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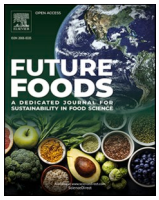

Protein extraction from oat: Isoelectric precipitation and water-based extraction

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ABSTRACT

Protein extraction by alkaline extraction-isoelectric precipitation (AE-IEP) uses alkaline conditions that contribute to off-colour formation, through auto-oxidation of inherent phenolic compounds. We aimed to develop an alternative protein extraction process yielding comparable protein content to AE-IEP while avoiding alkaline conditions. It was hypothesised that a water-based extraction (WBE) of oats would yield lightly coloured native proteins. Protein contents and yields were determined using a newly established nitrogen-to-protein conversion factor of 5.12, based on the amino acid composition. WBE led to a comparable protein content to AE-IEP (69.5 % vs 70.1 % dry matter (DM)), but a 2.5 times lower yield (0.27 g/g protein DM). SDS-PAGE and quantitative proteomics revealed a protein composition of 60 % globulins and 40 % prolamins in both concentrates. A similar protein nativity was observed using differential scanning calorimetry. The WBE yielded in a lighter-coloured protein powder. The low protein yield of water-based extraction is currently a limitation for industrial applications. Nevertheless, this simple process can be used as a pre-treatment for AE-IEP to produce a more neutral-coloured powder. This study expands the knowledge on protein extraction from oats by comparing novel extraction methods with classic AE-IEP and establishing the nitrogen-to-protein conversion factor for oats.

AE-IEP	Alkaline extraction-isoelectric precipitation
IPF	Insoluble protein fraction
SPF	Soluble protein fraction
SRF	Starch-rich fraction
WBE	Water-based extraction

1. Introduction

Oat proteins have become of interest as a novel functional ingredient due to their high protein content of 12–20 g/100 g on dry matter (DM) (Mel and Malalgoda, 2022). The protein content varies depending on the genotype, environmental factors, and agricultural practices (Mel and Malalgoda, 2022). Additionally, the variation in reported protein content is caused by using different protein determination methods and nitrogen-to-protein conversion factors (N-factors). In the past, an N-factor of 6.25 was assumed for many food proteins, which was later adapted to 5.5 for oat proteins (Jones, 1931; Tkachuk, 1969). Other N-factors reported for oats were 5.4 by Heathcote (1950), while Mossé (1990) reported an N-factor with upper and lower limit of 5.52 and 5.31. However, these values do not take into account the different compositions of food proteins and the presence of other nitrogen-containing compounds, such as avenanthramides, which are oat-specific phenolamides. Despite the rising application of oat as a protein source, there has been no recent research verifying the N-factor of oats.

Industrially available plant proteins such as soy and pea are commonly extracted through the wet fractionation process referred to as 'alkaline extraction-isoelectric precipitation' (AE-IEP) (Schutyser and van der Goot, 2011). This extraction process includes bringing the proteins into solution by increasing the pH to alkaline conditions (above pH 8.0), followed by precipitation of the proteins at their isoelectric point (~4–5.5 for oat) (Kumar et al., 2021). This process typically results in a protein content of at least 64 % (DM) and a yield of more than 50 % (DM) for pulses (Boye et al., 2010a). For oat protein extracted by AE-IEP

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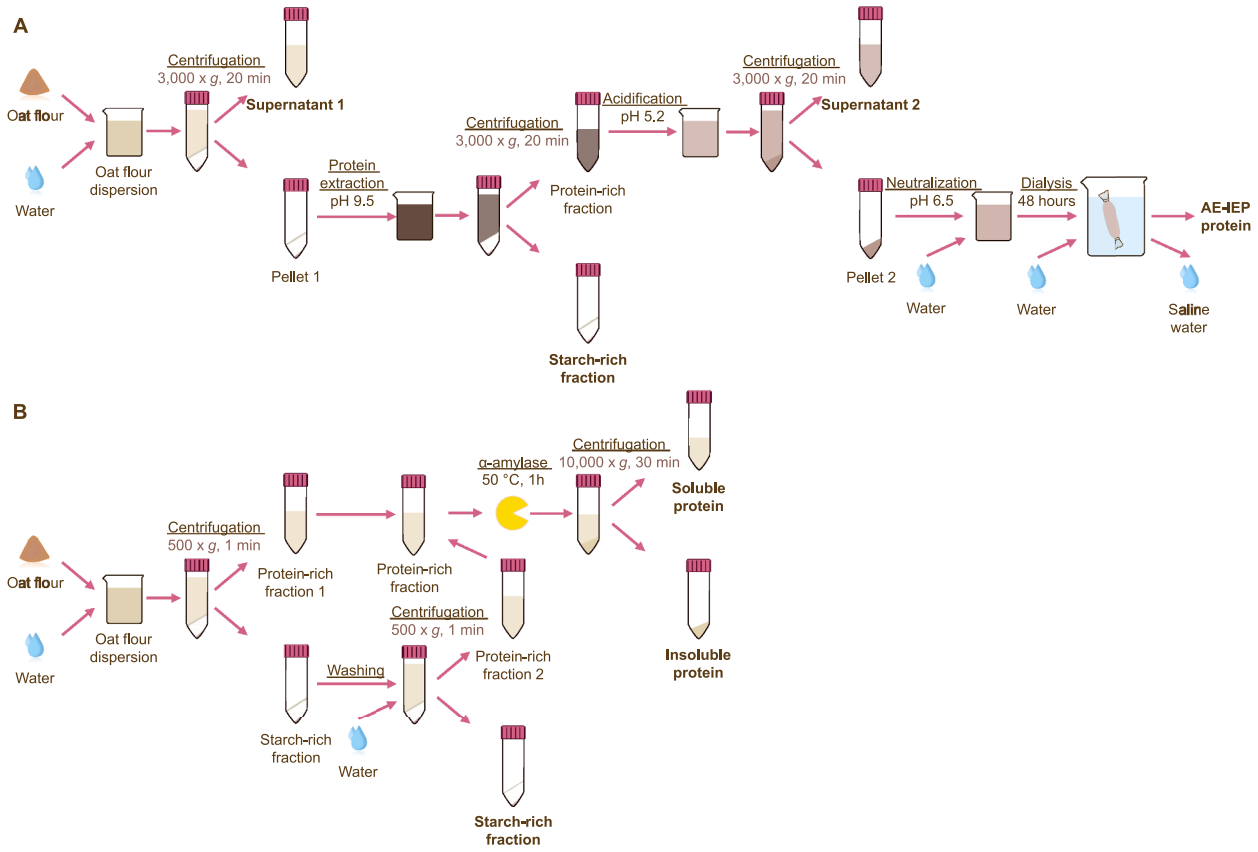


Fig. 1. Schematic overview of conventional wet fractionation using alkaline extraction-isoelectric precipitation (A) and mild wet fractionation using a water-based extraction (B) of oat protein from oat flour. The sample colours and layers after centrifugation represent the colours and layers observed during fractionation.

a similar protein content of 65% (DM) was reported by Ma (1983), with a yield above 60% (DM). Liu (2014) adapted this method and fractionated oats into four fractions, including a protein fraction with a higher protein content and protein yield of 72.4–92.6 % (DM) and 60.4–72.1 % (DM), respectively.

However, alkaline conditions could induce auto-oxidation of the phenolic compounds inherently present in oats and their subsequent polymerisation to dark pigments (X. Zhou et al., 2021). Alternatively, the oxidised phenolic compounds can conjugate with proteins, which can also result in off-colour formation (Zhang et al., 2021). Oats contain phenolic compounds, such as the hydroxycinnamic acids caffeic acid, ferulic acid, *p*-coumaric acid, and avenanthramides. The phenolic compound content ranges between 394.8 – 1518.6 μg/g (fresh weight) (Soycan et al., 2019). Another disadvantage of the alkaline conditions used in AE-IEP is potential changes in the nativity of the proteins, which consequently also changes their functional properties (Tanger et al., 2020). For soy protein it was found that the precipitation step formed protein aggregates (Verfaillie et al., 2023). Besides the disadvantages of alkaline conditions, AE-IEP uses high quantities of chemicals, such as sodium hydroxide and hydrogen chloride. These chemicals in turn form salts, which need to be removed at the end of the process, resulting in large quantities of saline water. Additionally, after protein extraction, the wet samples need to be dried, which is an energy-inefficient process (Schutyser and van der Goot, 2011). Therefore, alternative protein extraction methods should be investigated to obtain native and unmodified proteins and to reduce the ecological footprint of the process.

