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Original article

The investigation of the functional properties of single and mixed milk/lupine protein systems

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Summary Proteins play an important role in the human diet. While animal proteins have traditionally been a dietary staple due to their techno-functional properties, factors such as cost, availability and sustainability concerns have driven the search for alternative protein sources. In this study, the techno-functional properties of lupine proteins were studied. Protein isolates were shown to exhibit superior properties, with α-La outperforming WPI and LPI in foaming and gelation properties. The combination of WPI and LPI displayed greater stability to pH changes, which is an important factor for the structural integrity of food products, contributing to quality, customer acceptance and shelf-life. The findings of this study can contribute to existing knowledge on the functional properties of proteins from plant sources such as lupine, which can therefore help to enhance its usage and alleviate the overdependence on soy as an alternative protein.

Keywords Alternative proteins, functional properties, lupine protein, mixed system protein extraction, sustainability, whey protein.

Introduction

Proteins play a vital role in the human diet and foods as both nutritional and techno-functional ingredients (Grossmann & Weiss, 2021). Among these, proteins derived from animal sources, like eggs and milk, are widely utilised in food applications due to their functional properties such as gelation, emulsification, and their ability to contribute to foam ability and stability. However, the production of proteins from animal sources does come at a cost due to its impact on the environment. Therefore, the search for potential replacers for animal proteins that are sustainable, cheaper and environmentally friendly has resulted in the exploration and incorporation of alternative proteins in different food product formulations (Hoehnel et al., 2022).

The increasing demand for as well as cost of proteins has led to an increased interest in the utilisation of proteins from plant sources as partial replacers of animal proteins. This is a challenging task in product development, given that replacing one protein with another can result in products that have different sensorial properties, such as texture, taste and flavour, compared to the original formulations (Munialo & Vriesekoop, 2023). Therefore, before replacing animal proteins by plant proteins, it is critical to investigate and understand the consequences for the rheological and structural properties in specific protein mixtures.

There is an increasing interest in the utilisation of plant-derived proteins as ingredients in foods to address the cost, sustainability concerns and the shortage of nutritious food for the growing population (Munialo & Vriesekoop, 2023). Legumes are of particular interest as alternative sources of protein due to their high protein content (~20% in dry matter), mainly consisting of globulin (~70%) and albumin (~20%) proteins (Grasberger *et al.*, 2023). Most commonly utilised legume protein sources within food include fava, soy, pea and lupine. Soy protein continues to be used in most formulations that require alternative proteins such as plant-based meat alternatives (Cai *et al.*, 2002). However, there continues to be a competition for the use of soy as both animal feed and human food and this calls for the search and continued exploration of other plant-based proteins that not only can be used as potential replacers of animal proteins but also alleviate the pressure on soy proteins.

Protein from *Lupinus angustifolius*, also known as narrow-leaved blue lupine, is of growing interest as an alternative protein source due to its high protein, high dietary fibre, low starch and low fat, in addition to its

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increasing cultivation within Europe (Al-Ali *et al.*, 2021). Lupine seeds contain 30–42% protein, of which 87% are globulins (Grasberger *et al.*, 2023). While there is limited research on the emulsifying ability and interfacial properties of lupin proteins, few studies suggest that lupin proteins exhibit good emulsifying properties (Albe-Slabi *et al.*, 2022).

There are important knowledge gaps in the utilisation of lupin protein isolates in food products, as the processing functionality of lupine protein is not fully understood. Additionally, the nutritional profile of plant proteins often requires careful formulation to ensure an appropriate amino acid profile (Duranti & Cerletti, 1979). In addition to the macronutrients, dairy ingredients can also provide important nutritional functionalities to our diets due to the presence of various micronutrients in a complex matrix (Lemus-Conejo *et al.*, 2023). Thus, the utilisation of plant-dairy protein blends in food formulations would address not only sustainability concerns, cost of protein, but also lead to nutritional benefits (Pereira *et al.*, 2022).

Materials and methods

Protein extraction and characterisation

Commercial lupine protein flour (which will subsequently be referred to as lupine protein) was used as it was sourced from the producer without further purification or extraction steps. Given the fact that this sample contained only 39% protein, an extraction step was used to isolate the soluble fractions of the proteins, and this will subsequently be referred to as lupine protein isolate (LPI). LPI was extracted from lupine protein flour using two methods, *i.e.* acidic and alkaline extraction, based on the outlined protocol by (Vogelsang-O'Dwyer *et al.*, 2020; Lo *et al.*, 2021; Shrestha *et al.*, 2021) with slight modifications. LPI with the highest protein content was used for SDS-PAGE, gelation, foaming and emulsification.

