Designing a sustainable oleosome aqueous extraction

-A new way to make emulsion-based foods-

María Juliana Romero Guzmán

Propositions

1. The efficiencies of extraction processes should be measured in total extracted functionality, not in total mass of extracted components (this thesis).
2. Retaining the natural structures in oilseeds is better than re-creating them (this thesis).
3. Scientific progress is stimulated by combining cultures (gs).
4. The emphasis on scientific reputation impedes scientific revolutions (gs).
5. The globalized society is threatened by its lack of humaneness (g).
6. Food tells more than language about a country's culture (g).

Propositions belonging to the thesis, entitled

Designing a sustainable oleosome aqueous extraction

-A new way to make emulsion-based foods-

María Juliana Romero Guzmán

Designing a sustainable oleosome aqueous extraction

-A new way to make emulsion-based foods-

María Juliana Romero Guzmán

Thesis Committee

Promotor
Prof. Dr R. M. Boom
Professor of Food Process Engineering
Wageningen University & Research

Co-promotor
Dr C.V. Nikiforidis
Assistant professor, Biobased Chemistry and Technology
Wageningen University & Research

Other members
Prof. Dr Vincenzo Fogliano, Wageningen University & Research
Prof. Dr Jasper van der Gucht, Wageningen University & Research
Prof. Dr Valeria Garmin, TU Delft, Delft
Dr. Michiel Meeuse, Unilever, Wageningen

This research was conducted under the auspices of Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences).

Designing a sustainable oleosome aqueous extraction

-A new way to make emulsion-based foods-

María Juliana Romero Guzmán

Thesis

submitted in fulfilment of the requirement for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Thursday 9th of April 2020
at 11 a.m. in the Aula.

María Juliana Romero Guzmán

Designing a sustainable oleosome aqueous extraction
A new way to make emulsion-based foods
183 pages

PhD thesis Wageningen University, Wageningen, the Netherlands (2020)
With references, with summary in English

DOI: https://doi.org/10.18174/510424
ISBN: 978-94-6395-265-1

Table of contents

1

Introduction

1.1 The food industry within the food chain

Food is an essential part of human life and its supply is closely entwined with our social arrangements and our interaction with the environment. Fig. 1.1 depicts the current food chain in a simplified way. While agriculture has been tremendously successful in producing sufficient food for the 7.5 billion people living at this moment, it also accounts for around 30% of the global energy consumption and 92% of the human water footprint [1]. Moreover, the way in which we are using these agricultural resources is not always efficient, like when creating food products from them [2]. This misuse of resources is relevant, especially since the assurance of food supply for the 9.5 billion people expected around 2050 is not given. As if this would not be enough, this is occurring in a time in which it is expected that climate change will have a negative impact on agricultural productivity. Therefore in line with the SDGs (Sustainable Development Goals) proposed by several United Nations Members [3], it is important to not just provide production systems for foods that are safe and tasty with a prolonged shelf-life, but also to make more efficient use of the crops while implementing greener processes [4].

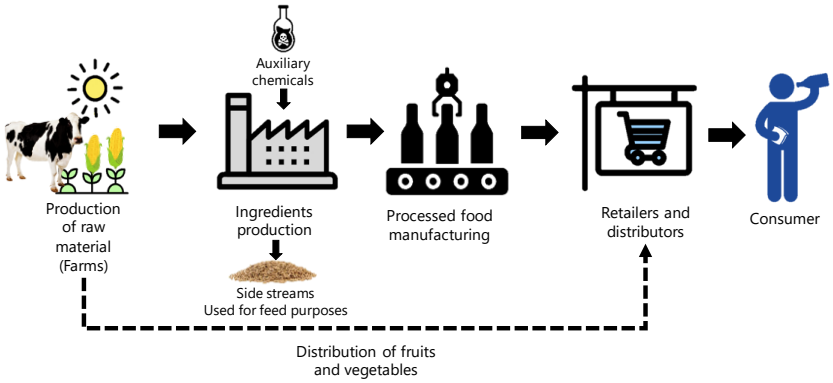

Fig. 1.1 An overview of the food chain from raw material to consumer.

