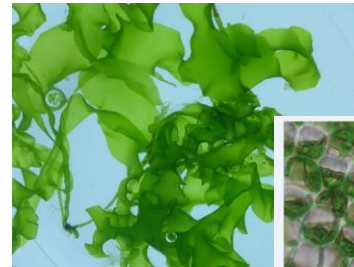
**0.6-1.2 ton protein/ha/year**

# But, protein extraction more challenging from seaweed than e.g. beans!



Protein in storage vacuoles (PSVs)

Schmidt et al. Plant Physiology, May 2011, Vol. 156, pp. 330–345



Photosynthetic!

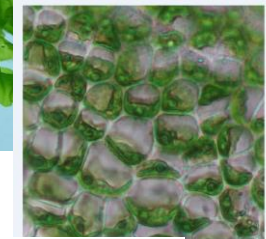
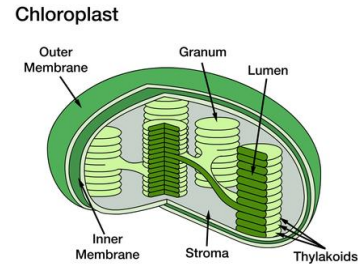
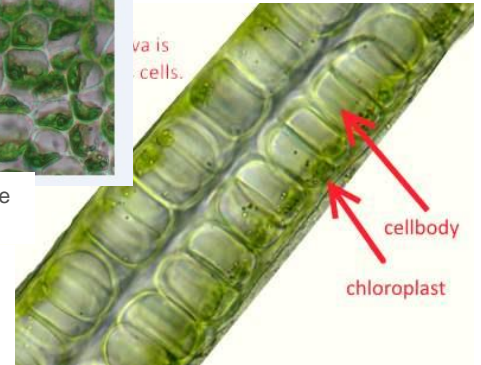


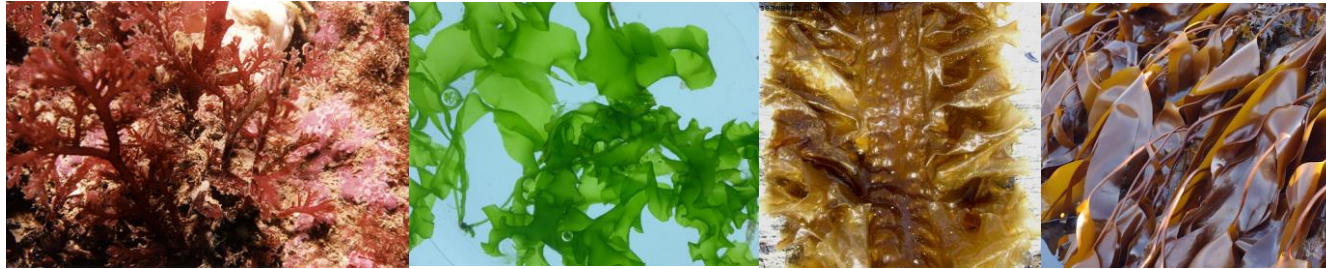
Photo: Sophie Steinhagen



Proteins mainly in the form of enzymes and structural proteins

Steinhagen, et al. (2019). European Journal of Phycology, DOI: 10.1080/09670262.2019.1597925  
<https://pixels.com/featured/chloroplast-from-red-alga-griffithsia-sp-dennis-kunkel-microscopyscience-photo-library.html>  
<https://awhitebiology.weebly.com/chloroplasts.html>

