POLITECNICO
MILANO 1863

Circular Materials from Proteins

Maurizio Galimberti,
Vincenzina Barbera, Alberto Bottari, Ivana Dragojlov, Elisa Fasoli,
Davide Gentile, Edoardo Testa, Simone Vesentini

Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "G. Natta"

CPAC Rome Workshop 2023
Rome (I), March 20-22, 2023

Outline of the Presentation

☞ Why materials from proteins?

☞ What types of proteins?

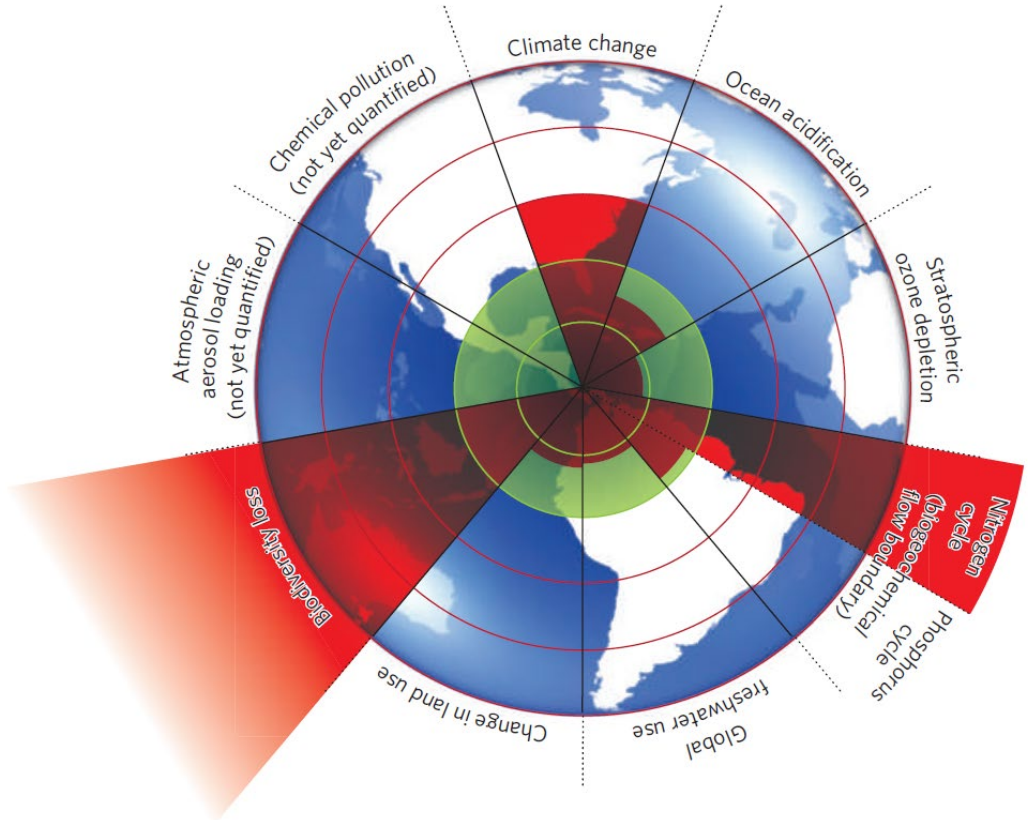
☞ The strategy @ ISCaMaP - Polimi

☞ Proteins for materials

Why materials from proteins?

What types of proteins?

Planetary boundaries that must not be transgressed

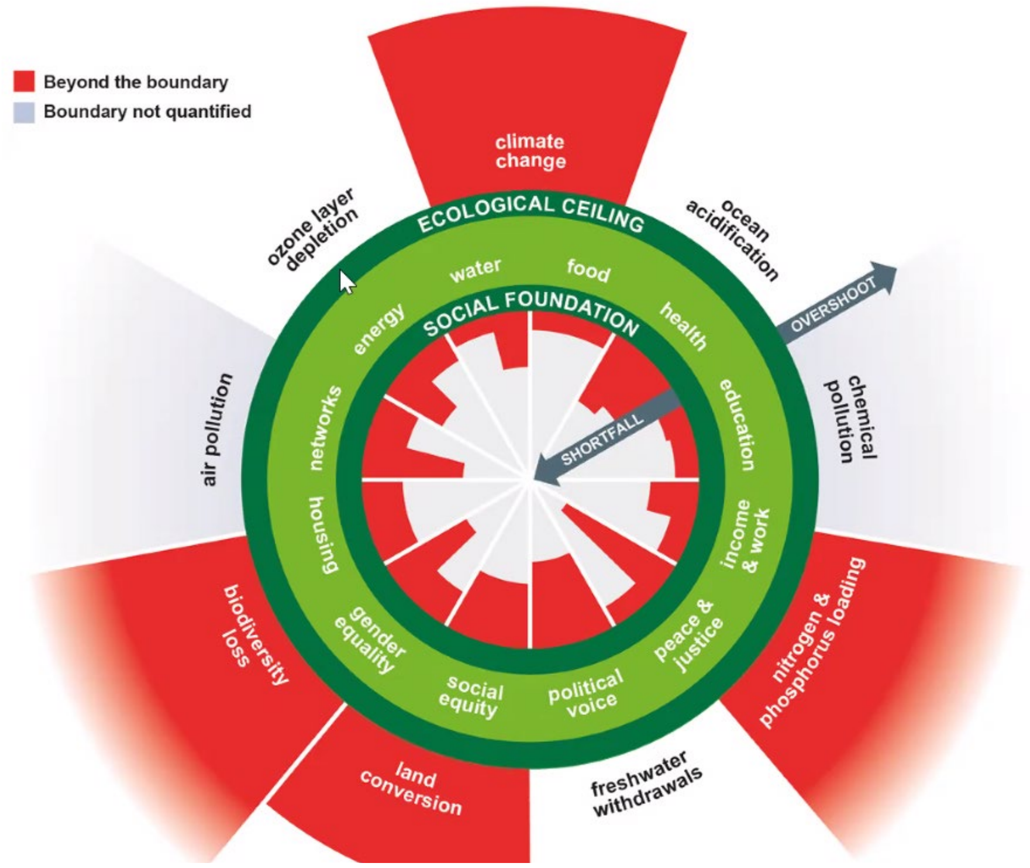


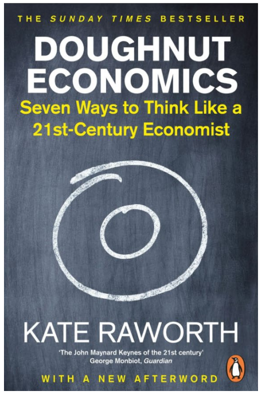
safe operating

space for nine planetary systems

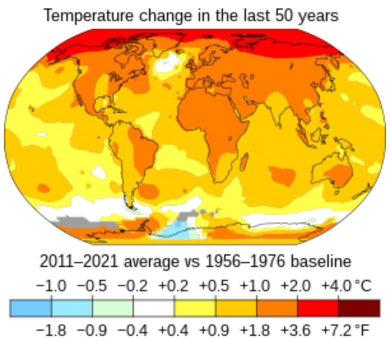
Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F. S., Lambin, E. F., ... & Foley, J. A. (2009). A safe operating space for humanity. *Nature*, *461*(7263), 472-475.

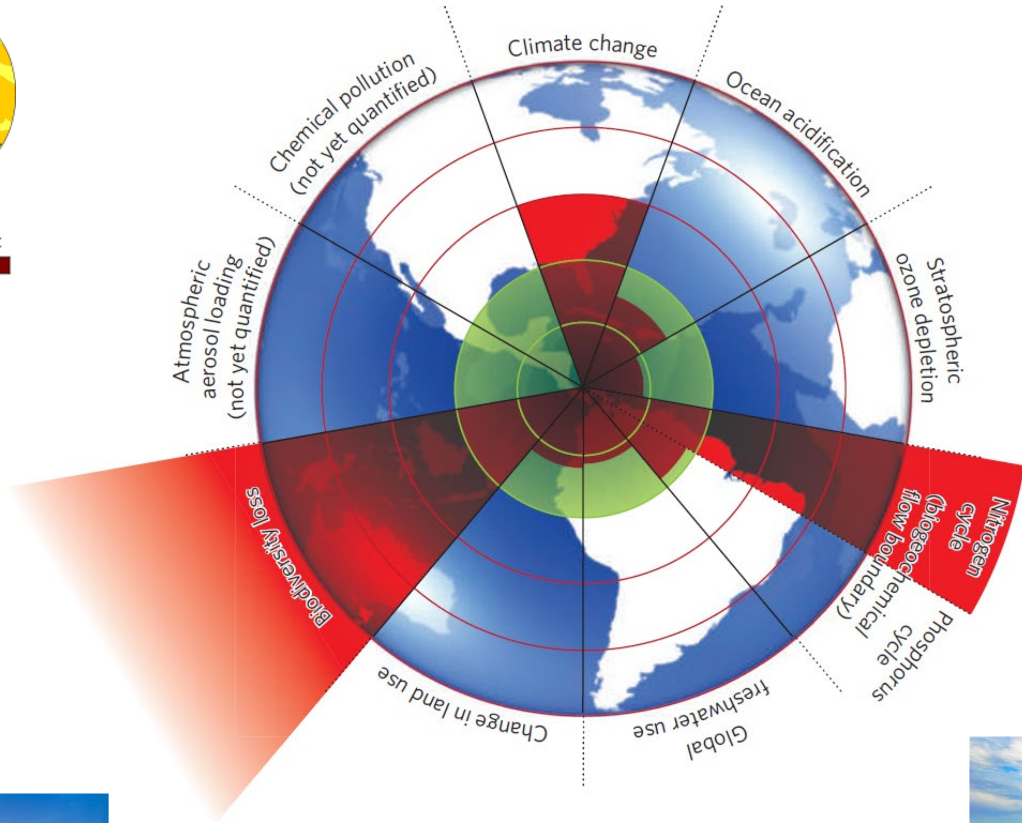
Planetary boundaries and the Doughnut economics





To exploit wastes, to reduce the environmental impact





Proteins from …

Industry sector

Food:	~ 400	Mton	
Textiles and apparels:	~ 0.35	Mton	(sericin and wasted wool)
Microbiology/Pharmacology:	~ 0.02	Mton	

Plants

Wasted soy proteins	~ 150	Mton	(most abundant)

Living organisms (from waste materials, excluding for example meet as food)

Animals

Keratin from feathers	~ 40	Mton	(most abundant)

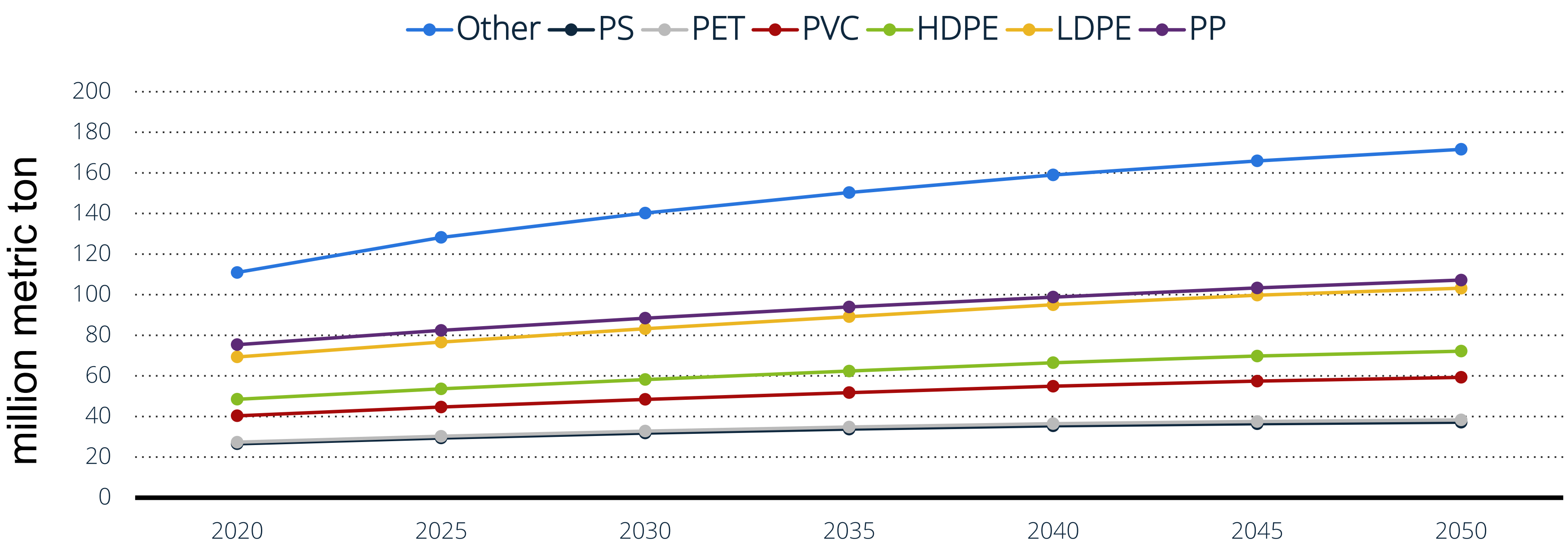
Single cell: micro-algae, Yeasts, Bacteria, Fungi

Algae proteins.	Not available	~ 0.4 Mtons (by 2030, expected)

Insects

From BSF	~ 0.01	Mton	~ 0.5 Mtons (by 2030, expected)

The most important thermoplastic polymers



https://www.statista.com/statistics/1192886/thermoplastics-production-volume-by-type-globally/

Proteins from …

Industry sector

Food:	~ 400	Mton	
Textiles and apparels:	~ 0.35	Mton	(sericin and wasted wool)
Microbiology/Pharmacology:	~ 0.02	Mton	

Plants

wasted soy proteins	~ 150	Mton	(most abundant)

Living organisms (from waste materials, excluding for example meet as food)

Animals

Keratin from feathers	~ 40	Mton	(most abundant)

Single cell: Micro-algae, Yeasts, Bacteria, Fungi

Algae proteins.	Not available	~ 0.4 Mtons (by 2030, expected)

Insects

From BSF	~ 0.01	Mton	~ 0.5 Mtons (by 2030, expected)

Proteins from food. The negative impacts of the Food Industry

Intensive production of animal food accounts for 12% of total GHG

Intensive plant production strongly and negatively impacts on N and P cycles

Both industries abuse of fresh water, lands and contribute in their contamination

The food cycle: strongly inefficient

1.3 Billion Tonn of waste from the food cycle are discarded every year

30% of all food produced

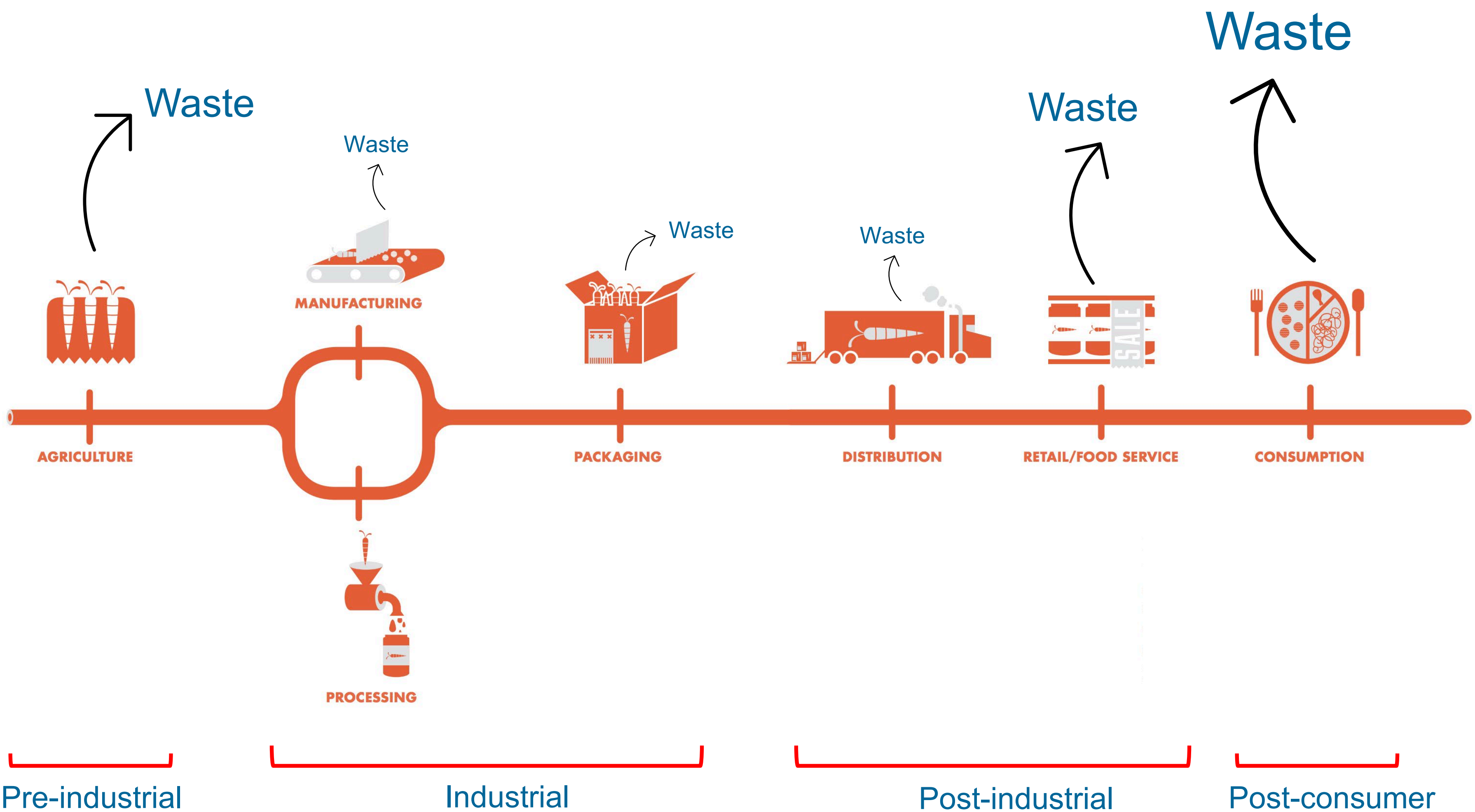
↓

8−10 % of global greenhouse gas emissions (GHG)

https://www.theguardian.com/environment/2016/jul/14/from-field-to-fork-the-six-stages-of-wasting-food