Next to the distribution of fresh products (i.e. fruits and vegetables), industrialized food processing to produce processed food (i.e. bread, canned soups, meat-replacers) can be roughly divided into two steps (Fig. 1.1). The first step is the production of ingredients and the second is the assembly of these ingredients into final products [2]. In the first step, the raw materials are refined into basic building blocks such as oil, protein, flour and starch [5]–[7]. The processes required to derive these refined ingredients often include high temperatures, extreme pH values and auxiliary chemicals such as hexane[8], [9]. This happens because many of these processes are primarily designed to extract from seeds and grains as much as possible of one major component, and do not take into account adverse effects on the other valuable biomass components [10]. Moreover, most isolation processes are not just optimized for high yields, but also for high purity of the produced ingredient(s), often at serious cost of resources and leading to the production of toxic side streams.

The reason behind this purity and refinement of ingredients comes hand in hand with one of the aims of the food industry, which is to manufacture products with constant quality [11]. However, it is noteworthy that during the second stage of the production of food products, these refined ingredients are re-combined with components that oftentimes were also present in the raw materials from which they were derived. Examples of these mixtures are: fat and sugar to make chocolate, starch and protein to make soups, or water and oil, which are mixed to create one of the most common structures in food products, emulsions.

1.2 Food Emulsions

A wide range of food products such as yogurt, cheese, spreads, mayonnaise, chocolate and ice-cream are examples of emulsions [11]. The creation of a

finely dispersed suspension of droplets of one phase into the other, either as oil-in-water or as water-in-oil emulsions, requires stabilization of the instable interface between the two phases [12]. This can be done by incorporation of stabilizing emulsifiers with amphiphilic properties [13]. Due to their nature, emulsifiers are adsorbed at the interface between oil and water and depending on their classification, their presence delays/hinders the destabilization of the formed droplets. In Fig. 1.2 a representation of an oil droplet stabilized by some food-grade emulsifiers [14], [15] is shown.

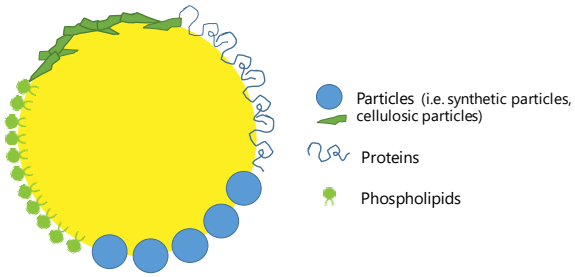

Fig. 1.2 Oil droplet and examples of food-grade emulsifiers.

The microstructure of the emulsion and the type of emulsifier are essential for emulsion stability and emulsion formation [11][16]. In addition, the emulsifier and interface are important for the chemical stability of the emulsion [17], [18]. There is however an increasing scepticism against these additives amongst consumers and in that light it is interesting to note that vegetable oils in their original biological matrix of the oil-bearing seeds, are contained inside structures that provide good protection against physical and chemical stresses [19][20]. These structures are named oleosomes and are the main focus of this thesis.

1.3 Oleosomes oil storage organelles: a natural emulsion

Oil from oil-bearing seeds is present within structures named oleosomes or oil bodies (Fig. 1.3). Their physiological role is the storage and protection of metabolic energy, this means that the fatty acids stored in their core needs to stay inert until the seed requires them (i.e. during germination) [21]. Hence, oleosomes are naturally stabilized against physical and chemical stresses [19], [22]. Their interface protects their lipid core with a monolayer of phospholipids to which proteins such as oleosin, caleosin and steroleosin are embedded [22]–[24]. These natural structures strongly resemble manufactured emulsions and due to the presence of these proteins, the oleosome surface is partly hydrophilic [25]. Therefore oleosomes can be extracted using water as extraction medium [26], [27]. This extraction yields oleosomes dispersed in water and depending on the processing conditions other components such as proteins interact with them at their interface [28], [29].