# Swedish initiatives to cultivate and use seaweed for multiple products



GÖTEBORGS  
UNIVERSITET

Linnéuniversitetet



LUNDS  
UNIVERSITET



**CirkAlg**

seafarm  
2019 9.11.11

Makroalger för ett biobaserat samhälle



STIFTELSEN för STRATEGISK FORSKNING

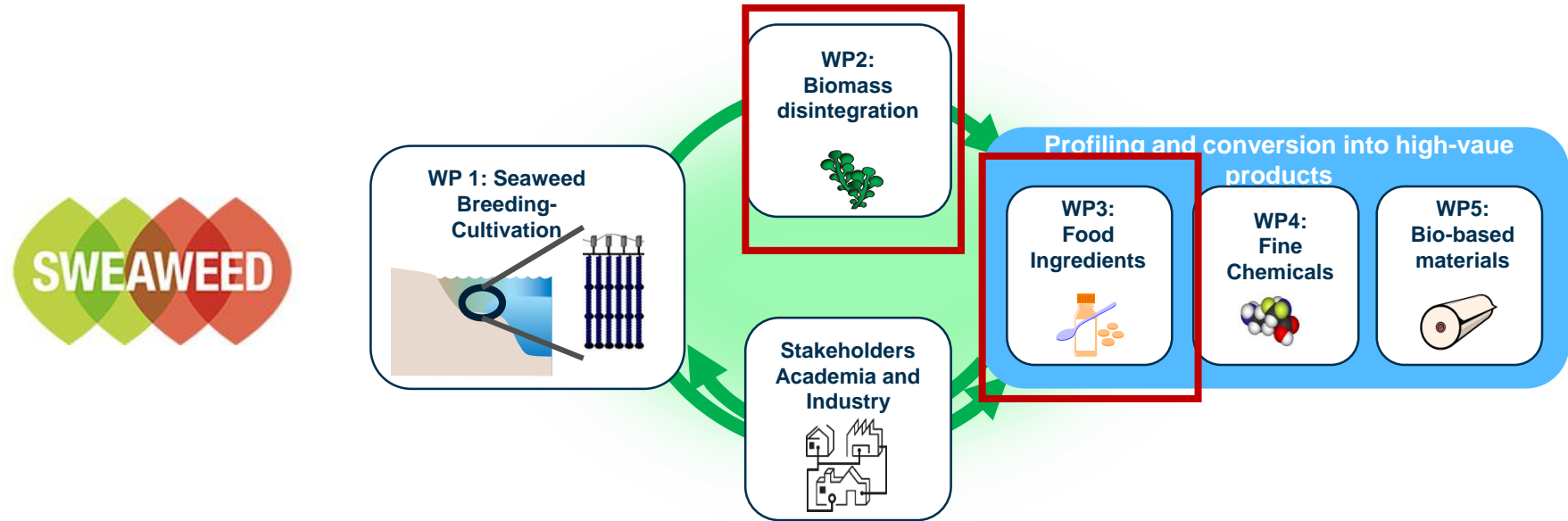
**FORMAS**



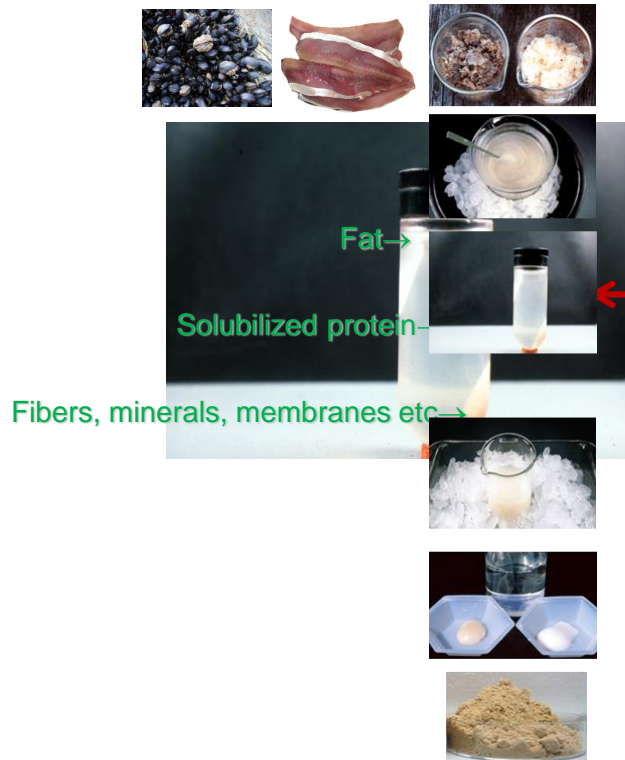
Ingrid Undeland, Chalmers



# Seaweed proteins –a contribution to the protein shift?



# pH-shift process for concentrating of protein



Homogenizing in water  
algae + water ( $\geq 1:3$ )

Protein solubilization  
pH  $\rightarrow$   $\sim 12$

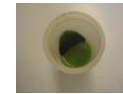
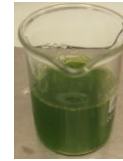
Centrifugation

Precipitation  
pH  $\rightarrow$   $\sim 2$

Centrifugation

Protein isolate

Drying



## We have evaluated:

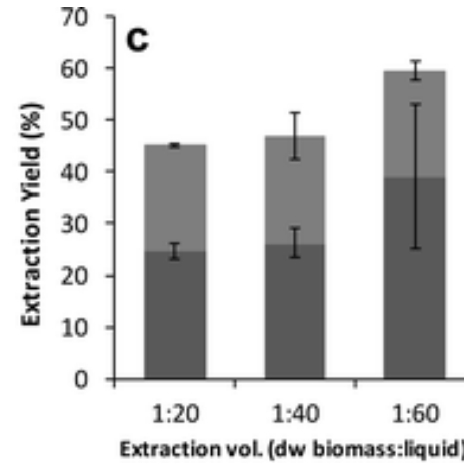
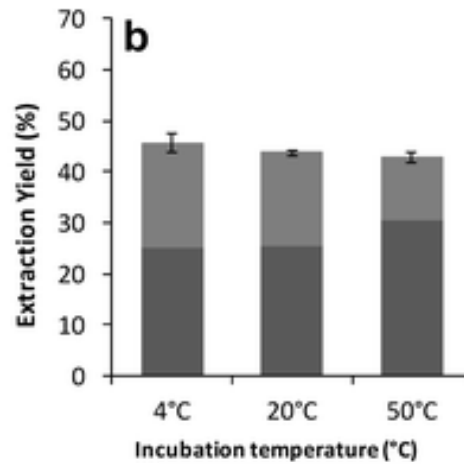
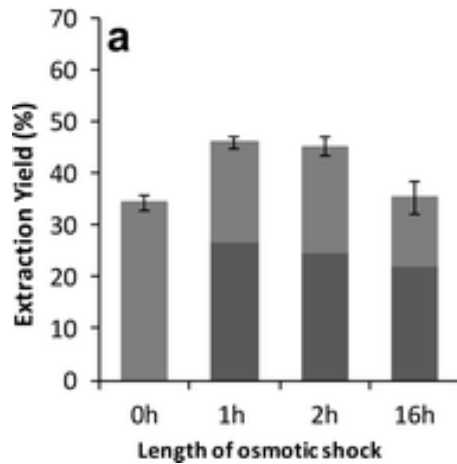
- Season
- Preservation
- Water volume
- Osmotic shock
- Enzymes
- Temperature/time

