



# Controlled oleosome extraction to produce a plant-based mayonnaise-like emulsion using solely rapeseed seeds

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## ABSTRACT

Oleosomes are oil storage structures in seeds, consisting of triglycerides surrounded by a protein-phospholipid mixed monolayer. They can be extracted aqueously together with other seed components such as proteins and soluble fibers. The co-extracted biomolecules can affect the properties of the extracts. Nevertheless, it is possible to control the electrostatic and hydrophobic interactions between these biomolecules and oleosomes by adjusting the extraction conditions. Hence, our aim was to adjust the extraction conditions in order to recover a natural emulsion with a specific functionality: a plant-based mayonnaise-like product, derived solely from rapeseed seeds. By varying the pH of extraction, the droplet size was customized and by increasing the number of purification steps, the right amount of co-extracted material was removed. A combination of these conditions shifted the rheological properties of the obtained natural emulsion to a range similar to the benchmark mayonnaises. This work shows that it is feasible to produce a plant-based mayonnaise with an oil content ranging from 61.7 g/100g to 72.0 g/100g through a simple and continuous oleosome extraction process by controlling the interactions between oleosomes and co-extracted material.

## 1. Introduction

Oleosomes (also known as oil bodies) are micron-sized (0.3–4.0  $\mu\text{m}$ ) lipid storage structures found abundantly in oleaginous seeds such as rapeseed seeds (Barre, Simplicien, Cassan, Benoist, & Rougé, 2018). These lipid structures also present in nuts in which sizes up to 20  $\mu\text{m}$  have been reported (Dave, Ye, & Singh, 2019). Oleosomes consist of a triglycerides (TAGs) core, surrounded by a mixed monolayer of phospholipids and proteins such as oleosin, caleosin and steroleosin (Jolivet et al., 2009; Purkrtova, Jolivet, Miquel, & Chardot, 2008; Shimada & Hara-Nishimura, 2010; Tzen, 2012). The arrangement of these proteins at oleosomes interface renders them hydrophilic, and therefore they can be extracted using water as extraction medium (Iwanaga et al., 2007; Nikiforidis & Kiosseoglou, 2009). Due to their structure, oleosomes can potentially replace any kind of manufactured oil droplets broadly used (Zielbauer et al., 2018). For example the use of intact oleosomes was already suggested for dressings, sauces, dips, beverages, and desserts (Nikiforidis, Matsakidou, & Kiosseoglou, 2014), and specially as emulsifiers (Karefyllakis, Van Der Goot, & Nikiforidis, 2019; Ishii et al., 2017; Maurer et al., 2013).

The extraction of oleosomes starts with soaking the seeds in aqueous medium followed by blending or pressing to disrupt the cell walls and release the cellular material (Romero-Guzmán, Jung, Kyriakopoulou, Boom, & Nikiforidis, 2020). This extraction yields an emulsion that still contains exogenous storage proteins and cell wall components such as fibers and soluble carbohydrates (i.e. pectin). These materials influence the overall properties of the emulsion, and in case a purified oleosome emulsion is needed, the extrinsic material can be removed by repeated centrifugal washing steps (De Chirico, di Bari, Foster, & Gray, 2018; Millichip et al., 1996; Nikiforidis & Scholten, 2015).

The type and amount of co-extracted materials depend on the extraction conditions, such as the pH and ionic strength. These parameters will determine the composition (Nikiforidis, Biliaderis, & Kiosseoglou, 2012; Romero-Guzmán, Petris, et al., 2020; White et al., 2008) and thus the rheological properties of the emulsion. Moreover, the pH and ionic strength at which the oleosomes are washed to remove the extrinsic material can also influence the single size of the oleosomes and the size of their aggregates (Romero-Guzmán, Petris, et al., 2020). Hence, those parameters can be used to adapt the properties of the natural emulsion towards a specific application, instead of adapting the properties by

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using additives.

Undoubtedly, the use of oleosomes eliminates the need of a high-energy consuming emulsification step and the need of emulsifiers. In addition, the proteins-phospholipids naturally-occurring interface protects the oil from oxidation, rendering the use of antioxidant agents unnecessary (Kapchie, Yao, Hauck, Wang, & Murphy, 2013; Wijesundera & Shen, 2014). Recently, it has been discovered one can adapt the properties of these natural emulsions by tuning their interactions with the co-extracted material (Romero-Guzmán, Petris, et al., 2020). Therefore, it was hypothesized that the extraction process could be tailored to customize the properties of the natural emulsion towards a specific application, for example, plant-based emulsion products, such as a mayonnaise.

The rheological properties of mayonnaise are very distinctive and important for the acceptance by consumers (McClements, 2004; Scholten, 2017). Mayonnaise shows a semisolid and viscoelastic behaviour, also known as plastic (McClements, 2004). Many emulsion characteristics such as droplet size and the interactions between droplets play an important role in its viscoelastic behaviour (Scholten, 2017). Therefore rheological characterization is a common quality control parameter of mayonnaise (Liu, Xu, & Guo, 2007).

