



Protein hydrolysis of *Camelina sativa* meals: A study of molecular weight distribution and emulsifying properties

Pau Sentís-Moré^{a,b}, Nàdia Ortega-Olivé^b, Ivan Robles-Rodríguez^b, Aritz Royo-Esnal^a, Maria-Paz Romero-Fabregat^{a,*}

^a University of Lleida, Food Technology Department. AGROTECNIO Center, Lleida, Spain

^b Eurecat, Centre Tecnològic de Catalunya, Unitat de Nutrició i Salut, Reus, 43204, Spain

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ABSTRACT

Camelina is an ancient oilseed known for its healthy oil composition and high crude protein content. Efforts have recently been made to reintroduced camelina in Europe and North America. This study aimed to propose an innovative strategy for valorising the camelina meal, a by-product obtained after oil extraction. Specifically, the investigation focused on the hydrolysis of protein directly applied to camelina meal studying two enzymes (Alcalase® and Protamex®) across three *Camelina* varieties (Calena, Vera and Sonny). Protein hydrolysates were successfully produced, with a maximum weight yield of 47%, and achieved values above 49 g/100 g in crude protein content and 58% in protein yield. The study revealed that Alcalase® and Protamex® produced similar protein molecular weight distribution but exhibited distinct technological properties. The emulsifying capacities of the protein hydrolysates were enhanced by Alcalase®, with the maximum emulsifying activity index reaching 36 m²/s. Conversely, Protamex® decreased the emulsifying capabilities of protein hydrolysates. To evaluate the applicability of the protein hydrolysates obtained, an oil-in-water emulsion was selected as a food application. The results suggested that camelina protein hydrolysates have the potential to serve as a new emulsifier ingredient. Furthermore, no significant differences were observed among the three camelina varieties in terms of protein yields or technological properties.

1. Introduction

Camelina sativa is an oilseed plant from the *Brassicaceae* family, an annual plant with a short life cycle. It is an ancient crop native from Europe and Central Asia, that experienced a significant decline during the Medieval Age (Mondor & Hernández-Álvarez, 2022). However, in recent times, camelina has made a resurgence in Europe and North America due to its adaptability to a wide range of environmental conditions, including cold and dry areas. Moreover, its capacity to thrive with low water and fertilizers requirements enhances to its economic viability (Berti et al., 2016). Researchers have also explored its potential as a valuable rotational crop (Ahmadvandi et al., 2021). *Camelina sativa* includes both spring and winter varieties, with spring varieties being the most widespread in the world (Kurasiak-Popowska et al., 2021).

Camelina crops are primarily cultivated for their oil, which is very attractive for its health properties and qualities. It contains a high amount of polyunsaturated fatty acids, such as ω -3 and ω -6, as well as

tocopherols and phenolic compounds (Abramovič et al., 2007; Ghobadi et al., 2021). Hexane solvent extraction is the conventional method for extracting camelina oil, although alternative methods such as cold press extraction or SC-CO₂ supercritical fluid extraction have been explored (Moslavac et al., 2014). While camelina oil has generally been recognized as safe (GRAS) for the human consumption by the US government (Food and Drug Administration, 2016), concerns have been raised in the EU regarding the presence of erucic acid and antinutrients (Panel on Contaminants in the Food Chain (CONTAM), 2009). Consequently, the utilization of camelina oil in food products and formulations remains limited. Other uses such as cosmetic applications (Ionescu et al., 2015; Kurasiak-Popowska et al., 2017) and biofuels are more prevalent (Arshad et al., 2022; Mondor & Hernández-Álvarez, 2022).

The by-product of camelina oil extraction, known as camelina meal, is highly valued as animal feed due to its residual oil content (approximately 10 to 15 g/100 g), containing fatty acids ω -3 and ω -6. Furthermore, it also contains significant amounts of protein (30 to 43 g/100 g)

* Corresponding author.

E-mail address: mariapaz.romero@udl.cat (M.-P. Romero-Fabregat).

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and crude fiber (10 to 15 g/100 g) (Berti et al., 2016; Ibrahim & El Habbasha, 2015). However, compared to other oilseed, camelina meal has received limited research attention (Li et al., 2014), with existing literature mainly focusing on its benefits in animal feed applications, such as improved egg production and quality (Aziza et al., 2013; Lolli et al., 2020), and increased polyunsaturated fatty acids (PUFA) in beef cattle (Cappelozza et al., 2012; Mondor & Hernández-Álvarez, 2022). Certain studies have explored the extraction of specific compounds from camelina meal, such as glucosinolates (Cao et al., 2015; Das et al., 2014) or protein fractions for their adhesion properties and potential application as bio-based wood adhesives (Li et al., 2015; Zhu et al., 2017).

Recent research efforts have focused on the protein fraction of camelina sativa meal. Li et al. (2014) identified glutelin as the main protein fraction, followed by globulin and albumin, highlighting camelina's well-balanced amino acid composition for human nutrition. High levels of glutamate (20% of total amino acids), aspartate (10%), leucine (8.3%), arginine (8.6%), and phenylalanine (5%) were found in camelina meal (Li et al., 2014). Other studies have investigated the isolation of whole proteins through various reagent steps and fractionation (Boyle et al., 2018; Sarv et al., 2017). Sarv et al. (2017) achieved 67 g protein/100 g dry basis in the protein precipitate, and 43 g protein/100 g dry bases in the protein solubilization, separating the polysaccharide fraction of mucilage before protein isolation to avoid interferences. Boyle et al. (2018) achieved a peak protein yield of 42% and protein content of 82 g/100 g in the protein isolates, although the weight yield was likely low due to multiple isolation stages. The structural and functional impact of the protein isolates obtained was also reviewed in the study of Boyle et al. (2018), and achieved emulsifying and foaming properties inferior to soy protein isolate.

