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RESEARCH ARTICLE

REVISED Scaling up from laboratory experiments to pilot trials to recover protein from tomato waste remaining after processing and exploring the use of proteolysis as a further purification step

[version 2; peer review: 2 approved with reservations, 3 not approved]
Previously titled: "A comparison of pilot scale trials to recover protein from tomato seeds and from tomato pomace"

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v2 **First published:** 16 Apr 2026, **6**:121
https://doi.org/10.12688/openreseurope.23437.1
Latest published: 09 Jul 2026, **6**:121
https://doi.org/10.12688/openreseurope.23437.2

Abstract

Background

Tomato seeds contain a significant source of protein with valorization potential. Seeds by weight comprise between 0.1% to 0.25% in whole tomatoes but are enriched in tomato pomace to 70%.

Methods

The procedure to recover protein from tomato seeds that had been separated from the tomato pomace was performed at pilot scale with 2.8 kg of seeds using the combination of high shear mixing and wet milling. At each step in the process, soluble protein was quantified using the Bradford assay. Further steps involved centrifugation, ultrafiltration and spray drying.

Results

The results revealed a protein extraction yield of 8.2% during the first extraction step and much lower protein concentrations in the subsequent steps. The additional steps were performed using only stirring in contrast to the first step where high shear mixing and wet milling were used. Four sequential extractions were pooled together

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Approval Status ✗ ✗ ✗ ? ?

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version 2 (revision) 09 Jul 2026			✗ view	? view	? view
version 1 16 Apr 2026	✗ view	✗ view			

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and the soluble protein yield was 19.2%. Protein concentration by ultrafiltration through a 10 kDa membrane reduced the expected recovery to 4 mg per g dry seeds. A final protein powder of 28 g was recovered using spray drying having a total protein content of 40%. Processing of the tomato pomace without separation of the seeds resulted in a lower protein yield of 0.9% when extractions were pooled together. A green protein powder of 42 g was recovered having a protein content of only 7%. Additional processing using hexane extraction and protease digestion of crude protein samples was also investigated.

Conclusions

These results revealed that highest protein recovery was obtained from the seeds that were separated from the tomato pomace and that high shear mixing was important in releasing the protein into the suspension.

Keywords
High shear mixing, wet milling, decanter, ultrafiltration, spray drying, bacteria

H2020 This article is included in the Horizon 2020 gateway.

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Any reports and responses or comments on the article can be found at the end of the article.

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Competing interests: No competing interests were disclosed.

Grant information: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 792050 (Development of novel functional proteins and bioactive ingredients from rapeseed, olive, tomato and citrus fruit side streams for applications in food, cosmetics, pet food and adhesives [Pro-Enrich]).

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How to cite this article: Baker P and Charlton A. **Scaling up from laboratory experiments to pilot trials to recover protein from tomato waste remaining after processing and exploring the use of proteolysis as a further purification step [version 2; peer review: 2 approved with reservations, 3 not approved]** Open Research Europe 2026, **6**:121 https://doi.org/10.12688/openreseurope.23437.2

First published: 16 Apr 2026, **6**:121 https://doi.org/10.12688/openreseurope.23437.1

REVISED **Amendments from Version 1**

The title has been changed to accurately reflect all aspects of the study. Additional information has been provided in the introduction on the quantities of tomatoes produced in different European countries and the quantities of tomato pomace processed globally. Furthermore, additional details have been provided on the composition of tomatoes and tomato pomace. Within the method section, additional details have been provided into the use and types of equipment as well as a clarification of why protease hydrolysis was used and why that particular type. The introduction section has been re-structured beginning with the quantities of tomatoes produced in Europe, followed by approaches to deseeding the tomato pomace and finally protein extraction from the seeds. In the results and discussion section, the data in Table 2 has been corrected, changed into percentages to allow ease of comparison with previous studies, and certain sections have been highlighted. In addition, previous data has been included in this table. Figure 1 has been modified to become clearer and certain points within the process have been highlighted to match those in Table 2. Five additional references have been added to support the extra comments. Finally, all calculated data has been made available in the university repository.

Any further responses from the reviewers can be found at the end of the article

Introduction

Globally between the years 2006 and 2016, two nations in Europe were among the ten highest tomato producers, Spain (4.7 million tonnes (mT)) and Italy (6.4 mT) (Lu et al., 2019). In 2022, 16.9 mT of tomatoes were grown in Europe especially in the southern countries: Spain (6.8 mT), Italy (4.9 mT) and Portugal (1.9 mT) with 3.3 mT grown in the remaining European countries (Almeida et al., 2024). About 44% of tomatoes produced in Europe are processed in five different European countries resulting in the production of tomato juice at different levels of concentration leaving behind a pulp described as tomato pomace (AgroTimes, 2026) (Table 1). Consequently, large quantities of waste by-products are generated during production such as rotten tomatoes, unsaleable (misshapen and unripe) tomatoes and during processing such as tomato pomace which is comprised of peels and seeds when creating tomato paste. The composition of the discarded tomatoes along with attached greenery comprise of cellulose (50%), lignin (19%), hemicellulose (14%) and pectin (5%) (Fritsch et al., 2017). After processing, the tomato pomace has a combination of cellulose and lignin (22%), protein (14–20%) and pectin (5–10%). However, there is potential to upscale this biomass by recovering the seeds which itself can be used as a food additive (Roy et al., 2022; Rana & Kapoor, 2025) or processed further to recover its protein. The tomato seeds are a good source of protein which in one study involving the assessment of two different tomato seed

Table 1. Global processing of tomatoes by 50 major companies.

Country	Processed (million tonnes)	Number of companies	Number of factories
China	6.871	10	42
USA	9.749	11	19
Spain	2.72	6	15
Italy	2.656	6	28
Japan	1.783	1	6
Turkey	1.465	4	14
Portugal	1.412	1	5
Brazil	1.14	3	6
Tunisa	0.552	2	3
Chile	0.52	1	1
Ukraine	0.49	1	2
Peru	0.33	1	2
Egypt	0.31	1	3
Greece	0.225	1	3
Canada	0.21	1	2
Europe	7.503	15	53
World	30.433	50	151

Data in table from AgroTimes, 2025.

cultivars at different stages in the growth cycle, which revealed a protein content associated with non-defatted seeds to be 23% (g per 100 g dry weight) and 27% with defatted seeds (Brodowski & Geisman, 1980).

Sedimentation has often been used to separate the seeds from tomato pomace (Kaur et al., 2005; Meshkani et al., 2016; Miklavčič Višnjevec et al., 2021) and from whole tomatoes (Sarkar & Kaul, 2014). However, it is possible to recover the seeds from whole tomatoes by filtration through sieves with holes sufficiently large enough to allow the passage of the tomato seeds. The presence of a gelatinous layer surrounding the tomato seeds allows the seeds to readily pass through these holes whereas the tomato peel remains trapped on the surface of the sieve. This was demonstrated with 20 kg whole cherry tomatoes to recover 53 g of seeds (Baker et al., 2022) and from 100 kg salad-type whole tomatoes to recover 110 g of seeds (Baker, unpublished). Whether this process would be effective at a much larger scale remains to be determined as the larger quantity of seeds could prevent passage through the holes. However, the process to extract tomato juice from tomatoes has resulted in tomato pomace containing seeds where the gelatinous layer has been removed, and these seeds could only be separated from the remaining tomato pomace by sedimentation (Miklavčič Višnjevec et al., 2021). The compositional analysis of tomato pomace compared with whole tomatoes has revealed differences in the fibre content resulting in a lower hemicellulose content and higher lignin and lipid contents, often by two-fold or more (Almeida et al., 2024). The seed content associated with the tomato pomace (at 53%) based on of dry weight (Liadakis et al., 1995) is high in contrast to regular tomatoes at 2% (Baker, unpublished).

