AMYLOID-LIKE SELF-ASSEMBLING OF PROTEIN FROM SOLDIER FLY AND BIOPLASTICS OBTAINED THEREFROM

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Materiali da Proteine

BIOECONOMY DIALOGUES 2025
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The Group

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ISCaMaP

Innovative Sustainable Chemistry and Materials and Proteins Group

Natural sources

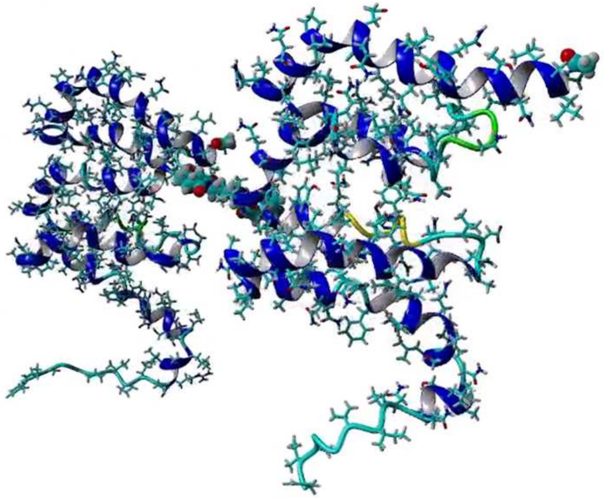
Wastes and EOL materials

Building blocks
for chemical platforms

Chemicals and Materials

Outline of the Presentation

- ☞ Proteins for materials
- ☞ Films
- ☞ Amiloyd fibrils
- ☞ Thermoset material

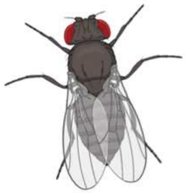


Protein waste sources

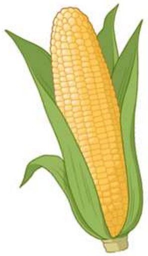
	INDUSTRY/SECTOR	BYPRODUCT QUANTITY	VALORIZABLE PROTEINS
ANIMAL BASED	Dairy industry	240 mln tons/year[1]	2 mln tons/year[1]
	Meat industry	52.6 mln tons/year[3]	0.5 – 44.71 mln tons/year[3]
	Seafood industry	80-128 mln tons/year[1]	48 – 76.8 mln tons/year[1]
	BSF bioconversion	1.3 bil tons/year (OFMSW)[6]	8 mln tons/year
	Silk industry	-	0.05 mln tons/year[5]
PLANT BASED	Soy industry	3.58 bil tons/year (soy whey)[1] 14 mln tons/year (okara)[1]	4.76 – 29.36 mln tons/year[1] 2.93 – 5.47 mln tons/year[1]
	Rapeseed industry	40 mln tons/year[1]	11.2 – 18 mln tons/year[1]
	Sunflower industry	20 mln tons/year[1]	4 – 6 mln tons/year[1]
	Corn industry	68.1 mln tons/year (corn gluten meal)[1,4]	47.67 mln tons/year[1]
	Rice industry	70 mln tons/year (rice kernel)[1]	10.5 mln tons/year[1]

From OFMSW to proteins from BSF

Far from the objective of being exhaustive, these are the **waste-derived proteins explored** so far:



Black soldier fly proteins (BSF)



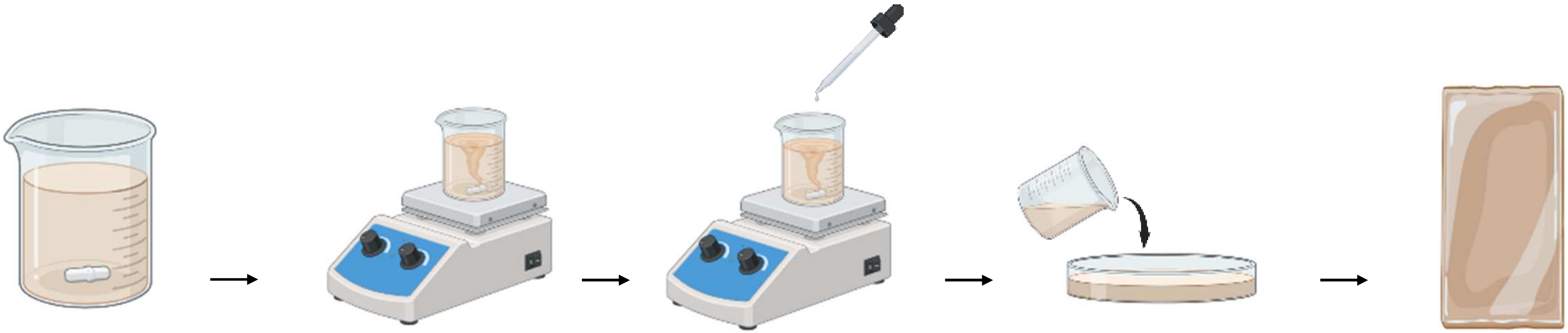
Zein

Protein sources

PROTEIN	ORIGIN and TYPE	REASONS FOR ITS APPLICATION	PRODUCTION PER YEAR	PURCHASE COST
Zein	Plant-derived protein. Mainly obtained from byproducts of corn industrial processing	As a food industry byproduct, is highly abundant and readily available.	500 tons/year[10]	250 $ per kg
BSF proteins	Animal-derived protein. Obtained from the freeze-dried biomass of BSF larvae or pupae	BSF proteins are a product of an innovative circular bioeconomy chain for the valorization of OFMSW.	OFMSW: 1300 million tons/year BSF protein extraction yield: 0.6% (potentially up to 8 million tons/year)	Not available

Proteins for films

Proteins film-forming protocols



Solubilization of proteins in aqueous solution → Solution stirring (with or w/o heating) → Glycerol addition and solution stirring → Pouring of the solution into the mold → Film drying and removal from the mold

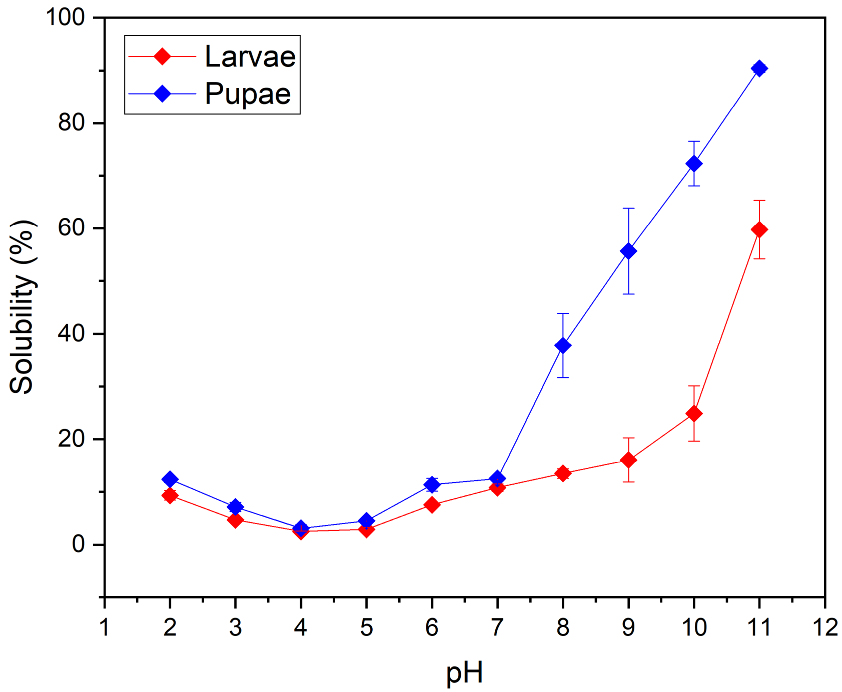
5% w/v

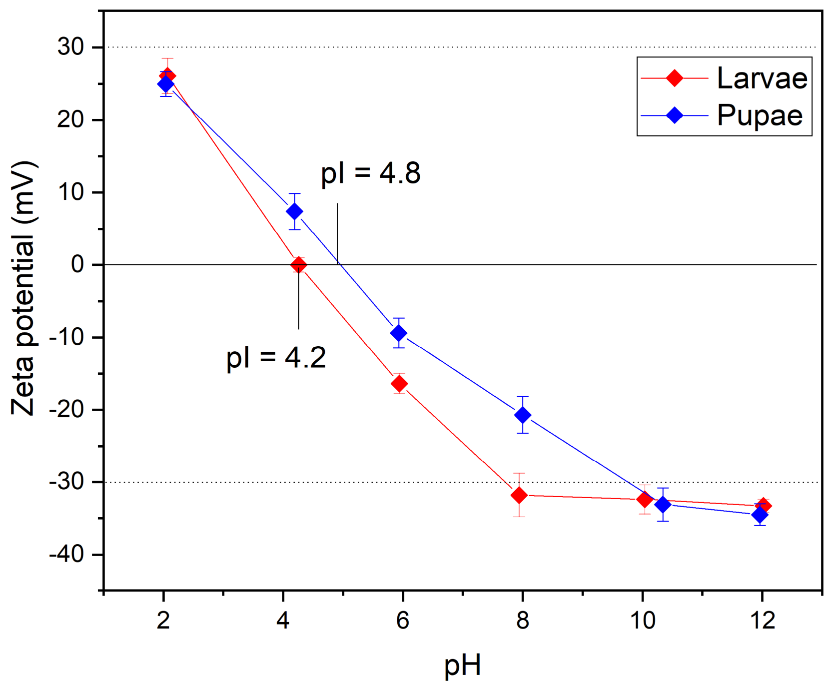
RT – 85°C

pH = 2 - 11

Bruno, D.; Orlando, M.; Testa, E.; Miino, M. C.; Pesaro, G.; Miceli, M.; Pollegioni, L.; Barbera, V.; Fasoli, E.; Draghi, L.; Baltrocchi, A. P. D.; Ferronato, N.; Seri, R.; Maggi, E.; Caccia, S.; Casartelli, M.; Molla, G.; Galimberti, M. S.; Torretta, V.; Vezzulli, A.; Tettamanti, G. Valorization of Organic Waste through Black Soldier Fly: On the Way of a Real Circular Bioeconomy Process. Waste Manage. 2025, 191, 123−134.

