Efficient single-step rapeseed oleosome extraction using twin-screw press

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A B S T R A C T

Oil in seeds is encapsulated in oleosomes, which are small lipid droplets surrounded by a phospholipid-protein monolayer. The currently proposed method to extract intact oleosomes includes mixing seeds with alkaline media in a ratio 1:7, batch blending and filtering. In this work, we propose the use of a twin-screw press to perform the oleosome extraction at pH 7. The results show that similarly to blender extraction, twin-screw press recovers ~60% of the oleosomes; however the twin-screw press is able to achieve this yield even when just pure water is used. While in the blender extraction, the yield depends on ionic strength and pH of the extraction media, when using twin-screw press, the oleosome extraction yield predominantly depends on the mechanical forces. These shear forces are able to break the cell walls and release the cellular material while maintaining the integrity of oleosomes. The oleosomes extracted with twin-screw press have similar characteristics than those obtained by the blending process. Overall, twin-screw press seems a promising alternative to scale-up the oleosome aqueous extraction, especially as neutral pH can be used and the water usage is significantly reduced. Additionally, preliminary results showed that the yield can increase up to 90 wt%.

1. Introduction

Vegetable oil is stored in intracellular organelles named oleosomes. Oleosomes consist of a core of triglycerides surrounded by a monolayer of phospholipids and proteins (Tzen, 2012). This structure provides oleosomes with great stability against physical and chemical stresses (Huang, 1992; Purkrtova et al., 2008). The hydrophilic nature of the oleosome interface allows their extraction by aqueous solvents, forming a natural and stable oil in water emulsion. The properties of the obtained emulsion can be customized by the composition of the aqueous extraction media, which influences the interactions of the oleosome interface and the co-extracted proteins (Romero-Guzmán et al., 2020). This, in turn, influences the oleosome solubility and hence their extractability (Iwanaga et al., 2007; Nikiforidis and Kiosseoglou, 2009). Oleosome extraction is currently performed by soaking the oilseeds at a ratio of 1:7, followed by blending and filtration. A series of centrifugation cycles recovers a cream rich in oleosomes with characteristics very similar to those of engineered emulsions (Nikiforidis and Kiosseoglou, 2009; Rosenthal et al., 1998, 1996). This extraction procedure requires nevertheless a large amount of water, which makes upscaling difficult (Kapchie et al., 2011).

Hence, alternative technologies that could deal with the mentioned requirements are necessary. A possible technology to replace the current batch-blending method is the use of a continuous twin-screw press. This technology is available at an industrial scale and is commonly used in the food industry for grinding, liquid/solid extraction and liquid/solid separation (Thiyam-Hollaender et al., 2012; Uitterhaegen and Evon, 2017). While it has been successfully used for aqueous oil extraction (Evon et al., 2010, 2007; Uitterhaegen and Evon, 2017) we think that the twin-screw press has the potential to obtain oleosomes as the blender and filtering process.

Therefore, the aim of this work is to compare the oleosome twin-screw extraction to the current lab-scale blending-based process on its extraction efficiency and the characteristics of the extracted oleosomes, using aqueous media with different composition and ionic strength.

2. Materials and methods

2.1. Materials

Rapeseeds (*Alizze*) were purchased from a seed producer. The used seeds are food grade as they do not contain euricic acid and have a low

glucosinolate content (13 mg/100 g). Their composition is: 9.0% ± 1.2 moisture, 36.0 wt % ± 1.3 of oil and 18.0 wt % ± 0.7 of protein in wet basis. All the chemicals were obtained in analytical grade from Sigma-Aldrich (St. Louis, MO, USA). Solutions and dispersions were made with ultra-pure water (MilliQ) obtained by a Merck Millipore device (Darmstadt, Germany).

2.2. Aqueous extraction of oleosomes by blender or by twin-screw press

All extractions were performed in batches of 100 g of seeds. Based on the work of De Chirico et al. (2018), the optimized extraction conditions for rapeseed, such as soaking time and settings during blending, were chosen. Prior to the extraction, the seeds were soaked for 16 h at 4 °C using a solution of either $NaHCO_3$ (0.1 mol/L) adjusted to pH 9.5, KCl (0.2 mol/L) adjusted to pH 7 or H_2O also adjusted to pH 7. The low temperature suggested during the soaking time has proved to supressed the enzymatic activity and microbial growth (De Chirico et al., 2018; Simon et al., 1976; Valero et al., 2009). The pH was adjusted with a solution of NaOH (1.0 mol/L) for the alkaline solution and NaOH (0.1 mol/L) for pH 7. A SevenMulti™ dual meter pH/conductivity (Mettler Toledo, Greifensee, Switzerland) was used to monitor the pH. The seed:solution ratio was 1:1 by weight. The pre-soaked seeds were then used for the extractions either with a kitchen blender (2.2.1) or with a twin-screw press (2.2.2).

2.2.1. Extraction with the blender method

For the lab-scale blender extraction, the ratio of pre-soaked seeds and extraction media was adjusted to 1:7 based on dry weight of the initial amount of seeds, both seeds and extraction media were kept cooled until the moment of extraction. The seeds and the media were blended (Thermomix Vorwerk, Germany) for 90 s at 7200 rpm. The obtained slurry was filtered using 2 layers of cheesecloth with a pore size of ~150 μm (GEFU®, Eslohe, Germany). The filtrate constituted the initial oleosome extract, while the remaining solids constituted the cake. After filtration the filtrate was immediately cooled down to 4 °C.

