Red Clover as a Novel Source of Protein for Human Consumption: Processing Using High Moisture Extrusion

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Potential use of UK grasslands

~40% of the UK is **pastures** and semi-natural **grasslands**, home to forage crops (grasses and legumes)

Main use

- Grown for animal grazing or conserved as hay/silage

Research into other applications

- Modified fibres and cellulose
- Fertilisers
- Biogas and bioethanol
- Lactic acid and plastics

Human consumption

- Not suitable for direct consumption: unpalatable and high in undigestible fibres
- Requires extraction, fractionation, purification and/or chemical and biological conversion

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ibers

- Biorefinery of forage crops to separate key fractions

Red Clover (*Trifolium Pratense*)

- Leguminous forage crop commonly fed to livestock in the form of silage.
- Used as a rotational crop
- Leaves and stems contain the most protein
- The crude protein content averages between 15-30% (dry weight) compared to soybeans having 44-49%.

Attributes

•Amino acids
•Sugars
•Minerals
•Vitamins
•Polyphenols
•Grows quick and the UK is favourable
•Fix nitrogen

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Soy vs. Leaf Protein

	Clean label	Protein content	Allergenicity	GMO free	Environmental sustainability
Leaf Protein 					
Soy Protein 					

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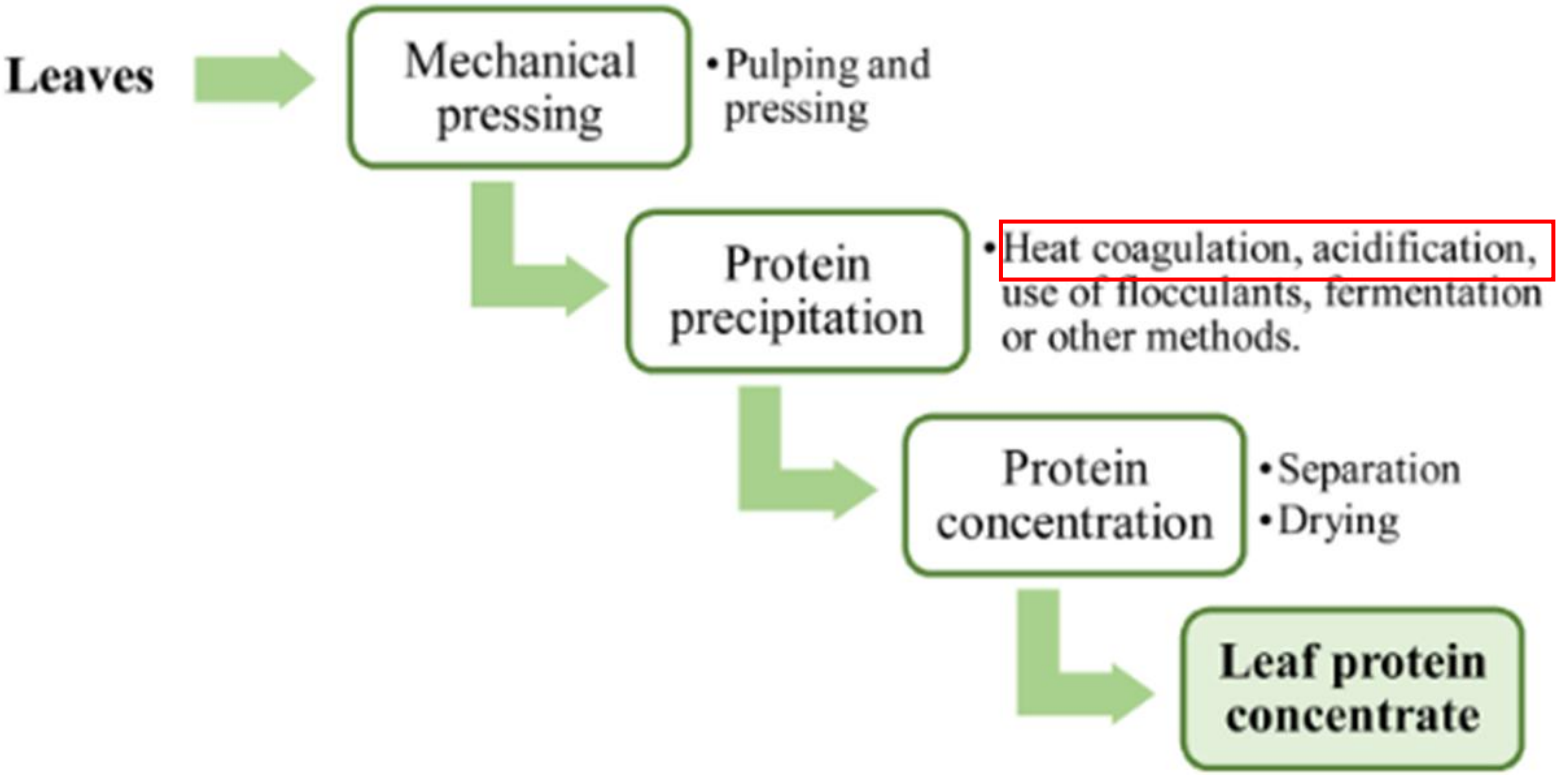
Isolation of protein from forage crops (Red Clover)



