



Valorisation of proteins from palm kernel meal

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ABSTRACT

A process to extract proteins from palm kernel meal was investigated. A sequence of unit operations was implemented and optimized in order to ensure an optimal process efficacy. It has been determined that among all the enzymes that were tested, Alcalase had the highest capacity to hydrolyse proteins and solubilize them in solution, especially after using 5% dry matter of PKM as a starting material. Thus, 80% of the proteins were solubilized in the supernatant after Alcalase treatment, but only 60% of the proteins were recovered in the permeate after applying ultrafiltration (300 kDa), with a purity of 60%. Functionality tests revealed that the protein fraction obtained had a very high solubility and a mild foaming and emulsification capacity.

1. Introduction

Protein consumption has increased significantly in both the feed and food industries over the last decade. Oil seeds, such as soy and rapeseed, are significant contributors to protein requirements. After removing the oil, they produce a considerable amount of side products (press cakes) that are rich in protein. As a result, oilseed cakes and meals are an excellent alternative protein source to meet the growing need for protein.

Palm oil is one of the most frequently utilized vegetable oils on the planet. Its vast industry produced 75 million metric tons in 2021 and is predicted to produce 240 million metric tons by 2050 (Corley, 2009). Indonesia and Malaysia are the world's leading palm oil producers (producing more than 80% of global output), followed by Thailand, Colombia, Nigeria, and other nations (Ofori-Boateng and Lee, 2013). Nonetheless, the palm oil business has been chastised across the world for its harmful environmental effect. Large forest areas have been sacrificed to provide more space for oil palm tree cultivation, resulting in 1) deforestation, 2) loss of biodiversity due to monoculture, 3) jeopardizing endangered animal species, 4) destruction of natural habitats, and 5) significant disruption of the local environment. As a result, producers have been rightfully pressed to take significant steps to regulate palm oil tree agriculture and to develop a long-term sustainable solution, such as management and efficient use of natural resources for production (Gesteiro et al., 2019).

Palm oil production creates millions of tons of palm kernel meal

(PKM), a by-product of the palm kernel oil extraction process. Currently, the majority of PKM produced is shipped at a cheap cost to Europe, where it is often used to feed livestock such as cattle, pigs, and poultry (Nwokolo et al., 1976; Agunbiade et al., 1999; Ng and Chen, 2002; Carvalho et al., 2006; Sundu et al., 2006; Azizi et al., 2021). Furthermore, the cheap cost and availability of PKM piques people's curiosity in its possible usage as fish feed. However, employing PKM just to feed animals diminishes its actual worth because PKM contains useful components such as proteins that may be valorised into more valuable end products such as food (Baladrán-Quintana et al., 2019).

PKM has been studied in some studies, and their proteins have been thoroughly characterised and nutritionally evaluated (Chang et al., 2014; Ezieshi and Olomu, 2007). They determined that PKM is an attractive source of high quality proteins since it contains 12–21% protein (Sundu et al., 2006) with a percentage ratio of essential to total amino acids of 36%. That ratio is the appropriate level considered by the WHO for an ideal protein quality for infants (WHO, 2007; Chang et al., 2014; Faridah et al., 2020). Furthermore, few research have focused on extracting proteins from PKM using either the Osborne protein fractionation method (Chang et al., 2014) or by extracting the proteins by using an alkaline solution (Chang et al., 2014; Arifin et al., 2009). Both techniques are effective in extracting soluble proteins from biomass, however they are ineffective at extracting non-soluble proteins (50% of total proteins in PKM). As a result, it is beneficial to use a technology that extracts both soluble and non-soluble proteins in order to maximize the final yield while minimizing the loss of a valuable component.

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The primary goal of this research was to extract as many proteins as possible from PKM, and purify them. Multiple unit operations and associated parameters were examined in order to determine the optimal conditions for extracting and purifying the proteins. The primary functionality of the obtained PKM protein-rich fractions, including solubility, emulsification, foaming and viscoelastic property, was examined and evaluated.

2. Materials and methods

2.1. Enzymatic treatment

A reactor was used to conduct the enzymatic hydrolysis on Palm Kernel Meal at 2 different dry weight (5% and 10% d.w.). For optimal results, the pH was adjusted to 8 using 2 N NaOH, and the temperature set to 60 °C. Then 0.2% or 2% w/w of enzymes was added (4 different enzymes were tested), and stirred for 2 or 4 h. Subsequently, the reaction was stopped, and the enzyme inactivation process was triggered by increasing the temperature to 90 °C for 10 min. After cooling, the solution was centrifuged during 20 min at 20 °C, 17,000g. The supernatant was collected afterwards, and proteins were quantified by using Kjeldahl method.