Solubility and surface charge



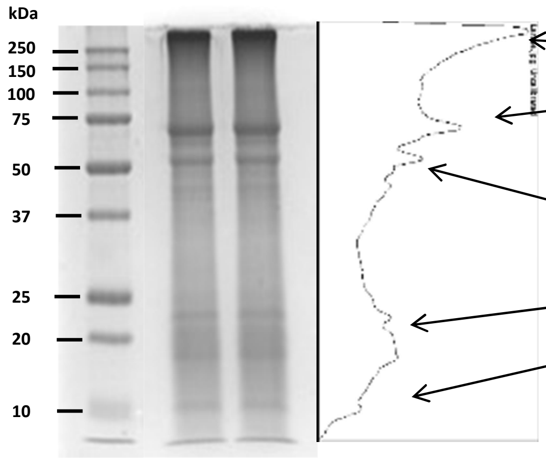


Proteomic analysis

Molecular weight

SDS-Page

Larvae (s-OFMSW)



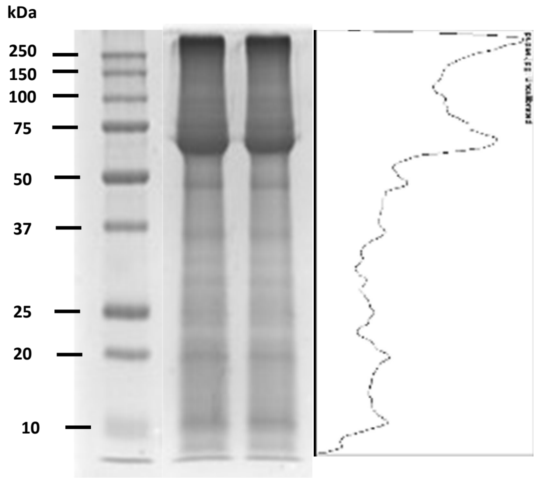
intense bands

above 250 kD
around 75 kD

smaller bands

around
50 kDa
20 kDa
10 kDa

Pupae (s-OFMSW)



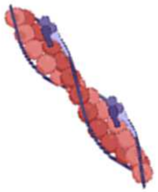
In larvae extracts band intensity was lower, especially in the region around 75 kDa and above

Proteomic analysis

Molecular weight

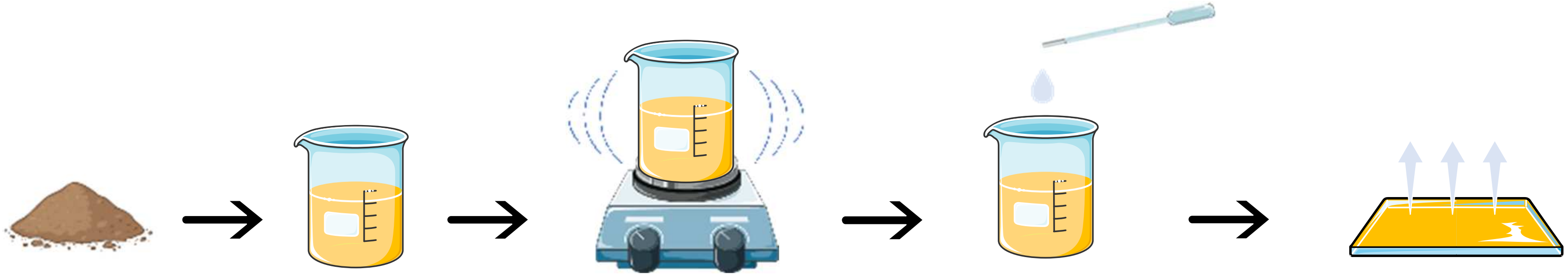
nLC-MS/MS

Protein	Accession number	MW (kDa)	Mascot Score
Tropomyosin-2	Q1HPQ0	33	1523
Tropomyosin-2 (Isoform 3)	Q1HPU0-3	33	1276
Tropomyosin-1, muscle specific	Q1HPU0	33	901
Actin-2, muscle-specific	P45885	42	648
Actin-87E	P10981	42	624
ATP-Synthase	Q05825	54	603
Troponin C	P47949	18	345
Arginine Kinase	P48610	40	231
Troponin 1	P36188	30	201
Tropomyosin Lep s 1.0101	Q8T380	33	57



Structural (muscolar) proteins

Preparation of film

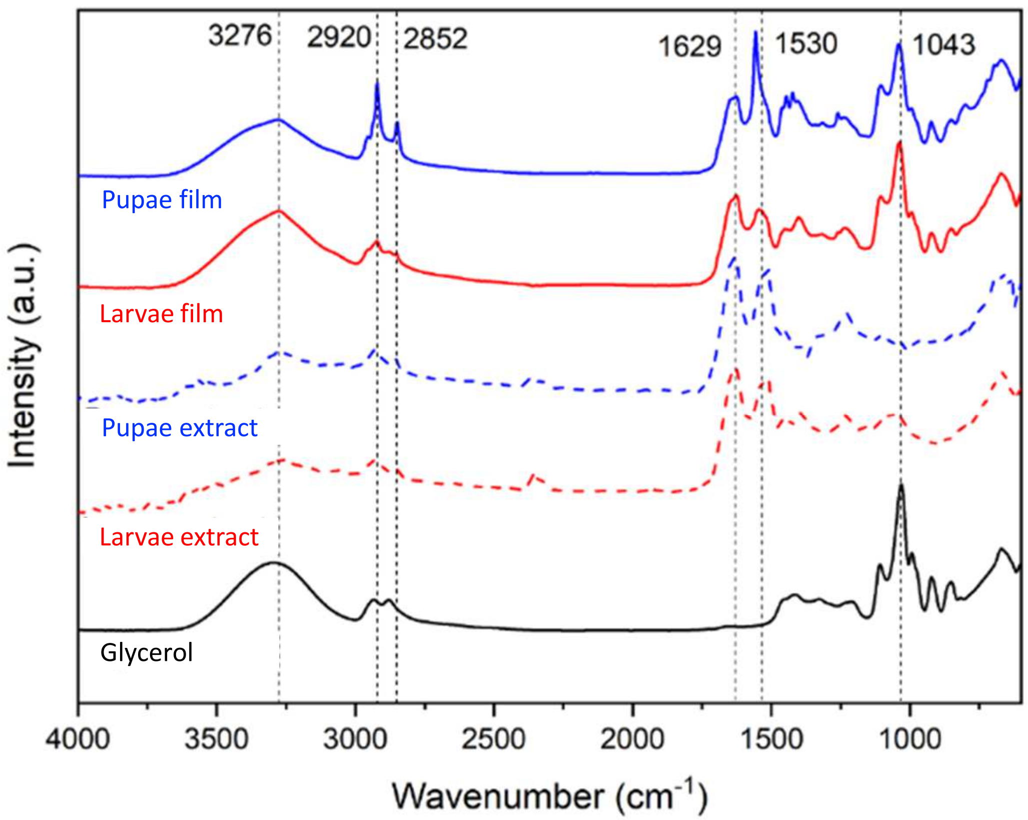


BSF protein extract → Water dispersion (Cprotein = 50 mg/mL, pH 11.5) → Homogenization and heating (80°C, 20 min) → Glycerol (50% w/w on extract weight) → Casting (48-72h)

Recipe

BSF extract [mg]	Milli-Q water [mL]	NaOH 0.1 M [mL]	Glycerol [mg]
250	4.5	0.5	125

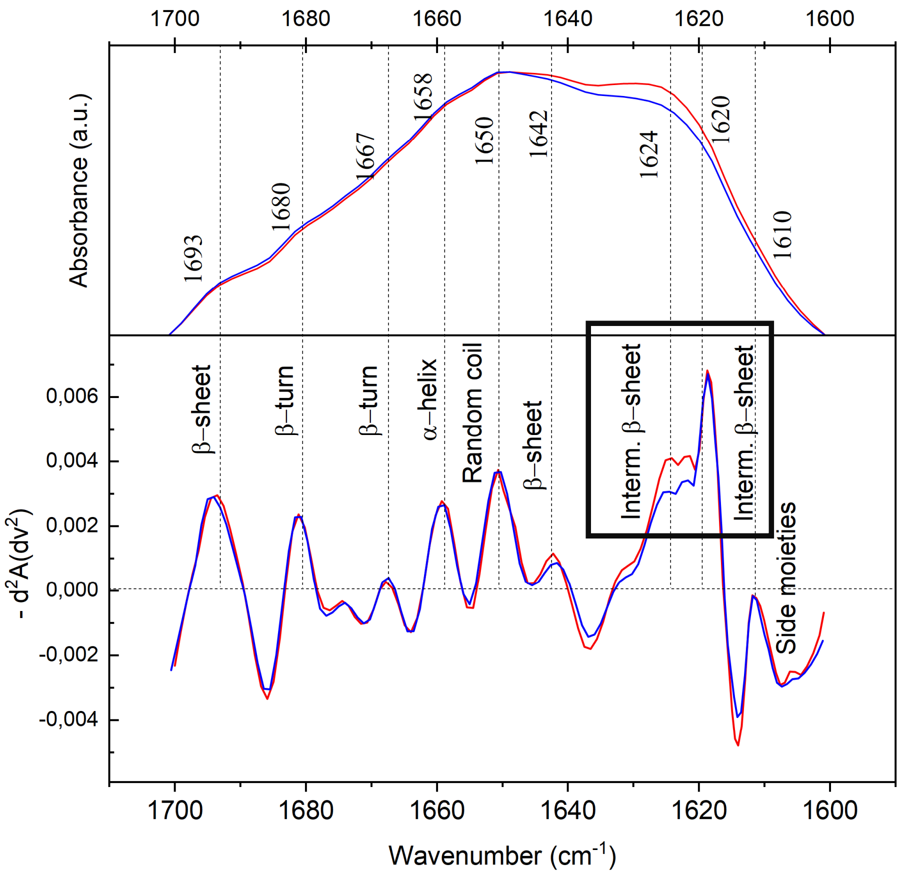
Film. IR analysis



Amide I and Amide II modes in bioplastics and raw protein extracts, with peaks occurring at 1629 cm^{-1} and 1530 cm^{-1}