Therefore, in this work, we investigate the feasibility of producing a plant-based mayonnaise-like product by better controlling the oleosome extraction process conditions. Followed by a comparison between the rheological behaviour of the obtained plant-based products with commercial benchmarks.

## 2. Materials and methods

### 2.1. Materials

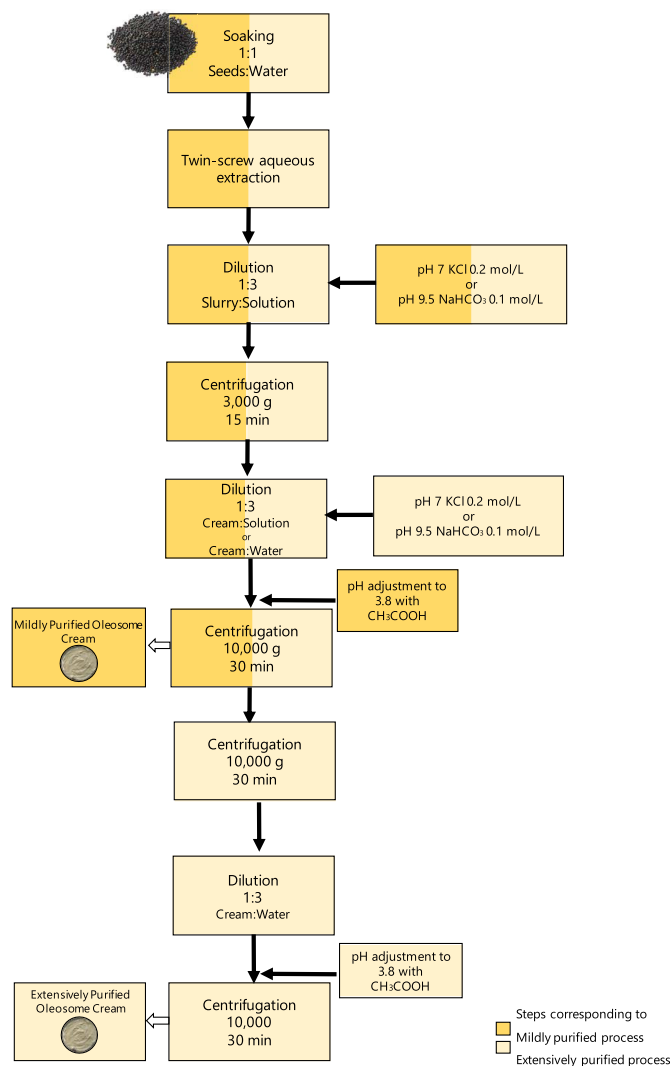
Rapeseed seeds (type Alizze) used in this study were kindly provided by a seed producer. The composition of the seeds was as follows: moisture  $9.0 \pm 1.2$  g/100 g, oil  $36.0 \pm 1.3$  g/100 g, and protein  $18.0 \pm 0.7$  g/100 g. Moreover, the seeds contained 13 mg/100g of glucosinolates and no erucic acid. Deionized water was obtained with a Milli-Q purification system (Merck Millipore, USA). KCl was purchased from Merck (Merck, Germany). Petroleum ether was obtained from Fisher Scientific (JT Baker Fisher Scientific, USA). All other chemicals were obtained in analytical grade from Sigma-Aldrich (St. Louis, MO, USA). The mayonnaises used as benchmark were: Hellmann's® with olive oil, AH Halvanaise® and Remia® Mayolijn, more information about them can be found in Appendix 1.

### 2.2. Extraction of oleosomes and recovery of oleosome-rich creams

All extractions were performed in batches of 100 g of seeds. Prior to the extraction, the seeds were soaked in a solution of either 0.1 mol/L  $\text{NaHCO}_3$  (pH adjusted to 9.5 with 1.0 mol/L NaOH) or 0.2 mol/L KCl (pH adjusted to 7 with 0.1 mol/L NaOH) for 16 h at 4 °C. The soaking conditions were obtained from De Chirico et al. (2018) and Romero-Guzmán, Jung et al., (2020). The mass ratio of seeds to the solution was 1:1. The soaked seeds were then processed with a lab-scale twin-screw press (Angel 7500 extractor), maintaining the seeds-to-solution ratio of 1:1 from the soaking step. Two streams were recovered: (1) the press cake, which was recovered at the end of the press and (2) a concentrated slurry (the first coarse extract) which was recovered along the device. For further oleosome recovery, three times more of the same solution (0.1 mol/L  $\text{NaHCO}_3$  solution at pH 9.5, or 0.2 mol/L KCl solution at pH 7) was added to the first extract. This diluted emulsion was then subjected to concentration and/or purification by centrifugation.

#### 2.2.1. Mild purification

The diluted first coarse extract was centrifuged at 3,000 g, 4 °C for 15 min. An oleosome rich cream layer was recovered and drained from the excess of media using filter paper. The collected oleosome cream



**Fig. 1.** Scheme for the recovery of concentrated oleosome creams either mildly purified (●) or extensively purified (○), extracted at pH 7 with KCl 0.2 mol/L or pH 9.5 with  $\text{NaHCO}_3$  0.1 mol/L.

was subsequently dispersed in water (1:3 w/v) and the pH was adjusted to 3.8 with  $\text{CH}_3\text{COOH}$  1.0 mol/L as the desired product was mayonnaise. A second centrifugation followed at 10,000 g, 4 °C for 30 min (Sorval Lynx 4000 Centrifuge, Thermo Scientific USA). The oleosome rich cream layer was drained again from the excess of media with the aid of a spoon and a filter paper. The obtained cream was collected and analysed for its composition and physical properties.

