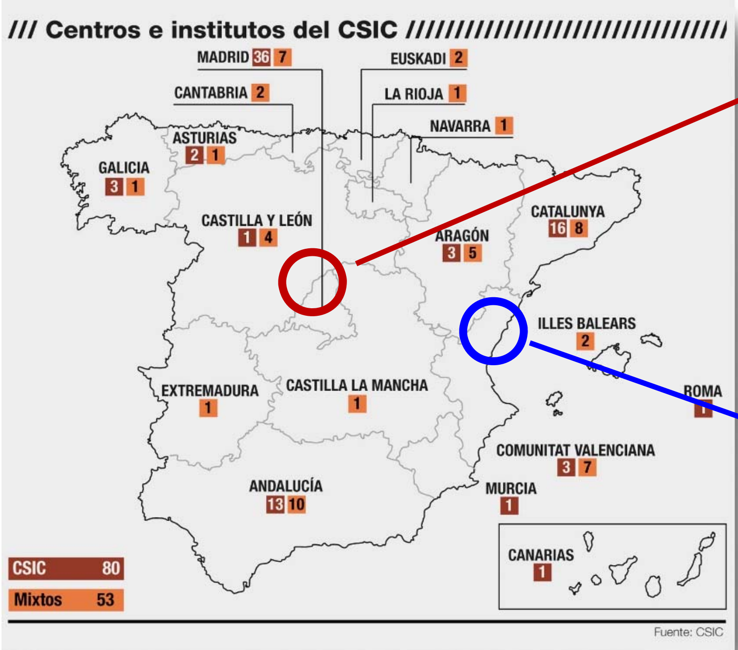
The potential of small angle scattering techniques to study the structure of polysaccharide gels

CSIC

CIAL
INSTITUTO DE INVESTIGACIÓN
EN CIENCIAS DE LA ALIMENTACIÓN

Marta Martínez-Sanz
marta.martinez@csic.es

Spanish Council for Scientific Research (CSIC)



Institute of Food Science Research (CIAL)

- Impact of food structure and polysaccharide-protein interactions on gastrointestinal digestion
- Development of polysaccharide-protein products resistant to gastric digestion

Institute of Agrochemistry and Food Technology (IATA)

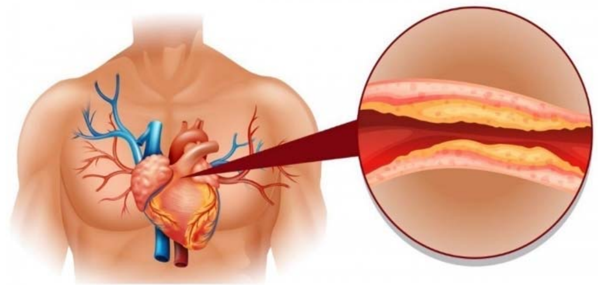
- Extraction of polysaccharides from marine biomass
- Development of polysaccharide-based gel-like structures with interest for food industry

University of Queensland (**UQ**)

Plant Cell Walls
ARC Centre of Excellence

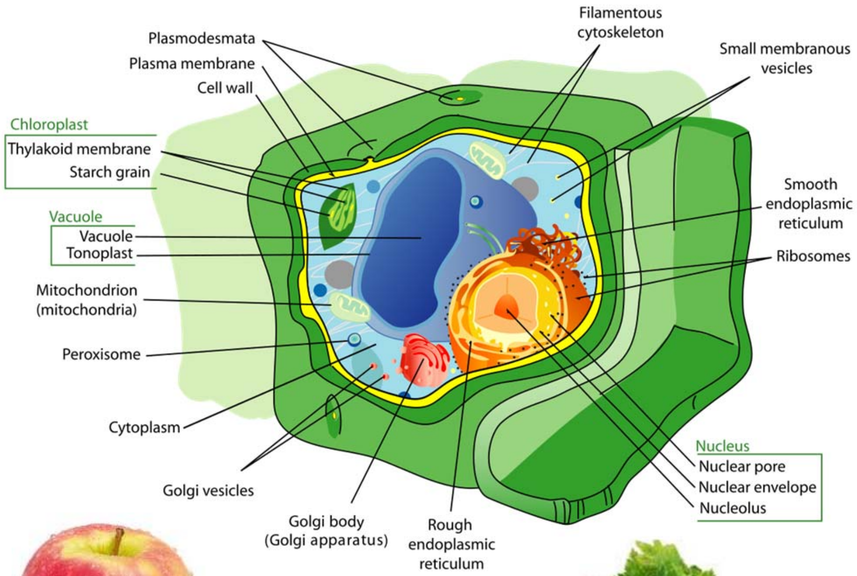
Australian Nuclear Science and Technology Organisation (**ANSTO**)

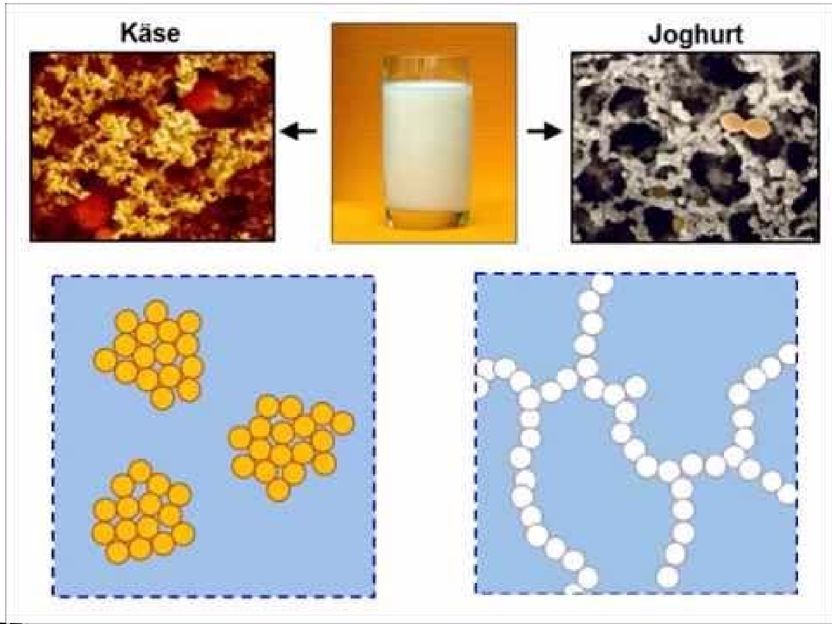
Scattering techniques in Food Science



- Demands on the food market push for the development of **new products**: functional foods, minimally processed foods, dietetic foods, products for specific dietary requirements…
- Consumers are more concerned about the relationship between **diet and health**
- Knowledge of **food structure** in essential from an industrial (design of new products) and scientific (nutrition and health impact) perspective

Scattering techniques in Food Science

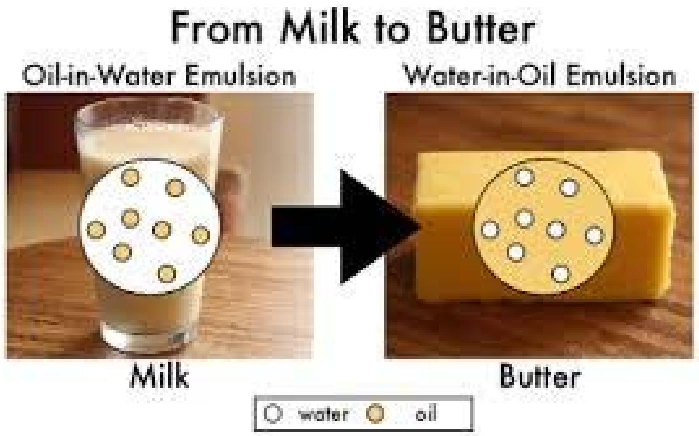


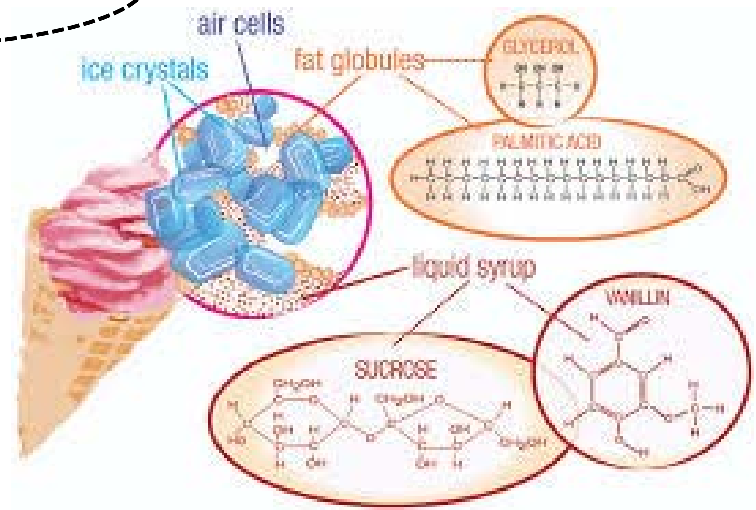


Polysaccharides

Proteins

Fats



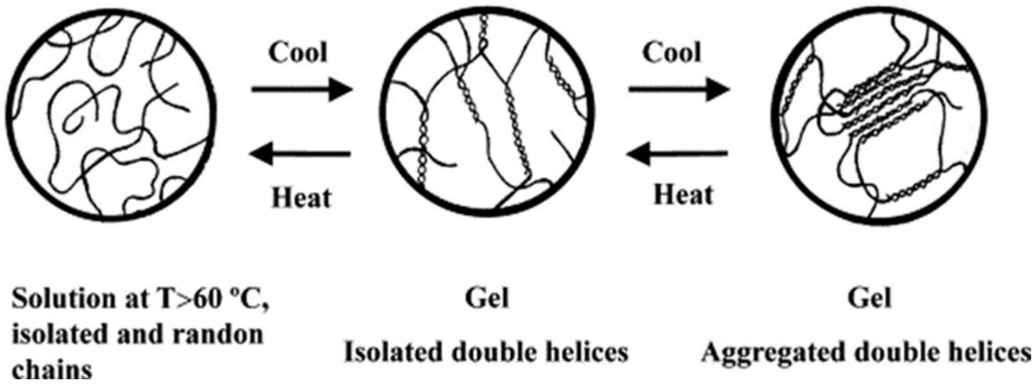


Polysaccharides

- Major components in **fruits and vegetables**
- **Food ingredients**: thickeners, gelling agents, encapsulants, additives for low calorie products, etc

