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VALPRO path.

NEW VALUE LANDSCAPES FOR PLANT PROTEIN PATHWAYS

Deliverable 2.4: Report on composition of new coproducts and ingredients developed in IPSs

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Co-funded by the European Union

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Abstract:	This is the updated document of the summary of compositional analysis carried out on the ingredients, side streams and selected products studied in VALPRO. The data serve as the foundation for the business cases and story boards of the project. The updated information in this document is in blue font.
Update	Second update of Deliverable 2.3

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List of Abbreviations and Acronyms	
ATP	Alpine-Turboplex-Feinstsichter

Table 1: List of abbreviations and acronyms

EXECUTIVE SUMMARY

In this first iteration of Deliverable 2.3, we report on the findings in relation to the composition of the side streams studied within the IPSs. The second iteration adds more data to the report, from the analyses carried out from June 2024 to June 2025.

Specifically in this document, we highlight the compositional differences of side streams processed by dry fractionation, adding chickpea and pea composition to lupin, as model crops. Samples were obtained from pilot scale experiments and compared with commercially available fractions – to ensure relevance to current market realities. Due to the underutilized nature of the carbohydrate fraction in the side stream, its composition was also analyzed. Furthermore, in the second revision work reports on potential utilization of the fiber rich or starch rich fraction by summarizing the composition of food and non-food (packaging) prototypes; to reduce waste side streams. Composition of protein ingredient prototypes is also reported in this document.

1. INTRODUCTION

In transitioning from harvest to final product, protein-rich grain undergoes various processes, resulting in changes in composition as well as nutritional and technological functionality. In VALPRO Path, WP2 supports the innovation platforms, which have been designed in industrial settings, with all the necessary processing and analysis testing completed in WP2 to demonstrate potential innovations as well as to provide analytical information on product composition. In this deliverable, we report on the compositional data related to product and ingredient prototypes, as well as data on isolations and fractionations. A summary of the methods used was provided in Del. 2.3 version 1.

2. RESULTS

3.1 Analysis of co-streams during dry fractionation of Blue Lupin

As part of IPS1 activities focused on “de-centralized technologies”, dry fractionation was carried out at the pilot scale facilities of ALPINE. As an outcome of the process, two fractions, a coarse (depleted in protein) and a fine (protein concentrate) were obtained. This process was evaluated for blue lupin, which are narrow leafed in structure and can contain crude protein values of 28 – 38%, dependent on the variety grown

For the milling process a ZPS impact mill was used, together with a Turboplex fine classifier (ATP). This equipment is well suited to fine grinding of soft to medium-hard materials with a steep grain size distribution.

In the first milling step, the ground material was dosed into the grinding chamber where it hits a rotating grinding disk with a vertical drive shaft (rotor). The grinding tools on the grinding disk cause comminution through particle impact and give the material to be ground a centrifugal acceleration for further impact against the fixed grinding track, which lines the inside of the grinding chamber. The impact comminution between the grinding tools and the grinding track continues until the air flow added below the grinding disc, which rises between the grinding disc and the grinding track, entrains the particles.

The air flow then passes through a horizontally arranged classifier wheel, whereby the particles undergo further classification with a defined separation limit. Only the fine material content comes out of the mill using the pneumatic conveyor. The coarse material is rejected by the classifier wheel

and repeatedly goes through the shredding process between the grinding tool and the grinding track until the desired fineness is achieved. The mill is adjusted primarily via the speed of the sifting wheel.

Through this milling step, two batches of samples were produced using two milling classifier speeds (2200 rpm and 1700 rpm). As expected, the higher speed generated a finer product (batch 1 was finer than batch 2). Results of the two milling steps, producing the two product batches subjected to dry milling, are shown in Table 2.

	Batch 1	**Batch 2**
Classifier speed	2200 rpm	1700 rpm
Amount of lupin used	296 kg	237 kg
Throughput ZPS 200	127 kg/h	142 kg/h

Table 2. Process parameters in the milling of blue lupin samples with a ZPS equipment, generating samples 1 and 2 from whole blue lupin grain.

The Turboplex fine classifier is central to the technology tested at ALPINE, and a schematic representation is shown in (Fig. 1) and youtube video linked in the figure legend. In this process, the air flow is only shown in simplified form. The fine fraction also passes through a sifting wheel, and various speeds were tested.

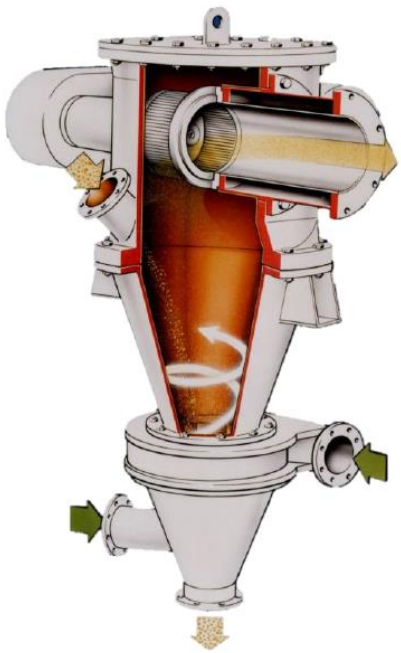


Fig. 1. Schematic of the air classifier used. For the detailed explanation a video (produced by the manufacturer for marketing purposes) of the process can be seen here: https://www.youtube.com/watch?v=Sfsf2ig5QLM.

Various tests were performed to optimize the conditions of separation. Table 3 shows only the data collected from the optimized tests.

The milled flour was classified at three different speeds: 3500, 4500 and 5500 rpm. The process parameters measured are summarized in Table 3 (These values will be shared also with WP1, for supply chain optimization.

