



EXTRACTION AND SEPARATION

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24-03-2021

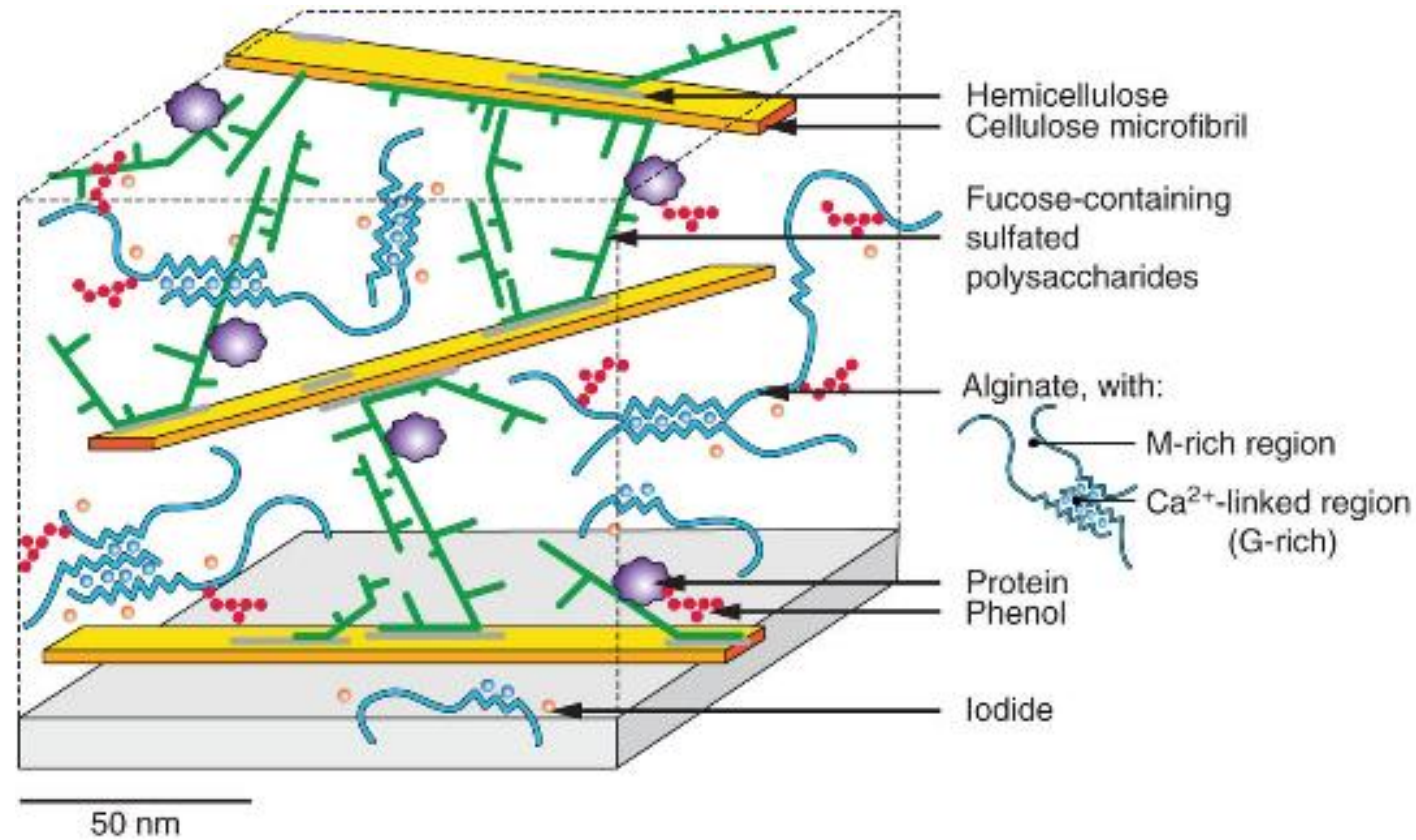
MacroCascade Final Conference



Objectives

1. Development of scalable and sustainable extraction/separation methods
2. For the production of multiple products from brown seaweed or seaweed residues (biorefinery approach)
3. By means of (enzyme aided) physicochemical/mechanical methods
4. Targeted (intermediate) products: 1 alginate, 2 fucoidan, 3 mannitol, 4 laminarin, 5 protein, 6 polyphenols

Structure-function relationships



Deniaud-Bouet, E., G. Michel, T. Tonon, B. Kloareg, C. Herve and N. Kervarec 2014. Chemical and enzymatic fractionation of cell walls from Fucales: insights into the structure of the extracellular matrix of brown algae. *Ann Bot* 114: 1203-1216.

Composition *Sacch. Lat. (literature)*

<i>Saccharina Latissima</i>	Autumn wt% DM	Spring wt% DM	Lit. example wt% DM
1 Alginate	12	20	21.5
2 Fucoidan	5	5	0.8
3 Mannitol	22	6	17.6
4 Laminarin	24	1	5.0
5 Protein	6	13	10.6
6 Polyphenols	1-3	1-3	0.6
Cellulose	8	8	
Minerals/ash	23	36	26.2
Other	0	11	

* Stevant, P., 2017, J. Appl. Phycol.: Ahead of Print; Schiener, P., 2016, Biomass Convers. Biorefin.: Ahead of Print.