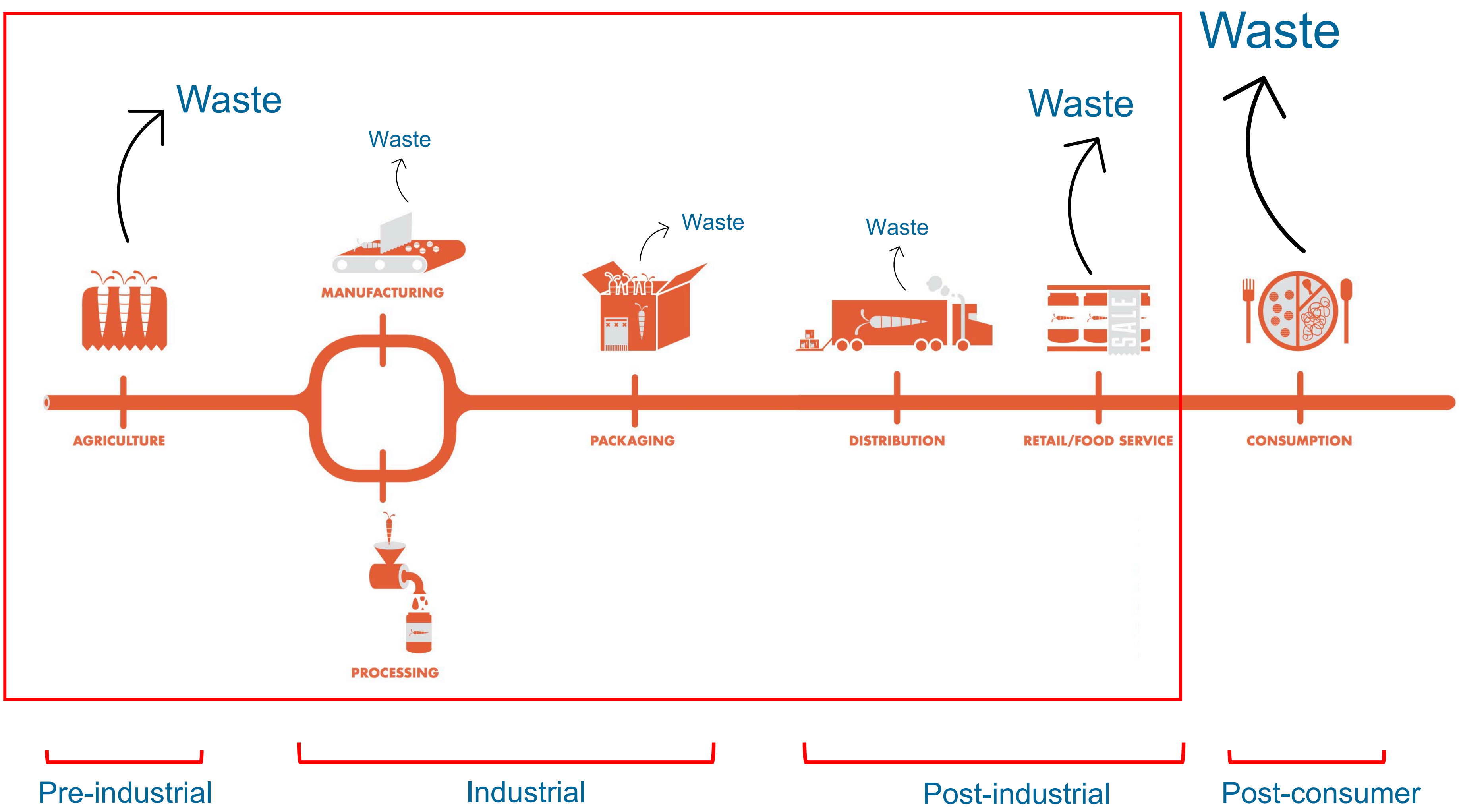
https://climate.nasa.gov/effects/

Where do food wastes come from?



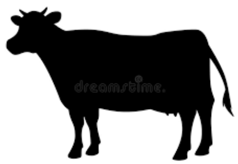
https://www.canr.msu.edu/news/modeling-an-equitable-michigan-food-system

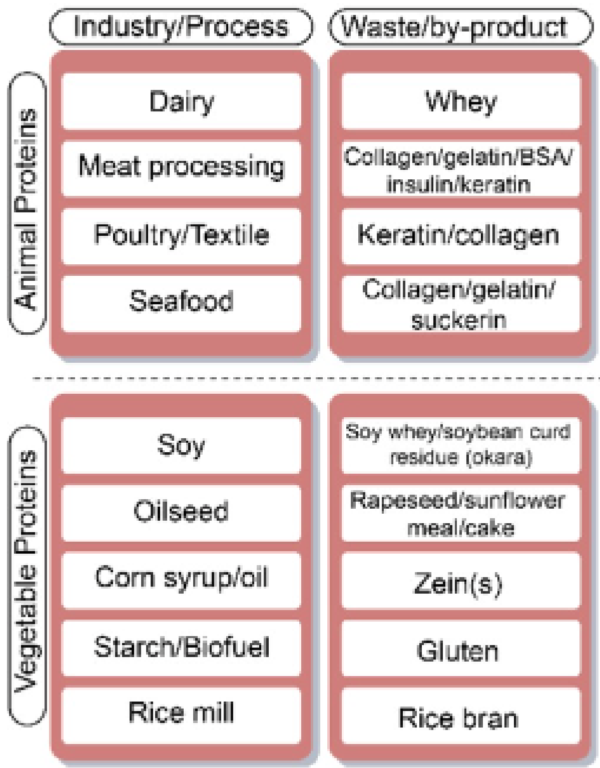
Where do food wastes come from?



https://www.canr.msu.edu/news/modeling-an-equitable-michigan-food-system

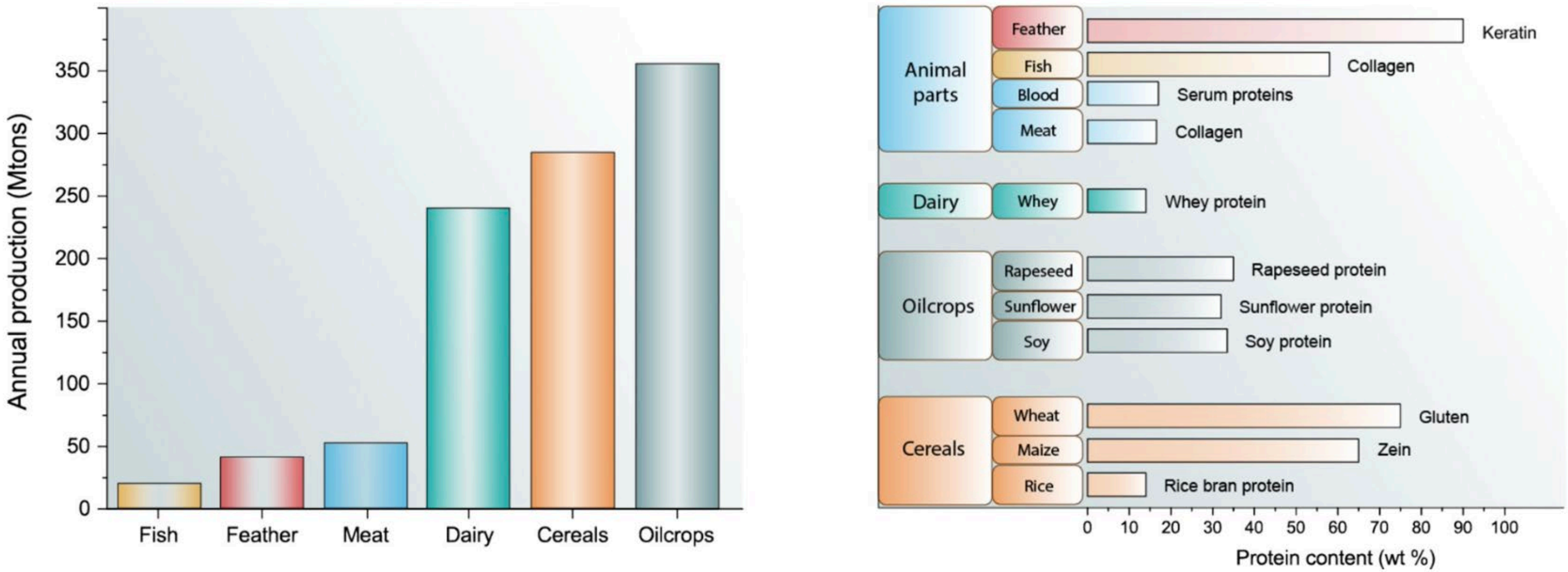
Proteins from the pre-customer food waste





Chem. Rev. 2023, 123, 2112–2154

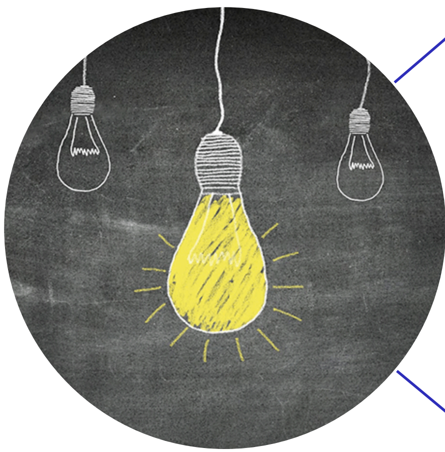
Protein-rich waste sources from the food industry



The Food Industry is the major producer of protein-rich waste sources

Chem. Rev. 2023, 123, 2112−2154

What to do with the food waste?

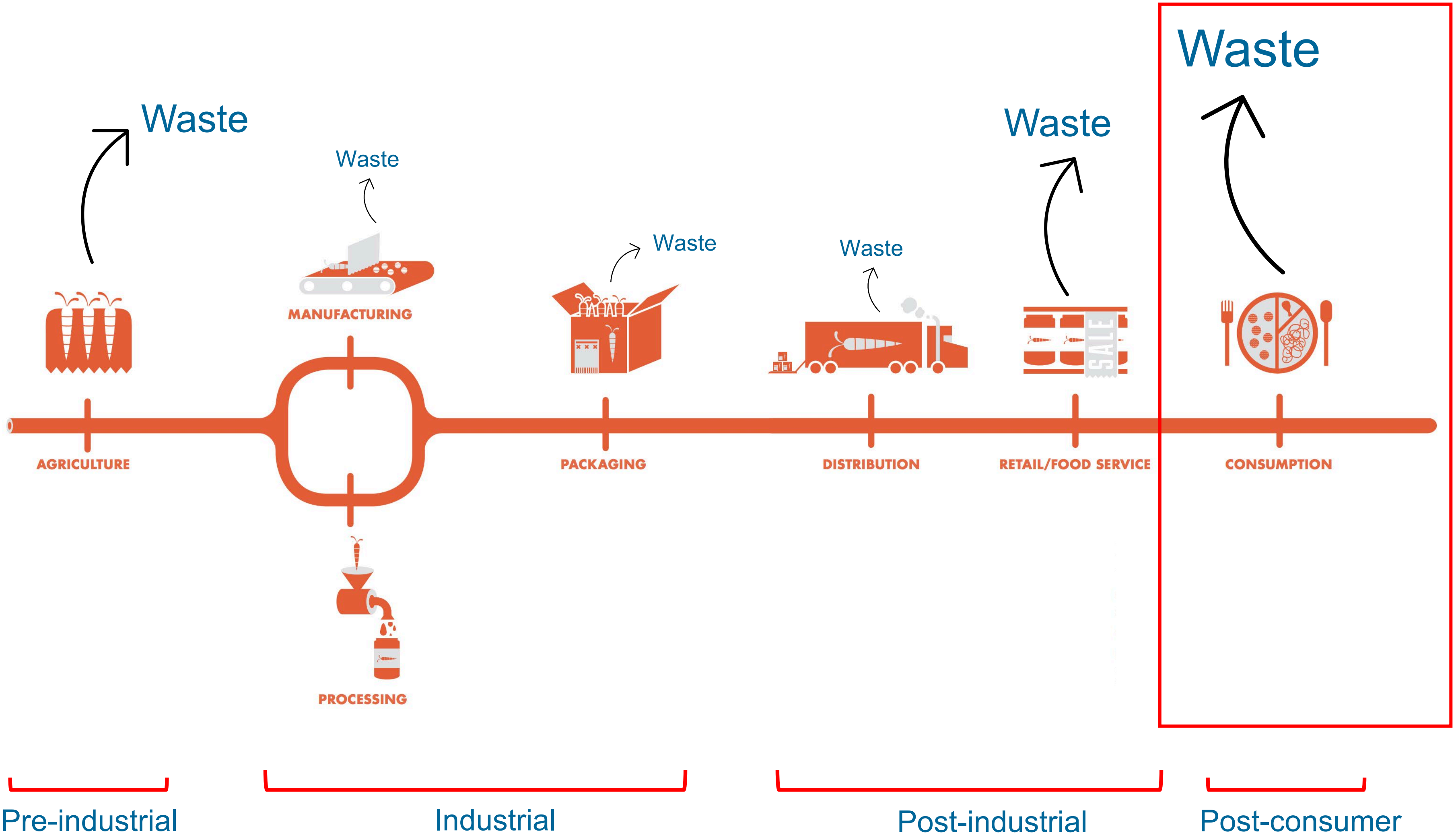


Eliminating the Food Cycle
- Impossible

Revaluating food waste
- Re-entering proteins from food wastes in the food cycle

Upcycling food waste
- Substituting synthetic plastics with protein-based bioplastics

Where do food wastes come from?



https://www.canr.msu.edu/news/modeling-an-equitable-michigan-food-system

Increasing amount of waste is produced on the planet

Yearly production of municipal solid waste (MSW):
estimated to achieve 2.5 billion tonn.

More than one third: food and kitchen waste:
so-called organic fraction of municipal solid waste: OFMSW.

This amount is expected to increase by 70% within 2050,
both in high-income and low-income countries

FAO. Global food losses and food waste - Extent, causes and prevention. Food and agriculture organization of the United Nations, Rome, 1-37, 2011. http://www.fao.org/3/a-i2697e.pdf,
Wilson D.C., Rodic L., Modak P., Soos R., Carpintero Rogero A., Velis C., Iyer M., Simonett O. Global waste management outlook. Report. United Nations Environment Programme, 1-346]

[https://www.epa.gov/sustainability. Access 2020.02.27].

Organic **F**raction of **M**unicipal **S**olid **W**aste

Organic **F**raction of **M**unicipal **S**olid **W**aste
(**OFMSW**)

To give value to OFMSW. Proteins from insects

Black Soldier Fly

Organic **F**raction of **M**unicipal **S**olid **W**aste (**OFMSW**)

↓

Insects proteins

To give value to OFMSW

☞ to feed larvae of insects such as the black soldier fly with OFMSW

Reduction of waste volume and production of protein-rich biomass.

European Commission Regulation No 2017/893:
allowed a partial use of the processed animal proteins from insects.

BSF larvae are a source of chitin, lipids, and antimicrobial peptides

Proteins and Materials obtained without using soil and water

BSF-based bioconversion
is considered as one of the most promising technologies
for organic waste processing and valorization

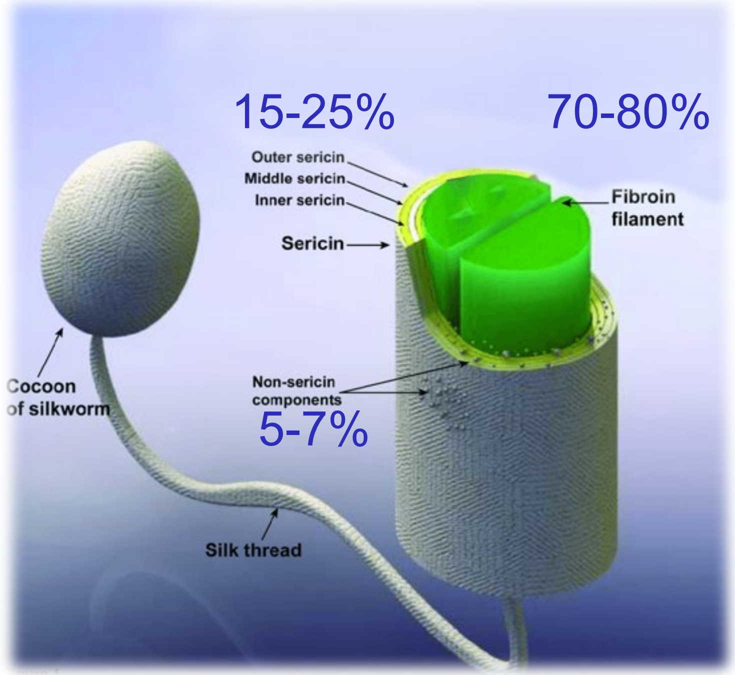
Gold M., Tomberlin J.K., Diener S., Zurbrügg C., Mathys A. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review. Waste Management, 82: 302-318, 2018. https://doi.org/10.1016/j.wasman.2018.10.022].