2.2.2. Extraction with twin-screw press

The pre-soaked seeds (1:1 seed:solution by weight) were taken out of the fridge (4 °C) and directly processed with a lab-scale twin-screw press

(Angel 7500, Naarden The Netherlands). Due to the short processing time ~10 s, the temperature of the extract did not change much. In Fig. 1 an image of the lab-scale twin-screw press used for the extraction is depicted. The velocity of the rotation of the screws could not be adjusted, so it was kept constant to 82 rpm. Two streams were recovered from the press: a press cake and a concentrated slurry, which was the oleosome-rich extract. For a fair comparison between the two extraction methods; however, an additional step was introduced. The collected extract was diluted to a ratio of 1:7 by weight using the corresponding cooled extraction solution ($NaHCO_3$ 0.1 mol/L solution at pH 9.5, or KCl 0.2 mol/L solution at pH 7 or H_2O), which resulted in a stream here after referred as the first extract. Subsequently, the same oleosome recovery procedure was followed as with the blender-isolated oleosomes.

2.3. Isolation of oleosomes

Isolation of the oleosomes from the first extract was performed by centrifugation at 3000 g, 4 °C for 15 min, followed by a second centrifugation, 10,000 g, 4 °C for 30 min (Sorval Lynx 4000 Centrifuge, Thermo Scientific USA). The oleosome rich cream layer was then drained from the excess of solution using filter paper. The collected cream was subsequently dispersed in one of the three solutions (0.1 mol/L $NaHCO_3$, 0.2 mol/L KCl or pure water) at a weight ratio of 1:4 and centrifuged at 10,000 g, 4 °C for 30 min. The cream was collected and analysed for its composition and physical properties.

2.4. Characterization of the streams

2.4.1. Moisture content

To determine the moisture content of the cake and the oleosome cream, 1 g was dried with a Moisture Analyser (Leicester, UK) at 90 °C until constant weight. The drying time varied from 10 to 40 min, depending on the sample. The % of moisture was determined as the weight difference between the initial and the dehydrated sample, divided by the initial mass of the sample.

2.4.2. Lipid content

The lipid content of dried samples was determined by Soxhlet extraction with petroleum ether (B-811 Buchi Extractor, Switzerland).
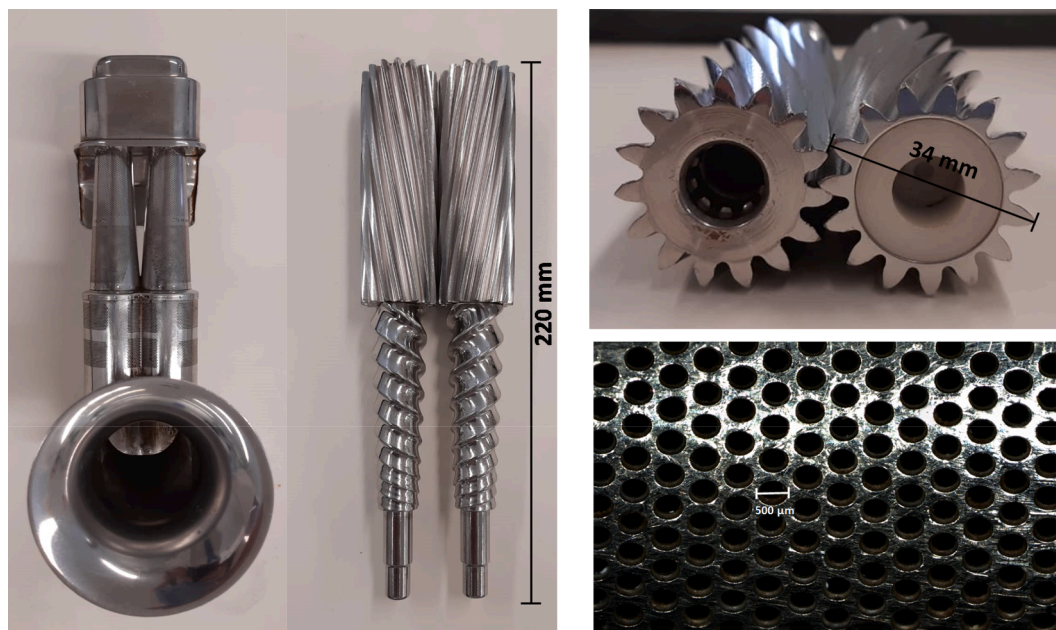

Fig. 1. Twin-screw case and filter (left), screws profile (centre), screws diameter (top right), zoom-in on the sieve attached to the case (bottom right) used for the oleosome extraction.

The analyses were performed in triplicates for each sample. The oleosome extraction yield was calculated based on the difference between the amount of oil in the initial seeds (36.5 ± 1.3) and the amount of oil in the cake. This calculation assumed that all the extracted oil was expected to be either in the form of native oleosomes or emulsified oil.

2.4.3. Protein content

The protein content was determined using the Dumas method. The protein content was quantified using a conversion factor of 5.7 for the nitrogen content. The protein extraction yield was calculated based on the difference between the protein content remaining in the cake and the initial protein content in the seeds (18.5 ± 0.7).