The next step is the recovery of protein from the tomato seeds and one important parameter in this process is pH which revealed a significant increase in yield from 5% to 14% between pH 7 and pH 9 followed by a gradual increase from pH 9 to 12 (Piyakina & Yunusov, 1995). Consequently, most studies have focused on using an alkaline extraction process to recover the protein from defatted tomato seeds at pH 8 in 0.1 M NaCl at 50°C (Sarkar & Kaul, 2014), in 1% NaOH (Sogi et al., 2005) and 1 N NaOH at pH 9 (Latlief & Knorr, 1983). One of the limitations associated with protein recovery at high pH is the loss of the three-dimensional structure of the protein that could affect functional properties such as colour (Rana et al. 2025). Consequently, some studies have extracted protein from tomato seeds directly (Latlief & Knorr, 1983; Sarkar & Kaul, 2014) while other studies have defatted the seed meal with petroleum ether (Rana et al., 2025) or with hexane (Sogi et al., 2005). However, defatting tomato seeds with petroleum ether resulted in reduced protein recovery (Piyakina & Yunusov, 1995), whereas defatting with chloroform and methanol appeared to result in higher protein recovery (Brodowski & Geisman, 1980). Furthermore, defatting of the seed meal requires the use of large quantities of organic solvent at the start of the process.

The aim of this study was to scale-up from previous smaller scale laboratory experiments to pilot scale using the fixed equipment available at the pilot plant. Previous studies have made comparisons in protein recovery at laboratory scale to pilot-scale from pre-treated pea revealing a slight improvement in protein yield from 60% to 94% (Schmidt et al., 2022). More recently, pilot scale protein extractions have also been performed with rapeseed (Ahlström et al., 2022), lentil (Joehnke et al., 2021) and hempseed (Helstad et al., 2022). In our study, modifications were necessary during upscaling to account for the increased volume during the process. The pilot facilities offered the opportunity to concentrate the protein using ultrafiltration and to recover the protein using spray drying instead of acid precipitation. As the research progressed and discovering that only some of the protein was being recovered, the use of protease treatment was explored as an additional step.

Materials

Tomato pomace (50 kg) formed by pressing Roma tomatoes grown on farms by the conglomerate, Agrofusion in Kherson, Ukraine and sedimentation and was shipped frozen from Agrofusion, Kherson, Ukraine, once separated from the tomato juice. Deseeding was achieved by immersion in 150 L hot water at 50°C for 20 minutes in an electric tilting kettle with programmable controls (Firex Cucimix CBTE), skimming off floating pomace and skins from the surface and collecting the resuspended seeds on a sieve (Miklavčič Višnjevec et al., 2021). The deseeded pomace was concentrated by passing through a juicer (Hobart vegetable preparation machine) to remove excess water and retain the pomace.

Pilot scale extraction from tomato seeds

The seeds (2.815 kg wet weight equivalent to 809 g in dry weight) were resuspended in 10 L water and stirred at 160 rpm. An outline of the procedure is shown in Figure 1 which was performed over two days. The suspension was at pH 5.5 and at about 20°C at the start of the experiment. The seeds were transported to Bangor University laboratory and high-shear mixed for five minutes at 8000 rpm progressing with the attachment of three different workheads, (General purpose disintegrating head, square hole high shear screen and emulsor screen, Silverson, UK). This process began with the workhead having the largest holes (5 mm) and completing with the workhead with the smallest holes (1 mm). High shear mixing was used initially instead of colloidal wet milling because the large quantity of seeds was too much for the wet mill to initially process. After high shear mixing, the suspensions were transported to the pilot scale facilities and another 10 L

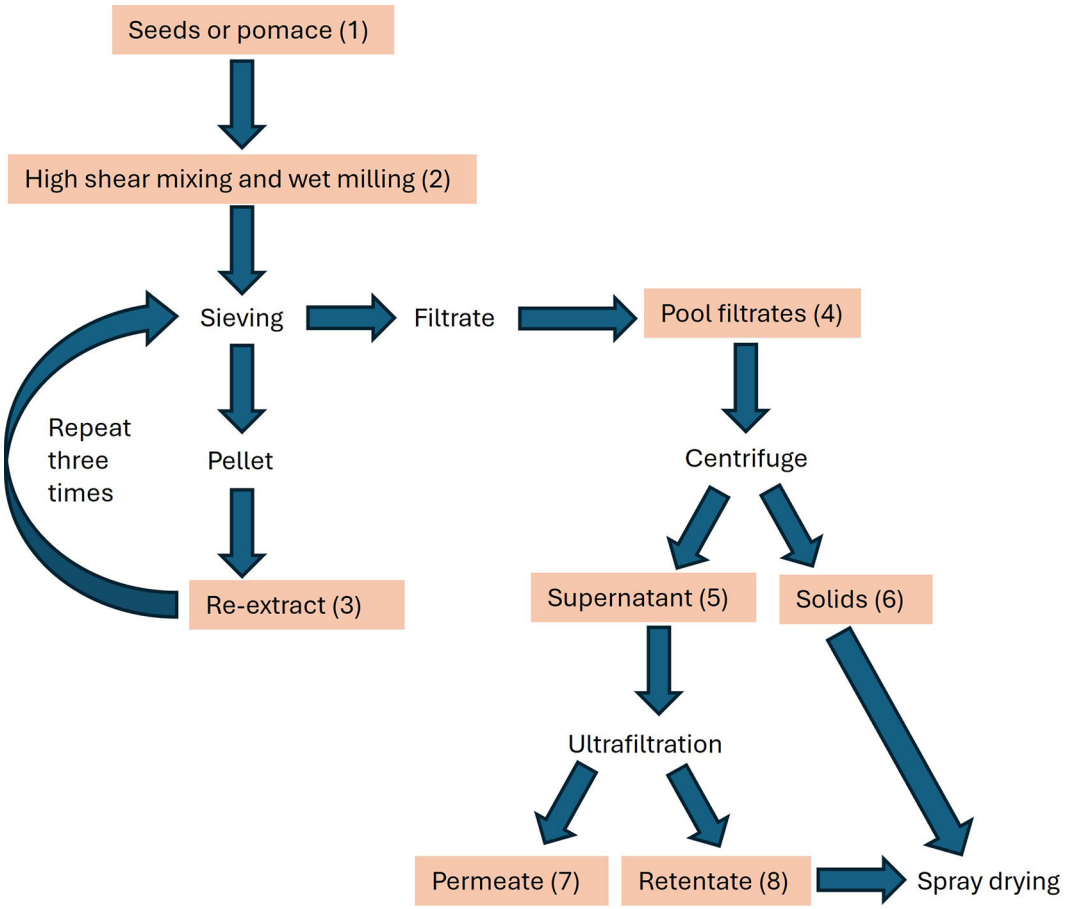

Figure 1. Schematic diagram of pilot process to extract protein from tomato seeds and pomace. The process was similar with both substrates except where shown in brackets.

water was added to the suspension which passed from one 150 L stirred tank through a wet mill (model no. MZ50, FrymaKoruma) into another 150 L stirred tank over 30 min where the cone size of the wet mill was set at 0.5 mm at the beginning and decreased to 0.1 mm. The remaining suspension was adjusted to pH 8 with 2 M sodium hydroxide (500 g, Fisher Scientific, UK, product code 15433117) and the suspension was stirred at 160 rpm for 1 h at room temperature. The suspension was then filtered through 250 µm sieve and the filtrate was retained. The sieved material was resuspended in 20 L water and adjusted to pH 8 with 2 M sodium hydroxide. The process of resuspending the material collected on the sieve in 20 L water, adjusting to pH 8, stirring for 30 min and sieving again to collect another filtrate was repeated altogether three times. The filtrates were pooled together, stirred and adjusted to pH 5 with 3 M phosphoric acid (2.5 L, Fisher Scientific, UK, product code 10698133) and left overnight at room temperature. Acidification to pH 4 was avoided to prevent protein precipitation. The filtrate was separated by a stack disc centrifuge (model no. SB 7–06–576, GEA Westfalia, Germany) into a centrate and an enriched solids suspension that was stored at 4°C. The centrate was ultrafiltered for six hours through a 10 kDa polyether sulphone, ultrafiltration membrane (Axium Process Ltd., UK) at 30°C resulting in 60 L permeate containing soluble compounds without protein and 20 L retentate containing the concentrated protein. This retentate was high shear mixed with the emulsor screen for 5 min at 7000 rpm in 5 L batches and then passed through the spray dryer (Spray dryer model no. S1, Anhydro, Denmark) at a temperature between 90–100°C at the rate of 1 L per hour. A sample of this crude protein (1 g) was resuspended in 200 ml hexane for 1 h at 70°C and then the 95% n-hexane (2.5 L, Fisher Scientific, product code 10783601) was decanted. The pellet was completely dried by rotary evaporation (Rotavapor R100, Buchi, Switzerland), resuspended in deionized water and freeze-dried.