Protein quality and quantity tests

- SDS-PAGE
- Protein yield (DUMAS and mass balance)
- In-vitro protein digestibility
- Amino acid profile
- Protein functional properties
- Phenolics

Santamaría-Fernández & Lübeck, 2020

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Pilot-scale protein isolation and quantification

Three protein fractions:

- **RCWP**- acetone/ethanol wash t0 removed the green colour
- **RCGP1**- freeze-dried after being frozen for 1 day
- **RCCP2**- freeze-dried after being frozen for 1 week

RCWP **RCGP1** **RCGP2**

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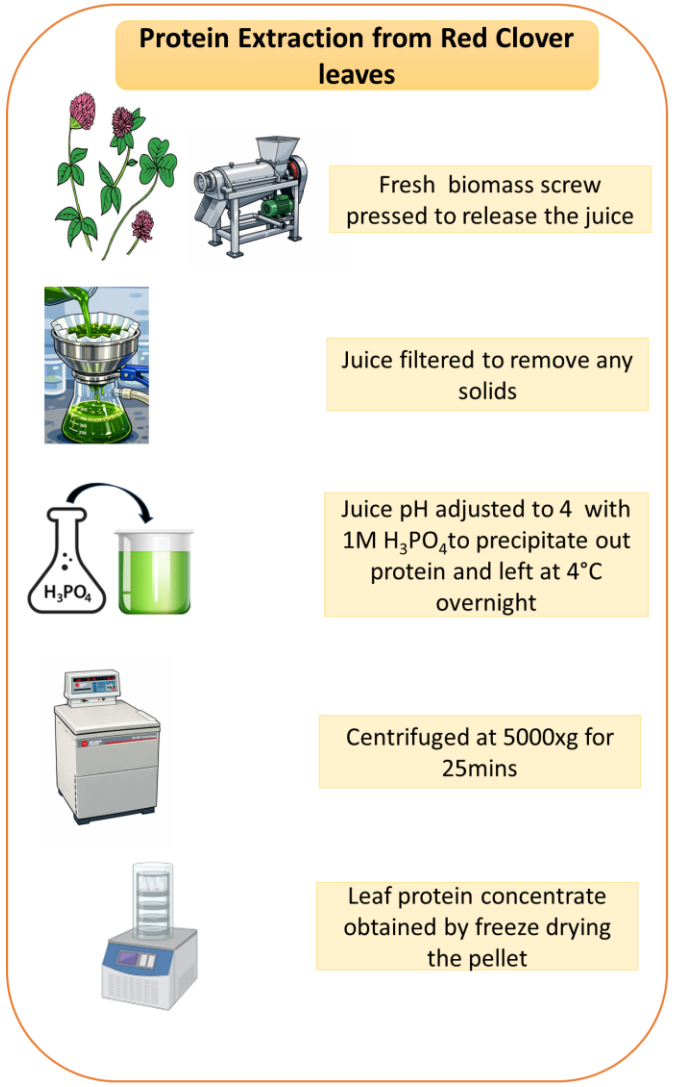
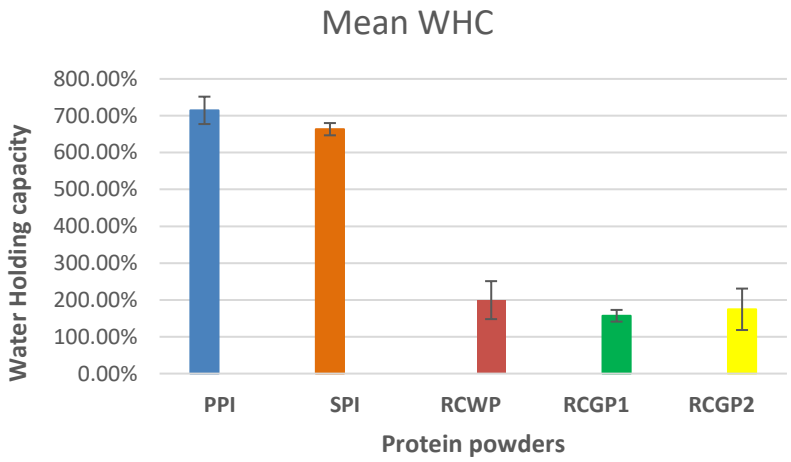
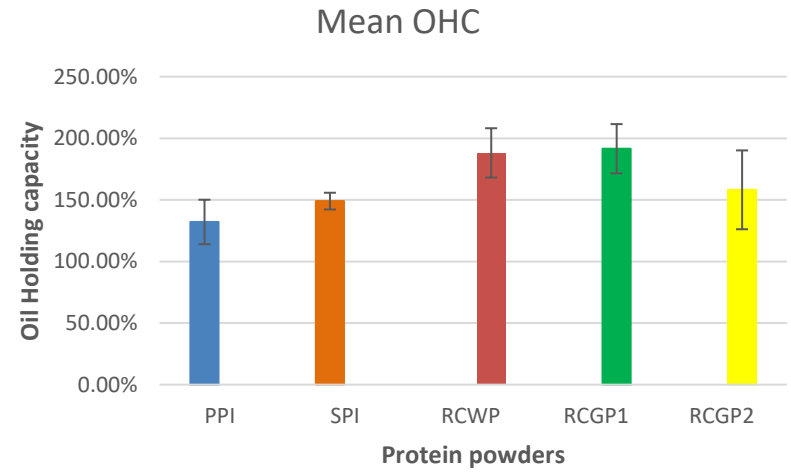


Proximate composition and Protein Functionality

Test sample	RCGP1	RCGP2	RCWP(RCFP)	SP1 90	PPI
Protein (%)	51.69±1.51	50.84±1.14	71.80±1.67	88.89±0.30	81.5
Moisture (%)	3.37 ± 0.09	3.51 ± 0.27	4.23 ± 0.18	3.00	7±1
Fat (%)	5.19	5.29	0	0.75±0.25	0.4
Ash (%)	6.6	6	7.2	5±1	4.8
WSC (%)	2.98	3.24	0.5	0.56	7.2
Starch (%)	0.72 ± 0.14	0.68 ± 0.03	0.77 ± 0.00	0.25 ± 0.01	
pH	3.98	4.03	4.55	7.63	7.27