1 Alginate

- Biomass
 - Alginate major component in *Sacch Lat* (12-20 wt%)
 - Structural carbohydrate, associated with phenolic compounds in cell wall
 - High molecular weight molecule (Mw 10.000-600.000 g/mol)
 - Divalent (Ca and Mg) salts and alginic acid insoluble, monovalent (Na and K) salts soluble
- Extraction
 - Industrial process, well known. Alkaline ion-exchange (Ca^{2+} to Na^{+}) and purification
 - Research will focus on fate other components. Minor adjustments to extraction process, e.g. no use of acids to prevent hydrolysis of laminarin and fucoidan

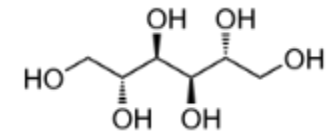
2 Fucoidan

- Biomass
 - Fucoidan minor component in *Sacch Lat* (1-5 wt%)
 - Structural carbohydrate, tightly associated with proteins and cellulose in cell wall
 - High molecular weight molecule ($M_w \approx 20.000$ g/mol)
- Extraction
 - Mild aqueous conditions to obtain native fucoidan (hot water or acid treatment)
 - Bioactivity related to M_w /sulphate content/position sulfate ester group
 - Isolation procedure should avoid loss of sulphate and structural alterations

3 Mannitol

- Biomass

- Mannitol major component in *Sacch Lat* (6-22 wt%)
- Storage carbohydrate (food reserve), content varies with seasons.
- Sugar alcohol (polyol), water soluble, Mw 182 g/mol



- Extraction

- Cold/hot water extraction, ethanol extraction
- Leaches from seaweed in fresh water

4 Laminarin

- Biomass
 - Laminarin minor/major component in *Sacch Lat* (1-24 wt%), content varies with seasons
 - Storage carbohydrate (food reserve), located in vacuoles present in cells.
 - Glucan polymer, DP 20-30 (Mw 3.000-5.000 g/mol)
- Extraction
 - Mild aqueous conditions to obtain native laminarin (cold/hot water or acid treatment)
 - Solubility and bioactivity related to Mw/branching

5 Proteins

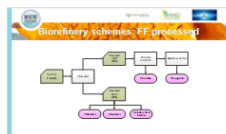
- Biomass
 - Protein major component in *Sacch Lat* (6-13 wt%)
 - Soluble proteins and membrane proteins (tightly associated with fucoidan and polyphenols in cell wall)
- Extraction
 - Protein extraction
 - Alkaline extraction (pH 12), acid precipitation (pH 2), $T < 50\text{ }^{\circ}\text{C}$ to prevent denaturation
 - Production of protein-enriched fractions by removal of other compounds
 - Enzymatic (proteases) to peptides/amino acids

6 Polyphenols

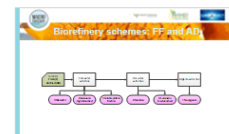
- Biomass
 - Polyphenols minor component in *Sacch Lat* (0.6 wt%)
 - *Ascophyllum Nodosum* reported as high polyphenol accumulating species (4.6 wt%)
 - Tightly associated with proteins and carbohydrates in cell wall
Knowledge on polyphenol extraction valuable in separation of fucoidan and proteins
- Extraction
 - Polar solvents (acetone, alcohol, water). Acetone inhibits interaction between polyphenols and proteins during extraction
 - Novel techniques water based (PLE, SC-CO₂)

Seaweeds received July 2017

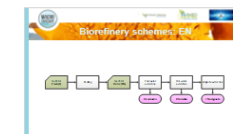
Fresh Frozen (FF)



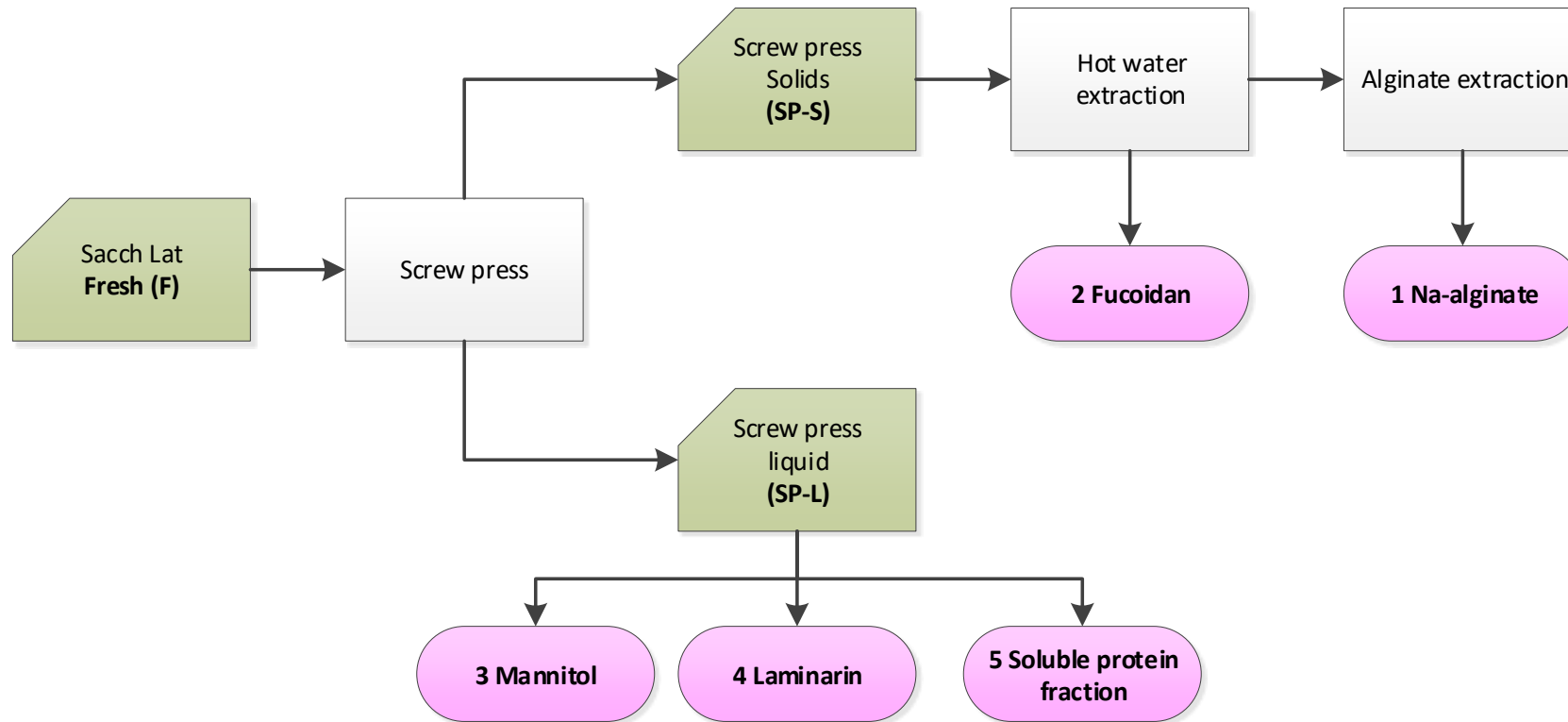
Air Dried (AD)