2.5. Protein profile characterization

The protein profile was analysed qualitatively by SDS polyacrylamide gel electrophoresis using a Bio-Rad MiniProtean cell (Bio-Rad Laboratories Inc., Hercules, USA). Two types of buffers were used to unfold the proteins (Nikiforidis and Kiosseoglou, 2009). Buffer 1 consisted of Tris-HCl (50.0 mmol/L), Urea (5.0 mol/L), 1 wt % SDS and 4 wt % 2-mercaptoethanol. Buffer 2 consisted of Tris-HCl (125.0 mmol/L), Urea (5.0 mol/L), 1 wt % SDS, 20 wt % Glycerol and 4 wt% 2-mercaptoethanol. The creams were dispersed in ultra-pure water (1:100 wt/v.) and combined with Buffer 1 (1:1 by volume) and agitated for 15 min at room temperature. Each sample was rested for 15 min before buffer 2 was added. The samples were vortexed once more for 15 min and rested for another 15 min. Afterwards, the samples were heated at 90 °C for 5 min and kept at −20 °C overnight. Before the samples were loaded onto the gel, 3 freeze-thaw cycles were applied. 20 μL of each sample were loaded on a 12% Tris–HCl SDS-ready gel, size range of 10–200 kDa; plus 10 μL of Pre-Stained Protein Standard (Bio-Rad Laboratories Inc., Hercules, USA). The electrophoresis was carried out at 200 V for about 30 min. Subsequently, the gel was stained with Bio-safe Coomassie Stain (Bio-Rad Laboratories Inc., Hercules, USA).

2.6. Particle size distribution determination

The particle size of the creams was measured by a static laser light scattering (Malvern Master Sizer 3000, Malvern Instruments, UK). The refractive index used was 1.43. The oleosome cream was first dissolved in ultra-pure water at a 1:10 (weight to volume). An aliquot of the dissolved cream was added in the device, filled with ultra-pure water at pH 6.5. Each sample was measured in triplicate and expressed with differential particle size distributions.

2.7. Oleosome zeta-potential determination

Dynamic light scattering (DLS Zetasizer Nano ZS, Malvern Instruments Ltd, UK) was used to analyse the ζ-potential of the samples. The creams were diluted 1000 times (oil-base) with ultra-pure water. After the dilution, the pH of the dispersions was adjusted manually in a range of 3–9 with either a HCl (1.0 mol/L) or a NaOH (1.0 mol/L) solution. The refractive indices used were 1.43 for the dispersed phase and 1.33 for the continuous phase.

2.8. Microscopy

Light microscopy images were captured using a Zeiss Axioscope microscope (Carl Zeiss Micro Imaging, Inc., Thornwood, NY). The oleosome cream was first dissolved in ultra-pure water to 1:10 (weight to volume) which was then further diluted 1:100 (volume based).

2.9. Statistical analysis

A one way analysis of variance (ANOVA) and a LSD post-hoc significance test were applied to assess the differences among the extraction yields w.r.t. the extraction method and the recirculation steps. The analyses were performed with IBM SPSS statistics 23 software. Differences were considered to be significant at $p < 0.05$.

3. Results and discussion

3.1. Oleosome and protein extraction yields

Aqueous oleosome extraction differs from other known oil extraction procedures, such as dry-pressing (Matthäus, 2015), aqueous oil extraction (Campbell and Glatz, 2009) and aqueous enzymatic oil extraction (Mat Yusoff et al., 2015). To extract oil, oleosome disruption is necessary, which is achieved by employing intensive conditions, like dry-pressing, high temperature pre-treatments, and organic solvent extraction (De Moura et al., 2008; Dickey et al., 2008; Evon et al., 2007; Moreau et al., 2004). On the other hand, for the retrieval of intact oleosomes mild conditions (soaking blending, filtering) are used (De Chirico et al., 2018; Iwanaga et al., 2007; Nikiforidis and Kiosseoglou, 2009).

Understanding the nature and structure of oleosomes allows the selection of proper extraction conditions. Oleosomes are surrounded by a phospholipid monolayer and proteins, which equip them with characteristics similar to those of micron-sized protein particles (Maurer et al., 2013; Zielbauer et al., 2018). In example, the charge profile with pH changes follows the same s-shaped pattern like seed storage proteins (Adams et al., 2012; De Chirico et al., 2018; Nikiforidis and Kiosseoglou, 2009). Therefore, similar to proteins, oleosomes can be extracted in alkaline media or media with high ionic strength, where they are highly charged (−40 to −70 mV) and soluble (De Chirico et al., 2018; Nikiforidis and Kiosseoglou, 2009; Romero-Guzmán et al., 2020). In a previous study, we compared the oleosome extraction at alkaline pH with the extraction at neutral pH, with the presence of salt. Therefore, to validate the effect of the mechanical forces in the twin screw press, we used similar extraction media (Romero-Guzmán et al., 2020). More specifically, we used three different extraction solutions: (1) alkaline conditions at pH 9.5 with NaHCO$_3$ (0.1 mol/L), (2) neutral conditions with KCl (0.2 mol/L), and (3) neutral conditions using ultra-pure water. The two first extraction media (1) and (2) have been reported to solubilize oleosomes efficiently and enhance the extraction yield, relative to pure water (3), which in our previous study was used as a reference (Romero-Guzmán et al., 2020). The extraction yields are given in Fig. 2.