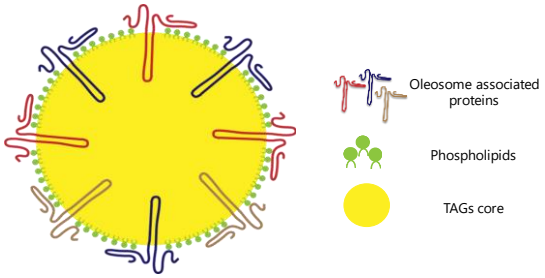

Fig. 1.3 Oleosome structure and its components

1.4 Conventional extraction of vegetable oil

The conventional oil extraction for refined oil production (Fig. 1.4) aims to destroy the oleosomes by either dry-pressing them with mechanical forces (e.g. using a single-screw press), or by combining this mechanical pressing

with a solvent (i.e. hexane) extraction. This extraction process is long, consists of many harsh steps and it needs to be followed by extensive refinement to yield a food-grade oil, while producing a big side stream (cake) with low functionality [7].

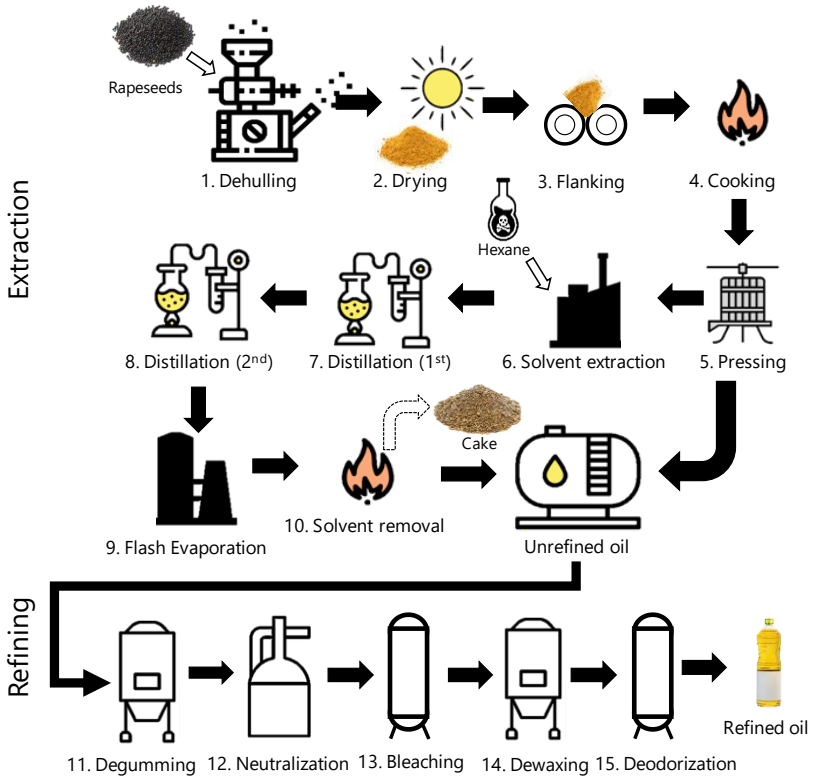

Fig. 1.4 Conventional extraction of refined oil

1.5 Oleosome aqueous extraction

The extraction of oleosomes is simpler and consists of 4 steps (Fig. 1.5): (1) soaking of the seeds, (2) cell-lysis, (3) solid-liquid separation and (4) liquid-liquid separation. The soaking step aims to soften the seed components to ease the release of material. The breakage of the cell walls is conventionally preformed in a blender and it aims to break the cell walls and release all their cellular content. The solid-liquid separation encompasses the separation of

non-soluble and soluble components, yielding an opaque dispersion which contains oleosomes, proteins, some soluble carbohydrates and very fine pieces of insoluble ones. Finally, the liquid-liquid separation is performed by centrifugation and is used to recover a concentrated cream rich in oleosomes [30]–[32].