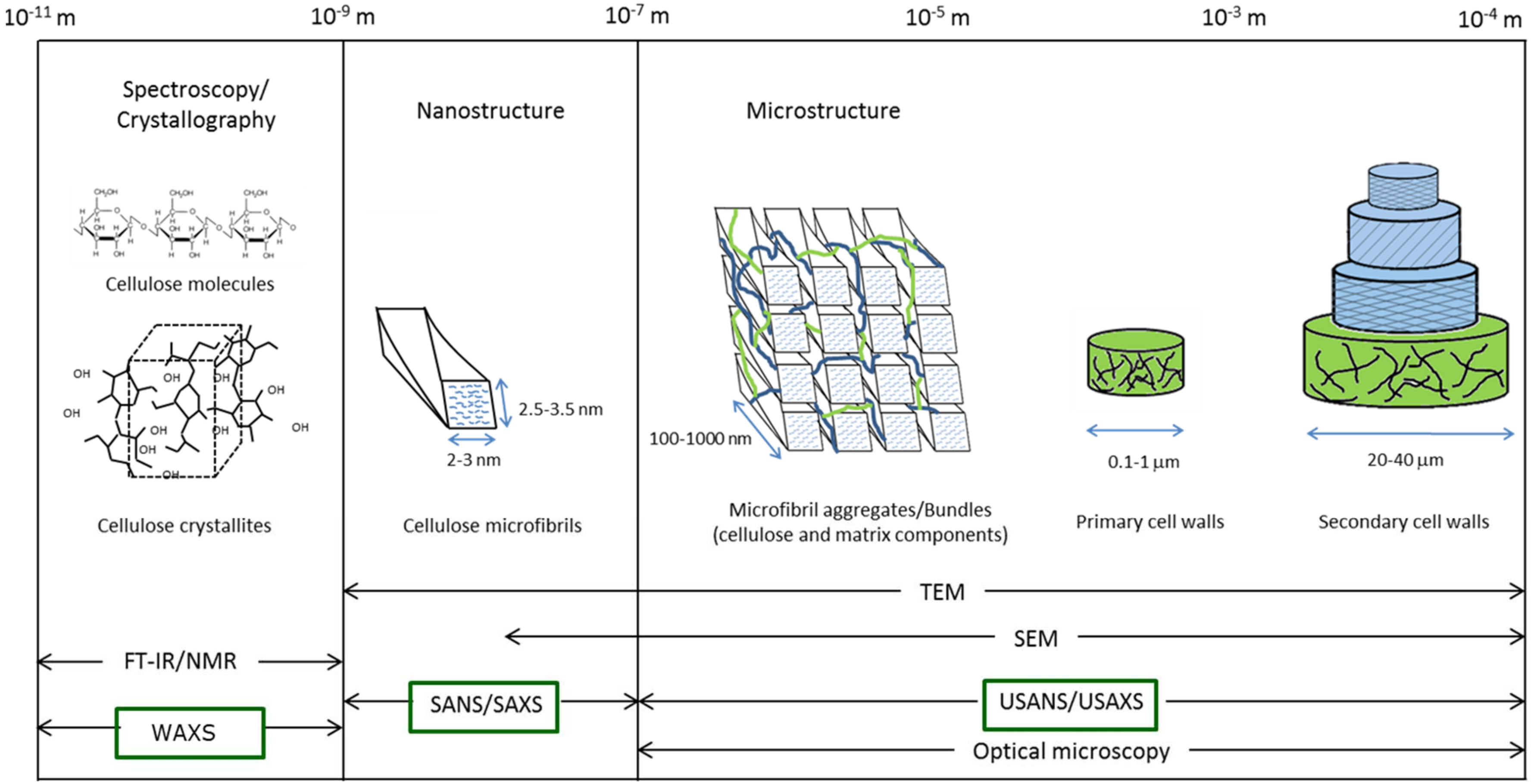
Source	Examples
Higher plants	Cellulose, hemicelluloses, starches, pectins
Seaweed	Agars, alginates, carragenaans
Microorganisms	Xanthans, pullulan, gellan, curdlan

- Many of them are found in nature in **hydrated systems**
- Might present **crystalline** (impermeable to water) or **amorphous** (water-soluble) structures



Scattering techniques in Food Science

Cellulose

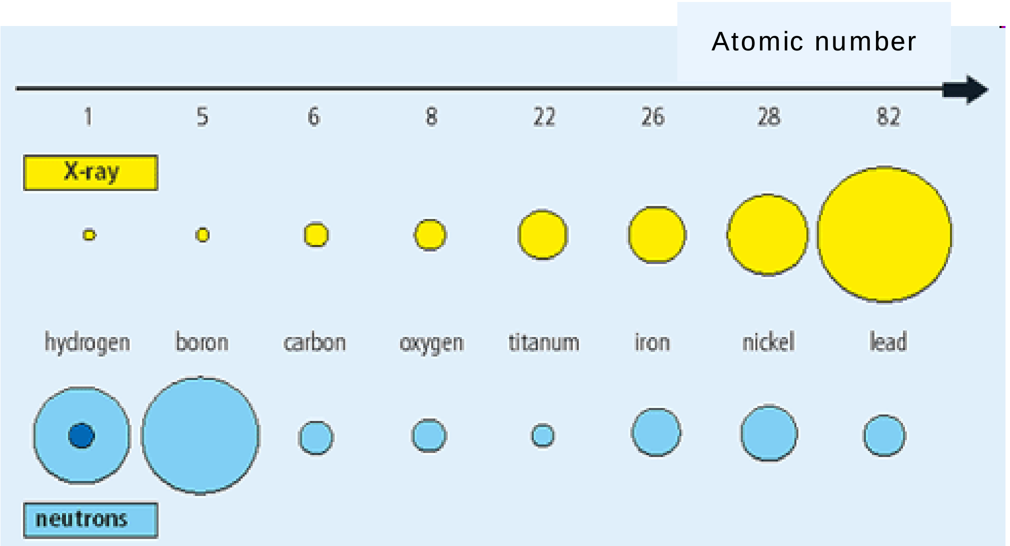


Martínez-Sanz, M., Gidley, M. J., & Gilbert, E. P. *Carbohydrate polymers* (2015)

Scattering techniques in Food Science

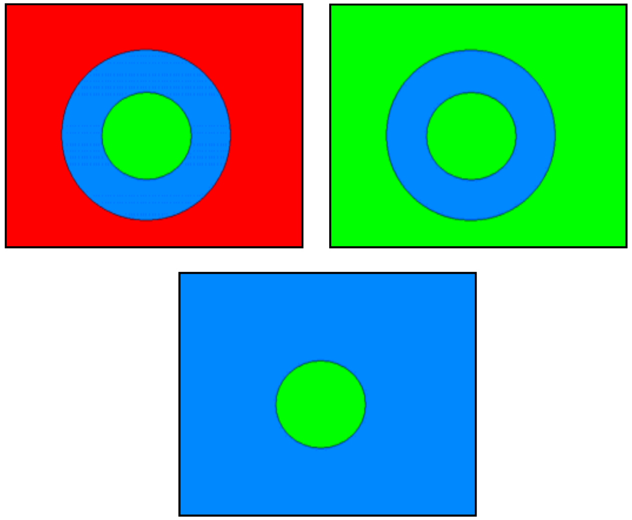
X-Rays

- Scattered by the electrons present in an atom
  → Sensitive to variations in electron density
- Scattering intensity increases with atomic number
- Hydrogens are almost "invisible"



Neutrons

- Scattered by the atomic nuclei
- Different scattering intensity for isotopes of the same element (H vs D)
- Very sensitive to hydrogens
- Phase differentiation by "contrast variation"

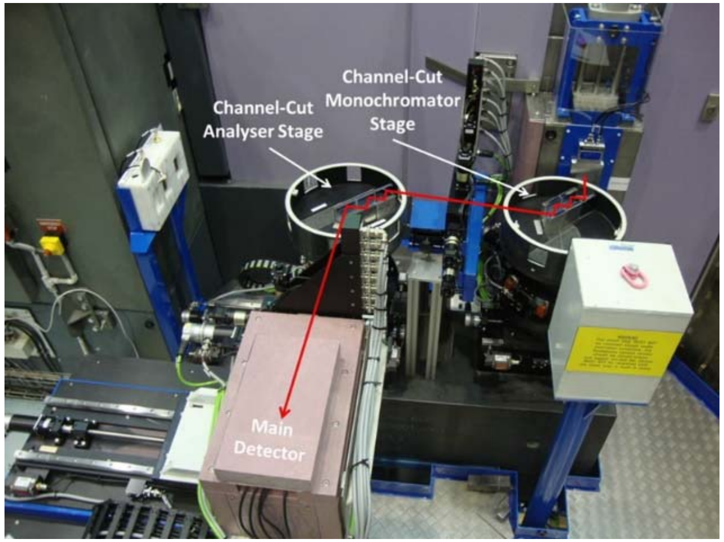


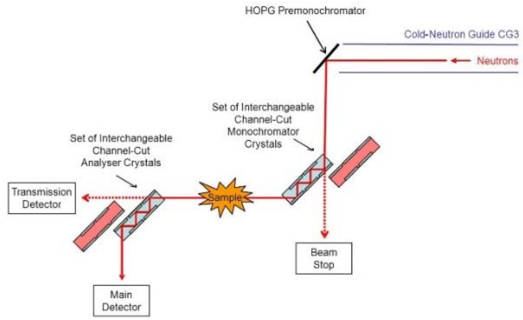
Source of radiation selected on the basis of sample composition and structural features to be probed