Film. IR analysis

second derivative band narrowing

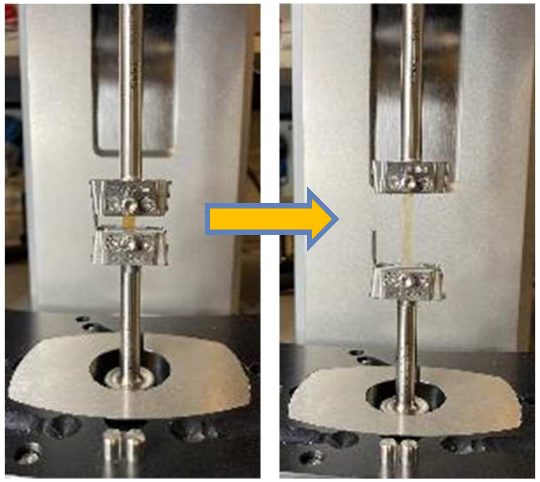


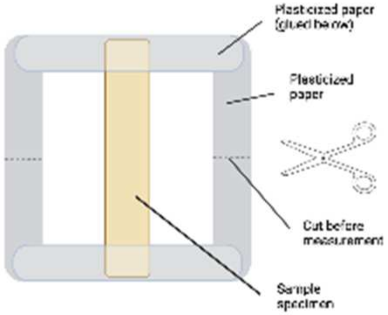
Secondary structure	Larvae (%)	Pupae(%)
β-sheet	19.4	18.1
β-turn	10.7	11.5
α-helix	11.1	11.9
Random coil	14.8	15.9
Interm. β-sheet	44.0	42.7
Total	**100.0**	**100.0**

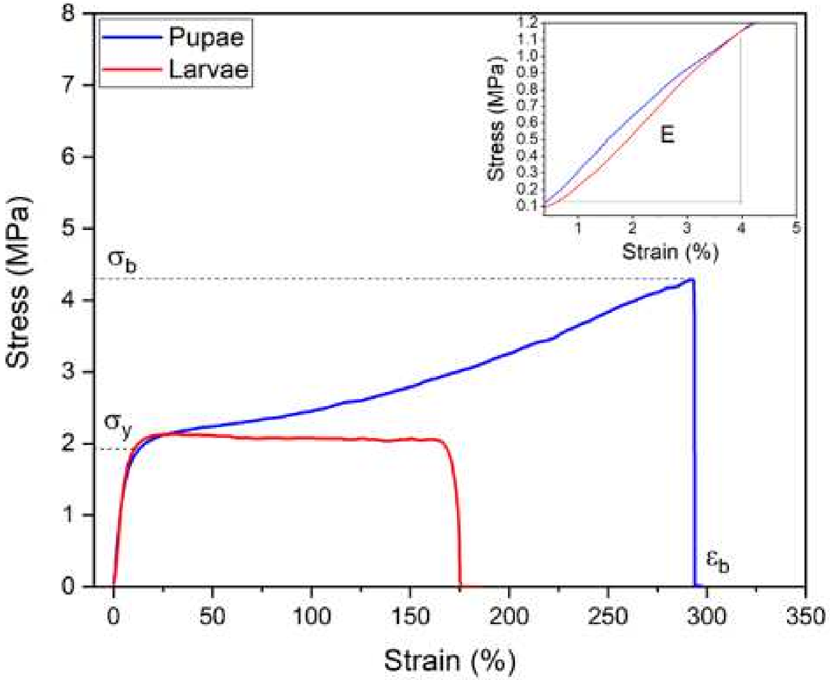
common protein mixtures usually present a profile of the raw Amide I spectra centered around α-helix and random coils

the high relative content of β-sheets and intermolecular β-sheet in BSF protein-based films may be indicative of a high potential of BSF proteins towards a particular type of aggregation