2.2. Ultrafiltration

This unit operation was implemented in the biorefinery process to further purify the crude protein fraction by eliminating the undesirable components obtained in the supernatant. Hence, for the protein fractions obtained after enzymatic treatment, three membranes cut-offs were tested (100 kDa, 300 kDa and 1000 kDa) as part of the optimization process of ultrafiltration. The transmembrane pressure applied on all membranes was 1.3 bar together with continuous stirring of the medium that is subject to ultrafiltration.

2.3. Protein quantification

Protein nitrogen was quantified by the Kjeldahl method (Gerhardt Analytical Systems - Germany) for the samples obtained after extraction. Dried samples of 200 mg were digested by means of sulfuric acid and high temperature (420 °C) in a KJELDATHERM® block heating system. Once the digestion step was completed, the samples were transferred to a VAPODEST® 50s fully automated system in terms of dilution, filling and titration. The standard conversion factor of 6.25 was used to calculate the total protein from total nitrogen.

2.4. Functionality tests

2.4.1. Solubility and water holding capacity

The solubility and water holding capacity (WHC) of PKM (retentate and permeate 300 kDa) were determined at different pHs and at two temperatures (unheated at room temperature and heated in a water bath at 90 °C for 30 min). The solubility of materials was determined as the weight fraction of materials found in the supernatant after centrifugation at 4000g for 45 min. The WHC of materials was defined as the ratio of the weight of water and the weight of dry materials found in the pellet after centrifugation.

2.4.2. Emulsification

Emulsifying experiments were performed to get an impression of the emulsifying capacity and stability of PKM (retentate and permeate 300 kDa) at pH 3 and its natural pH. The emulsions of 3% d.w. of PKM retentate or permeate and 1.5% d.w. of sunflower oil (obtained from a local supermarket) were prepared in two steps. These mixtures were first mixed using a high-speed mixer Ultra-Turrax (T25 digital ULTRA-TURRAX®, IKA®-Werke GmbH & CO. KG, Staufen, Germany) at a mixing speed of 13.500 rpm for 60 s per 100 g of sample. Consecutively,

samples were homogenised in a LabScope homogeniser (Delta Instruments, the Netherlands) for 2 min with an average pressure of 160 bar. The emulsions were stored at room temperature until phase separation was observed. The stability of the emulsion was evaluated visually: images of the emulsions were taken to evaluate their stability.

2.4.3. Foaming

Foaming experiments were performed to get an impression of the foamability and stability of PKM (retentate and permeate 300 kDa) at its natural pH. 15 mL of PKM permeate or retentate solution (2% d.w.) was mixed using a high-speed mixer Ultra-Turrax at a mixing speed of 8000 rpm for 5 min. The stability of the foam was evaluated visually: images of the foams were taken every 10 min until the foam volume was reduced to half (Lomakina and Míková, 2006).

The foam capacity (FC) was calculated as:

$$FC (\%) = (FV/ILV) \times 100\%$$

FV = volume of foam (mL)

ILV = volume of the initial PKM permeate or retentate solution

2.4.4. Rheology and viscosity

The viscosity (flow curves) and rheological properties (frequency sweeps) of 20% d.w. suspensions of the PKM (retentate and permeate 300 kDa) were determined at two temperatures (20 °C and 90 °C) using a DHR2 rheometer (TA Instruments, United States). To determine suitable strains for the frequency sweeps, amplitude sweeps were performed and amplitudes in the linear range were selected from those. The amplitude sweeps, frequency sweeps and flow curves (shear rate range: 0.01–1000s⁻¹) were performed consecutively for the measurements at 20 °C. For the measurements at 90 °C, the DHR2 was cleaned and refilled in between the three different determinations. A soak time of 1 min was applied before and in between the measurements.

2.5. Statistical analysis

All experiments were conducted in duplicates. Statistical analyses were carried out using Minitab 17 software. ANOVA test was carried out and measurements of duplicates for each sample were reproducible for ±5% of their respective mean values.