In summary, for the acidic extraction method, 16% (w/w) lupine protein flour was reconstituted in 600 mL RO water to make a protein slurry which was left to hydrate overnight at 4°C. Subsequently, the pH of the slurry was adjusted to 4.5 with 1 M HCl and under continuous stirring at room temperature (20 ± 2°C) for 30 min. The slurry was then centrifuged at 4400 rpm for 30 min using an Eppendorf™ 5702 Low-Speed Centrifuge (UK) to remove insoluble materials. The supernatant was collected, followed by adjusting the pH and a second centrifugation step where this time round, the pellet was collected, and the supernatant discarded. The pellet was then redispersed in 180 mL RO water, followed by the adjustment of the pH to 7 with 1 M NaOH and incubation of the samples at room temperature (20 ± 2°C) for 30 min under continuous stirring. Subsequently, the protein-rich supernatant was centrifuged at 4400 rpm (20°C, 25 min) to remove the remaining traces of insoluble materials, which yielded LPI-A. A similar procedure was followed for alkaline extraction, except for the protein being adjusted to pH 9 using 1M NaOH at the start of the extraction process (LPI-B).

To be able to quantify the protein content of soluble lupine proteins (LPI-A and LPI-B), the Bradford method was used with Bio-Rad Protein Assay Dye Reagent, RO water as a blank and BSA as a reference/control protein according to the protocol described by (Bradford, 1976; Kruger, 2002). In summary, 1 mL of reagent was mixed with 2 mL of each protein sample as well as the BSA standards. The mixtures were vortexed and incubated at room temperature for 5 min, and the absorbency at 595 nm was determined. A calibration curve was obtained using 0, 200, 400, 600, 800 and 1000 μM BSA concentrations and the protein yield was calculated using eqn 1. All measurements were performed in duplicate.

The protein yield was calculated as shown in eqn 1.

$$\frac{\text{Protein obtained (g)}}{\text{Weight of flour (g)} \times \text{Protein content (\%)}} \times 100 \quad (1)$$

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) was used for the determination of the molecular weight distribution of protein samples under reducing conditions according to the method by Laemmli (1970). Ten mg ml^{-1} protein solutions (50 μL) were mixed in a 1:1 ratio with Laemmli 2x concentration sample buffer. Subsequently, these mixtures were vortexed, and heated at 95°C for 5 min. Next, 20 μL of sample and 10 μL of protein ladder marker were loaded onto the wells of the precast gel. Electrophoresis conditions were 80 V at 10 mA throughout at 20°C ~ 60 min running time in Tris/Glycine/SDS buffer. The electrophoretograms were then scanned using Bio-Rad Gel Doc E2 Imager (Bio-Rad Laboratories Ltd., Watford, UK) and the acquired images were analysed by the associated Image Lab software.

Preparation of protein gels

The pH and ionic concentration play a significant role in the nature and types of gels that can be formed. Divalent cations have been reported to be more effective gel formers than monovalent cations (Tang *et al.*, 1996). Thus, to study the impact of pH and ionic strength on lupine protein and LPI-A and LPI-B, gelation was carried out at pH 4 and 7 and in the presence or absence of a divalent salt ($CaCl_2$). The selection of $CaCl_2$ was based on the fact that marginally soluble calcium salts such as

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$CaCl_2$ have been reported to form crosslink in gels (Harding *et al.*, 2011). The gelation properties for the lupine proteins were assessed using procedures previously described (Puyol *et al.*, 2001; Napieraj *et al.*, 2022), with modifications. In summary, the pH of lupine protein, LPI-A and LPI-B solutions at 10%, 15% and 20% was at either pH 7 or pH 4. The solutions were stirred for 30 min before the addition of 0.5% (w/w) of $CaCl_2$ and further stirred for 5 min. Gelation was performed in 20 mL syringes (20 mm in diameter), lubricated with paraffin oil and closed airtight to reduce air bubble formation during heating at 95°C for 30 min in a temperature-controlled water bath. Following the heating step, the gels were cooled at room temperature (±20°C) followed by visual inspection to check for gel formation. The gels prepared from WPI, α-la and lupine protein at 10%, 15% and 20% (w/w) concentration were assigned a score ranging from 0 to 5 based on their appearance and perceived firmness. For consistency, a scoring rubric (Table 1) was developed to define the criteria for each score, the total of which was later analysed where 0 is absence of gel formation, 1 is a puree-like texture lacking ability to maintain shape, 2 is a puree-like texture that is able to maintain shape, 3 is a material of tremulous consistency, 4 is a homogeneous gel structure and 5 is an elastic or friable homogeneous structure. To compare the gel strength, WPI, α-la and a mixture of WPI and LPI as well as WPI and lupine protein gels were made according to the procedure described above.