Film from BSF proteins. Tensile properties



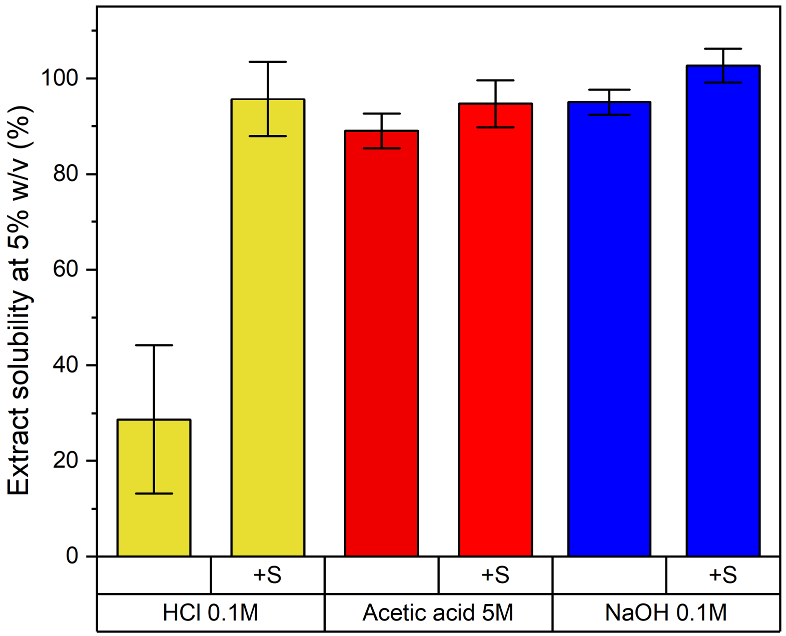




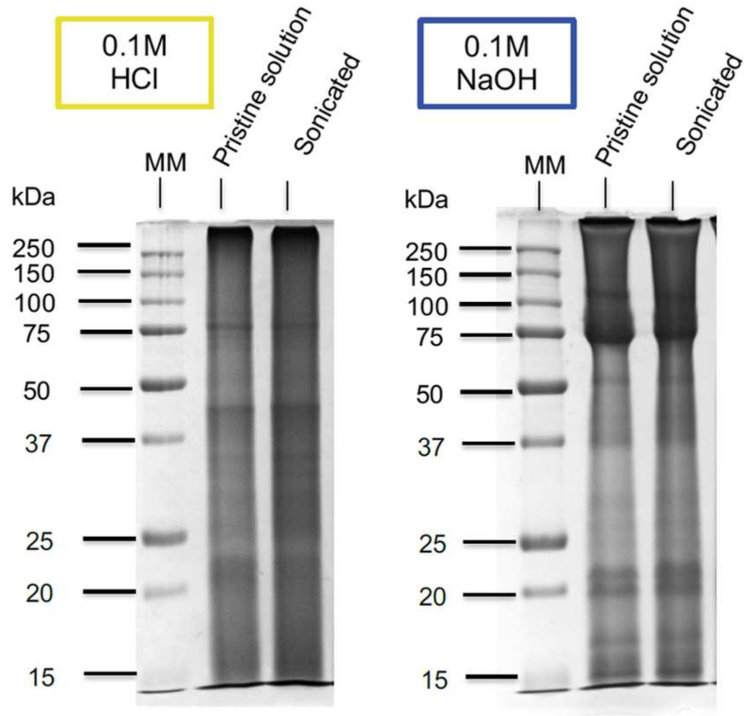
Amiloyd fibrils from BSF proteins

Proteomic analysis

Solubility and Molecular weight



+ S = ultrasonication

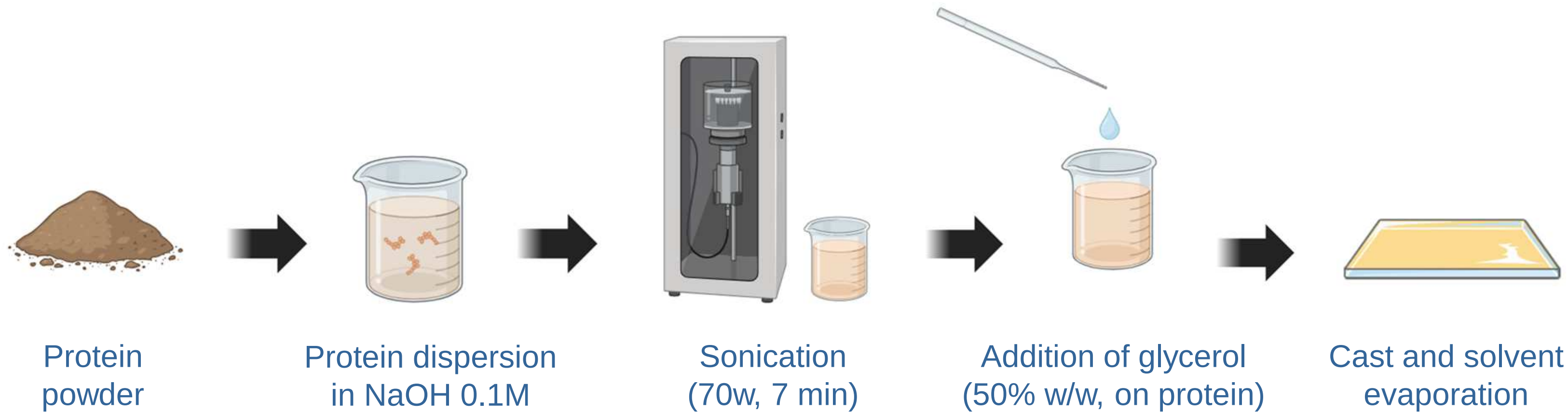


the ultrasonication step did not significantly alter

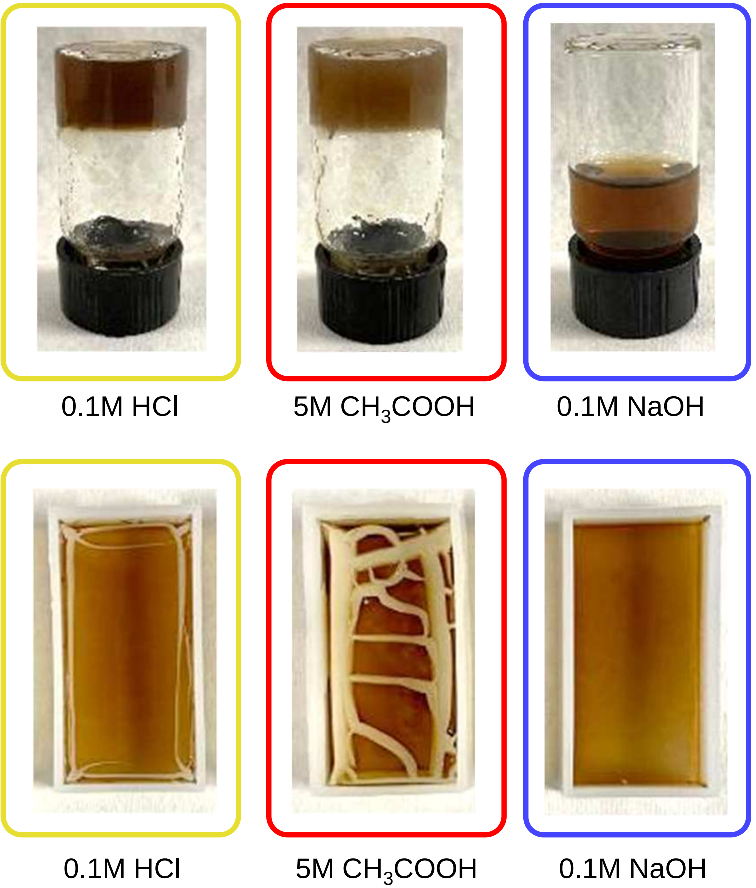
the MW profile of the samples, either in alkaline or acidic water

Edoardo Testa, Elisa Fasoli, Paola Rizzo, Morena Casartelli, Gianluca Molla, Gianluca Tettamanti and Maurizio Galimberti. Amyloid-Like Self-Assembling of Black Soldier Fly Proteins and Development of Bioplastics, *ACS Sustain. Chem. Eng.*, doi.org/10.1021/acssuschemeng.5c07418.

Preparation of films

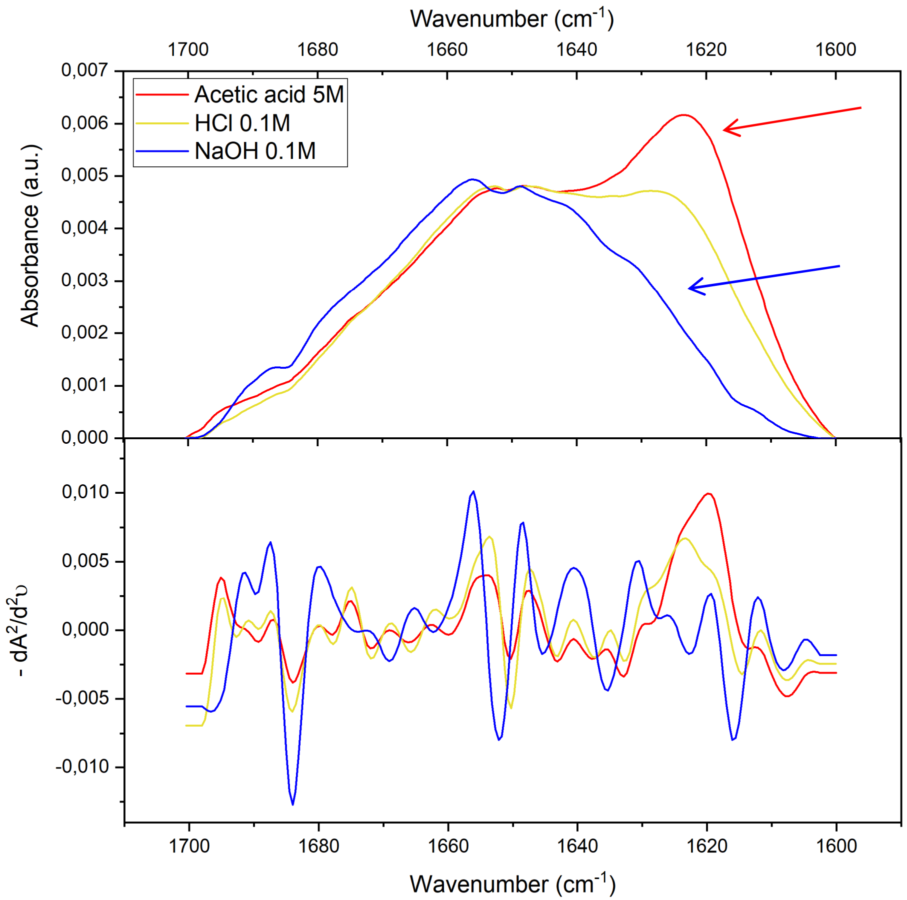


Preparation of films



After solvent evaporation,
only proteins from the 0.1M NaOH solution
led to homogeneous and free-standing films.

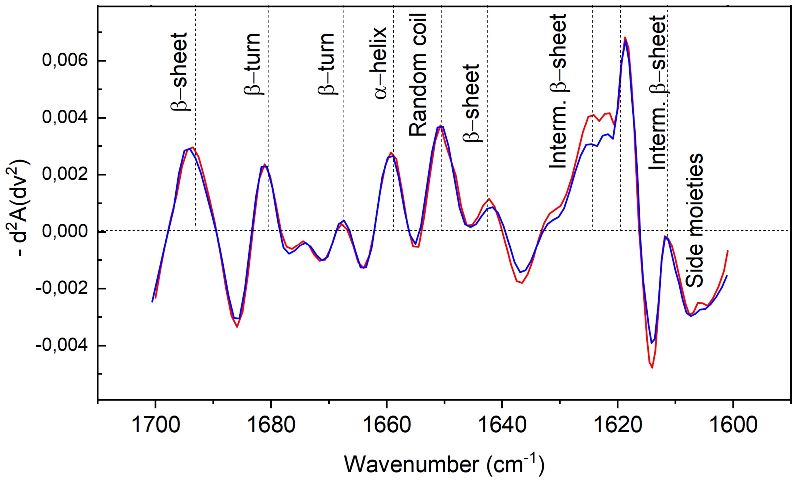
Preparation of films. Intermolecular aggregation between β-sheets