Proteins from textiles and apparels. The environmental impact

Fibroin

and

Sericin



400,000 tons of dry cocoons produced worldwide

↓

50,000 tons of sericin discarded in the water system

↓

Environmental problems

The strategy @ ISCaMaP - Polimi

ISCaMaP

Innovative Sustainable Chemistry and Materials and Proteins Group

Natural sources

Wastes and residues

↓

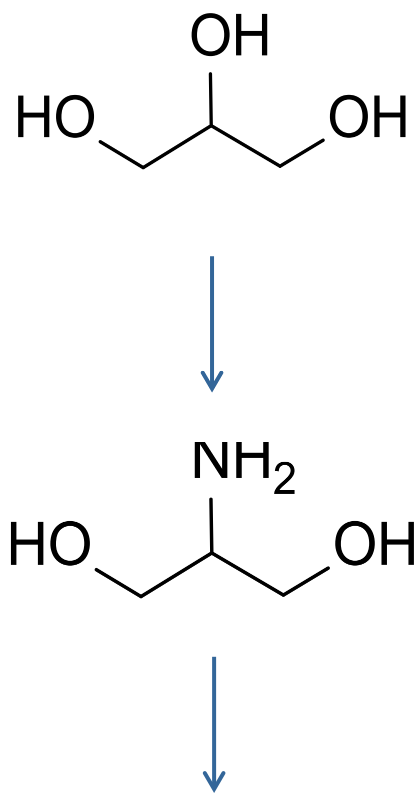
Building blocks
for chemical platforms

↓

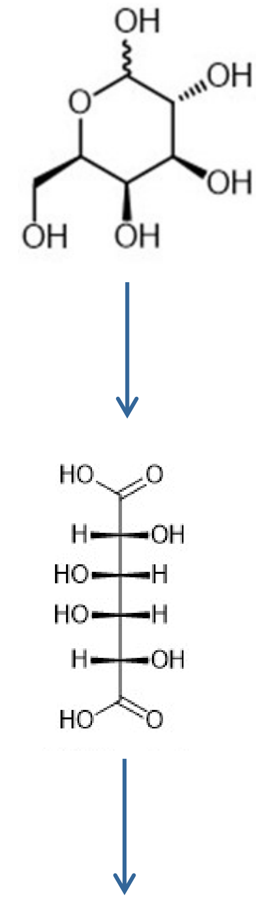
Chemicals and Materials

The ISCaMaP Group. Some examples

C3 and C6 building blocks

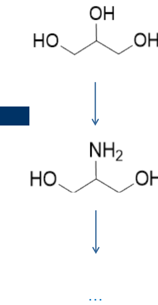


...



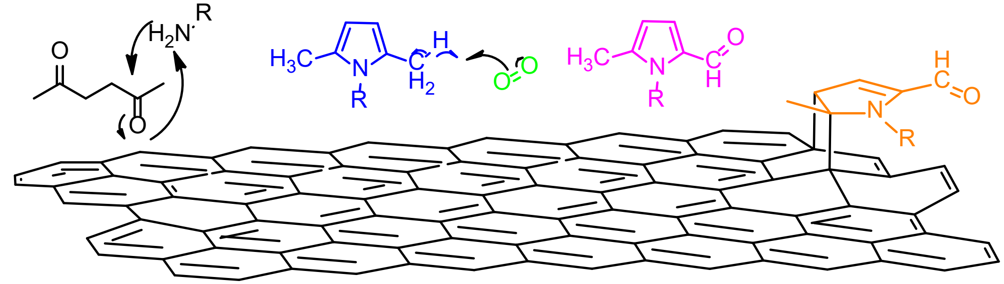
...

Reaction of the pyrrole compound with an sp² carbon allotrope



A + B / Carbon allotrope

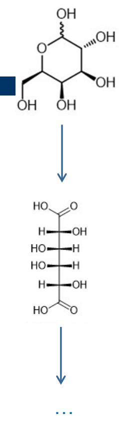
Functionalized carbon allotropes

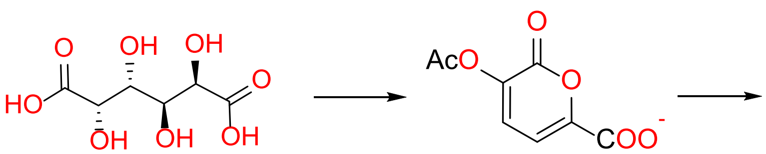


Rubber composites for tyres

V. Barbera, A. Citterio, M. Galimberti, G. Leonardi, R. Sebastiano, S.U. Shisodia, A.M. Valerio. *US10329253B2*
M. Galimberti, V. Barbera, R. Sebastiano, A. Citterio, G. Leonardi, A.M. Valerio. *US10160652B2*
M. Galimberti, V. Barbera, R. Sebastiano, A. Truscello, A.M. Valerio. *EP3180379B1*
M. Galimberti, V. Barbera, *EP3538511A1*
M. Galimberti, V. Barbera, *EP3538481A1*

Synthesis of Pyrone Derivatives from Aldaric Acids @ ISCaMaP





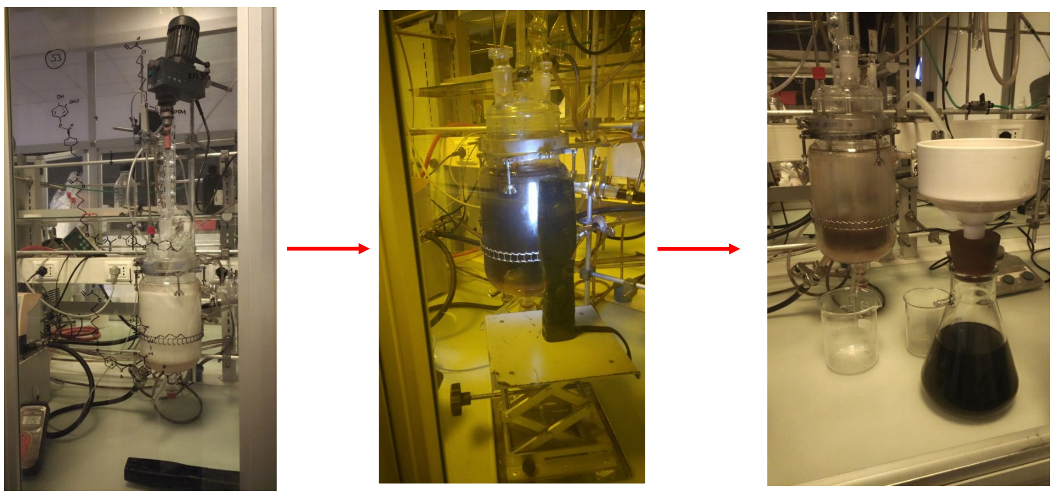
Galactaric acid

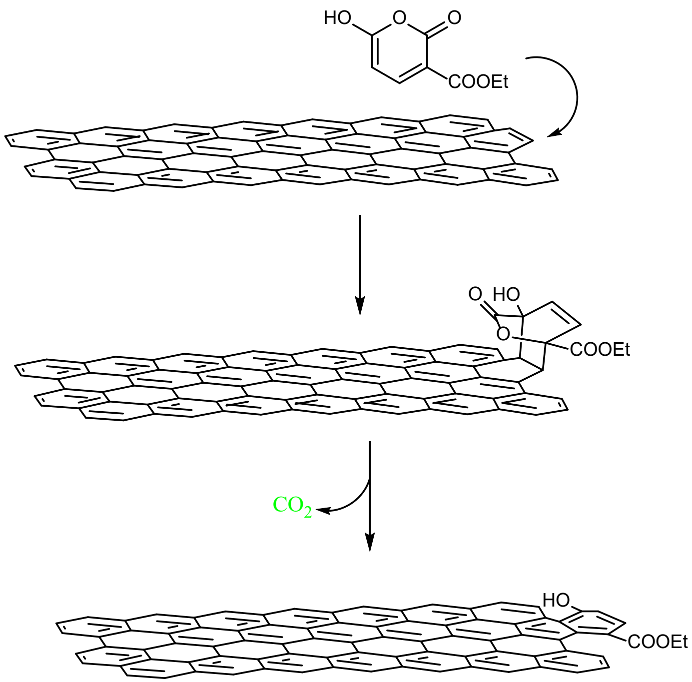
GAB-OAc

DEC

Yield
up to 73%

Yield
up to 90%

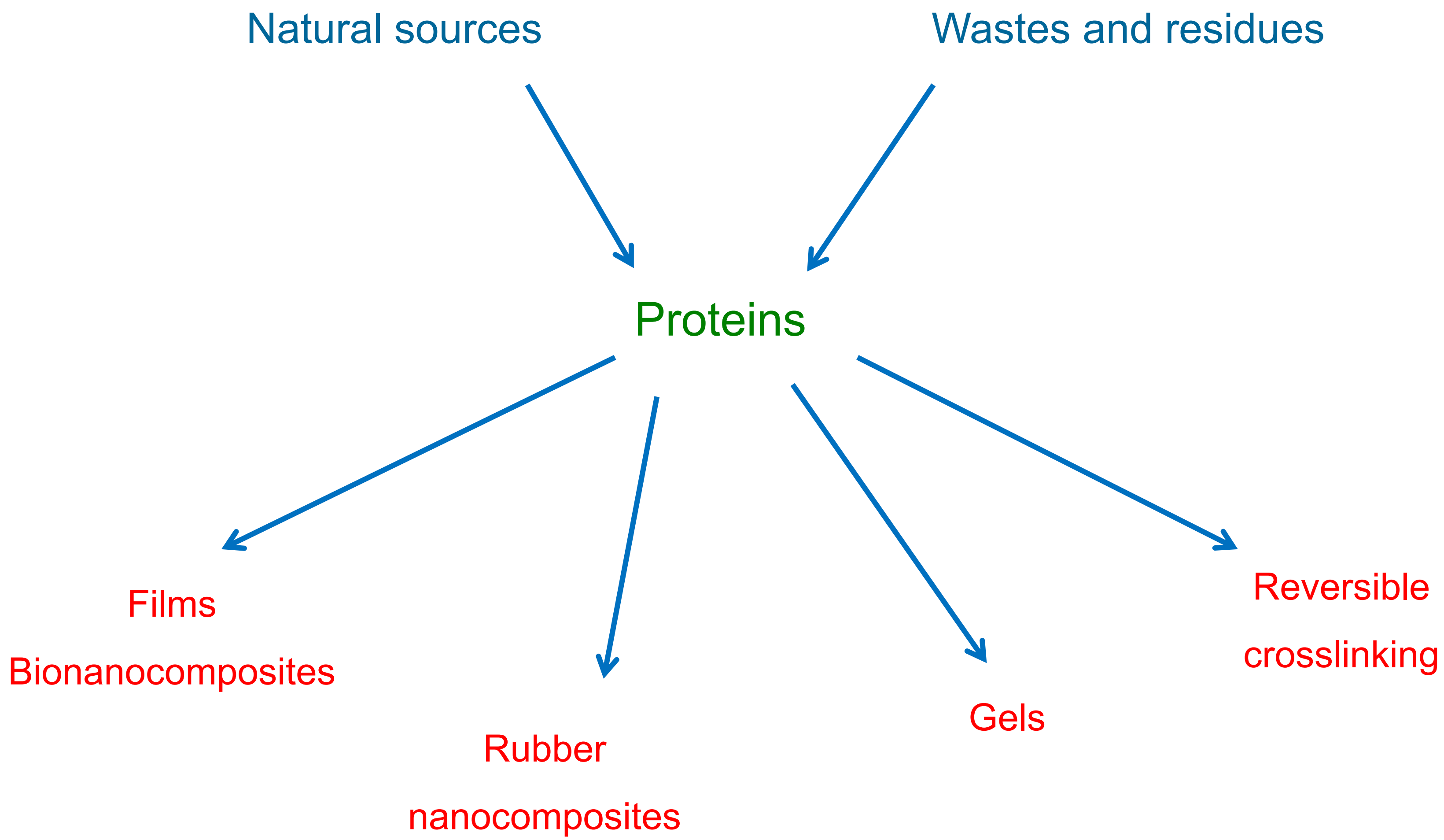




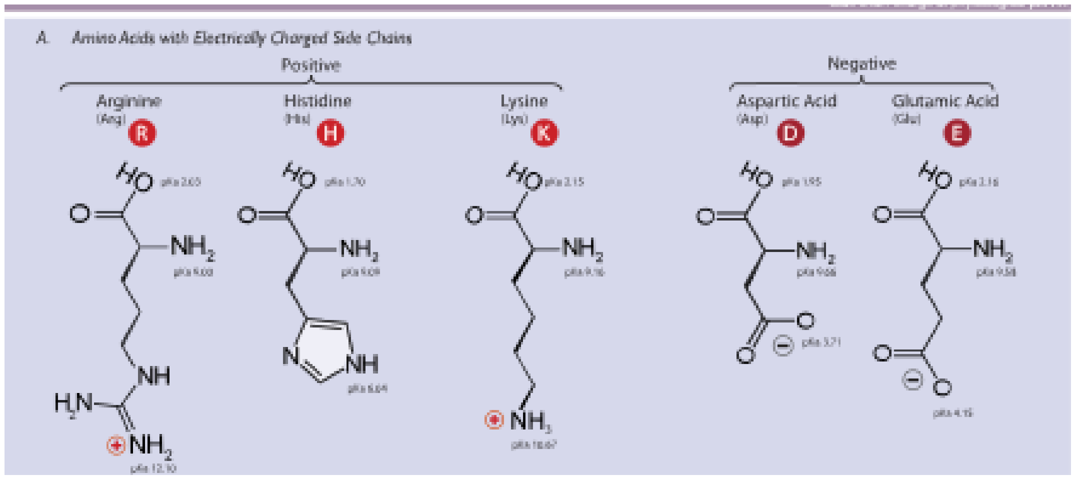
One Pot, 2 hours, Yield = 75%

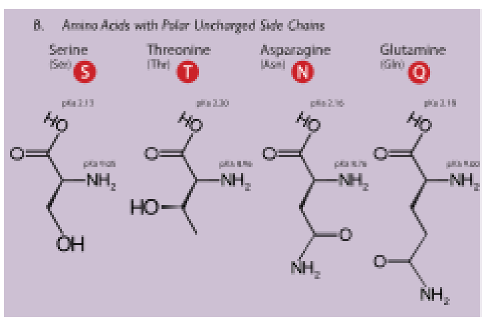
Anti-oxidants

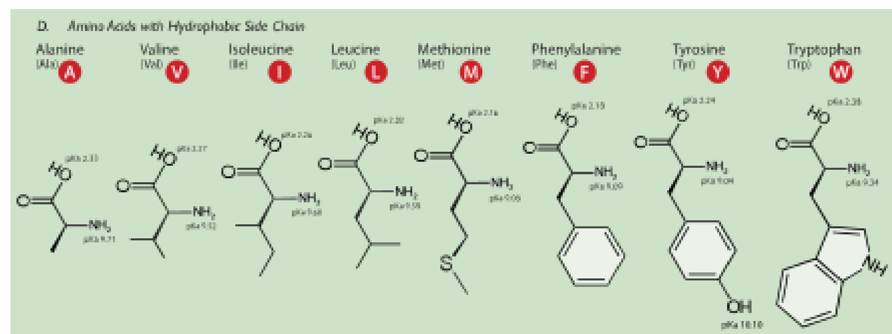
Catalysis for degradation of recalcitrant pollutants

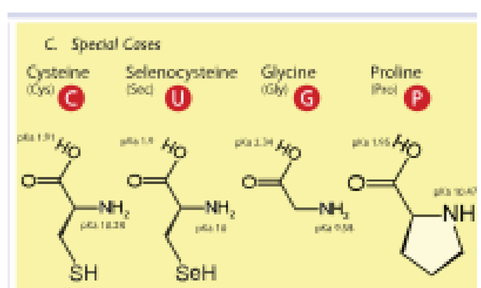

Natural sources
Wastes and residues
Proteins
Films
Bionanocomposites
Rubber
nanocomposites
Gels
Reversible
crosslinking

21 proteinogenic aminoacids



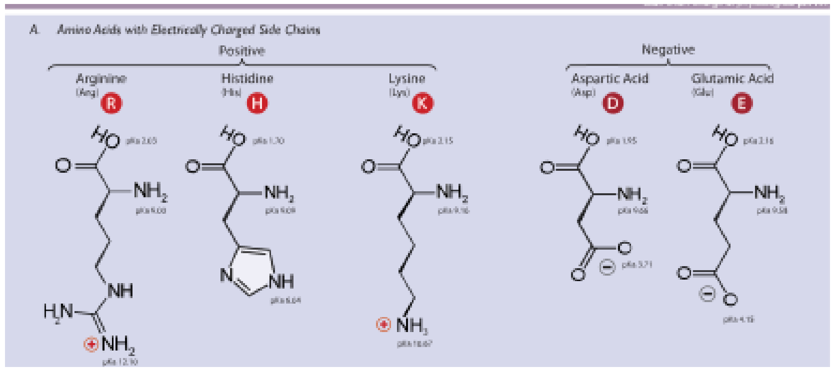


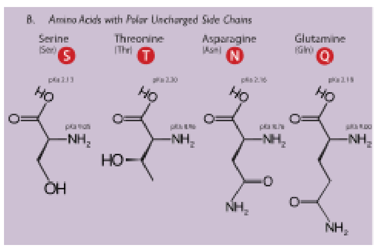


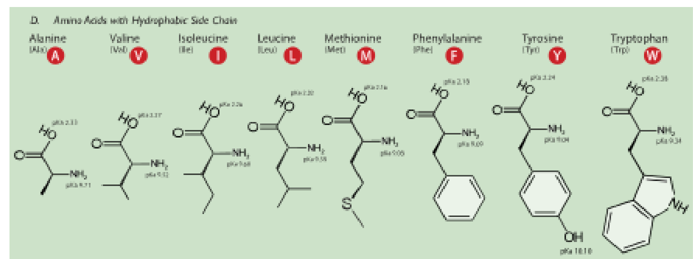


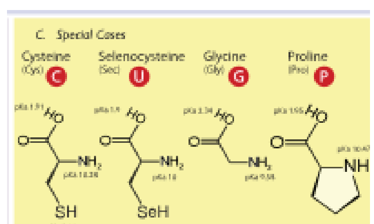
At physiologycal pH = 7.4

21 proteinogenic aminoacids









Circular materials. CRM: back to monomers

Research on Proteins at *ISCaMaP*

Natural sources → Proteins

Wastes and residues → Proteins

Proteins

- Characterization
- Chemical modification
- Preparation of nanocomposites
- Chemical recycling to monomers

Proteins from insects for films and bionanocomposites

RICH

Turning **R**ubbish **I**nto biobased materials: a sustainable **CH**ain for the full valorization of organic waste