An alternative protein extraction method is dry fractionation, e.g. by air classification, which does not involve the use of water or chemicals. This is expected to reduce the extent of phenolic oxidation and the subsequent phenolic polymerisation and/or protein-phenolic conjugation. Another advantage of air classification is that it eliminates the energy-intensive drying step. In this process, the proteins are separated from the other constituents based on their particle sizes and densities (Boye et al., 2010b). Despite being a more sustainable alternative, dry fractionation often results in lower protein purities than wet fractionation (Boye et al., 2010b). Dry fractionation using air classification has not yet been extensively tested for oat protein fractionation.

Mild wet fractionation is another alternative for protein extraction, in which the principles of wet and dry fractionation are combined. In this process, the plant flour is blended with water and separated based on gravitational forces. Protein purities previously obtained were between 54 % and 67 % (DM) for pulses (Boye et al., 2010b). In a recent study, Möller et al. (2022) investigated mild wet fractionation of yellow peas and obtained a soluble protein content and yield of 75 % and 65 % (DM), respectively, which is comparable to AE-IEP. In contrast to AE-IEP, mild wet fractionation uses water only, minimising the chance of phenolic oxidation and subsequent phenolic polymerisation and/or protein-phenolic conjugation, and elimination of the saline waste stream. This process has not yet been tested for oat protein extraction. Milder extraction methods could eventually be used in industry to produce native proteins while also eliminating the saline waste stream.

This study aims to develop a protein extraction process for oats to obtain a protein concentrate with high protein content and yield while preserving the protein nativity and preventing off-colour formation. As oats are a source of phenolic compounds, it was hypothesised that the milder conditions applied during dry fractionation or water-based extraction would retain the light colour and protein nativity of the oat protein better, while still providing sufficient protein purity and yield. To investigate this, the protein content, yield, composition, nativity, and off-colour of a mild wet fractionation method were compared to conventional alkaline extraction isoelectric precipitation. Additionally, the potential of dry fractionation for oat protein was explored.

2. Materials and methods

2.1. Materials

Non-heat treated dehulled oat groats were purchased from Vandinter Semo (Scheemda, The Netherlands) and were stored in an airtight container at –20 °C until use. *n*-Hexane for defatting was obtained from Actu-All chemicals BV (Oss, The Netherlands). Hydrochloric acid and sodium hydroxide pellets used for protein extraction were obtained from Avantor (Radnor, PA, USA) and Merck KGaA (Darmstadt, Germany), respectively. α-Amylase from *Bacillus licheniformis* (A3403, 500 units/mg according to the supplier), trypsin from porcine pancreas (T0303–1 G, 13,000–20,000 BAEE units/mg protein according to the supplier), Folin-Ciocalteu reagent, and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All water used was obtained from a Milli-Q water purification system (Merck). All other chemicals were used as received.

2.2. Oat flour preparation

Whole oat groats were defatted with *n*-hexane for 7 h in a Soxhlet extractor to improve the milling efficiency. After defatting, the oat groats were milled using a Hosokawa Multimill (Hirakata, Osaka, Japan), equipped with a ZPS fine impact mill with a classifier wheel speed of 5000 rpm, ZPS speed of 5500 rpm, an airflow of 50 m^3/h, and feed rate of 1 kg/h. This yielded the defatted oat flour, further referred to as oat flour, which was stored in airtight containers at room temperature until further use.

2.3. Protein extraction

Oat protein was extracted from oat flour via three different methods: conventional wet fractionation through alkaline extraction-isoelectric precipitation (AE-IEP), dry fractionation with air classification, and mild wet fractionation with a water-based extraction (WBE).

2.3.1. Conventional wet fractionation using alkaline extraction-isoelectric precipitation (AE-IEP)

Oat protein extraction through AE-IEP was based on previously published methods by Liu (2014) and Ma (1983) (Fig. 1A). To extract the proteins, oat flour was dispersed in a 1:6.7 (*w/w*) flour:water ratio and stirred at room temperature for 1 h. The dispersion was centrifuged at 3000 × *g* for 20 min. The pellet was redispersed in 1:5.5 (*w/w*) flour: water ratio (based on initial flour weight), and the pH was adjusted to 9.5 with 1 M NaOH. The alkaline dispersion was stirred for 1 h to bring the proteins into solution. The non-soluble fraction was removed by centrifugation at 3000 × *g* for 20 min. Subsequently, the supernatant containing the proteins was acidified with 1 M HCl to pH 5.2, which is the isoelectric point of oat protein. This was followed by centrifugation at 3000 × *g* for 20 min. The precipitate was redispersed in a 1:10 (*w/w*) ratio pellet:water and neutralized to pH 6.5 with NaOH to restore the pH's natural charge and to redisperse the protein. This dispersion was dialysed (cellulose; 8 kDa cut off; Biodesign, New York, USA) against demi-water at 4 °C for 48 h. The extraction resulted in four fractions, supernatant 1, starch-rich fraction, supernatant 2, and the AE-IEP protein. All fractions were lyophilized and stored in airtight containers at room temperature.

2.3.2. Dry fractionation with air classification

Dry fractionation with air classification was based on the method of Sibakov et al. (2011). The oat flour was re-milled into a fine flour using a Hosokawa Multimill, equipped with a UPZ pin mill with a UPZ speed of 17,800 rpm, air flow of 55 m^3/h, and feed rate of 1 kg/h. The fine oat flour was air-classified with the Hosokawa Multimill equipped with an ATP50 Hosokawa-Alpine classifier. The classification was performed with a classifier wheel speed of 4000 rpm, an airflow of 55 m^3/h and a feed rate of 1 kg/h to obtain a fine fraction (Fine fraction 1) and a coarse fraction (Coarse fraction 1). Fine fraction 1 was air classified again with a classifier wheel speed of 10,000 rpm, an airflow of 75 m^3/h, and a feed rate of 1 kg/h to obtain a final fine fraction (Fine fraction 2) and another coarse fraction (Coarse fraction 2).

2.3.3. Mild wet fractionation using a water-based extraction (WBE)

Oat proteins were extracted with water using a method that was based on Möller et al. (2022) with modifications (Fig. 1B). To extract the proteins, an oat flour dispersion of 10% (*w/w*) was stirred overnight at room temperature to fully hydrate all particles. After hydration, the oat flour dispersion was centrifuged at 500 × *g* for 1 min to obtain a protein-rich (supernatant) and a starch-rich fraction (pellet). To increase the protein yield, the starch-rich fraction was washed by redispersing it in water at 20 % (*w/w*) (based on initial flour weight) and stirred at room temperature for 1 h. Subsequently, the redispersed starch-rich fraction was centrifuged at 500 × *g* for 1 min to obtain a second protein-rich fraction and a washed starch-rich fraction. The first and second protein-rich fractions were combined into one protein-rich fraction. This combined fraction was incubated with α-amylase, 60 U/g starting material, at 50 °C for 1 h in a shaking water bath to degrade the remaining starch. Afterwards, the soluble and insoluble proteins were separated by centrifugation at 10,000 × *g* for 30 min. This extraction resulted in three fractions, which will be designated as the starch-rich fraction, the soluble protein fraction, and the insoluble protein fraction throughout the manuscript. All fractions were lyophilized and stored in airtight containers at room temperature.

2.4. Compositional analysis

The protein content of the starting material and fractions were determined by measuring the total nitrogen content using the Dumas method (Flash EA 1112 N analyser, Thermo Scientific), according to the manufacturer's protocol. The protein content was calculated using a nitrogen-to-protein conversion factor (N-factor) of 5.12, based on the amino acid composition (Section 2.5.3) and expressed as percentage on dry matter (DM). Additionally, the protein yield was expressed as g/g protein also based on DM.

Moisture and ash content were determined by drying the samples at 105 °C and 550 °C overnight, respectively. Starch and β-glucan content were determined using total starch and β-glucan assay kits (Megazyme, Wicklow, Ireland), according to manufacturer protocols. Fat content was measured by using a Soxtherm (SOXTHERM Unit SOX416, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) with *n*-hexane, according to manufacturer's protocol.