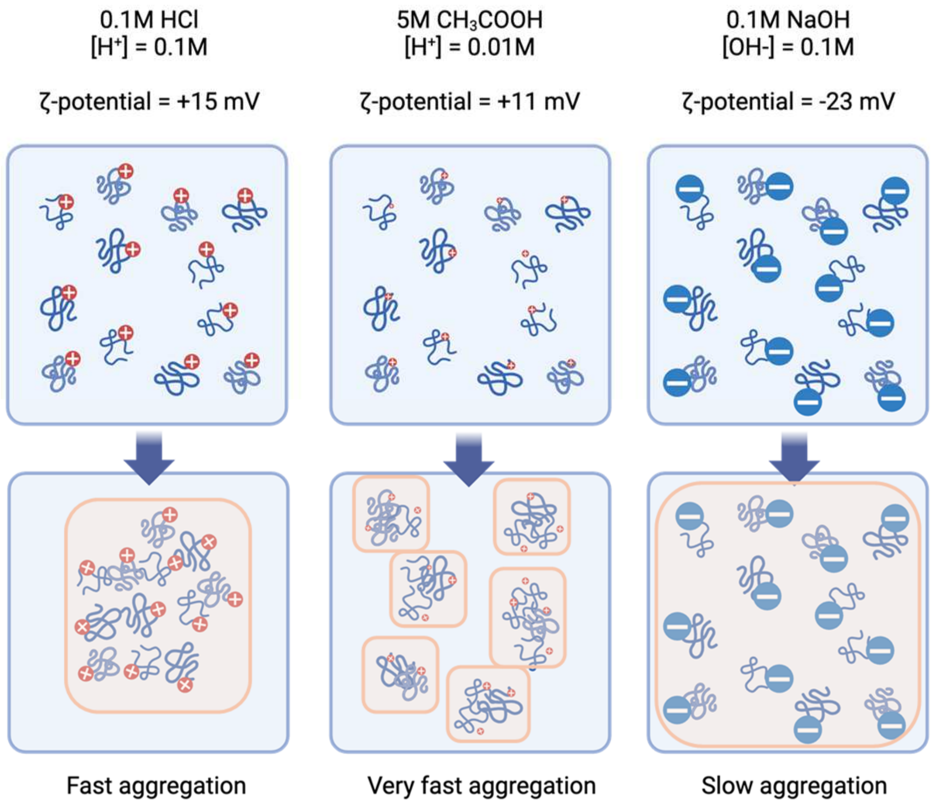
With acetic acid,

a significantly higher ATR-FTIR signal around 1620 cm^{-1}

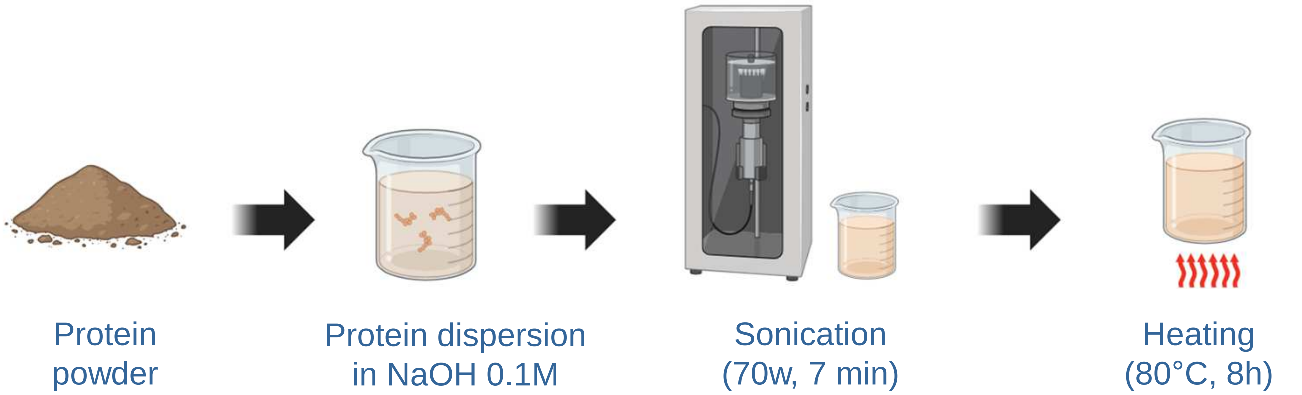
The lowest intensity with NaOH



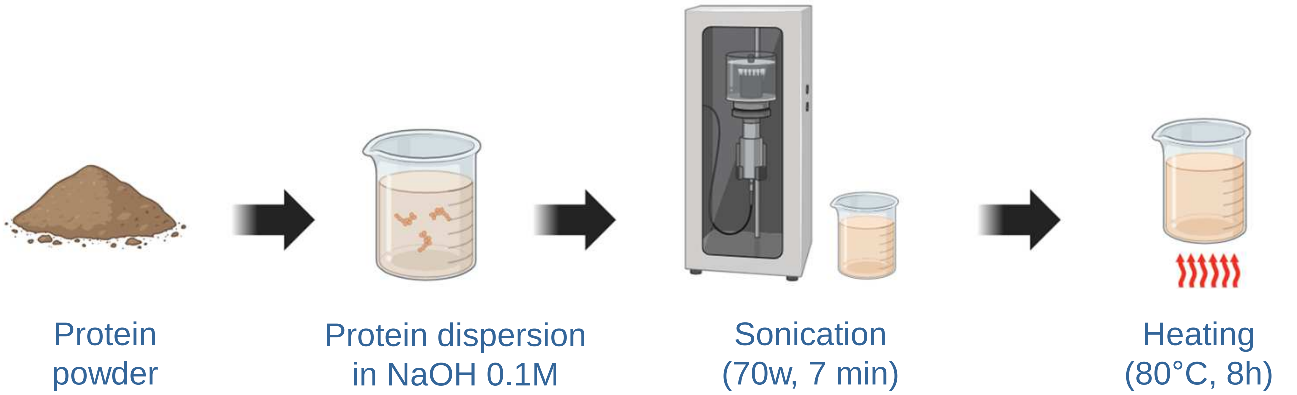
Preparation of films. Intermolecular aggregation between β-sheets

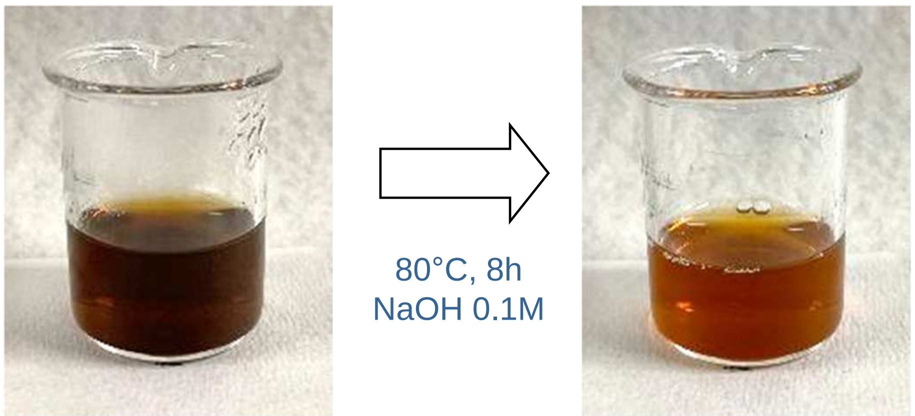


Procedure for fibrillation



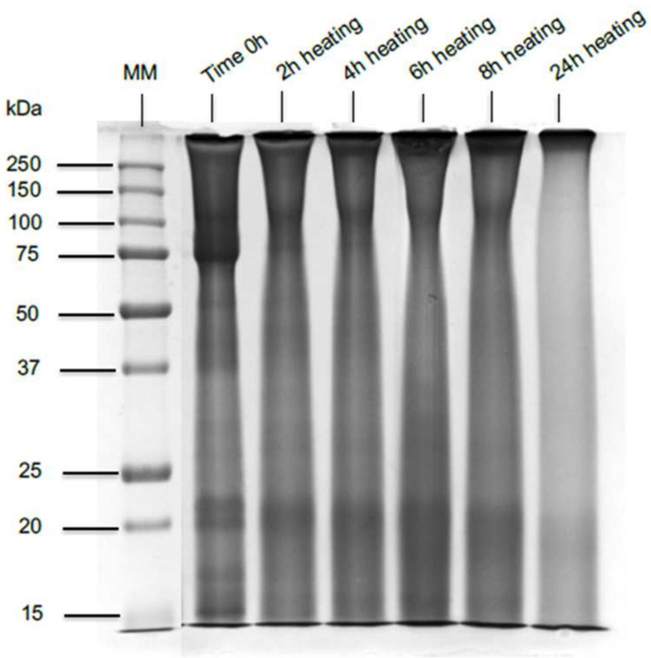
Procedure for fibrillation





Proteomic analysis

Molecular weight

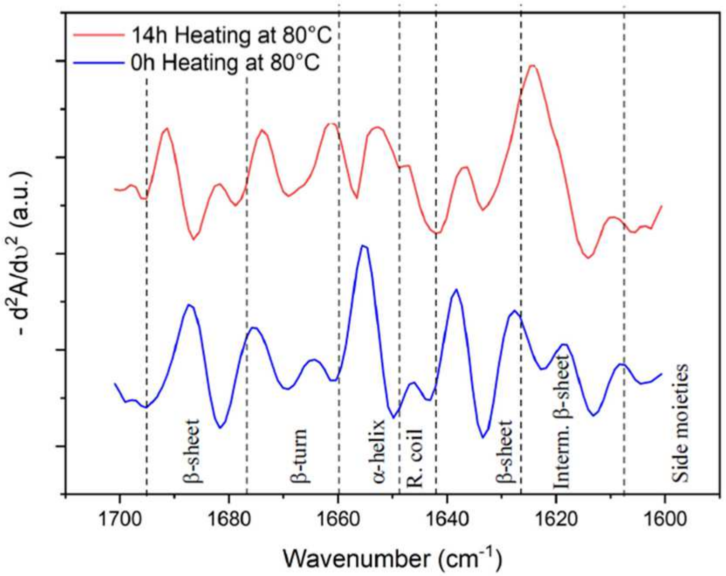


The predominant band around 75 kDa disappeared after 2h heating

Bands in the region between 50-37 kDa and below 20 kDa faded as the heating time increased.

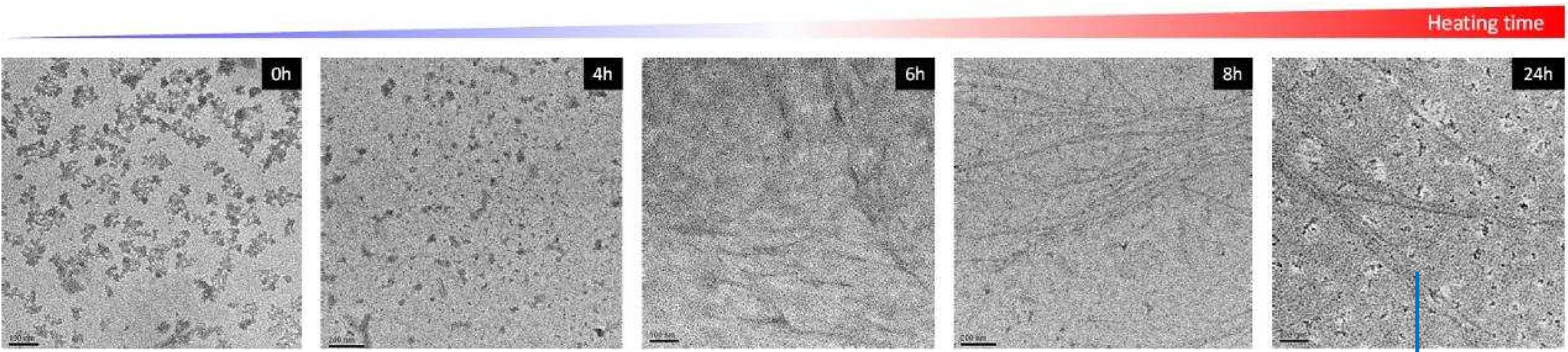
Bands around 20 kDa appeared slightly downgraded

The intermolecular aggregation



Peak centre (cm^{-1})	Secondary structure	Untreated film	Cured film
1695-1685 | 1642-1628	**β-sheets**	48,4	12,4
1680-1660	**β-turns**	11,9	26,2
1659-1652	**α-helices**	33,6	16,5
1650-1644	**Random coils**	0,0	1,8
1625-1620	**Intermolecular β-sheets**	6,1	43,1
< 1615	**Side moieties**	1,4	0,0
		100,0	**100,0**