- Freeze thawing

- Nutrient profile
- Functionality
- Bioavailability

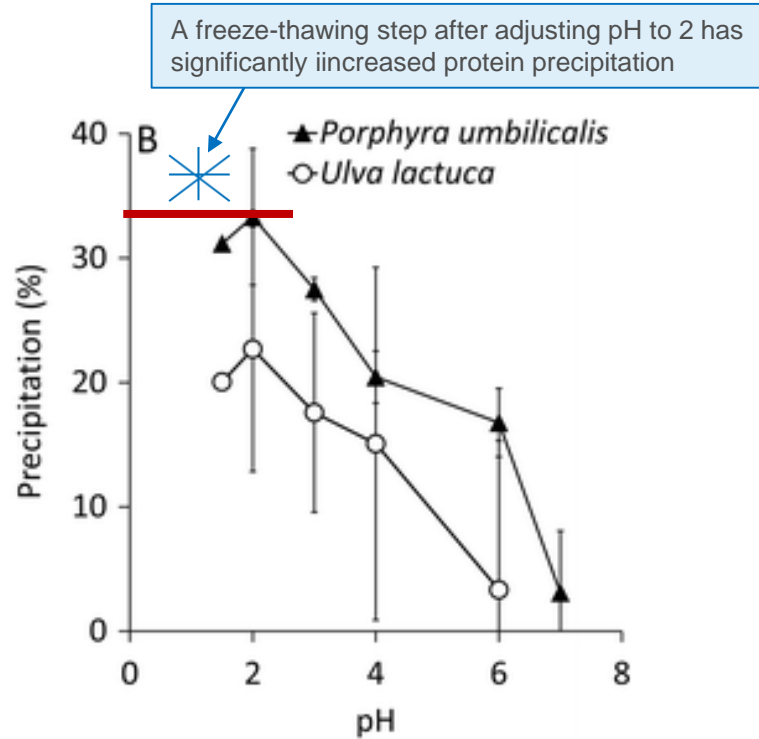
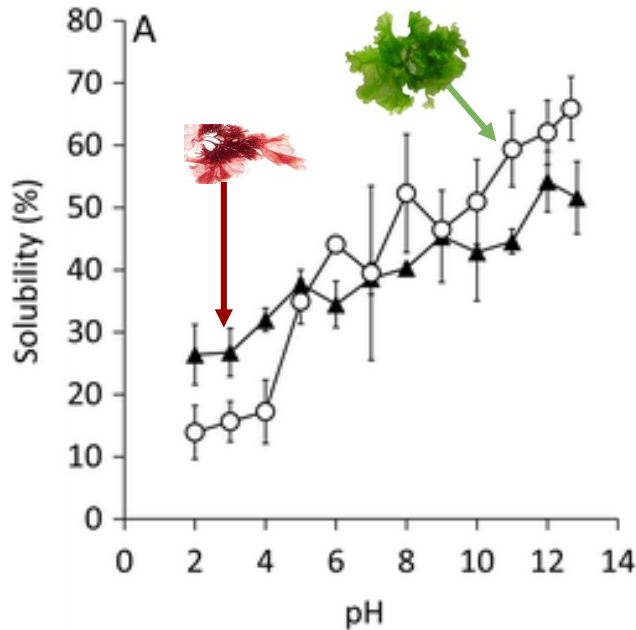
- Comparison to other methods

## Effect of osmotic shock, temperature and water volume on extraction yield





## Solubility and precipitation as a function of pH





## Production of protein extracts from Swedish red, green, and brown seaweeds, *Porphyra umbilicalis* Kützinger, *Ulva lactuca* Linnaeus, and *Saccharina latissima* (Linnaeus) J. V. Lamouroux using three different methods

Hanna Harrysson<sup>1</sup> · Maria Hayes<sup>2</sup> · Friederike Eimer<sup>3</sup> · Nils-Gunnar Carlsson<sup>1</sup> · Gunilla B. Toth<sup>3</sup> · Ingrid Undeland<sup>1</sup>

Received: 5 December 2017 / Revised and accepted: 16 April 2018 / Published online: 28 April 2018  
© The Author(s) 2018