3. Results and discussion

3.1. Process development and optimisation

The primary composition of PKM was 15 ± 0.03% proteins, 50 ± 0.07% carbohydrates, 8 ± 0.01% lipids, 16 ± 0.01% fibres, and 9 ± 0.01% ash. As it is described in the graphical abstract, the process consists of extracting the maximum amount of proteins from Palm Kernel Meal (PKM). Subsequently, the crude protein extract is purified by means of ultrafiltration. The sequence of unit operations employed has been tested step-by-step with its corresponding parameters in order to find the optimal conditions for a highly efficient extraction and purification process. Indeed, the outcomes of the process were closely monitored by measuring the extraction and filtration efficiency, and by measuring the protein yield after each step of the process.

The results displayed in Fig. 1 showed that Alcalase was the most efficient in extracting proteins up to 75% (w/w). The yield of proteins released after using Neutrase and Umamizyme was statistically similar with up to 20% (w/w), whereas Newlase was the least efficient with up to 15% of total proteins (w/w).

The purity of the protein was also monitored, and the highest purity obtained in the supernatant was 55% for Alcalase, followed by Neutrase and Umamizyme with 25%, and the lowest was for Newlase with 14% (Fig. 1). The purity of protein for PKM is lower than expected, but this

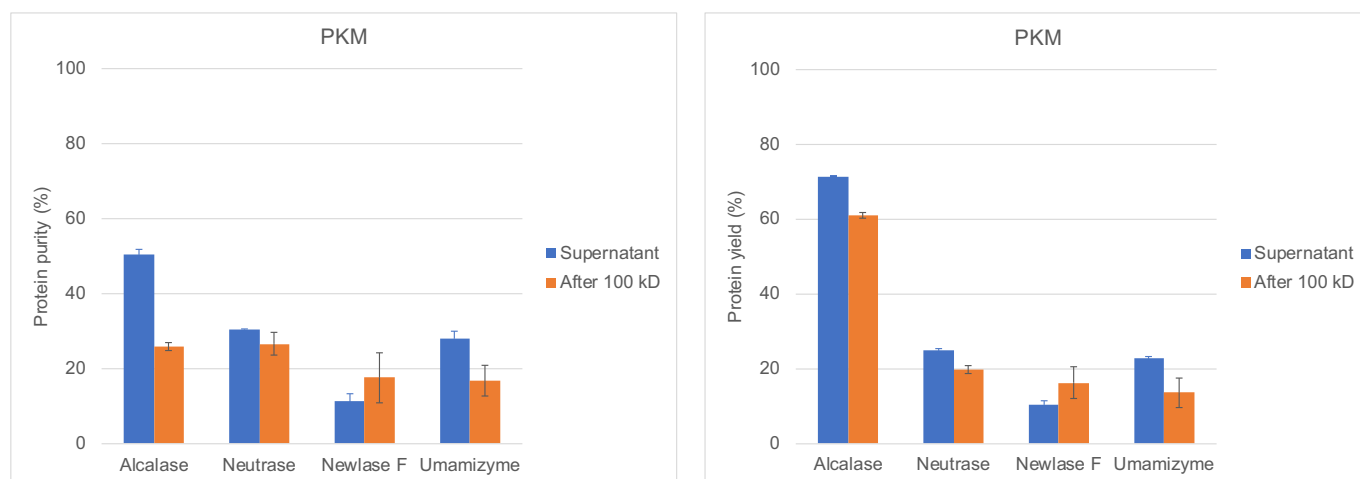


Fig. 1. Protein yield in the supernatant and the filtrate (100 kDa membrane) after testing multiple enzymes on PKM. Results represent the standard deviation of duplicated tests for each enzyme (\pm SD).

can be due to the presence of a significant amount of sugars in the supernatant. These sugars are soluble in water and can pass through the membrane into the permeate, thereby reducing the purity of proteins in the permeate.

The main reason to select proteases was to only hydrolyse the soluble proteins, but also the proteins that are non-soluble due to their covalent linkage to non-soluble polysaccharides. Looking at the results, it has been established that the best enzyme was Alcalase which showed the highest protein yield and purity all along the process. Alcalase has an efficient capacity to hydrolyse proteins into small peptides or even amino acids, however, this does not imply that the protein recovered after Alcalase extraction have maintained all their functional properties. Thus, if the end product requires functional proteins, a trade-off should be found between the selection of an enzyme with a certain degree of hydrolysis capacity and the desired yield/purity of the proteins in the downstream process.

The supernatant of each enzyme was submitted to ultrafiltration by using a 100 kDa membrane (Fig. 2). The results showed that the highest amount of proteins recovered in the permeate was for Alcalase with 60% which represents 80% of total proteins obtained in the supernatant. On the other hand, for the other three enzymes, the protein yield in the permeate was statistically equivalent ($p > 0.05$) and did not exceed 20%, which accounts to 27% of the total proteins obtained in the supernatant.