Batches	Batch 1 (milling speed 2200 rpm)		Batch 2 (milling speed 1700 rpm)		
Test number	11	12	13	14	15
Classifier speed (min-1)	**4500**	**3500**	**5500**	**4500**	**3500**
Amount of fines (kg)	8	14.5	14.1	46.3	15.7
Amount of coarse fraction (kg)	65	83	79	57.2	45
Total amount of product (kg)	73	97.5	93.1	103.5	60.7
Throughput ATP 200 (kg/h)	146	163	186	248	146
Yield of fines (%)	11	14.9	15.1	44.7	25.9

Table 3. Process results of the trial conducted on dry fractionation of blue lupin. Two separate batches were tested (see Table 2 for grinding conditions). Each column represents a classifier speed condition. The Yields of fines are based on total amount processed. Note: only tests from 11-15 are shown, as the rest were not successful trials and used only to fine tune testing conditions.

Table 3 shows the yields (weight/weight) of fines for total amount of product used in the dry fractionation process. Results clearly varied in yield due to differences in milling (prior step, batch differences) and classifier speed. The best ratio between the coarse and fine fraction was achieved for sample 14, with a medium classifier speed of 4500 rpm and starting from the finer sample 3. Hence, this ratio is achievable, and due to the pilot scale conditions used, also scalable to a larger process.

Values of protein composition, as well as data on the carbohydrate composition of the fine fraction of sample 14 (optimized separation) are shown in Table 4. In brief, protein in the coarse fraction decreased from 40 to 36%, while it increased to 55% in the fine fraction during the separation, due to the density gradient between the fiber and protein components in the flour.

Based on the data of sample 14, it is therefore possible to conclude that on a protein basis, and based on a rough estimate of the mass balance of the process, with an input of 41 kg of protein in the process, about 60% of the protein was recovered in the fine fraction, during lupin separation. This is according to what reported in literature for other crops.

The carbohydrate profile of these samples was also analyzed in detail and is shown in Table 5. Arabinogalactan was the main carbohydrate component in the lupin fractions, followed by starch and pectin. The hydrolysis method (TFA hydrolysis, 2 M TFA, 120 °C) used ensured no cellulose was taken into account in the total glucose method, so glucose can be ascribed to starch or mixed linkage beta-glucans. Differences in the abundance of the carbohydrates are apparent between the fine fractions of the dry fractionated samples (11, 12, 14 and 15) and the starting seed (sample 1) or the ground material (2 and 3). In these fine fractions, the total carbohydrate content is reduced in favor of protein enrichment. However, this decrease is not equal for all carbohydrate constituents. While arabinogalatan is substantially reduced, the proportion of starch in these fines is preserved. This may actually be positive as it increases the caloric and nutritious value of the fines, both from the protein and carbohydrate point of view.

As sample 14 was the best performing in terms of protein content in the fine fraction and overall yields, this sample was further analysed for its gross composition. Figure 2 reveals a high content in fat (9.4 %), linked to the technical difficulties experienced in the fractionation. Ash content (4.9 %) was similar to that of whole seeds while lignin contents was enriched in the fine fraction (8.4 %).

Sample	Protein – based on N (%)		Lipids (%)	Klason lignin (%)	Carbs (%)	Ash (%)
Original seed	38.9 ± 0.5					
Batch 1 (flour)	40.0 ± 0.2					
Batch 2 (flour)	41.1 ± 0.2					
Fraction number	***Coarse Fraction***	***Fine Fraction***				
L11	38.2 ± 0.2	55.7 ± 0.1	---	---	---	---
L12	35.6 ± 0.2	54.2 ± 0.1	---	---	---	---
L13	40.2 ± 0.1	55.2 ± 0.3	---	---	---	---
L14	35.8 ± 0.2	55.7 ± 0.1	9.4 ± 0.1	8.4 ± 0.8	11.0 ± 0.8	4.9 ± 0.0
L15	36.9 ± 0.1	54.5 ± 0.4	---	---	---	---

Table 4. Analytical data for protein and carbohydrates for the fine and coarse fraction. Protein was measured using 6.25 conversion factor and fat with Soxlet method.

Carbohydrate Content (µg/mg)							
Sample	Rhamnose	Arabinose	Galactose	Glucose	Xilose	Galacturonic Ac.	Glucuronic Ac.
L	6.1 ± 0.2	19.4 ± 1.4	110.7 ± 2.0	37.1± 2.0	7.5 ± 1.5	12.6 ± 0.9	0.0
Coarse fraction1	6.7 ± 0.1	20.3 ± 0.2	116.8 ± 0.4	31.7 ± 0.7	6.2 ± 0.0	14.3 ± 0.3	12.6 ± 0.5
Coarse fraction 2	6.3 ± 0.1	18.4 ± 0.4	113.0 ± 2.6	30.0 ± 0.3	5.5 ± 0.1	12.9 ± 0.9	9.7 ± 3.6
L11	2.3 ± 0.1	10.1 ± 0.2	65.6 ± 2.2	45.3 ± 3.2	3.1 ± 0.3	6.1 ± 0.2	10.3 ± 5.6
L12	1.9 ± 0.1	8.3 ± 0.2	57.3 ± 1.3	29.7 ± 0.9	2.8 ± 0.2	4.8 ± 0.5	n/a
L14	2.1 ± 0.2	8.3 ± 0.3	54.5 ± 1.1	24.0 ± 3	2.4 ± 0.1	4.8 ± 0.3	14.0 ± 2.5
L15	2.7 ± 0.8	10.4 ± 0.1	65.7 ± 0.6	22.1 ± 2.5	1.9 ± 1.2	6.1 ± 0.5	19.4 ± 4.0

Table 5.Carbohydrate profile of blue lupin seed, ground material (2 and 3) and fine fractions after dry fractionation (11-15)

Further lab and pilot scale testing was performed in 2025, to test dry fractionation of chickpea and to compare the values in composition to those of commercial samples to be used as benchmarks.