Ensiled (EN)



Biorefinery schemes: FF processed



Seaweeds received July 2017

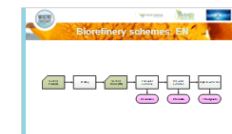
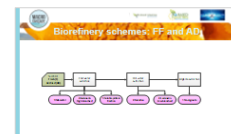
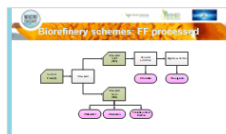
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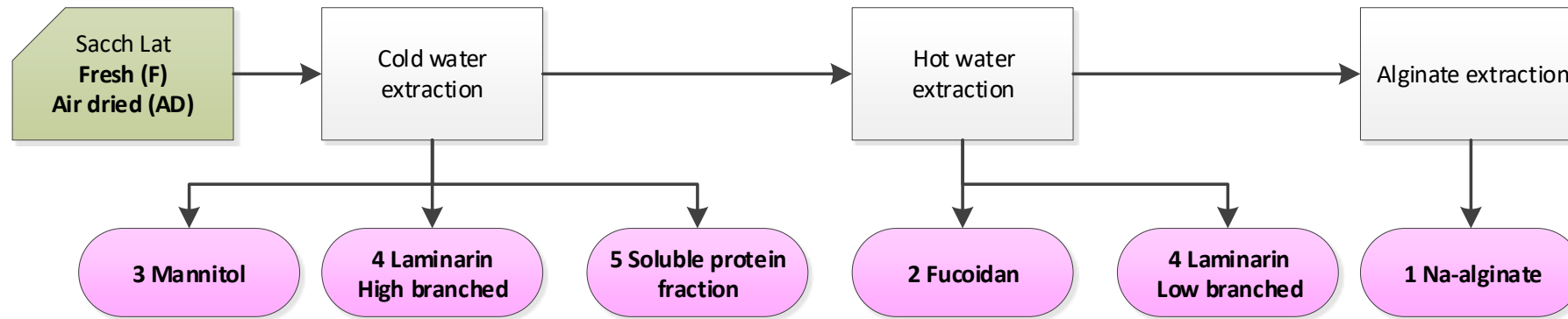
Air Dried (AD)



Ensiled (EN)

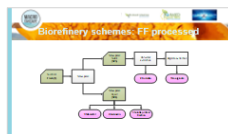


Biorefinery schemes: FF and AD

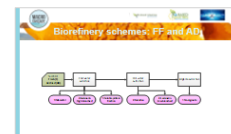


Seaweeds received July 2017

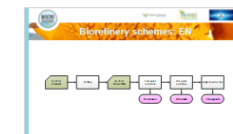
Fresh Frozen (FF)



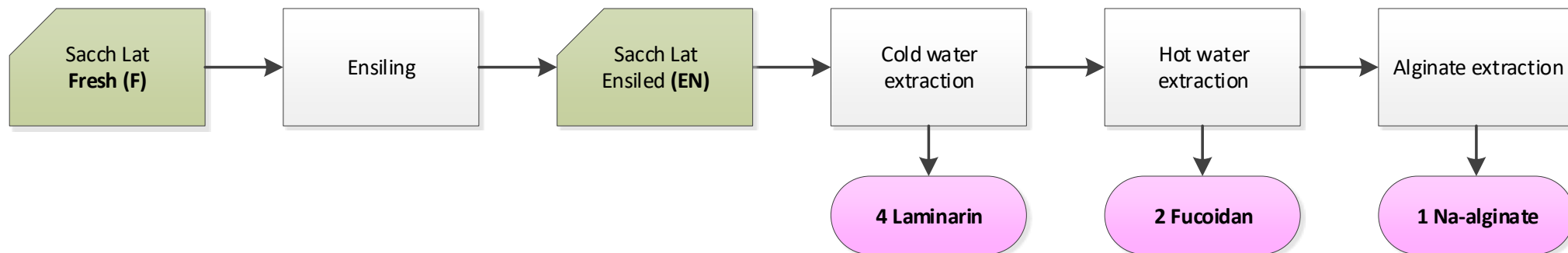
Air Dried (AD)