The purity of proteins in the permeate was also measured after ultrafiltration. The highest purity was obtained with Alcalase and Neutrase with 24% and the lowest was for Newlase and Umamizyme with 18%.

Given that Alcalase was selected due to its capacity to extract the highest amount of proteins, multiple membrane cut-offs were tested to optimize the ultrafiltration process (Fig. 2). The cut off of the membrane did not seem to have a significant effect to increase the amount of proteins in the permeate. Regardless which cut off was used, the yield of proteins was 60% in the permeate. However, the relative difference between all the membranes tested was statistically equivalent ($p > 0.05$) between the 100 kDa and 1000 kDa, but the 300 kDa membrane seems to yield the highest protein purity. This implies that the most suitable membrane cut off to filtrate the proteins of PKM is 300 kDa. Fig. 3 also shows us that regardless which membrane cut-off was employed, the purity of the proteins decreased in the permeate. This is due to multiple factors, among them is the polarisation layer that forms on the surface of the membrane, which leads to the slowing of the flux rate and the retention of some components including proteins. Moreover, glycoproteins are simultaneously retained due to their covalent linkages with large polysaccharides that did not pass through the membrane (Heaney-Kieras et al., 1977; Liu et al., 2005; Safi et al., 2017). Another explanation is that the supernatant is composed of a complex blend of unequally charged proteins (Safi et al., 2017), which leads to a strong

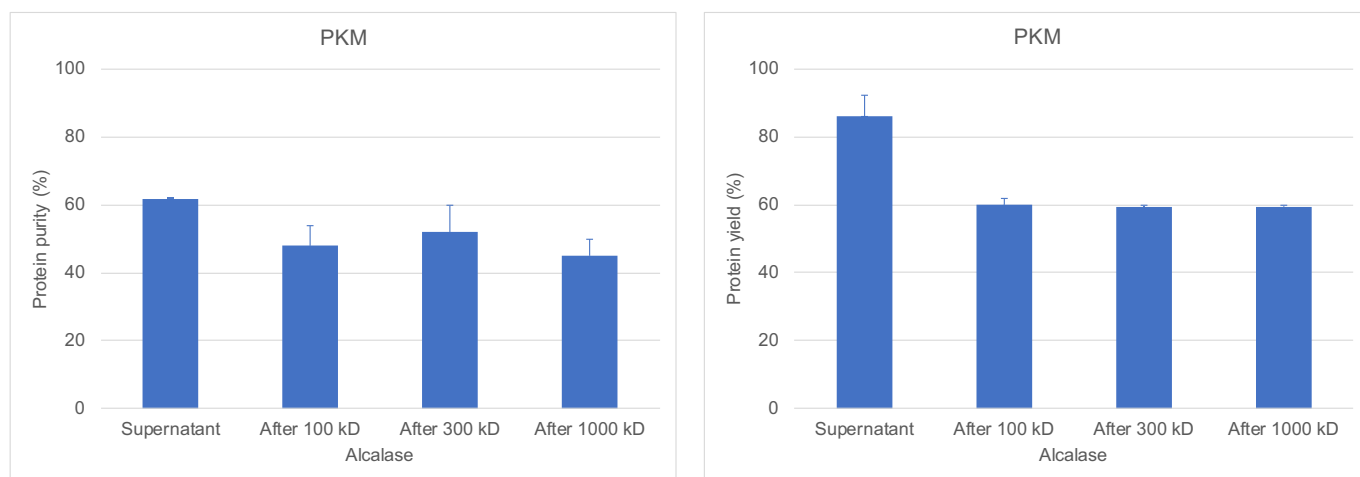


Fig. 2. Testing different membrane cut-offs on the protein extract obtained after Alcalase treatment. Results represent the standard deviation of duplicated tests for each enzyme (\pm SD).