Sample	Protein (%)	Carbohydrates (%)	Starch (%)	Amylose (%)	Fat (%)	Minerals (%)	Moisture (%)
Pea coarse fraction Vestkorn	7.8	82.0	59.3	19.2	1.1	1.6	7.4
Pea coarse fraction Peila	12.5	72.7	52.4	16.0	1.7	2.1	11.
Faba coarse fraction Vestkorn	12.6	76.4	29.4	9.8	1.3	7.7	1.9
Chickpea coarse fraction Peila	13.3	69.1	48	14.5	4.9	2.5	10.1
Pea fine fraction Vestkorn	39.5	35.6	7.7	4.9	4.5	5.3	15.2
Pea fine fraction Peila	44	35.8	7.2	5.0	5.4	6.15	8.7
Faba fine fraction Vestkorn	48.5	28.2	9.3	6.0	3.03	14.4	5.8
Chickpea fine fraction Peila	35.5	37.7	9	6.2	9.8	5.42	11.5

Table 6. compositional analysis of fine and coarse fractions from pea and chickpea, commercially available (Vestkorn) and prepared in the pilot plant at Alpine (Peila). Note that protein conversion factor used is 5.4.

3.2 Composition of isolates prepared by wet fractionation.

In an effort to evaluate the yields of proteins recovered from a wet fractionation, Fava, chickpea, and lupine flour were processed into protein isolates using the alkaline solubilisation-isoelectric precipitation process, to compare their nutritional profiles, yield, and protein recovery. This activity was part of WP2, processing and was carried out to provide context to the potential creation of more or less refined ingredients, and to evaluate the difference between wet and dry fractionated ingredients. Flours were first subjected to alkaline solubilisation at pH 11, followed by centrifugation to collect the supernatant. This supernatant was then adjusted to pH 4.4 to precipitate the protein, which was centrifuged further. The precipitated proteins were washed, neutralised, lyophilised, and milled into powder for analysis. The yield of protein isolates was calculated as a percentage of the initial flour weight, and protein recovery was assessed by comparing the protein content in the isolates to that in the original flour.

	Chickpea		Fava		Lupine	
	Flour	Isolate	Flour	Isolate	Flour	Isolate
Protein	20.14%	72.37%	25.85%	90.12%	39.46%	82.67%
Fat	5.54%	15.35%	0.60%	1.92%	1.60%	8.28%
Moisture	12.14%	4.44%	12.38 %	3.17%	9.64 %	7.35%

Yield	21%	21%	32%
Protein recovery	74%	71%	72%

Table 7. Nutritional Composition, Yield, and Protein Recovery of Fava, Chickpea, and Lupine Flours and Isolates

Table 6 shows the nutritional composition of the ingredients (protein isolates) prepared by wet fractionation, along with the yield and protein recovery for each legume. As expected, protein content was significantly higher in the isolates (>70%) compared to the flours (<40%) across all three legumes. The fava isolate showed the highest protein concentration (90%), attributable to its lower fat content compared to chickpea and lupine. This trend was anticipated, as fat content acts as a diluting agent in protein isolates. These findings indicate the necessity of a defatting step for sources with more than 1% fat to produce high-protein isolates. The yield and protein recovery percentages also showed fava as the most efficient in protein recovery, followed by chickpea and lupine, further highlighting the impact of fat content on protein isolation efficiency and final product quality. In conclusion, Fava's naturally low-fat content makes it a superior candidate for protein isolate production without an extra defatting step.

3.3 Analysis of protein solubility through hydrodynamic cavitation

Hydrodynamic cavitation was assessed to maximise protein release and improve extractability. This was carried out in the context of WP2, in an effort to create less side stream in the process of extraction. Raw fava flour was mixed with water at 5% w/w for both treatments. In one treatment, the mixture was only stirred, while in the other, it was subjected to hydrodynamic cavitation using an APV Cavitator (SPX, Denmark) operating at 800 L/h. The physical stability of the samples was evaluated qualitatively by observing phase separation after 12 hours. Both treatments were then centrifuged at 1000 g for 10 min, and the serum and pellet fractions analysed for protein content and moisture to determine the protein yield relative to the pre-centrifugation mixture. Protein content was determined using the Dumas method (conversion factor = 6.25) and moisture by CEM SMART Trac II system.

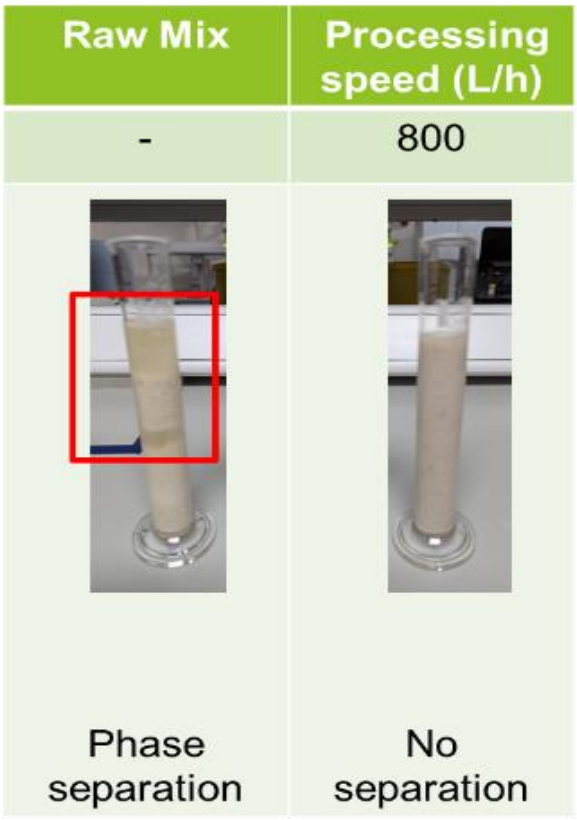


Fig. 2. Physical stability of the faba raw mix and processed with the cavitation after 12 hours

The physical stability of the faba suspension produced at 800 L/h was notably improved, showing enhanced resistance to phase separation after 12 hours when compared to the raw faba flour mix (Fig. 3). This observation suggests that cavitation at 800 L/h contributed to the stabilization of the suspension.