Table 1 shows the visual representation of the gels obtained following the heating of the protein solution in the presence or absence of salt. Some protein solutions were able to form self-supporting gels and the deformation properties and texture profile analysis (TPA) was carried out on these gels. The gels were cut into 2 cm × 2 cm cylindrical blocks and the deformation properties and TPA analyses were performed using Brookfield CT3 Texture Analyser (AMETEK Brookfield, Middleboro, MA, USA) with a 4.5 kg load with compression flat TA25/1000 probe at a constant velocity cycle of 60 mm min^{-1} (Test Target – Distance 10 mm and Trigger Load – 0.049 N). Results were analysed using the associated TexturePro CT V1.3 Build 17 software.

Foaming properties of the proteins

The foaming ability and stability of the proteins were evaluated using a method adapted from (Lonchamp *et al.*, 2022) at varying pH and ionic strength. Protein solutions were reconstituted at 1% (w/w) concentration and a final volume of 15 mL for each sample, *i.e.*, Lupine protein, LPI-A, LPI-B and WPI. The solutions were frothed for 1 min using a handheld K8 Milk Frother with a 3.7 V motor voltage. The initial foam height was measured and is defined as the foaming ability, followed by 5 min interval measurements until the foam collapsed, which is defined herein as the foam stability. The experiment was performed in duplicate, with two replicates analysed for each sample.

Emulsifying properties of the proteins

The emulsifying properties of the proteins at 1% (w/w) concentration in oil-in-water emulsions were evaluated using previously described procedures by D'Agostina *et al.* (2005) and Bader *et al.* (2011a, 2011b), with adaptations. Protein solutions were combined with sunflower oil at 20°C in 50 mL polypropylene conical centrifuge tubes, achieving a 3:1 water:oil volume fraction. The mixtures were then vigorously shaken for 1 min, and the height of the emulsion layer was measured every 1 min until a stable oil-in-water phase was observed. The experiment was repeated in duplicate, with two replicates of each sample analysed.

Statistical evaluations

Statistical analysis of the TPA data was carried out using the SPSS Statistics 28.0 software (IBM, Armonk, NY, USA). A Multivariate General Linear Model was performed to analyse all TPA parameters. A full-factorial model incorporating homogeneity tests and *post hoc* Tukey's honestly significant difference test was performed for Protein and Concentration. The Shapiro–Wilk test was applied to assess the normality of the data distribution. The Kruskal–Wallis and Mann–Whitney tests were employed to identify significant differences ($P < 0.05$) between various proteins and the impact of pH and salt addition on the functional properties.

The means, standard deviations and standard errors for the gelation, foaming and emulsification results were calculated and visualised using Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA).

Results and discussion

Quantification of the protein content

Figure 1 compares the two fractionation methods for LPI yield, with error bars representing standard error derived from the standard deviation within each sample set. The findings indicate that LPI-A displayed a protein content of 61.6 mg mL^{-1} and a yield of 86.4% ± 0.7. On the other hand, LPI-B exhibited a protein content of 47.4 mg mL^{-1} and a yield of 74.1 ± 0.8%. These results suggest that the extractions process resulted in a higher amount of soluble protein in lupine protein, especially in LPI-A, compared to the protein content found in the dried flour.

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Table 1 Scoring rubric for gel analysis.

	Subjective scoring rubric for gelation experiment					
Score	**0**	**1**	**2**	**3**	**4**	**5**
Appearance						
	i.e. 10% LPI-A without salt	*i.e.* 20% lupine protein with $CaCl_2$	*i.e.* 15% lupine protein without salt	*i.e.* 20% α-la without $CaCl_2$	*i.e.* 20% WPI without salt	*i.e.* 20% WPI with $CacCl_2$
Score description/ reasoning	An aqueous solution with no gel formation observed	A puree-like texture lacking the ability to maintain its cylindrical form once extracted from the syringe	A puree-like texture with the ability to maintain the formed cylindrical configuration once extracted from the syringe	A material of tremulous consistency yet retaining its form and having minimal moisture loss	A homogeneous gel structure with an even and glossy appearance across the surface, demonstrating adequate self-support that conforms effortlessly to the cylindrical shape of the syringe	A homogeneous structure which is either exceptionally elastic or of an excessively friable nature breaks down in an attempt to cut the gel. Minimal to no moisture loss
	Some elements of coagulation present yet possess insufficient autostability - disintegrating once extracted from the syringe		Yet, the fragile material is prone to disintegration upon touch and prone to a severe release of moisture content	The texture is notably more stable/tender, with potential indicators of friability	The gel is easy to cut through, displaying minimal moisture loss. Possible disintegration of the gel into robust structures	