2.5. Protein characterisation

2.5.1. Protein composition by SDS-PAGE

The composition of the oat proteins was determined by using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), under reducing (0.1 M dithiothreitol (DTT)) and non-reducing conditions. Samples were dispersed at 0.2 % (*w/v*) based on protein content (DM) in water and were diluted with sample buffer with and without DTT. This was followed by heating at 100 °C for 10 min and centrifugation at 14,000 × *g* for 1 min. The samples were loaded into gels (Mini-PROTEAN TGX stain-free precast gels, Bio-Rad Laboratories, Lunteren, The Netherlands) and separated on a Mini-PROTEAN tetra cell at 200 V. The protein bands were visualised with Bio-Safe Coomassie blue stain (Bio-Rad Laboratories). The molecular weight of the bands were assigned based on a standard protein marker (Precision plus protein standards Dual colour). Scanning and analysis of the gels were performed with a densitometer (GS-900, Bio-Rad laboratories) and Image Lab software v6.0 (Bio-Rad laboratories), respectively.

2.5.2. Protein composition by ultra-high performance liquid chromatography-photodiode array-mass spectrometry (UPLC-PDA-MS)

The protein composition was further analysed using a quantitative proteomics approach similar to Vreeke et al. (2023). AE-IEP protein and insoluble protein (IPF) from WBE were dispersed at 1 % (*w/v*) in 100 mM Tris–HCl buffer pH 7.5 and hydrolysed using porcine trypsin at an enzyme-to-substrate ratio of 1:25 at 40 °C for 2 h. The hydrolysates were incubated with 20 mM dithiothreitol to reduce the disulphide bridges, acidified to LC conditions and centrifuged at 14,000 × *g* for 10 min. The supernatants were analysed with the UPLC-MS using the same conditions and settings as described by Vreeke et al. (2023). The mass spectra were matched to peptide sequences using UNIFI software v1.9.4 according to the data-processing guideline of Vreeke et al. (2022). The following protein variant sequences were exported from Uniprot (excluding signal peptide) and considered in the analysis: oat 12S storage globulin-1 (P12615), oat storage globulin-2 (P14812), avenin (P27919), avenin-3 (P80356), avenin-F (Q09097), avenin-E (Q09114). No unique peptides were identified for the proteins ubiquitin (P69310) and tubulin (P25862 and Q38771), so these proteins were excluded in quantification. The absolute concentration of each peptide was calculated based on its UV absorbance at 214 nm and a predicted molar extinction coefficient as described by Kuipers & Gruppen (2007). The relative protein variant concentrations were calculated from the peptide concentrations using calculation approach I from Vreeke et al. (2023).

2.5.3. Amino acid composition

The amino acid composition was analysed by Triskelion (Utrecht, The Netherlands), with a validated method based on the ISO-4214 method and ISO-13,904 method for tryptophan. The N-factors were calculated according to Mossé (1990). The summed weight of all amino acid residues of a fraction minus the mass of 1 water molecule per mole amino acid was divided by the total nitrogen content of the same fraction (see supplementary data Table A.1).

2.5.4. Protein nativity determination

The protein nativity was determined by investigating the thermal stability of the proteins, using differential scanning calorimetry (DSC) (Durowoju et al., 2017). To avoid solubility issues between the different fractions, dry protein samples were measured in hermetically sealed stainless steel high-volume pans (TA instruments, New Castle, DE, USA). Samples were equilibrated for 1 min at 20 °C, followed by heating to 140 °C at a rate of 5 °C/min using a DSC-250 (TA instruments). After 1 min of equilibration at 140 °C the samples were cooled down to 20 °C at a cooling rate of 20 °C/min. Subsequently, the same heating and cooling process was repeated for the same sample to confirm protein denaturation. The results were analysed using TRIOS data analysis software (TA instruments). The change in enthalpy was corrected for sample weight and protein concentration (DM) and given in J/g protein.

2.6. Colour measurement

The colour of the obtained protein powders was measured as CIE-LAB values using a port-up dual-beam benchtop spectrophotometer (Hunterlab Colorflex EZ; Hunter Associates Laboratory Inc., Reston, VA). The colours were expressed as *L*- (lightness) *a*- (red/green), and *b*- (blue/yellow) values.

2.7. Unbound phenolic compound content

2.7.1. Unbound phenolic compound extraction

Unbound phenolic compounds were extracted from the resulting extraction fractions with three cycles of 80% aqueous methanol in a 1:6.7 (*w/v*) ratio, with centrifugation at 5000 × *g* for 10 min between each cycle. The supernatants were pooled together and used to determine the total phenolic compound content.

Table 1
Composition of oat flour and various fractions obtained from alkaline extraction-isoelectric precipitation (AE-IEP), and water-based extraction (WBE) of oat protein. Values are reported as means and standard deviations of analytical replicates ($n = 3$ for protein and ash content and $n = 2$ for starch and β-glucan content) and are expressed as percentages based on dry matter. Abbreviations: SRF, starch-rich fraction; SPF, soluble protein fraction; IPF, insoluble protein fraction.

	Protein (%)	Starch (%)	β-glucan (%)	Ash (%)	Other
Oat flour	11.0 ± 0.35	58.7 ± 11.74	2.7 ± 0.32	1.7 ± 0.09	26.0 ± 4.97
Alkaline extraction-Isoelectric precipitation					
SRF_{AE-IEP}	1.8 ± 0.14	81.9 ± 1.11	1.2 ± 0.02	11.3 ± 1.2	3.9 ± 0.53
Supernatant 1	24.3 ± 2.02	<LOD [1]	14.2 ± 0.99	1.1 ± 0.06	60.4 ± 0.94
Supernatant 2	19.6 ± 0.17	<LOD [1]	18.0 ± 0.72	31.5 ± 3.2	30.8 ± 1.29
AE-IEP protein	70.1 ± 1.03	0.1 ± 0.55	<LOD [1]	19.1 ± 0.53	10.7 ± 0.23
Water-based extraction					
SRF_{WBE}	6.6 ± 0.42	74.3 ± 0.37	0.94 ± 0.00	1.1 ± 0.03	17.1 ± 0.19
SPF	19.6 ± 0.06	<LOD1	9.9 ± 1.32	11.6 ± 1.0	58.9 ± 0.53
IPF	69.5 ± 0.80	<LOD [1]	0.20 ± 0.03	1.1 ± 1.10	29.2 ± 0.45

[1] Below the limit of detection.

2.7.2. Total phenolic compound content by Folin-Ciocalteu

The total unbound phenolic compound content was determined with the Folin-Ciocalteu method, adapted from Ainsworth & Gillespie (2007), and expressed as gallic acid equivalent (GAE) in mg/g sample. To 20 µL sample or calibrant 1.6 mL water was added, followed by 100 µL Folin Ciocalteu reagent and 300 µL 20% (*w/v*) sodium carbonate. The samples and calibrants were incubated at 60 °C for 45 min and cooled down to room temperature after which the absorbance was measured at 765 nm.

2.8. Statistical analysis

Significance of differences was determined by ANOVA followed by Tukey's honest significant differences post hoc test using R Studio v.4.4.2. with dplyr, readxl, multcomp, and multcopview library packages. Significance was defined as $p < 0.05$.

3. Results and discussion

Oat protein was extracted using air classification, water-based extraction and conventional AE-IEP method. The proteins obtained by water-based extraction were compared to those obtained via the reference AE-IEP method with respect to the protein content, composition, yield, nativity, amino acid composition, and colour. Non-heat treated oats were used as starting material, as heat treatment could change the protein nativity and extractability (Arntfield and Murray, 1981; Runyon et al., 2015). Fig. 1 visualises a schematic overview of the conventional AE-IEP and water-based protein extraction method. Milled oat groats had a fat content of 13.0 % (DM) and were defatted using *n*-hexane to produce oat flour. This oat flour was composed of 11.0 % protein, 58.7 % starch, 2.7 % β-glucan and 1.7% ash (DM). The measured protein content was slightly lower than typically described in literature (12–20% DM) (Mel and Malalgoda, 2022). The difference can be attributed to variations in oat plant variety and cultivation, as well as the difference in the used N-factor. In this research, the N-factor of oat protein was determined to be 5.12, based on the amino acid composition. This factor was used for all protein calculations. To illustrate the impact of using a different N-factor, the protein content would have been 13.4% instead of 11% if the incorrect N-factor of 6.25 was used.