Protein hydrolysates (HP) are produced by the action of a protease enzyme in a matrix that contains a protein fraction. It has been used for the valorisation of co- and by-products with a high protein content. An increase in weight yield and protein content for oilseed meals has been achieved in other works (Cejudo-Bastante et al., 2022; Sentís-Moré et al., 2022). Different proteases have been commonly used for enzymatic hydrolysis, such as Alcalase®, a mix of endopeptidases that cleaves peptide bonds at hydrophobic amino acids; Neutrase®, that can cleavage peptides bond with amino groups (Leucine and Phenylalanine); and Flavourzyme® and Protamex®, a mix of endo and exopeptidases, both enzymes are less used in enzymatic hydrolysis modification (Tacias-Pascacio et al., 2020; Vollet Marson et al., 2019). The modification of structures during enzymatic hydrolysis depends on the cleavage sites of enzymes (endo and exopeptidases). Therefore, applying the appropriate enzyme under optimal conditions can lead to an increase in technological properties, as many studies have shown in other sources (Aluko & McIntosh, 2005; Wang et al., 2022; Zheng et al., 2015). Boyle et al. (2018) already studied enzyme Protease M without favourable results on the protein functionality. In fact, Boyle et al. (2018) concluded that more research was needed to investigate the effect of hydrolysis on camelina meal protein, using several enzymes and identifying the optimal hydrolysis conditions.

Emulsification is one of the most important processes in food manufacturing. Oil-in-water (O/W) emulsions are commonly used in the food industry and are stabilized by proteins based on their amphiphilic properties. Some vegetal proteins have limited water solubility and therefore their emulsifying properties are limited (McClements & Gumus, 2016); therefore, its structure must be modified to increase its technological properties.

Therefore, the present work aimed to fill the gap in research on the production of protein hydrolysates and the modulation of their technological properties from camelina meal by using different enzymes and conditions. The hypothesis would be that the action of the enzymes would improve the emulsifying properties. In summary, the objectives of the present study were (1) to increase research on the *Camelina sativa* protein, applying a sustainable and scalable procedure to the camelina meal by-product from different varieties, (2) to study the effect of

enzymatic hydrolysis on the production of protein hydrolysates and their technological properties, applying two enzymes in their optimal extraction conditions, and finally (3) to test the effect of applying the hydrolysates as emulsifier substitutes in a mayonnaise product. The knowledge gained with the current work could be used to define the binomial enzyme-hydrolysis conditions for obtaining a potential camelina protein hydrolysate as emulsifier ingredient.

2. Materials and methods

2.1. Material

Camelina seeds of the varieties Vera, Calena and Sonny were kindly provided by Camelina Company Spain SL (Madrid, Spain), University of Bologna (Bologna, Italy) and KWS Saat SE (Einbeck, Germany), respectively. These varieties were produced in the area of Lleida (Spain) and detailed information about the crop management is described in Codina-Pascual et al. (2022). Camelina oil and meal were obtained by pressing at Reinartz GmbH & Co. KG (Neuss, Germany). Camelina meals were nutritionally characterized to determine crude protein by Kjeldahl combustion in a Bloc Digest and PRO-NITRO A distiller (Selecta, Barcelona, Spain), fiber content by enzymatic kit (Megazyme, Bray, Ireland), fat content in a Soxhlet apparatus (Selecta, Barcelona, Spain), moisture by heating to 105 °C, the ash content by incineration in a Select-Horn muffle furnace (Selecta, Barcelona, Spain) and finally carbohydrate content, calculated by weight difference in relation to the other macronutrients. The mucilage fraction was analysed in camelina seeds of the Calena variety, following the procedure of Sarv et al. (2017).

2.2. Extraction process

Briefly, an M20 Universal mill (IKA, Staufen, Germany) was used to grind the camelina meals and then passed through a 0.5 mm sieve (Haver & Boecker, Oelde, Germany) as pre-treatment. Camelina meals were dispersed in distilled water at a ratio of 1:10 (w:v). Two different enzymes were used, and the pH was pre-adjusted based on the optimal enzyme conditions (pH 8.0 for Alcalase® (Novozymes, Bagsvaerd, Denmark) and pH 7.0 for Protamex® (Novozymes, Bagsvaerd, Denmark)). Both enzymes were added in an enzyme/substrate ratio of 0.4 AU/g crude protein. Hydrolysis was performed for 2 h in a shaking laboratory bath (Selecta, Barcelona, Spain) at 50 °C. A blank was made for each extraction condition, without the addition of enzyme, leading to 2 more samples for each variety. At the end of the hydrolysis, the enzymes were inactivated by heating for 15 min in a bath at 80 °C. In the solid/liquid separation stage, centrifugation at 7083×g (8000 rpm) for 10 min at room temperature with a Universal Centrifuge 320 R (Rotor 1620A, Hettich Zentrifugen, Tuttlingen, Germany). The liquid phases were freeze-dried in a Lyobetta 10 equipment (Telstar, Barcelona, Spain) and stored at −20 °C until analysis.

2.3. Extraction yield

Performance parameters were calculated to evaluate the extraction yield of the different samples. The weight yield was calculated using Equation (1).

$$\text{Weight Yield (\%)} = \frac{\text{Final weight of the protein hydrolysate}}{\text{Initial weight of the source}} \times 100 \quad (1)$$

In parallel, the protein yield was calculated with Equation (2).

$$\text{Protein Yield (\%)} = \frac{\text{Final protein weight of the protein hydrolysate}}{\text{Initial protein weight of the source}} \times 100 \quad (2)$$

Both were expressed in percentage (%). All data were expressed as the mean ± the standard deviation of two replicates.