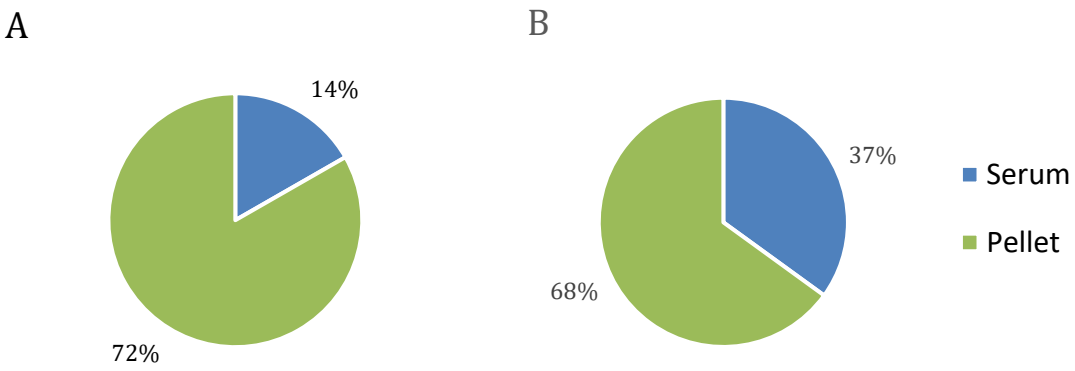


Fig. 3. Protein yield in serum and pellet fraction of stirred faba mix (A) and after hydrodynamic cavitation (B)

Figure 3 summarizes the amount of protein recovered in the serum phase after cavitation. In the raw mix, the protein content recovered in the mix was 14% of the total extracted, with most of the material left in the precipitate. After cavitation, the protein content extracted in the serum phase reached 37%

of the total, decreasing the amount of protein present in the side stream. The results indicate that the cavitation treatment effectively increased the amount of extracted soluble proteins, raising the protein content from 0.42 to 0.70 g/g solids. This improvement is likely attributed to the disruption of the starch matrix, facilitating the release of proteins resulting in a higher protein yield.

These findings suggest that operating the cavitator at 800 L/h is ideal for producing stable faba suspensions, which could be used as an intermediate step in a protein extraction process to obtain a concentrate. Additionally, future tests involving dry fractionate could provide insights into whether cavitation can effectively solubilize proteins, thereby facilitating the separation of protein and starch and potentially improving overall yield and efficiency in protein extraction processes.

Methods for compositional analysis

The following methods were used for the compositional analysis for all experiments:

- Crude protein content was estimated by Dumas analysis using a LECO FP-528 N analyser (Leco Instruments UK Ltd., Cheshire, UK), with a nitrogen-to-protein conversion factor of 6.25
- The moisture and fat content were determined using a bench-top nuclear magnetic resonance (NMR) instrument (SMART Trac Fat Analyser; CEM Corporation USA).
- The ash content of the samples was analysed using a Leco TGA701 gravimetric oven (Leco Instruments UK Ltd., Cheshire, UK).

Wet extraction of fava bean, chickpea and Lupin seeds

Fava bean, chickpea, pea, and lupine seeds were dehulled and processed into flours and protein isolates using the alkaline solubilisation–isoelectric precipitation method. Chickpea and lupine flours were defatted using hexane prior to protein extraction. Flours were first subjected to alkaline solubilisation at pH 11, followed by centrifugation to recover the supernatant. The recollected supernatant was then acidified to pH 4.5 to precipitate the protein, except for lupine (pH 4.95). The precipitated proteins were collected by centrifugation. The fibre extract from alkalinisation and the protein isolates from acidification were washed, neutralised, lyophilised, and milled into powders. Hulls, flours, defatted flours, fibre extract and protein isolate were analysed for compositional analysis.

Production of novel fava bean products

The composition of faba bean products obtained through wet milling, dry fractionation, and roasting was analysed.

Wet milling involved milling whole faba beans using a colloid mill, followed by drying with VOMM drying technology to produce a flour.

Dry fractionation products, including a protein concentrate and a starch-rich fraction, were kindly donated by a commercial company. These fractions were derived from dehulled faba bean flour using air classification.

Roasted samples were prepared by subjecting dehulled faba beans to VOMM drying at two temperatures: 155 °C for mild toasting and 180 °C for high toasting.

Peanut oil and cake varieties

Peanut oil and cake were produced using a OW510-Inox oil press model. Deshelled peanuts were fed into the press, where mechanical cold pressing was carried out. The oil was collected directly from the outlet, while the remaining solid residue, known as

3.4 Composition of peanut shell side stream.

In the harvest of peanuts, the shell is a considerable waste stream with around a third of the whole biomass weight. Peanut shell samples were obtained from IPS1 and were analyzed at CSIC for their gross composition and carbohydrate profile. The gross composition is depicted in Table 7. As can be observed, the main constituents are carbohydrates and lignin. Of note is still the high amount of protein (9%). This amount of protein needs to be further evaluated, as it was estimated from N values, and may or may not be a viable source of protein.

Component	%
Lipids	5.3 ± 0.0
Protein	9.2 ± 0.5
Klason lignin	47.9 ± 6.6
Carbohydrates	53.8 ± 7.3
Ash	2.8 ± 0.1

Table 8. Peanut shell composition.

Table 8 summarizes the carbohydrate profile in the peanut shell waste. Glucose arising mainly from cellulose was the main carbohydrate component, followed by hemicelluloses (arabinoxylan, xyloglucan) and pectin. The very high lignin contents, slightly higher than common woody tissues and cellulose content, makes it a potential reinforcing agent in food contact materials (i.e. packaging).