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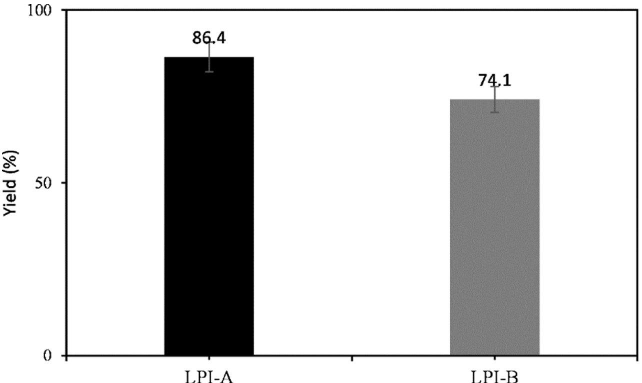

Figure 1 Comparison of lupine protein isolate (LPI) yield using two fractionation methods with error bars derived from the standard error calculated from standard deviation within each sample set.

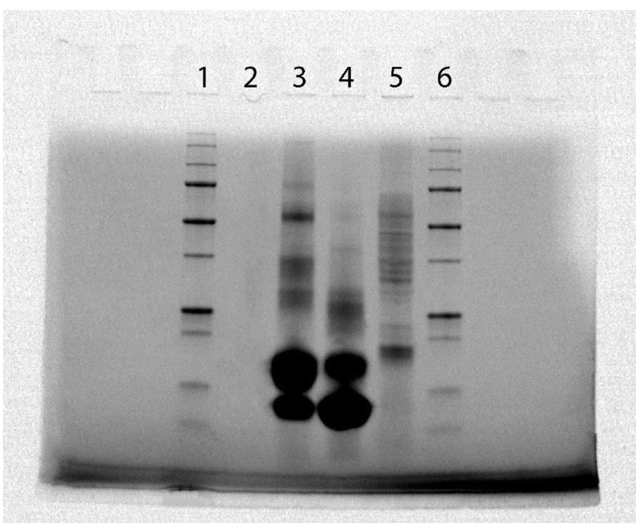

Figure 2 Molecular weight distribution of whey protein isolates (WPI), α-La and lupine protein isolate (LPI). Lane 3 represents the molecular weight distribution of WPI, whereas lanes 4, 5 and 6 are the α-La, LPI and the standard marker, respectively.

These results are consistent with previous research on lupine protein conducted by Bader *et al.* (2011a, 2011b) and Fontanari *et al.* (2011), who reported protein yields between 36% and 82% for both extraction methods. However, other studies have shown different outcomes, with acid precipitation resulting in a lower 20% protein recovery yield, as demonstrated by Chew (2003). This discrepancy may be attributed to the different protein quantification methods used; Fontanari *et al.* employed the Bradford method, while Chew *et al.* relied on measuring the protein content at 280 nm of the spray-dried protein powder after acid precipitation extraction followed by ultrafiltration. Drying of proteins can result in changes in the protein structure, such as denaturation and protein unfolding, and this could impact the solubility of the proteins (Munialo *et al.*, 2022). Furthermore, the application of ultrafiltration could result in the fractionation of the proteins and allow for only given sizes to pass through the filtration membrane, and this can impact the quantification of the protein content. The protein content of WPI and α-la were 90% and 92%, respectively, and these samples were used as they were without determining the protein concentration.

Molecular weight assessment by SDS-PAGE

The seed storage proteins of lupine are α-conglutin (11S globulin, legumin), β-conglutin (7S globulin, vicilin), γ-conglutin (7S basic globulin) and δ-conglutin (2S albumin) (Foley *et al.*, 2011). The SDS-PAGE gel (Fig. 2) displayed numerous heterogeneous protein bands in the samples analysed, with molecular weights ranging from 10 to 70 kDa. For the whey proteins (wells 5–6), five distinct protein clusters were identified: one at ~ 55 kDa, two bands between 25 and 37 kDa, and two highly intense bands between 10 and 20 kDa. In the case of LPI (well 7), a highly intense band was observed at ~ 18 kDa, accompanied by a primary, less intense, heterogeneous band cluster between ~ 30 and 70 kDa. No bands were detected below 18 kDa for LPI.