#### 2.2.2. Extensive purification

The cream obtained above was once more submitted to centrifugation for 30 min at 10,000 g and 4 °C. The cream was then drained from the excess of media using filter paper and subsequently dispersed in water at 1:3 w/v, the pH of this dispersion was also adjusted to 3.8 with  $\text{CH}_3\text{COOH}$  as this is the pH of mayonnaise and again centrifuged at 10,000 g, 4 °C for 30 min. The obtained cream was collected and analysed for its composition and physical properties. The scheme of the recovery of both mildly purified and extensively purified oleosome creams at either pH 7 with KCl 0.2 mol/L or pH 9.5  $\text{NaHCO}_3$  0.1 mol/L is shown in Fig. 1.

## 2.3. Characterization of the streams

### 2.3.1. Moisture content

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

### 2.3.2. Lipid content

The lipid content of dry samples was determined by Soxhlet extraction with petroleum ether (B-811 Buchi Extractor, Switzerland). All analyses were performed in duplicate.

### 2.3.3. Protein content

The protein content of dry and defatted samples was determined using the Dumas method. N<sub>2</sub> was quantified; a conversion factor of 5.5 (Lindeboom & Wanasundara, 2007) was used to convert it to protein. All analyses were performed in duplicate.

## 2.4. Protein profile characterization

The protein profile of the samples was analysed qualitatively by SDS polyacrylamide gel electrophoresis using a Bio-Rad MiniProtean cell (Bio-Rad Laboratories Inc., Hercules, USA). 2 types of buffers were used to unfold the proteins (Nikiforidis & Kiosseoglou, 2009). Buffer 1 consisted of Tris-HCl (50.0 mmol/L), urea (5.0 mol/L), 1 g/100 mL SDS and 4 g/100 mL 2-mercaptoethanol. Buffer 2 consisted of Tris-HCl (125.0 mmol/L), urea (5.0 mol/L), 1 g/100 mL SDS, 20 g/100 mL glycerol and g/100 mL 2-mercaptoethanol. The creams were dispersed in ultra-pure water (1:100 w/v), were 1:1 combined with buffer 1 and agitated for 15 min at room temperature. Each sample rested for 15 min, after which 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 to break the emulsion and to avoid oleosome associated proteins stayed interacting with the oil phase and hindering their diffusion through the gel. 20 µL of each sample was loaded on a 12% Tris-HCl SDS-ready gel, size range of 10–200 kDa; and 10 µL of Pre-Stained Protein Standard (Bio-Rad Laboratories Inc., Hercules, USA) were loaded. 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).

## 2.5. Zeta-potential determination

Dynamic light scattering (DLS Zetasizer Nano ZS, Malvern Instruments Ltd, UK) was used to determine the ζ-potential. The creams were dispersed 1000 times (oil-base) in ultra-pure water. After the dilution, the pH of the dispersions was adjusted manually to a value in the range of 3–9 with either HCl (1.0 mol/L) or NaOH (1.0 mol/L) solution. The refractive indices used were 1.47 for the dispersed phase and 1.33 for the continuous phase.

## 2.6. Particle size distribution determination

The particle size of the creams was measured with static laser light scattering (Malvern Master Sizer 3000, Malvern Instruments, UK). The refractive index used was 1.47. To perform the analysis the oleosome cream was first dispersed in ultra-pure water at a 1:10 (w/v). Each sample was measured in triplicate. The distributions were expressed in volumetric particle size distributions.

## 2.7. CLSM microscopy

The creams were diluted in ultra-pure water at 1:100 (v/v). 1 mL/100 mL of Coumarin-6 was added to the solutions. The samples were observed with a Carl-Zeiss LSM 200 (Zeiss, Germany) with a 100x oil lens and the 488 and 633 nm laser.

## 2.8. Rheological measurements

The rheological properties of the creams and benchmark mayonnaises were measured with an Anton Paar Rheometer MCR502 (Anton Paar, Austria). Every measurement was performed at 20 °C with a gap of 1.5 mm. To determine the viscoelastic behaviour of the mayonnaises, small deformation oscillatory measurements G' (storage modulus) and G'' (loss modulus) were performed. During this test, a constant deformation of 5% was used. The oscillation frequency was decreased from 20 to 0.5 Hz with logarithmic steps.