Component	µg/mg
Rhamnose	14.1 ± 3.2
Arabinose	30.8 ± 6.7
Galactose	14.4 ± 2.6
Glucose	283.9 ± 30.2
Xilose	129.3 ± 14.0
Galacturonic Ac.	61.2 ± 14.4
Glucuronic Ac.	2.6 ± 0.5
Fucose	2.3 ± 0.5

Table 9 Carbohydrate profile of peanut shell.

3.5 VALORISATION OF SIDE STREAMS (PEANUT SHELL AND PEA PODS): THERMOPLASTIC BIOCOMPOSITES AS VALUE -ADDED MATERIAL

Samples of **Peanut Shells (PS)** and residual biomass from pea crops were received. The PS were processes as such, while the **Pea Pods (PP)** were separated from the residual biomass prior to processing. Stalks, leaves and roots from pea crop were not used.

The PS and PP biomasses were washed, dried and subsequently milled and sieved to a size of < 2 mm before performing the compositional analysis.

The samples were further micronized to a particle size of < 250 μm (PS) and < 500 μm (PP). Additionally, the particle size distribution of the micronized PP was assessed using a sieving machine with a series of sieves sizes (250, 125 and 90 μm). It was not possible to assess the particle size distribution of the PS, since the micronized material tended to agglomerate during sieving.

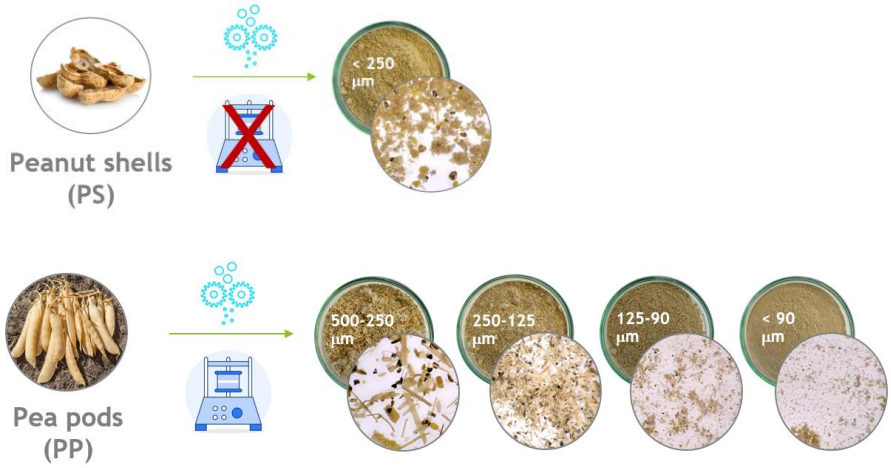


Fig. 4. Schematic representation of the residual biomass samples processing. Peanut Shells (PS): micronized to < 250 μm. Pea Pods (PP): micronized to < 500 μm and further sieved into different particle size fractions.

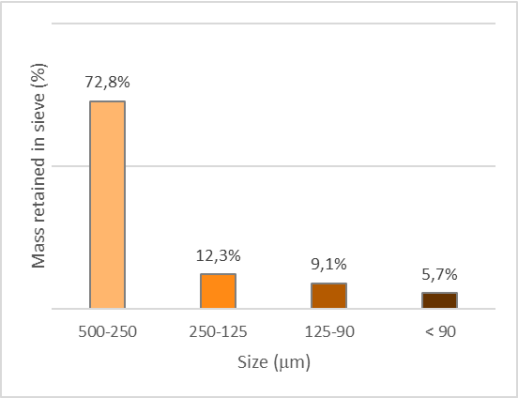


Fig. 5. Particle size distribution of the micronized Pea Pods (PP).

The initially milled biomasses (particle size < 2 mm) were used for compositional analysis, with protein calculated with a 6.25 conversion factor. The carbohydrate composition was ascertained after acid

methanolysis of the dry samples followed by quantification of the monosaccharides by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

The lignin content was analysed following the TAPPI 222 (lignin insoluble) and 250 (lignin soluble) standards. the lipid content was determined using the Soxhlet extraction with hexane according to AOAC method 2003.06.

Table 10. Compositional analysis of Peanut Shells year 2025 harvest (PS).

COMPOSITION (w/w %)	**PEANUT SHELL**
Non-volatile solids	97.97 ± 0.19
	Dry sample
Ashes	3.27 ± 0.01
Lipids	4.57 ± 0.11
Protein	9.37 ± 0.29
Lignin	0.36 ± 0.01
Cellulose	39.15 ± 1.99
SOLUBLE SUGARS	25.35
Of which:	
Fucose	0.69 ± 0.04
Rhamnose	4.16 ± 0.36
Arabinose	8.49 ± 0.43
Galactose	3.63 ± 0.09
Glucose	15.54 ± 0.51
Manose	0.00
Xilose	51.46 ± 3.01
Galacturonic Acid	15.37 ± 1.82
Glucuronic Acid	0.66 ± 0.03
TOTAL	79.07