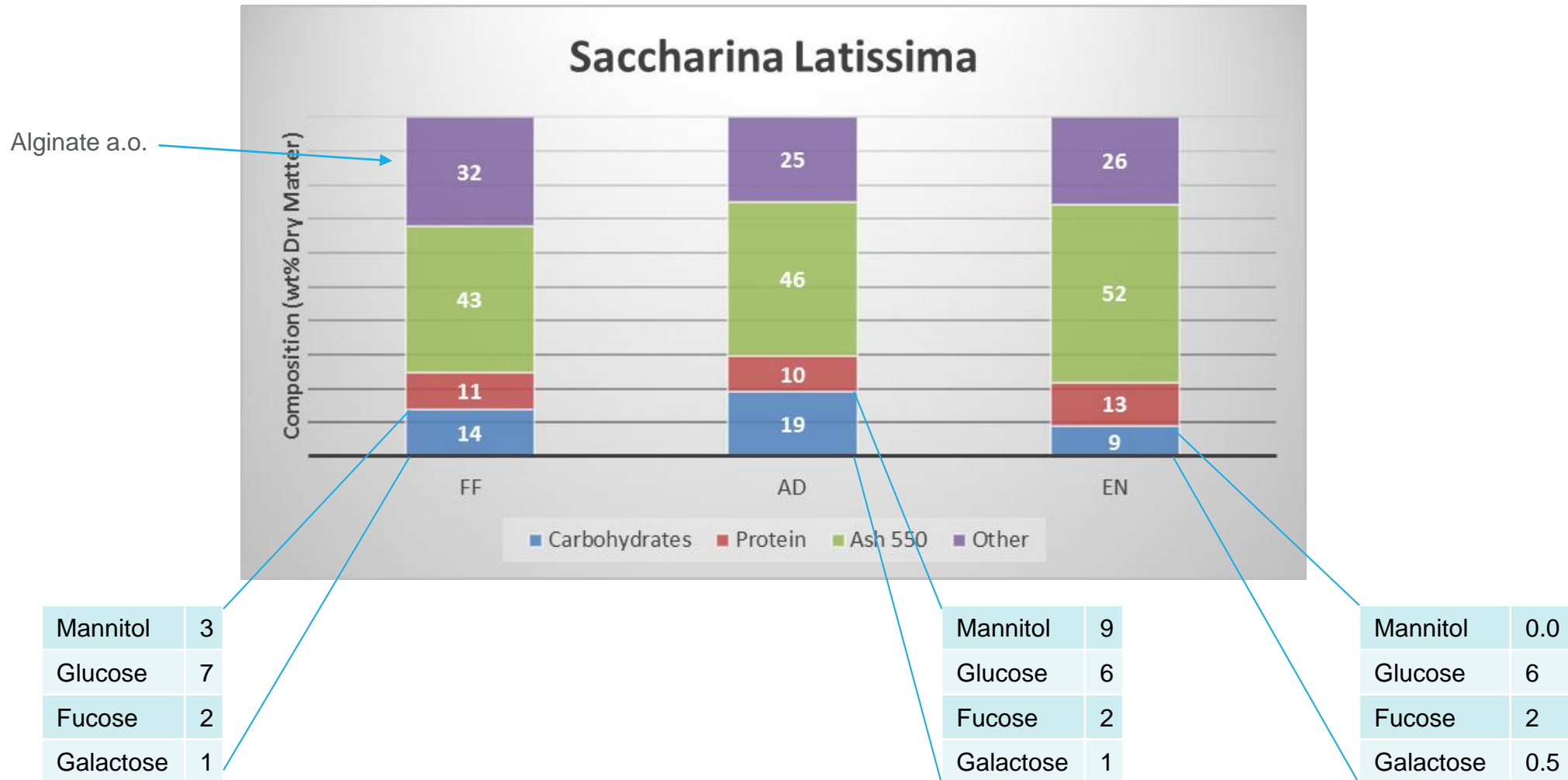
Ensiled (EN)