## 2.9. Statistic analysis

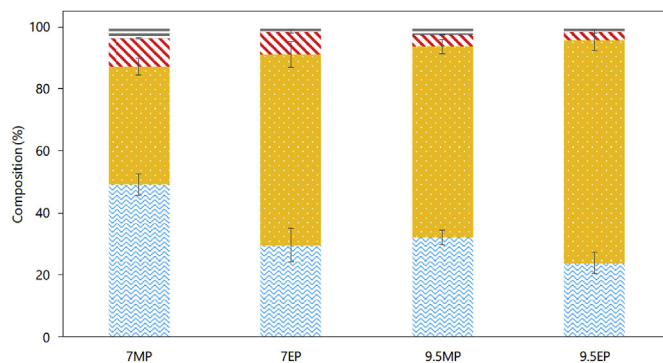
One-way analysis of variance (ANOVA) and LSD post-hoc significance test were applied to assess differences among the composition of the oleosome creams. Analyses were performed with the IBM SPSS statistics 23 software. Differences were significant when  $p < 0.05$ .

## 3. Results and discussion

### 3.1. Oleosome cream composition

The oleosomes were extracted with two different aqueous media: (1) at pH 9.5 NaHCO<sub>3</sub> 0.1 mol/L and (2) at pH 7 KCl 0.2 mol/L. Additionally, to obtain a final cream at the pH of a conventional mayonnaise (pH 3.8) the last centrifugation for all the treatments was performed at pH 3.8. The recovered creams were classified depending on the number of centrifugation cycles, as mildly purified (1 centrifugation cycle) or extensively purified creams (3 centrifugation cycles) as it is depicted in Fig. 1. Abbreviations were assigned for all the treatments as follows: 9.5MP (extracted at pH 9.5 NaHCO<sub>3</sub> 0.1 mol/L with mild purification), 9.5EP (extracted at pH 9.5 NaHCO<sub>3</sub> 0.1 mol/L with extensive purification), 7MP (extracted at pH 7 KCl 0.2 mol/L with mild purification), and 7EP (extracted at pH 7 KCl 0.2 mol/L with extensive purification). The compositions of the obtained creams are shown in Fig. 2.

The 7MP and 7EP creams were clearly richer in proteins and other components than 9.5MP and 9.5EP. The 7MP cream had the highest protein content (9.29 g/100 g), while 9.5EP cream had the lowest



**Fig. 2.** Composition of the oleosome creams obtained with KCl 0.2 mol/L at pH 7 mildly purified (7MP) and extensively purified (7EP) and with NaHCO<sub>3</sub> 0.1 mol/L at pH 9.5 mildly purified (9.5MP) and extensively purified (9.5EP). Each of the components is indicated as follows: Water (■), Oil (■), Protein (■) and Others (■).

protein content (2.64 g/100 g). In addition, the 9.5EP cream had the highest oil content (~72.03 g/100 g), while 7MP had the lowest oil content (~38.07 g/100 g). The material classified as “others” was composed by soluble carbohydrates and ashes (Eriksson, Westerlund, & Åman, 1994; Voragen, Coenen, Verhoef, & Schols, 2009). Among the soluble carbohydrates, pectin was expected to be one of the most abundant ones as it represents around 4–5 g/100g of the initial weight of rapeseed (Nacz & Shahidi, 1990). The content of these materials in the recovered creams was 3.56 g/100g and 2.39 g/100 g, for 7MP and 9.5MP creams; while 7EP and 9.5EP creams had 1.58 and 1.56 g/100 g, respectively. Overall, the creams processed at milder conditions contained higher amounts of co-extracted extrinsic material than their counterparts.

At alkaline conditions oleosomes interface is negatively charged (Tzen, Lie, & Huang, 1992) and hence, the repulsion between oleosomes and co-extracted materials is enhanced. This repulsion favours their separation, increasing the oleosome purity (De Chirico et al., 2018). On the contrary, the creams recovered at neutral conditions are recovered close to their isoelectric point and hence interacting with more co-extracted material. In our previous work, we reported that these co-extracted proteins interacted with the oleosome interface components via electrostatic or hydrophobic interactions (Romero-Guzmán, Petris, et al., 2020). However, the number of centrifugation cycles was effective in removing the co-extracted proteins, which could mean that the aqueous media at neutral pH (0.2 mol/L KCl) also affected these interactions in a lower degree which was observed just after several centrifugation steps.

Furthermore, a clear relation between the moisture content and co-extracted material was observed (Fig. 3). The water holding capacity of plant proteins is typically between 2 and 4 g/g; specifically, it has been reported for rapeseed protein concentrate a value of 3.4 g/g and 3.10 g/g for rapeseed protein isolate (Ohlson & Anjou, 1979). This physical characteristic is defined as the ability of a food structure to prevent water from being released from the three-dimensional structure of the protein (Zayas & Zayas, 1997). However, as no release of water was observed on the samples, the moisture content in the creams was also attributed to the presence of proteins and carbohydrates. These two components could also hinder the drainage of water from the cream, by creating a network in which water was entrapped, leading to an increase in the final water content (Chang et al., 2017).