The oleosome extraction yields obtained by using blending at pH 9.5 (NaHCO$_3$ 0.1 mol/L) and at pH 7 (KCl 0.2 mol/L) were similar at ~64 wt %, which was attributed to the increases in solubilization due to the ionic environments created by pH or increased ionic strength (Nikiforidis and Kiosseoglou, 2009; Romero-Guzmán et al., 2020). The extraction yield using pure water was lower at 43 wt%. The low ionic strength of pure water did not affect the interactions of the oleosomes with the co-extracted material, leading to lower solubilization of oleosomes and therefore lower extraction yield (Romero-Guzmán et al., 2020).

In the case of twin-screw press extraction, the yields were less dependent on the extraction solution; as similar yields were obtained for all three media (~60 wt %). This suggested that the extraction in the twin-screw press is mechanistically different from the blender-cheese cloth extraction. It has been reported that using the twin-screw press already leads to efficient cell-lysis (Uitterhaegen and Evon, 2017). However, the sieve attached to the twin-screw press (~500 μm pore size) allows bigger particles to pass through compared to those formed with the blender knife and separated with the cheese-cloth (~150 μm pore size). Furthermore, to investigate the mass transfer of other components during the pressing step, the amount of extracted proteins was analysed as well (Fig. 3). It would be expected that the media extraction conditions (pH and ionic strength) can influence the solubilization and hence the extraction of storage proteins (Kramer et al., 2012). However, Fig. 3 shows that the mass transfer of the extraction is not affected by the media. The extraction of proteins is mostly mastered by the mechanical
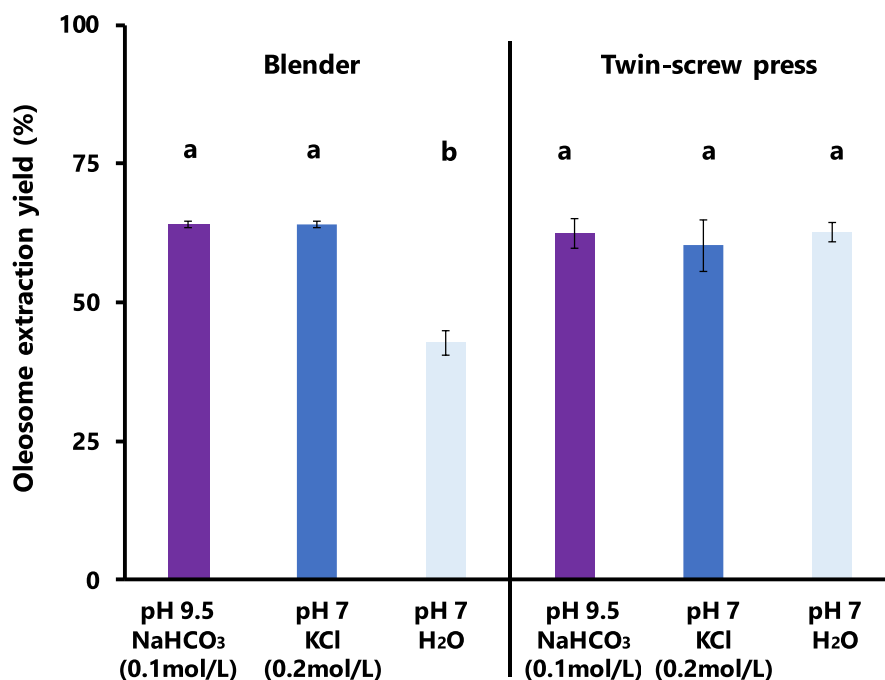
Fig. 2. Comparison of the yield (%) of oleosomes obtained from the extraction performed either with the twin-screw press of with the blender at either alkaline conditions (pH 9.5 NaHCO$_3$ 0.1 mol/L) or neutral conditions (pH 7 KCl 0.2 mol/L or H$_2$O). An ANOVA statistical analysis was performed with a $p < 0.05$.

forces in the twin-screw press.

The similar yields obtained for all the extraction media suggested that similar cell wall breakage (Rosenthal et al., 1998) and diffusion of cellular components (Passos et al., 2009) took place for all the twin-screw extractions. Probably due to the limited amount of aqueous media, the solubilization of the material was limited and the mechanical forces created in the screws controlled the release of cellular material. Therefore, using a twin-screw press mechanism can lead to significantly lower water and chemicals use. In Fig. 4 we present our hypothesis on the mechanisms of extraction of each of the examined devices.

The oleosome extracts from both extraction procedures are suitable to use as ingredients for emulsion systems. To achieve this, an

appropriate heat treatment (90 °C, 30 min), would be necessary to deactivate co-extracted endogenous enzymes such as lipase and lipoxygenase (Chen et al., 2012). Nevertheless, in order to analyse in depth the effect of the extraction methods on oleosome properties, the oleosomes where further isolated.

3.2. Effect of the extraction method on the recovered oleosomes

Intact oleosomes have excellent chemical stability and may well have specific nutritional properties, depending on their degree of integrity and the amount of proteins that are co-extracted (Nikiforidis et al., 2014). It was therefore important to assess the physical stability and properties of the oleosomes obtained with both processes. For this, the extracts obtained with the twin-screw press and the blender were diluted towards the same solid:solution ratio and centrifuged to concentrate the oleosomes.

3.2.1. Oleosome cream composition

Table 1 summarises the composition of the obtained oleosome creams, regarding oil, protein and moisture compositions.