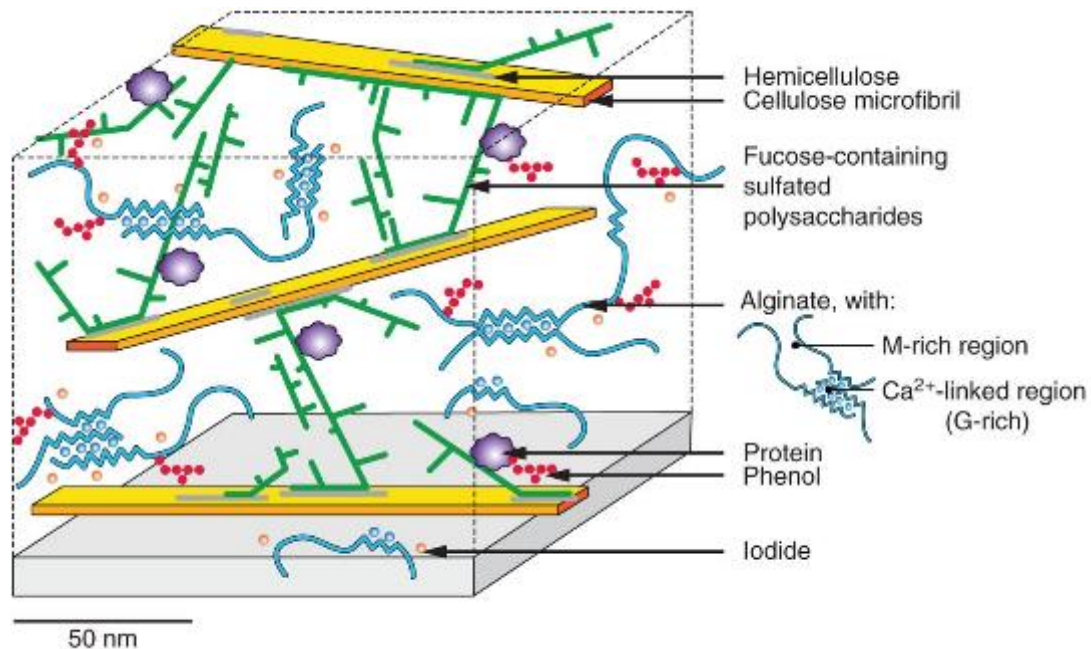
Biorefinery schemes: EN



Seaweed composition



Alginate



- Biomass

- Alginate major component in *Sacch Lat* (12-20 wt%)
- Divalent (Ca and Mg) salts and alginic acid insoluble, monovalent (Na and K) salts soluble

- Extraction

- Industrial process, well known. Alkaline ion-exchange (Ca²⁺ to Na⁺) followed by purification
- Research focus on DSP and fate of other components

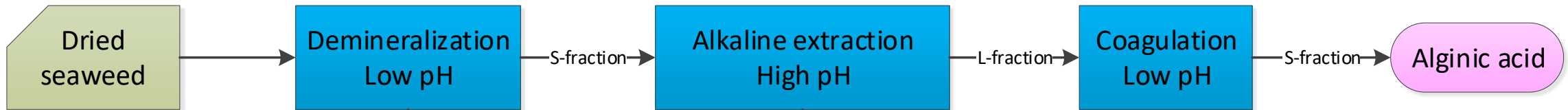
Alginate extraction

Ca-alginate

H-alginate

Na-alginate

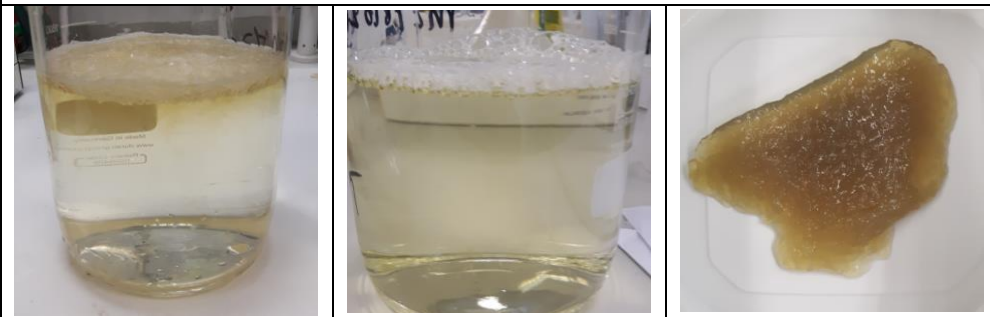
H-alginate



DSP: Acid precipitation vs diafiltration

- Acid precipitation
 - Large volumes of acid required to precipitate the alginate
 - Washing and neutralization
 - Loss of alginate
 - Addition of sodium carbonate to increase pH adds salt to the sample
- Diafiltration
 - Separation of components based on molecular size to obtain a pure solution
 - Washing and purification of alginate without the use of acid