Tettamanti Gianluca, Molla Gianluca, Pollegioni Loredano, Morena Casartelli, Daniele Bruno, Marco Bonelli, Ulrich Giese

Proteins from BSF for films and bionanocomposites

Hermetia illucens
(**BSF**)

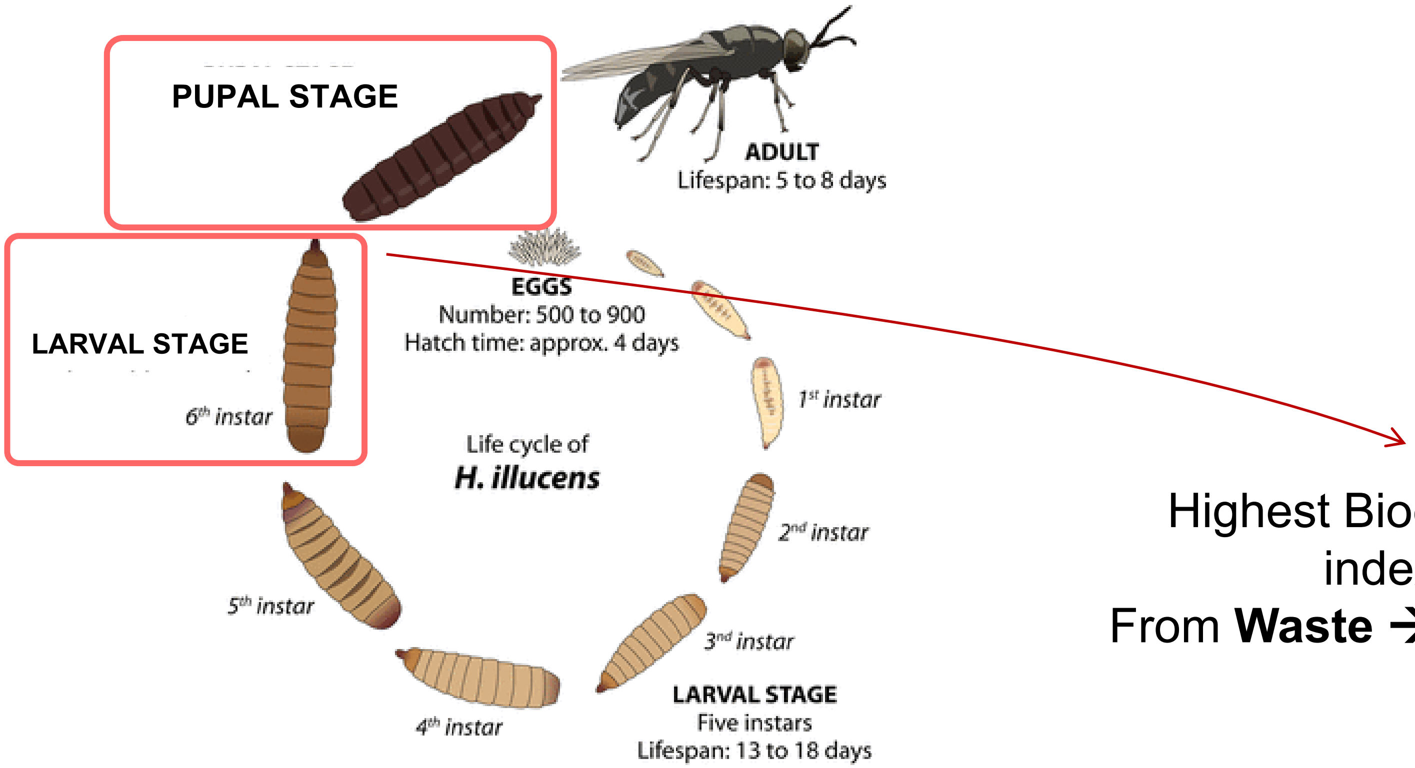
Organic **F**raction of **M**unicipal **S**olid **W**aste
(**OFMSW**)

Proteins

Project

Protein-based
bioplastics

Proteins from BSF for films and bionanocomposites



Highest Bioconversion indexes
From **Waste** → To **Proteins**

Polymers (Basel)., vol. 11, no. 2, 2019; *J. Sci. Food Agric.*, vol. 101(11) 4506–4513, 2021,; *Environ. Eng. Manag. J.*, 18(10), 2123–2131, 2019; *Agronomy*, 10 (7), 1–10, 2020; *Waste and Biomass Valorization*, (12) 9, 5121–5130, 2021; *Food Res. Int.*, 115, 116–125, 2019.

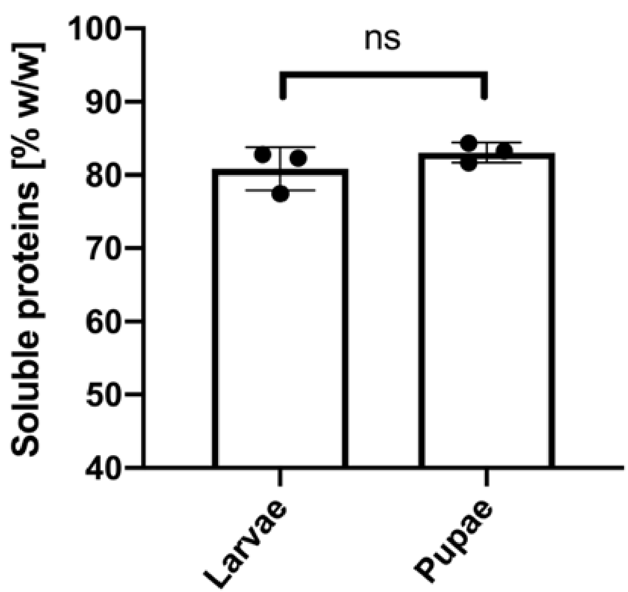
Proteins from BSF. Characterization

BCA

TNBSA assay

SDS-PAGE

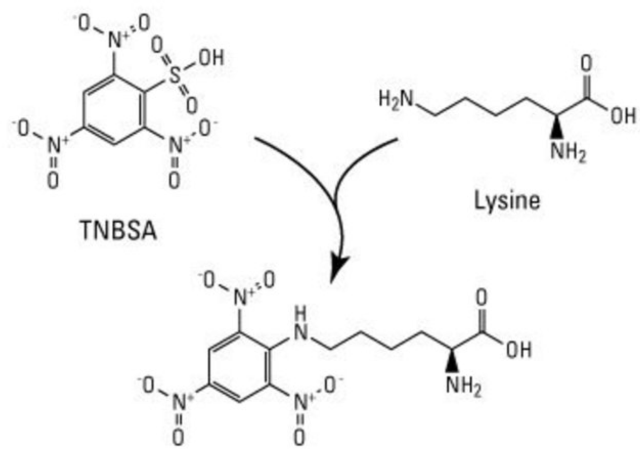
Nano LC-MS/MS



	Larvae	Pupae
Mean [%]	80,84	83,05
SD [%]	2,93	1,36

Proteins from BSF. Characterization

BCA | **TNBSA assay** | SDS-PAGE | Nano LC-MS/MS



Primary amine titration

$$M_n \propto \frac{1}{\mathrm{NH_2}}$$

	NH_2 [mmol/g]				
	200 µg/mL	100 µg/mL	50 µg/mL	**Mean**	**SD**
Larvae extracts	0,45	0,47	0,48	0,47	±0,02
Pupae extracts	0,35	0,34	0,30	0,33	±0,01

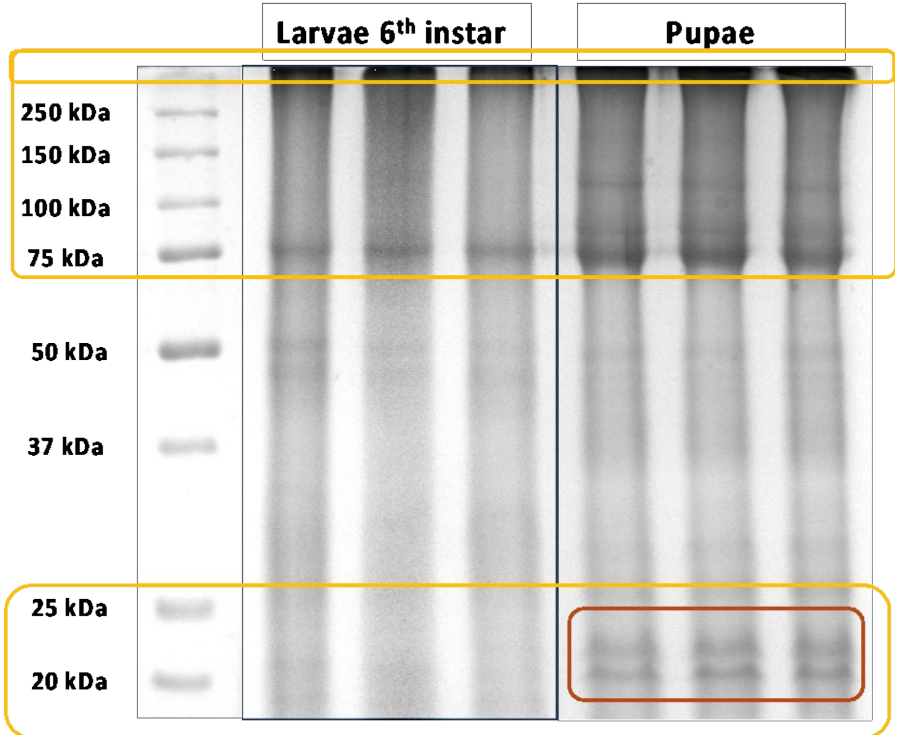
Proteins from BSF. Characterization

BCA

TNBSA assay

SDS-PAGE

Nano LC-MS/MS



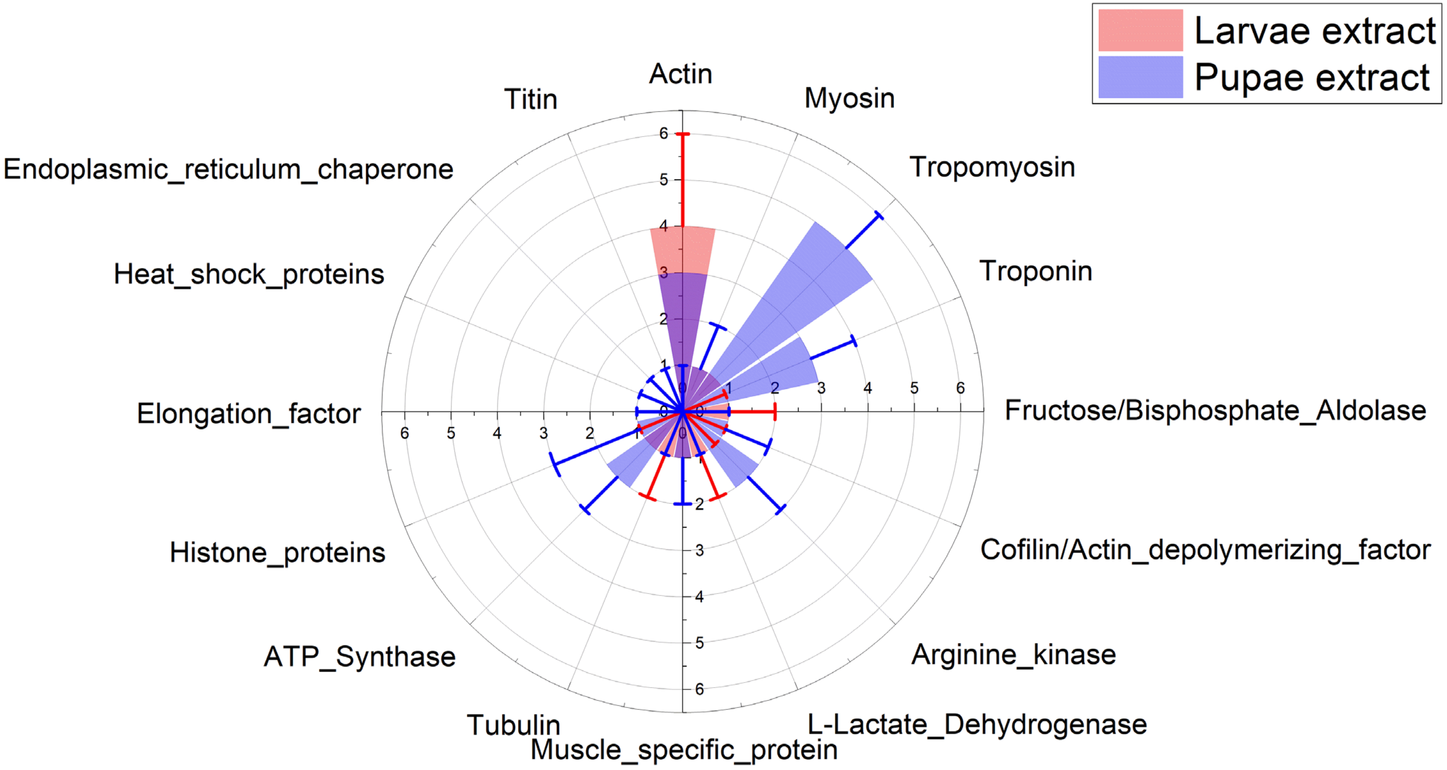
Proteins from BSF. Characterization

BCA

TNBSA assay

SDS-PAGE

Nano LC-MS/MS



Proteins from BSF. Film preparation

Protein denaturation

NaOH + 80°C, 20 min

→

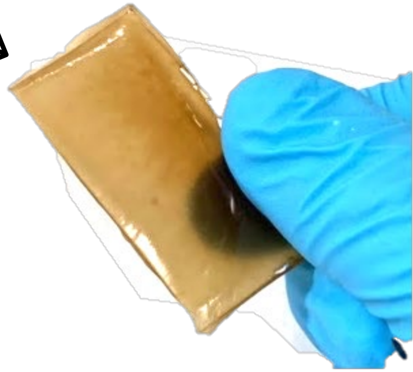
Addition of **glycerol**

33% wt.

→

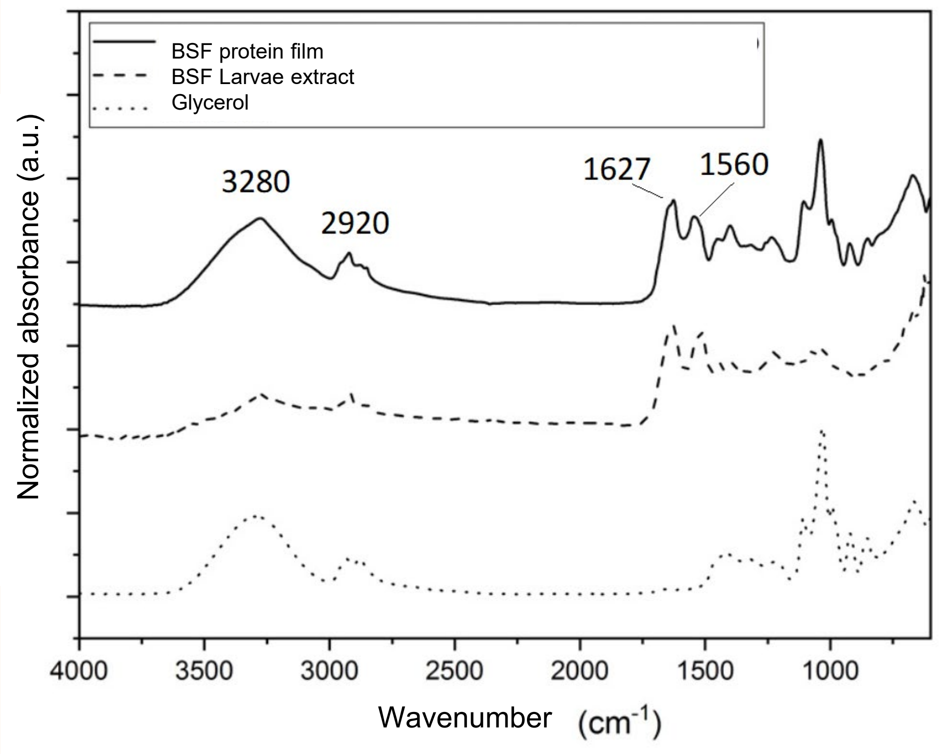
Solvent casting

R.T., 48h



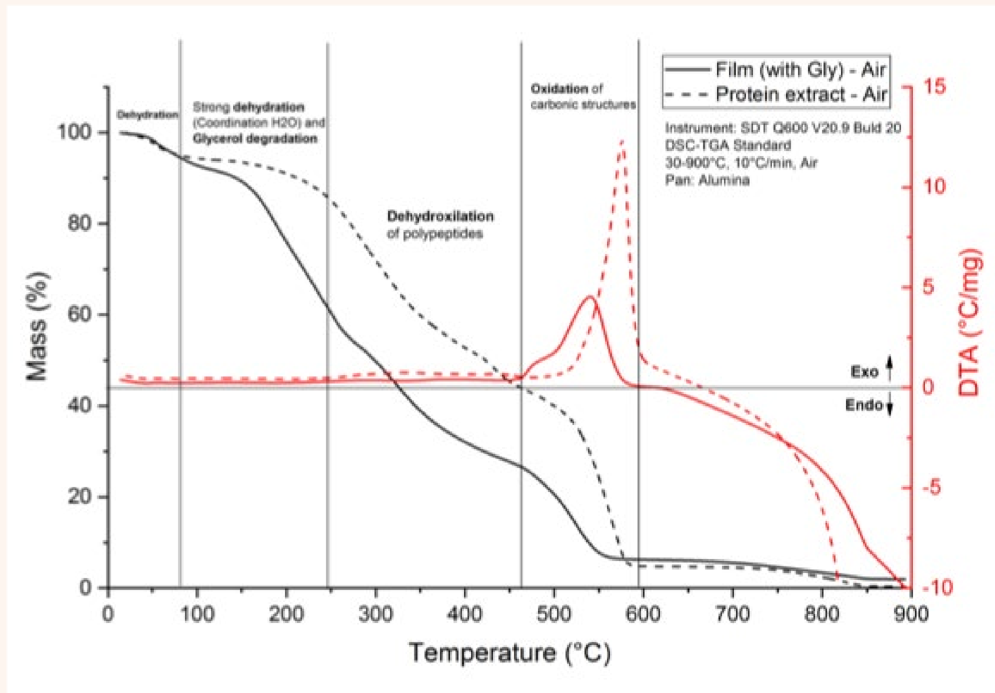
Proteins from BSF. Film characterization