### 3.2. Oleosome interfacial proteins and surface charge

The electrophoretic patterns of the 4 different creams are presented in Fig. 4. Besides the oleosome surface proteins, co-extracted storage proteins were present as well. As it has been previously reported (De Chirico et al., 2018) and can also be seen at the gels, the amount of co-extracted storage proteins recovered with oleosome creams depends on the pH of extraction and the extent of purification (Romero-Guzmán,

Jung, et al., 2020). At a pH closer to the isoelectric point of the proteins and oleosomes (IEP = 4–5), weak electrostatic repulsive forces are in place and oleosome-storage protein complexes are formed due to electrostatic and hydrophobic interactions (Romero-Guzmán, Petris, et al., 2020). The storage proteins are cruciferin (globulin) and napin (albumin) (Wanasundara, 2011). These proteins are in situ in structures named protein bodies and they represent around 70% w/w of the total amount of proteins present in the rapeseed (Perera, McIntosh, & Wanasundara, 2016).

Oleosome interfacial proteins are oleosins, caleosins and steroleosins. Oleosins accounts for up to 80% of the oleosome surface. The two other oleosome-bound proteins, steroleosin and caleosin could be expected at 42 kDa and 27 kDa, respectively, but cannot be distinguished due to the presence of other proteins (Lin, Tai, Peng, & Tzen, 2002; Næsted et al., 2000).

Regarding the effect of the different treatments, in case of the creams extracted at alkaline conditions (9.5MP and 9.5EP) (Fig. 4 bands 1 and 2), most of the proteins at the oleosomes interface are oleosins (~18 kDa). At neutral extraction conditions, the final protein composition strongly depends on the extent of purification (Fig. 4 bands 3 and 4). In the extensively purified cream 7EP (Fig. 4 band 4) mostly oleosins are observed (~18 kDa), while storage proteins can be observed in the mildly purified cream 7MP (Fig. 4 band 3). Interestingly, the steroleosin and caleosin bands are stronger in the mildly purified creams 9.5MP and 7MP (Fig. 4 bands 1 and 3), which suggests that also these mixed monolayer-bound proteins were partially removed during the purification process (Cao et al., 2015). This, could be explained due to the effect of cations on the oleosome mixed monolayer, which was previously studied (Romero-Guzmán, Petris, et al., 2020). From this work it was suggested that both monovalent and divalent cations influenced the stability of the oleosomes, probably related to some re-configurations of the proteins from their mixed monolayer.

The zeta-potential measurements (Fig. 5) show that both mildly purified creams have a zero charge point close to pH 4, which corresponds to an average between the IEP of most food proteins (IEP~5) and polysaccharides (IEP~3.5) such as pectin and other soluble carbohydrates. These soluble carbohydrates form complex coacervates at acidic pH, being a pH between 3.6 and 4.5 the optimum pH range for interactions pectin-proteins to occur (Flutto, 2004). This range of pH coincide with the pH at which the creams were lastly recovered, suggesting the presence of these coacervates in the mildly refined creams (7MP and 9.5MP). On the other hand, the extensively purified creams (7EP and 9.5EP) show a zero charge point very close to the one of native and pure rapeseed oleosomes (pH ~ 6) (De Chirico et al., 2018; Tzen, 1992), showing that mostly oleosins are present at their interface. This supports the earlier observation, which suggest that the extensive purification effectively removes the extraneous material adhering to the oleosome surface, independently of the pH of extraction.

### 3.3. Oleosome size distribution and microstructure

The oil droplet size distribution (Fig. 6) has a big impact on the rheological properties of the final product (McClements, 2004). Therefore, it was important to analyse the effect of the oleosome recovery process on oleosome size distribution.

The particle size of native oleosomes was expected to be below 2.0 µm (Tzen, Cao, Laurent, Ratnayake, & Huang, 1993; Ying et al., 2015). However, none of the creams showed this size distribution. The particle size distributions of the mildly refined creams (7MP and 9.5MP) ranged between 10 and 100 µm. In contrast, the extensively purified creams (7EP and 9.5EP) had a bimodal size distribution. One of the peaks correspond to pure oleosomes (~1 µm) and the other one to aggregates and or coalesced droplets (~10 µm) depending on the pH of extraction, as it is depicted by the CLSM micrographs.

The presence of extraneous material clearly influenced the aggregation behaviour of the oleosomes (Romero-Guzmán, Petris, et al.,