2.4. Protein hydrolysates characterisation

2.4.1. Amino acid (AA) analysis

Amino acids content was measured in the camelina meal sources and protein hydrolysates. Briefly, 100 mg of each sample was weighted and 10 mL of 6N HCl (Sigma-Aldrich, Saint Louis, MO, USA) was added to ensure a complete hydrolyzation at 110 °C for 24 h. An aliquot of 50 µL was separated and evaporated completely. The amino acids analysis was performed by ultra-performance liquid chromatography with instrumental XEVO-TQS (Waters, Milford, MA, USA) using an electrospray ionization (ESI) operated in positive mode. Samples were analysed in the ACQUITY UPLC® BEH Amide 1.7 µm column (2.1 x 100 mm) (Waters, Milford, USA) with a binary phase at a flow rate of 0.5 mL/min. The eluent A was acetonitrile with formic acid 0.15% and B was 10 mM ammonium formate with formic acid 0.15% (Sigma-Aldrich, Saint Louis, MO, USA). The elution gradient at 35 °C was: 0–6 min, 80%–20% B; 6–7.5 min, 75%–25% B; 7.5–9 min, 70%–30% B; 9–12 min, 25%–65% B; 12–14 min 80%–20% B. Data acquisition was carried out by MassLynx v4.1 software (Waters, Milford, MA, USA).

The amino acid yield was calculated with Equation (3).

$$AA \text{ Yield (\%)} = \frac{\text{Total AAs in protein hydrolysate} \times \text{weight yield}}{\text{Total AAs in camelina cultivar}} \times 100 \quad (3)$$

2.4.2. Molecular weight distribution (MWD)

The molecular weight distribution (MWD) of HP was analysed by high-performance liquid chromatography (Waters, Milford, USA) according to Bougateg et al. (2020), with some modifications. Samples were analysed in the XBridge BEH 125Å size exclusion chromatography (SEC) column of 3.5 µm diameter and 7.8 x 300 mm length (Waters, Milford, USA) with the mobile phase of acetonitrile 30% with trifluoroacetic acid 0.1% (Sigma-Aldrich, Saint Louis, MO, USA). Peaks signals were detected at 214 nm with a Waters 2996 Photodiode Array Detector (Waters, Milford, USA). The molecular weight standards used were Aprotinin (6511 Da), Insulin (5733 Da), Insulin Chain B (3495 Da), Angiotensin (1046 Da), and L-Tryptophan (204 Da). The calibration curve and data were obtained with Empower 3 software (Waters, Milford, USA). MWD results in all samples are expressed as percentages in groups of different molecular weights (MW): i) >3000 Da, ii) 3000 to 1000 Da, iii) 1000 to 500 Da, iv) 500 to 200 Da, v) <200 Da.

2.4.3. Determination of emulsion activity and stability indexes

Emulsion properties were determined according to Pearce & Kinsella, 1978 with modifications. In summary, 120 mg of camelina meal HP was solubilised in 12 mL of distilled water and with a vortex. Sunflower oil (4 mL) was added, and the mixture was emulsified with a Polytron PT 10-35 (Kinematica, Malters, Switzerland) at 14,000 rpm. Then, 50 µL of the bottom part of the emulsion was added to 10 mL of 0.1% (w/v) sodium dodecyl sulphate (SDS) (Sigma-Aldrich, Saint Louis, MO, USA) and absorbance was measured in the spectrophotometer at 500 nm, at times 0 and 10 min, to obtain Abs_0 and Abs_{10} , respectively. Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI) were calculated with the Equations (4) and (5).

$$EAI \left(m^2 / g \right) = \frac{2 \times 2.303 \times Abs_0 \times DF}{C \times \phi \times 10000} \quad (4)$$

$$ESI \left(min \right) = \frac{Abs_0}{Abs_0 - Abs_{10}} \times (\Delta t_{10} - \Delta t_0) \quad (5)$$

Where DF is the dilution factor of the samples, C is the protein mass and ϕ is the volume fraction of the oil in the emulsion.

2.5. Emulsion

2.5.1. Emulsion production

Camelina protein hydrolysates were applied as an emulsifier in a mayonnaise product. Formulation A was made with 25 g of cow milk, 50 g of sunflower oil, 1.25 g of lemon juice, 0.25 g of salt and 0.38 g of the emulsifying agent. Formulation B was made with 25 g of cow milk, 35 g of sunflower oil, 1.25 g of lemon juice, 0.25 g of salt and 0.38 g of the emulsifying agent. The emulsifying agent was soy lecithin in the control formulation or protein hydrolysate on the test samples.

2.5.2. Emulsion characterisation

Two indices were used to evaluate the emulsions. The creaming index (CI) was calculated adapted from (Ali & EL Said, 2020), introducing 10 g of the mayonnaise sample in a graduated tube, and the emulsion high was recorded after 1 and 5 days. CI was calculated using Equation (6).

$$\text{Creaming Index (CI)} = \frac{\text{Final height of the emulsion}}{\text{Initial height of the emulsion}} \quad (6)$$

Emulsion stability (ES) was calculated by placing 10 g of the emulsion sample in a tube, heating for 30 min at 80 °C. The samples were then centrifugated at 2767 × g (5000 rpm) for 30 min at room temperature with a Universal Centrifuge 320 R (Rotor 1620A, Hettich Zentrifugen, Tuttlingen, Germany). The determination was made with the Equation (7).

$$\text{Emulsion stability (ES)} = \frac{\text{Weight of the pellet}}{\text{Initial weight of the emulsion}} \times 100 \quad (7)$$

2.6. Statistical analysis

The results are presented as mean of at least two replicates. The statistical analysis of the full factorial was carried out using JMP® Pro 16.0.0 (SAS Institute, Cary, USA). Appropriate least square means were compared using the t -test after the F -tests had been performed. The difference between the samples was significant when $p < 0.01$.