Chemical groups: ATR FT-IR profiles: 4000-600 cm^{-1}



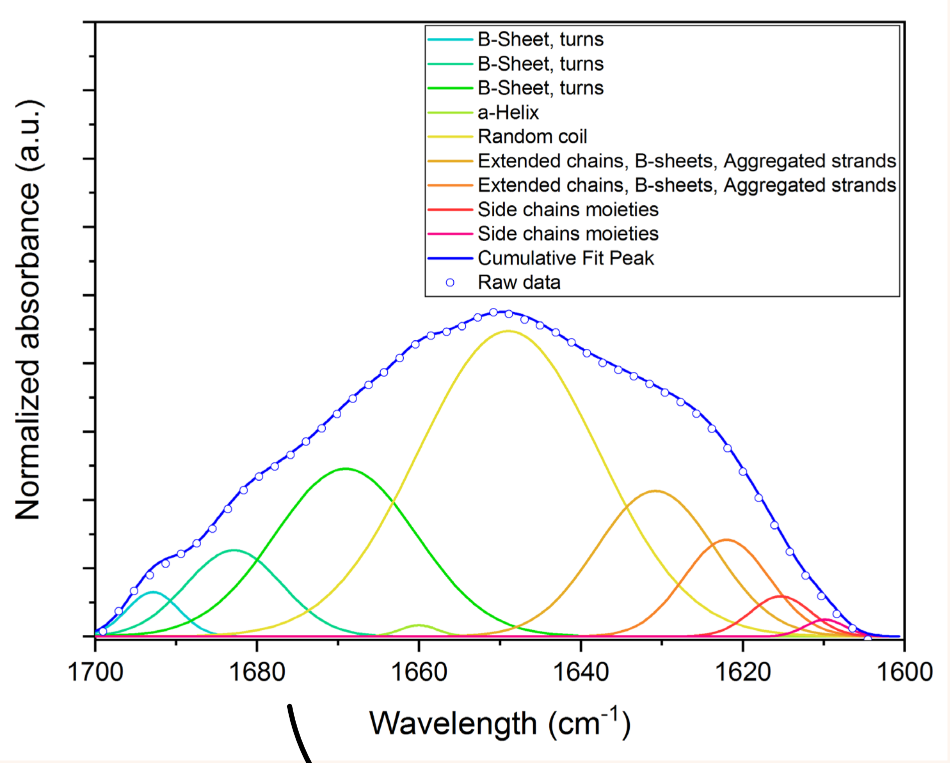
Proteins from BSF. Film characterization

Thermal stability: TGA/DTA anlyses



Proteins from BSF. Film characterization

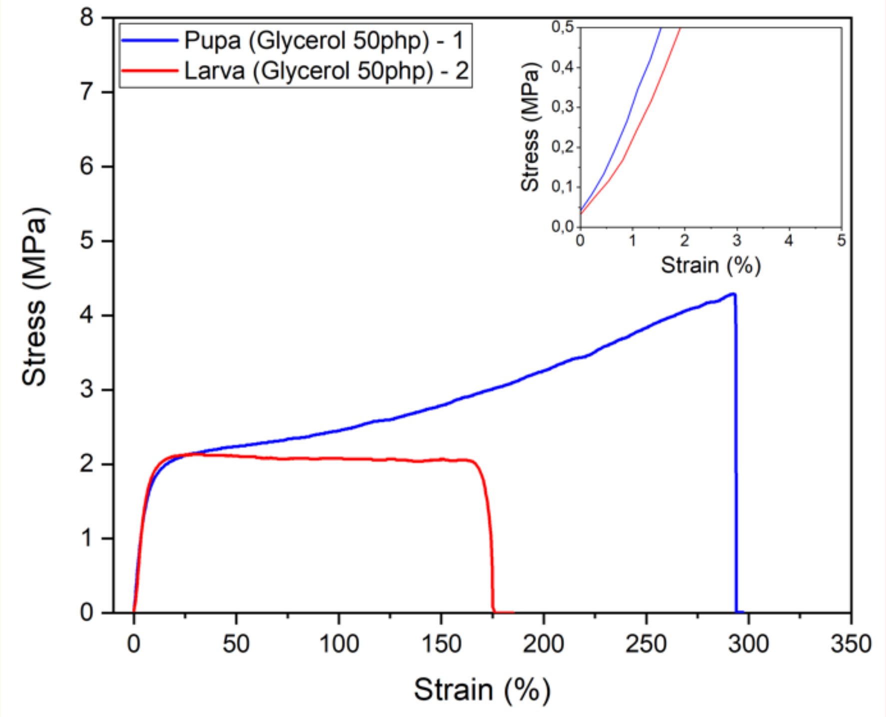
Secondary structures: ATR FT-IR profiles: 1700-1600 cm^{-1}



Secondary structure	□ sheet, turns	□helix	Random coil	□sheet, aggregated	Side chain moieties
[%]	28.98	0.35	46.45	21.59	2.63

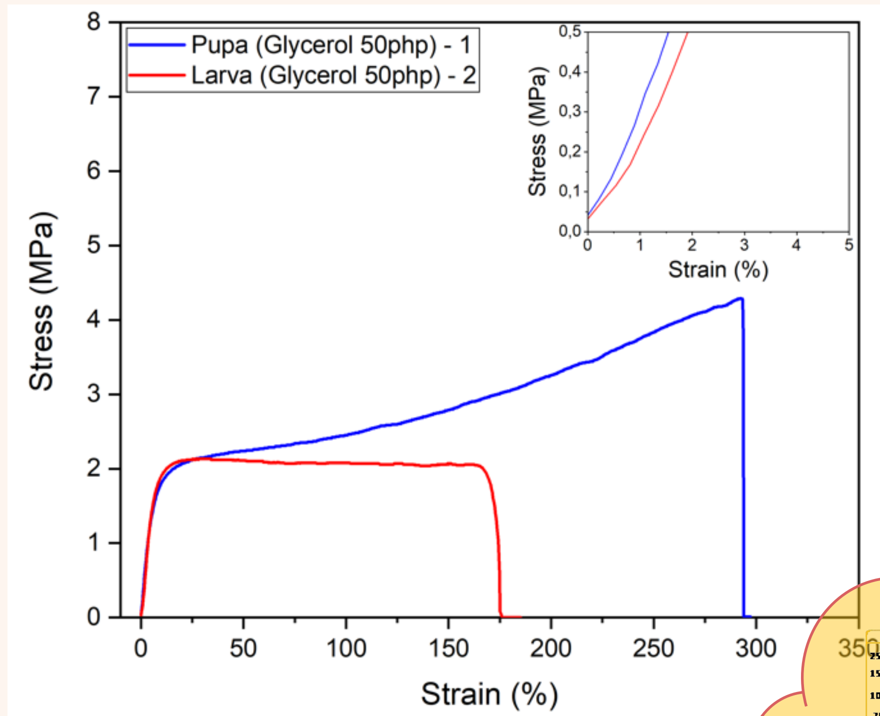
Proteins from BSF. Film characterization

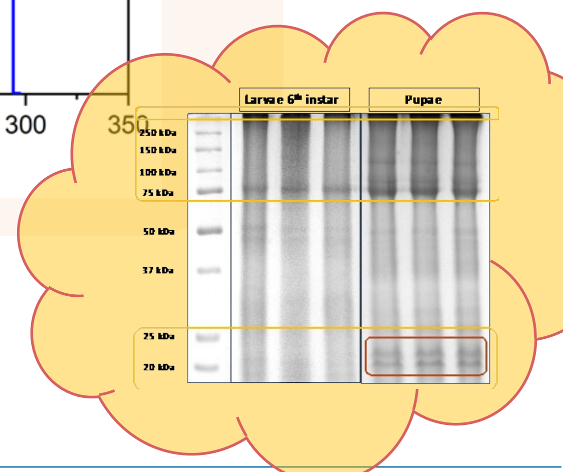
Mechanical tests – Stress/strain



Proteins from BSF. Film: structure – property correlation

Mechanical tests – Stress/strain





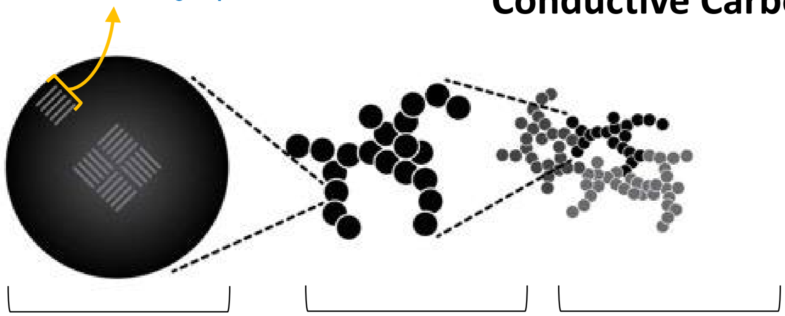
Proteins from BSF. Bionanocomposites

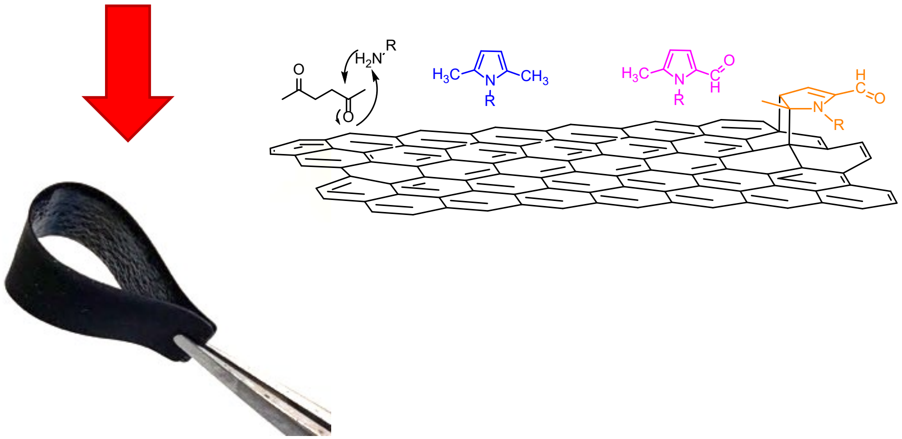
Conductive protein-based bio-nanocomposites for flexible electronics

BSF extract

+

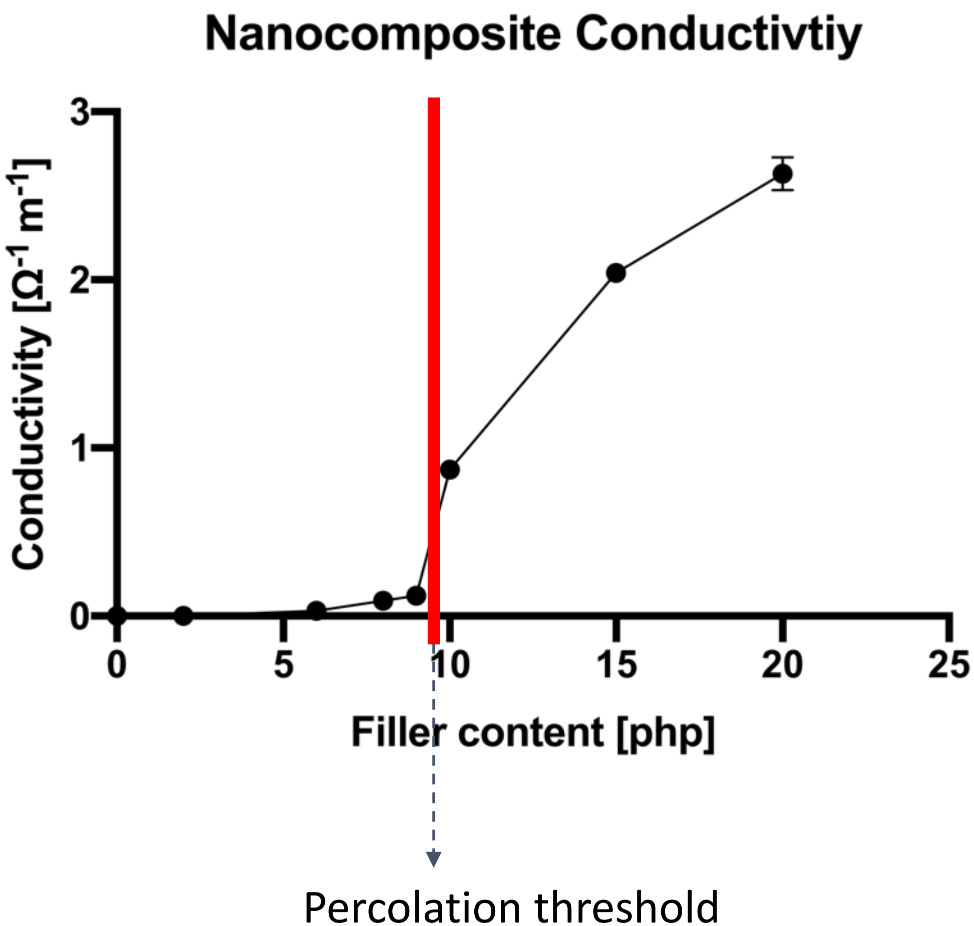
Functionalized
Conductive Carbon Black





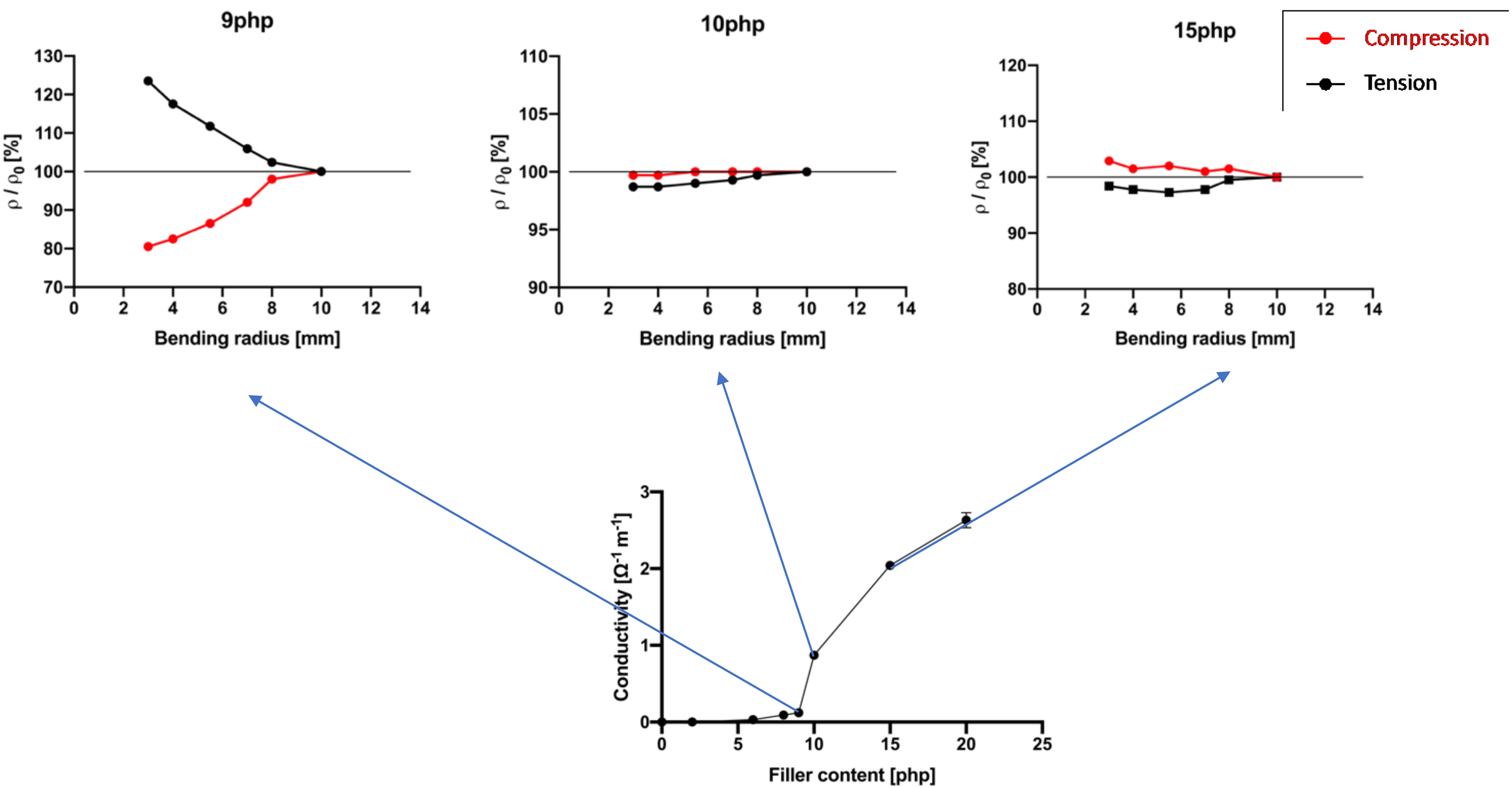
M. Galimberti, V. Barbera, E. Testa, E. Fasoli, G. Tettamanti, D. Bruno, G. Molla, M. Orlando, L. Pollegioni, M. Casartelli - "*Materiale bio-nanocomposito" - Patent application No. 102022000019020, Sep. 16, 2022*