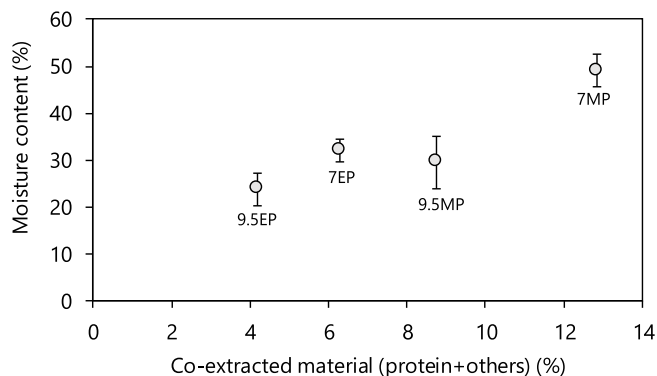
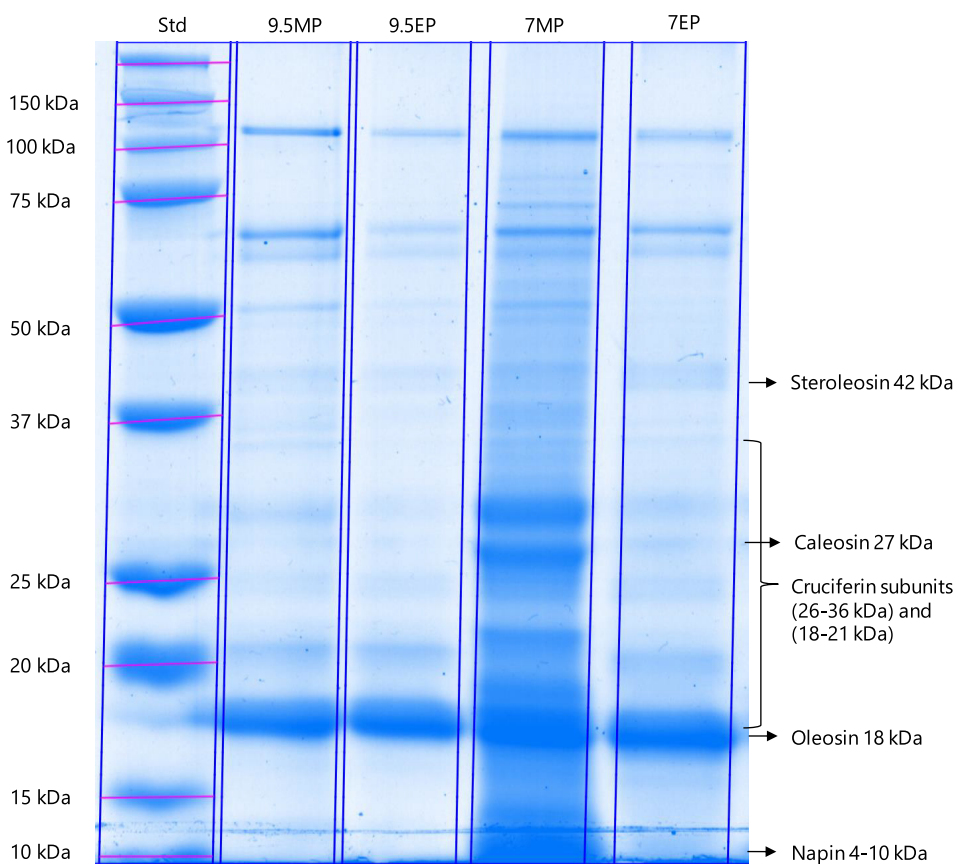
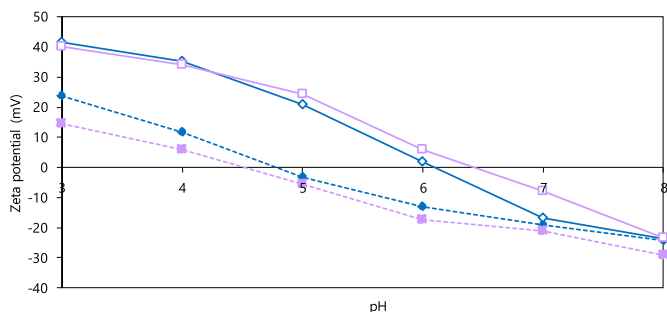


Fig. 3. Relation between the percentage of co-extracted material and the moisture content of the different oleosome creams.



**Fig. 4.** SDS-PAGE of the different oleosome creams obtained with  $\text{NaHCO}_3$  0.1 mol/L at pH 9.5 after a mild purification 9.5MP (1) or an extensive purification 9.5EP (2) and with KCl 0.2 mol/L at pH 7 after a mild purification 7MP (3) or an extensive purification 7EP (4).



**Fig. 5.** Dependence upon pH of the  $\zeta$ -potential of the oleosome creams obtained with KCl 0.2 mol/L at pH 7 extensively purified 7EP (○) and mildly purified 7MP (●) and with  $\text{NaHCO}_3$  0.1 mol/L at pH 9.5 extensively purified 9.5EP (□) and mildly purified 9.5MP (■).

2020); hence, both mildly purified samples had most of their oleosomes aggregated. Remarkably is that the amount of extraneous material (Fig. 2) was not relevant for their aggregation behaviour (Fig. 6). For example, the mildly purified cream extracted at pH 9.5 (9.5MP) has as much co-extracted material as the purified one extracted at pH 7 (7EP); however, these creams had a different aggregation behaviour. We hypothesized that big molecules were in charge of this aggregation; as they were easily removed by the purification steps (Geerts, Mienis, Nikiforidis, van der Padt, & van der Goot, 2017).