LPI-A was found to contain two glycinin proteins with molecular weights like those of β-conglutin and α-conglutin (~ 17–64 kDa). These results align with findings by Berghout *et al.* (2014) as well as Fontanari *et al.* (2011), where lupine proteins, including the *L. albus* species examined in the current study, were reported to be primarily composed of globulins, specifically conglutins, with the molecular weights ranging from 20 to 90 kDa.

Whey proteins are known for their varying molecular weights and include major proteins such as β-lactoglobulin (18 kDa), α-lactalbumin (14 kDa), bovine serum albumin (approximately 65 kDa) and immunoglobulins (~ 150 kDa). The study observed bands likely corresponding to these proteins, with two particularly intense bands between 10–20 kDa, which may represent β-lactoglobulin and α-lactalbumin. This is consistent with previous studies on whey proteins (Lai *et al.*, 2015; Grasberger *et al.*, 2023).

Gelation properties

Visual observation of gelation

Overall, both the pH and the presence of $CaCl_2$ had a statistically significant influence on gel formation ($P < 0.05$), with the firmest gels formed at pH 7 in the presence of 0.5% (w/w) $CaCl_2$ (Some results summarised in Table 1 and supported by the texture

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Table 2 Comparison of texture profile analysis (TPA) measurements for different gel formulations.

	Protein						
	WPI	α-La			Lupin	WPI + Lupin	
TPA parameters	With salt	Without salt	With salt	Without salt	With salt	With salt	Without salt
Deformation of hardness (mm)	9.99 ± 0.01	9.98 ± 0.01	9.98 ± 0.01	9.99 ± 0.01	9.99 ± 0.01	7.72 ± 0.01	6.13 ± 0.01
Springiness (mm)	7.67 ± 0.03	9.46 ± 0.26	8.39 ± 0.12	8.93 ± 0.00	1.87 ± 0.00	6.39 ± 0.43	7.83 ± 0.00
Springiness index	0.76 ± 0.01	0.94 ± 0.02	0.83 ± 0.02	0.89 ± 0.01	0.19 ± 0.01	0.65 ± 0.07	0.79 ± 0.01
Adhesiveness (mJ)	0.06 ± 0.05	0.06 ± 0.05	0.13 ± 0.05	0.01 ± 0.01	0.43 ± 0.01	0.98 ± 0.16	0.31 ± 0.00
Adhesive force (N)	0.05 ± 0.00	0.06 ± 0.03	0.06 ± 0.04	0.02 ± 0.01	0.15 ± 0.00	0.16 ± 0.05	0.17 ± 0.01
Hardness cycle 1 (N)	17.66 ± 0.01	21.89 ± 14.50	7.08 ± 16.25	17.20 ± 0.05	0.50 ± 0.02	4.84 ± 4.57	7.17 ± 0.00
Hardness cycle 2 (N)	16.95 ± 0.00	19.42 ± 13.88	6.57 ± 13.82	15.14 ± 0.01	0.44 ± 0.01	2.37 ± 4.21	4.43 ± 0.01
Cohesiveness	0.71 ± 0.00	0.85 ± 0.01	0.76 ± 0.05	0.77 ± 0.01	0.17 ± 0.01	0.24 ± 0.02	0.28 ± 0.00
Gumminess (N)	12.35 ± 0.01	17.91 ± 10.02	5.47 ± 12.80	13.24 ± 0.01	0.08 ± 0.01	1.18 ± 3.65	1.99 ± 0.01
Chewiness (mJ)	96.40 ± 0.01	167.97 ± 78.71	47.27 ± 118.9	118.23 ± 0.03	0.16 ± 0.00	7.54 ± 33.17	15.56 ± 0.00
Chewiness index (N)	9.63 ± 0.01	16.75 ± 7.84	4.79 ± 11.81	11.78 ± 0.01	0.02 ± 0.02	0.77 ± 3.34	1.54 ± 0.02

The data are presented as averages of duplicate measurements ± SD.

