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Pilot scale extraction of protein from cold and hot-pressed rapeseed cake: Preliminary studies on the effect of upstream mechanical processing

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Abstract

It is important from a techno-economic perspective to understand the challenges associated with developing scalable processes to extract functional proteins from rapeseed cake for applications as functional food ingredients. One of these challenges is~~n~~ to develop a better understanding of the link between upstream mechanical processing of the rapeseed cake and the yield of extracted protein. An initial trial with cold pressed rapeseed cake (CPR) indicated that wet milling had no impact on protein yield. Measurement of protein yields with the Bradford assay indicated that 14% would be recovered. ~~with the of ~ 48 mg protein per g dry biomass obtained and determined using the Bradford assay.~~ The total protein recovered (~~19 mg per g dry biomass~~5.4% by Kjeldahl analysis) contained a glucosinolate and phytate content that was similar to the rapeseed cake at the start of the process. The fate of the protein extraction was followed during a pilot scale trial with hot-pressed rapeseed cake (HPR), using only stirring followed by sieving, ~~revealed~~ revealing an increase in protein ~~concentrations~~yields, as determined by the Bradford assay, from 8.0%~~39 mg per g dry biomass~~ at the start to 22.6%~~57 mg per g dry biomass~~ at the end of processing. In addition, only 16% of HPR was solubilized. A similar pilot scale trial with HPR using wet milling and stirring followed by decanting also showed an increase in protein concentration from 15.3%~~37 mg per g dry biomass~~ at the start to 23.7%~~66 mg per g dry biomass~~ at the end of processing. In addition, 38% of HPR was solubilized. This study provides an insight into what the effect of upstream mechanical processing (wet milling) has in releasing protein from rapeseed cake in order to improve process efficiency. This could have important consequences for upscaling and commercial extraction of rapeseed cake protein.

Keywords

Colloidal wet milling, rapeseed cake, functional proteins, solubilization

1. Introduction

Colloidal wet milling involves forcing suspended material in water into a conical stator under atmospheric pressure and through the gap between a rapidly spinning toothed rotator and the stator. The rotator can be raised or lowered to change the gap size that in turn will affect the particle size of the material being processed, through a process of hydraulic shearing. Wet milling is commonly used in processing a range of food materials, including cereal crops to recover starch, although it can be energy intensive and requiring soaking beforehand for substrates containing low moisture contents (Wronkowska, 2016). A study on rice flour revealed that the protein content in the remaining material was lower after wet milling compared with dry milling (Leewatchararongjaroen and Anuntagool, 2016), indicating that wet milling might be more effective than dry milling. In relation to rapeseed, wet milling has been described in a patent as an important process step to recover protein from the residual cake following oil separation and in combination with enzymatic treatment (Kvist et al., 2005). A more recent study into releasing carbohydrates from rapeseed meal using wet milling found that the particle size of rapeseed meal was reduced from 520 µm to 135 µm, which was accompanied by minor reductions in protein ~~yield~~ from 33% to 30% in the remaining rapeseed meal (Pustjens et al., 2012). These studies have shown that wet milling resulted in reduced particle size and that the protein content of the remaining material after processing. No information has been reported on protein concentrations after each wet milling cycle and this could be an important factor in relation to process optimization and the number of cycles required

One of the other key technical challenges linked to protein recovery from rapeseed cake is the use of elevated temperature for the extraction of the residual oil, using organic solvents that can cause denaturing of the proteins, thereby reducing functionality and limiting their application. In addition to denaturation, the degradation of proteins during high temperature processing can cause scorching, loss of bulkiness and poor moisture absorption, which are undesirable characteristics in high quality food products. Rapeseed cake is formed by pressing and crushing rapeseeds to recover Canola oil, resulting in flat pieces of caked material. Initially, the rapeseeds are roasted at 160°C, adjusted to a

moisture content of between 5-10% and then cold pressed at 60 ± 10°C, or hot pressed at higher temperatures 90 ± 10°C, to yield hot oil and a protein rich cake (Siger et al., 2017). While the lower temperatures used during cold pressing may result in protein with retained functional properties, most of the global production of Canola oil occurs through hot-pressing (Östbring et al., 2020). The protein derived from HPR cake provides a source of functional ingredients for applications in the food sector with ideal gelation, emulsification, and foaming properties (Mupondwa et al., 2018). ~~Typical~~ It has been described that cold pressed rapeseed cake contains 17% residual oil which was reduced to less than 5%~~and 95% of this can be removed~~ by hexane extraction at 130°C ~~will reduce this to 5%~~ to produce rapeseed meal for one use as animal feed in chicken broilers (Kasprzak et al., 2016). Another study revealed that 32% of the oil remained in cold pressed rapeseed cake and less than 1% remained after solvent extraction (Mosenthil et al., 2016).