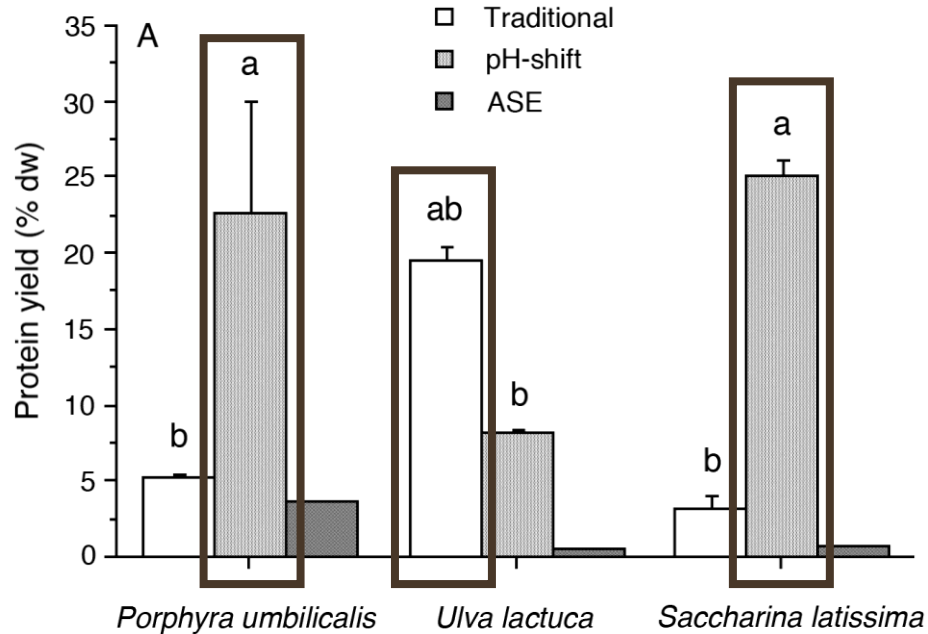
### Abstract

The demand for vegetable proteins increases globally and seaweeds are considered novel and promising protein sources. However, the tough polysaccharide-rich cell walls and the abundance of polyphenols reduce the extractability and digestibility of seaweed proteins. Therefore, food grade, scalable, and environmentally friendly protein extraction techniques are required. To date, little work has been carried out on developing such methods taking into consideration the structural differences between seaweed species. In this work, three different protein extraction methods were applied to three Swedish seaweeds (*Porphyra umbilicalis*, *Ulva lactuca*, and *Saccharina latissima*). These methods included (I) a traditional method using sonication in water and subsequent ammonium sulfate-induced protein precipitation, (II) the pH-shift protein extraction method using alkaline protein solubilization followed by isoelectric precipitation, and (III) the accelerated solvent extraction (ASE®) method where proteins are extracted after pre-removal of lipids and phlorotannins. The highest protein yields were achieved using the pH-shift method applied to *P. umbilicalis* ( $22.6 \pm 7.3\%$ ) and *S. latissima* ( $25.1 \pm 0.9\%$ ). The traditional method resulted in the greatest protein yield when applied to *U. lactuca* ( $19.6 \pm 0.8\%$ ). However, the protein concentration in the produced extracts was highest for all three species using the pH-shift method ( $71.0 \pm 3.7\%$ ,  $51.2 \pm 2.1\%$ , and  $40.7 \pm 0.5\%$  for *P. umbilicalis*, *U. lactuca*, and *S. latissima*, respectively). In addition, the pH-shift method was found to concentrate the fatty acids in *U. lactuca* and *S. latissima* by 2.2 and 1.6 times, respectively. The pH-shift method can therefore be considered a promising strategy for producing seaweed protein ingredients for use in food and feed.

**Keywords** Seaweed · Protein extraction · Ammonium sulfate precipitation · pH shift · Accelerated solvent extraction (ASE®) · Amino acids