Pilot scale extraction from tomato pomace

Frozen tomato pomace (2.806 kg equivalent to 0.703 kg in dry weight) was thawed in 20 L hot tap water and then high shear mixed in the Bangor University laboratory using each of the workheads for 5 min at 7000 rpm in batches of 5 L as described in the previous section. The resuspended pomace was transported to the pilot scale facilities and was colloidal wet milled using a cone setting of 0.1 mm for 30 minutes at room temperature and then passed through a 250 µm sieve.

The pellet was resuspended in another 20 L tap water and colloidal wet milled for 30 min, then sieved and the filtrate was retained. The pellet was resuspended in another 10 L tap water and colloidal wet milled for 30 min, sieved and filtered. This was repeated a further two times resulting in two filtrates. The filtrates were combined and stirred. The pooled filtrate was passed through the stack disc centrifuge to remove the solids which was stored at 4°C. The centrate was concentrated by ultrafiltration using a 10 kDa membrane resulting in a large volume of permeate waste. This suspension was high shear mixed with the emulsor screen attached for 5 min at 7000 rpm in 5 L batches and then passed through the spray dryer at temperatures between 90–100°C.

Protease treatment of tomato seed fractions

In a separate laboratory experiment, proteolysis of extracted tomato seed protein was explored. Extracts from seeds will contain peptides and amino acids which will pass through a 10 kDa ultrafilter membrane and become lost. The intention would be to treat the seed extract with a protease and to collect all of the proteinaceous material by sequential ultrafiltration through 10 kDa and 300 Da ultrafilter membranes and to concentrate the final permeate by spray drying. Therefore, the aim of this protease treatment was to confirm that a significant quantity of peptides and amino acids could be formed. Crude protein was extracted from tomato seeds (10.5 g wet weight equivalent to 3 g in dry weight) as previously described (Miklavčič Višnjevec et al., 2021). Briefly, the seeds were immersed in 500 ml deionized water and high shear mixed (Silverson mixer, UK) for 15 min at 7000 rpm. The seed hull solid was collected by sedimentation, and the crude protein suspension was decanted into another vessel. The pellet was resuspended in deionized water (50 ml) and a sample (1 ml) was removed to determine protein and peptides content before protease treatment. The pH of the remaining suspension was checked to ensure that it was between pH 5.5 to 7.5, the optimal pH range of the enzyme, and Neutrase (100 µl) (100 ml, NCBE, UK) was added. A sample (1 ml) was removed from the supernatant (100 ml) was checked to determine protein and peptide content and then Neutrase (20 µl) was added. The enzyme, which could be bought in small quantities, was active within the pH range of the tomato extracts without requirement pH adjustment. Both the resuspended pellet and supernatants were incubated at 50°C on the bottle roller (Fisher Scientific, UK, product code 15258854) at 80 rpm for 1 h and then incubated at 85°C for 15 min with occasional shaking to deactivate the enzyme. Finally, the suspensions were cooled to room temperature in order to determine the protein and peptide concentrations using the Bradford reagent (500 ml, Fisher Scientific, UK, product code 15424109) and modified Kaiser test kit (50 ml of each of three components, Merck, product code 12161503). The resuspended seed hulls were centrifuged (Avanti JXN-30 centrifuge, Beckman Coulter, Germany) at 4320 × g for 5 min. The protein concentrations were determined using the Bradford assay while peptide and amino acid concentrations using the modified Kaiser assay. Controls were performed using Neutrase (100 µl) immersed in 100 ml in water only. The protein, peptide and amino acid concentrations were calculated by subtracting the appropriate controls depending on whether protease was used.

Characterisation of protein samples

Sample collection

The volume of effluent was measured at each stage and was used in determining the protein contents. Samples (10 ml) were periodically collected during the process steps (Figure 1).

Protein content

Protein contents were determined using the Bradford assay and measured in a plate reader (BioTek Epoch microplate spectrophotometer, Agilent Technologies, USA) at 595 nm. A standard curve was created using bovine serum albumin which was dissolved in deionized water at 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml.

Bacterial enumeration

Bacterial populations were determined by serial dilution in 0.1% (w/v) sodium chloride (500 g, Fisher Scientific, UK, product code 10616082) solution and plating onto nutrient agar (500 g, Fisher Scientific, UK, product code 10219252). The plates were incubated at 25°C for one week and colonies were counted.

Amino acid and peptide content

The modified Kaiser assay was used to determine the presence of amino acids and peptides in the resuspended fraction and in the filtrate revealing that considerably more peptides were found in the filtrate. The latter assay was performed using 910 µl of sample, neat or diluted, with the addition of components of the Kaiser test [30 µl 0.2 mg potassium cyanide and 66% (v/v) pyridine, 30 µl 5% ninhydrin in n-butanol, and 30 µl 200% phenol in n-butanol]. The suspension was then incubated at 105°C for 5 min and then cooled to room temperature. Samples of 100 µl were measured in the plate reader at

570 nm. A standard curve was created using proteose peptone which had been enzymatically treated was prepared at 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml.

Statistical analysis

All of the experiments were performed only once thus precluding the possibility of using standard statistical tests without replicates.

Results and discussion

The suspension of tomato seeds was at pH 5.5 at the start and almost no protein was detected (Table 2). Protein concentrations in the filtrate had increased that corresponded with the disintegration of the seeds. High shear mixing was necessary in order to reduce the particle size of the seeds because the large quantity of seeds was too much for the wet mill to process that resulted in a back-up of material. The protein concentration in the filtrate had increased as expected and resuspension of the remaining material collected on the sieve was at pH 7.31 which was adjusted to pH 8. The adjustment to pH 8 resulted in no change in the concentration of soluble protein in contrast to a previous study that revealed the quantity of protein extracted increased from pH 7 to pH 9 (Piyakina & Yunusov, 1995). This may be influenced by the presence of oil that has been shown to result in a 15% lower protein yield (Brodowski & Geisman, 1980), as a result of the good oil emulsion properties of this protein between pH 6 and 8 (Sarkar et al., 2016). Consequently, the presence of oil, albeit at low quantities, will result in some of the protein becoming associated with the oil and may be undetectable using the Bradford assay. The resuspensions of the remaining material collected on the sieve were at pH 7.51 on the third cycle and pH 7.67 on the fourth cycle and were readjusted to pH 8 each time. The protein concentrations recovered after the first and subsequent stirring cycles revealed considerably lower quantities were being recovered. This yield was lower than expected compared with laboratory trials (Table 3) where the yields appeared to be three-fold higher. Lower yields were obtained during repeated extractions in the large-scale trial (800 g) compared with the laboratory experiment (200 g). This could be attributed to the use of high shear mixing only in the laboratory experiments and not in the large-scale trial. The failure to use additional wet milling during subsequent extraction cycles was likely to be one of the contributing factors to reduced protein recovery. It would be expected that a further reduction in particle size would increase surface area supporting the transfer of proteins closer to the surface into the suspension. In addition, it was evident in a previous study

Table 2. Protein concentrations and bacterial populations during each of the processing steps from tomato seeds and protein concentrations from tomato pomace comprising both seeds and skins.