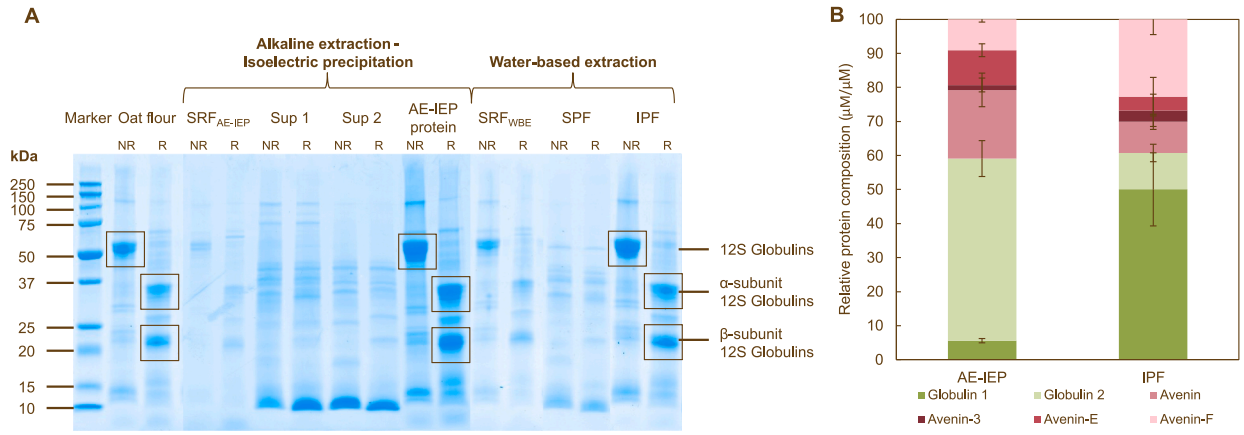


Fig. 2. (A) SDS-PAGE gel of the fractions obtained with AE-IEP and WBE, with NR and R denoting the non-reduced and reduced lanes, respectively. From left to right: protein marker; oat flour; starch-rich fraction, supernatant 1, supernatant 2, and alkaline extraction-isoelectric precipitated protein from AE-IEP; starch-rich fraction, insoluble protein fraction, and soluble protein fraction from WBE. (B) Relative protein composition μM/μM of oat protein obtained with IEP and the insoluble protein fraction (IPF) obtained with WBE. Values are reported as means and standard deviations of analytical replicates ($n = 2$).

3.1. Protein extraction and composition

3.1.1. Conventional alkaline extraction-isoelectric precipitation produces oat protein concentrates

Conventional protein extraction using alkaline extraction-isoelectric precipitation (AE-IEP) on oat flour resulted in a protein concentrate with a protein content of 70.1 % (Table 1) and a protein yield of 0.66 g/g protein. These results were comparable to the findings of Liu (2014), who found a protein content of 59.3–75.9 % DM and a protein yield of 0.5–0.6 g/ g protein. Note that these values were corrected with our calculated N-factor of 5.12 to make it a fair comparison.

Other than protein, the concentrate contained mainly ash (19.1 % DM). During AE-IEP, the pH of the dispersions was adjusted to alkaline and acidic conditions with NaOH and HCl, which formed NaCl (salt). Removal of this salt is necessary, as the presence of salt can influence the protein's functional properties, such as the solubility (Ragab et al., 2004). Depending on the salt type and concentration, salting in or out can occur. Salting in increases the protein solubility by shielding the charges, while salting out reduces the hydration and thus decreases the protein solubility (Arakawa and Timasheff, 1985). Besides protein, AE-IEP resulted in a starch-rich fraction (SRF $_{AE-IEP}$), supernatant 1 and supernatant 2. Although supernatant 1 and 2 consisted of 24.3 % and 19.6 % protein, respectively, both had a low protein yield of 0.03 and 0.04 g/g protein.

The schematic overview of AE-IEP presented in Fig. 1A visualises the colours observed during AE-IEP. The oat flour dispersion started as a beige turbid dispersion. Upon increasing the pH to 9.5, the dispersion immediately changed into a dark brown suspension. This colour change could be an indication of phenolic compound oxidation to quinones and subsequent polymerisation to dark pigments and/or conjugation to the protein (Zhang et al., 2021; X. Zhou et al., 2021). After centrifugation and acidification of the supernatant, the colour of the supernatant changed to light greyish-brown. This indicates that the dark pigments were co-extracted during protein extraction. *Re*-dispersing of the pellet, neutralising the pH, dialysis, and freeze drying resulted in a greyish-brown protein concentrate (see Section 3.3. for further discussion of the colour).

3.1.2. Dry fractionation with air classification

Several attempts were made to concentrate protein using air classification (see supplementary data Figure A.1). In the case of other crops, like peas, cowpeas, and mung beans, air classification can lead to an increase in the protein content in one of the fractions (Schlangen et al., 2022). However, air classification of oats did not lead to a (fine) fraction with a protein content higher than the starting material (13.0 %) (see supplementary data Table A.2). This poor separation can be attributed to the overlapping size of the protein (0.3 – 5 μm) and starch (3–10 μm) particles in oats (Bechtel and Pomeranz, 1981; Sayar and White, 2011). As separation in air classification is based on differences in particle size and density, overlapping particle sizes hinder separation. The particle size of the defatted oat flour had a unimodal distribution with a D [3,4] of 4.17 μm (data not shown), which is comparable to the size of the protein bodies and starch particles (Bechtel and Pomeranz, 1981; Sayar and White, 2011). Based on a previous study by Sibakov et al. (2011), using supercritical carbon dioxide instead of *n*-hexane for defatting could possibly improve separation. Further investigation of sample preparation prior to air classification is needed to successfully extract proteins from oats. Based on our results, it was concluded that air fractionation on defatted oat flour with hexane was not a suitable method to concentrate oat proteins.

3.1.3. Mild wet fractionation using a water-based extraction produces oat protein concentrates

Water-based extraction (WBE) of oat flour resulted in three fractions, which were designated as insoluble protein fraction (IPF), soluble protein fraction (SPF), and a starch-rich fraction (SRF $_{WBE}$). The composition of each fraction is presented in Table 1. The insoluble protein fraction had a protein content of 69.5%, which is comparable to AE-IEP protein. In contrast, the soluble protein fraction had a protein content of 19.6 %. The addition of the α-amylase treatment step increased the protein content of the IPF by degrading residual starch into glucose. The formed glucose remained in solution and in turn, reduced the protein content of the SPF. This extraction process resulted in a protein yield of 0.27 and 0.02 g/g protein for IPF and SPF, respectively.

Fig. 1B visualises the colours observed during the WBE, as well as the different layers observed in the pellet after centrifugation. Centrifugation led to a pellet consisting of three layers. The bottom layer had a beige colour with some darker specs, of which it was speculated that this layer consisted of insoluble fibres. This was followed by a white starch layer and at the top a light beige-grey protein layer. After washing the starch-rich fraction, the top layer of the pellet appeared to have decreased in size, indicating that part of the remaining protein fraction was transferred to the protein-rich fraction showing the protein fraction going into solution.

3.2. Comparing protein composition and amino acid composition of AE-IEP and WBE proteins

3.2.1. Protein composition

Electrophoretic profiles of all fractions under reducing and non-

Table 2
Amino acid composition and calculated nitrogen-to-protein conversion factor of oat flour, IEP protein, IPF, and SPF. Values are reported as means and standard deviations ($n = 2$) and are expressed as percentages based on dry matter.