Some of the previously reported pilot scale studies have described the protein extraction from 10-15 kg rapeseed meal, which was treated with hexane to remove most of the oil followed by desolventization and toasting of rapeseed cake (Fauduet et al., 1995; Mosenthin et al., 2016; Thobani & Diosady, 1997). However, ~~the process to produce rapeseed meal requires desolventization and toasting of rapeseed cake to remove any remaining oil, but~~ large quantities of rapeseed cake are industrially produced by the company supplying hot-pressed rapeseed in this project as the final end by-product. Furthermore, the elevated temperatures used during toasting to recover hexane can result in loss of functionality, negatively impacting use of this material for many food ingredient applications (Mupondwa et al., 2018). Therefore, it is important to develop a valorisation strategy for the protein obtained from rapeseed cake, although to our knowledge only one previous pilot scale study has reported details of this process, under acidic conditions (pH 2) and without any additional mechanical processing (Rehder et al., 2017). However, a few laboratory studies have described protein extraction from rapeseed cake combined with different mechanical treatments without hexane extraction (Boukroufa et al., 2017), and with supercritical carbon dioxide extraction (Rommi et al., 2015). Nevertheless, the presence of unextracted oil in rapeseed cake will present problems

during protein extraction due to the formation of emulsions and this could interfere with the downstream extraction and separation process.

The use of wet milling at pilot scale was an important part in the recovery of starch and gluten protein from corn kernels (Singh et al., 1997; Vignaux et al., 2006). However, all these laboratory scale experiments were developed to optimize starch recovery from corn kernels, rather than protein recovery from rapeseed cake.

The aim of this study was to evaluate protein concentrations during upstream mechanical processing of cold pressed rapeseed cake (CPR - which was not treated with hexane to remove the residual oil) by wet milling and then to recover protein, where the anti-nutritional properties associated with this protein were evaluated. It was evident during this trial that wet milling did not appear to result in an increase in protein concentrations. Therefore, further trials focussed on the effect of upstream wet milling on the release of protein from the HPR at pilot scale during the incubation phase and to investigate sieving wet rapeseed cake to improve insoluble protein recovery. Furthermore, previous reports have shown some success in separating fragmented dry rapeseed hulls from rapeseed kernels by dry sieving prior to further processing (Campbell et al., 2016; Hansen et al., 2017).

2. Methods

2.1 Materials and equipment

Cold pressed rapeseed cake (CPR) was sourced from the UK while hot-pressed rapeseed cake (HPR) was supplied by Emmelev Ltd, Denmark. The HPR suspension was processed in two heated, continuously stirred tank reactors (150L) set up in series and linked to a colloidal wet mill (FrymaKoruma , model no. MZ50). The contents of each tank were pumped through the colloidal wet mill and then back into the tanks to continue incubation at the required temperature. The flow from the reactor was then pumped into a decanting centrifuge (GEA Westfalia model UCD 205-00-32) in order to separate the solid from the liquid (centrate), and the latter was then pumped back into the

reactor. The flow from the reactor was then diverted to a disc stack centrifuge (GEA Westfalia, model no. SB 7-06-576) and a suspension of enriched solids was collected, while the flow of the solid-free centrate was returned to the other reactor. In one of the trials and instead of using the decanting centrifuge, the suspension was gradually passed through a $1m^2$ sieve containing 250 µm holes in order to collect the fragmented hulls on the sieve. The material collected on the sieve was periodically removed to facilitate faster sieving.

During the CPR trial, the solid-free centrate (supernatant) obtained from the clarifier was passed through a 10 kDa, polyether sulphone, ultrafiltration membrane (Axium Process Ltd., UK) at 30°C. Ultrafiltration for 7 h resulted in 30 L of retentate which was spray dried at 200°C (Anydro model no. S1) and 60 L of permeate which was discarded because no protein was present in this fraction. The protein contents of the samples collected by spray drying were determined in duplicate samples (0.3 g) using Kjeldahl analysis.

2.2 Pilot scale trials

Before beginning each trial, the dry weight content of a sample of rapeseed cake (5 g) was determined, by drying in a moisture analyser at 105°C until a water loss rate of less than 20 mg water per minute was obtained. In each trial (Fig. 1) which were each performed without replication, rapeseed cake (1.2 or 13 kg) was soaked for 24 h at ~20°C in water (90 L) and then resuspended with stirring (160 rpm, 50°C, 2 h for CPR or 3 h for HPR). The suspension was pH 4.5 at the start each trial and was adjusted to the desired pH ~~7~~ with 2.5 M sodium hydroxide. Samples of CPR (1 L) and HPR (15 ml) were collected (Fig. 1- grey arrows). In two of the trials, the suspension was then passed through the colloidal wet mill with a cone gap of 0.5 mm at the start of the experiment and reduced to 0.1 mm towards the third cycle, when material became easier to feed through the mill. In one of the trials, colloidal wet milling was not used. The suspension was continuously stirred at 160 rpm during all of the trials.

