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Membrane chromatography to fractionate plant proteins from rapeseed and pea seed: a promising approach

Objective: evaluate the feasibility of replacing resin chromatography with membrane techniques for the purification of plant globulins from oleaginous and pulse proteins sources.

7S & 11S globulins from pea

The fractionation of proteins extracted from pea flour (protein content = 21.5%, dry basis) on chromatography resin (HitrapFF Q Cytiva, 1mL) was compared with membrane fractionation (Mustang® Q XT Acrodisc® Pall, 0.86mL) and electrophoresis of the fractions eluted by NaCl gradient allowed the identification of proteins present in each peak.

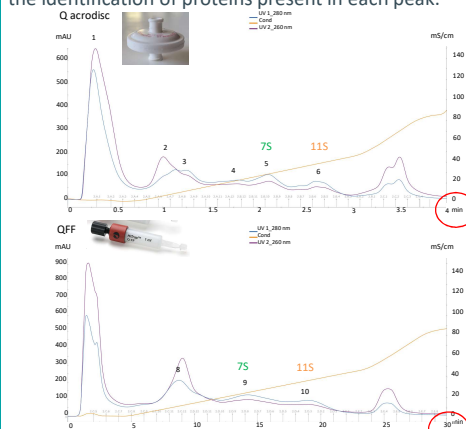


Figure: Separation of pea proteins using anion exchange. Chromatogram of membrane (Mustang Q acrodisc at 8mL/min) and resin chromatography (QFF at 1mL/min) (binding buffer: 50 mM Tris, pH 8.5; eluting buffer: 50 mM Tris, pH 8.5 1M NaCl; linear elution gradient length: 20 mL)

The scale-up was evaluated using the Sartobind® Q 20mL membrane (Sartorius) with step elution. Multiple cycles were repeated, with cleaning steps between them, to produce larger amounts of protein: from 110g of pea flour, 3g of 7S and 0.7g of 11S globulins were produced in 13 runs lasting approximately 4h.

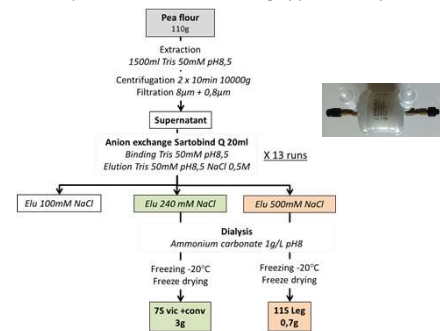


Figure: Schematic of the preparatory-scale purification processes for pea 7S and 11S globulin.

Table: Fixation capacity of pea globulins per hour per mL of column.

	Resin Membrane		
	Column volume (ml)	200	20
Total purification time (h)	6	4	
Purified 7S amount (g)	9	3	
Purified 11S amount (g)	3	0,7	
7S capacity (mg / h / ml column)	8	38	
11S capacity (mg / h / ml column)	3	9	

↪ Separation of 7S and 11S globulins: 4 min for membrane, 30 min for resin.
↪ Binding capacity: 45 mg of protein/mL of membrane ≈ resin.

↪ Productivity increased with membrane compared to resin at preparative scale.

12S globulin & 2S albumin from rapeseed

The proteins were extracted from rapeseed meal (protein content = 38.6%, dry basis) then desalted. Their fractionation on chromatography resin (HitrapFF SP Cytiva, 1mL) was compared with membrane fractionation (Mustang® S Acrodisc® Pall, 0.86mL) and electrophoresis of the fractions eluted by NaCl gradient allowed the identification of proteins present.

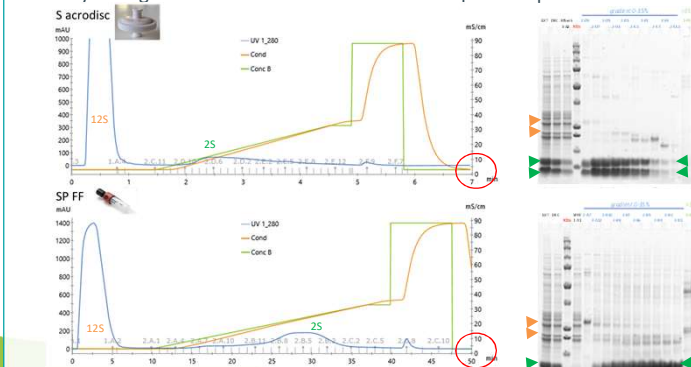


Figure: Separation of rapeseed proteins using cation exchange. Chromatogram of membrane (Mustang S acrodisc at 8mL/min) and resin chromatography (SP FF at 1mL/min) (binding buffer: 50 mM Tris, pH 8.5; eluting buffer: 50 mM Tris, pH 8.5 1M NaCl; linear elution gradient length: 28 mL)

Figure: SDS-PAGE of eluted fractions stained by Coomassie blue. 12S in orange and 2S green

The membrane binding capacity was measured by continuous feeding until saturation was achieved, as determined by a UV trace that asymptotically flattened out → 4 mg of protein/mL of membrane.

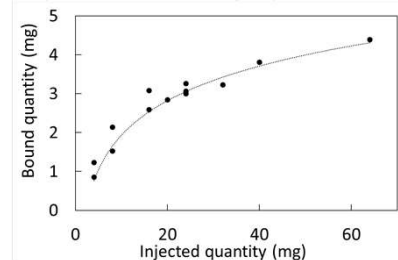


Figure: 2S albumin equilibrium adsorption on Mustang® S acrodisc membrane.

↪ Lower binding capacity for 2S albumin in the membrane compared to resin.

↪ Upscale on Sartobind® 20mL allowed the production of 5g of 2S albumin and 11g of 12S globulins

Conclusion

Membrane technology advantages: high flow rates, low pressure drop and ease of scale-up.

↪ Efficient fractionation of plant proteins in a shorter time compared to resin chromatography, thus increasing productivity.

↪ Interest of the process to produce food ingredients at industrial scale.