Scattering techniques in Food Science

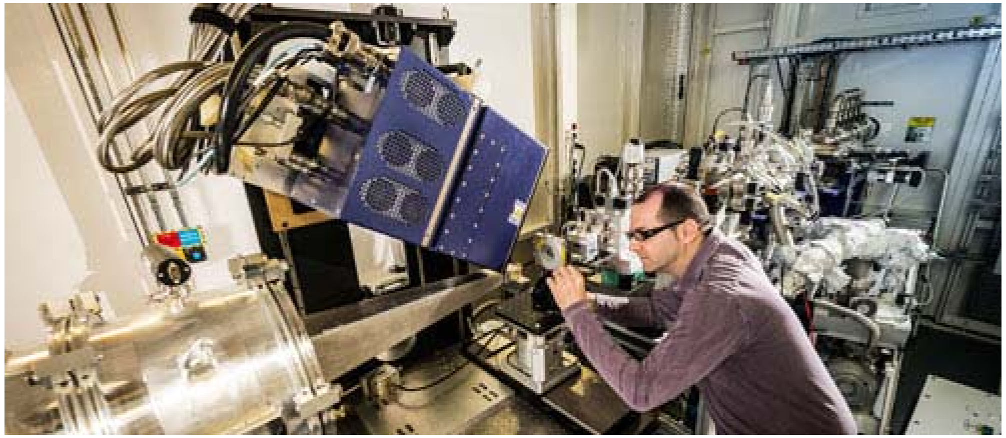
SANS

USANS





ansto
Nuclear-based science benefiting all Australians

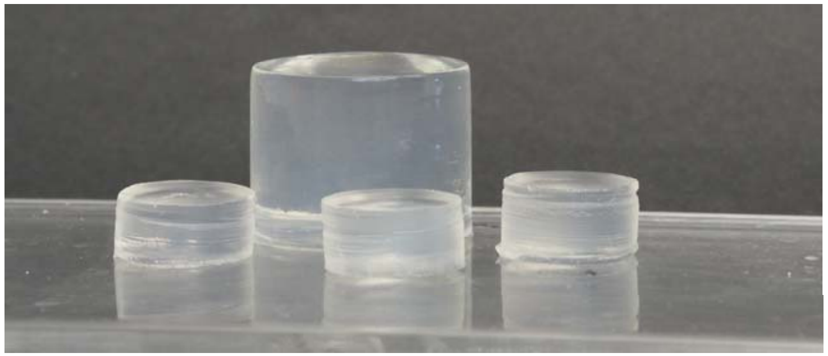


ALBA

Synchrotron
Simultaneous SAXS / WAXS

BL11 - NCD-SWEET

AGAR-BASED HYDROGELS



Martínez-Sanz et al. *Carb. Polym.*(2020)

iata
Instituto de Agroquímica y Tecnología de Alimentos

CHALMERS UNIVERSITY OF TECHNOLOGY

Nofima

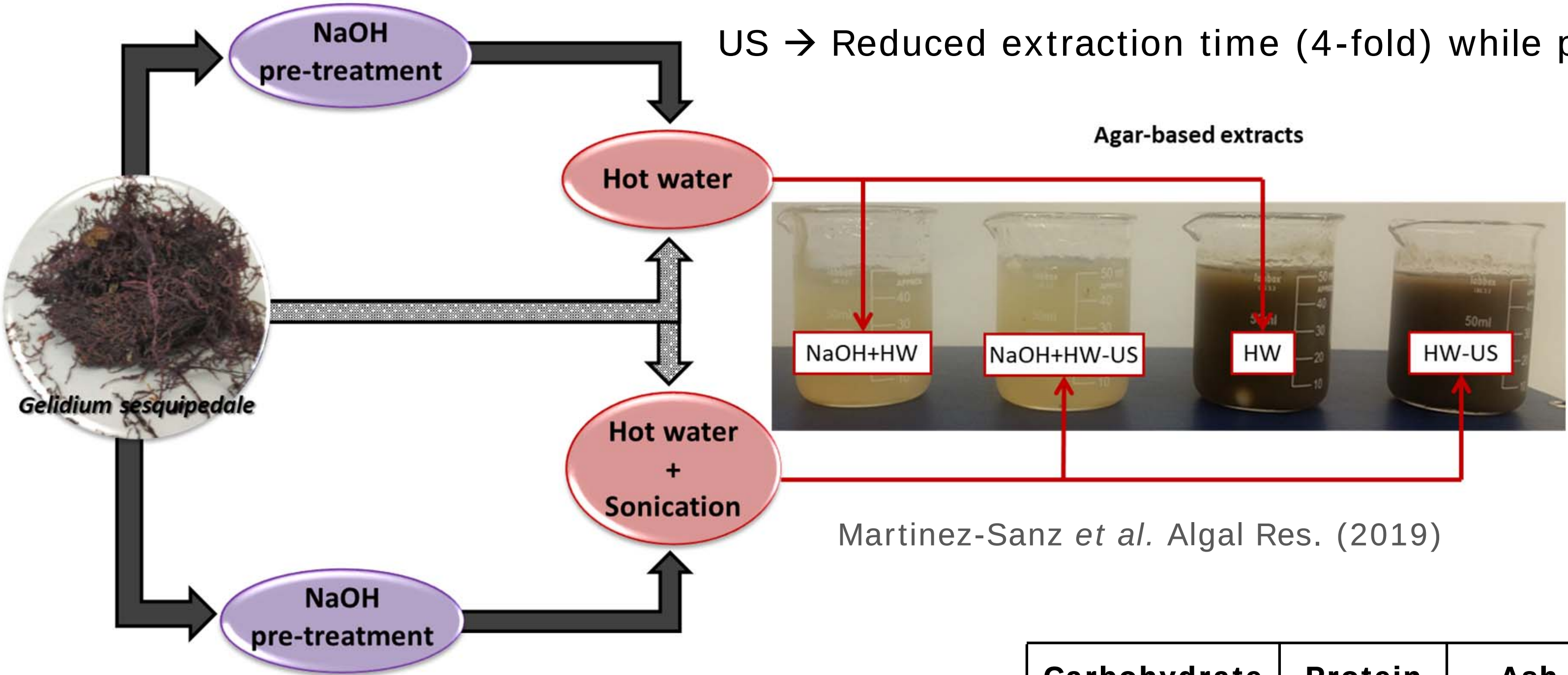
ansto
Nuclear-based science benefiting all Australians

ALBA

Agar-based hydrogels

Production of **agar-based extracts** from *Gelidium* using simplified extraction protocols

US → Reduced extraction time (4-fold) while preserving yield



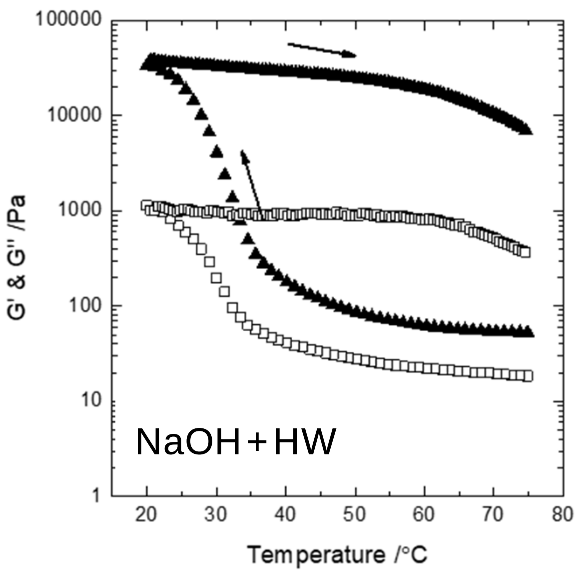
Martinez-Sanz *et al.* Algal Res. (2019)

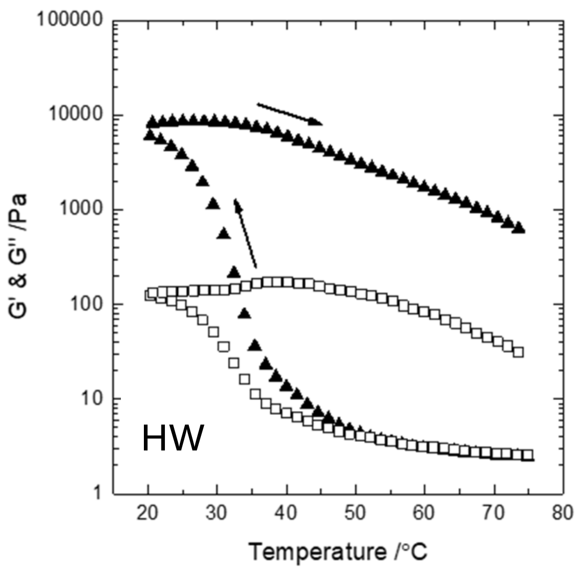
- Pre-treatment produced more purified agars, but led to partial degradation (lower M_w)
- **Non-purified extracts** are richer in proteins, minerals and polyphenols → ↑↑ **Bioactivity**

	Carbohydrate (%)	Protein (%)	Ash (%)	Mw (kDa)
Commercial	83	1.5	4.3	196
HW	42	**10.6**	**34.7**	840
HW-US	30	**11.3**	**36.4**	485
NaOH+HW	66	4.0	11.9	487
NaOH+HW-US	51	7.3	13.9	265

Agar-based hydrogels

- Gelation mediated through the formation of agarose **double helices** and **bundles** upon cooling



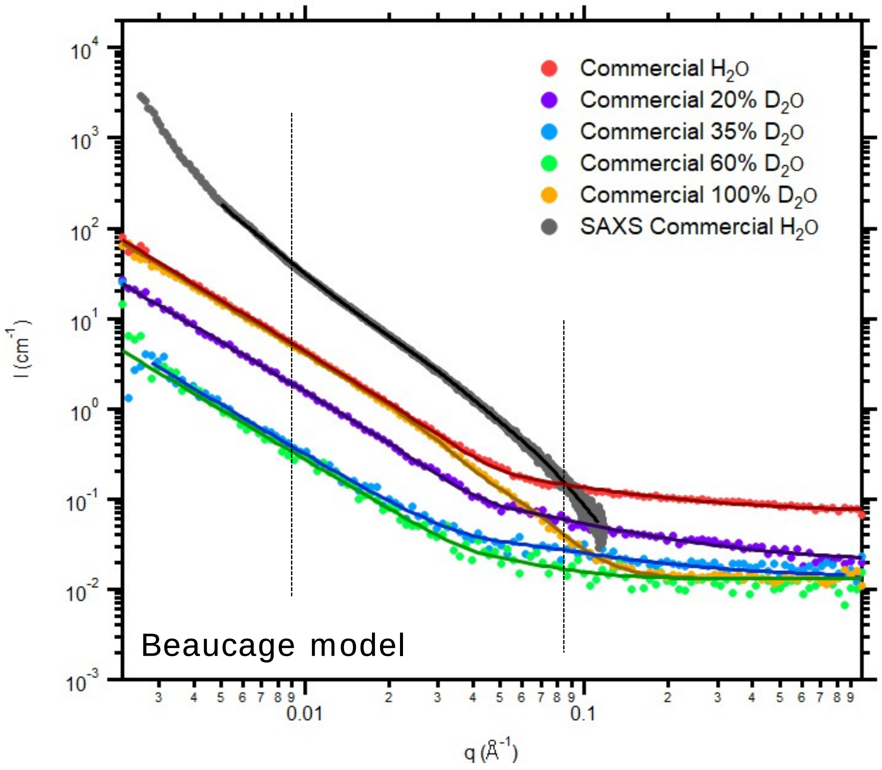


- **Similar gelling T** and mobility of cross-linking points
- Non-purified extracts form **softer gels**