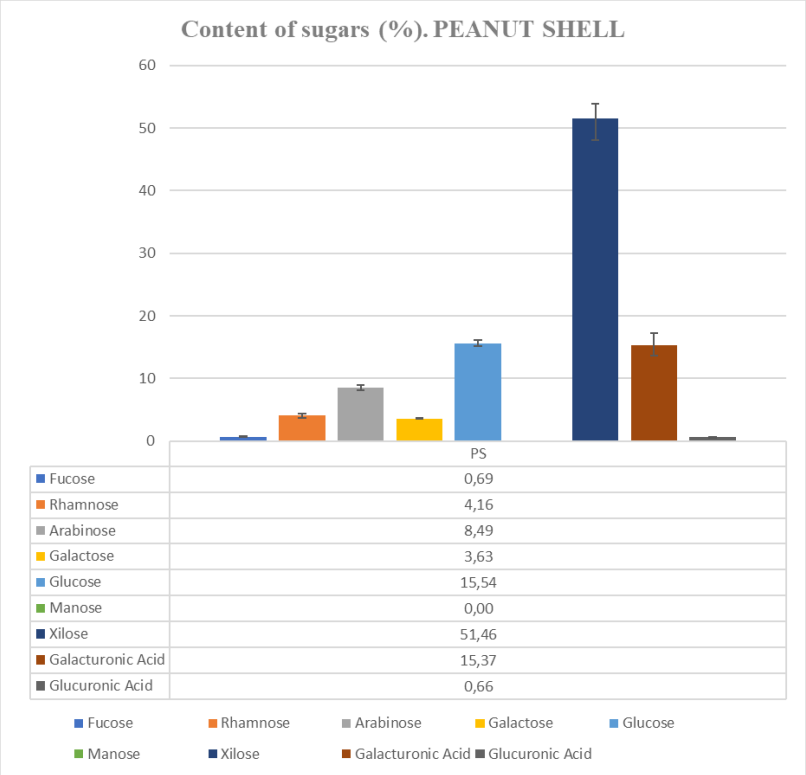


Fig. 6. Soluble monosaccharide content of Peanut Shells (PS).

Table 11. Compositional analysis of Pea Pods (PP).

COMPOSITION (w/w %)	PEA PODS
Non-volatile solids	94.40 ± 0.27
	Dry sample
Ashes	4.17 ± 0.37
Lipids	1.17 ± 0.05
Protein	7.76 ± 0.03
Lignin	0.22 ± 0.01
Cellulose	38.82 ± 2.20
SOLUBLE SUGARS	33.70
Of which:	
Fucose	0.69 ± 0.04
Rhamnose	3.59 ± 0.20
Arabinose	5.73 ± 1.24
Galactose	8.96 ± 0.50
Glucose	19.23 ± 1.55
Manose	1.99 ± 0.31
Xilose	37.77 ± 3.38
Galacturonic Acid	21.24 ± 3.00

Glucuronic Acid	0.81 ± 0.02
TOTAL	85.84

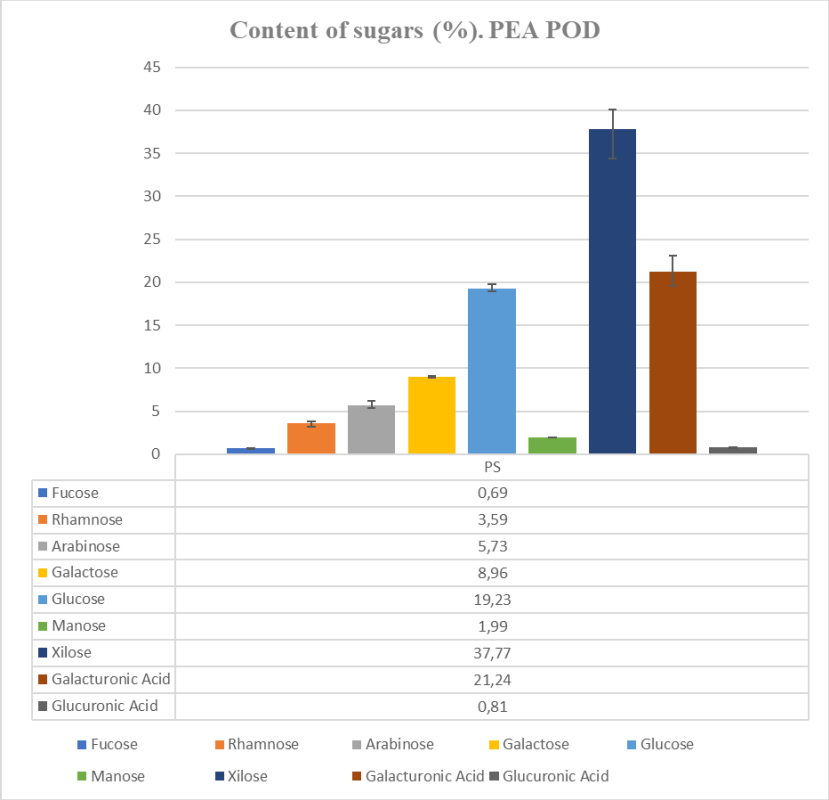


Fig. 7. Soluble monosaccharide content of Pea Pods (PP).

Two types of polymers were used as thermoplastic matrix for the preparation of biocomposites by melt-compounding:

- A blend of two **biopolyesters (BP)**: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and polybutylene adipate-co-terephthalate (PBAT).
- **STARCH** (potato-sourced) plasticized with glycerol.

The biocomposite formulations were prepared by adding the different side-stream biomasses (PS and PP) as fillers using a co-rotative twin-screw mini-compounder at a temperature of 190 °C (BP) or 130 °C (STARCH) and a rotation speed of 100 rpm for 2 min. About 500 µm thick films were obtained by compression moulding in a hydraulic press.

Injection-moulded specimens were also obtained from the biocomposites based on BP matrix.

The formulations descriptions and their visual appearance are shown in Fig. 8:



Fig. 8 . Thermoplastic biocomposites with Biopolyester (BP) or STARCH matrix and Peanut Shell (PS) or Pea Pods (PP fillers.

The biopolyester-based biocomposites obtained were analyzed for their mechanical properties, following an internal method based on ASTM standard method D882.10 (ASTM, 2001). Pre-conditioned specimens at 53% RH (10 mm wide by 80 mm long and thickness of 500 ± 50 µm) were mounted in film-extension grips of a texture analyzer (universal testing machine) with a distance of 50 mm between grips and stretched at 50 mm min^{-1} until break. The tensile parameters were determined from the stress-strain curves, estimated from the force-distance data obtained for the different films. At least 7 replicates per film formulation were tested.