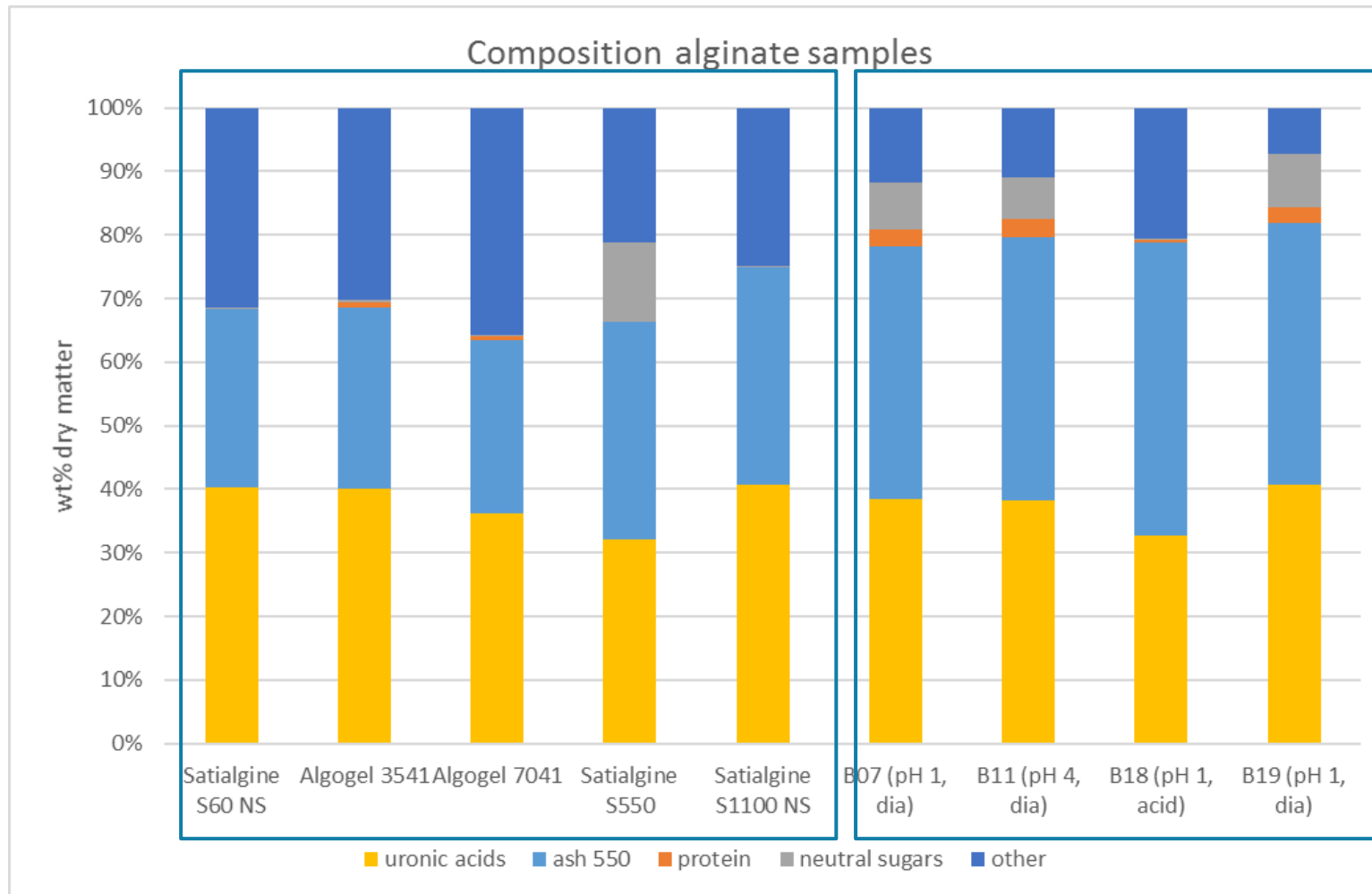
Alginate coagulation



Diafiltration: retentate, freeze dried, solution



Alginate total composition

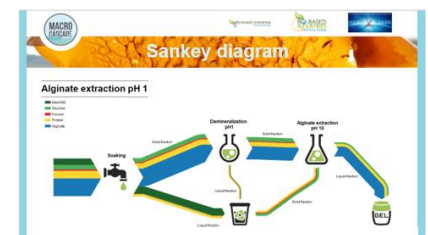


Not only free salts

Underestimation

Alginate conclusions

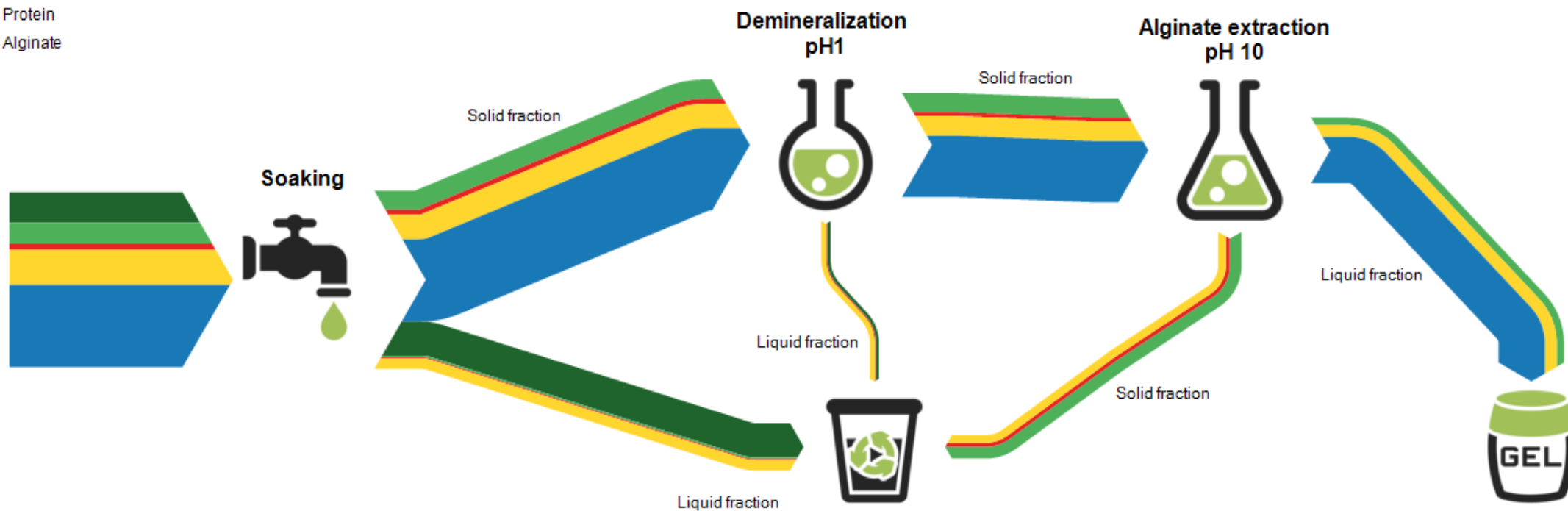
- Alginate extraction
 - Mode of action: divalent (Ca and Mg) salts and alginic acid are insoluble, monovalent (Na and K) salts soluble
 - pH1 vs pH4: Molecular weight pH4 sample seems higher, need less salts
 - Acid vs diafiltration: acid precipitation removes residual neutral carbohydrates and protein
- Biorefinery approach
 - Large part of mannitol is liberated during water soaking step
 - Fucose (fucoidan) detected in low concentrations in several fractions
 - Protein detected in several fractions, reasonable amount in solid residue after alginate extraction