		Amino acid content (%)			
		Oat flour	AE-IEP protein	Insoluble protein	Soluble protein
Hydrophilic	Arginine	7.3 ± 0.01	7.92 ± 0	7.74 ± 0.01	4.22 ± 0.14
	Asparagine & Aspartic acid	8.35 ± 0.28	7.94 ± 0.01	8.07 ± 0.02	10.13 ± 0.26
	Glutamine & Glutamic acid	20.43 ± 0.1	22.79 ± 0.01	23.09 ± 0.07	13.33 ± 0.06
	Histidine	2.16 ± 0.02	2.41 ± 0.01	2.39 ± 0.01	1.33 ± 0.02
	Lysine	4.21 ± 0.03	3.87 ± 0.01	3.69 ± 0.04	4.47 ± 0.08
	Serine	5.24 ± 0.11	4.41 ± 0.01	4.39 ± 0	7.43 ± 0.04
	Threonine	3.53 ± 0.07	3.38 ± 0	3.35 ± 0.01	3.9 ± 0.02
	Tyrosine	4.21 ± 0.05	3.99 ± 0.04	3.97 ± 0	5.73 ± 0.11
	Sum	55.42 ± 0.23	56.71 ± 0.03	56.7 ± 0.06	50.54 ± 0.22
Hydrophobic	Alanine	4.96 ± 0.02	4.57 ± 0.05	4.42 ± 0.06	6.34 ± 0.15
	Cystine & Cysteine	3.47 ± 0.04	2.11 ± 0.05	2.23 ± 0.03	9 ± 0.09
	Glycine	5.24 ± 0.02	4.27 ± 0.01	4.18 ± 0.01	9.39 ± 0.12
	Isoleucine	4.18 ± 0.15	4.43 ± 0.02	4.52 ± 0.02	2.46 ± 0.17
	Leucine	7.61 ± 0.12	8.03 ± 0.04	8.01 ± 0.02	5.58 ± 0.07
	Methionine	1.55 ± 0.03	1.89 ± 0.02	1.85 ± 0	1.36 ± 0.09
	Phenylalanine	5.41 ± 0.18	5.82 ± 0.01	5.94 ± 0.01	2.72 ± 0
	Proline	5.39 ± 0.02	5.23 ± 0.01	5.25 ± 0.04	6.19 ± 0.05
	Tryptophan	1.37 ± 0.02	1.25 ± 0.02	1.28 ± 0.03	2.04 ± 0.05
	Valine	5.4 ± 0.04	5.69 ± 0.01	5.63 ± 0.01	4.37 ± 0.03
	Sum	44.58 ± 0.19	43.29 ± 0.05	43.3 ± 0.05	49.46 ± 0.16
Nitrogen-to-protein conversion factor		5.17 ± 0.07	5.09 ± 0.10	5.14 ± 0.05	4.6 ± 0.07

reducing conditions are presented in Fig. 2A. The IPF obtained from WBE and alkaline extraction-isoelectric precipitated protein showed similar protein compositions under reducing and non-reducing conditions. Both concentrates had a dominant protein band at approximately 54 kDa under non-reducing conditions. After reduction, this protein band was replaced with two bands at approximately 32 kDa and 22 kDa. These bands match with the fact that the hexameric 12S globulins from oat consist of six subunits (54 kDa each), which dissociate into their α- and β-subunits (32 and 22 kDa) upon reduction (Klose and Arendt, 2012; Mäkinen et al., 2016; Peterson, 1978). These globulins are characterized as poorly soluble in water (Loponen et al., 2007), indicating that both protein concentrates contain mainly water-insoluble proteins, as expected.

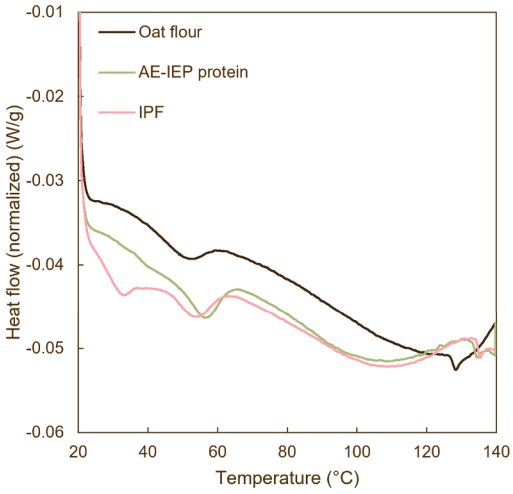
Other protein bands are visible in the range of 10–17, 20–27, and 36–47 kDa, which match with the water-soluble oat albumins and alcohol-soluble prolamins (Klose and Arendt, 2012). These bands are more prominent in the SPF obtained from WBE and supernatant 1 and 2 from AE-AE-IEP.

Besides electrophoretic profiling, the protein composition of AE-IEP protein and the IPF were compared using a quantitative UPLC-PDA-MS approach. Fig. 2B shows the relative protein composition of both protein concentrates. This measurement confirms that the globulins were the major protein in both concentrates and the oat-specific prolamins, i.e. avenins, constituted the remaining 40%. Interestingly, the ratio of globulin 1 and 2 differs between the concentrates, but the total ratio of globulins to prolamins is similar. The difference in globulin 1 and 2 content largely explains the difference in protein yield between the two concentrates. AE-IEP protein consists of approximately 50 % globulin 2, indicating that IPF misses ~50% of the protein, which is the approximate yield difference between the two concentrates.

Based on the electrophoretic profiles it can be concluded that WBE separates oat proteins in a primarily water-soluble and a primarily water-insoluble protein fraction. The relative protein composition showed a comparable ratio of globulins and avenins in AE-IEP and WBE, indicating that both methods extract similar proteins.

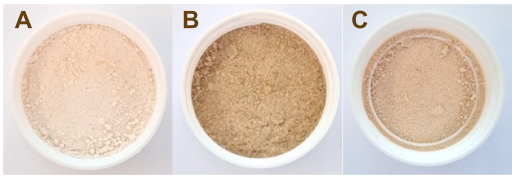
3.2.2. *Amino acid composition and nitrogen-to-protein conversion factor*

Similar to the results of the protein composition, the amino acid composition of AE-IEP extracted protein and the IPF were comparable. Table 2 presents the amino acid composition, separated by hydrophobic and hydrophilic amino acids, of oat flour, AE-IEP protein, IPF, and SPF as percentages. From the amino acid composition and nitrogen content, the nitrogen-to-protein conversion factor (N-factor) was calculated (see supplementary data Table A.1). Due to the different protein and amino



	Onset temperature (°C)	Peak temperature (°C)	Enthalpy ΔH (J/g protein)	Full Width at Half Maximum $\Delta T_{1/2}$
Oat flour	43.7	51.6	4.301	11.46
	126.9	128.2	0.888	2.88
AE-IEP	48.1	56.4	1.019	9.57
protein	133.4	134.4	0.085	1.69
	46.2	54.1	0.869	8.91
IPF	134.4	135.3	0.108	1.54

Fig. 3. Differential scanning calorimetry thermograms of oat flour, AE-IEP protein and IPF with their peak temperatures, enthalpy change (ΔH), and full width at half maximum ($\Delta T_{1/2}$).



	A	B	C
L	84.16 ± 0.07 ^{c}	59.89 ± 0.01 ^{a}	67.45 ± 0.10 ^{b}
a	0.46 ± 0.03 ^{c}	1.21 ± 0.04 ^{b}	1.45 ± 0.03 ^{a}
b	10.88 ± 0.22 ^{c}	18.22 ± 0.27 ^{b}	20.27 ± 0.15 ^{a}

Fig. 4. Oat flour (A), oat protein concentrate obtained with alkaline extraction-isoelectric precipitation (B) and insoluble protein fraction obtained with water-based extraction of oat flour (C). The colours are expressed as CIE-LAB values, with *L* denoting the lightness, *a* red/green hue, and *b* blue/yellow hues. Values are presented as means and standard deviations of analytical replicates ($n = 3$), with different superscript letters representing statistical difference within a row ($p < 0.05$).

acid composition, the calculated N-factor differs between the fractions. Oat flour, AE-IEP protein, and IPF have a comparable N-factor of ~5.1, while SPF has a lower N-factor of 4.6. The lower N-factor of the SPF can be attributed to the different amino acid composition, as well as the presence of the nitrogen-containing components such as the avenanthramides (see Section 3.3). Since the AE-IEP protein and IPF were the protein concentrates of interest, the average N-factor of AE-IEP protein and IPF, 5.12 was used to calculate the protein content.

3.2.3. WBE results in similar protein nativity as AE-IEP

Protein nativity for AE-IEP protein and IPF were investigated with DSC. The thermograms of the first temperature ramp are summarised in Fig. 3 (see supplementary data Figure A.2 for thermograms with two temperature ramps). For both AE-IEP and IPF, two endothermic peaks were observed at approximately 55 °C and 135 °C, with comparable enthalpies (Fig. 3). After repeating the temperature ramp, both peaks disappeared, which indicates thermal protein denaturation. Kumar et al. (2021) and Mel & Malalgoda (2022) reported a denaturation temperature of approximately 110 °C for oat globulins. The difference in measured denaturation temperatures could be attributed to the different state in measurement, dry or wet, as well as the presence of multiple proteins and impurities. As seen in the protein composition (Section 3.2), both AE-IEP protein and IPF mainly contained oat globulins, but also some prolamins. Other impurities such as carbohydrates can also influence the measured denaturation temperature and change in enthalpy (Relkin, 1994). The endothermic peaks observed around 55 °C could correspond to starch gelation, as observed by Lazaridou et al. (2003) and M. Zhou et al. (1998). However, based on our composition analysis, both protein concentrates had a starch content below the limit of detection (Table 1). An explanation could be the presence of resistant starch, which was not properly broken down into glucose by the enzymes of the assay. This would have led to an underestimation of the true starch content.