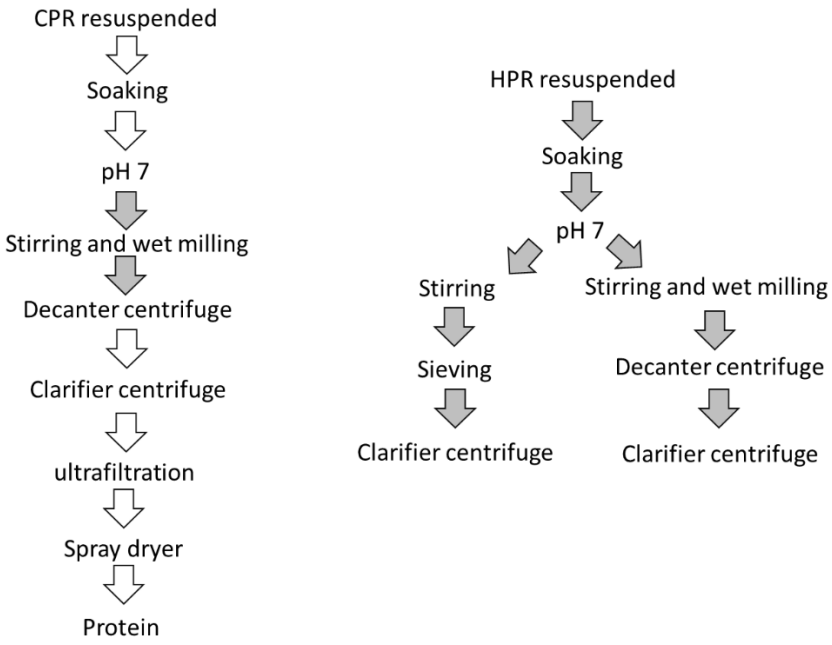

Fig. 1. Process flow diagram for the pilot scale protein extraction from CPR (three day trial) and HPR (one day trial)

(three trials conducted - sampling indicated by the grey arrows, non-sampling indicated by the white arrows. The final protein collected from CPR was evaluated for protein and anti-nutritional contents. All trials were performed without replication).

There were minor differences between the trials, which are shown in Table 1. The remaining solids in both HPR trials were collected by the disc stack centrifuge (clarifier) and further concentrated by centrifuging at 6000 xg 15 min. The supernatant was combined with the centrate in both trials and all of the solids collected in each trial which combined and thoroughly mixed. The collected solid was weighed, and the dry weight content of triplicate samples (5 g) was determined by moisture analysis.

2.3 Determination of protein concentrations

Protein analysis was performed on the supernatant collected after centrifuging the samples (4000 x g, 2 min), using the Bradford assay at 595 nm and after diluting the samples appropriately (Baker, 2020). The P1 protein concentrations were calculated based on the volume of water added to resuspend the HPR. The P2 protein concentrations were more accurate calculations based on subtracting the water associated with the remaining HPR from the quantity of water added to resuspend the HPR. Any water added to the pilot trial to wash through remaining rapeseed cake was accurately monitored and used in the calculations.

2.4 Determination of bacterial populations

The bacterial populations were determined aseptically by serially diluting each of the collected the samples ten-fold in 0.85% (w/v) NaCl, which had been autoclaved (15 mins, 121°C) and cooled to ambient temperature. The samples were mixed for 20 seconds on a multi-tube Vortex mixer (Grant V-32) after each serial dilution and 100 µl of each dilution was transferred onto spread plates containing R2A medium. The plates were incubated at 20°C for one week, then the colonies were counted, and populations were calculated. The whole process was completed within one day at ambient temperature (~ 20°C).

2.5 Assay determinations phytic acid

Phytic acid concentrations were determined using an assay kit (Megazyme), after the phytic acids had been solubilized from duplicate 1 g samples in 20 ml 0.66 M HCl (Megazyme Manual). The assay contains phytase that degrades phytic acids into myo-inositol polyphosphates and then into free phosphates using alkaline phosphatase. The phosphates react with ammonium molybdate and acids to form a blue coloured organometallic complex and the absorbance of this was measured in a plate reader at 655 nm.