Conductivity of protein-based bio-nanocomposites



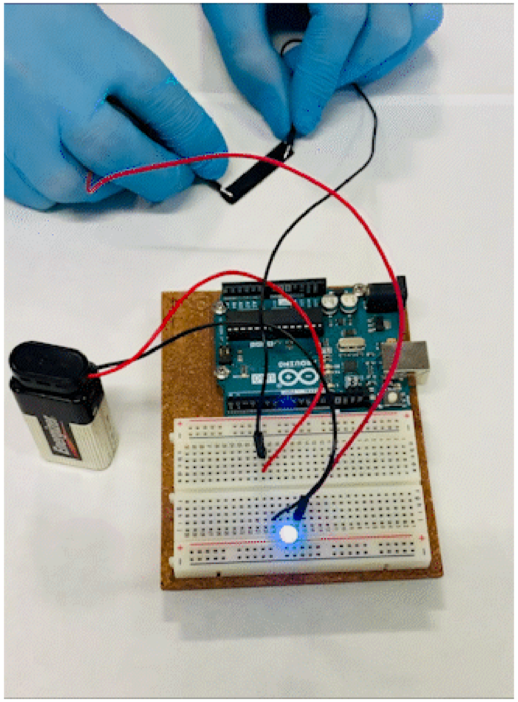
Proteins from BSF. Bionanocomposites

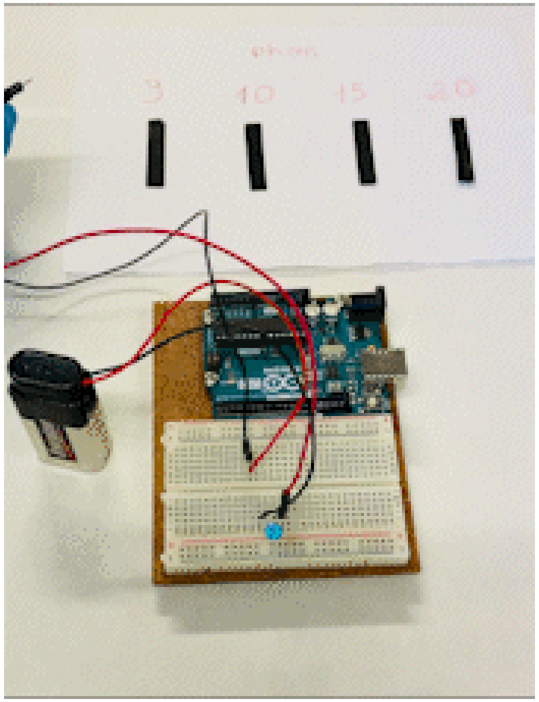
Conductivity of protein-based bio-nanocomposites vs. bending radius



Proteins from BSF. Bionanocomposites

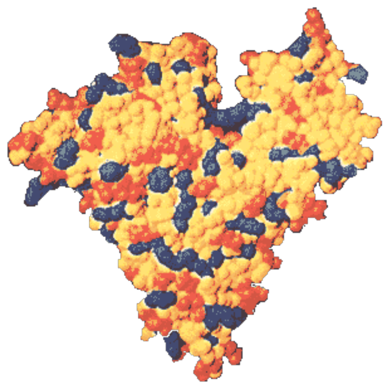
Conductive protein-based bio-nanocomposites for flexible electronics





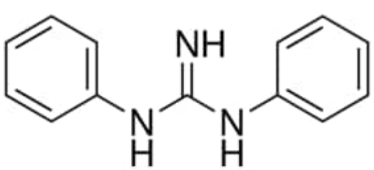
ECOTRON European Project

Proteins as accelerators of sulphur based vulcanization



BSA, Egg White

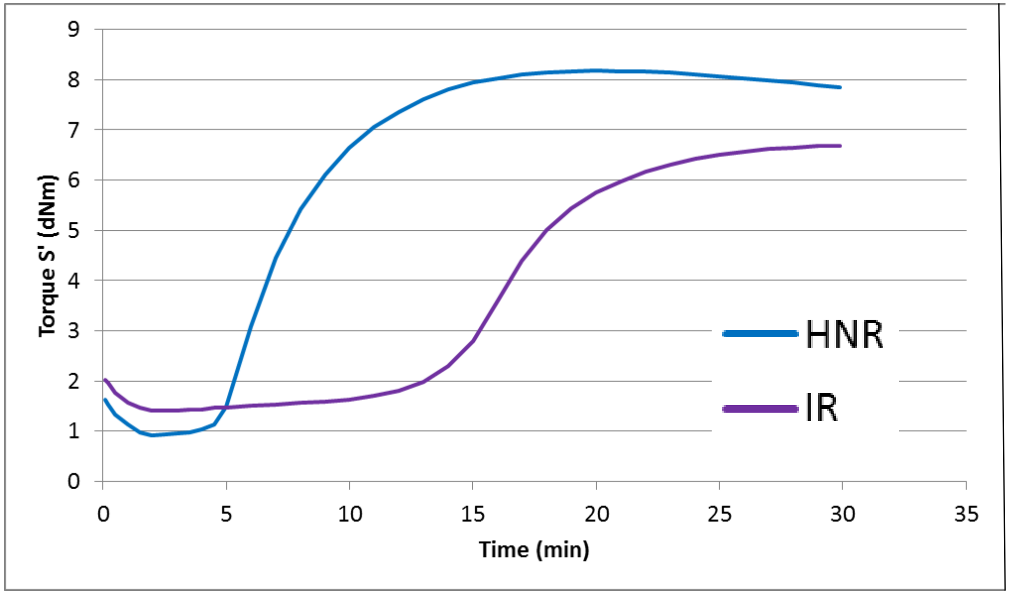
In place of oil based amines



Sulphur based crosslinking of poly(1,4-cis-isoprene)

Ingredients	phr
Rubber[a]	100.0
ZnO	6.0
Stearic Acids	0.5
Sulphur	3.5
TBBS	0.7

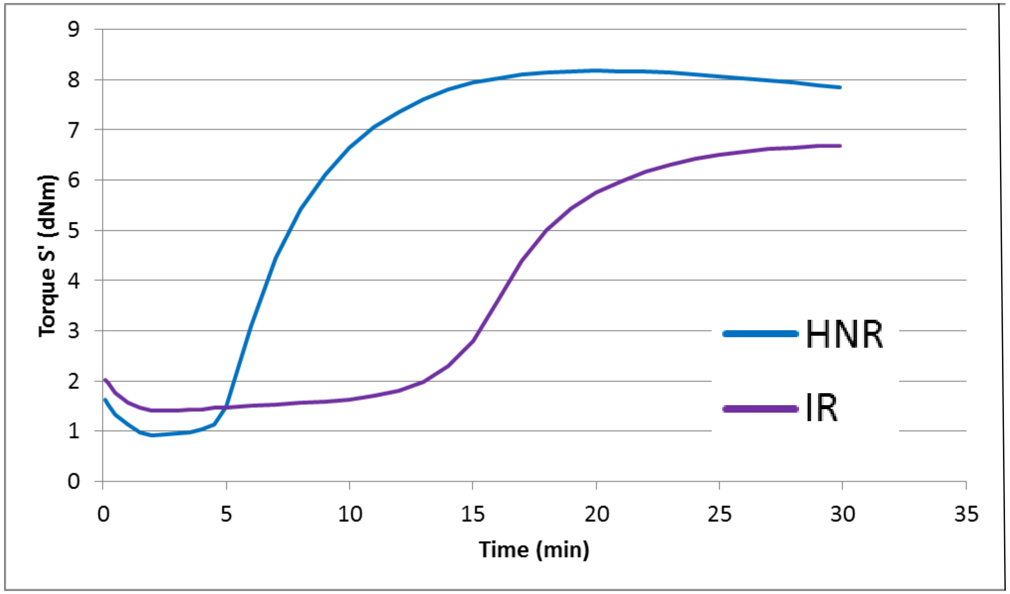
ASTM D 3184 – 89 FORMULATION



Sulphur based crosslinking of poly(1,4-cis-isoprene)

Ingredients	phr
Rubber[a]	100.0
ZnO	6.0
Stearic Acids	0.5
Sulphur	3.5
TBBS	0.7

ASTM D 3184 – 89 FORMULATION



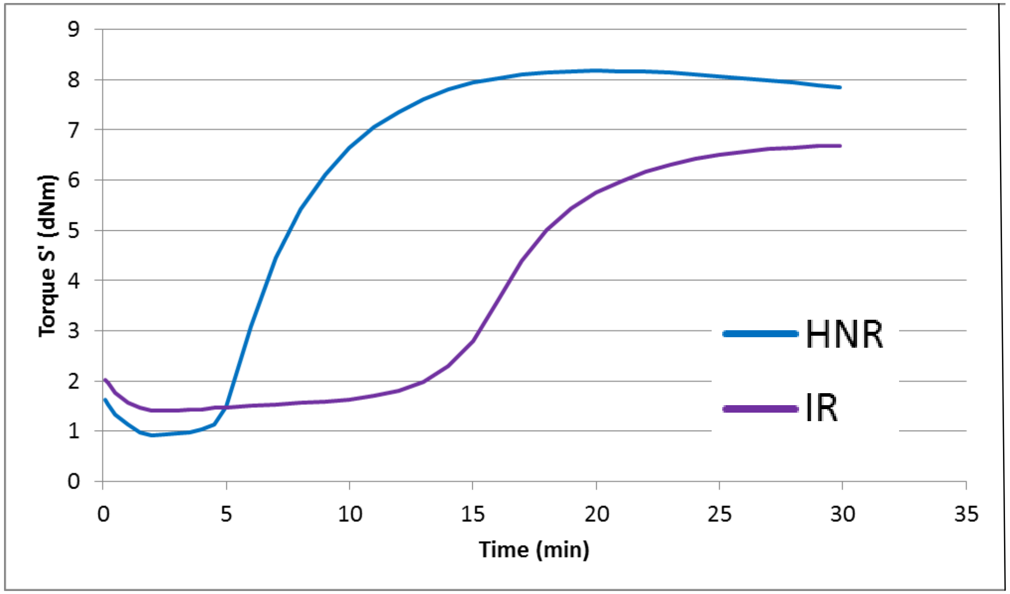
Faster sulphur based vulcanization of NR
with respect to synthetic diene rubbers,
is in general attributed to the presence of proteins
and also to nitrogenous bases that can be in NR latex.

Gregg E. C. Jr., Macey J. H., Rubber Chemistry and Technology,1973, 46, 47-66.

Sulphur based crosslinking of poly(1,4-cis-isoprene)

Ingredients	phr
Rubber[a]	100.0
ZnO	6.0
Stearic Acids	0.5
Sulphur	3.5
TBBS	0.7

ASTM D 3184 – 89 FORMULATION



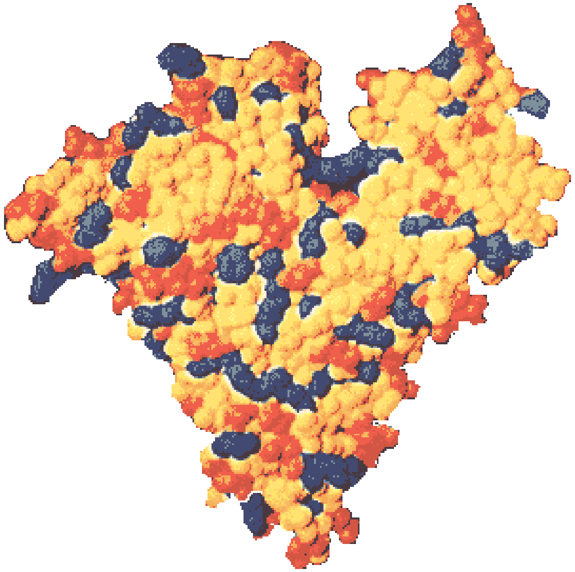
Proteins in Natural Rubber

Isolectric Point (pI): 4.0 - 4.6

J. Sansatsadeekul, J. Sakdapipanich P. Rojruthai Journal of Bioscience and Bioengineering VOL. 111 No. 6, 628–634, 2011

Proteins. Bovine serum albumin (BSA)

Main aminoacids:	Alanine; Valine; Leucine; Cysteine; Threonine; Lysine (≈ 47% by mole)	
	Histidine, Lysine, Arginine	(≈ 17% by mole)
	Aspartic acid; Glutamic acid	(≈ 17% by mole)
Nitrogen content:	≈ 16% by mass	
Isoelectric point:	4.7	

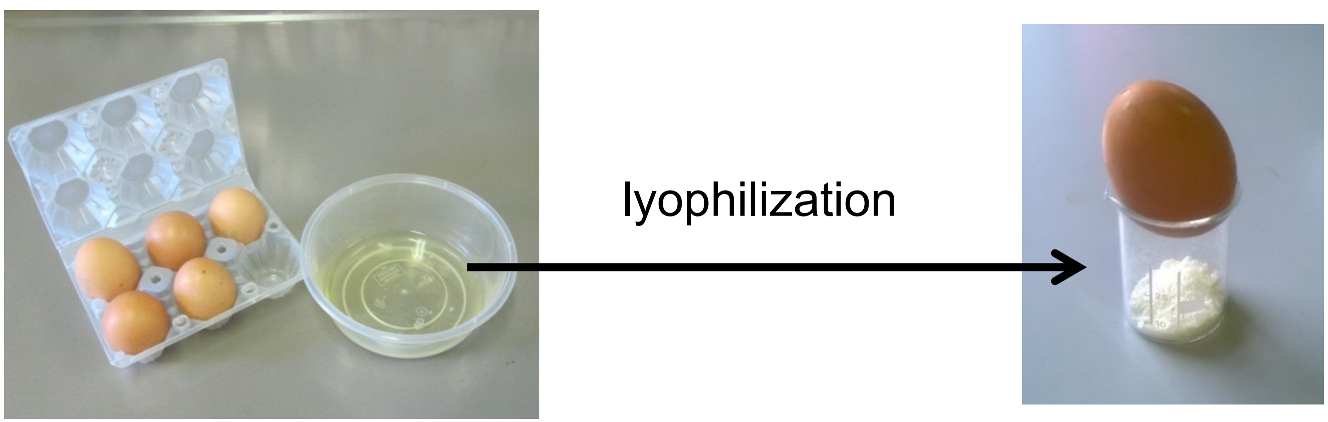


Proteins. Egg White (EW)

Main proteins: Ovoalbumin (54%); Conalbumin; Ovomucoid; Lysozyme; Globulins; Ovomucin

Nitrogen content: ≈ 15% by mass

Ovoalbumin Isoelectric point: 4.7



Composites based on NR, IR and BSA

	NR	NR + 1BSA	IR	IR + 1BSA	IR + 2BSA
IR[a]	0.0	0.0	100	100	100
NR[b]	100	100	0.0	0.0	0.0
EW	0.0	0.0	0.0	0.0	0.0
BSA	0.0	2.6	0.0	2.6	5.2