- No melting upon heating
- Large syneresis

Agar-based hydrogels

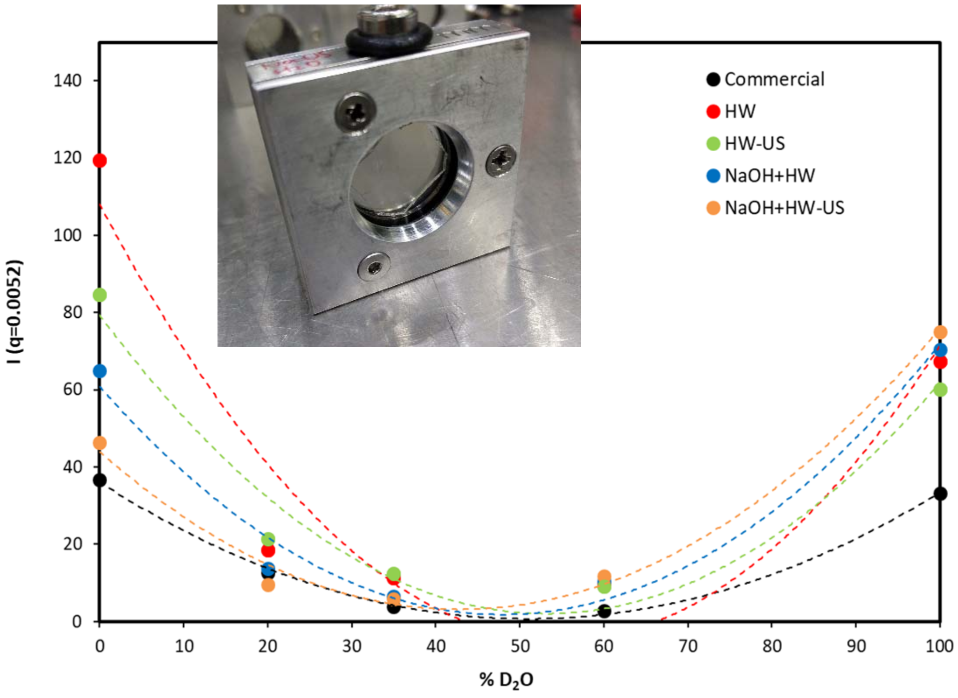
SAXS + SANS contrast variation of gels



Aggregation of agarose double helices:

Rg=5-6 nm for the non-purified agars

Rg=7-10 nm for the purified agars



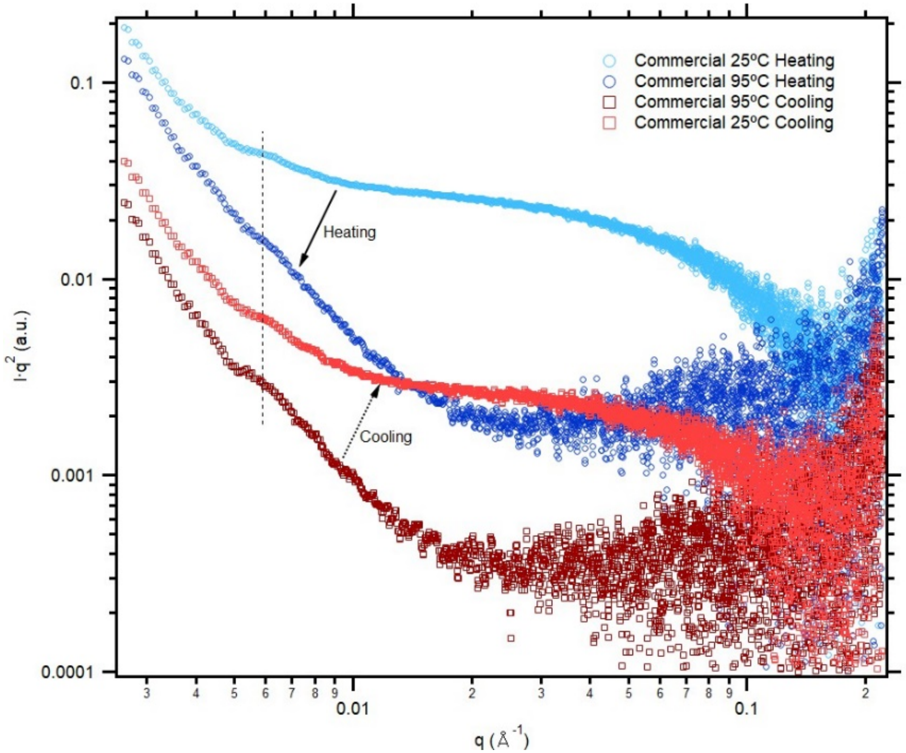
Greater deviation from the theoretical behaviour in the non-purified agars due to (1) OH/OD exchange and (2) impurities

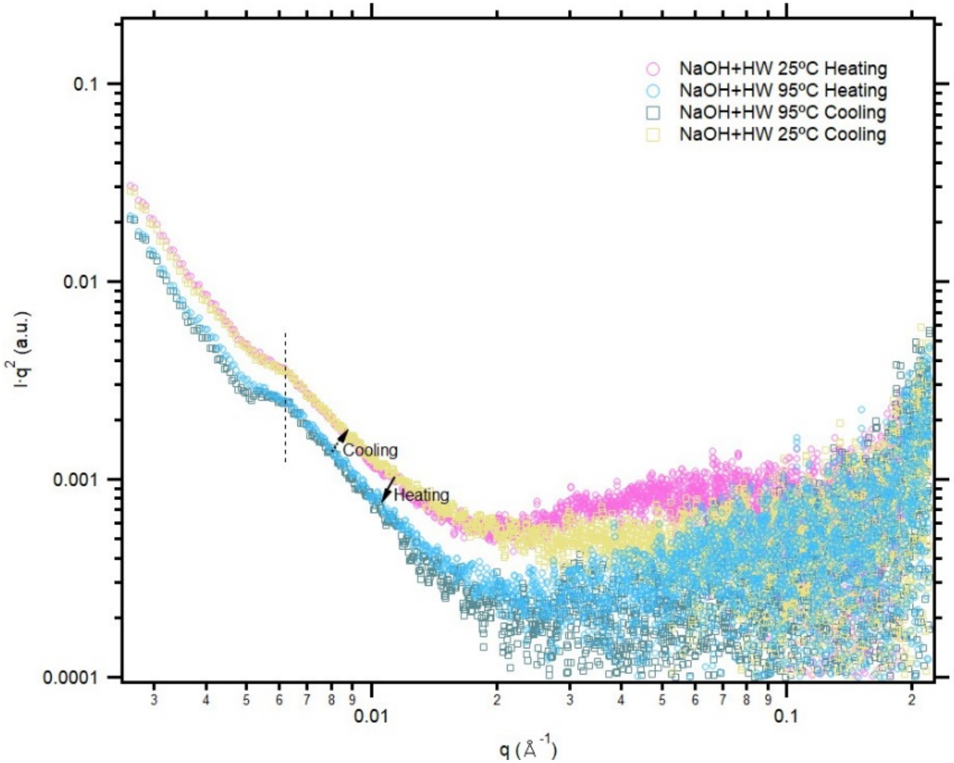
SLD Agarose = $2.16 \cdot 10^{10}$ cm^{-2}

	Commercial	HW	HW-US	NaOH+HW	NaOH+HW-US
Contrast match (% D_2O)	51.0	54.9	53.2	47.8	42.8
SLD (10^{10} cm^{-2})	2.96	3.23	3.11	2.74	2.39