Step in procedure	Step in Figure 1	Concentration (percentage of total protein in seeds)		Bacterial population of seeds
		Seeds	Pomace*	(Log cell counts per g DM)
RESUSPENSION				
At start	1	0.2	0. 27	6.48
After high shear mixing and wet milling	2	8.7	0. 37	6.87
Adjustment to pH 8	2	8.7		ND
EXTRACTION AND SIEVING				
First	3	8.2	0. 41	6.82
Second	3	0.69	0. 17	6.15
Third	3	0.33	0. 073	6.90
Fourth	3	0.20	ND	6.59
Pooled, adjust to pH 5	4	19.2	0. 90	6.18
STACK DISK CENTRIFUGATION				
Effluent	5	16.5	1.1	4.66
Solids	6	2.9	0. 064	8.54
ULTRAFILTRATION				
Permeate	7	0.067	ND	8.32
Retentate	8	1.5	ND	7.55

*These values were calculated which accounted for the proportion of seeds in the tomato pomace thereby allowing direct comparison with the seed extraction.

Table 3. Protein extraction from wet tomato seeds using consecutive high shear mixing extractions using different starting quantities.

	Protein concentration (mg per g DM of original seeds)			
Sample (based on dry weight)	1 g[1]	200 g[2]	800 g[3]	ND[4]
High shear mixing speed	6000	6000	7000	0
Initial extraction volume (ml)	500	4000	20,000	ND
Liquid/solid ratio (ml/g)	500	20	25	ND
Hulls	11.6	27.3	ND	
First extract	87.7	52.2	22.4	78.5
Second extract	ND	40.6	1.9	20.4[5,6]
Third extract	ND	42.5	0.9	7.7[5,7]
Fourth extract	ND	ND	ND	4.7[5,8]
Total extracts	87.7	135.3	25.2	111.3

Quantities expressed in terms of dry weight.
ND not described or not determined.
[1]Baker 2022, unpublished.
[2]Data presented in Miklavčič Višnjevec et al., 2021.
[3]This work.
[4]Piyakina and Yunusov, 1995.
[5]Pleshkov, 1968.
[6]Extraction in 0.5 M NaCl.
[7]Extraction in 70% ethanol.
[8]Extraction in 0.2% NaOH.

that the presence of plant oils during the recovery of dietary fibres from olive pomace had a profound negative effect in pilot scale extraction compared with the laboratory experiments (Baker et al., 2023). The laboratory study revealed that optimal protein recovery was achieved with a high liquid-to-seed material of 500:1 during the first step (Table 3). Previous research has shown that a much lower ratio of 50:1 was sufficiently effective and that increasing this ratio did not increase protein recovery level (Sari et al., 2015). Nevertheless, the use of larger quantities of liquid becomes difficult to handle during the initial extraction to achieve effective disintegration during high shear mixing. The smaller volumes can be pooled together for storage in a larger vessel before proceeding to the next step in the process. In this large-scale trial, the pooling of four extractions would result in a ratio of 80:1, higher than the recommended 50:1 ratio. The first step in both laboratory experiments (700 g of wet tomato seed material) and the pilot scale extraction was similar involving high shear mixing and similar ratios of liquid to solid. However, the yield of protein was two-fold lower in the pilot scale extraction compared with the laboratory extraction indicating that another factor had affected the recovery of protein. This may be attributed to the higher quantity of seed mass of the pilot scale extraction resulting in more resistance to disintegration during high shear mixing compared with the smaller quantities used during the laboratory experiments.

The bacterial population during the pilot-scale processing steps remained low and appeared to decrease during the subsequent extraction steps as any bacteria remaining with the equipment was washed away (Table 2). However, the use of mild acidification to prohibit microbial growth during overnight storage was unsuccessful resulting in an increase in the bacterial population. The suspension showed a minor increase from pH 5.03 to pH 5.37 after 15 h on the following day. Ideally, continuous processing would have been preferential and instead the protein extracts were stored at 4°C during the subsequent trial with tomato pomace. The soluble protein content at this stage was higher compared with the sum of the soluble protein in the four separate filtrates which was perhaps caused by protease activity associated with the bacteria releasing insoluble protein that was not measured. The bacterial population remained relatively stable thereafter, but the soluble protein concentrations appeared to have decreased by the end of ultrafiltration. There are several possible explanations for the decrease in protein such as degradation caused by bacterial contamination or the presence of oils causing the proteins to adhere to the surface of the ultrafilter membrane. The protein concentrations associated with the solids enriched suspension collected by the stack disc centrifuge were also low because it is likely that most of these proteins were insoluble. The predicted soluble protein yield was highest with the pooled filtrates at 7.7% per g dry mass of tomato seeds and subsequently decreased to 0.6%. However, this calculated protein yield did not take into account insoluble protein associated with solids enriched suspension collected by the stack disc centrifuge, which was not measured. Rather than using ultrafiltration to concentrate the protein, acid precipitation followed by centrifugation has been previously used in the recovery of protein from rapeseed (Helstad et al., 2022). Consequently, a protein yield of 57% and a protein content of 91% was obtained when using alkaline extraction at pH 10.5. The crude protein yield after spray

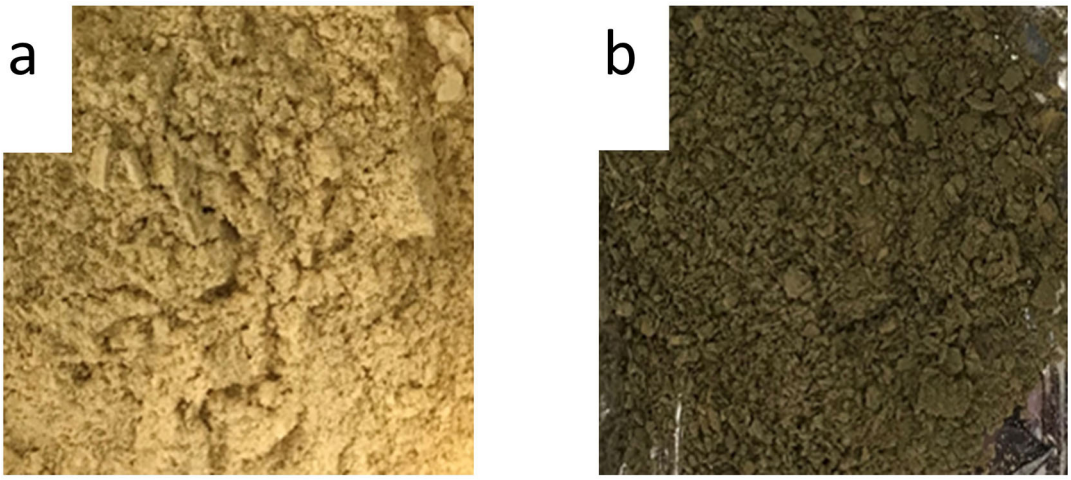

Figure 2. Final spray dried protein extracts from (a) tomato seeds and from (b) tomato pomace.

drying in our study was 21.88 g (2.7% of seeds DM) and appeared light yellow (Figure 2a). The powder contained a protein content of 40.3% as determined by Kjeldahl analysis using the standard conversion factor of 6.25 and 35% as determined using the Bradford assay. The protein yield was calculated as 2.6% g protein extracted per g tomato seed protein when using the value determined by Kjeldahl analysis. In another study on defatted tomato seeds, a higher protein yield of 27.4% g extracted protein per g tomato seed protein was obtained which had a higher protein content of 92% (w/w) from defatted tomato seeds (Sarkar & Kaul, 2014) compared with our study. The study used a similar technique, expect heat was applied (50°) that could result in more protein being extracted and higher centrifuging speeds were obtained on a much smaller sample size (40 g), which could result in a higher protein content. Protein recovery from lupin seeds appeared to be more effective due to the ease of dehulling resulting in kernels having a much higher initial protein content of 60% (Burgos-Díaz et al., 2019). Consequently, the use of more alkaline conditions and similarly high centrifugation speeds to remove remaining seed particles resulted in a protein content of 82% (w/w). While it is important to recover as much of the protein from tomato seeds, the low protein content of 40% (w/w) in our study would have limited applications in foods at that concentration. It would be described as a flour with a lower economic value in food applications compared with protein isolates having a protein content above 90% (Sari et al., 2015) and would require further processing to remove associated components. This yield was lower than expected compared with laboratory trials (Table 3) where the yields appeared to be three-fold higher. Hexane extraction of the crude protein to remove fats and oils resulted in an increase in the protein content from 35% to 47% as determined using the Bradford assay. The protein extract before hexane extraction appeared pale yellow whereas another study revealed that alkaline extraction of tomato seed protein may appeared darker possibly caused by the presence of phenolics despite having a higher protein content of 84% (Rana et al., 2025).