"1" = Nitrogen content in NR

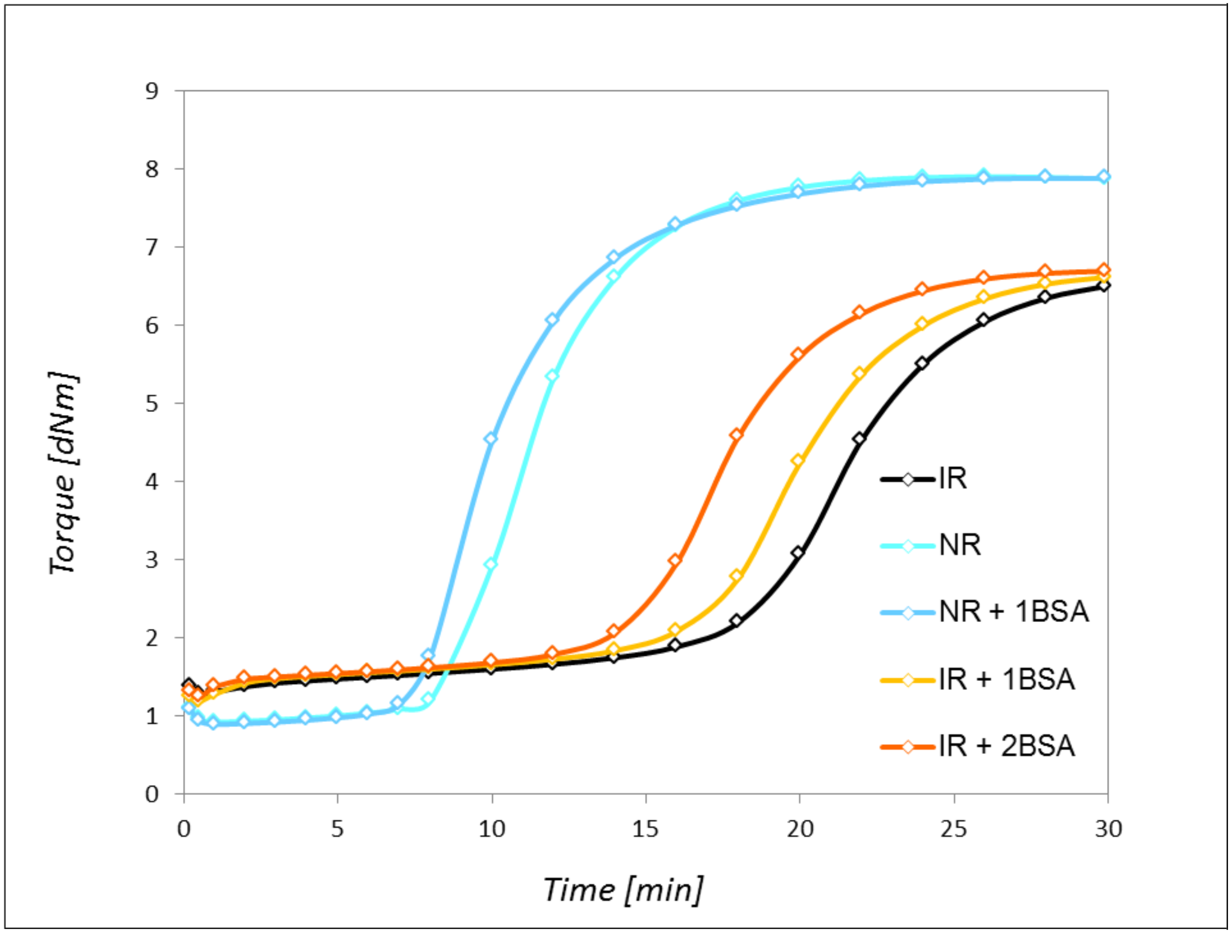
"2" = Twice the Nitrogen content in NR

Other ingredients: ZnO 5.0, stearic acid 2.0, TBBS 0.7, sulphur 2.25

[a] SMR GP from Lee Rubber [b] SKI III from Nizhnekamskneftechim Export

Composites based on NR, IR and BSA

Sulphur based crosslinking - Rheometric curves



Composites based on NR, IR and BSA, EW

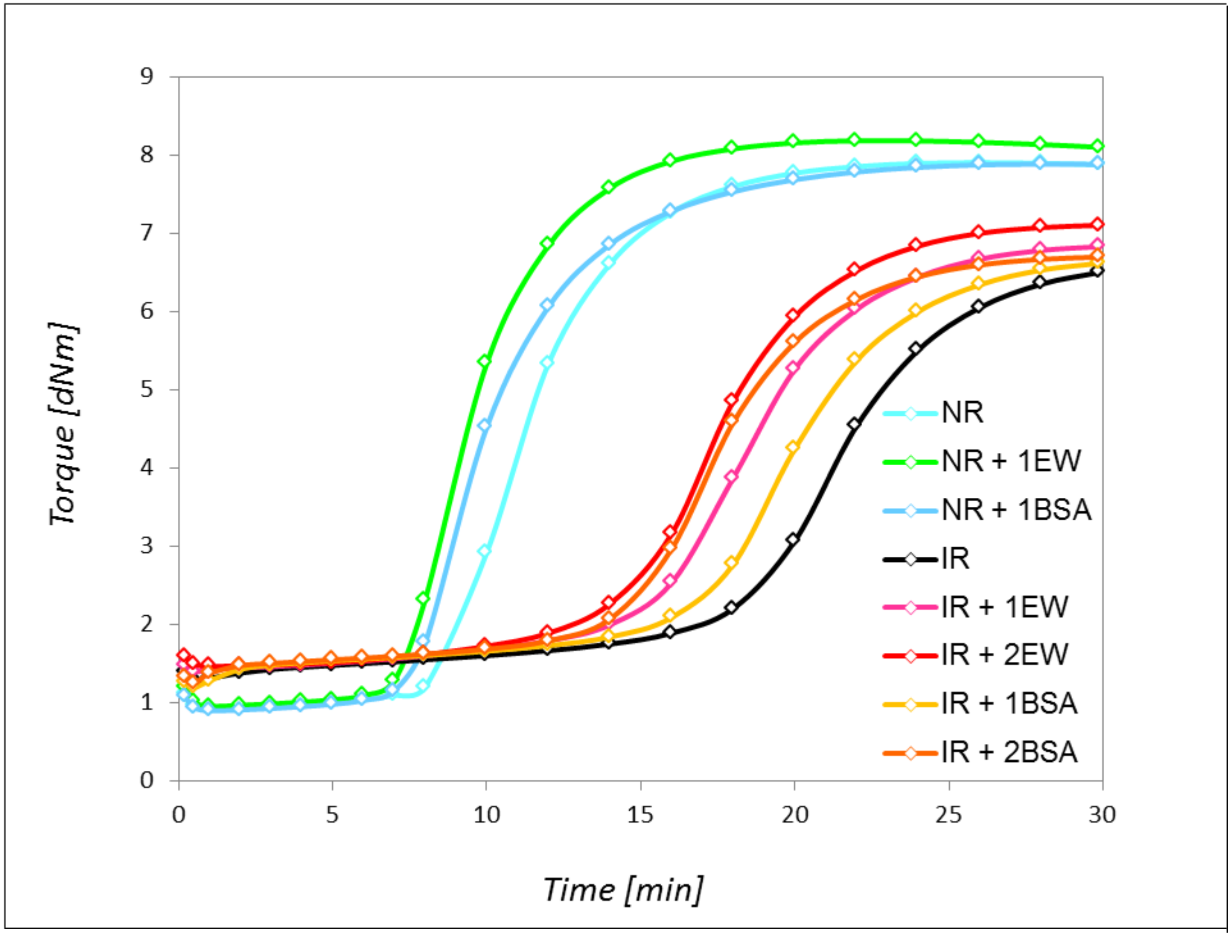
	NR	NR + 1BSA	NR + 1EW	IR	IR + 1BSA	IR + 2BSA	IR + 1EW	IR + 2EW
IR[a]	0.0	0.0	0.0	100	100	100	100	100
NR[b]	100	100	100	0.0	0.0	0.0	0.0	0.0
EW	0.0	0.0	2.8	0.0	0.0	0.0	2.8	5.6
BSA	0.0	2.6	0.0	0.0	2.6	5.2	0.0	0.0

"1" = Nitrogen content in NR

"2" = Twice the Nitrogen content in NR

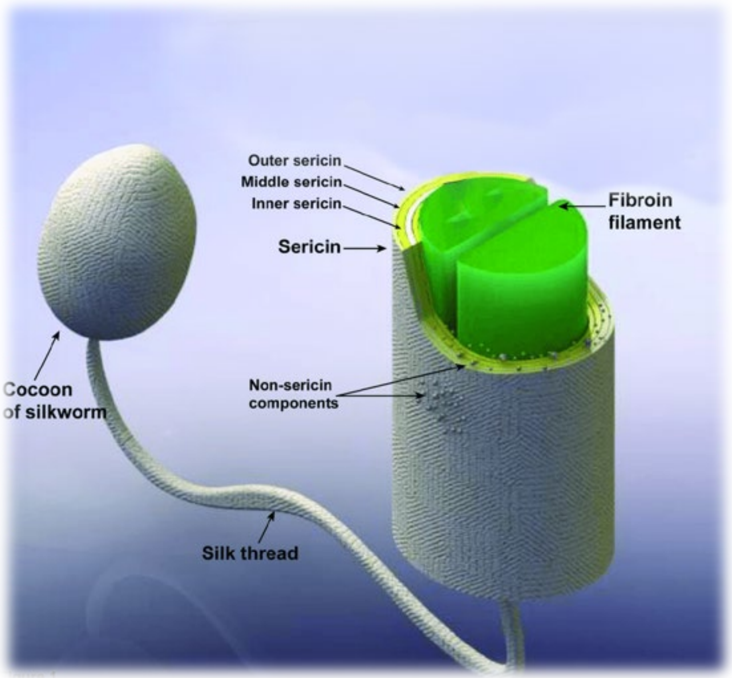
Composites based on NR, IR and BSA, EW

Sulphur based crosslinking - Rheometric curves

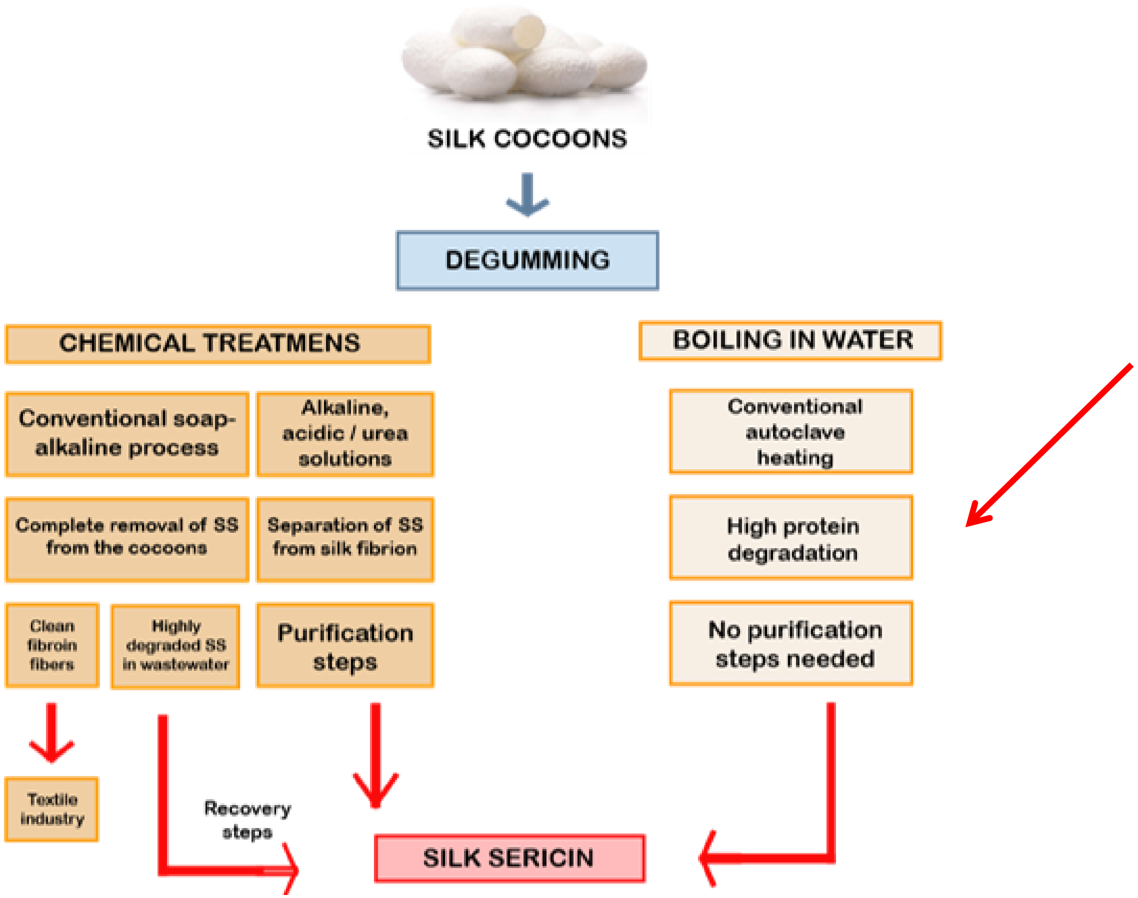


M. Galimberti, V. Barbera et al Rrubber World 2019

Sericin for gels preparation



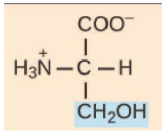
Sericin. Extraction



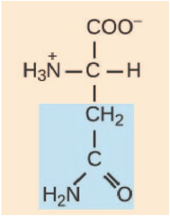
Sericin. Chemical composition

Polar charged side chains (hydrophilic)	
Aspartic acid	16-18
Glutammic acid	5-6
Arginine	2.9-3.2
Lysine	2.1-2.7
Hystidine	1.1-1.3

Polar amino acids (hydrophilic)	
Serine	26-34
Tyrosine	3-4
Threonine	7-8



Serine

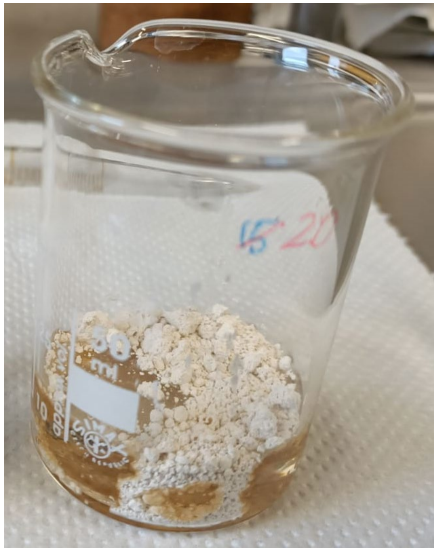


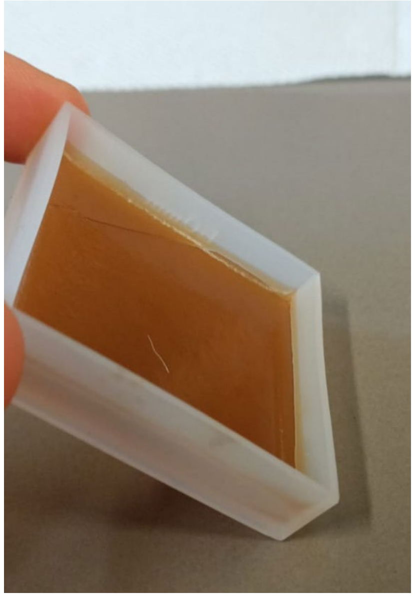
Aspartic acid

Non-polar amino acids (hydrophobic)	
Glycine	15-20
Alanine	4-7
Valine	3-4
Isoleucine	0.6-1
Leucine	1-1.5
Phenylalanine	0.3-0.7
Proline	0.5-0.8

Sericin. Gels preparation

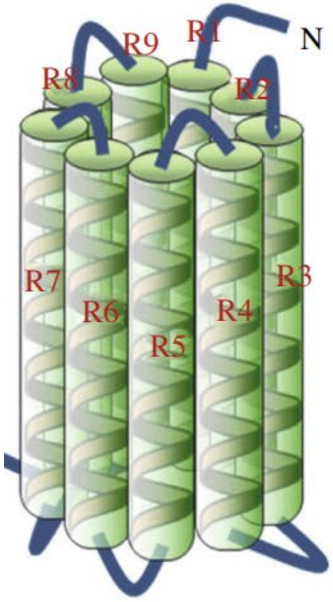
Sericin powder → (Boling water, 10 minutes) → Solubilization → (Denaturation) → Casting





ECOTRON European Project

Reversible crosslinking of proteins



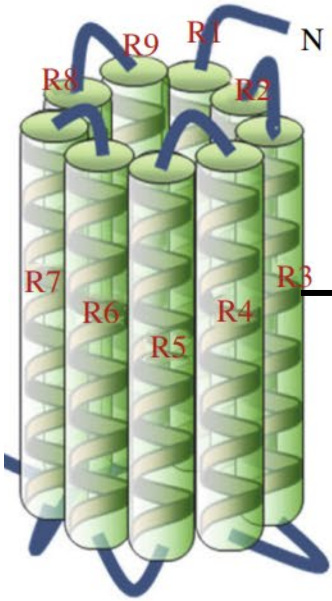
Protein

Reversible

crosslinking

Circular thermoset material

Reversible crosslinking of zein



DIENE

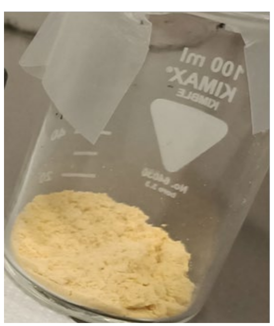
DIENOPHILE
(crosslinking)

Diels–Alder reaction

Δ T

Retro
Diels–Alder reaction

Zein

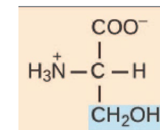


ECOTRON European Project

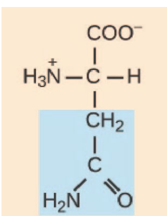
Zein. Chemical composition

Polar charged side chains (hydrophilic)	
Aspartic acid	4-5
Glutammic acid	21-26
Arginine	1-4
Lysine	0
Hystidine	1.1-1.3

Polar amino acids (hydrophilic)	
Serine	7-7.5
Tyrosine	5
Threonine	2-3



Serine



Aspartic acid

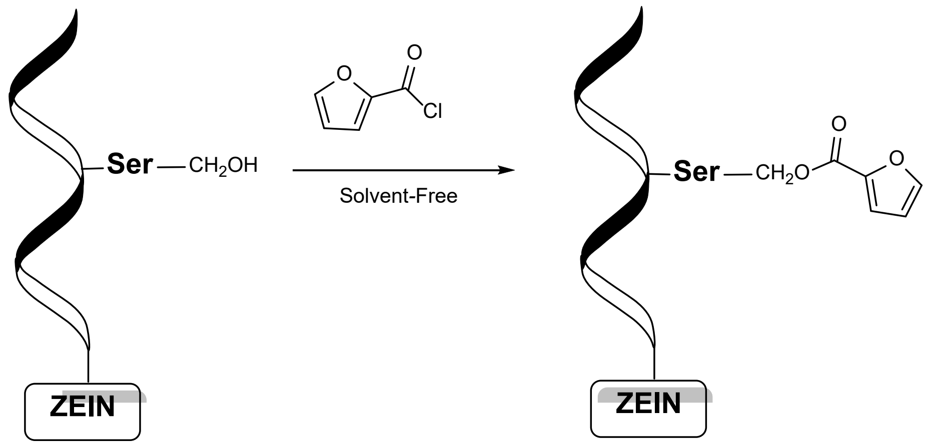
Non-polar amino acids (hydrophobic)	
Glycine	0-0.5
Alanine	8-10
Valine	3-4
Isoleucine	5-6
Leucine	19-21
Phenylalanine	7
Proline	9-10

Shukla, R., & Cheryan, M. (2001). Zein: the industrial protein from corn. *Industrial crops and products*, *13*(3), 171-192.