3. Results and discussion

3.1. Source characterisation

The composition results of the camelina meal for the three varieties are presented in Table 1. The crude protein content ranged from 36 to 39 g/100 g, which aligns with previous reports ranging from 30 to 43 g crude protein/100 g (Berti et al., 2016). The camelina meals exhibited also high fiber content, ranging from 25 to 29 g/100 g, with a fat fraction of around 10 g/100 g. Regarding the amino acid analysis, the total amino acid content was significantly ($p < 0.01$) higher in the Calena variety, followed by the Vera and Sonny varieties. The camelina meals were particularly rich in glutamic acid (5.3 to 7.2 g/100 g) and aspartic acid (2.3 to 3.3 g/100 g) (Table S1). These amino acids compositions are consistent with previous findings on camelina meal (Li et al., 2014). Other studies have focused on the amino acid profile of camelina, but were done directly on seeds or groats (Bátrina et al., 2020; Zubr, 2003),

Table 1

Camelina meal composition on different parameters: macronutrients of moisture, crude protein, fat, carbohydrates, fiber and ash in g/100 g; and total amino acids in g/100 g.

Variety	Parameters (g/100 g)						
	Moisture	Crude Protein	Fat	Carbohydrates	Fiber	Ash	Total Amino Acids
Calena	9.2 ^a	36.5 ^a	10.5 ^a	9.0 ^a	29.0 ^a	5.8 ^a	34.9 ^a
Vera	9.1 ^a	38.5 ^a	10.1 ^a	11.3 ^a	25.2 ^a	5.8 ^a	30.2 ^b
Sonny	9.1 ^a	35.8 ^a	10.6 ^a	11.0 ^a	27.5 ^a	6.0 ^a	26.2 ^c
SED	0.39	0.47	0.81	0.75	0.24	0.89	0.25

The results are expressed as Mean of measurements, and results not connected by the same letter are significantly different ($p < 0.01$). Statistics made by column. SED = Standard Error of the Difference.

Table 2

Results of the production of protein hydrolysates from different camelina meal varieties: weight yield (%), crude protein (g/100 g), protein yield (%), total amino acids (g/100 g) and amino acid yield (%).

Sample		Weight Yield (%)	Crude Protein (g/100 g)	Protein Yield (%)	Total amino acids (g/100 g)	Amino acid Yield (%)
Calena Meal						
pH 7	None	25.1 ^e	44.0 ^{cd}	29.7 ^f	31.5 ^f	22.8 ^j
	Protamex®	45.4 ^a	47.1 ^{abc}	57.6 ^a	42.1 ^a	55.3 ^c
pH 8	None	32.8 ^c	48.7 ^{ab}	43.0 ^c	42.0 ^{ab}	39.8 ^{fg}
	Alcalase®	40.3 ^b	49.3 ^a	53.2 ^b	38.4 ^{cd}	44.5 ^{de}
Vera Meal						
pH 7	None	17.7 ^g	44.2 ^{cd}	20.7 ^g	20.8 ^g	12.4 ^k
	Protamex®	47.0 ^a	46.4 ^{abc}	57.5 ^a	38.5 ^{cd}	60.5 ^b
pH 8	None	26.6 ^{de}	48.6 ^{ab}	34.0 ^e	36.1 ^{de}	32.1 ^h
	Alcalase®	33.7 ^c	49.0 ^{ab}	43.5 ^c	38.5 ^{cd}	43.4 ^{ef}
Sonny Meal						
pH 7	None	20.8 ^f	42.4 ^d	23.8 ^g	34.7 ^e	27.9 ⁱ
	Protamex®	46.1 ^a	46.0 ^{bc}	57.0 ^{ab}	39.4 ^{bc}	70.0 ^a
pH 8	None	28.8 ^d	48.9 ^{ab}	38.0 ^d	34.8 ^e	38.7 ^g
	Alcalase®	35.4 ^c	47.1 ^{abc}	44.8 ^c	35.1 ^e	47.8 ^d
SED		0.28	0.36	0.21	0.25	0.40

The results are expressed as Mean of measurements, and results not connected by the same letter are significantly different ($p < 0.01$). Statistics made by column. SED = Standard Error of the Difference.

in which the results reported the same dominant amino acids as those described in our work. In the carbohydrates fraction, it is worth noting the amount of mucilage present in camelina seeds, which measured 6.9 g/100 g in camelina seeds, consistent with previous reports (Ibrahim & El Habbasha, 2015; Zubr, 2010).

When comparing the three varieties in this study, the Vera variety had a higher crude protein content compared to the other two varieties, with a significant difference ($p < 0.01$). In terms of other macronutrient fractions, the results were similar among the varieties, with only slight differences observed in fiber and fat content for the Vera variety and lower carbohydrate content for the Calena variety. However, these differences were not statistically significant ($p < 0.01$) (Table 1).