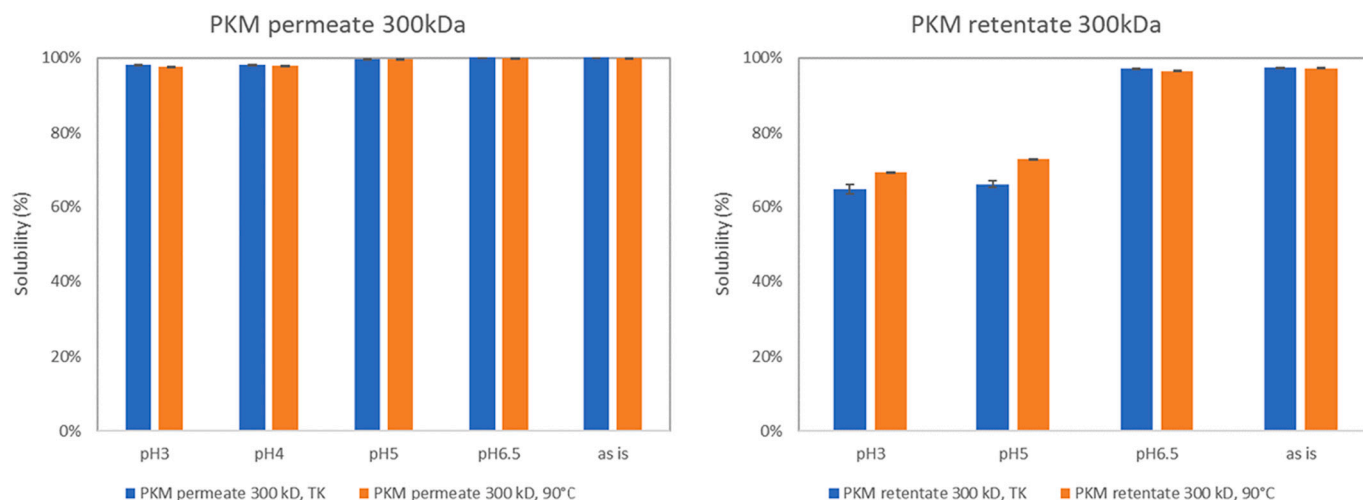


Fig. 3. Solubility of 5% wt suspensions of PKM 300 kDa permeate (left) and retentate (right) as a function of pH and at two temperatures (room temperature: blue bars and 90 °C: orange bars). Results represent the standard deviation of duplicated tests for each enzyme (\pm SD). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

interaction between the positively and negatively charged proteins that form aggregates of large molecular weight (Ursu et al., 2014; van den Berg and Smolders, 1990). This renders them large enough to be retained by the membranes and contributes to the increase in resistance due to the concentrated layer formed near the membrane interface (van den Berg and Smolders, 1990).

Alcalase showed high efficiency in hydrolysing proteins into small molecular weight peptides or amino acids. Thus, given the small molecular weight of the hydrolysed proteins, it was expected that the majority of the hydrolysed proteins will be pass through the membrane and into the permeates of all the tested membranes. However, against all odds, the permeates of contained a lower-than-expected amount of proteins. In previous studies, the phenomena of protein loss during ultrafiltration have been defined as adsorptive fouling (Safi et al., 2017; Susanto et al., 2008). These studies performed UF experiments by using a polyethersulfone membrane on protein-polysaccharide mixtures and they found that membranes with a large molecular weight were more prone to a decline in the flux rate due to adsorptive fouling of polysaccharides. This implies that in addition to the polarisation layer, it is possible that for large membranes cut-offs, a fraction of retained molecules can penetrate into the membrane pores and contribute to the membrane fouling as well as to the lower yield of proteins in the permeate (Safi et al., 2017; De la Torre et al., 2009; Susanto et al., 2008).

To further optimize the process, several other parameters were tested such as Alcalase concentration, stirring time and dry weight of the biomass. Results showed that the higher the enzyme concentration (2% w/w) and the stirring time (4 h), the higher the yield of proteins in the supernatant, but not by much. Furthermore, the extraction process seems to yield more proteins - with some slight differences - while using 5% dry weight of PKM. Nevertheless, the protein purity was statistically similar ($p > 0.05$) regardless which amount of PKM was employed, which enzyme concentration was used and which stirring time was selected.

It is worthwhile noting that the best parameters selected do not specifically mean that they are the best in terms of cost efficiency and sustainability. This implies that if the statistical differences are not very high, it would be better to select the lowest enzyme concentration in order to lower the process cost, and select the lower stirring time to reduce the energy consumption while running the process.

3.2. Functionality tests

3.2.1. Solubility and water holding capacity

The solubility and WHC of PKM permeate and retentate were measured at a variety of pH levels, including pH 3, 4, 5, 6.5, and natural pH. The pH of 5% d.w. PKM permeate and retentate in their natural state (pH as is) was 7.8 and 7.7, respectively. According to the results, the proteins extracted from the 300 kDa permeate were exceedingly soluble (almost 100%) at all pH tested. Heating the protein solutions at 90 °C for 30 min had no influence on the materials' solubility. A solubility of near 100% in a wide range of pH indicating that proteins present in PKM permeate were in hydrolysed forms as no isoelectric values, the pH value at which the net charge of a protein becomes zero, causing aggregation and precipitation were detected.