profile analysis results in Gel texture profile analysis section). It was also determined that pH 4 provided a more favourable environment for gelation without the addition of salt for all proteins. Protein concentration showed no statistically significant impact on gelation ($P > 0.05$), as firm gels were formed with 10%, 15% and 20% (w/w) protein concentrations for WPI and α-La, which, overall, did not have a significant difference ($P > 0.05$) in their properties. LPI-A and LPI-B failed to contribute to the formation of self-supporting gels (Results not shown). The mixture of WPI and LPI-A or LPI-B, as well as with lupine protein at a ratio of 1:1 10% and 15% (w/w), resulted in the formation of firm and self-supporting gels. The iso-electric point (IEP) of lupine protein is 4.5, whereas the IEP for whey proteins does range from 4.8 to 6. Given that the gels in this work were prepared at pH 4 and 7, pH 4 would be below the IEP for both proteins, where they will both assume a positive charge and ordinarily would like to repel each other, and this should have resulted in segregative phase separation. However, given the presence of salt, some of the charges could be screened, and this could result in attraction between the proteins and strengthening of the networks. However, no significant difference ($P > 0.05$) was observed in response to changing conditions.

Gel texture profile analysis

Table 2 summarises the TPA results generated for gels produced as a function of concentration variation and the presence of $CaCl_2$. Multivariate test results demonstrate that both concentration variation and the presence of $CaCl_2$ significantly ($P < 0.05$) affect the majority of TPA parameters, and all have equal variances across groups, meeting the assumption of homogeneity of variances. However, it was found that the Deformation of Hardness is not influenced by concentration changes ($P > 0.05$) and that Hardness Cycles and Gumminess are not influenced by the addition of $CaCl_2$ ($P > 0.05$). Furthermore, the effect of protein on Deformation of Hardness does not depend on the level of Protein Concentration ($P > 0.05$), and the effects that Concentration has on Hardness Cycles, Deformation of Hardness, Springiness Index, Gumminess and Chewiness do not depend on the presence of salt and *vice versa*. Overall, lupine, whey and the synergy of WPI + Lupine protein are significantly different in their TPA parameters ($P > 0.05$), with some attributes being less significant.

In general, the study of the effects of pH and $CaCl_2$ on gel formation and gel strength revealed that salt significantly impacts gel formation, with pH 4 being the optimal environment for gelation without salt across all proteins. It was observed that at higher concentrations, lupine protein gels maintained a viscous, paste-like consistency after being sheared or broken. This is unlike for whey proteins that formed a self-supporting structure that was tough to cut and not prone to deformation. The results match with previous observations for whey proteins, where α-La has been reported to have better gelation ability at pH 4 than the whole WPI at variable pH (Wu *et al.*, 2023).

The observation also aligns with previous research on lupine protein gelation, which suggests that lupine protein gels are generally weaker and softer than whey or other alternative proteins such as soy, requiring higher protein concentrations to form stiffer gels (Duranti & Cerletti, 1979). The minimum gelling concentration reported in this study was 15% (w/w), which matched with the range of 11.5–16%, previously studied, indicating a considerable variation in gelling capabilities among LPIs (Mariotti *et al.*, 2002). Prior

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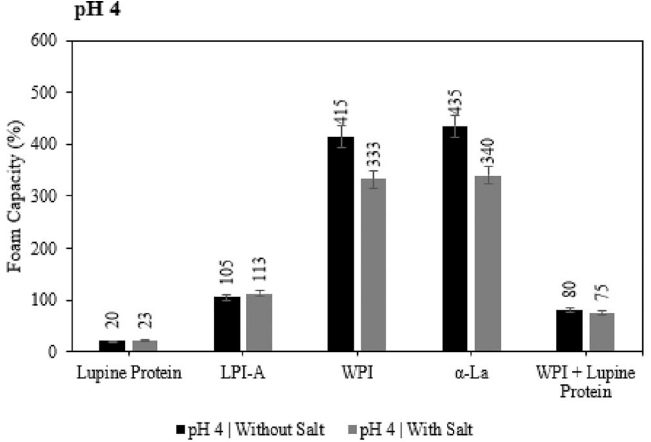

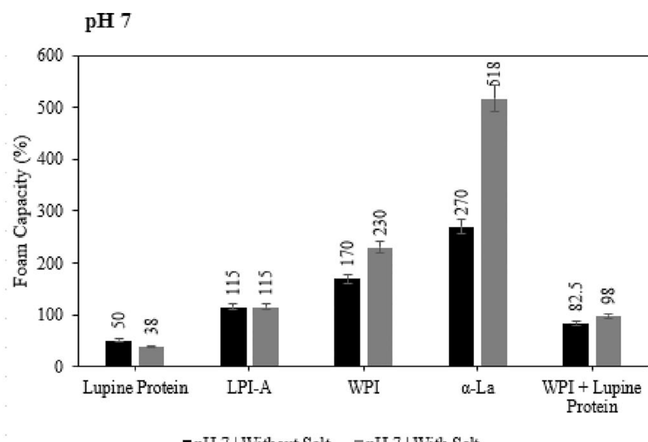

Figure 3 Protein foam capacity comparison at different pH levels.