## Effect of process method and species on protein yield



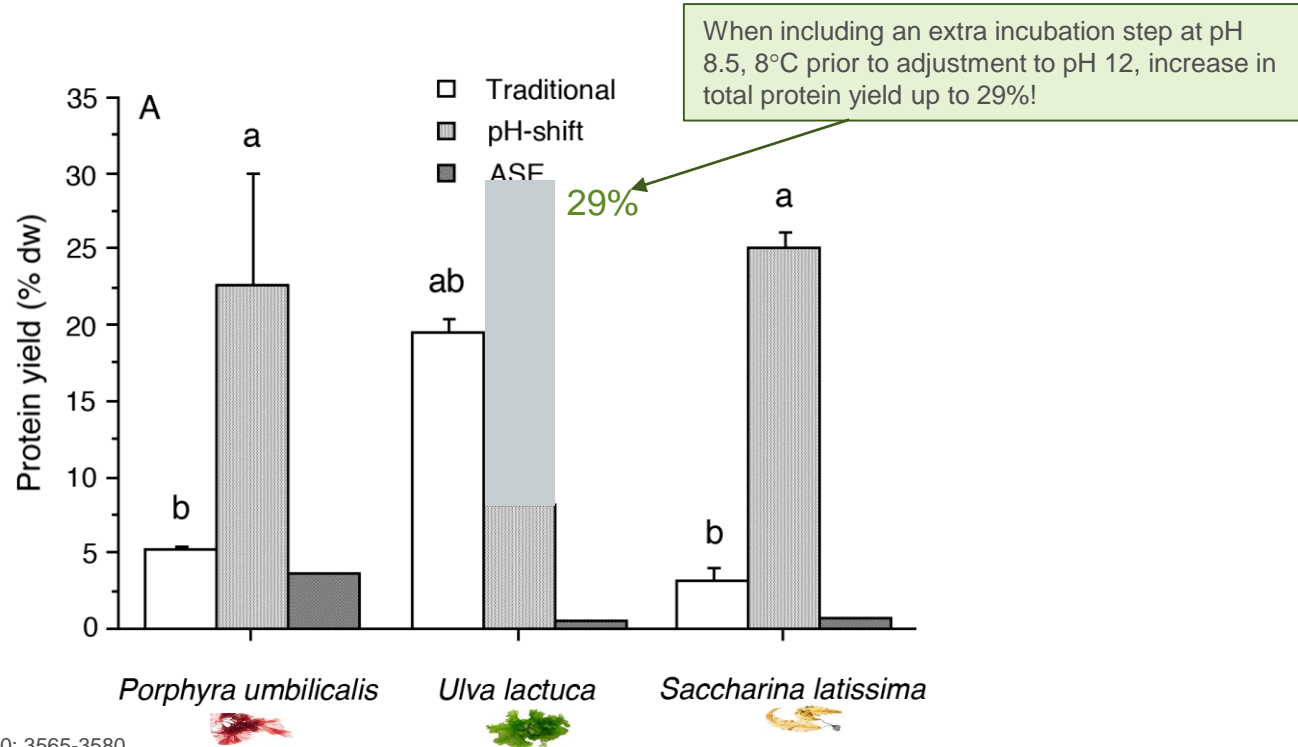


## Protein, fat and ash of the extracts

	$\Sigma$ Amino acids <sup>a</sup> (% dw)	$\Sigma$ Fatty acids <sup>b</sup> (% dw)	Ash <sup>c</sup> (% dw)	
	<b>Dry <i>Porphyra</i></b>	<b>31.8</b>	<b>2.5</b>	<b>23.2</b>
	Porphyra traditional	13.9	0.5	3.1
	Porphyra pH-shift	<b>71.0</b>	<b>2.2</b>	4.7
	Porphyra ASE	21.2	0.7	<b>46.3</b>
	<b>Dry <i>Ulva</i></b>	<b>19.6</b>	<b>2.3</b>	<b>26.0</b>
	Ulva traditional	10.7	0.7	1.1
	Ulva pH-shift	<b>51.2</b>	<b>5.0</b>	13.9
	Ulva ASE	13.0	2.1	<b>51.3</b>
	<b>Dry <i>Saccharina</i></b>	<b>10.1</b>	<b>2.1</b>	<b>49.6</b>
	Saccharina trad.	1.9	0.1	2.6
	Saccharina pH-shift	<b>40.7</b>	<b>3.3</b>	15.9
	Saccharina ASE	5.0	1.6	<b>67.7</b>

**EAA%:**  
40→42.5  
after pH-shift  
processing

## Effect of process method and species on protein yield



• Harrysson et al. *J Applied Phycol* (2018) 30: 3565-3580

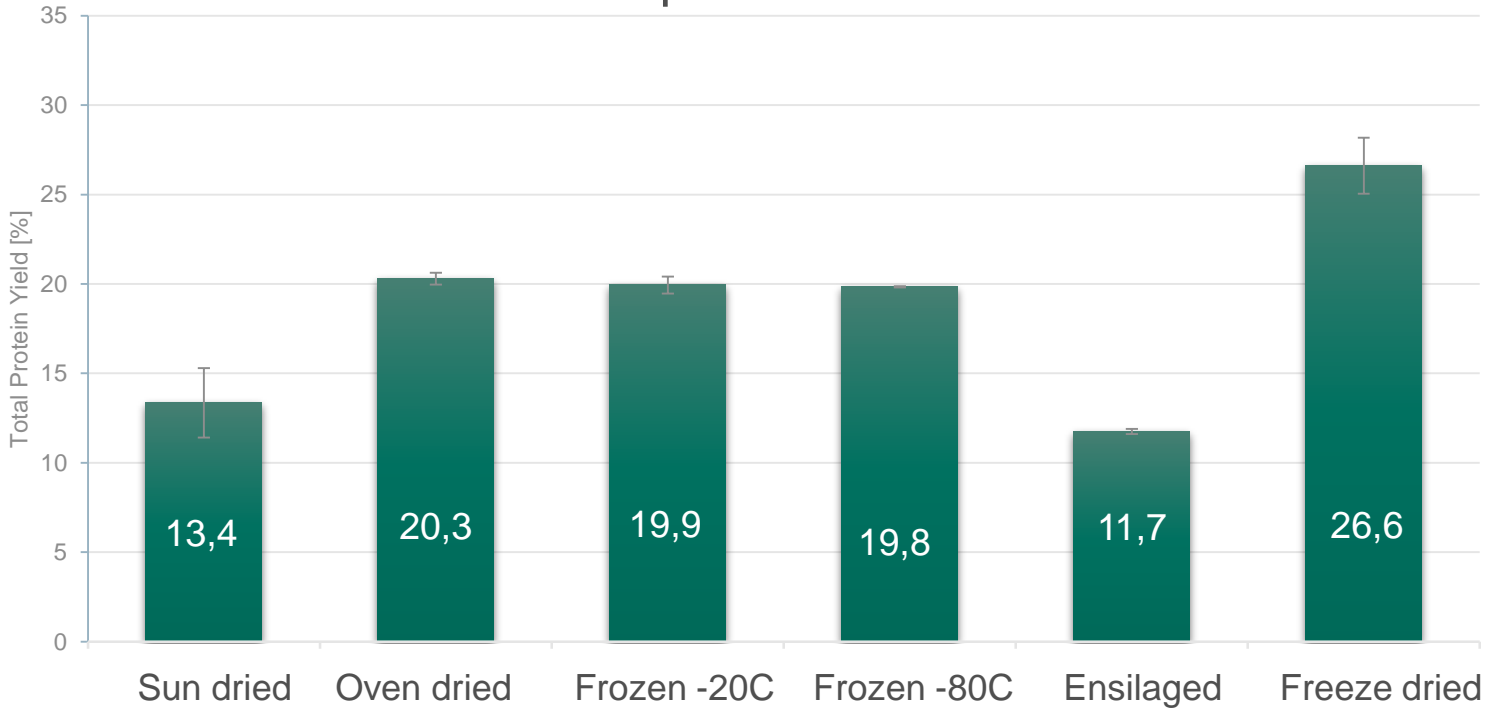
• Harrysson et al. Strategies for improving the protein yield in pH-shift processing of *Ulva lactuca* Linnaeus - Effects of ulvan lyases, pH-exposure time and temperature, *ACS Sustainable Chemistry & Engineering*. In Press 2019