The obtained creams had very similar compositions in both cases, indicating that the extraction process did not impact the composition of the final recovered oleosome-concentrated creams. Nevertheless, it is known that the extraction solution influences the composition; the use of pure water leads to more interactions between oleosomes and co-extracted proteins (Romero-Guzmán et al., 2020). While the mechanical pressure in the twin-screw press allowed a similar extraction yield by opening up the cells, it did not have an effect on the interactions between oleosomes and co-extracted material. This effect was undoubtedly still defined by the pH and ionic strength of the extraction media, which did not allow the breakage of ionic bonds nor hydrophobic interactions between oleosomes and the co-extracted proteins in their direct vicinity. This reinforces the idea that the main influence of the press forces is in the opening of the cells and release of the material, but the direct solubilization of the oleosomes was still lead by the aqueous media.
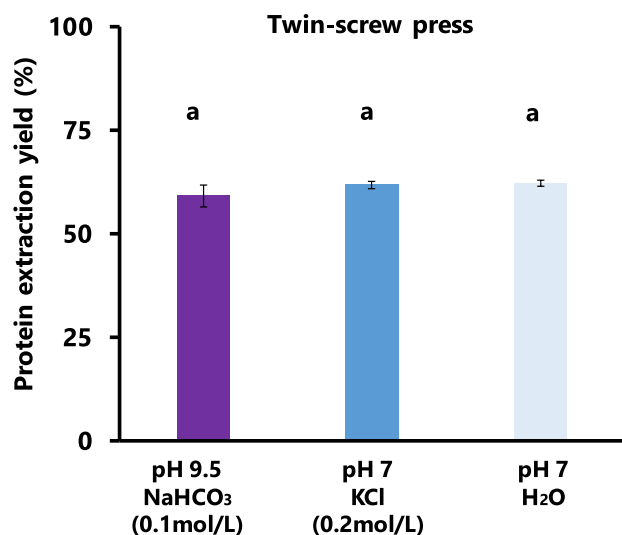

Fig. 3. Protein extraction yield (%) obtained with twin-screw press with each different extraction media either at alkaline (pH 9.5 NaHCO$_3$ 0.1 mol/L) or neutral conditions (pH 7 KCl 0.2 mol/L and H$_2$O). An ANOVA statistical analysis was performed with a $p < 0.05$.
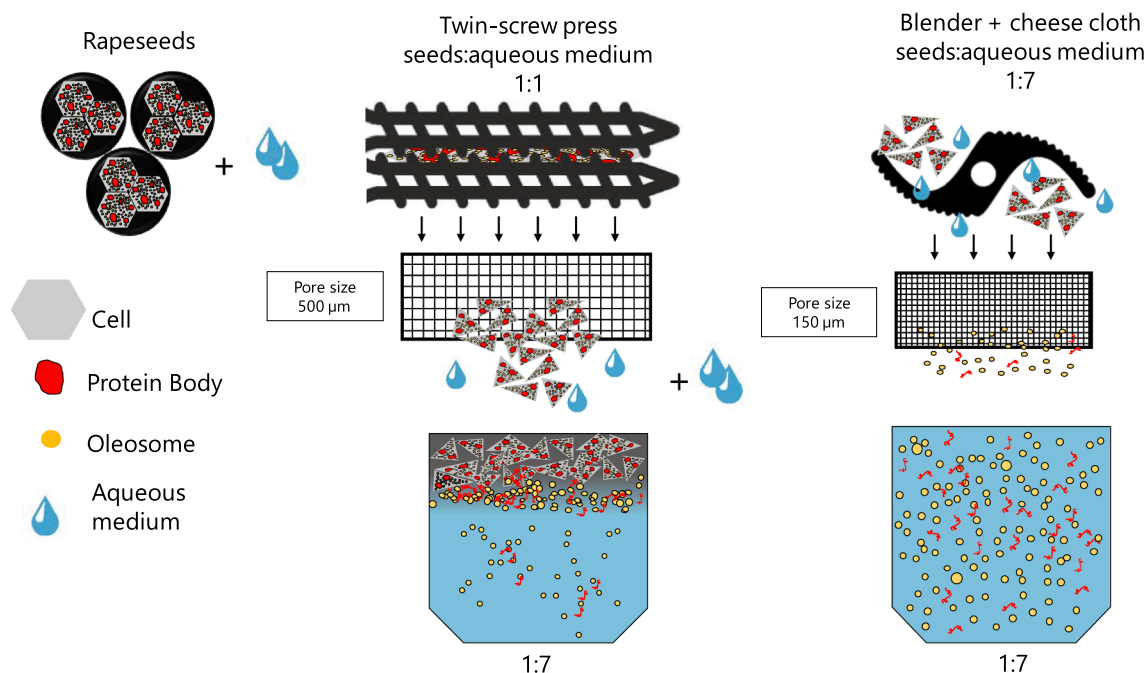
Fig. 4. Proposed mechanisms of extraction of oleosomes and proteins when using twin-screw press and blender extraction. A. During twin-screw press extraction, cell-lysis occurs and cellular material is released; however, due to the limited amount of aqueous media, the solubilization of the material is also limited. All material that passes through the sieve attached to the device (pore size ~500 μm) is mixed with additional aqueous medium. After this step the solubilization of the material is enhanced. B. During blender extraction the cells are disrupted inside the blender while due to the abundant aqueous medium (1:7 solid:water ratio), the solubilization of the material is happening parallel to the cell-lysis. Finally, mostly the soluble material passes through the cheese-cloth (pore size ~150 μm) and it is recovered.

Table 1
Composition of the creams obtained with either blender or twin-screw press at alkaline conditions (pH 9.5 NaHCO$_3$ 0.1 mol/L) or at neutral conditions (pH 7 KCl 0.2 mol/L or pH 7 H$_2$O).