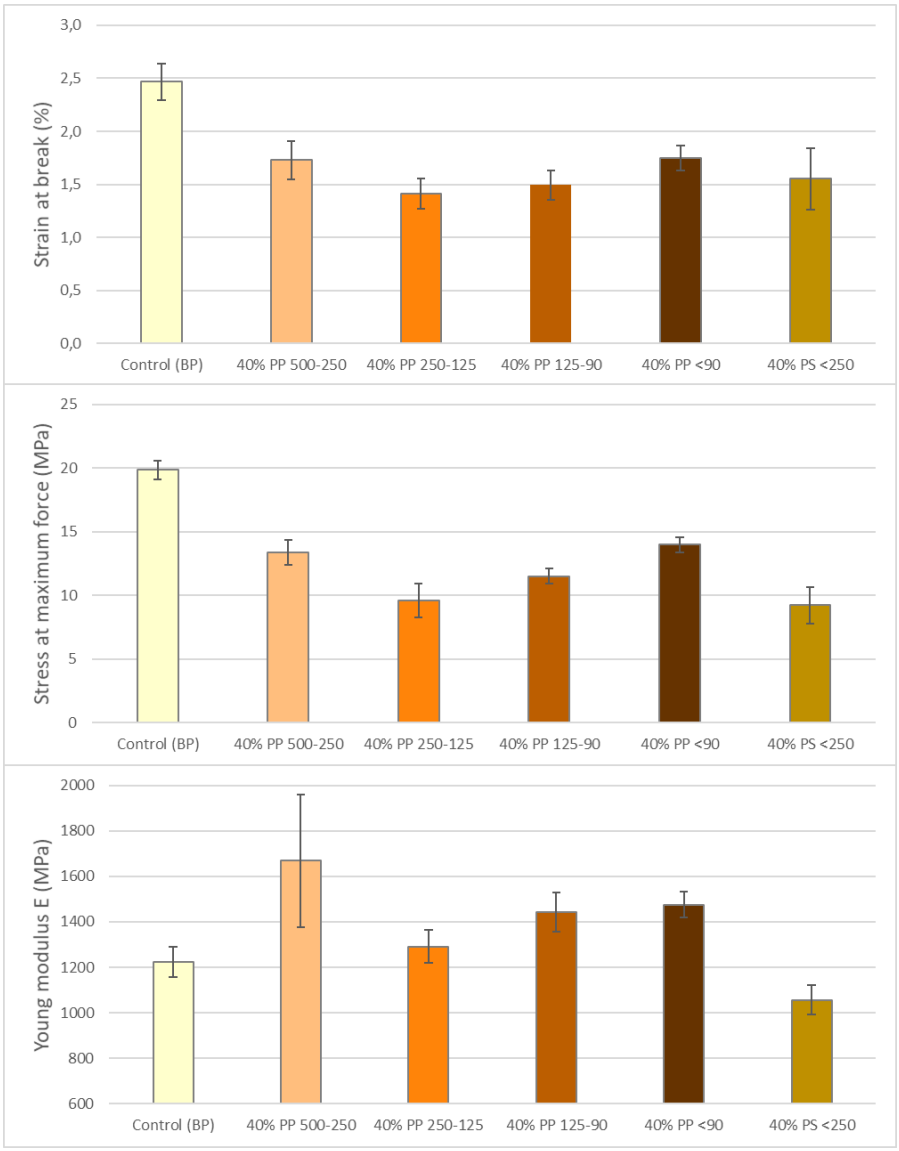


Fig. 9. Mechanical properties of the thermoplastic biocomposites based on Biopolyester (BP) matrix and Peanut Shell (PS) or Pea Pods (PP fillers.

In conclusion up to 40% of waste filler (PS or PP) could be incorporated into the biopolyester (BP) matrix through relatively easy melt-compounding. Both films and injection-moulded specimens could be obtained from the formulated biocomposites. The developed materials are brown-coloured and opaque. PP acted as a reinforcement, specially the highest particle size fraction (higher E), while PS did not enhance the mechanical properties of the matrix blend.

Considering their properties, and due to the biodegradable nature of the components, these materials are suitable for the production of e.g. single-use items for application in different sectors such as packaging or agriculture. Other transformation processes such as 3D-printing or thermoforming could be explored to produce a variety of articles.

ANNEX I

Analytical methodologies used by the Partners

1. Pre-treatment and moisture content of grains / flours

All samples must be dried to <15% relative humidity by the producers to ensure stability upon shipping and storage. The moisture content can then be determined gravimetrically after freeze-drying of the samples or after oven drying at 105°C until constant weight is achieved (ISO 18134).

Samples are milled to <0.5mm prior to analysis at the analysers' facilities (Teagasc, AU or CSIC).

2. Compositional analysis

2.1 Protein

The estimation of the protein content is done after quantification of total nitrogen. The total nitrogen is analysed based on the Dumas combustion method. The Dumas combustion method is an absolute method for the determination of the total nitrogen content in usually organic matrices. The sample is combusted at high temperature, and via subsequent oxidation and reduction, nitrogen is quantitatively converted to N2. This method is preferred to the Kjeldahl method due to a higher automation capacity which incurs in lower inter-laboratory variation and higher reproducibility.

Although the method includes inorganic nitrogen, the quantities expected in pulses compared to protein content are negligible. The conversion factor used in most of VALPRO is 6.25, as the typical conversion factor for foods, and there is not full agreement on the conversion factor for pulses proteins. However, as lower factors, ranging from 5.32 to 6.03, have also been suggested, whenever other factors are used it will be specifically reported.

Both elemental analysers or nitrogen analysers are used in VALPRO PATH, by partners TEAGASC, AU and CSIC. The quantities needed for the analysis range from 15-600 mg for a triplicate analysis, depending on the equipment used. Producers were therefore asked to provide at least 5 g of sample for this analysis. The samples are analysed in duplicate or triplicate.