Agar-based hydrogels

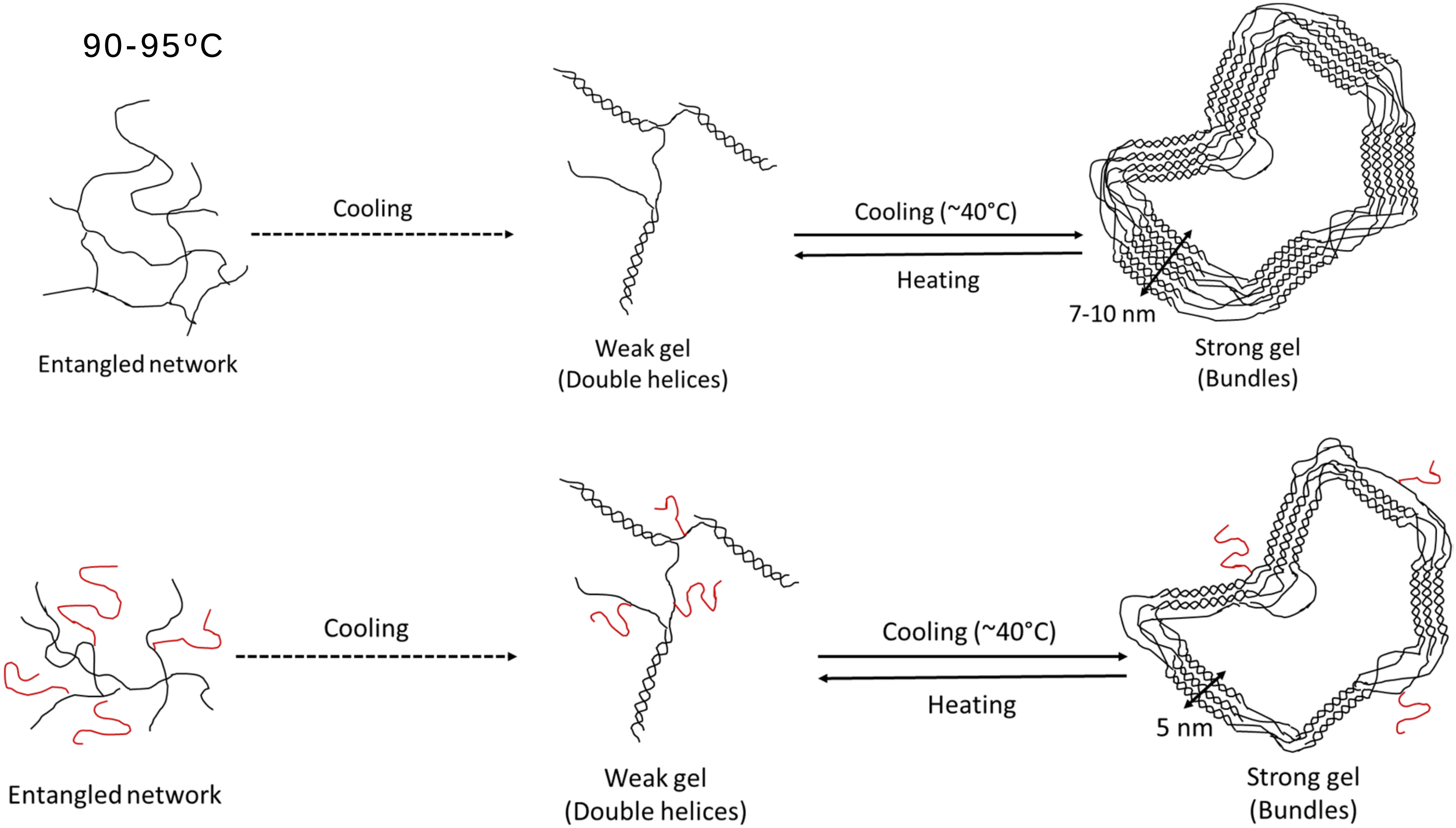
SAXS Temperature-resolved experiments





- No real melting transition but **disruption** of agarose **double-helix aggregates**.
- Aggregates are stable even at 95ºC in the more purified samples.
- The disruption of aggregates is **not completely reversible**.

Agar-based hydrogels



- ↑Agar content → Greater extent of chain association (thicker agarose bundles) → Stronger and stiffer hydrogels
- Impurities did not impair the interconnectivity in the hydrogel network

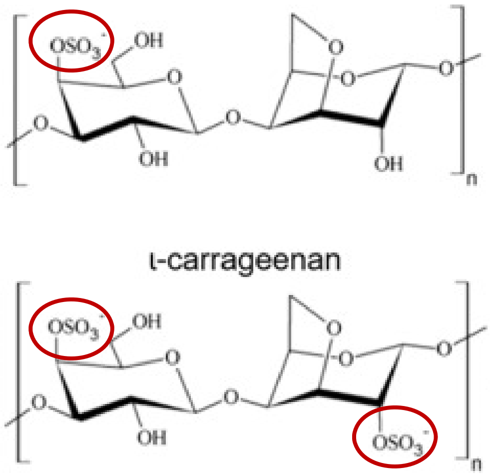
CARRAGEENAN HYDROGELS

Fontes-Candia et al. *Algal Res.* (2020)

Carrageenan hydrogels

Red seaweed

κ-carrageenan



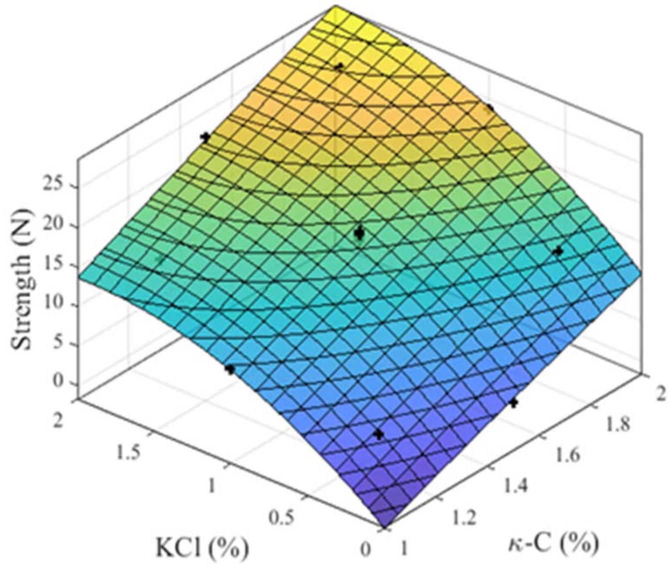
ι-carrageenan

H_2O

KCl

$CaCl_2$

Gel strength measurements



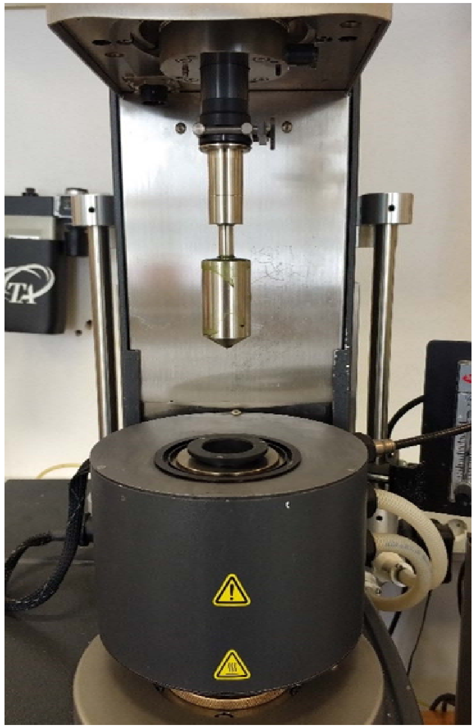
- **Carrageenan** and **salt concentration** have a strong impact on hydrogel strength
- ↑**sulphate content** in ι-C → **weaker hydrogels**
- K^+ had a greater impact in κ-C, whereas Ca^{2+} worked better for ι-C