studies have proposed that the inferior gelling ability of lupine protein, compared to soy protein, may result from its heat-stable nature and limited capacity to form new disulphide bonds (Berghout *et al*., 2015). Kiosseoglou *et al*. found that reheating lupine gels negated the strengthening effect of cooling, indicating that network formation and strengthening relied solely on physical interactions (1999). However, considering the age of the study, it is feasible to argue that a re-evaluation of the functionality is necessary as the advance in lupine isolation technology may influence LPI's gelation (Berghout *et al*., 2015). This was observed when the maximum temperature applied was 82°C; although the study also showed that more covalent interactions occurred in gels formed at 95°C, hence, this temperature was used in the present study to determine the ability of proteins to form a gel (Duranti & Cerletti, 1979; Mariotti *et al*., 2002).

Overall, both LPI-A and LPI-B formed weak gels composed of insoluble swollen microparticles, which cannot support their weight against gravity. This renders them unable to compete with other gel structure-forming proteins like whey protein. To expand the applications of lupine protein, its gelation properties need enhancement through modification.

Foaming properties

All proteins were able to produce stable foams at 1% (w/w) concentration. However, WPI exhibited a significantly higher ($P > 0.05$) foam capacity at both pH levels compared to other proteins (Fig. 3). The highest foam capacity was for α-La with 518% at pH 7 with salt. Nevertheless, both LPI-A and LPI-B foam capacity demonstrated the least significant variation ($P > 0.05$) with changes in pH and salt presence, maintaining an average foam capacity of 112%. Although the presence of salt had a statistically insignificant ($P > 0.05$) difference in foam capacity, the average foam capacity with salt was 14% higher than without.

The variations in pH and salt addition did not have a statistically significant influence ($P > 0.05$) on protein foam stability and stabilisation rate. The results in Fig. 3 show LPI-A to display a comparable foam capacity at both pH levels to α-La, which has the highest foam capacity at 518% at pH 7 with salt. WPI + Lupine Protein and α-La formed the most stable foams, with foam setting after 96 and 73 min, respectively, and had the slowest stabilisation rates.

All the proteins in this work were able to foam with whey proteins having significantly higher foam capacity at both pH levels compared to others. The foam capacity of the combination of WPI + Lupine Protein showed the least variation (80%) with changes in pH and salt presence. The results correlate with the findings of El-Adawy *et al*. (2001) and Hojilla-Evangelista *et al*. (2004), where it was found that the foaming capacity of lupine protein extracted *via* selective fractionation was around 70%. However, this contrasts with D'Agostina *et al*. (2005) research where the foaming capacity of lupine was 1800%. This discrepancy may be due to the method of protein isolation, as D'Agostina implemented an ultrafiltration step in acid extraction, resulting in variable protein subunit compositions (Hojilla-Evangelista *et al*., 2004).

Emulsifying properties

A statistical difference was observed between the emulsion set times for the tested proteins ($P < 0.05$), with LPI and WPI + Lupine taking the longest time to form oil-in-water emulsions and exhibiting the slowest stabilisation rates (Fig. 4). Variations in pH and salt

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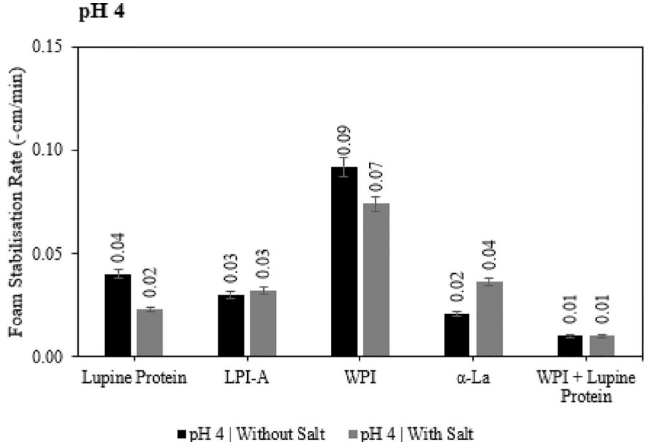