The concentration of the free phosphates was determined before using the phytase and this value was subtracted from total phosphates that were released following enzyme treatment.

2.6 Extraction of glucosinolates and analysis using HPLC-MS/MS

The extraction of glucosinolates followed the previously described procedure with some modification (Tolrà et al., 2000), using dried samples (10 mg), with 70% aqueous methanol in a boiling water bath for 5 min. After cooling and centrifugation (4000 x g, 5 min), the proteins in the supernatant were precipitated with 200 µL 0.1 M lead acetate and 200 µL 0.1 M barium acetate. After centrifugation (4000 x g, 5 min), the supernatants were filtered through Whatman No. 4 paper and evaporated to dryness at 35 ˚C. The pellets were resuspended in water (1 mL) and filtered through a 0.2 µm PA polyamide (Nylon) filters (Macherey-Nagel).

The glucosinolates were characterized using a high-pressure liquid chromatography system (HPLC), interfaced with a qTOF mass spectrometer (HPLC-ESI-QTOF / MS). The HPLC was equipped with a Poroshell 120 column (EC-C18; 2.7 µm; 3.0 × 150 mm). The elution gradient of water/ formic acid (99.05: 0.5, v/v) (A) and acetonitrile/ methanol (50: 50, v/v) (B) was performed as follows: from 30% A (water) and 70% B to 0% A and 100% B in 15 minutes. The separation was performed at a flow rate 0.5 mL/min, using 1 µL of injection volume. The separated compounds were first monitored using DAD (230 nm) and then MS scans were performed in the range m/z 40-1000, under the following conditions: capillary voltage, 2.5 kV; gas temperature 250 °C; drying gas 8 L/min; sheath gas temperature 375 °C; sheath gas flow 11 L/min. The MS acquisition was performed in negative ionisation mode according to a previously reported method (Mellon et al., 2001). Under these conditions, experimental data with an accuracy within ± 3 ppm was obtained. All data was processed using Qualitative Workflow B.08.00 and Qualitative Navigator B.080.00 software. The screening strategies used were as previously reported (Mellon et al., 2001; Song et al., 2005). The quantification was performed using a calibration graph prepared using sinigrin hydrate (Sigma Aldrich, SL-00290-10mg) by HPLC-ESI-QTOF. Regression coefficients were higher than 0.990 (5 point per calibration graphs). LOQ was determined as the signal-to-noise ratio of 10:1.

Table 1 Process steps during each of the pilot scale protein extraction trials

Step	Cold pressed rapeseed cake	Hot pressed rapeseed cake	
	Stirring and wet milling	Stirring without wet milling	Stirring and wet milling
1	In water	13 g sodium benzoate in water	100 g sodium benzoate in water
2	Soaking for 24 h	Soaking for 24 h	Soaking for 24 h
3	Suspension adjusted to pH 7	Suspension adjusted to pH 7	Suspension adjusted to pH 7
4	First wet milling cycle	Stirring for 45 min, readjust to pH 7	First wet milling cycle, readjust to pH 7
5	Second wet milling cycle	Stirring for 90 min, readjust to pH 7	Second wet milling cycle, readjust to pH 7
6	Third wet milling cycle	Stirring for 135 min, readjust to pH 7	Third wet milling cycle, readjust to pH 7
7	-	End of stirring for 180 min (3 h)	End of wet milling, 3 h
8	Decanter centrifuge	Suspension filtered through 250 µm sieve	Decanter centrifuge
9	Disc stack centrifuge	Disc stack centrifuge	Disc stack centrifuge

Protein content CPR 35% and HPR 18.8% by Kjeldahl analysis

3. Results and Discussion

3.1 Protein extraction from cold-pressed rapeseed cake

The theoretical yield of protein based on the Bradford assay measurements was ~~about~~ 48.6 ± 1.7 mg per g dry biomass ~~(Table 2)~~. The protein content of CPR was 35% as determined by Kjeldahl analysis and protein yields did not increase with each wet milling cycle (Table 2). The average protein yield that was calculated from the yields at each wet milling cycle was 14 ± 0.5%. Other trials using smaller quantities of CPR revealed similar trends showing no apparent increase in protein yields. The spray dried material had a protein content of 65.8% based on Kjeldahl analysis and calculation of the actual yield of solid extract was 19.5 mg per g dry biomass and 5.4% of the protein was recovered. The difference between soluble protein yields determined during wet milling and within the protein extract collected at the end, may be caused by losses during subsequent processing steps. The protein