3.2. Production of the protein hydrolysates

The production flowchart for protein hydrolysates was tested based on a previous study conducted by the research group (Sentís-Moré et al., 2022). However, challenges were encountered in the solid-liquid separation process due to a high-density liquid fraction after the hydrolysis. Consequently, the optimised solid-liquid separation process based on vacuum filtration was not suitable in camelina meal for optimal protein hydrolysate production. This issue may be attributed to the high fraction of carbohydrates in camelina meal. Although camelina meal has a reported low starch content of around 1 g/100 g (Ibrahim & El Habbasha, 2015), the presence of mucilage fraction, which has been reported in high quantities (6.7 g/100 g) (Zubr, 2010), and described as an interference in the protein extraction and purification of camelina seeds before the extraction of camelina oil (Li et al., 2014; Sarv et al., 2017). As the objective of this work is to apply a sustainable process to produce

protein hydrolysates from camelina varieties, the minimization of operation units and their scalability was an important factor. Consequently, it was decided to keep only one step and use centrifugation instead of vacuum for the recovery of solid-liquid fraction. Furthermore, protein hydrolysis also depends on the type of proteins in the source. Globular protein has been described as the most difficult type of protein to be hydrolysed (Wang et al., 2022). In the case of camelina, previous studies described only a globulin fraction of 15% of the total protein (Li et al., 2014); therefore it could be assumed that the hydrolysis with a single enzyme can be used without the need for complementary physical treatment required (Wang et al., 2022).

The pH and temperature extraction conditions were selected based on the optimal conditions for the two enzymes used: Alcalase® and Protamex® (Rotimi E. Aluko, 2018). The results of protein hydrolysates production are presented in Table 2, and the outcomes of the full factorial statistical analysis are provided in Table 3. Starting with the control samples, the pH had an effect on extraction with pH 8 samples exhibiting significantly higher weight yield values and crude protein content compared to pH 7 samples ($p < 0.01$), consequently affecting the protein yield. This indicates that the solubility of camelina protein is higher at pH 8 than at pH 7. These results are in correlation with the solubility findings in camelina protein isolates, where Ngo and Shahidi (2021) observed solubility at the isoelectric point (pH 4), which gradually increased as the pH rose.

Regarding the enzyme effect, Protamex® provided the highest weight yields, reaching 47% in the Vera variety. On the contrary, Alcalase® showed higher crude protein content, reaching 49 g/100 g. Protamex® also gave higher protein yields, achieving 58% for the Calena and Vera varieties. The impact of the enzyme on weight and protein

Table 3

Statistical results F ratio and p value of full factorial analysis on variety and enzyme effects, from the results of protein hydrolysate production and characterisation of Camelina meal.

Effect	Weight Yield		Crude Protein Content		Protein Yield		Total Amino Acids		Amino Acids Yield		EAI		ESI	
	F ratio	p	F ratio	p	F ratio	p	F ratio	p	F ratio	p	F ratio	p	F ratio	p
Variety	2.01	0.1681	0.60	0.5631	2.04	0.1652	1.21	0.3243	2.58	0.1088	0.98	0.3979	1.28	0.3061
Enzyme	64.06	<0.0001	1.89	0.1860	37.87	<0.0001	4.10	0.0381	41.00	<0.0001	131.04	<0.0001	1.23	0.3189
Variety*Enzyme	0.92	0.4763	0.09	0.9826	0.46	0.7631	0.83	0.5293	0.87	0.5026	0.27	0.8952	0.27	0.8923

Values in bold are significant ($p < 0.01$).

yield was also observed in other oilseed studies (Cejudo-Bastante et al., 2022; Sentís-Moré et al., 2022; Özdemir et al., 2022). In general, Alcalase® provided protein hydrolysates with high crude protein content while Protamex® with more weight yield. The enzyme effect on the two yields was significant ($p < 0.01$) but not in the crude protein content ($p > 0.01$), as showed by statistical analysis in the Table 3. The main differences were indicated in weight yield between the control samples and samples treated with the enzymes, corroborated in the F ratio (Table 3). It is worth mentioning that the enzyme Protamex® had a much greater action on weight yield, crude protein content and protein yield than Alcalase®, compared to its control results, which were very low at pH 7. Regarding the three varieties used in this study, no significant differences were observed between them, neither at pH 7 nor at pH 8 ($p > 0.01$) (Table 3); so, there are no major differences between them in terms of production of the different protein hydrolysates.

To the best of our knowledge and based on the literature review conducted, this is the first study where protein hydrolysis was directly performed on camelina meal without a prior protein isolation step. In fact, few studies have focused on camelina meal protein, thus our discussion primarily relies on a comparison with two identified works conducted by Li et al. (2014) and Boyle et al. (2018), which aimed to investigate different procedures and conditions for camelina protein isolation and characterization. Considering this, the weight yields obtained in the current work (up to 47%) were similar to those reported by Li et al. (2014) (48%). However, greater differences in crude protein content and protein yield were observed mainly due to the application of multiple stages for protein isolation and also for purification of protein fractions. In these two works, extracts of 82–87 g protein/100 g were obtained, which were much greater than our values (49 g crude protein/100 g). Regarding protein yield, different results were obtained, Li et al. (2014) obtained maximum values of 88%, as a consequence of the

multiple fractions recovery; while Boyle et al. (2018) achieved a maximum protein yield of 42%, lower than the 58% achieved in our study. The multiple steps applied by (Boyle et al., 2018) to obtain the purified protein extract may explain the protein loss through the process. In summary, the production of protein hydrolysate in our study, obtained in a single step and without removing the mucilage fraction, allowed us to achieve more weight of extracts than the control process (up to 47% of weight yield) with a high purity in crude protein (up to 49%), considerably increasing the initial protein value of camelina meal. In fact, higher protein yields were achieved than some of the previous camelina works, with a maximum of 58%.