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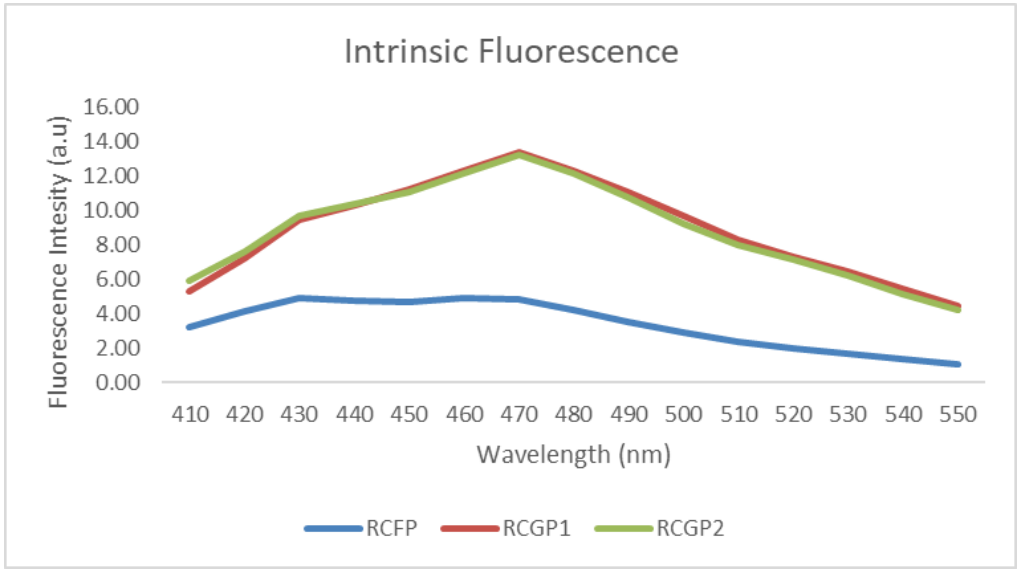
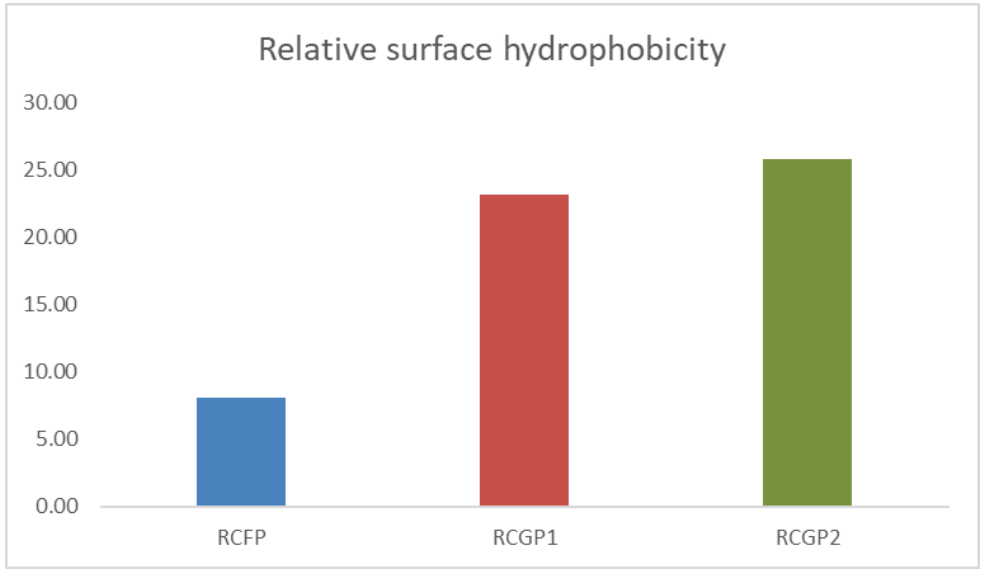
Protein functionality