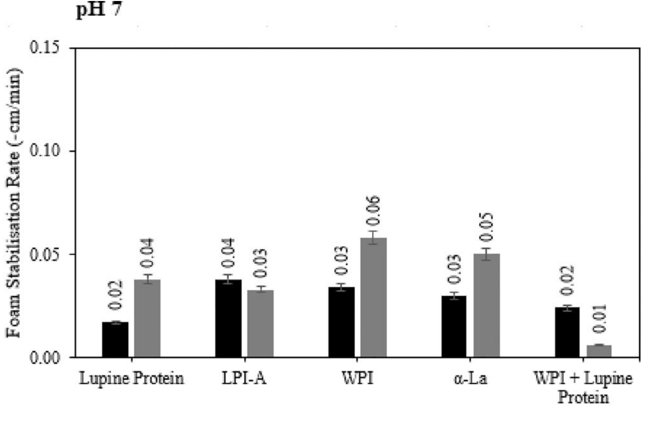
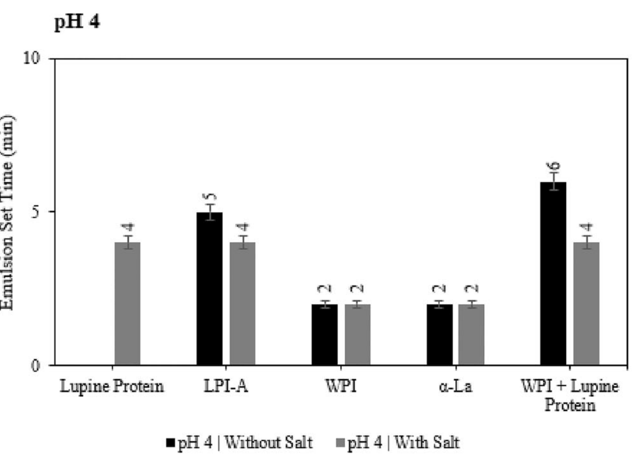


Figure 4 Protein foam stabilisation rate comparison at different pH levels.



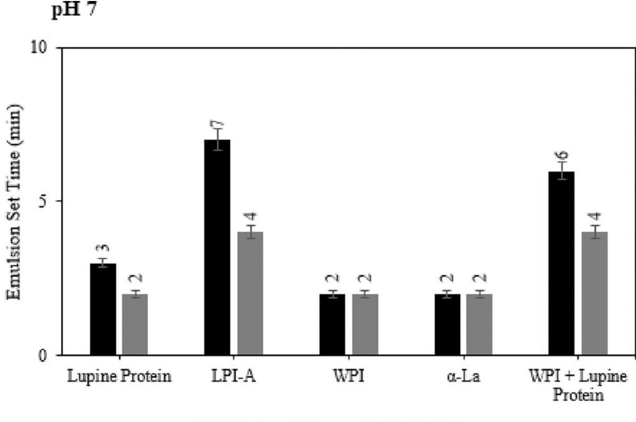


Figure 5 Protein emulsion stability comparison at different pH levels.

addition had no statistically significant impact on emulsification ($P > 0.05$).

The present study also examined emulsification properties of LPI and WPI mixed with oil at a 1:3 ratio found that LPI and WPI + Lupine Protein took the longest time to form oil-in-water emulsions and exhibited lower emulsion stability (Fig. 5). No significant impact of pH and salt variations on emulsification was observed. However, a control whey protein isolated by additional centrifugation exhibited superior functional properties compared to those isolated with salts.

Conclusion

This research focused on analysing the functional properties of lupine proteins in comparison to whey proteins. Both proteins demonstrated techno-functional properties like gelation and foaming when studied independently. However, this study highlights the complexity of whey and lupine proteins, which consist of multiple individual components that interact with each other, affecting the overall functional properties of the proteins.

The study revealed previously unreported gelling, foaming and emulsifying properties for WPI, LPI and the synergy of the two proteins. Gelation experiments demonstrated that lupine protein gels are generally weaker and softer than whey protein gels; however, when combined, they demonstrate exceptional stability and resistance to pH and $CaCl_2$ variations. It, therefore, emphasises the potential benefits of synergistic effects and functional stability under various conditions.

Future investigations will concentrate on refining extraction and purification techniques, as well as on the innovative application of composite gels in the food industry. The structural differences that could

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occur because of the variation in the salt concentrations will also be investigated.

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Author contributions

Maksim Ivanov: Investigation (lead); methodology (equal); visualization (lead); writing – original draft (lead). **Claire D. Munialo:** Conceptualization (lead); formal analysis (equal); methodology (equal); writing – review and editing (equal).

Conflict of interest statement

The authors have no conflicts of interest to declare.

Ethical statement

Ethics approval was not required for this research.

Peer review

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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