It was expected that AE-IEP would produce less native proteins, due to chemical modifications at alkaline pH during processing. However, AE-IEP and IPF had a similar change in enthalpy, indicating similar protein nativity. Verfaillie et al. (2023) investigated the effect of isoelectric precipitation steps of soy protein isolates on the protein nativity. It was observed that alkaline extraction and acid precipitation had limited effect on the degree of protein denaturation. The high degree of denaturation often observed in commercial soy protein isolates was attributed to an additional heat treatment to obtain a shelf-stable ingredient. Similarly, Loveday & Halim (2024), compared commercial mung bean and chickpea protein with laboratory-extracted protein. They observed that laboratory-extracted proteins had a lower degree of denaturation in comparison to proteins produced on an industrial scale,

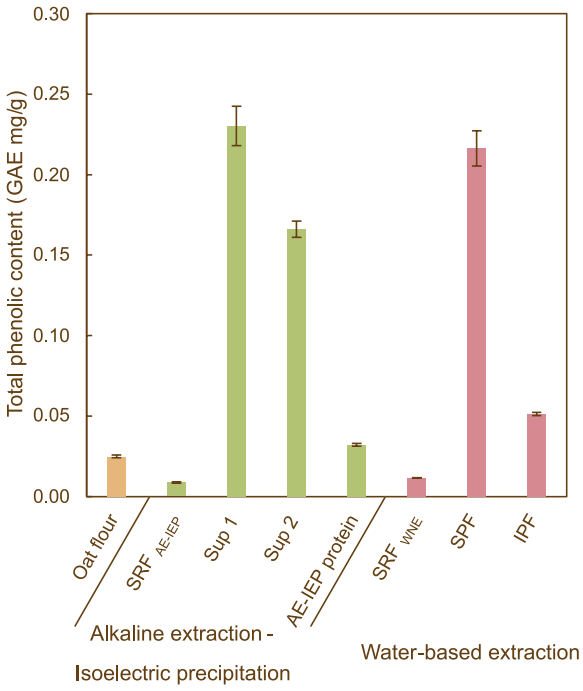


Fig. 5. Total phenolic compound content expressed as gallic acid equivalent (GAE) in mg/g sample of alkaline extraction-isoelectric precipitation (AE-IEP), and water-based extraction (WBE) of oat protein and their resulting fractions. Values are reported as means and standard deviations of analytical replicates ($n = 2$) and are expressed as percentages based on dry matter. Abbreviations: SRF, starch-rich fraction; Sup1, supernatant 1; Sup2, supernatant 2, SPF, soluble protein fraction; IPF, insoluble protein fraction.

and concluded that their alkaline extraction and freeze-drying processes were not representative of commercial isolation processes. This matches our observation that shifting the pH during AE-IEP of oat protein did not result in extensive protein denaturation.

3.3. Protein concentrate colours and total phenolic compound content

Although the DSC results showed a minimal difference in protein nativity between AE-IEP protein and IPF, the produced protein concentrate powders were different in colour. Fig. 4 presents the obtained protein powders and their *Lab*-values. AE-IEP protein and IPF both have a lower L-value than oat flour, indicating a darker colour. This darker colour was attributed to a combination of the absence of starch and the formation of off-colour due to the oxidation of phenolic compounds. The comparison of the protein concentrates revealed that AE-IEP protein had a smaller L-value and thus a darker colour than IPF. This darker colour could be an indication of the co-extraction of oxidised phenolic compounds, possibly followed by modification of the protein with these oxidation products. Laursen et al. (2024) also observed a darker colour upon alkaline extraction and attributed this colour to the co-extraction of other compounds, such as phenolic compounds. Depending on the type of protein and phenolic compounds, the reaction of oxidised phenolic compounds with proteins can lead to the formation of protein-phenolic conjugates. These conjugates can possess a brown colour, thereby contributing to the observed off-colour. Additionally, protein-phenolic conjugation can alter the functional properties of the proteins (Ozdal et al., 2013; Zhang et al., 2021; X. Zhou et al., 2021). Therefore, it is hypothesised that the darker colour of AE-IEP protein compared to IPF is caused by oxidation and co-extraction of inherent phenolic compounds and possibly the formation of protein-phenolic conjugates.

To gain more insight into the fate of the phenolic compounds during AE-IEP and WBE processes, the unbound phenolic compounds were

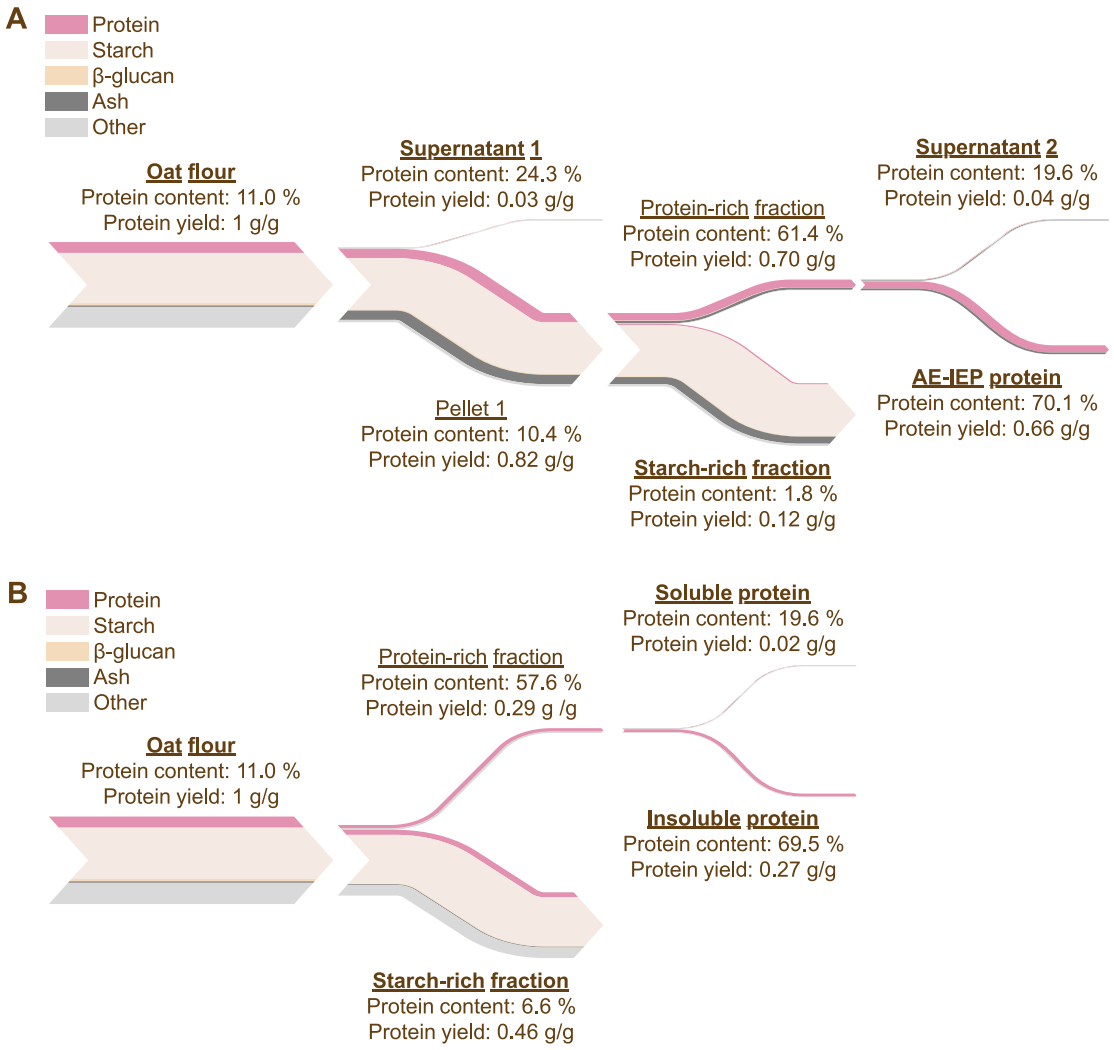


Fig. 6. Mass overview of the protein, starch, β-glucan, and ash streams, based on dry matter, during alkaline extraction-isoelectric precipitation (A) and water-based extraction (B) of oat protein extraction from oat flour.

extracted and the phenolic compound content was determined using the Folin-Ciocalteu assay. The total phenolic compound contents were expressed as gallic acid equivalents (GAE) in mg/g sample (Fig. 5). The supernatants of AE-IEP and SPF of WBE had the highest unbound phenolic compound content, while AE-IEP protein and IPF had a lower phenolic compound content of 0.03 and 0.05 GAE mg/g sample, respectively. During the dialysis step in the AE-IEP process, part of the unbound phenolic compounds could have been removed. Since AE-IEP protein had a darker colour than the IPF, this is an indication that auto-oxidation of phenolic compounds occurred, forming dark pigments e.g. through subsequent polymerisation and/or by interaction with proteins.