The processing of the whole tomato pomace was initiated using 2.806 kg in wet weight (833 g in dry weight) that comprised 70.4% seeds and 29.6% pomace. The pomace pellet collected on the sieve after the third and final filtration weighed 3 kg which had been squeezed by hand to remove as much water as possible. The tomato pomace after hand pressing had a dry weight content of 13%. Therefore, the remaining dry weight of material leftover after three successive extractions was 0.39 kg representing 47% of the original material at the start. The pooled filtrate was determined to be pH 4.20 and was stored in the fridge which decreased to pH 4.03 when left overnight. The protein content in the extracts was considerably lower than the extracts recovered from the separated seeds even after accounting for the proportion of seeds in the tomato pomace (Table 2). The spray dried protein extract weighed 42.2 g (6.0%) and contained a protein content of only 7% as determined using the Bradford assay, which reflected the lower protein content in tomato pomace. Furthermore, the spray dried crude protein appeared dark green (Figure 2b). Therefore, tomato seeds which contain most of the protein compared with the rest of the tomato pomace (Baker et al., 2022) must be separated from the remaining tomato pomace to efficiently recover protein in terms of yield and colour.

In an independent part of the study, the proportion of amino acids and peptides was assessed using a modified version of the Kaiser test. This test was originally designed to determine whether amino acids were present in a sample during protein synthesis (Kaiser et al., 1970). However, more recent studies have applied this method to quantify amino acids revealing most had similar levels of detection except cysteine and lysine which were barely detectable (Staußet al., 2024). Detection is based on the reaction of ninhydrin with primary amines (Jarre et al., 2014) and therefore peptides would have only one amine group despite having a much larger molecular weight. These semi-quantitative results revealed the

Table 4. Treatment of tomato seed extract and tomato seed hulls remaining after extraction with a protease and measurement of proteins and peptides.

Neutrase proteolysis	Sample	Concentration (mg/g dry seeds)		Yield (% of dry weight)	
		Protein	peptides	protein	peptides
Before	seed extract	52.5	51.4	18.2	18.8
	seed hull pellet	1.2	ND	0.4	
After	seed extract	26.4	173.2	9.2	60
	seed hull pellet	1.4	18.8	0.5	6.1

presence of amino acids and peptides were associated with the seed extract. It was assumed as shown with the soluble proteins that most of peptides would be soluble and found in the seed extract rather than remaining with the seed hull pellet. It was impossible to determine the separate proportions of amino acids and peptides. Proteolysis revealed that the total content of amino acids and peptides had increased assuming that the proportion of amino acids to peptides remained unchanged. This increase was accompanied by a decrease in the soluble protein content within the seed extract (Table 4). Likewise, amino acids and peptides were also found associated with the seed hull pellet after proteolysis, but soluble protein content associated with the seed hulls appeared to have shown a minor increase. It is highly probably that some insoluble protein remained with the seed hull and proteolysis may have solubilized some of this protein. A further reduction of the soluble protein associated with the seed extract could have been achieved by increased proteolysis incubation time. The recovery of soluble and insoluble proteins, and peptides from the suspension would require ultrafiltration through a 10 kD filter membrane followed by spray drying the retentate. The use of proteases in the recovery of protein from plant biomass appears to have the highest success in yields compared with the use of other enzymes (Baker and Charlton, 2020).

It is perhaps worth considering the value of an industry that could be created using the protein recovered from tomato pomace. This would be based on the values described by Almeida et al. (2024) within Europe of 16.9 million tonnes of tomatoes of which 40% are processed. Amounting to 6.76 million tonnes. It is possible to calculate the quantity of seeds based on the previous knowledge that 110 g of seeds was recovered from 100 kg tomatoes, which would amount to 7500 tonnes. The recovery of about 750 tonnes of crude protein could be achieved assuming a recovery yield of just under 10% was possible based on current estimates. Previous market research revealed that expected purchasing prices by different types of industry of protein flour, concentrates and isolates would be €3, €4–€5, €10–€14, respectively (Pinilla & de la Fuente, 2018). These values could be used to calculate the total income that could be generated. The low protein content of the crude tomato seed protein would be classified as a protein flour and therefore the combined European market value of protein from tomato seeds could be worth €2.25 million during 2018. However, this highly approximate income does not take into account investment and operational costs.

Despite the relatively low income associated with this protein source, one of the beneficial factors associated with tomato seed protein is the presence of a higher concentration of lysine, an essential amino acid, compared with proteins recovered from other major sources such as soy and corn (Rana et al., 2025; Mechmeche et al., 2017). In addition, tomato seed protein has been shown to have high oil absorption (Liadakis et al., 1998; Sogi et al., 2002). A further investigation into the oil emulsion property of tomato seed protein revealed that it was stable in the presence of sodium chloride, at above pH 4, at a temperature below 80°C, and in calcium chloride below 50 mM (Sarkar et al., 2016). These properties could make tomato seed protein more desirable in the market and that proteins recovered from other plant seeds such as rapeseed showed poor solubility in water (Nasrabadi et al., 2021).

Conclusions

A full pilot scale protein extraction from tomato seeds was performed which revealed problems within the process leading to low yields compared with initial laboratory experiments. Suggestions have been considered that could significantly increase protein yields such as the use of high shear mixing instead of wet milling. Furthermore, the presence of oils and fats associated with the seeds appeared to significantly impact protein concentration during ultrafiltration and it appears that protein precipitation by acidification would be more effective. At his stage, it would be possible to treat the crude protein extract with hexane to remove any remaining oils and fats before progressing to enzyme hydrolysis with protease, followed by ultrafiltration. This process would shift hexane extraction from the initial stage with tomato seeds to much later resulting in a major reduction in solvent usage. A comparison with pilot scale protein extraction from tomato pomace revealed a lower yield even when taking into account the lower proportion of seeds. Pilot scale extractions revealed that the protein yield from tomato seeds was higher than from tomato pomace (mixture of pomace and seeds) even when the lower proportion of seeds in the tomato pomace was considered.

Ethics and consent

Ethical approval and consent were not required.

Data availability

There is no additional data to the numbers represented in the tables of this research article. However, all raw data and calculations are provided as an electronic data set at the following website: tomato seed and pomace data.

https://pure.bangor.ac.uk/admin/workspace.xhtml

Data file: 2 g seeds – lab experiment, 200 g seeds – lab experiment, 800 g seeds – pilot trial, pomace – pilot trial, seeds – bacterial population and protease treated (only highlighted data used in this research article).

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Version 2

Reviewer Report 26 September 2026

https://doi.org/10.21956/openreseurope.26324.r80492

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?

Busra Oktar
University of Bologna, Bologna, Emilia-Romagna, Italy

This pilot-scale study addresses a relevant valorization problem and provides useful process-scale data. However, several mechanistic and methodological points require clarification before the reported yields and conclusions can be fully assessed. Rather than restating these as prescriptive comments, I list them below as specific questions for the authors.