could have different food applications depending on solubility, emulsification and gelling properties. Kjeldahl analysis provide an indication of ~~both soluble and insoluble~~total protein content (soluble and insoluble), but may also include other nitrogenous compounds, although this is unlikely to be significant in the current study. The Bradford assay can only quantify the presence of soluble proteins, with additional alkaline treatment required to measure some of the insoluble protein content. In addition, obtaining accurate measurements using the Bradford assay is difficult which can lead to overestimations. Furthermore, it is reasonable to assume that some losses occurred during processing and downstream separation of the protein and these losses could be determined by measuring the protein concentrations during each step. The conditions used in the current trials would be unlikely to cause protein denaturation, which occurs below pH 3 and temperatures above 60°C (Perera et al., 2016). The low protein yields are likely to be caused by oil interference with proteins and previous trials from CPR have shown yields ranging from 3.6 to 12.3% (Miklavčič Višnjevec et al., 2021). A study using rapeseed meal extracted with solvent to remove the residual oil, resulted in higher yields of 25% (Fauduet et al., 1995). In addition, it is likely that the extracted protein will contain oil that could be solvent extracted to remove the oil and increase protein content.

Table 2 Protein extraction yields from 1.2 kg CPR at pH 4.5 and at pH 5 and from 13 kg at pH 7

Process step	pH 4.5	pH 5	pH 7
3	7.0	14.6	13.6
4	8.2	15.8	14.7
5	5.9	14.9	13.9
6	6.4	15.0	13.9

(See Table 1 for description of each process step)

Only a small proportion (<10%) of phosphates present in CPR and extracted protein were soluble and most phosphates were released when treated with phytase, indicating the presence of phytic acid

(Table 3). Phytic acid constitutes one of the main anti-nutritional factors present in rapeseed that interferes with the uptake of minerals during food digestion and imparting a bitter flavour (Bell, 1993). ~~The use of phytase has been shown to be highly effective in recovering all the phytic acid from dehulled, solvent extracted rapeseed flour (Serraino & Thompson, 1984).~~ The reported levels of phytic acid range from 2.8-3.2 mg per g rapeseed meal (de Vries et al., 2014) to 28 mg per g defatted rapeseed meal (Akbari & Wu, 2015), where the variation may be attributed to slightly different assays used in the studies or variation between different rapeseed cultivars. Previous research has indicated that there is a higher reduction in phytic acid content to 50% in the protein extracts when compared with the rapeseed meal, although an acidification step was incorporated which assisted removal (Akbari & Wu, 2015). A comparison of the phytic acid contents within CPR and its protein extract derivative revealed a slightly higher content associated with CPR, although this was shown to be insignificant. ~~The phytic acid content in the CPR was slightly lower when compared with rapeseed kernels and higher compared with protein extract, but neither of these results were significantly different~~. ~~Previous research also determined a 50% reduction in phytic acid content in the protein extracts when compared with the rapeseed meal, although an acidification step was incorporated which assisted removal (Akbari & Wu, 2015).~~ The use of phytase has been shown to be highly effective in recovering all the phytic acid from dehulled, solvent extracted rapeseed flour (Serraino & Thompson, 1984).

Table 3 Determination of phytic acid by measuring phosphate concentrations and glucosinolates by measuring released sugars from CPR

	phosphate		phytic acid
	free	total	~~Phytic acid~~
CPR cake	0.11~~2~~ ± 0.~~00~~401	5.~~528~~ 53 ± 0.~~66~~567	5.42~~0~~ ± 0.66~~1~~
Protein extract	0.17~~2~~ ± 0.~~127~~13	3.~~477~~ 48 ± 2.87~~2~~	3.30~~0~~ ± 3.00~~0~~ (P = 0.43)

Values in mg/ g dry biomass except numbers in brackets represent t-tests on samples compared with CPR cake where $P < 0.01$ is very significant.

A previous study reported that the concentration of glucosinolates in rapeseed protein was one third of the concentration in rapeseed meal and ranging from 1.2- 1.4% g per dry g of biomass, although this decreased to 0.9% after one wet milling cycle (de Vries et al., 2014). One possible explanation for the decrease in glucosinolate content in the protein extract may be due to their solubility in water. However, the quantity of glucosinolates can show considerable variation from 3.9 - 87.5 µM per g dry biomass for different rapeseed varieties (Szydłowska-Czerniak et al., 2011; Yasumoto et al., 2010). Based on the major glucosinolates found in rapeseed such as progoitrin and gluconapin (Yasumoto et al., 2010) and their molecular weights, the glucosinolate concentration ranges from 1.5-34 mg per g between the different varieties. Our study showed the glucosinolate concentrations present in the protein extracts to be 0.6 mg per g of dry biomass, which is at the lower end of previously reported values.