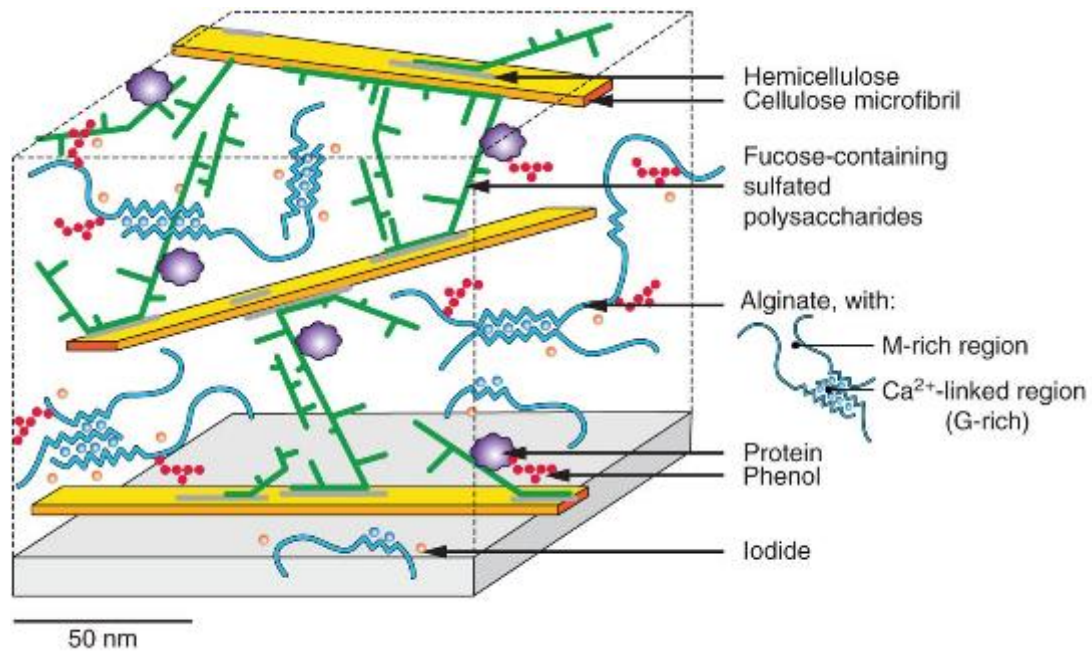
Sankey diagram

Alginate extraction pH 1

- Mannitol
- Glucose
- Fucose
- Protein
- Alginate



Protein

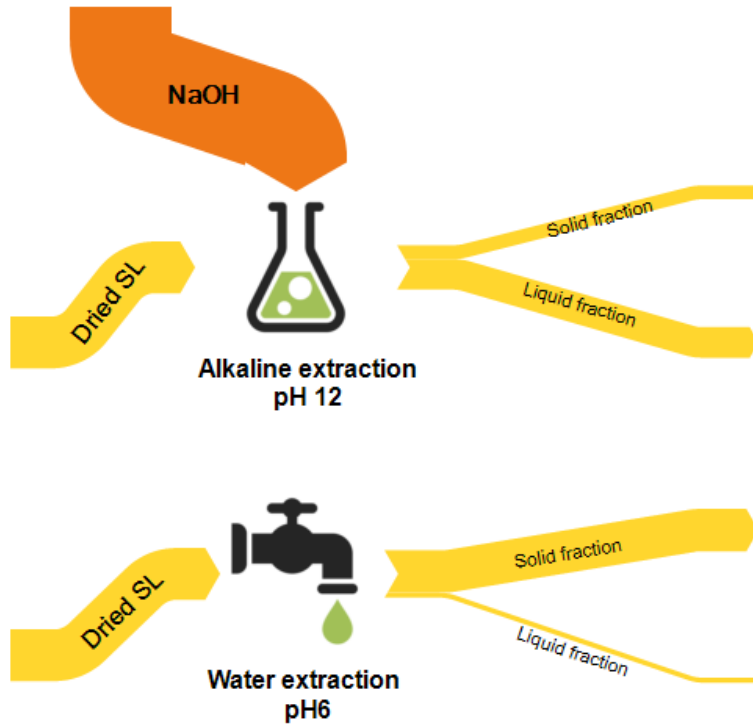


- Biomass
 - Soluble proteins
 - Membrane proteins, connected to fucoidan and phenols
- Extraction
 - Water
 - Acid
 - Alkaline
 - Enzymatic