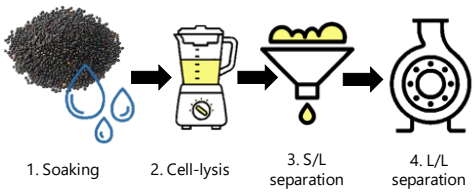

Fig. 1.5 Oleosome aqueous extraction

Differing from the manufactured emulsions, the size of the oleosomes and the type of materials situated at the interface can be customized depending on the type of seed used during the extraction [33] and the extraction conditions used during their recovery [27], [34]. Moreover, the extraction conditions also determine the yield of extraction of these structures [35].

1.6 New approach, new challenges

This aqueous extraction yields a natural oil-in-water emulsion, without needing a solvent extraction (i.e. hexane) and without energy-intensive emulsification steps such as high-pressure homogenization, as it is not necessary to re-create an emulsion from the extracted oil [36].

Since the natural oleosomes are similar to the emulsion-based foods that we produce from the refined oils, the current route from raw seed materials to assembled food is not completely logical. In the first stage of food processing, energy and resources are used for disrupting the native structures into purified oils as previously shown in Fig. 1.4; and in the second stage of food assembly,

energy and resources are again necessary to the assembly of the oil into an emulsion that is very similar to its original state inside the seeds (Fig. 1.6).

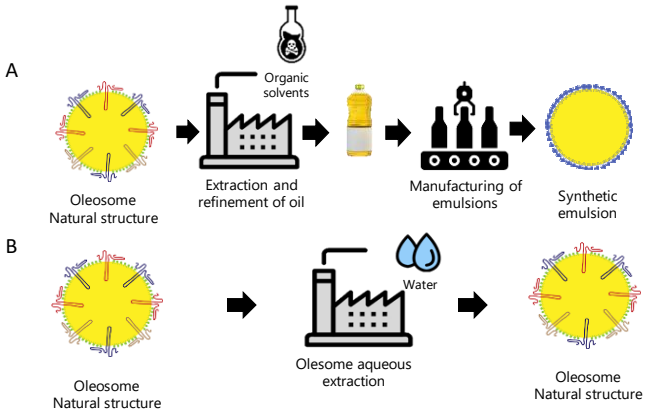

Fig. 1.6 Current production of emulsion-based food products (A), and proposed route using aqueous extraction of whole oleosomes (oil bodies) (B).

The use of intact oleosomes was already suggested for dressings, sauces, dips, beverages, and as carriers of hydrophobic compounds (i.e. flavours) [35]–[38]. Using these natural emulsions would eliminate the need for the energy intensive emulsification step [39], but would also make the addition of surfactants and stabilizers unnecessary as the natural phospholipid-protein monolayer has a similar function against physical and chemical destabilization [22], [26], [40], [41].

Even though oleosome extraction has clear advantages, it also has some drawbacks. Especially as this extraction process has been based on a lab-scale protocol to isolate pure oleosomes [19]. Therefore, its feasibility seems limited (i.e. production of expensive goods such as cosmetics). Below, we explained in more detail the challenges that oleosome aqueous extraction faces.

The oleosome extraction is generally performed at alkaline conditions [27], [42], [43], since at this pH the oleosome-associated proteins are negatively

charged, which increases their solubility and release [26], [27]. However, these pH conditions intensify the process as more auxiliary chemicals are used. Moreover, for some seeds these conditions also hinder the co-extraction of other components in their native state (e.g. proteins), favouring the covalent interactions between phenolic compounds and proteins [44], reducing their functionality. This trade-off to increase the yield is neither that positive as the achieved oil extraction yields at these conditions are still not as high as those obtained via solvent extraction [8], [27]. In addition, the soaking step lasts up to 24 h, which makes the process time consuming [42]. Moreover, oleosome aqueous extraction requires copious quantities of water [45], [46], to reduce the damage of oleosomes during their extraction. This necessity of large volumes of water in combination with the use of a technology employed for the extraction at lab-scale makes the up-scaling of the extraction process difficult [47]. Overall, all these factors limit the feasibility of the process and hence its real application at the industrial scale.