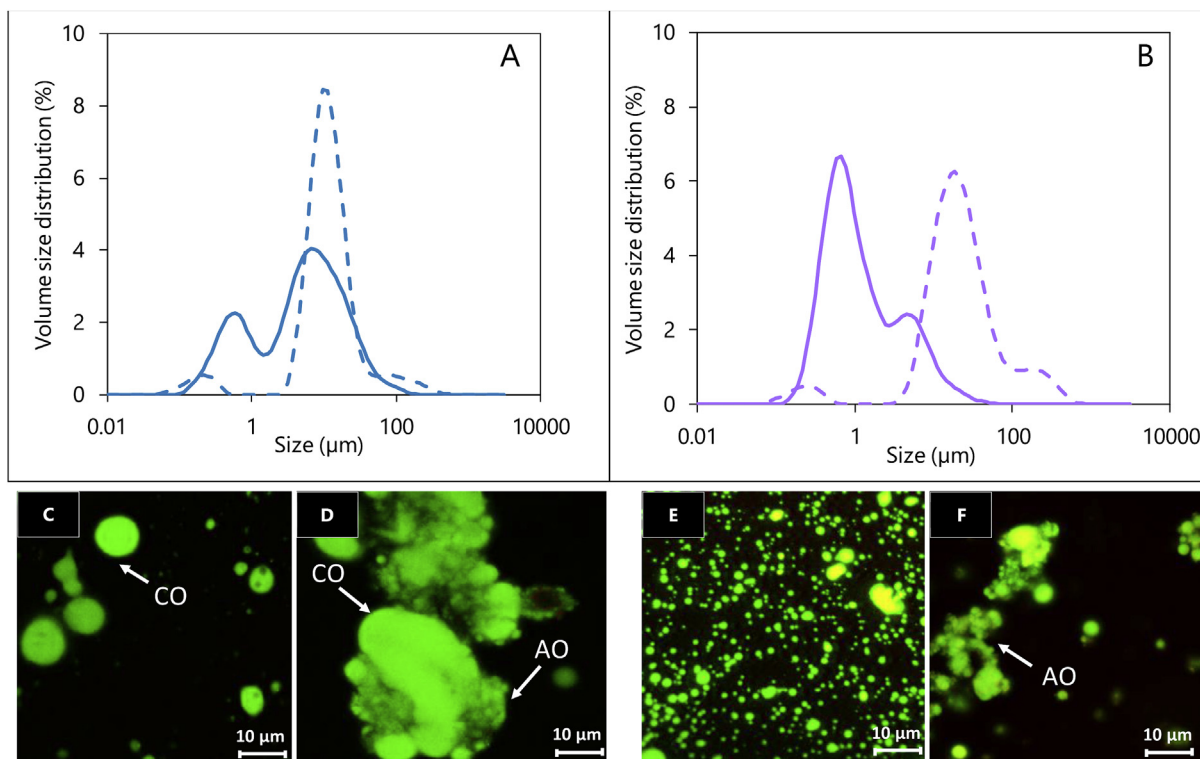
### 3.4. Rheological properties of oleosome cream

Finally, the rheological evaluation of the oleosome creams as potential vegan mayonnaise-like products was done by determining the storage modulus ( $G'$ ) and the loss modulus ( $G''$ ) of the obtained creams

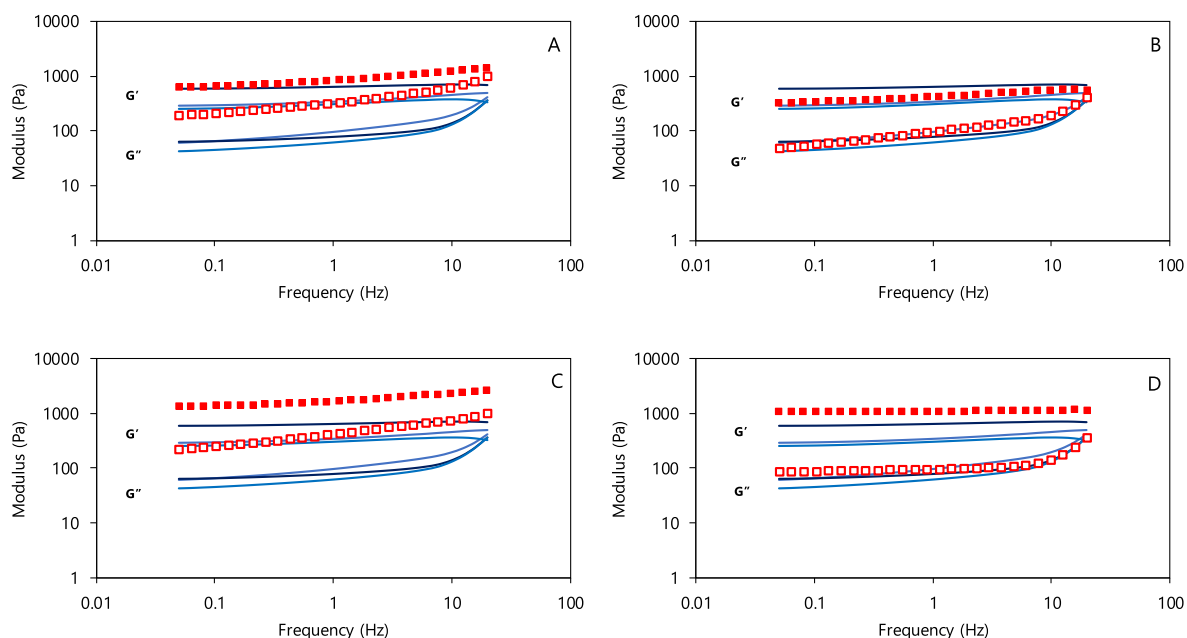
and compared them to 3 benchmark mayonnaises (Appendix 1) (Fig. 7).