# How to preserve seaweed post harvest?

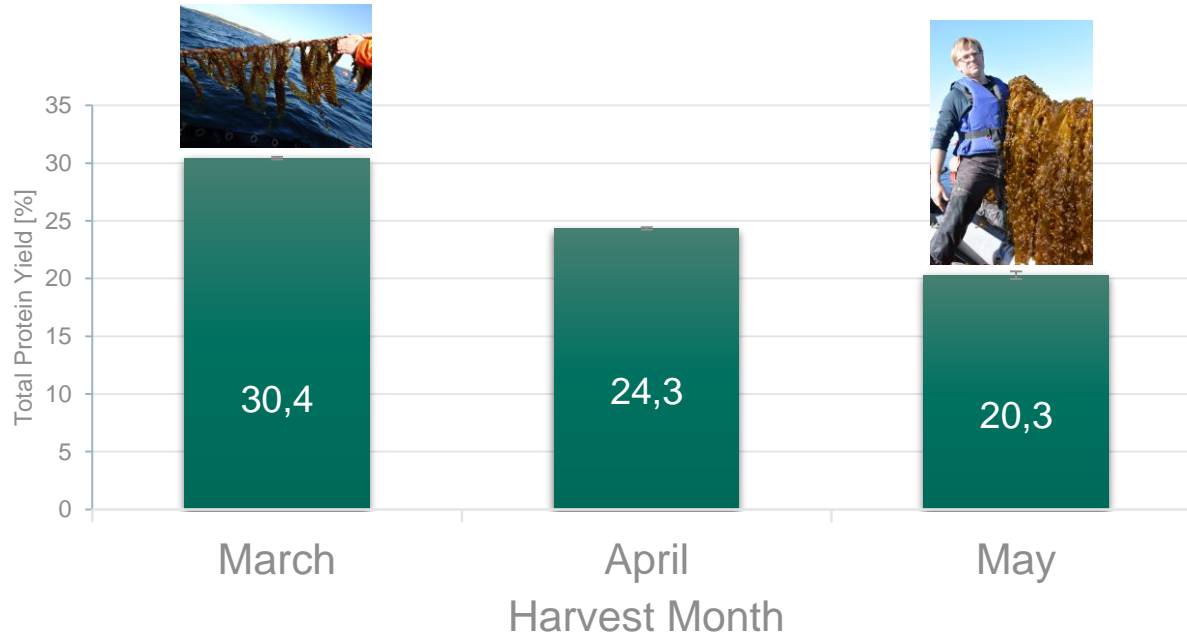


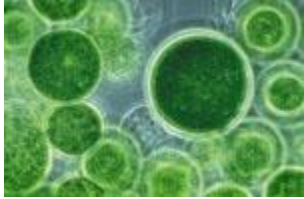


## Effect of seaweed preservation method



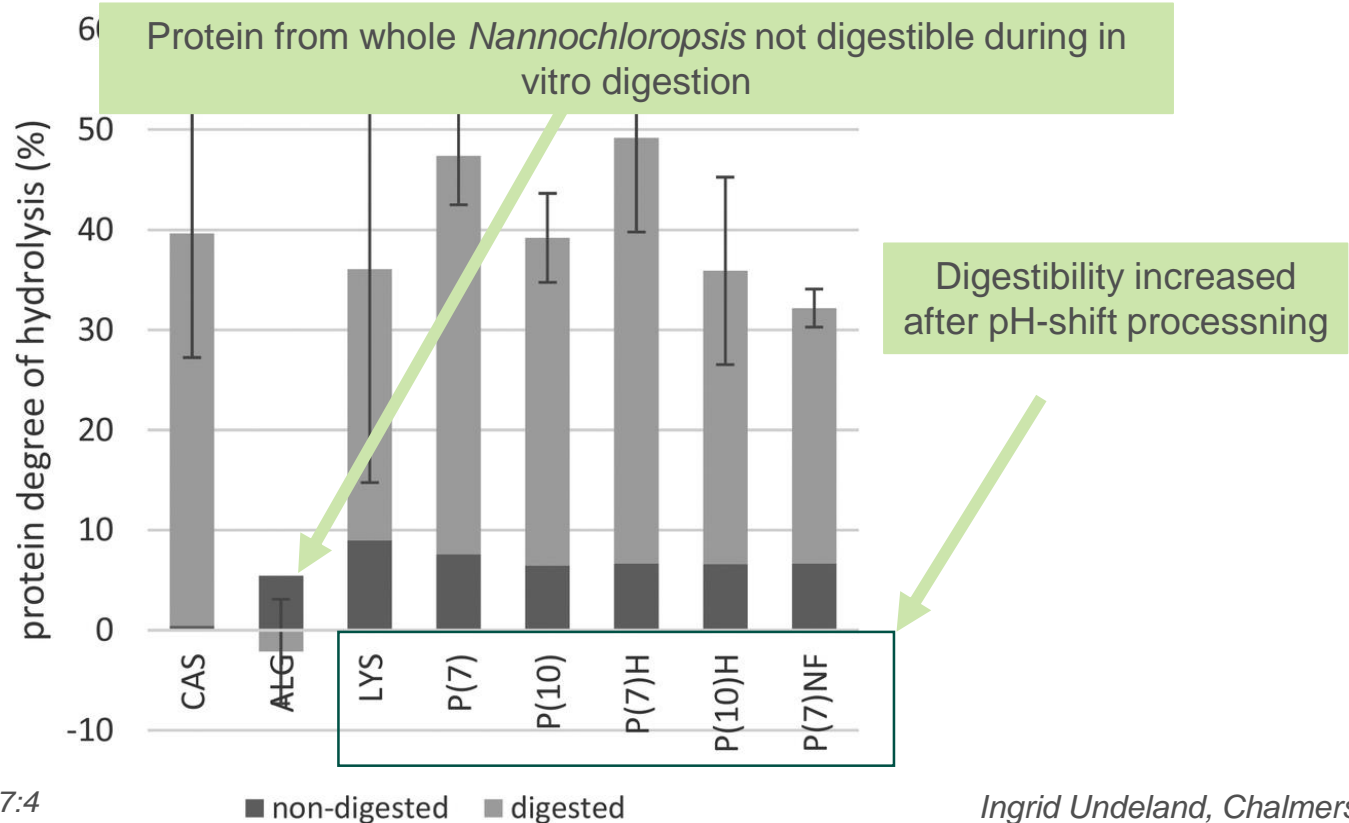
## Effect of harvest month





<https://www.andaluciaecologica.com/empresas/endesa-microalgas/>

## Digestibility of algae proteins?



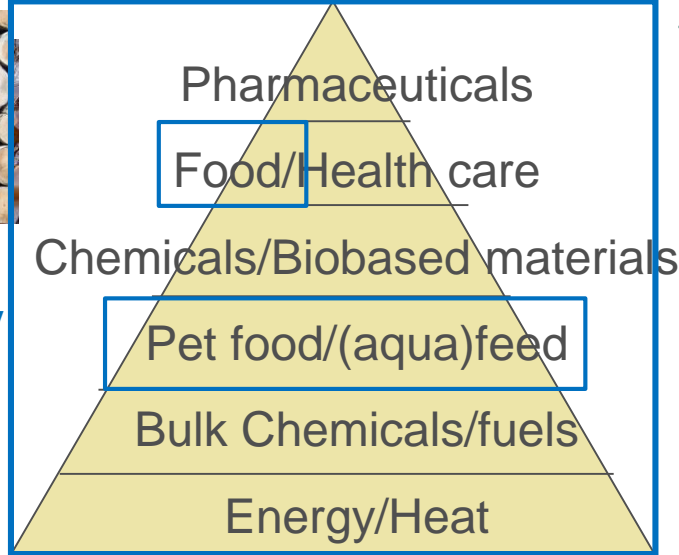


# The pH-shift process as part of a biorefinery



Biomass

The blue biorefinery

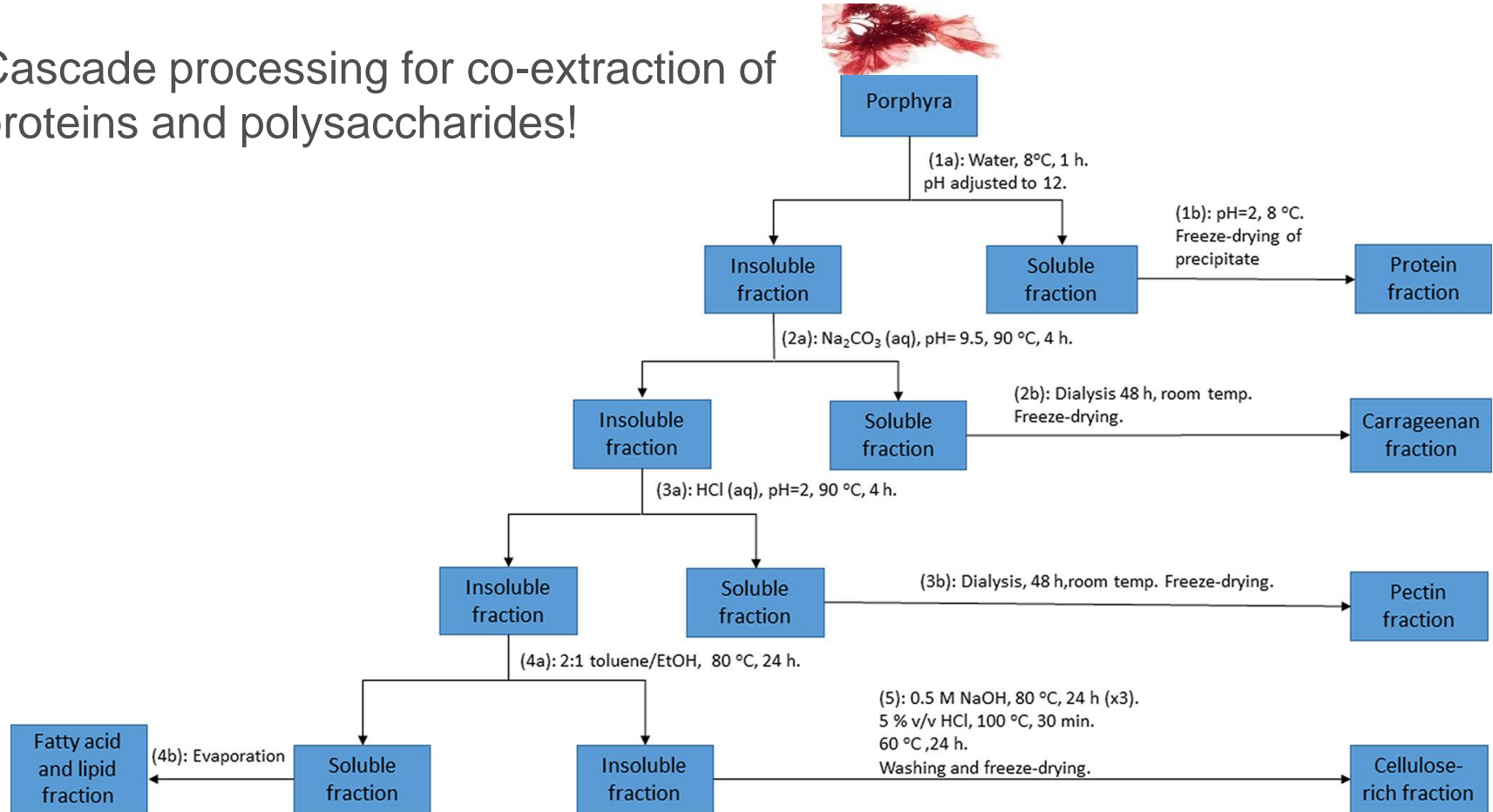


Volume

Value