The quantity of water that a particle can hold is measured by the WHC (weight of water divided by the dry weight of the pellet). The results were obtained under the specified circumstances (5% d.w., pH ranging from 3 to practically 8, room temperature or 90 °C), the WHC of the PKM permeates could not be calculated as almost all materials were in the supernatants.

The solubility of the 300 kDa retentates proteins was lower than that of PKM permeates (Fig. 3). The pH altered the solubility of PKM retentate: at acidic pHs, PKM retentate was less soluble than at neutral and natural pHs. The solubility of PKM was likewise unaffected by heating.

Fig. 4 shows the WHC of the PKM 300 kDa retentate pellets. The WHC of the pellet rose as the pH increased. The WHC of the pellets was lowered by heating the materials at 90 °C for 30 min. At natural pH (PKM retentate), the pellet has a high WHC (higher than 10). At these pHs, just a small fraction of the PKM 300 kDa retentate was found in the pellet (less than 10% d.w.).

3.2.2. Emulsification

The stability of PKM permeate or retentate-stabilized emulsions was monitored throughout time. Emulsions containing 1.5% d.w. of sunflower oil and 3% d.w. of PKM permeate and retentate at pH 3 were phase-separated soon after homogenisation. At this pH, phase separation of the emulsions with PKM permeate was slightly slower than that of the emulsions stabilized by PKM retentate.

The stability of the emulsions stabilized by PKM permeate and retentate was comparable at their natural pH after 24 h of storage at room temperature. At their natural pH, after 24 h at room temperature, both emulsions with PKM permeate and retentate had visible creaming and precipitation.

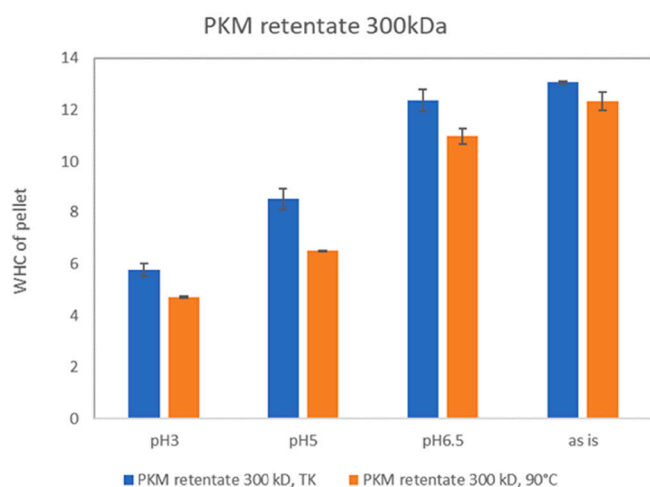


Fig. 4. Water Holding Capacity (WHC) of PKM 300 kDa retentate as a function of pH and at 2 temperatures (room temperature: blue Bars and 90 °C: orange bars). Results represent the standard deviation of duplicated tests for each enzyme (\pm SD). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Both PKM permeate and retentate showed better emulsification capacity at their natural pH (near neutral pH) than at pH 3. Their emulsification capacity and stability could be further investigated at different conditions such as other pH values, various ratios of PKM and oil, different mixing conditions (mixing speed and time) and application of homogenisation step.

3.2.3. Foaming

At its natural pH, the stability of foams stabilized by PKM permeate or retentate (2% d.w.) was monitored throughout time. PKM permeate foam exhibited a greater foam height than PKM retentate foam at $t =$

0 (immediately after mixing). The foam capacity (FC) of PKM permeate and retentate was 66% and 40% respectively. These FC values may be different when different mixing or whipping conditions are applied.

After 20 min at room temperature, the height of foam stabilized by PKM permeate was decreased to half, whereas foam stabilized by PKM retentate lost nearly half of its height after 10 min (Table 1). Note that the foam height was lower in the centre of the tube in the image of foam stabilized by PKM retentate after 10 min (not visible on image). PKM permeate also outperformed PKM retentate in terms of foaming and foam stability. After 1 h stored at room temperature, all foam bubbles disappeared from both samples.