The WBE process avoids alkaline conditions, which prevents auto-oxidation, thereby explaining the lighter colour of IPF. However, avoiding alkaline conditions does not eliminate oxidation entirely. Firstly, auto-oxidation can also occur at around neutral pH, albeit much more slowly than under alkaline conditions. Secondly, enzymatic oxidation of phenolic compounds can be catalysed by endogenous oxidative enzymes. Thus, to investigate the potential role of oxidative enzymes in off-colour formation, the catechol oxidase and tyrosinase activity of oat flour was investigated (see supplementary data Figure A.3). The results showed that the activities of these oxidative enzymes in oat flour were negligible. Therefore, it was concluded that endogenous oxidative enzyme activity does not contribute to off-colour formation during oat protein extraction.

It was concluded that the formation of off-colour during AE-IEP likely occurred to rapid auto-oxidation of phenolic compounds at alkaline conditions. During WBE the oxidation of phenolic compounds and resulting off-colour formation was limited because auto-oxidation is proceeds much more slowly at milder conditions (i.e. neutral pH) (Friedman and Jürgens, 2000).

3.4. *Comparing AE-IEP and WBE as oat protein extraction method*

In this study, three fractionation processes for oat flour were compared which differed in terms of process intensity, as well as, water and chemical consumption. The potentially most sustainable fractionation process, dry fractionation, did not result in protein enrichment probably due to the lack of difference in size and density of protein and starch particles after defatting with hexane. The application of the two wet fractionation processes, conventional AE-IEP and WBE resulted in oat protein concentrates of high purity. Both concentrates had similar protein composition and mainly contained oat globulins. AE-IEP protein and IPF were also comparable in amino acid composition and protein nativity. Fig. 6 shows the mass balances of both extractions as Sankey diagrams. Despite the similar high protein content, WBE gave a lower protein yield of 0.27 g/g protein than AE-IEP. The majority of the protein ended up in the starch-rich fraction (0.46 g/g protein). To increase

the protein yield, additional washing steps with water were investigated (see supplementary data Figure A.4). A first washing step increased the protein yield of the protein-rich fraction from 0.21 ± 0.01 g/g protein to 0.25 ± 0.07 g/g protein, a second washing step resulted in a protein yield to 0.36 ± 0.09 g/g protein, while a third and fourth washing steps resulted in a yield of 0.34 ± 0.01 [a] g/g protein and 0.35 ± 0.08 g/g protein, respectively. Thus, multiple washing steps did not increase the protein yield significantly, suggesting a limited solubility or dispersibility of oat protein in water at these conditions (pH 6.3 at room temperature). This limited dispersibility could also be an explanation for the different concentration ratio of globulin 1 and 2 between AE-IEP and IPF (Section 3.2.). To keep the WBE process simple, only one washing step was applied in the final process. AE-IEP resulted in a yield of 0.66 g/g protein which is similar to the yield obtained by Ma (1983).

Extraction of oat protein using a water-based extraction has not previously been investigated. In comparison to conventional AE-IEP, WBE has its advantages and disadvantages. The main advantage being a simple process that extracts the same proteins as AE-IEP but limits phenolic compound oxidation resulting in a lighter colour. Despite the lower yield, this water-based extraction can be used as a pre-treatment to extract part of the proteins before starch extraction. Another application for this process is to extract proteins with a low salt content, followed by further purification using salt or AE-IEP to produce concentrates or isolates with a higher salt content. The latter can be even further tuned by altering the high pH values used in AE-IEP. A lower pH than 9 might give a somewhat higher yield than pH 6.3 while keeping salt levels and the extend of phenolic compounds oxidation acceptable.

To improve the protein yield of WBE, several techniques could be considered to assist the protein extraction, such as ultrasonication-(Chen et al., 2018) or pulsed electric field (PEF). However, especially ultrasonication but also PEF can change the protein structure and functionality, potentially also through the increasing heat load in the process (Rafique et al., 2024; Sweers et al., 2025). Additionally, these techniques could lead to undesired coextraction of phenolic compounds from vegetable by making cells more permeable for various molecules including phenolic compounds (Medina-Torres et al., 2017). Thus, the effect of ultrasonication, PEF, or alternative techniques should be taken into account when exploring their application to enhance the protein yield of WBE.

Ethical statement

The authors declare that no studies in humans and animals have been carried out

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CRediT authorship contribution statement

Solange M.L. Ha: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wouter J.C. de Bruijn:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Gijs J.C. Vreeke:** Writing – review & editing, Formal analysis. **Marieke E. Bruins:** Writing – review & editing, Project administration, Funding acquisition. **Maaike Nieuwland:** Writing – review & editing, Project administration, Funding acquisition. **Atze Jan van der Goot:** Writing – review & editing, Supervision, Conceptualization. **Julia K. Keppler:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100591.

Data availability

Data will be made available on request.

References

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc 2 (4), 875–877. https://doi.org/10.1038/nprot.2007.102.

Arakawa, T., Timasheff, S.N., 1985. Theory of protein solubility. Methods in Enzymology, 114. Academic Press, pp. 49–77.

Arntfield, S.D., Murray, E.D., 1981. The influence of processing parameters on food protein functionality I. Differential scanning calorimetry as an indicator of protein denaturation. Canad. Instit. Food Sci. Technol. J. 14 (4), 289–294.

Bechtel, D.B., Pomeranz, Y., 1981. Ultrastructure and cytochemistry of mature oat (*Avena sativa* L.) endosperm. The aleurone layer and starchy endosperm. Cereal Chem 58 (1), 61–69.

Boye, J.I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., Rajamohamed, S.H., 2010a. Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. Food Res. Int. 43 (2), 537–546. https://doi.org/10.1016/j.foodres.2009.07.021.

Boye, J.I., Zare, F., Pletch, A., 2010b. Pulse proteins: processing, characterization, functional properties and applications in food and feed. Food Res. Int. 43 (2), 414–431. https://doi.org/10.1016/j.foodres.2009.09.003.

Chen, C., Wang, L., Wang, R., Luo, X., Li, Y., Li, J., Li, Y., Chen, Z., 2018. Ultrasound-assisted extraction from defatted oat (*Avena sativa* L.) bran to simultaneously enhance phenolic compounds and β-glucan contents: compositional and kinetic studies. J. Food Eng. 222, 1–10. https://doi.org/10.1016/j.jfoodeng.2017.11.002.

Durowoju, I.B., Bhandal, K.S., Hu, J., Carpick, B., Kirkitadze, M., 2017. Differential scanning calorimetry — A method for assessing the thermal stability and conformation of protein antigen. J. Visualiz. Exp. 2017 (121). https://doi.org/10.3791/55262.

Friedman, M., Jürgens, H.S., 2000. Effect of pH on the stability of plant phenolic compounds. J. Agric. Food Chem. 48 (6), 2101–2110. https://doi.org/10.1021/jf990489j.

Heathcote, J.G., 1950. The protein quality of oats. British Journal of Nutrition 4 (2–3), 145–154. https://doi.org/10.1079/BJN19500030.

Jones, D.B., 1931. Factors For Converting Percentages of Nitrogen in Foods and Feeds Into Percentages of Proteins, 183.

Klose, C., Arendt, E.K., 2012. Proteins in oats; their synthesis and changes during germination: a review. Crit Rev Food Sci Nutr 52 (7), 629–639. https://doi.org/10.1080/10408398.2010.504902.

Kuipers, B.J.H., Gruppen, H., 2007. Prediction of molar extinction coefficients of proteins and peptides using UV absorption of the constituent amino acids at 214 nm to enable quantitative reverse phase high-performance liquid chromatography-mass spectrometry analysis. J. Agric. Food Chem. 55 (14), 5445–5451. https://doi.org/10.1021/jf070337l.

Kumar, L., Sehrawat, R., Kong, Y., 2021. Oat proteins: a perspective on functional properties. LWT 152. https://doi.org/10.1016/j.lwt.2021.112307.