3.3. Protein hydrolysates characterisation

The protein profile of protein hydrolysates was analysed using two characterisations, MWD and amino acid composition. The size exclusion chromatography results are presented in the Fig. 1 as groups of different molecular weight fractions. It shows that enzymatic hydrolysis influences the distribution of the molecular weight, especially in the medium and small molecular weight fractions (3000 to 1000, 1000 to 500 and 500 to 200 Da) compared to samples without enzymatic treatment (controls), which had a higher fraction (>3000 Da), as can be corroborated in the chromatograms of the Vera variety for both enzymes used (Fig. 2). Although, fraction lower than 200 Da was altered in the analysis due to higher peaks located nearby. When comparing the control samples, differences between the two extraction pH levels were observed, likely related to the variation in crude protein content, as mentioned previously. The hydrolysis process at pH 8 allowed the solubilization of more proteins of >3000 Da. Regarding the comparison of MWD between both enzymes used, no major differences were reported. Additionally, there were no notable differences in MWD between the different

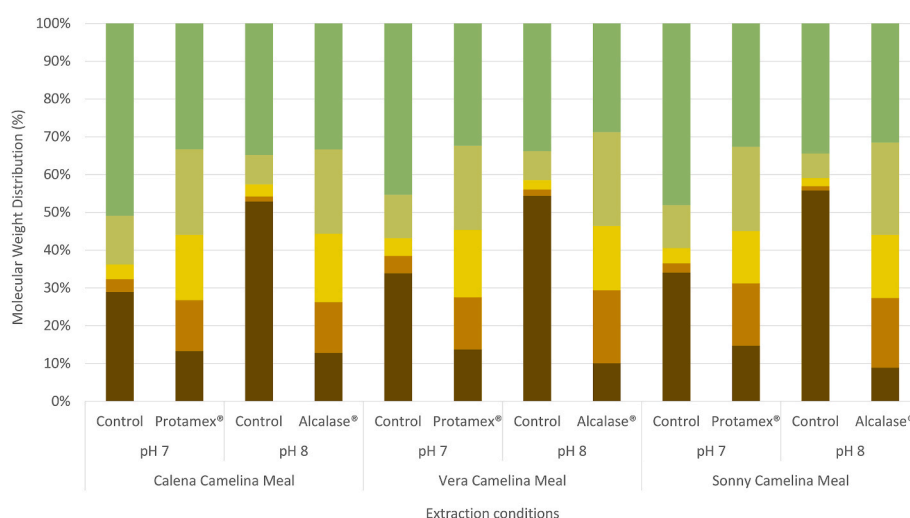


Fig. 1. Molecular weight distribution (%) of the protein hydrolysates from camelina meal of the Calena, Vera and Sonny varieties, by fractions: >3000 Da, 3000 to 1000 Da, 1000 to 500 Da, 500 to 200 Da, and <200 Da, on different extraction conditions. The calculation of the distribution of molecular weights was made by separation of the chromatogram in the area delimited by the valleys of the main chromatographic peaks.

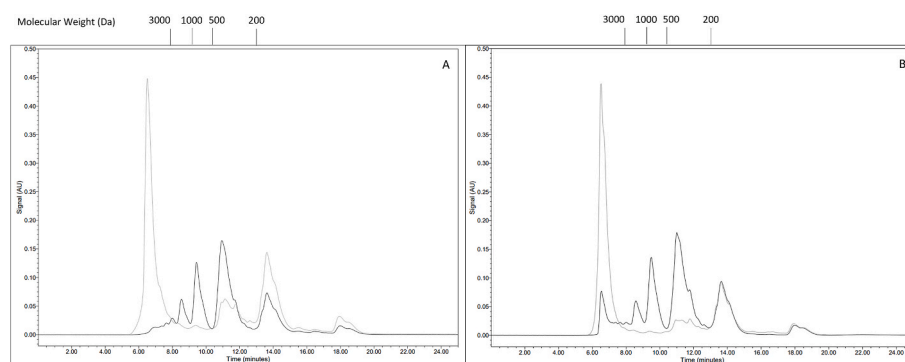


Fig. 2. Chromatogram comparison from the protein molecular weight distribution of protein hydrolysates from camelina meal of the Vera variety: A) Control pH 7 — and Protamex® pH 7. — B) Control pH 8 — and Alcalase® pH 8 —.

Table 4

Results of Emulsifying Activity Index (EAI) (m^2/g) and Emulsifying Stability Index (ESI) (min) of the protein hydrolysates of Camelina meal from different varieties.

Sample		Emulsifying Activity Index (m^2/g)	Emulsifying Stability Index (min)
Calena Meal			
pH 7	None	25.1 ^e	20.2 ^{cde}
	Protamex®	6.3 ^f	28.3 ^b
pH 8	None	30.9 ^{cd}	32.6 ^a
	Alcalase®	31.9 ^{bcd}	22.8 ^{cd}
Vera Meal			
pH 7	None	24.0 ^e	18.3 ^{ef}
	Protamex®	6.7 ^f	23.4 ^c
pH 8	None	30.2 ^d	31.8 ^a
	Alcalase®	34.9 ^{ab}	16.1 ^{fg}
Sonny Meal			
pH 7	None	25.8 ^e	13.6 ^g
	Protamex®	7.9 ^f	19.7 ^{de}
pH 8	None	33.7 ^{abc}	31.3 ^{ab}
	Alcalase®	35.5 ^a	19.6 ^{de}
SED		0.33	

The results are expressed as Mean of measurements, and results not connected by the same letter are significantly different ($p < 0.01$). Statistics made by column. SED = Standard Error of the Difference.

camelina varieties. However, the stronger hydrolysis action of Alcalase® on the high molecular weight fraction (3000 Da) observed in the control samples could be highlighted. In comparison (Li et al., 2014), also conducted HPLC analysis on the different protein fractions obtained and reported molecular weights higher than 29000 Da, confirming that only protein fraction isolation was achieved without hydrolysis.