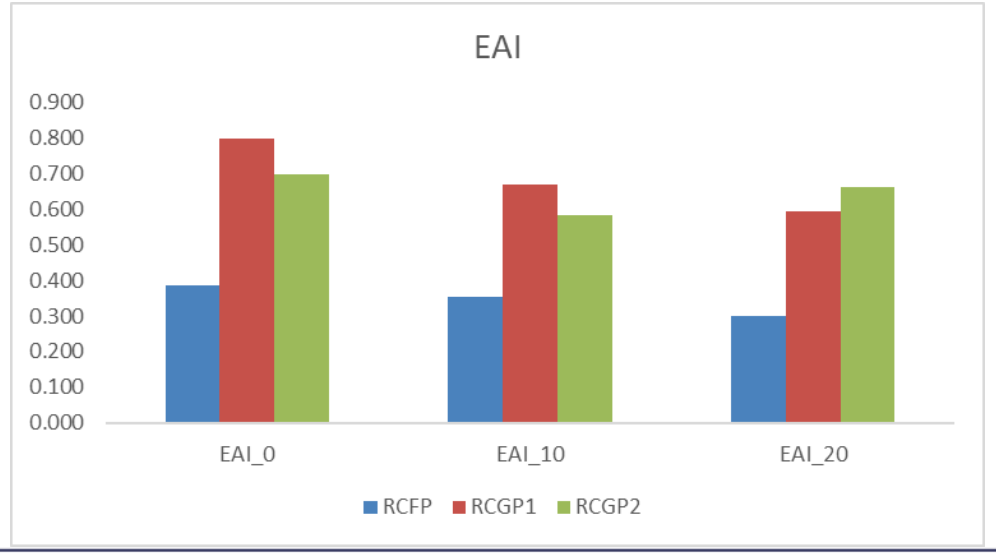




Emulsion Stability

Sample ID	ABS_0min	ABS_20min	ESI
RCWP/RCFP	0.032	0.027	66.31
RCGP1	0.085	0.064	39.79
RCGP2	0.083	0.067	53.34

Emulsion Capacity



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Rheology to assess gelation

Sample	G' (Pa)	G" (Pa)	Tan δ	Comment
RCGP1	0.201	0.215	1.07	No gel
RCGP2	0.388	0.411	1.061	No gel
RCFP	0.318	0.140	0.441	Weak gel

Rheology showed that RCP forms weak or no gels thus we blended 10% of RCP with 90% of PPI to assess if blending had an effect on both proteins.

Sample	G' (Pa)	G" (Pa)	Tan δ	Comment
PPI+RCGP1	8.10922E-06	2.12759E-06	0.262	Weak gel
PPI+RCGP2	1.71142E-07	3.46588E-08	0.203	Weak gel
PPI+-RCFP	0.00017	3.75037E-05	0.219	Weak gel

Blending affected both G' and G'' significantly ($G' > G''$,) and the RCP fractions that had no gelation previously formed weak and predominantly elastic gels.

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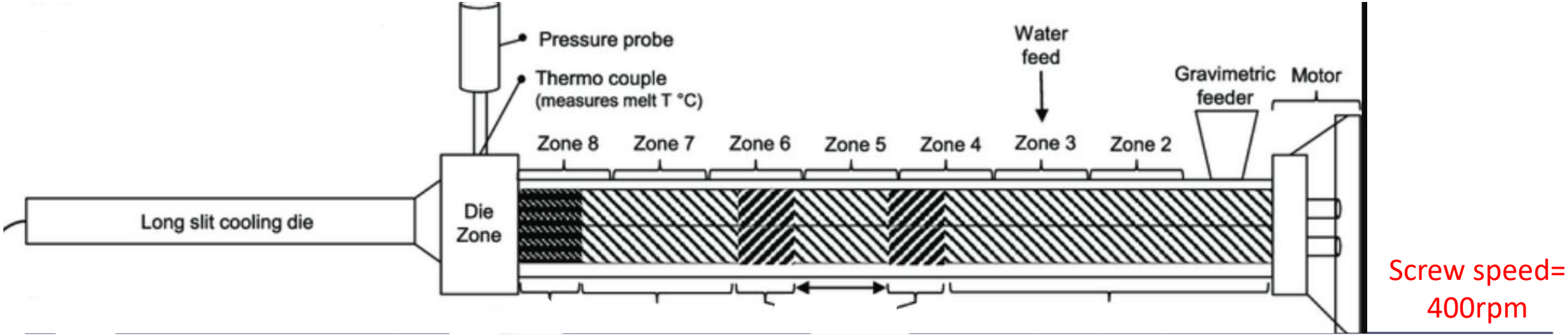
High Moisture Extrusion (HME)