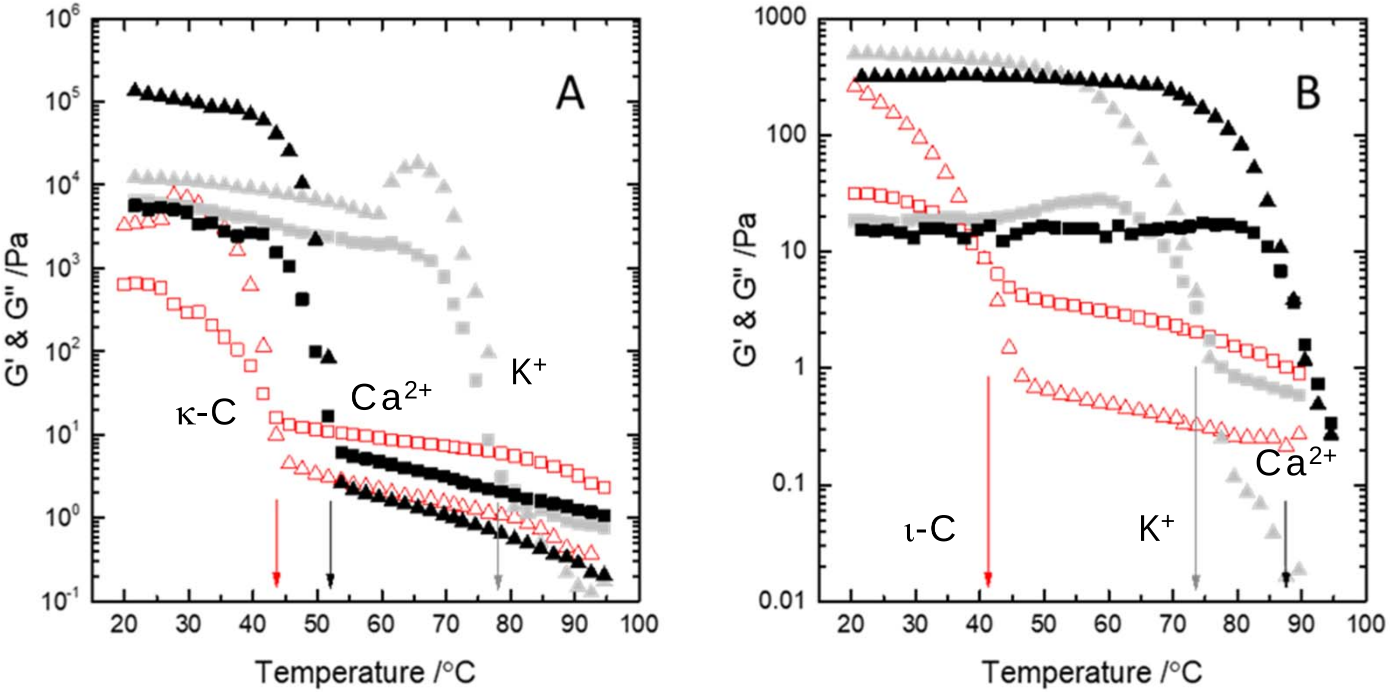
2% κ-C + 2% KCl → 29 N

2% ι-C + 1% $CaCl_2$ → 0.4 N

Carrageenan hydrogels

2% carrageenan + 1.5% salt



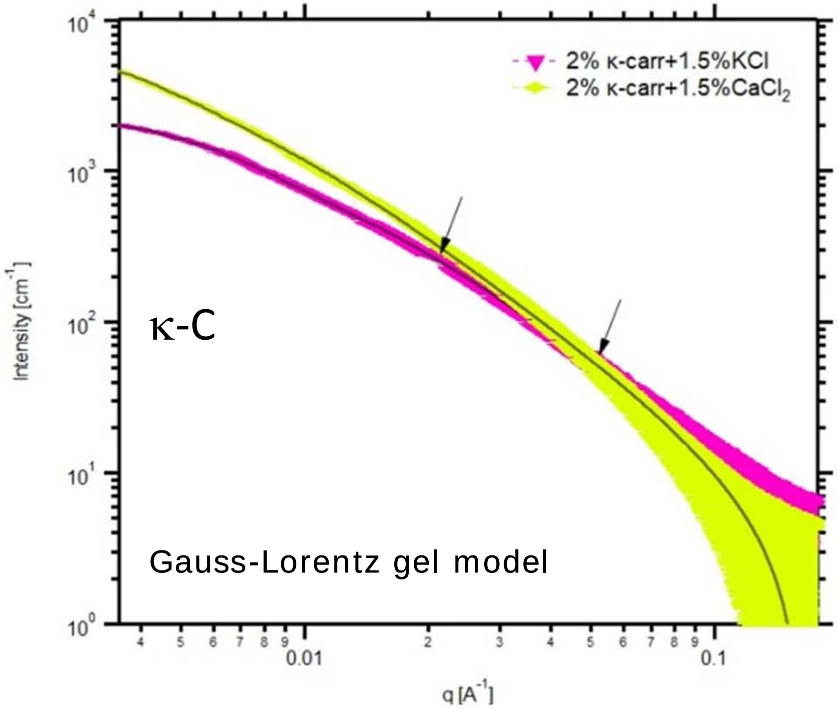


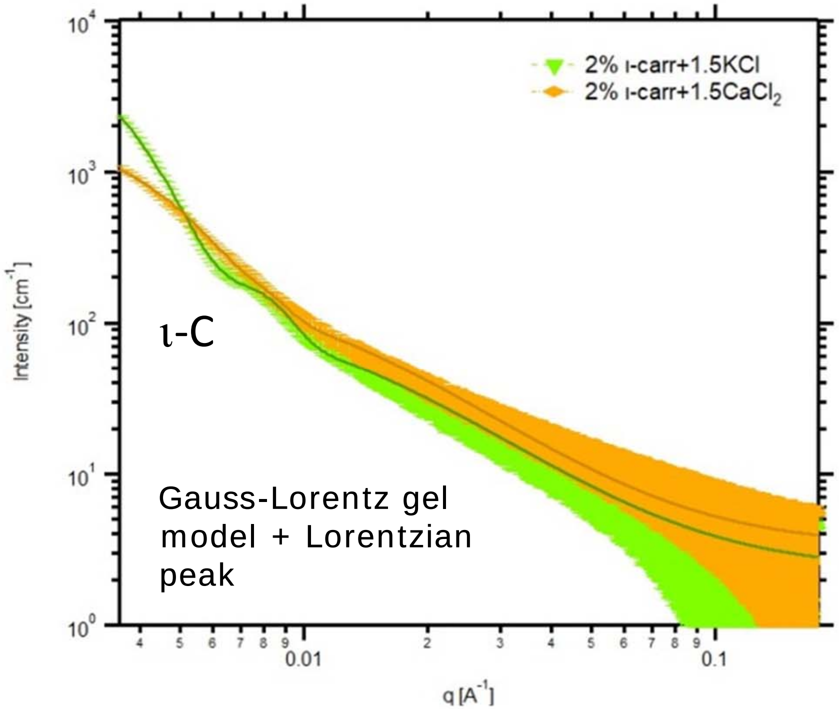
- Addition of salts → Promoted association of carrageenan chains → Higher gelation T

  → Greater hydrogel strength

- κ-C Tgel: κ-C < Ca^{2+} < K^{+}
- ι-C Tgel: ι-C < K^{+} < Ca^{2+}

Carrageenan hydrogels





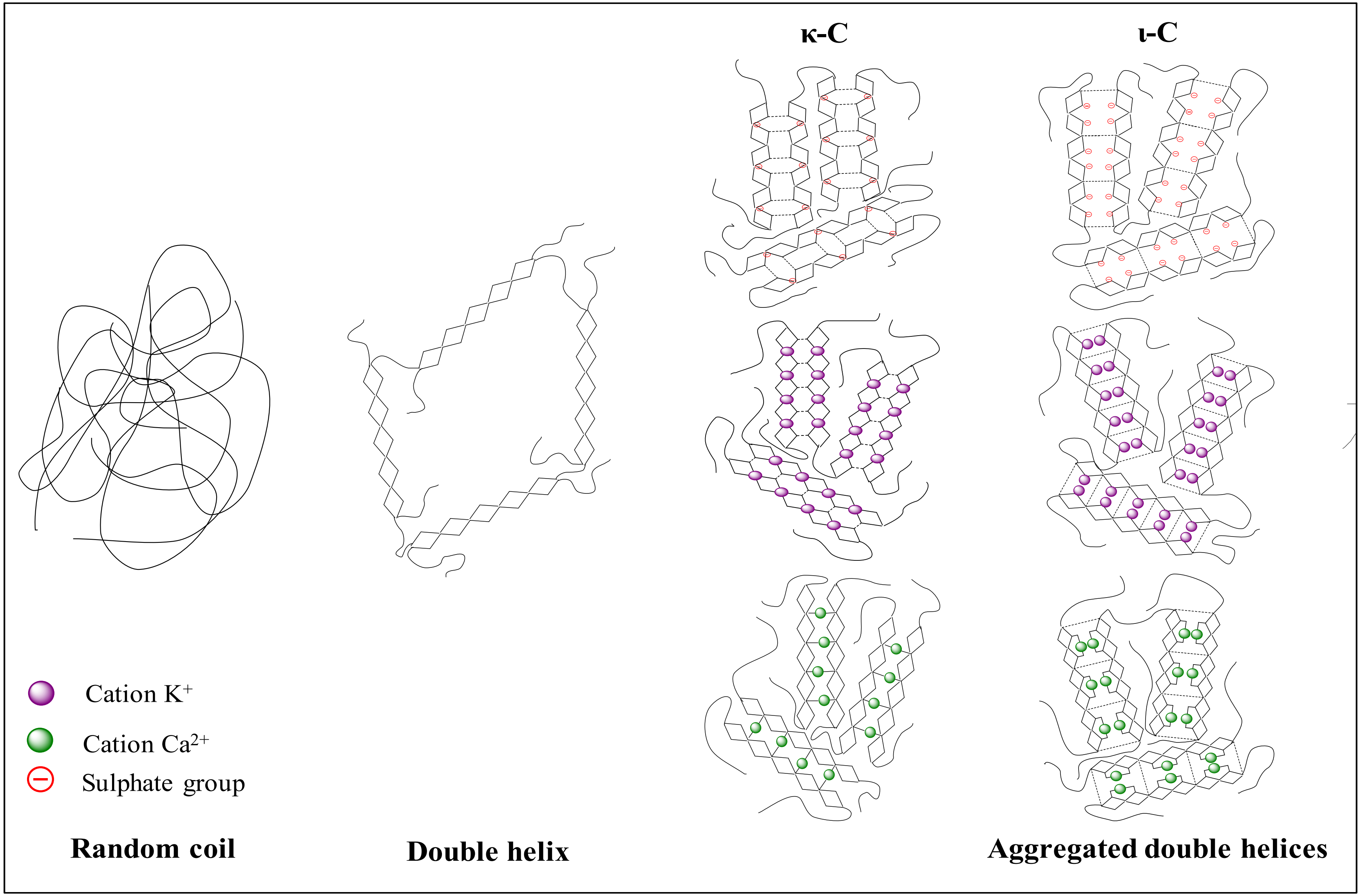
mesh size

double helix size

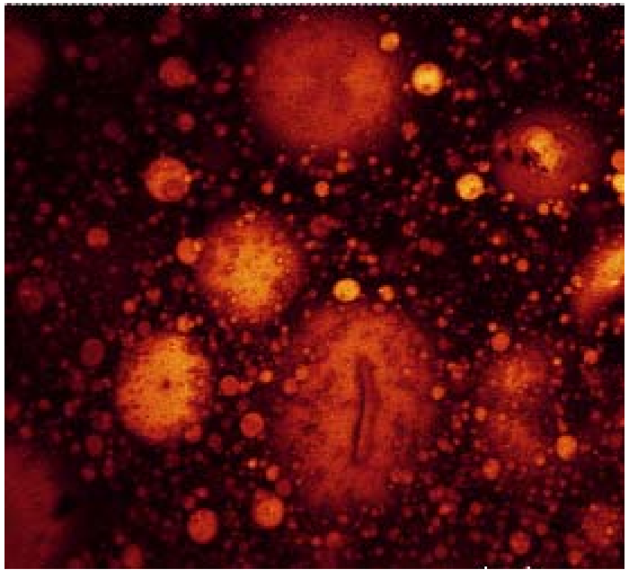
	2% κ-C + 1.5% KCl	2% κ-C + 1.5% $CaCl_2$	2% ι-C + 1.5% KCl	2% ι-C + 1.5% $CaCl_2$
I_G	1236	3091	11123	1910
Ξ (nm)	25.1	41	50.4	35.14
I_L	1314	4888	113	172
ξ (nm)	9.5	17.7	8.5	9.43
I_0	---	---	77	19
d (nm)	---	---	85.4	77.4

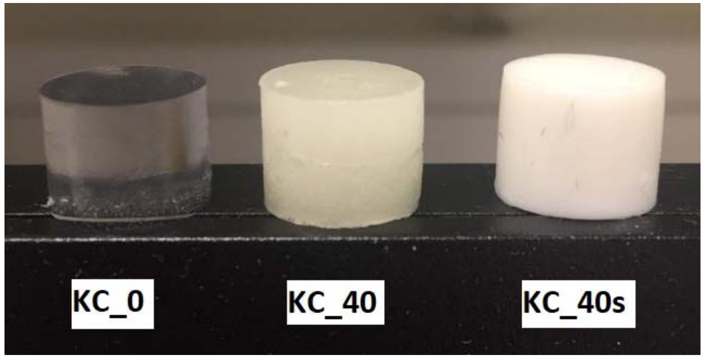
- More compact and ordered κ-C structures with K^+
- More packed ι-C structures with Ca^{2+}
- The nature of the bridges holding together the bundles is different depending on the carrageenan and the valency of the salt