Given the drawbacks of the current aqueous extraction process, it is important to further study the phenomena occurring during aqueous extraction, to obtain further insight towards an aqueous extraction route that would not be limited by the aspects discussed above.

1.7 Aim and outline of this thesis

The overall aim of this thesis is to design a sustainable and feasible process for oleosome aqueous extraction, that yields emulsions that are in principle suitable to use in consumer foods. To achieve this, it was chosen to (1) identify extraction process conditions that allow efficient use of resources and industrial application, and (2) relate those process conditions to the extraction yield of oleosomes and to their properties. The results are then compiled into

guidelines for designing the combination of extraction process and resulting emulsion, since the extracted emulsions should be stable and suitable for application into emulsion-based food products. Fig. 1.7 summarizes the challenges of the oleosome aqueous extraction and links them with the chapters of this thesis, where these challenges were addressed.

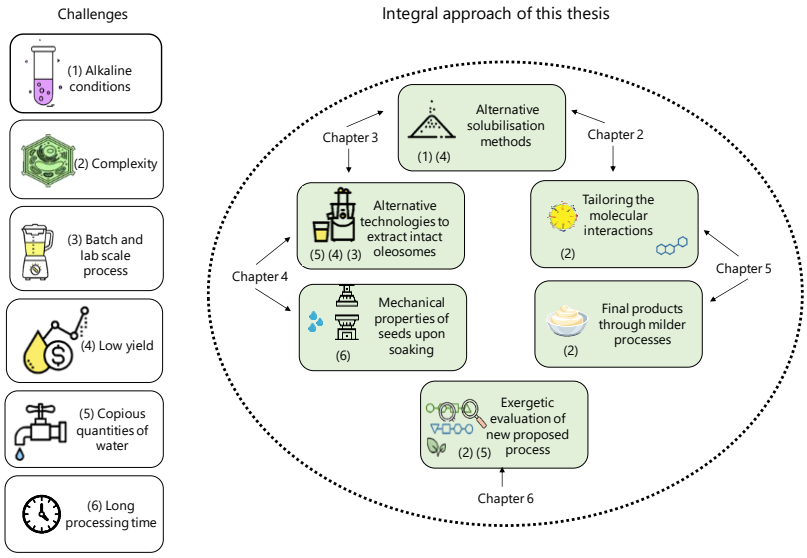

Fig. 1.7 Challenges of the current oleosome extraction process and the integral approach of this thesis, indicating where those challenges were addressed.

The challenges that were previously discussed are: (1) the use of alkaline conditions to achieve high extraction yields, (2) the lack of information about the interactions between oleosomes and the other molecules and structures during the extraction, (3) the limited knowledge about technologies that can be used during the extraction and that could recover intact oleosomes, (4) the trade-off between harsh conditions and low yields, (5) the big quantities of water required during the extraction process and finally (6) the long processing times required for the extraction. These challenges were addressed in the different chapters of this thesis as described below.

Chapter 2, is aimed at the first challenge, by avoiding the alkaline conditions typically used during oleosome extraction. As it was previously mentioned the oleosome interface is composed of proteins and phospholipids; however, many investigations suggest that the amphiphilic side of these interfacial proteins is exposed to the aqueous phase. Therefore, in this chapter it was hypothesized that oleosomes follow the rules of protein extraction. Thus, as an alternative to pH modification, the effect of different cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) was evaluated on the extraction yield and on the stability of the extracted oleosomes. All the cations improved the solubilization of oleosomes in comparison with pure water, but K^+ succeeded to extract the same amount of oleosomes than alkaline conditions. Additionally, the effect of these ions on the oleosomes interface also helped us to get more insights into the interactions of oleosomes and co-extracted material, which addressed also the second challenge. The effect of ions showed that the interactions between oleosomes and between oleosomes and other co-extracted material are not only electrostatic interactions but also hydrophobic.