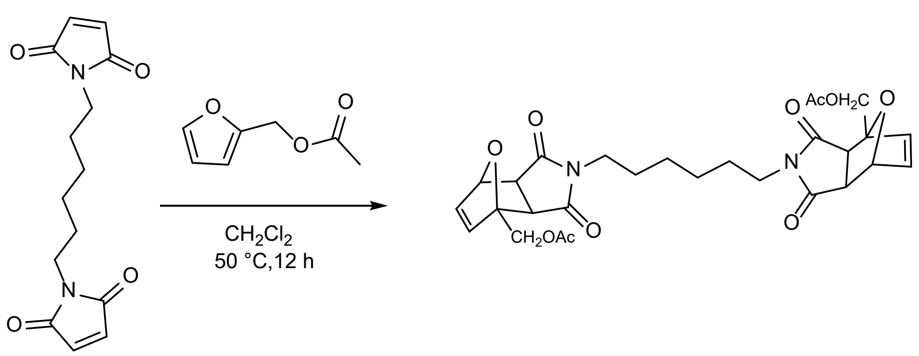
Reversible crosslinking of zein with Diels-Alder reaction

Preparation of the diene and the model compound

Diene: Diels-Alder reactive furan modified Zein

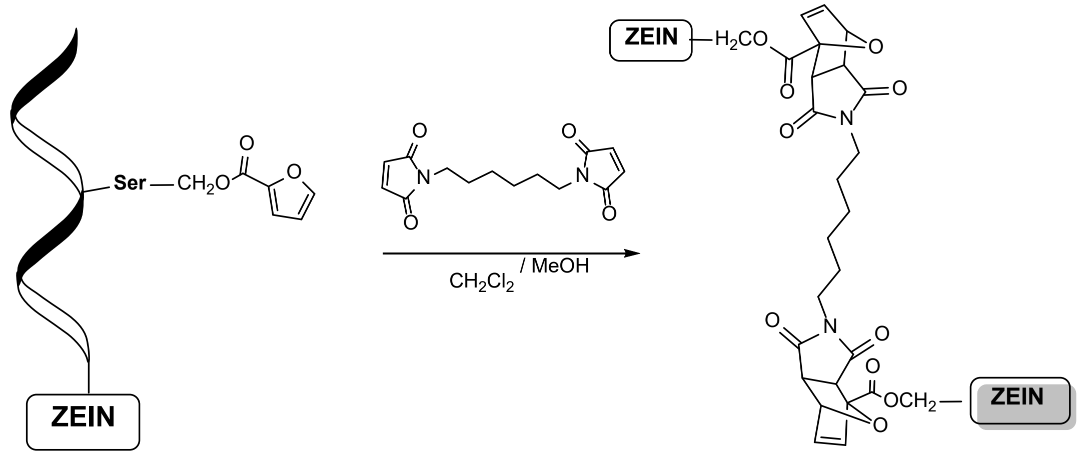


Model compound: 1,1'-(hexane-1,6-diyl)bis(1H-pyrrole-2,5-dione) + furan-2-ylmethyl acetate

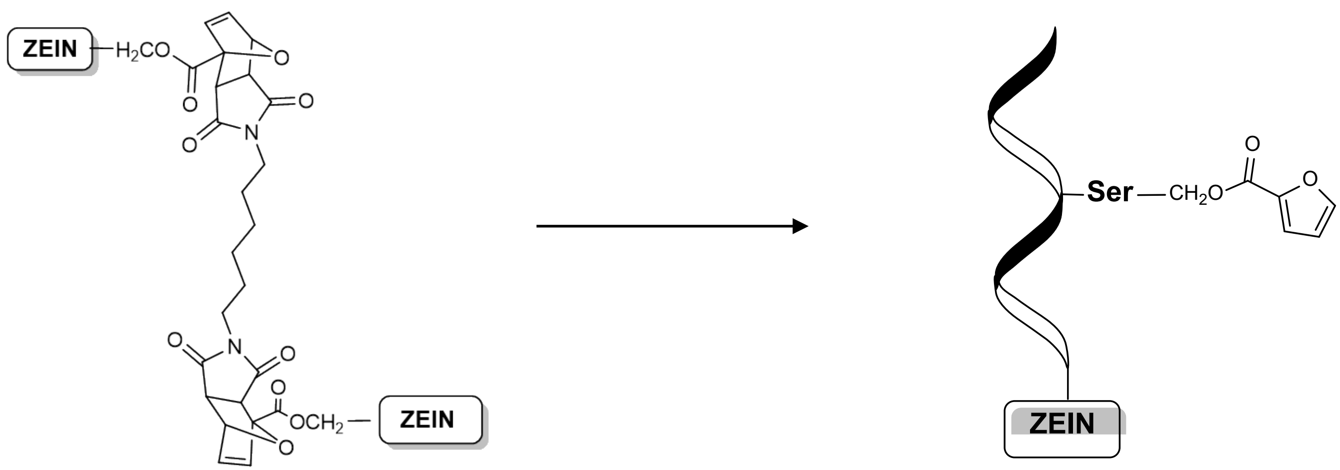


Reversible crosslinking of zein with Diels-Alder reaction

Crosslinked Furan modified zein



Retro Diels-Alder



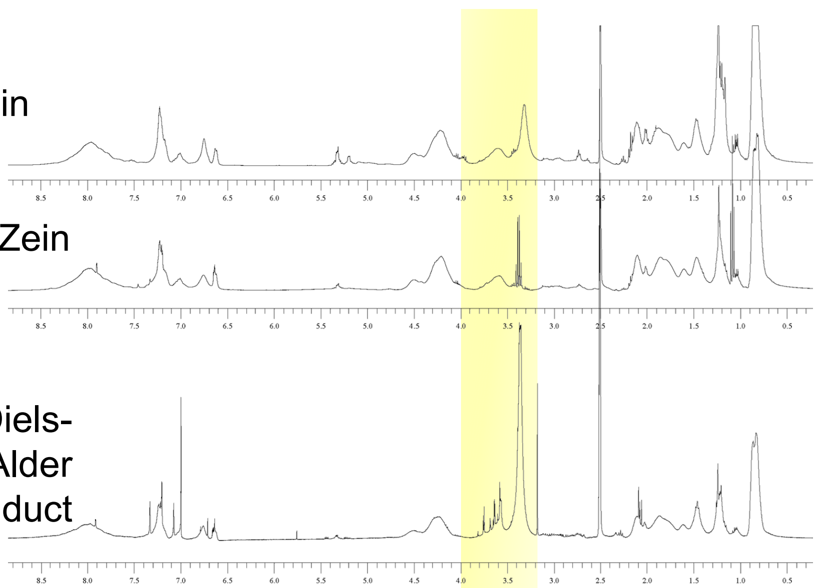
Reversible crosslinking of zein with Diels-Alder reaction

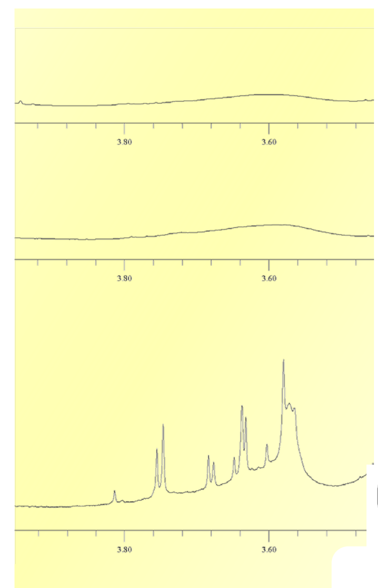
^{1}H-NMR

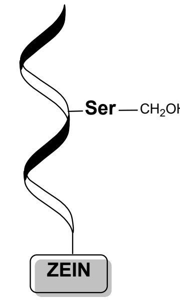
Zein

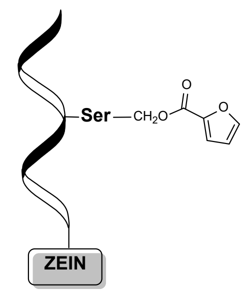
Furan-Zein

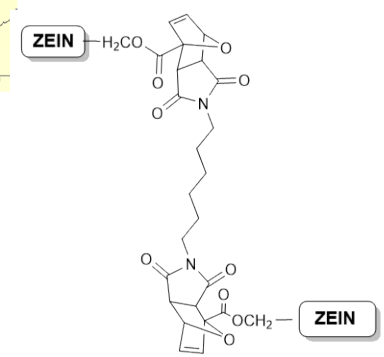
Diels-Alder adduct





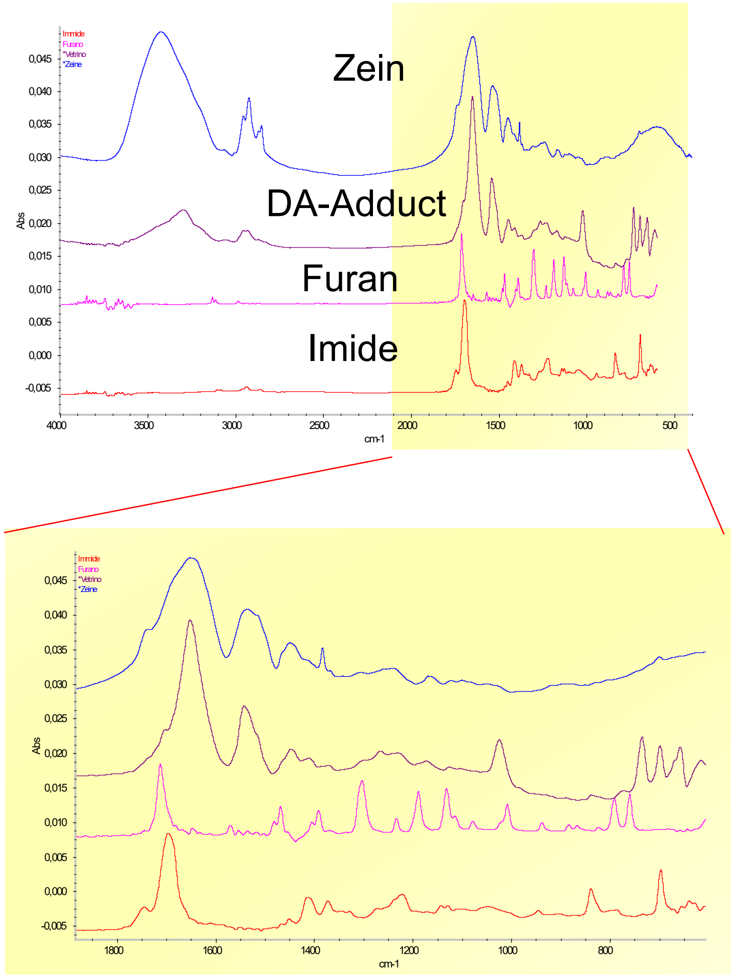




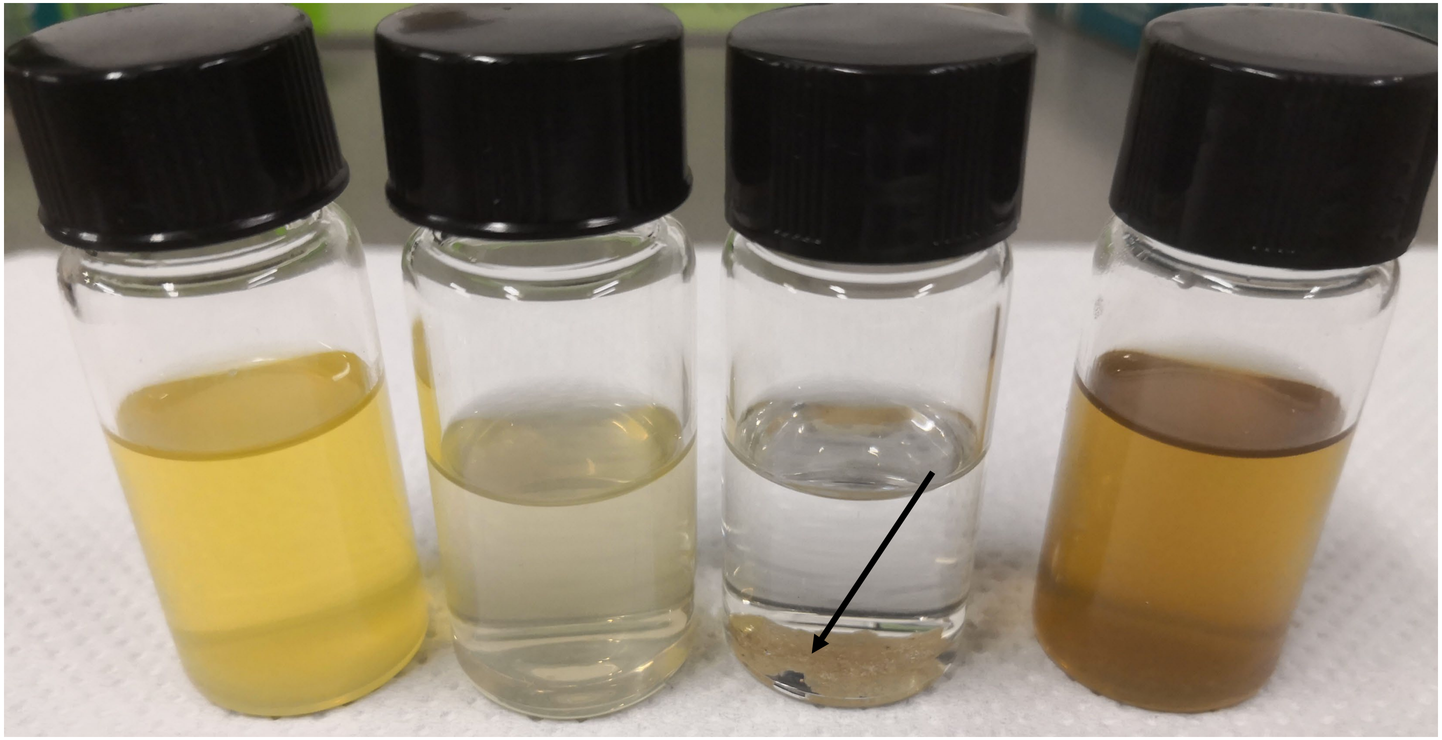


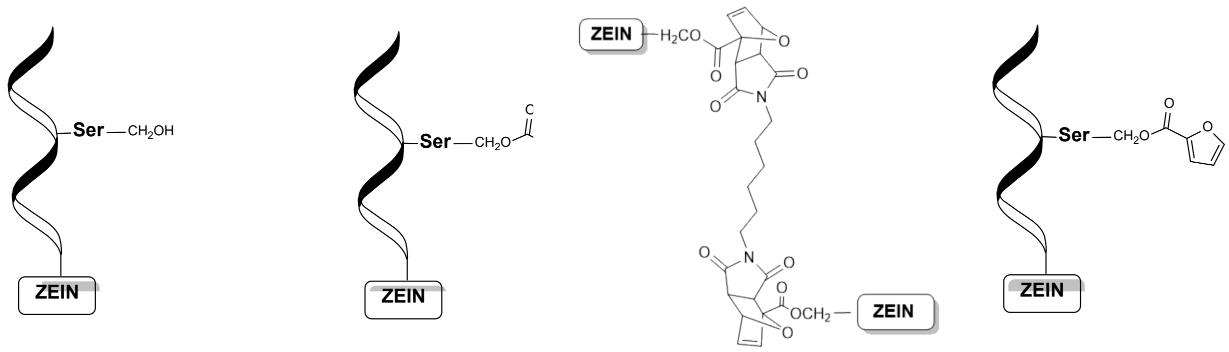
Reversible crosslinking of zein with Diels-Alder reaction

IR

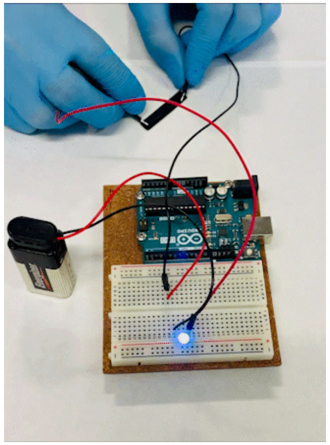


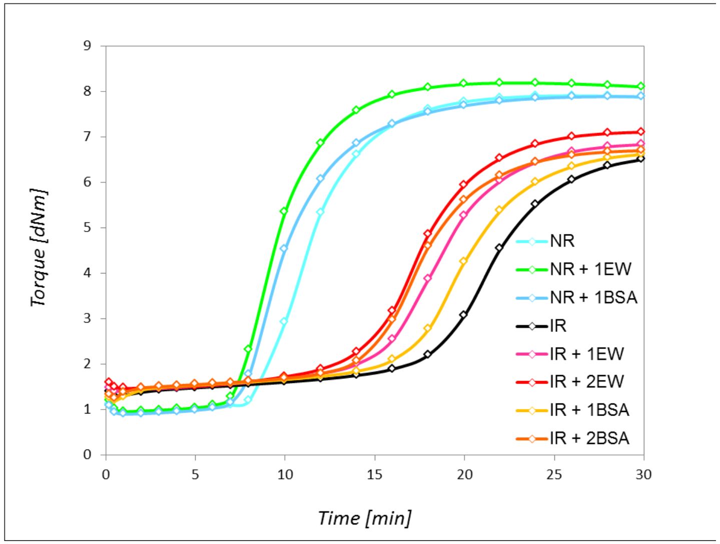
Reversible crosslinking of zein with Diels-Alder reaction

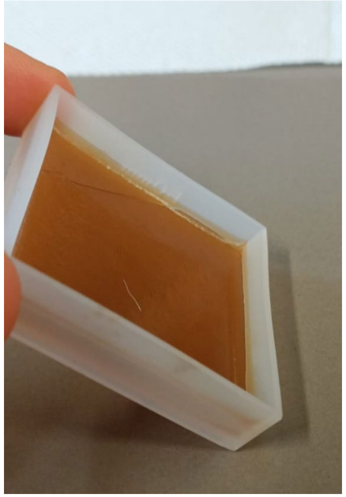


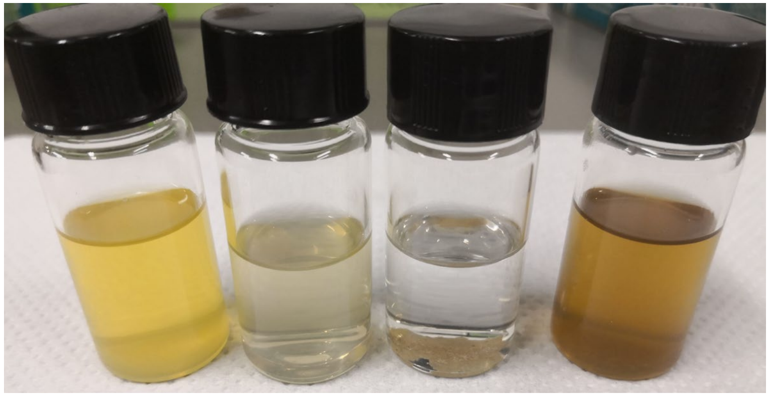


Conclusions









Acknowledgments

CARIPLO Foundation: RICH Project

European Commission: HORIZON Europe GAN.101070167 ECOTRON

European Union, Next generation EU. Ministero dell'Università e della Ricerca.
PNRR Mission 4 "Education and Research"
Extended Partnership MICS – Made in Italy Circolare e Sostenibile

Thanks for your attention!

CPAC Rome Workshop 2023
Rome (I), March 20-22, 2023