Questions for the Authors

1. Can the authors confirm whether the higher yield in the first extraction reflects high-shear mixing specifically, or the general “first-pass” depletion effect expected in any sequential extraction? Was a control isolating mixing intensity from extraction order tested? If not, please discuss this alternative explanation and its implications for the study’s conclusions.
2. What is the fat content of the starting seed material, and could residual lipid have limited protein solubilization during the initial aqueous extraction?
3. Were antinutrient or matrix-bound compounds in the seed material characterized, and could these have affected protein solubility or Bradford assay readings?
4. Given that many seed storage proteins are globulins requiring ionic strength for solubilization, was a salt-based extraction step considered, rather than relying solely on pH-shift (NaOH/phosphoric acid) extraction? Was the increasing ionic strength from repeated NaOH and phosphoric acid additions across the four sequential extractions monitored, and could this have contributed to the declining yield independent of mixing method?
5. What is the protein content of the “enriched solids suspension” retained after centrifugation, and of the ultrafiltration permeate? Were these confirmed analytically to be low in protein or assumed?
6. Was the whole-pomace process run under identical conditions (mixing/milling steps, number of pooled extractions) to the seed-separated process to ensure a fair comparison?
7. What nitrogen-to-protein conversion factor was used for the final powder’s total protein content, and how does this relate to the Bradford-based tracking used during processing?

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant protein extraction and valorisation of agri-food by-products; well placed to assess extraction methodology and yield-related aspects of this manuscript, but not the pilot-scale equipment engineering or microbiological analysis in depth.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 24 September 2026

https://doi.org/10.21956/openreseurope.26324.r80491

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?

Harshi Singhi
University of Illinois at Urbana-Champaign, Urbana, USA

This manuscript addresses an important topic: the pilot-scale recovery of protein from tomato seeds and tomato pomace. The scale-up perspective is valuable, and the study provides useful practical observations about the challenges associated with protein recovery. However, several methodological and interpretive issues need to be addressed before the conclusions can be fully supported.

1. The experiments were performed only once, which makes it difficult to assess variability or determine whether differences between processing steps are meaningful. The authors should clearly acknowledge this limitation and avoid making strong comparative conclusions.
2. Clearly report the basis (wet or dry weight) used for all calculations.

3. Several terms, including protein yield, recovery, protein concentration, and powder yield, appear to be used interchangeably. The authors should clearly define each calculation and provide respective equations.
4. Provide sufficient details of the protease treatment, including enzyme activity or enzyme-to-substrate ratio.
5. A process-flow diagram with protein recovery at each major step would improve the manuscript considerably.
6. Since the manuscript discusses potential food applications, characterization of basic functional properties of the recovered protein, such as solubility, water/oil-holding capacity, or emulsifying properties, would strengthen the study.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Extraction from tomato seeds

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 26 August 2026

https://doi.org/10.21956/openreseurope.26324.r79117

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Gebeyehu Ayele
Addis Ababa Science and Technology University, Addis Ababa, Addis Ababa, Ethiopia

Title: Scaling up from laboratory experiments to pilot trials to recover protein from tomato waste remaining after processing and exploring the use of proteolysis as a further purification step

Overall Assessment

The topic and the proposed title are interesting and potentially relevant, particularly because protein recovery from tomato waste is an important research direction for improving resource utilization and supporting the development of scalable protein-recovery processes. I particularly appreciate the emphasis on proteolysis as a further purification step, which gives the study a potentially distinctive process-oriented contribution. However, in its current form, I am not sufficiently confident that the manuscript has developed the scientific rationale, methodological rigor, characterization strategy, and scale-up analysis necessary to support its stated scope. The following points should therefore be addressed substantially before the manuscript can be considered further.

1. Alignment among the title, abstract, introduction, and main manuscript

There is a noticeable mismatch between the title and the actual emphasis of the manuscript. The central focus appears to be protein recovery from tomato waste, yet the Introduction is largely framed around tomato waste generation and waste-management considerations rather than establishing a strong scientific rationale for protein recovery, the challenges associated with recovering proteins from tomato waste, and the specific technological gap addressed by the study. Moreover, the title explicitly presents proteolysis as a further purification step, but this concept is not adequately introduced or scientifically justified in the Introduction. If proteolysis represents an important stage of the proposed recovery strategy, the authors should explain why it is necessary, what limitation of conventional protein recovery it is intended to overcome, and how it contributes to the final protein product. The same conceptual alignment should be maintained consistently throughout the Abstract, Introduction, Methods, Results, and Discussion.

2. Scientific strength of the abstract and overall manuscript

The Abstract requires substantial revision. In its present form, it does not provide a sufficiently strong scientific representation of the study and does not clearly communicate the research problem, methodological innovation or approach, principal quantitative findings, and implications for protein recovery and scale-up. More importantly, this weakness is not restricted to the Abstract; similar issues are evident throughout the manuscript. The manuscript should be reorganized around a clear scientific question and should move beyond general statements concerning tomato waste toward a rigorous evaluation of protein recovery efficiency, process performance, product quality, and scalability. The authors should ensure that the Abstract reflects the actual scientific contribution of the work rather than primarily describing the background and general importance of tomato waste valorization.

3. Clearly defined objective and scientific hypothesis

The objective of the study should be stated explicitly and precisely. At present, it is difficult to determine whether the primary objective is to develop a protein-recovery process, optimize protein yield, improve protein purity through proteolysis, characterize the recovered protein powder, or demonstrate the feasibility of scaling up the process. These are related but scientifically distinct objectives. The authors should clearly define the principal objective and, where appropriate, formulate specific sub-objectives or hypotheses. This is particularly important because the manuscript is positioned toward scale-up, and the experimental design and discussion should directly demonstrate how the reported results address that objective.

4. Clarity, reproducibility, and scientific status of the methods
The methodological description is not sufficiently clear to establish whether the procedures were developed by the authors, adapted from previously reported methods with minor modifications, or based on established/standardized analytical protocols. This distinction is important for reproducibility and for evaluating the scientific novelty of the work. For every major experimental and analytical procedure, the authors should clearly identify the methodological origin and specify any modifications introduced by the present study. Where standardized methods were used, the relevant standard or authoritative methodological reference should be provided. Where the authors developed a new procedure, the rationale, optimization basis, critical processing parameters, and validation strategy should be described adequately. Without this information, it is difficult to assess whether the reported recovery process is genuinely novel, appropriately adapted, or sufficiently reproducible for scale-up.
5. Insufficient characterization of the recovered protein powder
The characterization of the recovered protein powder is, in my view, one of the major weaknesses of the manuscript. If the central purpose is to maximize and evaluate protein recovery, the characterization should focus on parameters that are directly connected to recovery efficiency, protein quality, purity, functionality, and process suitability. The current characterization appears too limited to support the conclusions drawn from the study. For example, bacterial enumeration alone does not provide sufficient evidence regarding the quality or suitability of the recovered protein powder, particularly when only a limited number of additional physicochemical or compositional parameters are considered.
The authors should reconsider the characterization strategy from a process-development perspective. The reported analyses should allow the reader to understand not only how much protein was recovered, but also what type of protein product was obtained, how pure it is, what was lost during processing, and whether the recovered material has characteristics compatible with subsequent applications. Importantly, the authors should distinguish between measurements that are directly relevant to protein recovery and those that are merely supplementary quality-control parameters.
6. Discussion does not adequately address scale-up
The Discussion is currently too descriptive relative to the stated objective of scaling up protein recovery from tomato waste. A scale-up-oriented study should not simply restate the experimental findings; it should critically evaluate what the findings imply for larger-scale processing. The authors should discuss, based specifically on their experimental observations, the technical bottlenecks, process limitations, critical operating parameters, material handling challenges, downstream processing requirements, protein losses, reproducibility, and potential changes in process performance during scale-up.
The Discussion should also distinguish clearly between what has actually been demonstrated experimentally and what remains a future possibility. For example, if a particular recovery step performs well at laboratory scale, the authors should critically consider whether its performance is likely to remain stable at increased processing volumes and why. Issues such as mixing efficiency, mass transfer, enzyme or reagent requirements, residence time, solid–liquid separation, filtration/centrifugation capacity, energy consumption, water use, downstream concentration, drying, and process consistency may become increasingly important during scale-up. These aspects should be discussed only where they are relevant to the process investigated in the present study rather than introduced as generic scale-up considerations.
7. Discussion should be analytical rather than descriptive
The Discussion would benefit substantially from a more analytical structure. The authors should consistently ask why the observed result occurred, what process mechanism may explain it, what

limitation it reveals, and how that limitation could realistically be addressed. The discussion should therefore integrate three dimensions: limitations, opportunities, and technically plausible solutions. For example, where the current process achieves a certain recovery level but leaves substantial protein in the residual fraction, the authors should consider what this indicates about the extraction or purification mechanism and whether the remaining loss originates from incomplete solubilization, protein degradation, aggregation, or inefficient separation. Such analysis would make the manuscript considerably more informative for researchers interested in process development and scale-up.