3.2.4. Rheology and viscosity

At shear rates ranging from 0.01 to 1000s⁻¹, the viscosity of 20% d. w. PKM permeate or retentate was determined. The 300 kDa membrane permeate and retentate were both shear thinning, according to the results. Heated samples had a substantially higher shear thinning behaviour than unheated samples. Heated PKM samples were more viscous compared to the unheated PKM samples at lower shear rates (Fig. 5). PKM retentate samples had a somewhat greater viscosity than PKM permeate samples at the same shear rate and temperature (except at shear rate of 0.1 s⁻¹, 20 °C).

Prior to the frequency sweep experiments, amplitude sweep tests were performed to determine the limit of the non-destructive deformation range of the materials. From the results of these tests, an amplitude value of 1% was chosen for the frequency sweep tests. Frequency sweeps may provide information on the time-dependant behaviour of the materials: high frequencies simulate fast motion on short timescales, while low ones simulate slow motion on long timescales or even at rest. The storage and loss moduli of PKM permeate and PKM retentate were higher at 90 °C compared to 20 °C.

For samples at 20 °C, both storage and loss moduli were very low, in the similar range as that of vegetable oils (Yalcin et al., 2012). At lower frequencies, the storage moduli of both samples were higher than their loss moduli indicating a solid-like structure (Fig. 6). Deformations at

Table 1

Stability of foams formed and stabilized by PKM permeate or retentate at its natural pH.

Time (min)	t = 0	t = 5	t = 10	t = 15	t = 20	t = 60
PKM permeate						
PKM retentate						

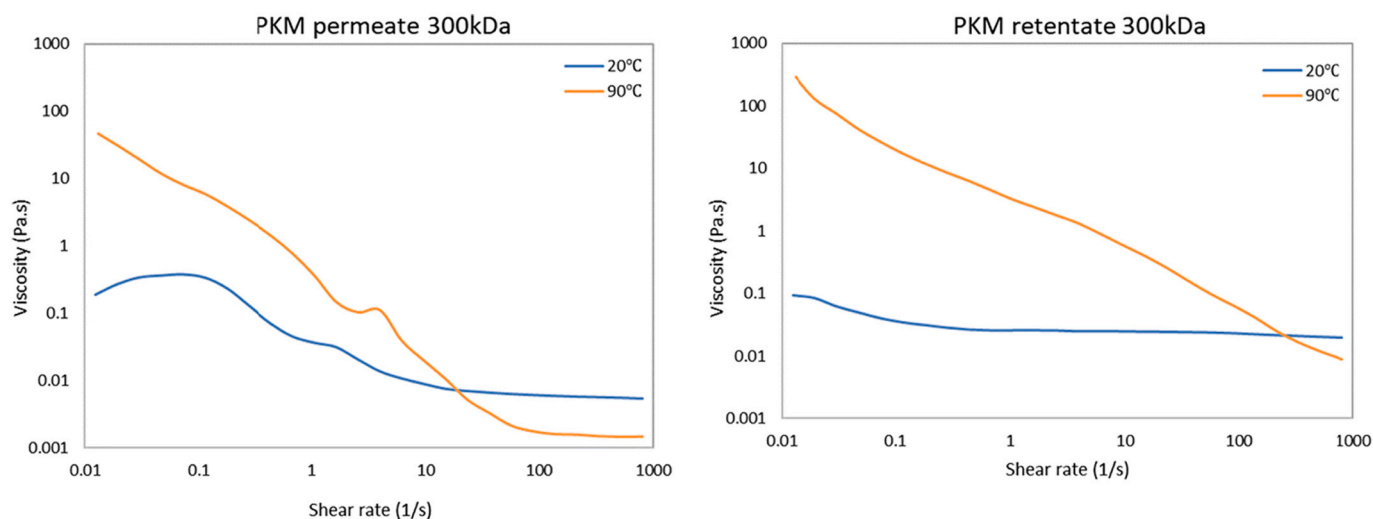


Fig. 5. Flow curves (shear rate 0.01–1000/s) of 20% wt of the PKM permeate (left) and retentate (right). The viscosity is plotted as a function of the shear rate at two temperatures (20 °C: blue lines and 90 °C: orange lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

