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# Preparative chromatography for purification of 7S globulin from pigeon pea seeds to improve foaming properties

**Objective :** To extract, purify, characterize and evaluate the functional properties of 7S globulin and compared to total protein extract from pigeon pea flour

## 7S purification

Pigeon pea is a protein-rich pulse (18-28 wt %) native to emerging countries in Asia and Africa. Globulins comprise the largest portion of pigeon pea proteins (around 60%), with the 7S fraction being the most abundant. From pigeon pea commercial flour, a purification strategy was optimized at laboratory scale. Then, a preparative scale procedure to purify the 7S globulin fraction was developed using **mild protein extraction** process and the following chromatographic steps: **desalting** of the protein extract; separation on an **anion exchange** column, and subsequent separation on a **gel filtration** column was established.

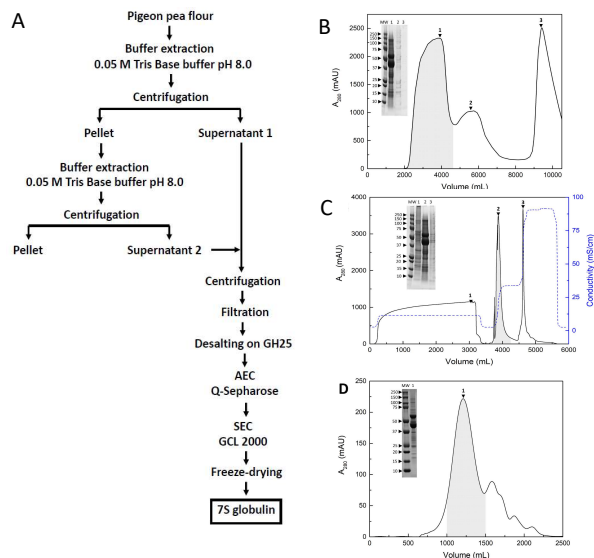


Figure A: Schematic of the preparative-scale purification processes of pigeon pea 7S globulin.

Figure BCD :

(B) Elution profile in the desalting column Cellufine GH25 (JNC).

(C) Elution profile in the anion exchange column DEAE FF (Cytiva).

(D) Elution profile in the size exclusion column Cellufine GCL2000 (JNC).

The inset shows SDS-PAGE analysis of numbered fractions. The gray filled peak corresponds to the 7S globulin fraction.

## Yield & purity

From 200 g of flour (~33.6 g proteins), 4.4 g of the purified 7S globulin fraction was obtained containing about 89 % protein in w/w dry matter.

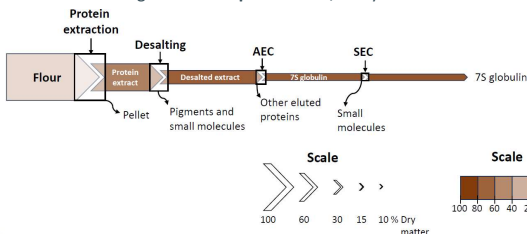


Figure: Distribution of dry matter and protein content during preparative-scale purification. AEC: anion exchange chromatography. SEC: size exclusion chromatography.

## Analytical characterization

The protein composition of the purified 7S globulin fraction was characterized by SDS-PAGE and proteomic analysis confirming purity and identification of **beta-conglycinin  $\alpha$ - (64 kDa) and  $\beta$ -chains (48 kDa)**.

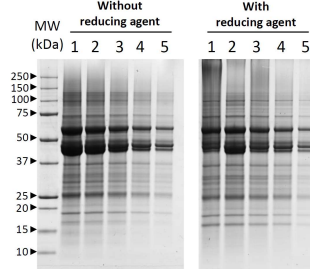


Figure: SDS-PAGE of pigeon pea 7S globulin in the presence or absence of reducing agent (2- $\beta$ -mercaptoethanol). Concentrations of 2.0, 1.5, 1.0, 0.5, and 0.25 mg/mL of proteins are indicated by the numbers 1, 2, 3, 4, and 5, respectively.

Table: Proteins identified on 7S globulin purified fraction by LC-MS/MS analysis

Band 64 kDa		Uniprot accession	Log (E-value)	MW
1	Beta-conglycinin, alpha chain	A0A151U6Q2	-239	64269
2	Glucose and ribitol dehydrogenase	A0A151U4W9	-27	31614
3	Glycinin	A0A151RNW3	-21	55550
4	Heat shock 70 kDa protein	P26413	-11	70835
Band 49 kDa		Uniprot accession	Log (E-value)	MW
1	Beta-conglycinin, beta chain	A0A151S2A5	-358	48585
2	Guanosine nucleotide diphosphate dissociation inhibitor	A0A151UC16	-25	49691
3	ATP-dependent RNA helicase	A0A072URR3	-14	48555
4	Glycinin	A0A151RNW3	-24	55550

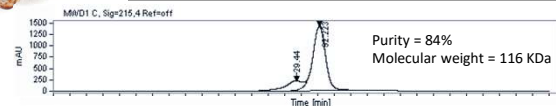


Figure: Size exclusion HPLC analysis of 7S globulin purified fraction in native conditions (PBS) at 214nm

## Foaming properties

The foamability of crude protein extracts (CPE-C), whey protein isolate (WPI) and purified 7S globulin fractions (7S) were evaluated using total and soluble protein suspensions using two connected syringes and making back and forth movements manually for 15 s.

7S globulin showed better foaming properties than the controls.

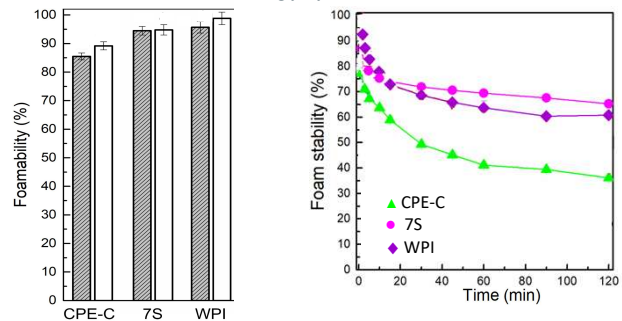


Figure: Foamability of crude protein extracts and purified 7S globulin solubilized at 4g/L (Grey: total protein suspensions, Blank: supernatant)

Figure: Foam stability over time of total protein suspensions.

## Conclusion:

The protocol used was first developed and optimized on a laboratory scale, showing reproducibility when transferred to the preparative scale. The **7S globulin purified by preparative chromatography at the level of grams** was mainly composed of  $\alpha$ - and  $\beta$ -chains of  $\beta$ -conglycinin. The **foamability and stability of foams stabilized by 7S globulin were higher** than those of crude extracts highlighting the potential of 7S globulin from pigeon pea for applications as a foaming agent in the food industry as an **alternative source to proteins of animal origin**.