HPLC analysis indicated that, the glucosinolate concentration was higher in the protein extract compared with the original CPR (Table 4). The increase in glucosinolates in the protein extract may occur during ultrafiltration because in one third volume of the original suspension remained (~30 L), which was spray dried. The whole suspension (~90 L) would contain all of the soluble glucosinolates, which would require further concentration by ultrafiltration to lower the quantity of glucosinolates remaining before spray drying. Altogether 16 different glucosinolate compounds were identified and eight showed higher concentrations in the protein extract compared with the original CPR.

Table 4 Concentrations of glucosinolates in the CPR at the start and protein extract presented as mg/kg

Name	CPR	Protein Extract
2-propenyl glucosinolate (sinigrin)	14.5	
2-hydroxy-3-butenyl glucosinolate (progoitrin)	Identified	336
3-butenyl glucosinolate (gluconapin)	25.3	168
3-methylthiopropyl-desulfoglucosinolate	4.6	
butyl glucosinolate	16.3	
4-hydroxybutyl glucosinolate	3.75	
4-pentenyl glucosinolate (glucobrassicanapin)	23.7	55.2
2-hydroxy-4-pentenyl glucosinolate (gluconapoliferin)	166.6	15
3-methylpentyl glucosinolate	62.7	
p-hydroxybenzyl glucosinolate (sinalbin)	123.8	203
2-Phenylethyl glucosinolate (gluconasturtiin)	identified	<2
(2R)-2-hydroxy-2-(4-hydroxyphenyl) ethyl glucosinolate	13.4	
2-phenylethyl glucosinolate (gluconasturtiin)	78.5	97.7
(2R)-2-hydroxy-2-phenylethyl glucosinolate	< 2	
7-Methylthioheptyl glucosinolate		16.2
8-Methylthiooctyl glucosinolate		109
TOTAL	**535.15**	**1098.9**

3.2 The effect of stirring without milling on protein concentrations recovered during processing

The protein content of the HPR was lower than CPR and was 19.1% as determined using Kjeldahl analysis. The HPR protein ~~concentrations~~ yields increased from 10.4%~~39 mg per g dry biomass~~ at the start of the experiment and after 24 h of soaking, to the maximum ~~concentration~~ yield of 25.0%~~57 mg per g dry biomass~~ after 180 minutes of stirring, representing an increase of 147% (Fig. 2). These protein ~~concentrations~~ yields (P1) considered the effect of HPR solubilization and the volume of

water associated with the remaining HPR whereas the other protein concentrations (P2) only considered the quantity of water added at the start of the experiment. ~~It was apparent that there was no major difference between protein concentrations calculated using different liquid volumes (P1 and P2).~~ The results obtained contrasted with the previous CPR trial that involved wet milling and which appeared to show only a minor increase based on protein concentrations calculated without considering CPR loss (Table 2) and other trials (Baker, unpublished). Higher protein yields were obtained with HPR compared with CPR, which were similar to previous trials on HPR ranging from 14.7 to 23.1% (Miklavčič Višnjevec et al., 2021). Previous published research has focused on the protein contents of the extracted material and of the residues, without measuring the protein concentrations during each stage of the process. Measurement of protein concentrations during each step in the process could assist in the optimization of the total protein yield across the whole process, by determining the number of wet milling cycles required to achieve this. It was assumed that the rate of HPR solubilization was linear during each timed interval of stirring, although ideally it would have been preferential to sieve the suspension after each interval and to determine the remaining weight of the sieved material and its moisture content. The use of sieving was explored because dry sieving has been shown to result in the separation of seed hulls and the rapeseed kernel enriched fraction which contained a protein content between 45-55% (Campbell et al., 2016; Hansen et al., 2017). Rapeseed meal (defatted rapeseed cake) has been shown to possess 20% rapeseed hulls which can be removed by dry sieving to increase the protein content of the remaining material (Carré et al., 2015). Therefore, sieving of wet cake has the potential to recover a coarse fibre fraction where all the seed hull fragments are collected on the sieve, while smaller kernel particles enriched with protein pass through the sieve. A previous trial using a smaller quantity (1.2 kg) of CPR cake showed that some separation was achieved when sieving wet CPR, resulting in an insoluble protein rich fraction that had passed through the sieve, with a protein content of 55% that could be enzymatically processed further at a later stage (Baker *et al,* unpublished). However, manual sieving of larger quantities of wet HPR with the available experimental set-up was slow (Fig. 2), although