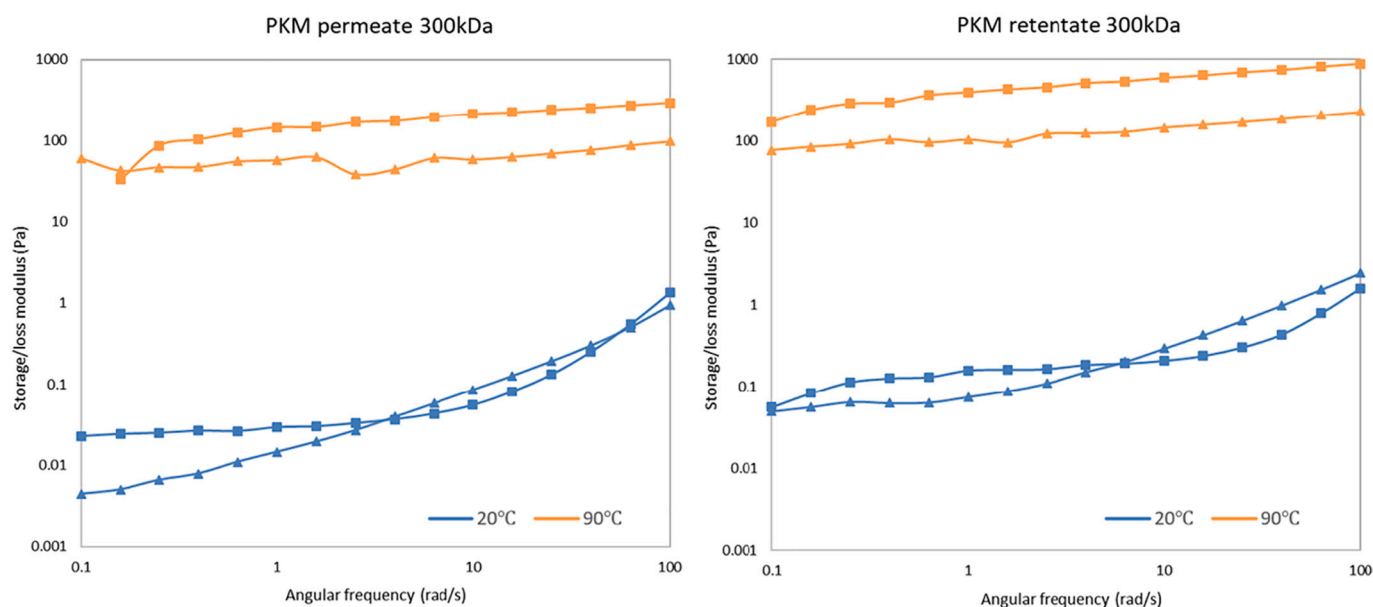


Fig. 6. Frequency sweeps (amplitude 1%, angular frequency 0.1–100 Rad/s) of 20% wt of the PKM permeate (left) and retentate (right). The storage modulus (squares) and loss modulus (triangles) are plotted as a function of angular frequency at two temperatures (20 °C: blue lines and 90 °C: orange lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

these frequencies were elastic. The loss and storage moduli were roughly equal at about 4 rad/s for PKM permeate and 6 rad/s for PKM retentate. Increase of frequencies beyond these values, deformations became viscous, and samples showed more liquid-like behaviour.

For samples at 90 °C, the storage moduli of both samples were higher than their loss moduli in the whole range of frequencies: samples behaved like viscoelastic soft gels.

Given the functional properties of the recovered proteins, it is plausible to suppose that after scaling up the process, some potential applications are possible. Protein beverages and protein-fortified vegan cheese are two examples of prospective uses. However, before manufacturing any goods, the bitterness of the hydrolysed proteins, as well as their safety, must be carefully evaluated. Toxicology tests should be carried out prior to the usage of these proteins.

4. Conclusions

The objective of the study was to develop a process to valorise the proteins of palm kernel meal and explore their functional properties. Multiple parameters were tested to optimize the extraction process and the results showed that the process was efficient enough to extract the majority of the proteins. However, protein losses were observed after applying ultrafiltration on the crude protein. Furthermore, multiple functionality tests were conducted and showed encouraging results such as high solubility and mild emulsification and foaming. Further development and techno-economic analyses will be required to validate the process and identify valorisation routes for the protein extract.

CRedit authorship contribution statement

Carl Safi: conceptualization, writing original draft preparation, submission process, supervising.

Nam-Phuong Humblet: conducting functionality tests, results assessment and writing.

Peter Geerdink: conceptualization and investigation.

Mira Theunissen: conducting functionality tests.

Brigit Beelen: conducting the process work, conducting all the analyses to validate the process, writing.

Juliën Voogt: conceptualization and investigation.

Wim Mulder: conceptualization, supervising, writing.

Declaration of competing interest

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

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