# Cascade processing for co-extraction of proteins and polysaccharides!



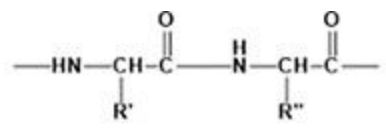
# BIOREFINING



*Porphyra umbilicalis*

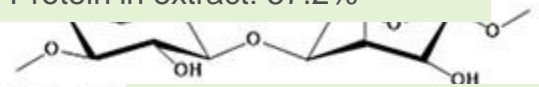
Changing the conventional method for carrageenan extraction from red seaweed (alkali+heat+ethanol precipitation) allowed multiple product production!

## PROTEINS



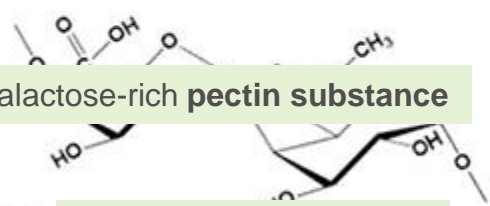
- Protein yield 26%
- Protein in extract: 57.2%

## CARRAGEENAN



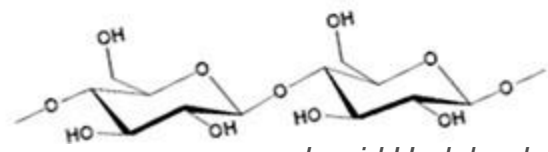
- HMW-type, gelled with K<sup>+</sup>

## PECTINS



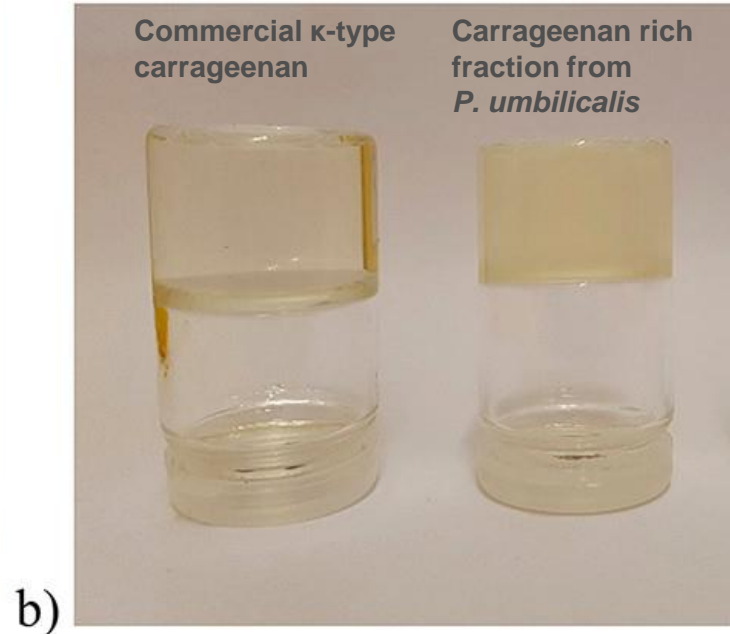
- Galactose-rich **pectin substance**

## CELLULOSE



- Low yield

# Carrageenan from the pH-shift residue of *Porphyra*





We need scalable and food grade methods which efficiently yields a concentrated, **multifunctional** and highly bioavailable seaweed protein isolate which are applicable on both wet and dried seaweed biomass

- pH-shift processing promising → concentrate with  $\leq 71\%$  protein &  $\leq 30\%$  protein yield
- Precipitation more challenging than solubilization!
- Season and preservation affects yield
- Possibilities for co-extraction of polysaccharides

Thanks!



*Ingrid Undeland, Chalmers*