The results of the amino acids analysis (Table S1) showed that camelina meal protein hydrolysates exhibited high levels of glutamic acid (ranging from 4.7 to 10 g/100 g) and aspartic acid (ranging from 2.8 to 5 g/100 g). As described in the source characterisation section (3.1), these results were also aligned with other amino acid results obtained in camelina meals and protein fractions (Li et al., 2014). Amino acid yield ranged from 43% to 70%, and the trends were similar to protein yield. The control samples showed a significant effect of pH, with greater recovery at pH 8 in all camelina varieties (Table 2), with a significant enzyme effect ($p < 0.01$) (Table 3). Furthermore, there was a significant enzyme effect ($p < 0.01$) on amino acid yields, with higher yields obtained from protein hydrolysates produced with Protamex® compared to those obtained with Alcalase® (Table 2).

3.4. Technological properties of protein hydrolysates

The objective of this work was to assess the improvement of

emulsifying properties in the protein hydrolysates obtained, which were evaluated using EAI and ESI, with the results presented in Table 4. The controls (samples without enzyme treatment) exhibited high EAI values. The influence of different extraction conditions on emulsifying properties could also be affirmed. For instance, the pH of extraction had a significant effect, with samples obtained at pH 7 generating extracts with significantly lower EAI compared to those obtained at pH 8 for all three varieties studied ($p < 0.01$). Additionally, the enzymes used in the production of protein hydrolysates resulted in distinct outcomes. On the one hand, Protamex® protein hydrolysates showed low EAI values, significantly lower than the control at the same pH ($p < 0.01$). On the other hand, the protein hydrolysates of Alcalase® showed better results than the control values and achieved the highest overall EAI value, reaching a maximum of 36 m^2/g . The significant differences in EAI with Alcalase® variety compared to its controls, were observed only in the Vera ($p < 0.01$), which could be attributed to the significantly higher crude protein content in the initial Vera camelina meal (Table 1). These differential results in EAI between the two enzymes could be explained by the differences in peptide bond cleavage mechanisms and the specificity of these enzymes towards camelina protein. In this sense, Alcalase® generated protein hydrolysates with higher emulsifying properties, while Protamex® had a decreasing effect on EAI, compared to the control samples at the same pH, mainly due to cleavage of endo-

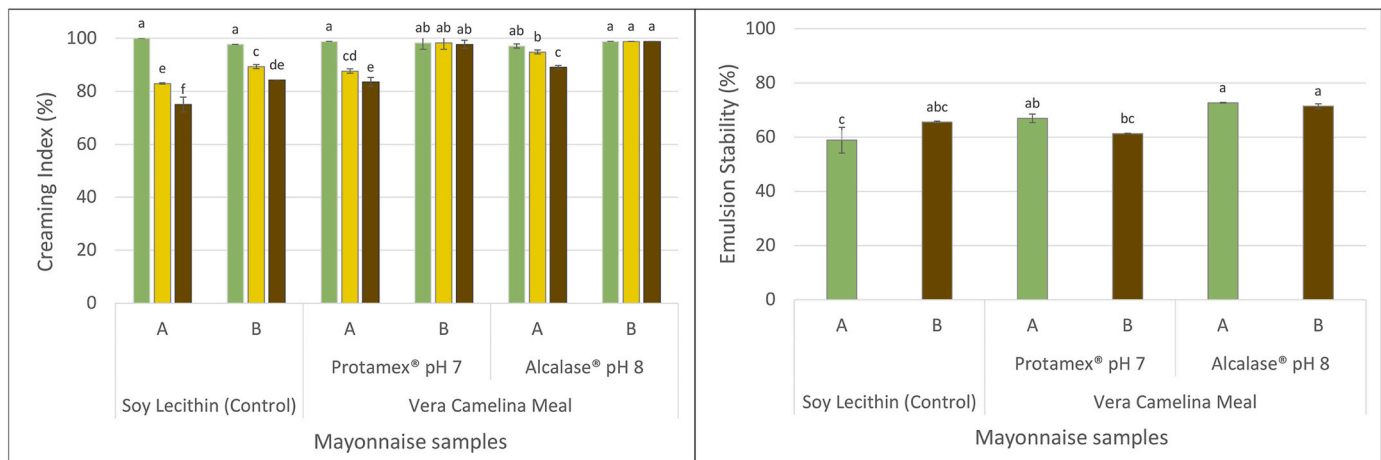


Fig. 3. Results of the characterisation on the emulsion samples: Soya lecithin (Control) and protein hydrolysates of Vera camelina meal of Protamex® pH 7 and Alcalase® pH 8 in two Formulations: A (25 g cow milk, 50 g sunflower oil, 1.25 g lemon juice, 0.25 g salt and 0.38 g emulsifying agent) and B (25 g cow milk, 35 g sunflower oil, 1.25 g lemon juice, 0.25 g salt and 0.38 g emulsifying agent). 3 A) Results of Creaming Index (CI) in different days: ■ Day 0, ■ Day 1 and ■ Day 5. B) Results of the Emulsion Stability (ES): ■ Formulation A and ■ Formulation B. The results are expressed as Mean of measurements with the standard deviations, and results not connected by the same letter are significantly different ($p < 0.01$).

and exo-peptide bonds.