Treatments		Oil (wt. %)	Protein (wt. %)	Water (wt. %)
Blender	pH 9.5 NaHCO$_3$ (0.1 mol/L)	70.6 ± 7.6[a]	3.6 ± 0.4[i]	25.8[x] ± 2.7[x]
	pH 7 KCl (0.2 mol/L)	62.6 ± 4.8[b]	3.8 ± 0.4[i]	30.2 ± 2.3[y]
	pH 7 H$_2$O	42.8 ± 2.8[c]	7.8 ± 1.2[ii]	29.4 ± 3.5[y]
Twin-screw press	pH 9.5 NaHCO$_3$ (0.1 mol/L)	71.3 ± 5.1[a]	4.5 ± 0.2[i]	26.2 ± 1.4[x]
	pH 7 KCl (0.2 mol/L)	68.1 ± 6.2[a]	5.2 ± 1.7[ii]	26.5 ± 2.3[x]
	pH 7 H$_2$O	45.0 ± 3.5[c]	8.5 ± 2.0[iii]	30.5 ± 3.3[y]

Values with different letters are significantly different with $p < 0.05$.

3.2.2. Effect of processing on oleosome protein interactions

The interfacial composition of the oleosomes was analysed in two ways. First, the proteins interacting with the oleosome interface were characterized with SDS Page; second, their zeta potential was measured.

The results of the SDS-page analyses are shown in Fig. 5. Extraction at alkaline conditions using the blender gave a single strong band at ~18 kDa, indicative of oleosin (Jolivet et al., 2011; Tzen, 2012), plus some minor bands around 9 kDa. The twin-screw press at the same alkaline conditions exhibited much more diverse proteins, quite similar to the patterns obtained at neutral conditions (both with and without KCl). For those extracts, there was evidence for the presence of both steroleosin and caleosin at 42 kDa and 27 kDa, respectively (Lin et al., 2002; Næsted et al., 2000). Under the reducing conditions during the gel analysis, the rapeseed storage proteins were monomerized. Consequently, cruciferin was monomerized from 250 kDa to 26–36 kDa and 18–21 kDa for the acidic and basic polypeptides, respectively (Zhao et al., 2016). Similarly, napin was reduced from 14 kDa to 4 kDa and
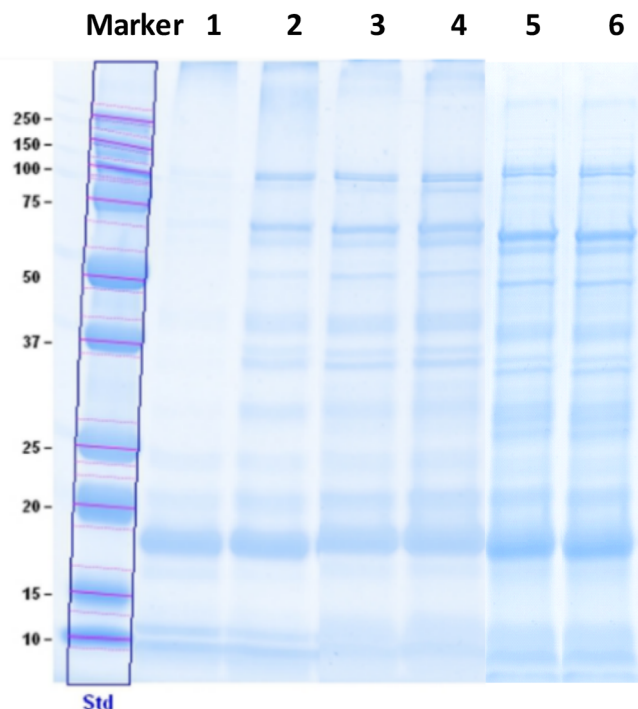

Fig. 5. SDS-PAGE of protein extracts from isolated oleosome creams extracted at pH 9.5 with NaHCO$_3$ (0.1 mol/L) using the blender (1) or the twin screw press (2), extracted at pH 7 with KCl (0.2 mol/L) using the blender (3) or the twin-screw press (4), and at pH 7 with H$_2$O using the blender (5) or the twin screw press (6).

9 kDa (Murphy and Cummins, 1989). Napin seemed to be more abundant than cruciferin at alkaline conditions, which could be due to its wider solubility at different pH in comparison to cruciferin (Perera et al., 2016).

We hypothesize that the additional proteins observed in the cream recovered with the twin-screw press using alkaline conditions (band 2) were co-extracted and entrapped by the oleosomes during the first centrifugation step. This could indicate that the step of adding aqueous media to the extracts after the twin-screw press was very short, thus, the co-extracted proteins were carried up with the oleosomes during centrifugation. On the contrary, in the blending process, the abundance of extraction media over a longer time prevented this protein recovery during centrifugation (Fig. 4). For the extractions at neutral conditions (Fig. 5 Bands 3–6), similar profiles were observed because the neutral conditions promote the interactions between the oleosomes and the proteins. Therefore, in these cases we did not observe differences between the twin-screw press and the blender extractions.

Despite the differences in the protein profile at alkaline conditions, the extraneous proteins that creamed with the oleosomes when using the twin-screw press did not affected the zeta potential (Fig. 6). Moreover, both extracts (twin-screw press and blender) with 0.1 mol/L NaHCO$_3$ showed a zero charge point of around pH 6.0 corresponding to the isoelectric point of the most abundant oleosome interfacial protein, oleosin (Tzen et al., 1993).