Amiloyd fibrils from BSF proteins. TEM analysis

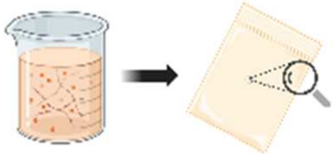


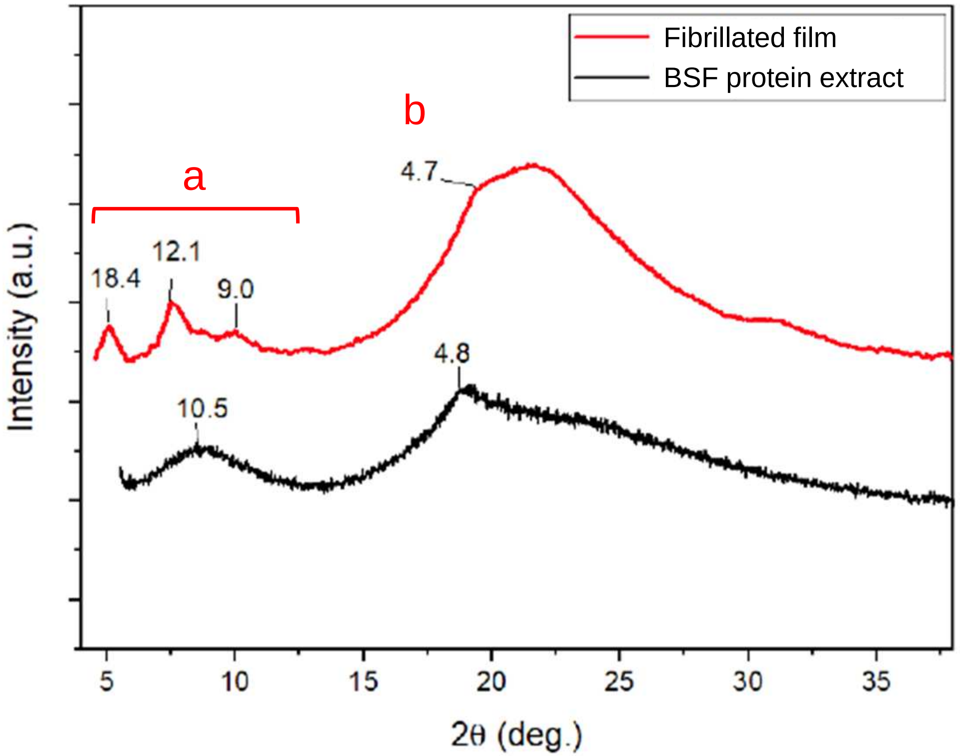
aggregates

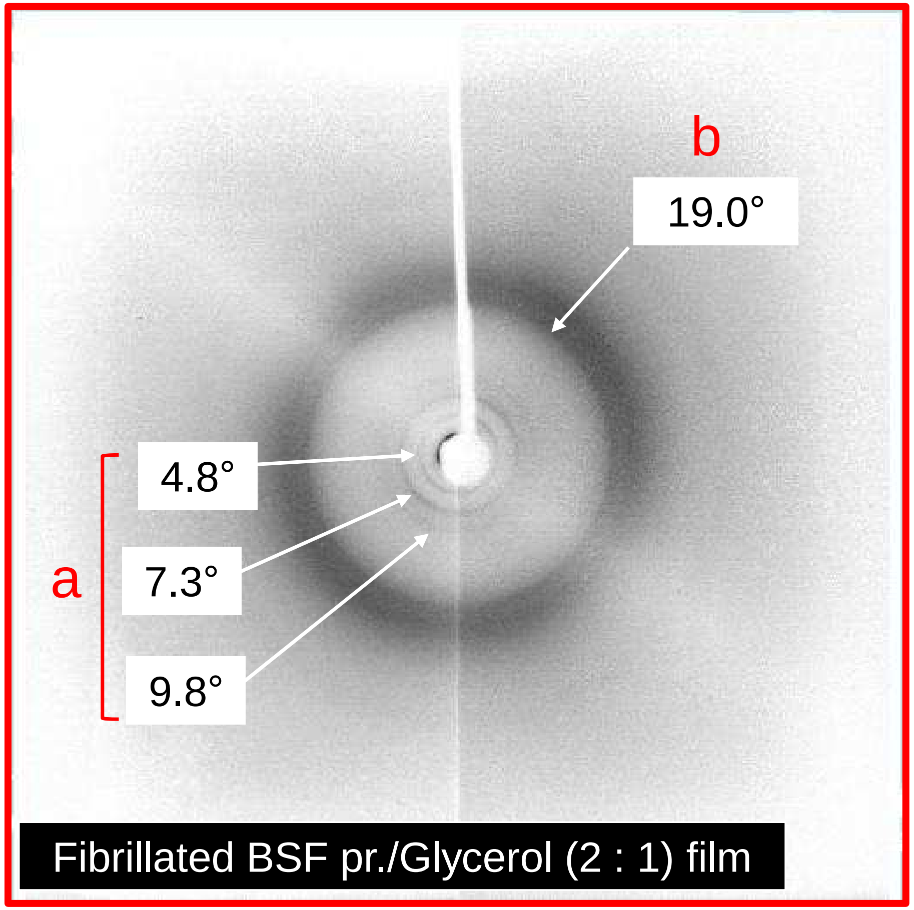
fibrillar aggregates

fibrillar structures
visible throughout the sample

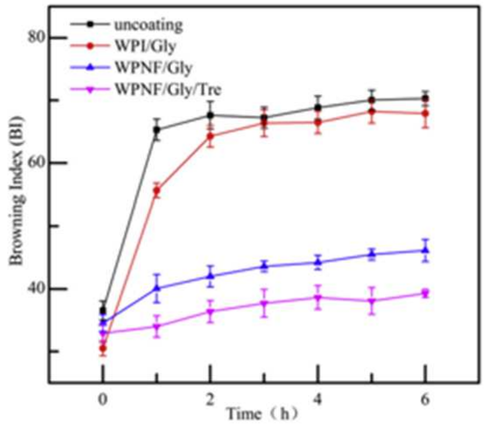
Amiloyd fibrils from BSF proteins. WAXD analysis of films



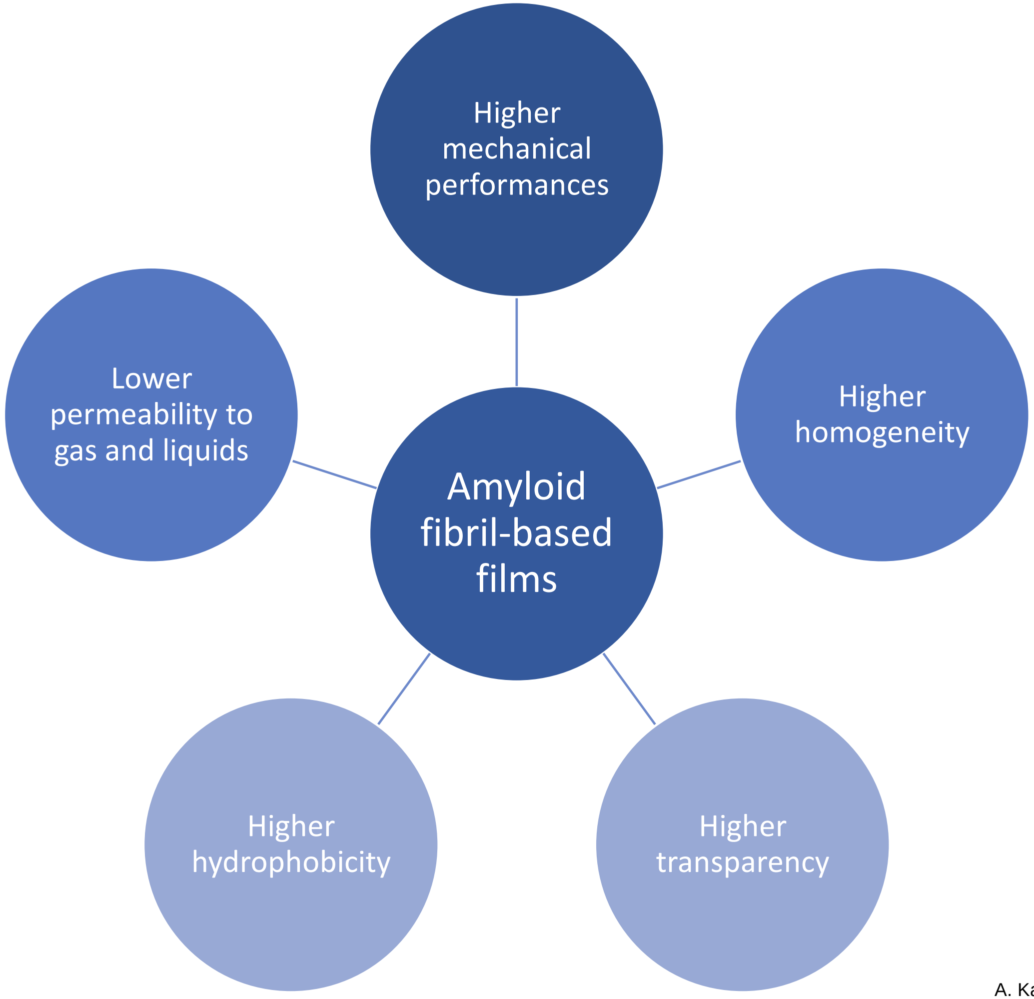


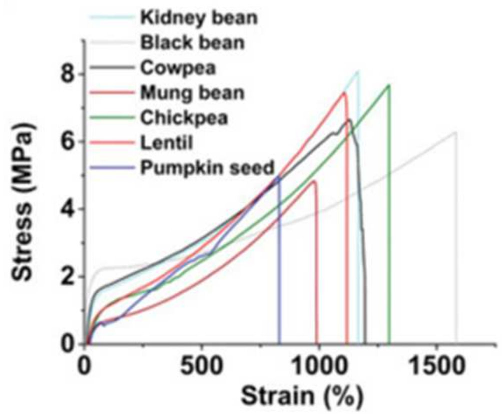


Amyloid fibrils!