Carrageenan hydrogels



POLYSACCHARIDE-BASED EMULSION GELS



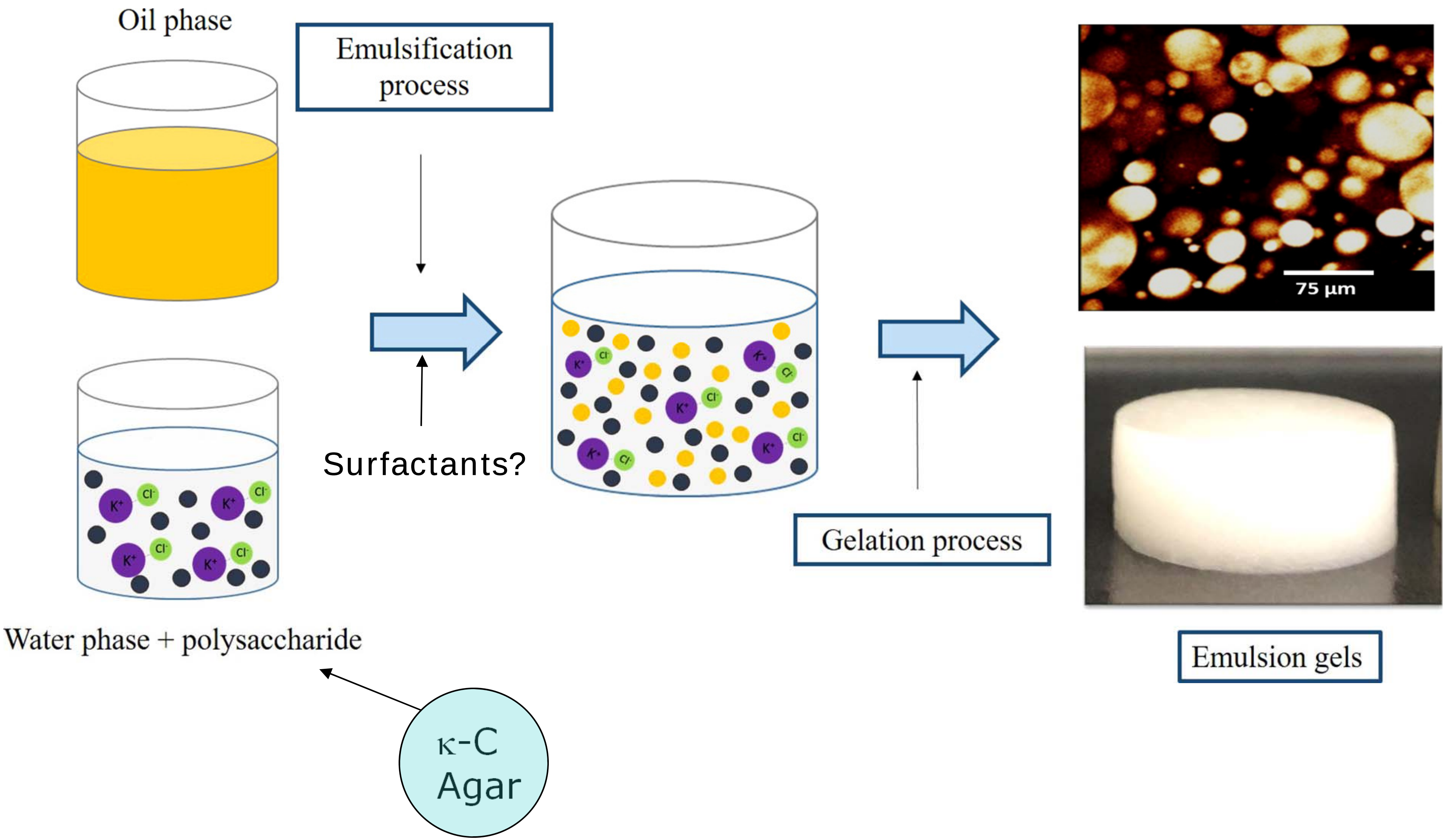


Fontes-Candia et al. *Algal Res.* (2020)

Fontes-Candia et al. *Carb. Polym.* (2021)