In the ESI, a pattern was also established in the results of the different varieties, although the effect of the variety and the enzyme were not significant, nor was the interaction ($p > 0.01$) (Table 3). The control samples at pH 8 achieved higher ESI results, exhibiting a significant difference ($p < 0.01$) compared to the control samples at pH 7 for all varieties studied. The Calena Meal variety obtained the maximum ESI at 33 min in the control sample at pH 8. Regarding the effect of enzyme treatment, Protamex® showed improved ESI results compared to its control samples at pH 7, while Alcalase® obtained worse results than the control at pH 8. Both differences were significant across all varieties studied ($p < 0.01$). These results could be directly related to MWD for control samples: controls extracted at pH 7 had lower fractions (> 3000 Da and from 3000 to 1000 Da) and had the lowest ESI values. In contrast, controls at pH 8 had higher molecular weight fractions in the MWD and showed the highest values of ESI.

In comparison to other studies, Ngo and Shahidi (2021) observed a correlation between EAI and pH, with the index increasing with pH and achieving the highest value at pH 10 (120 m²/g) using an ultrasonically assisted extraction method to recover camelina protein and evaluate its functional properties. Similar trends were obtained when comparing pH 7 and 8 in the present study. Some studies suggested that wide-range enzymes, such as Alcalase® and Protamex®, were more effective with non-protein isolated sources (Tacias-Pascacio et al., 2020), so the proposed process in this work, which avoids protein fraction isolation, is able to achieve similar yield values with also modulating and increasing technological properties (Wang et al., 2022).

3.5. Application of camelina meal protein hydrolysates on an emulsion

As there were no significant differences among the different varieties in any of the parameters studied (Table 3), protein hydrolysates obtained from the Vera meal variety were selected and applied to a commercial O/W emulsion product, a mayonnaise made with milk and sunflower oil. Protein hydrolysate from Protamex® at pH 7 and protein hydrolysate of Alcalase® at pH 8. The aim was to apply the protein hydrolysates as an emulsifying agent, so mayonnaises were compared with one made with soy lecithin, an emulsifying agent commonly used in the food industry. The results of CI and ES can be found in Fig. 3, comparing the two formulations used in each case. The statistical analysis results are presented in Table S2.

In the case of CI, the results showed a significantly better stability

($p < 0.01$) in the formulation B for all samples, likely due to the lower quantity of sunflower oil relative to the aqueous phase. Furthermore, both protein hydrolysates showed better CI compared to the soy lecithin emulsion in both formulations A and B, with significant differences observed on days 1 and 5 ($p < 0.01$). The CI remained above 90% in the emulsion using Vera Protamex® and Vera Alcalase® in formulation B even after 5 days from emulsion formation. Similar findings were reported in other studies, as in the work of Ali and El Said (2020), where egg was replaced with arabic gum in a vegan mayonnaise, resulting in better CI compared to the control samples.

In relation to emulsion stability, the emulsions made with protein hydrolysates had better emulsion stability than the soy lecithin emulsion, with significant differences observed only in formulation A ($p < 0.01$). This indicates that the protein hydrolysates from camelina meal were effective in forming and stabilizing an emulsion. Additionally, the emulsion made with Vera Alcalase® showed better stability than the one made with Vera Protamex®, with significant differences observed only in formulation B ($p < 0.01$).

For the application of camelina meal protein hydrolysates as an emulsifying agent in food products, further investigation is necessary to assess the presence of antinutrient such as glucosinolates, trypsin inhibitors and condensed tannins, as these molecules could be present in the protein hydrolysates (Panel on Contaminants in the Food Chain (CONTAM), 2009).

4. Conclusions

The protein hydrolysates were successfully produced from different varieties of Camelina meal, namely Calena, Vera, and Sonny, using two different enzymes: Alcalase® and Protamex®. For the production, a centrifugation method was employed for solid-liquid recovery, due to the thickness provided by the mucilage fraction. This fact though, did not affect the protein and weight yields. The enzymatic treatment increased the quantity of protein hydrolysate obtained and enhanced the crude protein content compared to their respective control samples. The MWD analysis revealed a change in the proportion of the lower molecular weight group, indicating the impact of enzymolysis on the molecular structure of the protein. The enzymatic treatment also influenced the technological properties of the hydrolysates, with Alcalase® exhibiting an increase in emulsifying activity while Protamex® showed a decrease. Protein hydrolysates from the Vera variety were tested on an emulsion as an emulsifying agent, with good results compared to soy lecithin, a common and

recognized ingredient used in the food industry. This suggests that camelina protein hydrolysates have promising emulsifying properties and could serve as a viable alternative to traditional emulsifiers.

Credit authorship contribution statement

Pau Sentís-Moré: Conceptualization, Methodology, Validation, Investigation, Writing – Original Draft. **Nàdia Ortega-Olivé:** Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Funding acquisition. **Ivan Robles-Rodriguez:** Methodology, Validation, Investigation. **Aritz Royo-Esnal:** Resources, Investigation, Funding acquisition. **Maria-Paz Romero-Fabregat:** Conceptualization, Validation, Resources, Writing – Review & Editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Experimental data are available in <https://doi.org/10.34810/data778>.

Protein hydrolysis of *Camelina sativa* meals: A study of molecular weight distribution and emulsifying properties (Original data) (Dataverse)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fbio.2023.103062>.

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