For the oleosomes extracted at neutral conditions with KCl (0.2 mol/L) or with H$_2$O, there was a clear shift to the left for all the recovered creams, irrespective of the extraction process (twin-screw press or blender). This shift can be attributed to the external material present at the oleosome interface, such as storage proteins or soluble polysaccharides coming from the mucilage of the rapeseed hulls (Eriksson et al., 1994), which can interact with the oleosome membrane (Tamayo Tenorio et al., 2017).

3.2.3. Oleosome size distribution

The physical stability of the oleosome creams was investigated by analysing the particle size distribution and by microscopy (Fig. 7).

The oleosomes extracted at pH 9.5 (Fig. 7A) were individual oil droplets with a d$_{3,2}$ of 0.59 μm and 0.76 μm. There was no discernible difference between the oleosomes recovered with the twin-screw press or the blender. The small shoulder at 3–10 μm is probably because of a slight association between some oleosomes. The oleosomes that were extracted at pH 7 with KCl (Fig. 7B) were extensively aggregated, leading to a d$_{3,2}$ of 11.9 μm and 15.4 μm. Once more, there was no difference between the oleosomes extracted with twin-screw press or blender. However, the microscopic analysis showed that the larger aggregates were composed of individual oleosomes with slightly bigger size than those obtained at pH 9.5. This is probably due to the effect of K$^+$ cations, which can slightly affect the coalesce rate of native

oleosomes when interacting with phospholipids at oleosomes' interface (Romero-Guzmán et al., 2020). For the case of extracts obtained with pure water, more aggregation was observed; up to the detection limit of the SLS system. This aggregation resulted in a larger water content of the cream obtained at neutral conditions (Table 1). Water is probably trapped within the aggregates due to a stronger network formation between co-extracted material and oleosomes (Nikiforidis and Scholten, 2015).

Our overall conclusion is therefore that the oleosomes remained intact under all extraction conditions, in spite of their being heavily aggregated when using neutral pH extraction media. It is of importance that many applications of oleosomes as emulsions will require attaining a minimum viscosity. Here, oleosome aggregation may be a positive aspect, as it will lead to higher viscosities with lower volume fractions of oleosomes (McClements, 2004). While this may not apply to each application, it is important that the extraction conditions may be adjusted to create the properties that are desired for specific applications.

3.3. Potential scalability of the oleosome extraction with a twin-screw press

In an attempt to achieve high extraction yields in the lab-scale twin-screw press, the obtained press cake was rehydrated (1:1) with pure water, and re-pressed through the twin-screw press. As a result, the overall oleosome yield reached 90 wt% ±2.4. This indicated that by increasing the mechanical forces, the extraction yield was significantly increased. Industrially, this could be achieved by the correct selection of length and gaps between the screws, which could lead to an increase in the residence time of the material and hence extraction time (Gautam and Choudhury, 1999). Moreover, the mild conditions used during this extraction, could favour the usage of the cake stream in products such as those in which other fibrous residues have succesfully been used (Lian et al., 2019; Montemayor-Mora et al., 2018).

Finally, we believe this technology could also be applied to other oilseeds and nuts. However, in order to address the effect on other oleosomes, specially due to their broad size range: 0.5–20 μm (Dave et al., 2019; Tzen and Huang, 1992) and since the mechanical forces seem to play an important role, and can be affected by the different composition of the oil-bearing material, further experiments and analysis are necessary.

4. Conclusion

Oleosome aqueous extraction was carried out by a twin-screw press at 1:1 solid to liquid ratio, and compared to blender extraction. The twin-screw extraction required six times less extraction media in comparison to the blender extraction. At alkaline conditions, the extraction
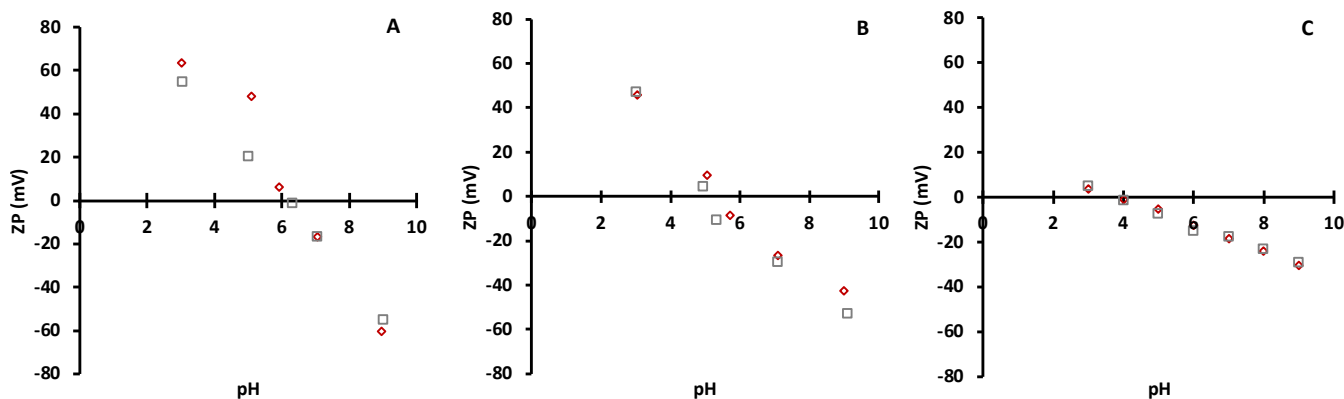