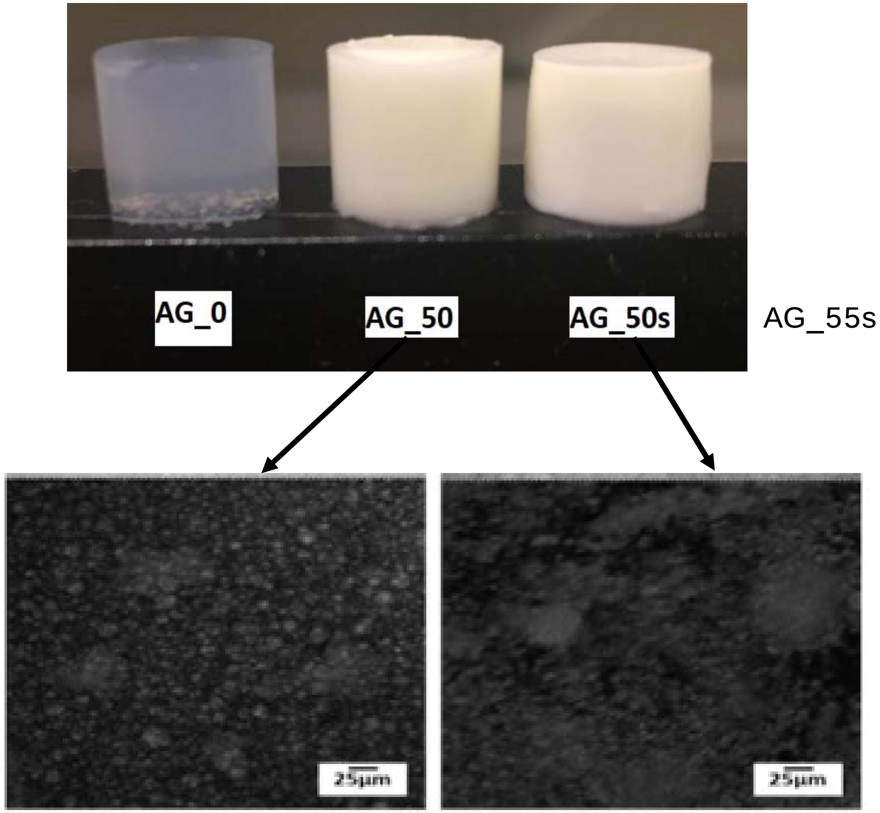
Emulsion gels

30-50 % w/w



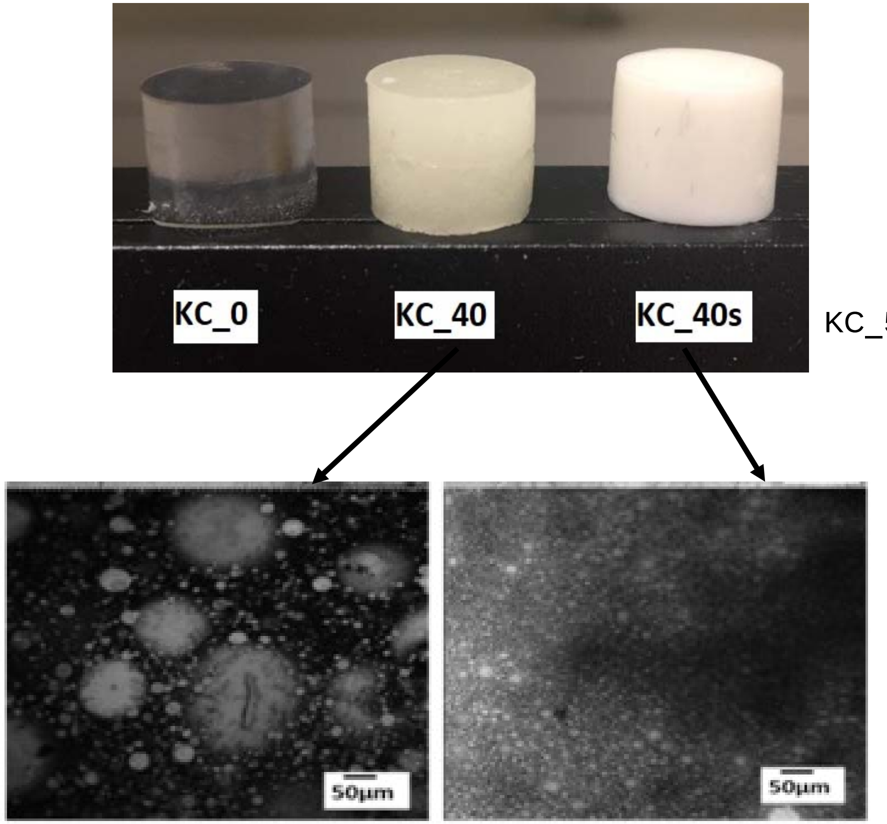
Emulsion gels

AGAR



AG_55s

κ-C

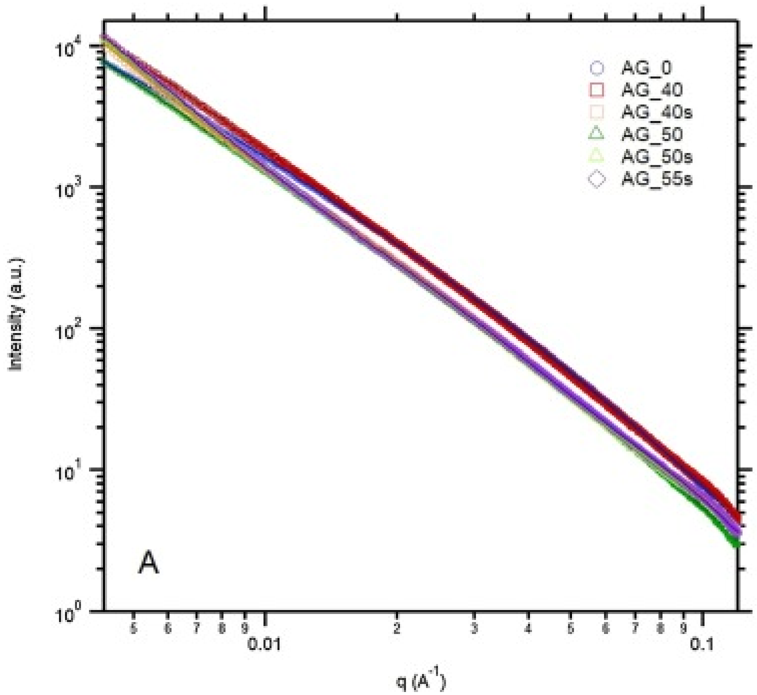


KC_50s

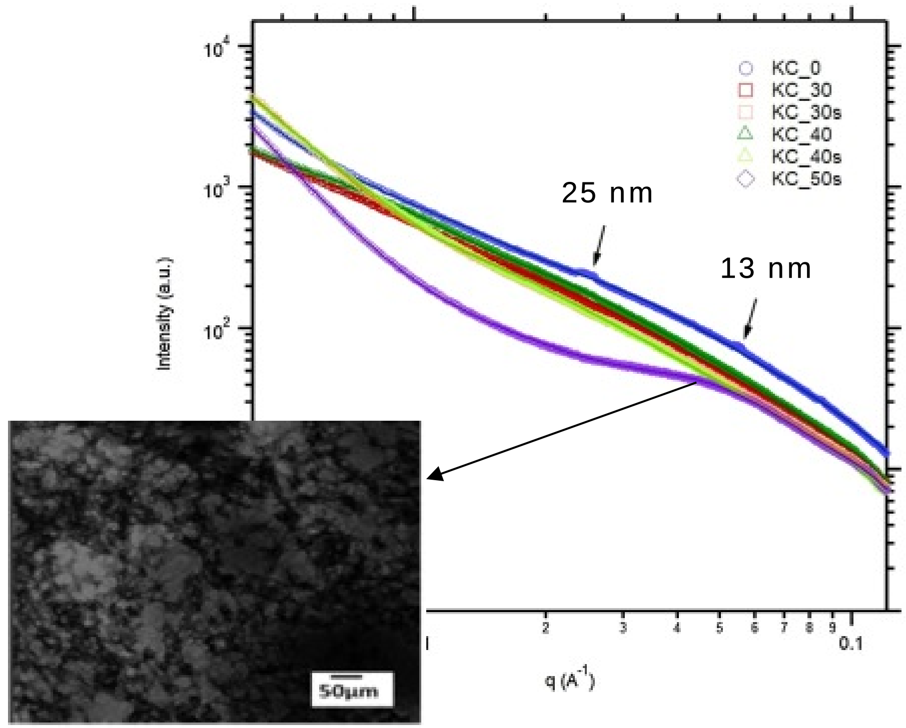
- ➢ Oil droplets distributed within the continuous gelling polysaccharide aqueous phase
- ➢ High **emulsifying properties** of **agar** → smaller and more homogeneous droplets
- ➢ Competition for the **droplet interface** between surfactants and agar → oil **coalescence**
- ➢ Decrease in the viscosity of κ-C with the addition of surfactants → better oil dispersion

Emulsion gels

AGAR



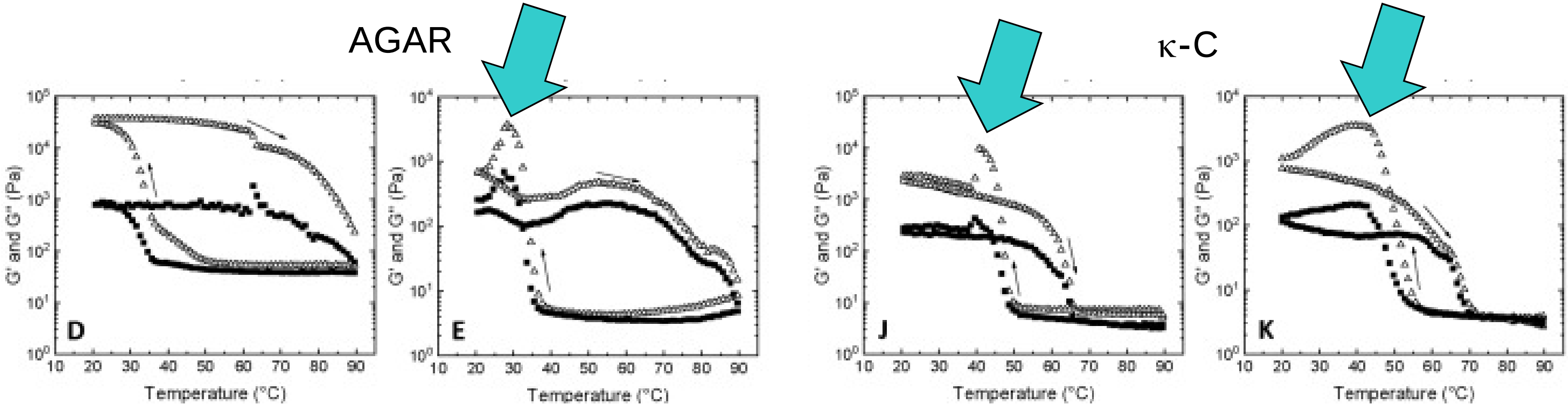
κ-C



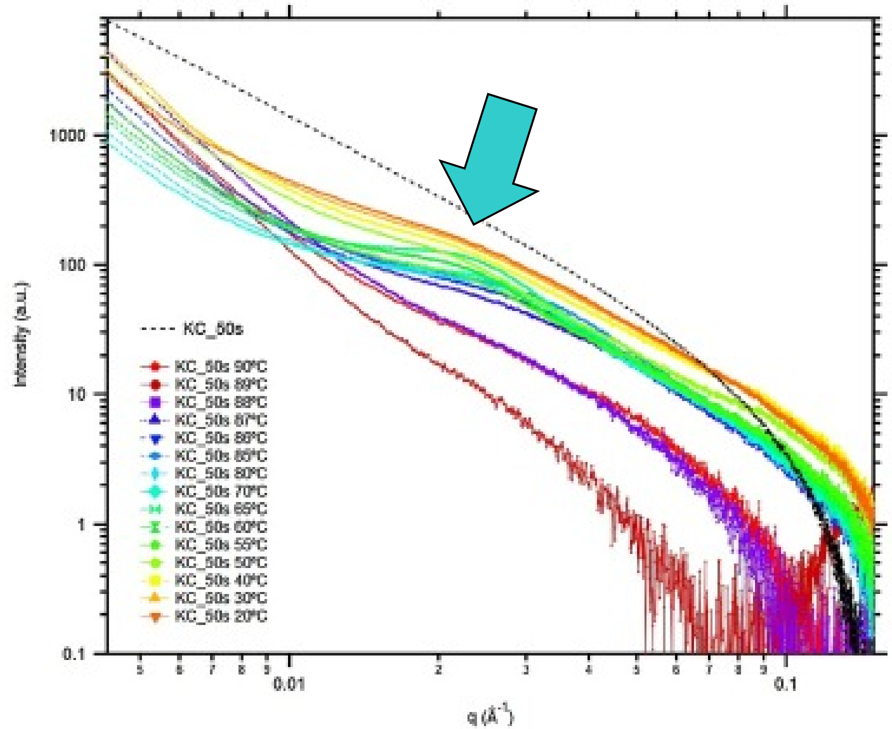
- Branched network structures
- Agar double helices ~ 5–6 nm
- The presence of oil does not have a strong impact on the gel network

- Less compact network structures
- Less packed κ-C bundles with oil
- κ-C double helices ~ 6 nm
- Formation of inverse emulsions?

Emulsion gels



SAXS (cooling ramp)

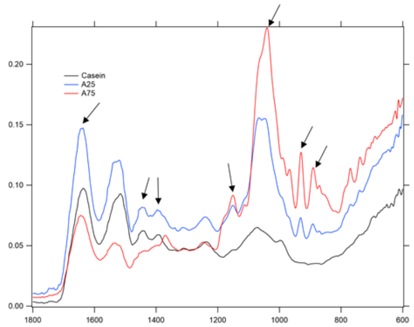


- Two-phase gelling (T_{gel1}~57ºC/T_{gel2}~37ºC) → (i) aqueous domain and (ii) agar located at the surface of the oil droplets
- One-phase gelling with surfactant (surfactant located at droplet surface)
- Formation of **metastable intermediate structure** which is disrupted upon further cooling

Ongoing work

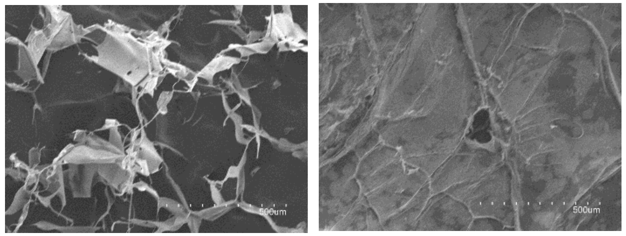
Polysaccharide gels to protect protein products during gastric digestion

Effect of the polysaccharide structure on the protein-polysaccharide interactions



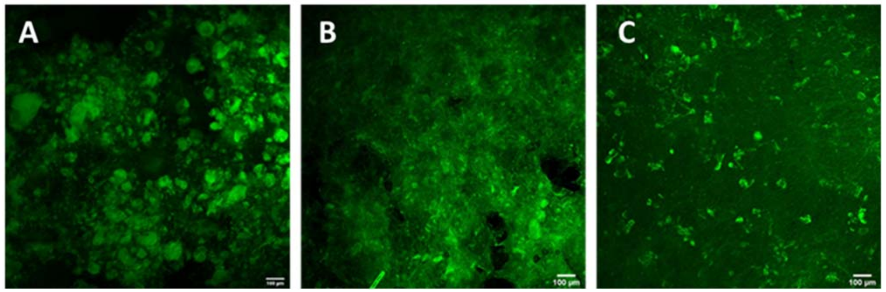
Development of different encapsulation structures

Hydrogels



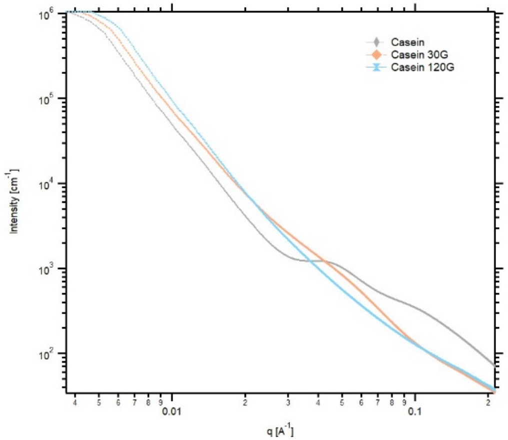
Casein/polysaccharide aerogels

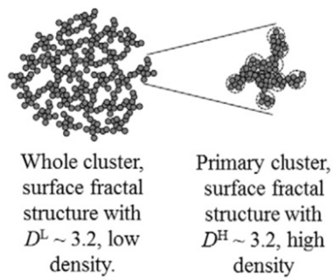
Implications on the digestión process



Casein/agar digestion products

Structural characterization by SAXS / SANS





Conclusions

- Scattering techniques are extremely useful to investigate the structure of food polysaccharides in their **native hydrated state**.
- **X-rays and neutrons are complementary** and their combination can provide a more exhaustive structural characterization.
- **Temperature-resolved** scattering **experiments** can be combined with **rheological characterization** to understand the **gelation mechanism** of polysaccharides.
- **In-situ digestion** experiments will be carried out in the future to understand the structural changes undergone by these polysaccharides upon human consumption.

Acknowledgements

Amparo López-Rubio
Cynthia Fontes-Candia

Anna Ström
Patricia López-Sánchez

Juan Carlos Martínez

Elliot Gilbert
Christine Rehm
Liliana de Campo
Anna Sokolova