8. Scale-up claims should be supported by the experimental evidence

Because the manuscript is presented in the context of scaling up protein recovery, the authors should be cautious about the strength of their claims. Laboratory-scale recovery does not by itself demonstrate scale-up feasibility. The manuscript should clearly distinguish proof of concept, process optimization, and demonstrated scale-up performance. If actual scale-up experiments were conducted, the authors should provide sufficient information to evaluate how process parameters and recovery performance changed with scale. If scale-up was not experimentally demonstrated, the conclusions should be appropriately qualified and the manuscript should present the work as providing a basis for future scale-up rather than implying that scalability has already been established.

9. Literature selection and recency

The literature base should also be updated and strengthened. I noticed the use of some substantially older references, including studies from 1983. Older references can certainly be appropriate when they represent foundational methodology or seminal findings, but they should not constitute major support for current claims regarding protein recovery technology, process optimization, food applications, or scale-up. The authors should replace outdated references where more recent and scientifically relevant evidence is available, while retaining older references only where their historical or methodological importance is justified.

Overall recommendation

Overall, I find the title promising and relevant, but I am not yet sufficiently confident in the manuscript as a whole. The major concern is not the research topic itself, but the lack of consistent scientific alignment between the stated focus on protein recovery and the way the study is introduced, methodologically described, characterized, and discussed. A substantial revision is needed to establish a clear objective, strengthen the scientific rationale, clarify methodological originality and reproducibility, expand the characterization of the recovered protein, and most importantly provide a rigorous, evidence-based discussion of the limitations and opportunities for scale-up. If these issues are addressed carefully, the manuscript could become considerably stronger and more convincing as a contribution to the field of protein recovery and tomato-waste valorization.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and does the work have academic merit?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Valorization of waste for single cell protein production

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Version 1

Reviewer Report 18 May 2026

https://doi.org/10.21956/openreseurope.25362.r74457

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Adilson Roberto Locali-Pereira
Universidade Nova de Lisboa Instituto de Tecnologia Quimica e Biologica (Ringgold ID: 98819), Oeiras, Lisbon, Portugal

The present study investigated the extraction of proteins from tomato by-products, more specifically tomato pomace and tomato seeds. Although the work has industrial relevance, several improvements are necessary to make the manuscript clearer before indexing.

Introduction

1) *"Tomato farming is a huge industry especially in southern European countries with Spain, Italy and Portugal producing more than half (Almeida et al., 2024)."* - I suggest adding data on tomato production at both the European level and specifically for the countries mentioned. I also suggest including numerical data regarding the production volume of tomato pomace;
2) The authors should add data on the proximate composition of tomato pomace and tomato seeds in the introduction;
3) *"In our study, modifications were necessary during upscaling to account for the increased volume during the process. The pilot facilities offered the opportunity to concentrate the protein using ultrafiltration and to recover the protein using spray drying instead of acid precipitation. The logistics of*

transporting tonnes of waste tomatoes from Spain to the UK was considered and found to be unviable. Consequently, a supplier of tomato pomace was found in Ukraine requiring the transport of only 50 kg which was logistically possible possessing a higher seed density." – This sentence seems somewhat misplaced at the end of the introduction. I suggest either removing it or moving it to the Results and Discussion section.

Methods

1) Please add the equipment used to mix the pomace with water (e.g., parameters, fabricator);
2) In Figure 1, the flowchart is somewhat confusing. I suggest separating the pomace and seed processes (perhaps using arrows in different colors) to facilitate visualization;
3) Which parameters were used in the spray dryer? For example, feed rate? Please specify;
4) It is somewhat unclear why the authors evaluated the action of the protease enzyme in the process, since this was not previously mentioned in the introduction or in the objectives of the study. Furthermore, why was this process evaluated only for the seeds? Was it due to the greater difficulty in releasing proteins from this matrix? If so, this should be clarified.
5) *"Collection and analysis of samples"* – I suggest a different title for this section, such as "Characterisation of protein extracts". Furthermore, for better readability, I recommend dividing the analyses performed into subsections (e.g., protein content, microbiological analyses, etc.).
6) Which N-to-P conversion factor was used for the protein content analysis?

Results and Discussion

1) *"Furthermore, tomato protein extract has been shown to interact with sunflower oil to form oil emulsions consisting of fine droplets measuring 533 nm at pH 5 (Sarkar et al., 2016).*" – This sentence is rather disconnected from the rest of the text and is not related to the main objective of the study;
2) Table 1 – To make the table clearer, I suggest adding highlighted headings for each main step (Mixing, Centrifugation, Filtration, etc.), with the respective cycles or details listed in rows below;
3) *"The suspension showed a minor increase from pH 5.03 to pH 5.37 on the following day."* – I suggest that the authors mention, perhaps in the Methods section, how long the entire process took.
4) The text occasionally mentions results obtained in a previous laboratory-scale study, but it is difficult to compare them with the data from the present work. I suggest presenting this comparison ("Lab scale vs. Large scale") in a table (perhaps in Table 2 itself).

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Food proteins; Plant proteins; Protein fractionation; Emulsions; Microencapsulation

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 08 Jun 2026

Paul Baker

Dear Dr Adilson Roberto Locali-Pereira, Thank you for taking the time to review my submitted review article and hope my replies adequately address your comments.

The present study investigated the extraction of proteins from tomato by-products, more specifically tomato pomace and tomato seeds. Although the work has industrial relevance, several improvements are necessary to make the manuscript clearer before indexing.

Introduction

1) "Tomato farming is a huge industry especially in southern European countries with Spain, Italy and Portugal producing more than half (Almeida et al., 2024)." - I suggest adding data on tomato production at both the European level and specifically for the countries mentioned. I also suggest including numerical data regarding the production volume of tomato pomace;

The introduction has been modified to include quantities of tomatoes produced as follows: "Tomato farming is a huge industry especially in southern European countries with Spain, Italy and Portugal producing 6.8, 4.9 and 1.9 million tonnes, respectively of the 16.9 million tonnes in Europe in 2022 (Almeida et al., 2024). These values showed minor changes from 2016 when Spain produced 4.7 million tonnes and Italy produced 6.4 million tonnes (Lu et al., 2019). About 44% of tomatoes produced in Europe are processed in five different European countries resulting in the production tomato juice at different levels of concentration leaving behind a pulp described as tomato pomace (AgroTimes, 2026) (Table 1)".
Table 1 has been included which lists quantities of tomatoes that are processed globally and by the number of companies involved in each country.

2) The authors should add data on the proximate composition of tomato pomace and tomato seeds in the introduction;
Details have been added as follows: "The composition of the discarded tomatoes along with attached greenery comprise of cellulose (50%), lignin (19%), hemicellulose (14%) and pectin (5%) (Fritsch et al., 2017). After processing, the tomato pomace has a combination of cellulose and lignin (22%), protein (14-20%) and pectin (5-10%)". *3) "In our study, modifications*

were necessary during upscaling to account for the increased volume during the process. The pilot facilities offered the opportunity to concentrate the protein using ultrafiltration and to recover the protein using spray drying instead of acid precipitation. The logistics of transporting tonnes of waste tomatoes from Spain to the UK was considered and found to be unviable. Consequently, a supplier of tomato pomace was found in Ukraine requiring the transport of only 50 kg which was logistically possible possessing a higher seed density." – This sentence seems somewhat misplaced at the end of the introduction. I suggest either removing it or moving it to the Results and Discussion section. This sentence has been removed.