All the obtained creams were viscoelastic systems as the  $G'$  of all the samples was greater than the  $G''$ , meaning that all the creams have a solid-like behaviour, as conventional mayonnaise (Harrison & Cunningham, 1985). The mildly purified samples show larger  $G'$  than the extensively purified creams, which indicates that the extraneous material increases the stiffness of the creams.  $G''$  is affected by impurities and the extraneous proteins which reinforce the network created by the closely packed dispersed phase (Nikiforidis et al., 2012). Moreover,  $G'$  indicates the strength of the network structure. The increase of  $G'$  in the mildly purified creams could be due to the presence of the previously mentioned coacervates. Their presence would explain the tighter structure and less deformability of the mildly purified creams (Muhoza, Xia, & Zhang, 2019).

The creams that achieved the rheological behaviour most similar to commercial mayonnaises were the extensively purified creams. Both creams resulted in the extraction of mostly individual oleosomes and with a low or almost absent amount of extraneous material. We believe their rheological behaviour was due to the close packing of the oleosomes that allows them to interact very strongly with one another forming a weak gel as in conventional mayonnaise (Depree & Savage, 2001). Specifically, the oleosome cream extensively purified and extracted at neutral conditions contained larger oil droplets than the extensively purified version extracted at alkaline pH (see Fig. 6). This increase in droplet size may reduce the  $G'$  which positively weakened the structure (Pal, 1996). In general, the extensively purified cream extracted at pH 7 had a perfect match of  $G'$  and  $G''$  with commercial mayonnaises.



**Fig. 6.** Particle size distribution of the oleosome creams obtained with (A) KCl 0.2 mol/L at pH 7 mildly purified 7MP (---) and extensively purified 7EP (—) and with (B) NaHCO<sub>3</sub> 0.1 mol/L at pH 9.5 mildly purified 9.5MP (---) and extensively purified 9.5EP (—). Confocal images of oleosome creams are: (C) 7EP, (D) 7MP, (E) 9.5EP and (F) 9.5MP. CO stands for Coalesced Oleosomes and AO stands for Aggregated Oleosomes. The used dye is Coumarin 6 and it is observed as green: oil. Scale bar 10 μm. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 7.** Storage modulus- $G'$  (■) and Loss modulus- $G''$  (□) of the creams obtained with KCl 0.2 mol/L at pH 7 mildly purified 7MP (A) and extensively purified 7EP (B) and with NaHCO<sub>3</sub> 0.1 mol/L at pH 9.5 mildly purified 9.5MP (C) and extensively purified 9.5EP (D). The blue lines represent 3 different benchmark mayonnaises. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

#### 4. Conclusion

We can conclude that it is possible to obtain a plant-based mayonnaise by controlling the conditions of the aqueous extraction process of oleosomes and co-extracted material. It is worth to mention that it is possible to customize the final application of the extracted emulsion by adjusting the extraction conditions. The pH of recovery and the extent of oleosome purification showed to have a relevant effect on the presence of co-extracted extraneous material, aggregation behaviour and droplet size distribution of the obtained creams. In one hand, the number of purification steps strongly influenced the amount of co-extracted material and hence the oleosome aggregation formation. While the pH of the extraction media also influenced the amount of co-extracted material but mostly it influenced the oil droplet size distribution. Regarding the evaluation of the rheological properties of the extracted creams, we showed that the aggregation of oleosomes had the biggest impact on the rheological properties. The aggregation of oleosomes lead to an increase of the G' of the recovered emulsions, which was explained as an effect of the coacervation of the soluble carbohydrates. The extensively purified creams, especially the one extracted at neutral conditions matched perfectly the rheological behaviour of commercial mayonnaises due to its particle size distribution and amount of co-extracted material.

Even though the results from this work are promising and may change the way in which emulsion-based products are manufactured, new challenges appear with this approach. One possible challenge is the consumer acceptability of the product. During the experiments, the obtained emulsion seemed to have similar organoleptic properties as compared to the commercial products. However, a complete sensorial evaluation is necessary to better understand if this is really the case, or if not how to work towards a satisfactory sensorial experience. Moreover, it is necessary to design an appropriate pasteurization process for the deactivation of enzymes and possible microbiological threats to ensure stability of the obtained-emulsion. Overall, we hope that the scientific community feels inspired to tackle these challenges and that the results presented in this work stimulate the development of more sustainable food production systems using solely plant-based ingredients.

#### CRediT authorship contribution statement

**María Juliana Romero-Guzmán:** Formal analysis, Writing - original draft. **Nienke Köllmann:** Formal analysis. **Lu Zhang:** Writing - original draft. **Remko M. Boom:** Writing - original draft. **Constantinos V. Nikiforidis:** Formal analysis, Writing - original draft.

#### Declaration of competing interest

The authors declare that there are no conflicts of interest.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2020.109120>.

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