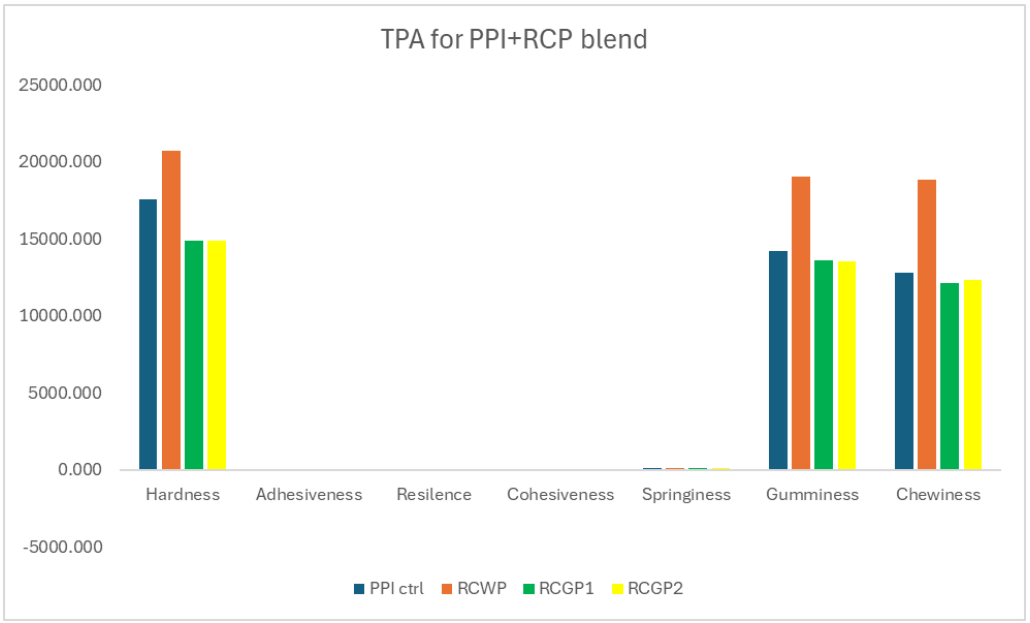
- RC protein was extruded as a blend with Pea Protein Isolate (PPI).
- It was 10% of RC protein and 90% PPI blend
- PPI was used as control
- This was extruded at the optimum conditions of the extruder;