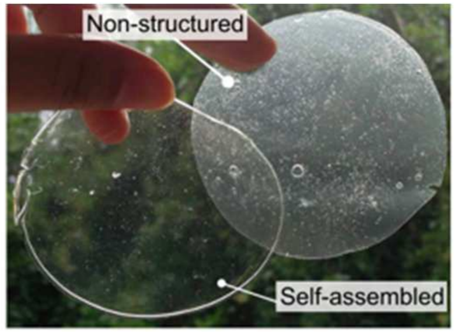
Z. Feng et al. / Food Hydrocolloids 79 (2018) 179e188





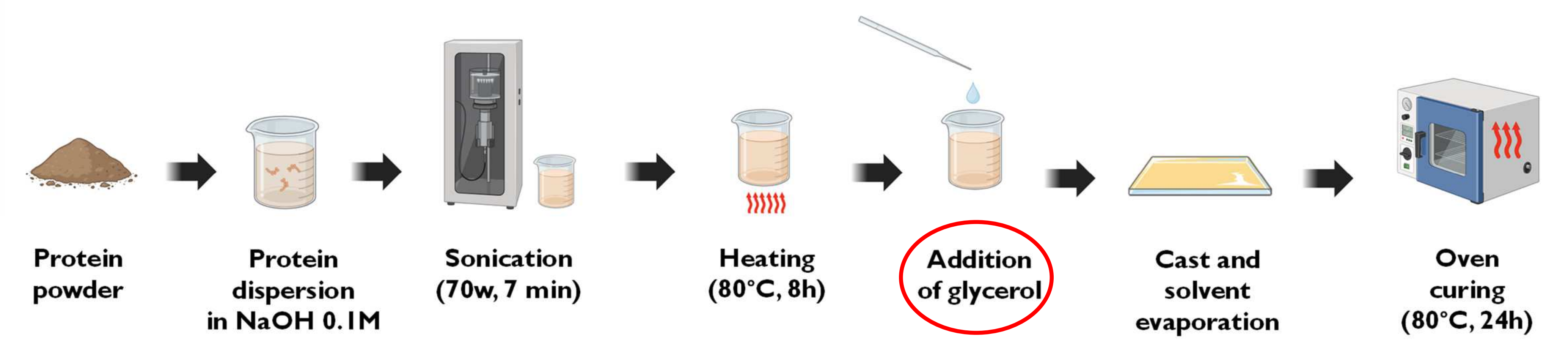
T. Li, J. Zhou, M. Peydayesh, Y. Yao, M. Bagnani, I. Kutzli, Z. Chen, L. Wang, R. Mezzenga, Plant Protein Amyloid Fibrils for Multifunctional Sustainable Materials. *Adv. Sustainable Syst.* 2023, 7 2200414.

M. Peydayesh, M. Bagnani, and R. Mezzenga, Sustainable Bioplastics from Amyloid Fibril-Biodegradable Polymer Blends, *ACS Sustain. Chem. Eng.*, vol. 9, no. 35, pp. 11916–11926, 2021, DOI: 10.1021/acssuschemeng.1c03937.

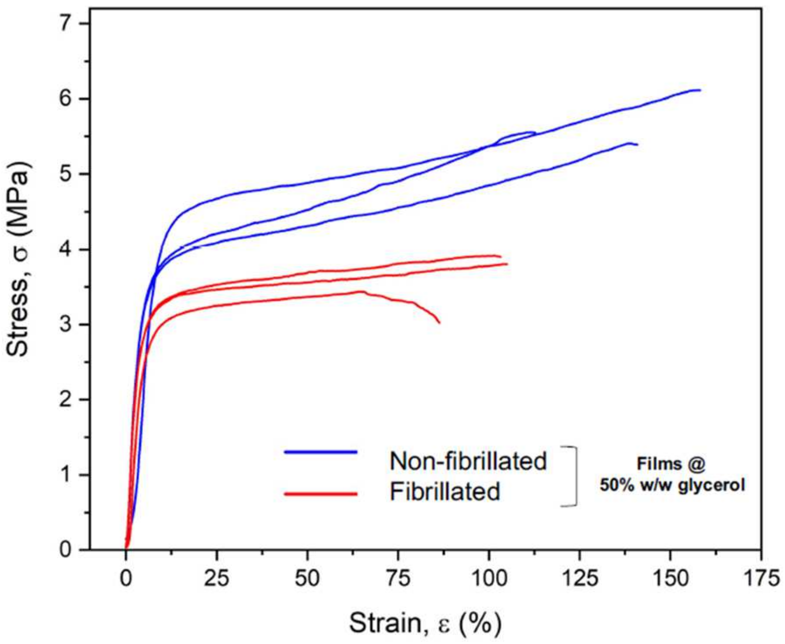


A. Kamada, M. Rodriguez-Garcia, F. S. Ruggeri, Y. Shen, A. Levin, and T. P. J. Knowles,, *Nat. Commun.*, vol. 12, no. 1, pp. 1–10, 2021, DOI: 10.1038/s41467-021-23813-6.

Proteins + glycerol. Preparation of films for mechanical properties

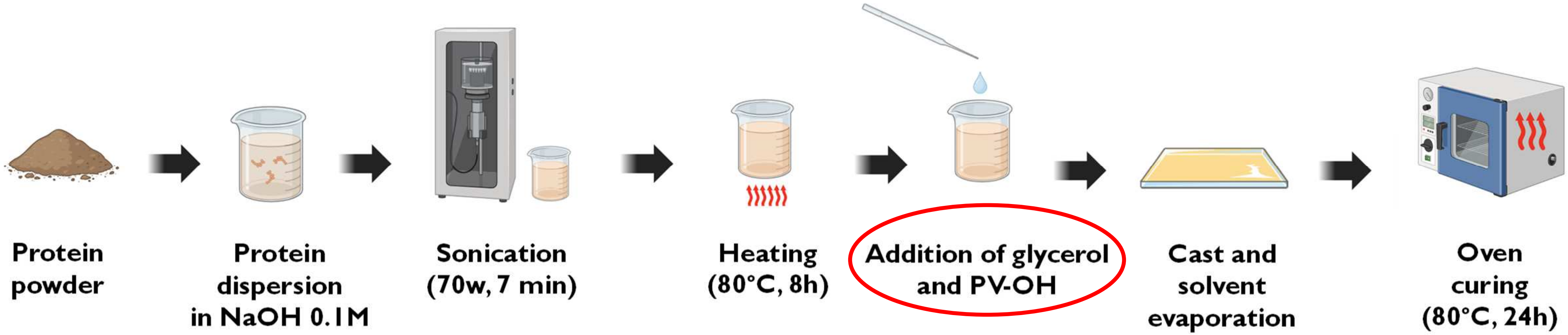


Proteins + glycerol. Preparation of films. Mechanical properties



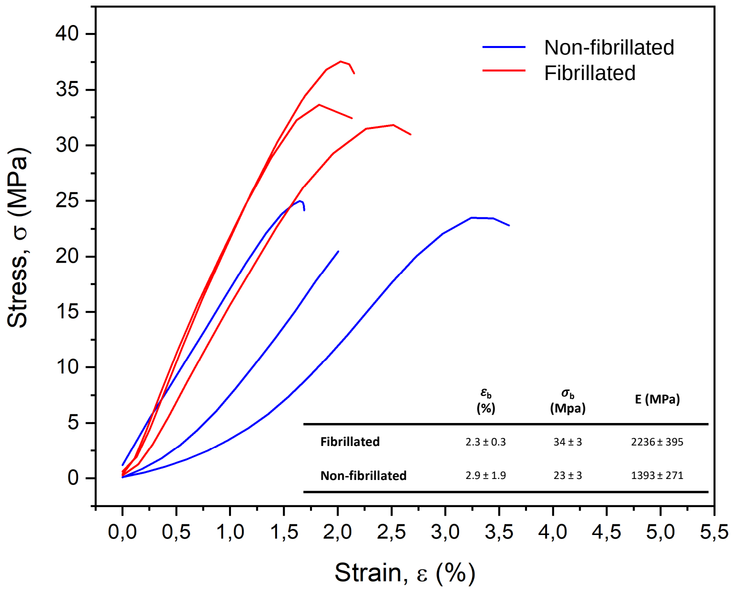
	ε_b (%)	σ_b (Mpa)	E (MPa)
Non-fibrillated	137 ± 23	6.0 ± 0.1	103 ± 26
Fibrillated	98 ± 10	4.0 ± 0.2	104 ± 9

Proteins + glycerol + PVOH. Preparation of films for mechanical properties



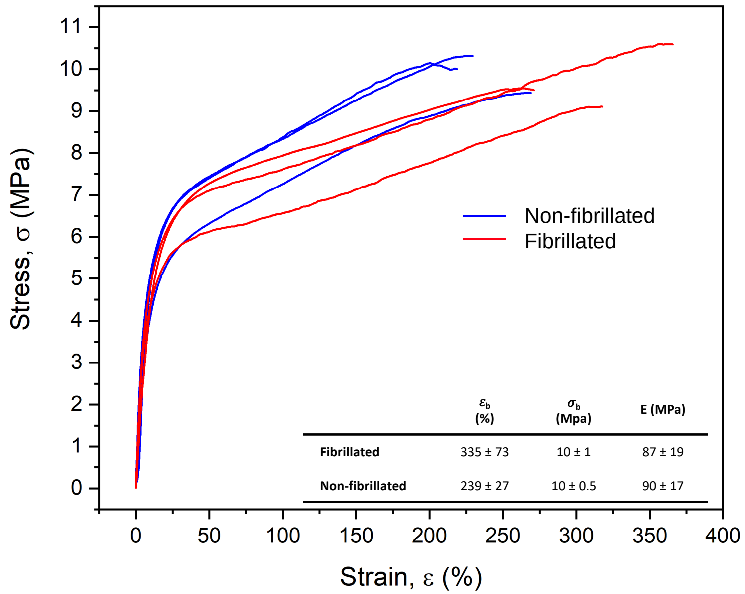
Proteins + glycerol + PVOH. Films. Mechanical properties