Sankey diagram

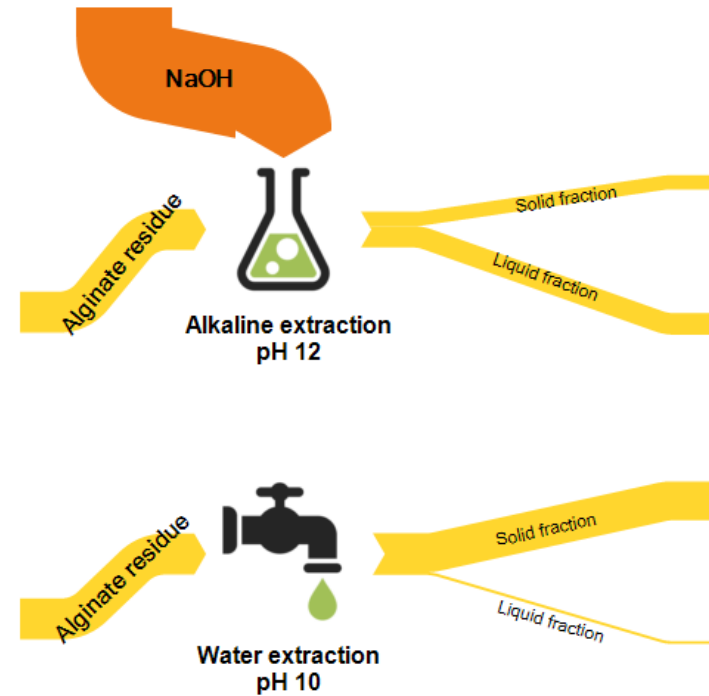
Protein extraction dried SL

Protein
NaOH



Protein extraction alginate residue

Protein
NaOH



Protein conclusions

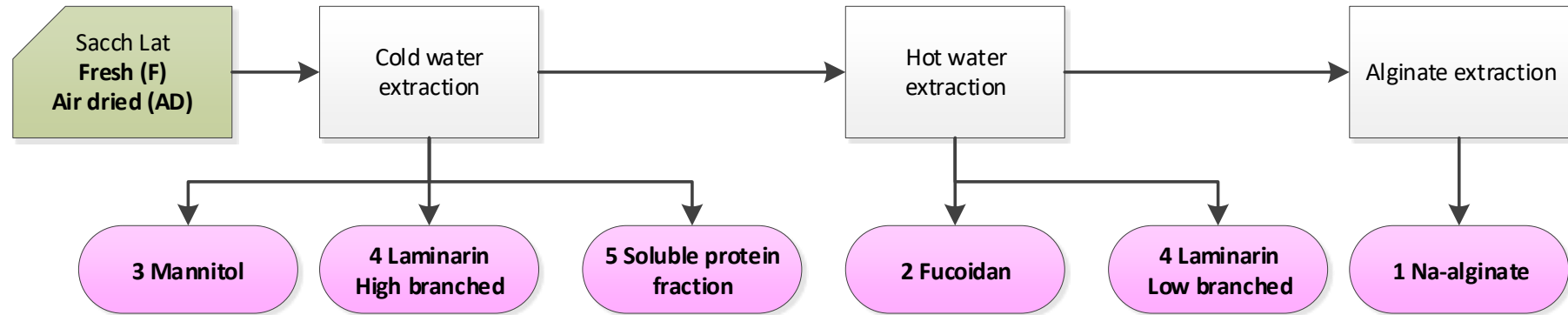
- Protein extractions
 - Soluble proteins
 - Easily extracted from seaweed with fresh water, followed by purification steps
 - Membrane bound proteins
 - Extracted under alkaline conditions, conditions need to be optimized. Proteins denatured.
 - Most optimal extraction method (acid, alkaline, enzymatic) depends on final application and desired product specifications
- Biorefinery approach
 - Proteins distributed in all fractions during alginate extraction
 - Soluble proteins from wash water or from press liquid, combined with mannitol
 - Membrane bound proteins from solid residue after alginate extraction
 - Simple washing with water results in protein-enriched fraction (25 wt% protein)
 - Advantage of alkaline extraction not clear yet

Achievements extractions

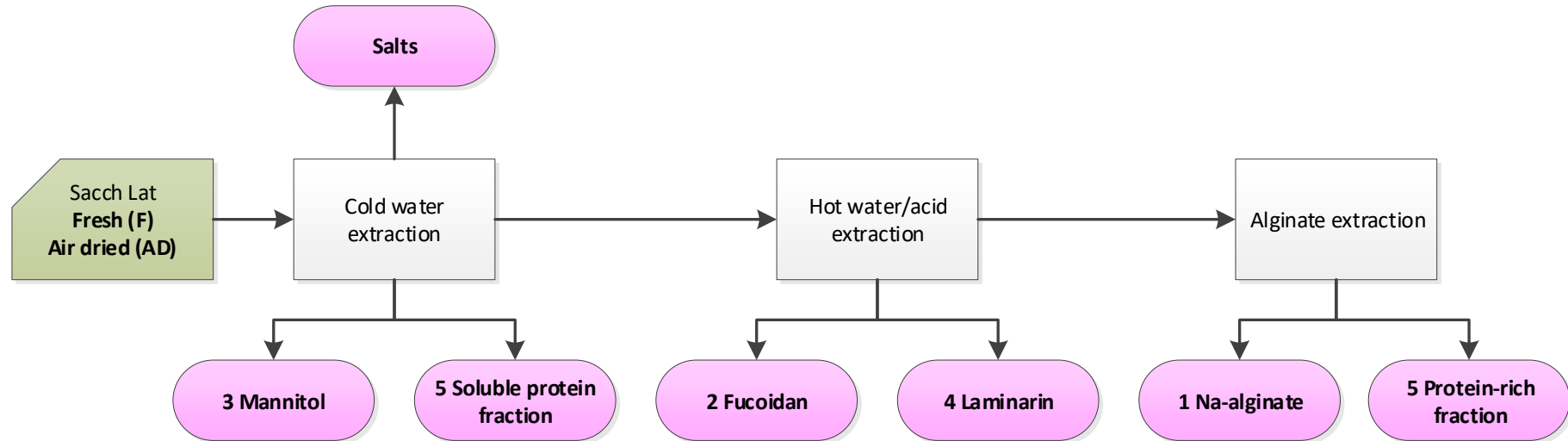
ORF seaweed	Alginate	Fucoidan	Mannitol	Laminarin	Protein	Polyphenols
Extractions studied	✓	✓	✓	✓	✓	✓
Component detected	✓	✓	✓	✓	✓	✓
Purified	✓	✓	✓	✗	✗	✓
Isolated (g-scale)	✓	✗	✓	✗	✓ Protein rich fraction	✓ (mg)

Biorefinery approach

Begin of the project



End of the project





Acknowledgement



This presentation is part of the Macro Cascade project.
This project has received funding from the European
Union's Horizon 2020 Bio-Based Industries Joint
Undertaking (BBI JU) under grant agreement No 720755

<https://www.macrocascade.eu/>