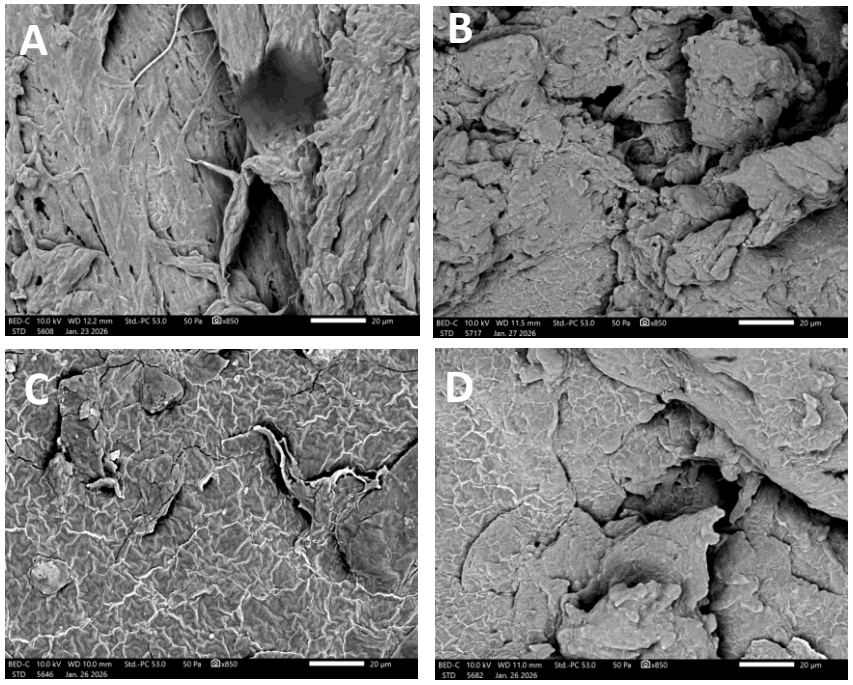


120°C 140 °C 150 °C 130°C 100°C 80°C 60°C 40°C

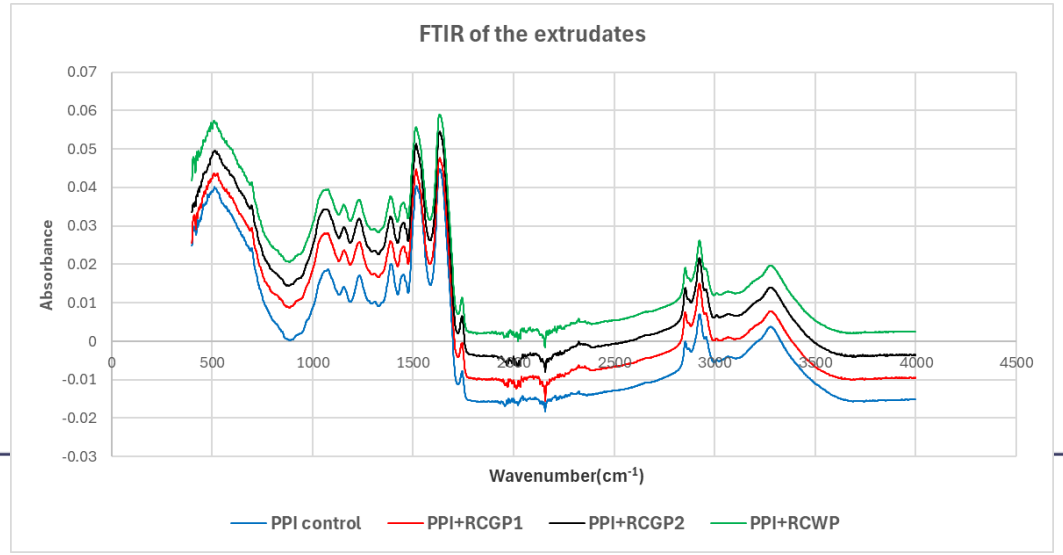
Texture Profile, Microstructure and Secondary Structure Analysis after Extrusion

PPI PPI+RCWP PPI+RCGP





A- PPI
B- PPI+RCWP
C-PPI+RCGP1
D-PPI+RCGP2



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Conclusions

1. The extraction method and other components such as phenolics affect the protein functionalities, thus how Rubisco is isolated from the leaves needs to be taken into consideration.

2. Rheology results for the RCP proteins show either no gelling or weak gels and this was due to less solubility of RCP protein in water, and this affected the PPI gelling as well in the blends.

3. Blending PPI with RCWP significantly affected the texture, appearance and microstructure of the extrudates (rougher texture), but RCGP only the appearance changed and to some extent the microstructure.

4. FTIR indicates no new chemical bonds or drastic secondary-structure changes, but some re-organisation or redistribution of existing structures.

5. Rubisco from RC still offers a promising alternative for human consumption but more robust protein isolation and purification method need to be developed to prevent compounds like phenolics, chlorophyll and PPO from interfering the protein functionalities.

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Future works:

GC-GC analysis and E-tongue to assess off flavours

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