Glycerol 0%



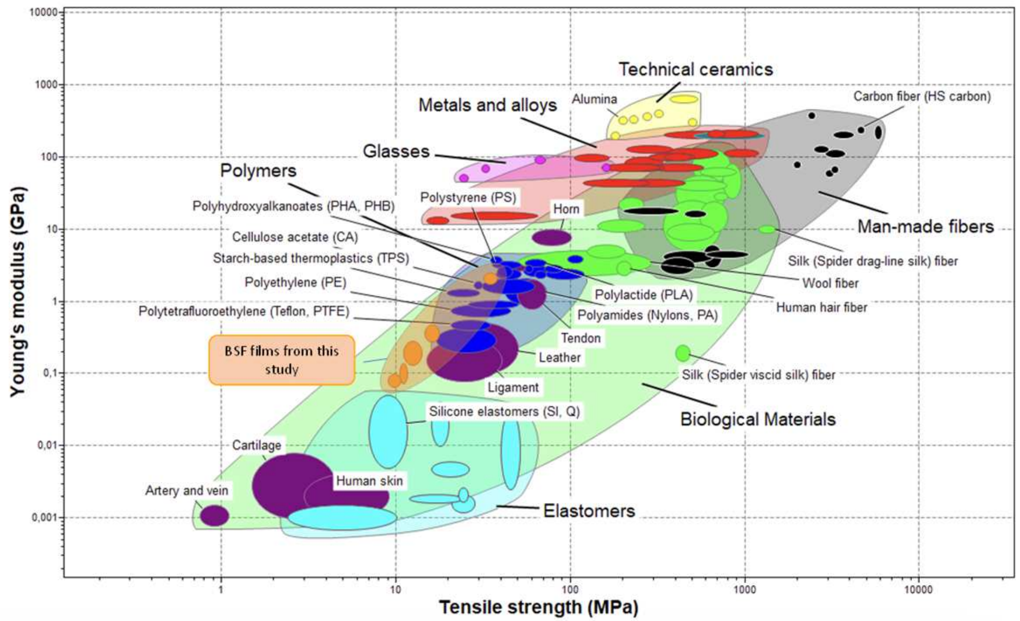
	ε_b (%)	σ_b (Mpa)	E (MPa)
Fibrillated	2.3 ± 0.3	34 ± 3	2236 ± 395
Non-fibrillated	2.9 ± 1.9	23 ± 3	1393 ± 271

Glycerol 50%

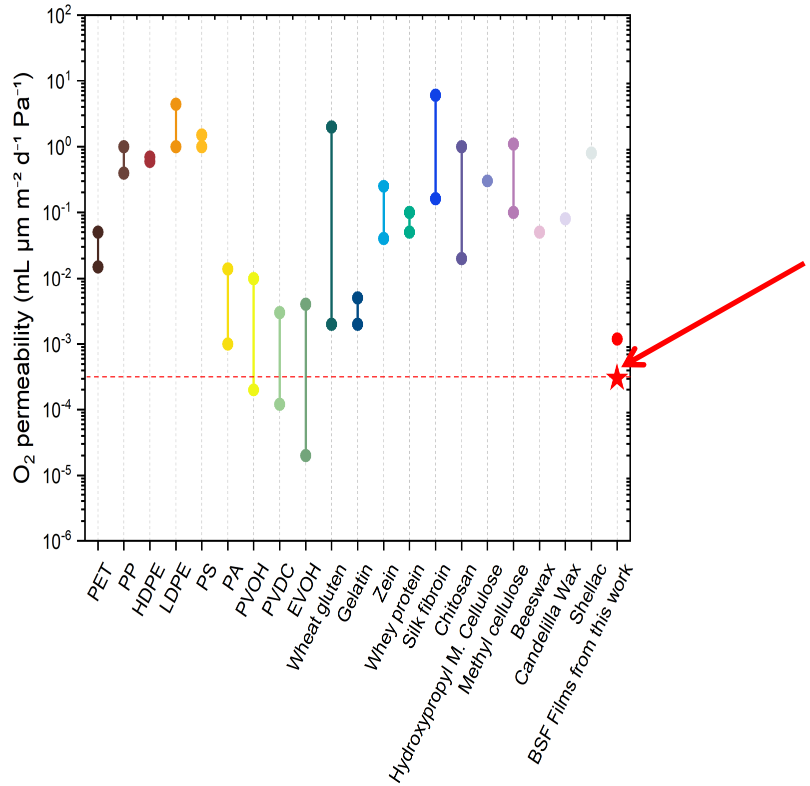


	ε_b (%)	σ_b (Mpa)	E (MPa)
Fibrillated	335 ± 73	10 ± 1	87 ± 19
Non-fibrillated	239 ± 27	10 ± 0.5	90 ± 17

Proteins + glycerol + PVOH. Films. Comparison with other materials

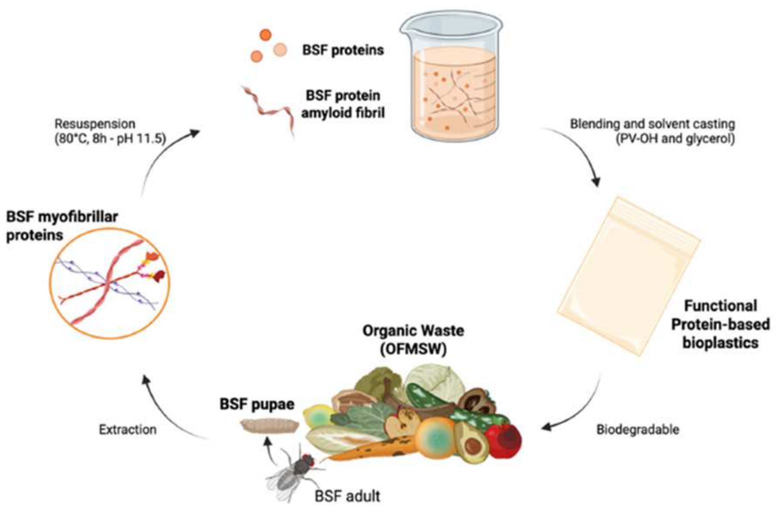


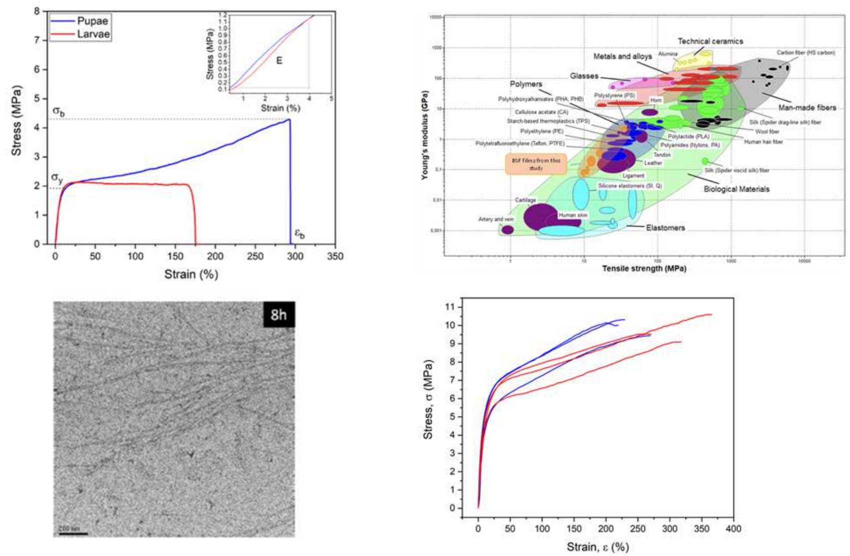
The mechanical properties of the BSF (af), PVOH, and glycerol-based films fall between those of common thermoplastic packaging materials (such as PE and TPS) and biological materials (such as tendons and leather), indicating an intermediate mechanical behavior between synthetic polymers and natural materials.



The films of this work showed very low O2 permeability values. They were comparable to those of polyamides, PVOH, PVDC and EVOH, considered today as the best performing barrier polymers for packaging applications.

Conclusions





•**Efficient Bioconversion**: *Hermetia illucens* (BSF) larvae effectively convert the organic fraction of municipal solid waste (OFMSW) into protein-rich biomass, with high substrate reduction and conversion rates.
•**High-Value Bioproduct Recovery**: Proteins, lipids, and chitin were extracted with high purity, with larvae providing higher protein yields and pupae containing more chitin.
•**Enhanced Bioplastic Performance**: Bioplastics produced from BSF proteins (both larvae and pupae) exhibited good elasticity and solvent resistance, showing potential for applications in flexible electronics and low-humidity packaging.
•**Environmental Impact**: Life cycle assessment (LCA) identified lipid extraction as the most environmentally impactful step, while using pupae reduced overall environmental impact compared to larvae.
•**Socioeconomic Feasibility**: The process offers economic advantages by reducing pre-treatment and transport costs compared to composting, supporting food security and the transition to a circular bioeconomy.
•**Bioplastics**:
BSF proteins are used to create biodegradable bioplastics through amyloid-like self-assembly in alkaline conditions.
•**Fibrillization**:
BSF proteins are solubilized in alkaline conditions (pH 11.5) and heated at 80°C, forming amyloid fibrils characterized by ThT fluorescence, SDS-PAGE, and TEM.
•**Film Production**:
Fibrillized proteins are mixed with PVOH and glycerol to produce films with strong mechanical and gas barrier properties, similar to conventional plastics.
•**Properties**:
The films show improved tensile strength, flexibility, and gas barrier performance. Glycerol enhances flexibility, while PVOH enables thermal weldability.

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Thanks for your attention!

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