Laursen, N.F., Atıl, G.U., Gregersen, S.B., Corredig, M., Christensen, C.H., 2024. Composition and functionality differences of oat protein concentrates: potential of less refined concentrates obtained by wet milling. Food Hydrocoll 155. https://doi.org/10.1016/j.foodhyd.2024.110187.

Lazaridou, A., Biliaderis, C.G., Izydorczyk, M.S., 2003. Molecular size effects on rheological properties of oat β-glucans in solution and gels. Food Hydrocoll 17 (5), 693–712. https://doi.org/10.1016/S0268-005X(03)00036-5.

Liu, K., 2014. Fractionation of oats into products enriched with protein, beta-glucan, starch, or other carbohydrates. J. Cereal Sci. 60 (2), 317–322. https://doi.org/10.1016/j.jcs.2014.06.002.
Loponen, J., Laine, P., Sontag-Strohm, T., Salovaara, H., 2007. Behaviour of oat globulins in lactic acid fermentation of oat bran. Eur. Food Res. Technol. 225 (1), 105–110. https://doi.org/10.1007/s00217-006-0387-9.
Loveday, S.M., Halim, C., 2024. Protein isolates made in the laboratory versus the factory: a case study with mung bean and chickpea. Sustain. Food Proteins 2 (2), 89–98. https://doi.org/10.1002/sfp2.1027.
Ma, C.-Y., 1983. Preparation, composition and functional properties of oat protein isolates. Canad. Instit. Food Sci. Technol. J. 16 (3), 201–205. https://doi.org/10.1016/S0315-5463(83)72208-X.
Mäkinen, O.E., Sozer, N., Ercili-Cura, D., Poutanen, K., 2016. Protein from oat: structure, processes, functionality, and nutrition. Sustainable Protein Sources. Elsevier Inc, pp. 105–119. https://doi.org/10.1016/B978-0-12-802778-3.00006-8.
Medina-Torres, N., Ayora-Talavera, T., Espinosa-Andrews, H., Sánchez-Contreras, A., Pacheco, N., 2017. Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources. Agronomy 7 (3). https://doi.org/10.3390/agronomy7030047.
Mel, R., Malalgoda, M., 2022. Oat protein as a novel protein ingredient: structure, functionality, and factors impacting utilization. Cereal Chem 99 (1), 21–36. https://doi.org/10.1002/cche.10488.
Möller, A.C., Li, J., van der Goot, A.J., van der Padt, A., 2022. A water-only process to fractionate yellow peas into its constituents. Innov. Food Sci. Emerg. Technol. 75. https://doi.org/10.1016/j.ifset.2021.102894.
Mossé, J., 1990. Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its different and determination. Variation according to species and to seed protein content. J. Agric. Food Chem. 38 (1), 18–24. https://doi.org/10.1021/jf00091a004.
Ozdal, T., Capanoglu, E., Altay, F., 2013. A review on protein-phenolic interactions and associated changes. Food Res. Int. 51, 954–970. https://doi.org/10.1016/j.foodres.2013.02.009.
Peterson, D.M., 1978. Subunit structure and composition of oat seed globulin. Plant Physiol. 62 (4), 506–509. https://doi.org/10.1104/pp.62.4.506.
Rafique, H., Peng, P., Hu, X., Saeed, K., Khalid, M.Z., Khalid, W., Morya, S., Alsulami, T., Mugabi, R., Nayik, G.A., 2024. Ultrasound-assisted modification of oat protein isolates: structural and functional enhancements. Ultrason Sonochem. 112. https://doi.org/10.1016/j.ultsonch.2024.107204.
Ragab, D.D.M., Babiker, E.E., Eltinay, A.H., 2004. Fractionation, solubility and functional properties of cowpea (Vigna unguiculata) proteins as affected by pH and/or salt concentration. Food Chem. 84 (2), 207–212. https://doi.org/10.1016/S0308-8146(03)00203-6.
Relkin, P., 1994. Differential scanning calorimetry: a useful tool for studying protein denaturation. Thermochim Acta 246 (2), 371–386. https://doi.org/10.1016/0040-6031(94)80103-7.
Runyon, J.R., Sunilkumar, B.A., Nilsson, L., Rascon, A., Bergenståhl, B., 2015. The effect of heat treatment on the soluble protein content of oats. J. Cereal Sci. 65, 119–124. https://doi.org/10.1016/j.jcs.2015.06.008.
Sayar, S., White, P.J., 2011. Oat starch: physicochemical properties and function. In: Webster, F.H., Wood, P.J. (Eds.), Oats Chemistry and Technology, 2nd Edition. AACC International Press, pp. 109–122. https://doi.org/10.1016/B978-1-891127-64-9.50012-9.
Schlangen, M., Taghian Dinani, S., Schutyser, M.A.I., van der Goot, A.J., 2022. Dry fractionation to produce functional fractions from mung bean, yellow pea and cowpea flour. Innov. Food Sci. Emerg. Technol. 78. https://doi.org/10.1016/j.ifset.2022.103018.
Schutyser, M.A.I., van der Goot, A.J., 2011. The potential of dry fractionation processes for sustainable plant protein production. Trends Food Sci. Technol 22, 154–164. https://doi.org/10.1016/j.tifs.2010.11.006.
Sibakov, J., Myllymäki, O., Holopainen, U., Kaukovirta-Norja, A., Hietaniemi, V., Pihlava, J.M., Poutanen, K., Lehtinen, P., 2011. Lipid removal enhances separation of oat grain cell wall material from starch and protein. J. Cereal Sci. 54 (1), 104–109. https://doi.org/10.1016/j.jcs.2011.04.003.
Soycan, G., Schär, M.Y., Kristek, A., Boberska, J., Alsharif, S.N.S., Corona, G., Shewry, P. R., Spencer, J.P.E., 2019. Composition and content of phenolic acids and avenanthramides in commercial oat products: are oats an important polyphenol source for consumers? Food Chem. X. 3. https://doi.org/10.1016/j.fochx.2019.100047.
Sweers, L.J.H., Mishyna, M., Ahrné, L.M., Boom, R.M., Fogliano, V., Patra, T., Lakemond, C.M.M., Keppler, J.K., 2025. Pulsed electric field processing of edible insect slurries induces thermally-assisted microbial inactivation. Curr. Res. Food Sci. 10. https://doi.org/10.1016/j.crfs.2024.100940.
Tanger, C., Engel, J., Kulozik, U., 2020. Influence of extraction conditions on the conformational alteration of pea protein extracted from pea flour. Food Hydrocoll. 107. https://doi.org/10.1016/j.foodhyd.2020.105949.
Tkachuk, R., 1969. Nitrogen-to-protein conversion factors for cereals and oilseed meals. Cereal Chem. 46, 419–424.
Verfaillie, D., Janssen, F., Van Royen, G., Wouters, A.G.B., 2023. A systematic study of the impact of the isoelectric precipitation process on the physical properties and protein composition of soy protein isolates. Food Res. Int. 163. https://doi.org/10.1016/j.foodres.2022.112177.
Vreeke, G.J.C., Lubbers, W., Vincken, J.P., Wierenga, P.A., 2022. A method to identify and quantify the complete peptide composition in protein hydrolysates. Anal. Chim. Acta 1201. https://doi.org/10.1016/j.aca.2022.339616.
Vreeke, G.J.C., Meijers, M.G.J., Vincken, J.P., Wierenga, P.A., 2023. Towards absolute quantification of protein genetic variants in Pisum sativum extracts. Anal. Biochem. 665. https://doi.org/10.1016/j.ab.2023.115048.
Zhang, Q., Cheng, Z., Wang, Y., Fu, L., 2021. Dietary protein-phenolic interactions: characterization, biochemical-physiological consequences, and potential food applications. Crit. Rev. Food Sci. Nutr. 61 (21), 3589–3615. https://doi.org/10.1080/10408398.2020.1803199.
Zhou, M., Robards, K., Glennie-Holmes, M., Helliwell, S., 1998. Structure and pasting properties of oat starch. Cereal Chemistry 75 (3), 273–281. https://doi.org/10.1094/CCHEM.1998.75.3.273.
Zhou, X., Iqbal, A., Li, J., Liu, C., Murtaza, A., Xu, X., Pan, S., Hu, W., 2021. Changes in browning degree and reducibility of polyphenols during autoxidation and enzymatic oxidation. Antioxidants 10 (11). https://doi.org/10.3390/antiox10111809.