2.2 Ashes

The amount of ash present in the VALPRO samples is also important, as it reflects the presence of minerals, but also potential anti-nutritionals such as phytates. Ashes are quantified after calcination in a muffle furnace, following the AOAC 932.135 standard. Around 1 g of the samples, at least in duplicate, is calcined in a muffled furnace at 550°C during at least 5 hours. Ashes are determined gravimetrically. Producers are therefore asked to provide 5g of sample (or at least 3g) for this analysis.

2.3 Lipids

Lipids may be quantified by two methods in VALPRO PATH.

The Soxhlet method is a widely accepted standard for food products, involving Sohlet extraction with petroleum ether or hexane, among other apolar solvents, the lipids gravimetrically determined from

the liquid organic phase. The standard method for crude fat determination in foods was used in this case, AOAC method 920.39.

As the lipid content in protein fractions obtained by the fractionations in VALPRO may be of a more complex nature, namely charged lipids such as phospholipids, glycolipids, in some instances the Folch method may be utilized. This is important, as lipids may hinder some of the technological functionalities of the protein, and therefore should be closely monitored in the selection of varieties, or processing treatments for yields. The analysis in this case implies the use of a mixture of chloroform and methanol in a solid-liquid extraction, the lipids gravimetrically determined from the liquid organic phase.

Producers are asked to provide 5g of sample (or at least 2g) for this analysis. The samples are analysed in duplicate or triplicate.

2.3 Polyphenol content

The total phenolic content is determined, in triplicate, by the Folin-Ciocalteau reagent as described by Singleton et al. (1999) with a slight modification. Briefly, Folin-Ciocalteau reagent was diluted 1:10 with distilled water and 1 mL mixed with 0.2 mL of each sample (5 mg/mL). 0.8 mL of Na_2CO_3 (75 mg/mL) are, then, added and the samples are heated up to 50 °C for 30 min. Finally, the absorbance values are read at 750 nm. The calibration curve is generated by using gallic acid as the standard and the total phenolic content expressed as mg of gallic acid equivalents (GAE)/g extract. Of note is that in VALPRO, particular studies may carry out LC-MS-MS identification and quantification (AU-FOOD), when more accurate determinations are needed.

2.4 Carbohydrate content

For a high through-put analysis of a high number of samples, the carbohydrate content can be estimated by subtraction from the previous components up to 100%.

2.4.1 Detailed carbohydrate composition

A more detailed analysis of the carbohydrate profile will be performed in many samples at CSIC, including the determination of all monosaccharide constituents. In these cases, the sum of all detected monosaccharides will be considered as the total carbohydrate contents. Samples are dried and milled before analysis (see above) and reported on a dry basis, and include the following steps.

1) **Soluble sugars and raffinose type antinutrients**

Both soluble sugars, such as sucrose, glucose, melibiose or fructose, and alfa-oligosaccharides, such as raffinose, stachyose and verbascose are extracted with water and quantified by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on an ICS-6000 equipment (Thermofisher). About 3 g of sample (at least 1 g) is needed for this analysis.

2) **Carbohydrate profile**

For all insoluble carbohydrates a profile serves to discern cellulose, hemicellulose and pectin components in the samples. It also gives information about the type and branching degree of pectin. The monosaccharide composition of the samples is evaluated after acid hydrolysis of the raw materials using trifluoroacetic acid, anhydrous acidic methanol or sulphuric acid, depending on the target polysaccharide and the nature of the sample (if recalcitrant fiber is present).

In general, A two-step sulfuric acid hydrolysis is performed by incubating 4 mg of sample with 250 µL of 72% H_2SO_4 at room temperature for 3 h; the solution is then diluted with 1250 µL of deionized water

and subsequently incubated at 100 °C for 3 hours. Unless otherwise indicated. The monosaccharides are then analyzed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) with a ICS-6000 system (Dionex) equipped with a CarboPac PA1 column (4 × 250 mm, Dionex).

TFA 2 M of 120 °C for 3 h will be used when the pectin and the cellulosic components are not expected to be prominent.

In the presence of a significant pectin amounts methanolysis should be carried out, then on freeze-dried samples (1 mg) incubated with 1 mL of 2 M HCl in dry methanol for 5 h at 100 °C. Samples are neutralized with pyridine, dried under a stream of air, and further hydrolyzed with 2 M TFA at 120 °C for 1 h. The samples were again dried under a stream of air and dissolved in water.

After hydrolysis, samples of known concentrations with mixtures of fucose, rhamnose, galactose, glucose, arabinose, xylose, mannose, galacturonic acid and glucuronic acid are used for calibration. All experiments are carried out in triplicate. The amount of samples needed for this analysis is about 0.05 g (at least 0.03 g).

3) Starch content

The starch content in the samples is estimated after digestion with a thermoresistant amylase + amyloglucosidase followed by a colorimetric reaction to quantify the glucose generated from the digested starch. This can be done using the enzymes available separately in our labs or using readily prepared kits for that purpose. Abouth 0.1 g of samples (at least 0.03 g) are needed. If necessary, the amylose and amylopectin content in the starch present in the samples can be estimated by precipitating the amylopectin fraction with concanavaline A. The supernatant is treated as in the protocol for the determination of starch and the results refer to the amylose content. The amylopectin content is calculated by subtracting the amylose contents to the total starch contents.

4) Soluble and insoluble lignin

Lignin is determined in samples where lignin is expected to be a major component, such as shells or other residues generated in the project, at CSIC. Insoluble or Klason lignin is determined following the TAPPI T222 om-06 standard method for woody tissues. It involves a gravimetric procedure after extracting resins and dissolving cellulose, hemicellulose and pectin with sulfuric acid. Soluble lignin can be determined spectrophotometrically from the dissolved material following the standard TAPPI UM-250.