was improved by sieving smaller quantities and regular removal of collected material from the surface of the sieve. It was determined that 84% of material was retained after sieving and this had a moisture content of 78.8%. It was presumed that the other 16% had dissolved during processing. Visual inspection of the sieved material indicated that it still contained plenty of water and inclusion of a decanting step after sieving could further aid recovery of the soluble protein trapped within the matrix. The loss in the remaining HPR was lower than in a previous study, where the quantity of rapeseed meal lost through solubilization determined to be 30% (Fauduet et al., 1995). It was evident that there was some insoluble material present even after clarification, which would contain additional insoluble protein and was not quantified. The bacterial populations were at 4.3 x 10^3 cells per ml at the start of the trial and appeared to slightly increase to 6.4 x 10^3 cells per ml after 3 h incubation and to 1.31 x 10^4 cells per ml after sieving, perhaps indicating the start of bacterial growth.

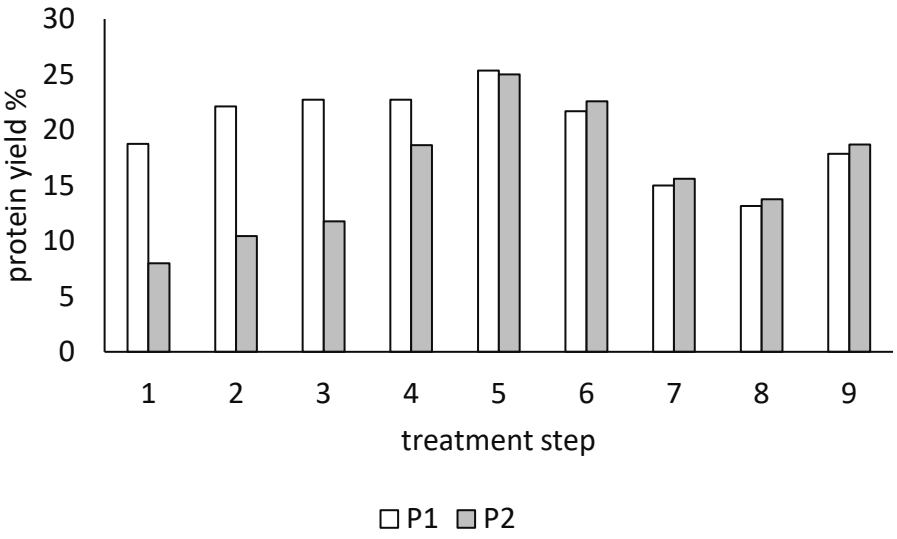

Fig. 2 Protein concentrations during HPR pilot scale trial using stirring without wet milling. The numbers on the x-axis refer to processing steps described in Table 1. P1 refers to protein calculated using liquid volumes at the start whereas P2 refers to a more accurate calculation of protein where water associated with HPR was subtracted from the liquid volume at the start.

3.3 The effect of combined wet milling and stirring on protein concentrations during processing

The protein ~~concentrations~~ yields increased from 15.3%~~37 mg per g dry biomass~~ at the start of the experiment after 24 h of soaking to a maximum of ~~66 mg per g dry biomass~~23.7% after 3 h of wet milling and stirring (Fig. 3). This was an increase of 167% based on protein concentrations that were calculated to account for the loss of HPR during wet milling (P1). The protein concentrations calculated based on volumes of water added at the start (P2) revealed only a minor increase that had similar trends to those in previous trials (Table 2). Therefore, the protein concentrations which were calculated by considering the impact of HPR solubilization showed significant increases compared with protein recovered at the start. These values were somewhat similar to those obtained with protein extraction of HPR using stirring without wet milling, perhaps indicating that wet milling had little effect in increasing protein concentrations. This correlates well with the previous studies on cold pressed rapeseed cake, which reported that particle size reduction had no effect on protein extraction (Rommi et al., 2015), although the mass of residual rapeseed cake after processing was not reported in that study. The bacterial population associated with the HPR suspension at the start of the experiment was 1.6 x 10^4 cells per ml, which had decreased to 4.0 x 10^3 cells per ml after 24 h of soaking at room temperature of about 20°C. The bacterial population appeared to increase slightly to 7.0 x 10^4 cells per ml when the suspension was adjusted to pH 7 and remained unchanged thereafter. It was unlikely that this increase was due to growth because the effect occurred immediately during pH adjustment and was perhaps due to detachment of bacteria from surfaces. These results indicate that the bacterial population was similar to those present in tap water and did not increase, possibly due to the presence of sodium benzoate acting as a preservative. However, after a further 24 h incubation at ~20°C the population had increased to 5 x

10^7 cells per ml. Protein extraction using a combination of wet milling and stirring indicated that 62.7% of the original material remained, implying that a considerable quantity of non-proteinaceous compounds was solubilized or had formed colloidal particles containing protein that could not be precipitated during decanting. It was evident that insoluble material was present even after clarification, which could not be measured using the Bradford assay. One of the advantages of using wet milling is the removal of phenolics and glucosinolates from the remaining material that would usually impart bitterness (Campbell et al., 2016). The decanted material had a lower moisture content of 70.1% compared with the sieved material. The presence of such a high concentration of dissolved solids in the centrate would contain protein that could be recovered using enzymatic processing but also challenges linked to downstream processing (ultrafiltration) and therefore a lower yield of remaining HPR that could be used as ruminant feed.