Methods

1) Please add the equipment used to mix the pomace with water (e.g., parameters, fabricator);

Further details have been added "Deseeding was achieved by immersion in 150 L hot water at 50°C for 20 minutes in an electric tilting kettle with programmable controls (Firex Cucimix CBTE)and skimming off floating pomace and skins from the surface and the resuspended seeds were collected on a sieve (Miklavčič Višnjevec et al., 2021), while the pomace was concentrated by passing through a juicer (Hobart vegetable preparation machine) to remove excess water and retain the pomace".

2) In Figure 1, the flowchart is somewhat confusing. I suggest separating the pomace and seed processes (perhaps using arrows in different colors) to facilitate visualization;

The flowchart has been modified to highlight certain sections that can be linked to Table 2. The separate designations of pomace and seeds has been removed. Further details are provided in the methods.

3) Which parameters were used in the spray dryer? For example, feed rate? Please specify; Feed rate has been added "at the rate of 1 L per hour" at the end of the sentence.

4) It is somewhat unclear why the authors evaluated the action of the protease enzyme in the process, since this was not previously mentioned in the introduction or in the objectives of the study.

Furthermore, why was this process evaluated only for the seeds? Was it due to the greater difficulty in releasing proteins from this matrix? If so, this should be clarified.
The sentences have been added "In a separate laboratory experiment, proteolysis of extracted tomato seed protein was explored. Extracts from seeds will contain peptides and amino acids which will pass through a 10 kDa ultrafilter membrane and become lost. The intention would be to treat the seed extract with a protease and to collect all of the proteinaceous material by sequential ultrafiltration through 10 kDa and 300 Da ultrafilter membranes and to concentrate the final permeate by spray drying. Therefore, the aim of this protease treatment was to confirm that a significant quantity of peptides and amino acids could be formed".

5) "Collection and analysis of samples" – I suggest a different title for this section, such as "Characterisation of protein extracts". Furthermore, for better readability, I recommend dividing the analyses performed into subsections (e.g., protein content, microbiological analyses, etc.). This section has been renamed and subdivided as has been suggested. *6) Which N-to-P conversion*

factor was used for the protein content analysis? The words "using the conversion factor of 6.25" have been included where Kjeldahl results were initially mentioned.

Results and Discussion

1) "Furthermore, tomato protein extract has been shown to interact with sunflower oil to form oil emulsions consisting of fine droplets measuring 533 nm at pH 5 (Sarkar et al., 2016)." – This sentence is rather disconnected from the rest of the text and is not related to the main objective of the study;

These sentences have been modified as follows: The presence of oil is another factor that resulted in a 15% lower protein yield (Brodowski & Geisman, 1980), which is likely due to the good oil emulsion properties of this protein between pH 6 and 8 (Sarkar et al., 2016). Consequently, the presence of oil, albeit at low quantities, will reduce the yield of recoverable protein.

2) Table 1 – To make the table clearer, I suggest adding highlighted headings for each main step (Mixing, Centrifugation, Filtration, etc.), with the respective cycles or details listed in rows below;

Highlighted section titles have been included as well as a step number which relates to Figure 1. Each of these step numbers have been highlighted in the figure.

3) "The suspension showed a minor increase from pH 5.03 to pH 5.37 on the following day." – I suggest that the authors mention, perhaps in the Methods section, how long the entire process took.

The following day has now been preceded with "after 15 h". In addition, the words "which was performed over two days" have been added at the end of line 106 in the methods section.

4) The text occasionally mentions results obtained in a previous laboratory-scale study, but it is difficult to compare them with the data from the present work. I suggest presenting this comparison ("Lab scale vs. Large scale") in a table (perhaps in Table 2 itself).

The study of Piyakina and Yunusov has been included in the table.

Is the work clearly and accurately presented and does it cite the current literature? Partly

Five additional relevant references have been included

Is the study design appropriate and does the work have academic merit? Partly

Changes have been made as suggested by the reviewer

Are sufficient details of methods and analysis provided to allow replication by others? Partly More details have been included

If applicable, is the statistical analysis and its interpretation appropriate? Not applicable

Are all the source data underlying the results available to ensure full reproducibility? Partly

All calculations have been made available in a repository, and details have been described.

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Report 23 April 2026

https://doi.org/10.21956/openreseurope.25362.r72608

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Bung-orn Hemung
Khon Kaen University, Nai Mueang, Khon Kaen, Thailand

This work is interesting trend of SDG and should be benefit for tomato processing plants. However, the way to convey information must be modified in order to meet the scientific article. The major comments are included.
1. Please modified title to represent the whole work and its objective. Is this the scaling up of protein extraction from tomato by-products?
2. Unit must be conformed to the journal style
3. Experimental design must be clearly state corresponding to the objective, especially why the protease has to be used? and why it has to be this type?
4. Comparative data must be re considered by rechecking the previous data.
5. Expression of protein recovery yield should be in % for facilitating the comparison.
6. Conclusion: please clarify this protein is from seed and pomace or from seed separated from pomace.
Revision according to these comments would lead to a fulfilment of manuscript prior indexing.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Food Science and Technology, Food Protein Chemistry, By-product Utilization, Food Innovation.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 08 Jun 2026

Paul Baker

Dear Dr Bung-orn Hemung, thank you for taking the time to review my submitted review article and hope my replies adequately address your comments.

This work is interesting trend of SDG and should be benefit for tomato processing plants. However, the way to convey information must be modified in order to meet the scientific article. The major comments are included.

1. Please modified title to represent the whole work and its objective. Is this the scaling up of protein extraction from tomato by-products?
The title has been modified to reflect the whole of the study as follows " Scaling up from laboratory experiments to pilot trials to recover protein from tomato waste remaining after processing and exploring the use of proteolysis as a further purification step".

2. Unit must be conformed to the journal style
Not clear which units are being referred to here, but changes have been made in Tables 2 and 4.

3. Experimental design must be clearly state corresponding to the objective, especially why the protease has to be used? and why it has to be this type?
A sentence has been included as follows" The enzyme, which could be bought in small quantities, was active within the pH range of the tomato extracts without requirement pH adjustment".

4. Comparative data must be re considered by rechecking the previous data.
I've checked the previous data and in Table 2 (which has now been relabelled as Table 3), Baker et al., 2022 is cited. However, this data was produced by ball milling and data for these values were not published. This citation has been updated.

5. Expression of protein recovery yield should be in % for facilitating the comparison.
The data in Table 2 and 4 have been adjusted to %. It was not possible to calculate % protein for Table 3 because protein content of reference 4 was unknown.

6. Conclusion: please clarify this protein is from seed and pomace or from seed separated from pomace.
The sentence has been added as follows "Pilot scale extractions revealed that the protein yield from tomato seeds was higher than from tomato pomace (mixture of pomace and seeds) even when the lower proportion of seeds in the tomato pomace was considered".

Revision according to these comments would lead to a fulfilment of manuscript prior indexing.
Is the work clearly and accurately presented and does it cite the current literature? Partly
Five additional relevant references have been included.

Is the study design appropriate and does the work have academic merit? Partly
Changes have been made as suggested by the reviewer
Are sufficient details of methods and analysis provided to allow replication by others? Partly
Additional details have been included.
If applicable, is the statistical analysis and its interpretation appropriate? Partly
A section has been added in the methods stating that no statistical analysis was performed and why.
Are all the source data underlying the results available to ensure full reproducibility? Partly
All calculations have been made available in a repository, and details have been described.
Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.