Fig. 6. Z-Potential of oleosome dispersions (1:100 wt/vol.) (A) extracted at pH 9.5 with NaHCO$_3$ (0.1 mol/L), (B) extracted at pH 7 with KCl (0.2 mol/L), and (C) extracted at pH 7 with H$_2$O with (◇) the blender or (□) the twin screw press in each case.
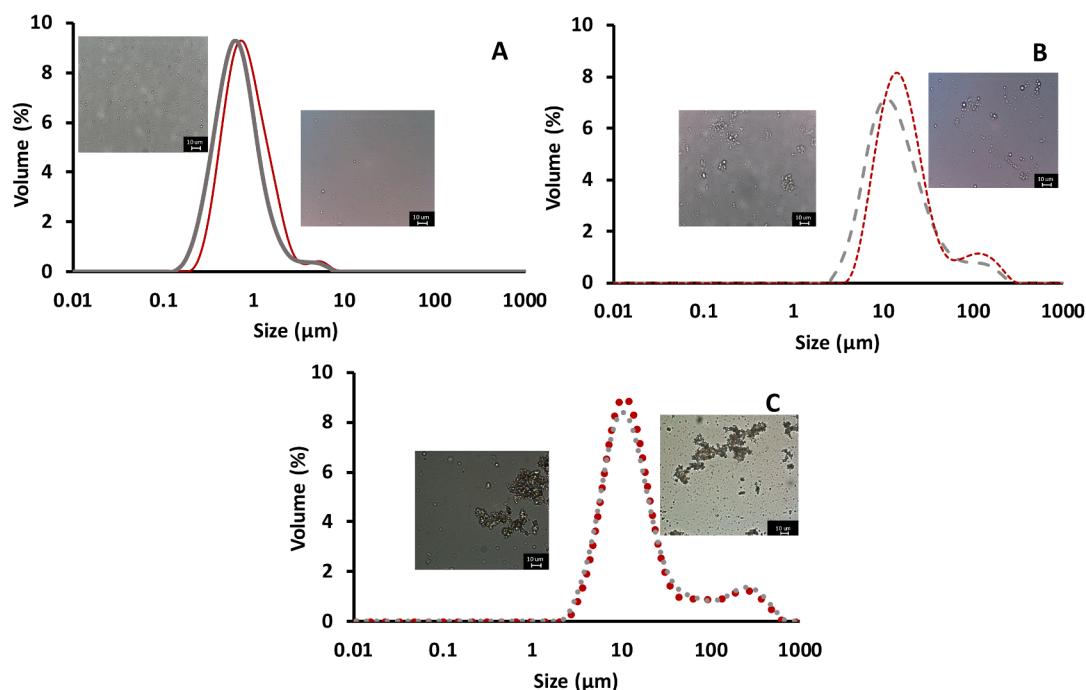
Fig. 7. Particle size distribution and corresponding microscopy images of washed oleosomes extracted (A) at pH 9.5 with NaHCO$_3$ (0.1 mol/L) using the blender (——) or the twin-screw press (——), (B) at pH 7 with KCl (0.2 mol/L) using the blender (- - -) or the twin-screw press (- - -) and (C) at pH 7 with H$_2$O using the blender ($\cdot\cdot\cdot$) or the twin-screw press ($\cdot\cdot\cdot$).

yields were similar with both investigated process steps, while, when using pure water as extraction media, significantly larger yields were obtained with the twin-screw press (43 wt % for blender vs 60 wt% with the twin-screw press). Additionally, there were no significant differences in extraction yield when different extraction media were used (0.1 mol/L NaHCO$_3$ at pH 9.5, 0.2 mol/L KCl, at pH 7, or H$_2$O) in the twin-screw press, showing that the mechanical forces in the twin screw press dominate the extraction dynamics and mechanism. The oleosome yield obtained with a single pass through the twin-screw, regardless the media was 60 wt % and it could be increased to 90 wt % with a second pass, indicating the potential of twin-screw press to increase the oleosome extraction yield.

The use of neutral conditions (with or without KCl) led to larger protein co-extraction and aggregation of the oleosomes. The oleosomes however remained intact, preserving their native characteristics. The mechanical forces during the twin-screw extraction were effective in opening the cells and release cellular material, but did not influence the solubilization of the oleosomes and most importantly, they did not disrupt the oleosomes.

The properties of the final oleosome suspension can be adjusted to the needs of their final application by adjusting the extraction conditions. Extraction at high pH gave a suspension of isolated oleosomes, while a neutral pH resulted in oleosome aggregates.

The twin-screw press is a unit operation that could also be used at industrial scales. The promising results generated in this work with a lab-scale twin-screw press could be used in order to further investigate the oleosome extraction mechanism in bigger scales and with different seeds.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Maria Juliana Romero Guzman: Conceived and designed the analysis, Collected the data, Contributed data or analysis tools, Performed the analysis, Wrote the paper, Louis Jung: Collected the data, Contributed data or analysis tools, Wrote the paper, Konstantina Kyriakopoulou: Contributed data or analysis tools, Performed the analysis, Wrote the paper, Remko Boom: Conceived and designed the analysis, Contributed data or analysis tools, Wrote the paper, Constantinos Nikiforidis: Conceived and designed the analysis, Contributed data or analysis tools, Wrote the paper, Other contribution Supervision.

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