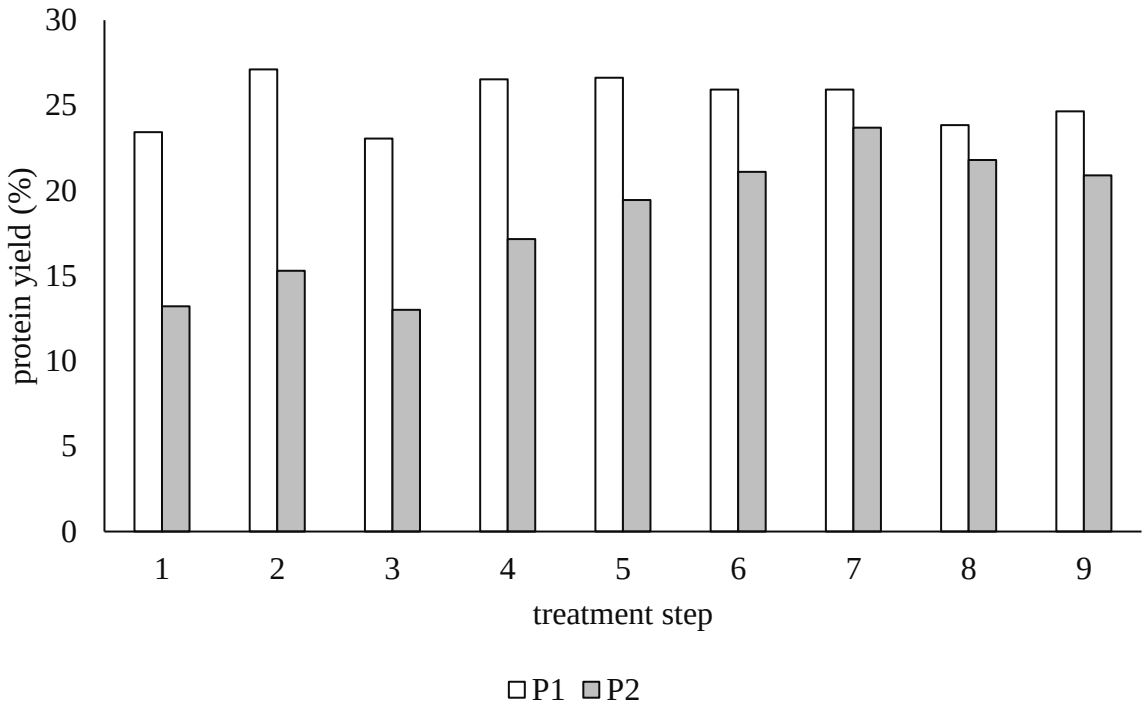

Fig. 3 Protein concentrations during HPR pilot scale trial using wet milling and stirring. The numbers on the x-axis refer to processing steps described in Table 1. P1 refers to protein calculated using liquid

volumes at the start whereas P2 refers to a more accurate calculation of protein where water associated with HPR was subtracted from the liquid volume at the start.

4. Conclusions

As part of this preliminary study, an initial trial with CPR resulted in the recovery of a solid protein extract that was evaluated for anti-nutritional properties. An evaluation of this trial indicated that quantity of rapeseed cake solubilization was important in calculating protein concentrations at each of the processing steps because partial solubilization of rapeseed cake would result in an increase in liquid volume. A further trial with HPR revealed that stirring without wet milling during protein fractionation resulted in an increase in protein concentrations, with only a minor reduction in the remaining quantity of HPR due to solubilization. A combination of wet milling and stirring of HPR showed similar levels of soluble proteins being released, when compared to processing without the use of wet milling, but was shown to be more effective in disintegrating the HPR. It is important to consider HPR solubilization and the effect on protein yield during wet milling, otherwise the measured protein concentrations did not show the expected increase and appeared to remain constant. These studies provide an opportunity to evaluate protein concentrations during each of the processing steps, leading towards optimization and an insight into the possible effect of wet milling in yield.

Data availability

The data will be made available on request

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