The third and the fifth challenges corresponding to the limited knowledge about technologies that could extract intact oleosomes and the large quantities of water required by the process were addressed by studying a new technology to perform the cell-lysis. In **chapter 3,** we compare the performance of the twin-screw press technology with the existing batch-wise process. When using a continuous twin-screw press, the oleosome extraction yields were similar to those obtained with the blender. However, differing from the blender extraction, these yields were unaffected by the aqueous media as water and alkaline media resulted in similar extraction yields. Finally, when using this technology, it was easy to increase the extraction yield up 90 wt.% while

reducing the water requirements seven-fold (i.e. from 1:7 to 1:1), addressing the third, the forth and the fifth challenge.

C**hapter 4**, is aimed at the sixth challenge, and investigated the effect of the soaking time on the mechanical properties of the swollen seeds, which in turn has major influence on the oleosome extraction. Based on the results, a shorter soaking time was proposed achieving similar oleosome extraction yields and without affecting the stability of the obtained oleosomes.

The process configuration and conditions of the extraction process influence the composition and properties of the final emulsion. This is investigated in **chapter 5**, in which emulsions were extracted with different rheological properties and compositions by only changing the extraction conditions.

In **chapter 6** the resource use of the process of oleosome extraction is compared to that in the conventional process of oil refining, using exergy analysis. While the conventional process is more efficient in its use of physical exergy (energy), the inherent degradation of the proteins in the press cake after solvent extraction, results in a poor efficiency with respect to chemical exergy (use of materials), which is much larger than the penalty on physical exergy. Therefore, oleosome extraction shows to be more efficient in its use of resources than the conventional process.

Finally, in **chapter 7** the learnings from the previous chapters are summarized and overall conclusions are discussed, relating back to the original aim and approach. An outlook is given towards the feasibility and industrial potential of oleosome aqueous extraction in relation to emulsion product quality. Aspects that should receive further research are indicated as well.

References

[1] N. Alexandratos. and J. Bruinsma, "World agriculture towards 2030/2050: the 2012 revision Global Perspective Studies Team FAO Agricultural Development Economics Division," *Agric. Dev. Econ. Food Agric. Organ. United Nations Viale delle Terme di Caracalla 00153 Rome, Italy*, 2012.

[2] A. J. Van Der Goot *et al.*, "Concepts for further sustainable production of foods," *J. Food Eng.* 2016.

[3] R. J. McInnes, "Sustainable development goals," in *The Wetland Book: I: Structure and Function, Management, and Methods*. 2018.

[4] J. I. Boye and Y. Arcand, "Current Trends in Green Technologies in Food Production and Processing," *Food Engineering Reviews*. 2013.

[5] W. F. Breuninger, K. Piyachomkwan, and K. Sriroth, "Tapioca/Cassava Starch: Production and Use," in *Starch*. 2009.

[6] A. Akbari and J. Wu, "An integrated method of isolating napin and cruciferin from defatted canola meal," *LWT - Food Sci. Technol.* 2015.

[7] E. G. Hammond, L. A. Johnson, C. Su, T. Wang, and P. J. White, "Soybean Oil," in *Bailey's Industrial Oil and Fat Products*, 6th edition., edited by F. Shahidi, John Wiley & Sons, New York. 2005.

[8] U. Thiyam-Hollaender, N. Eskin, and N. Michael, "*Canola and rapeseed: production, processing, food quality, and nutrition*," CRC Press, Boca Raton, Florida, USA. 2012.

[9] A. Rivera del Rio, M. Opazo-Navarrete, Y. Cepero-Betancourt, G. Tabilo-Munizaga, R. M. Boom, and A. E. M. Janssen, "Heat-induced changes in microstructure of spray-dried plant protein isolates and its implications on in vitro gastric digestion," *LWT*. 2019.

[10] A. Lomascolo, E. Uzan-Boukhris, J. C. Sigoillot, and F. Fine, "Rapeseed and sunflower meal: A review on biotechnology status and challenges," *Appl. Microbiol. Biotechnol.* 2012.

[11] D. J. McClements, "Critical review of techniques and methodologies for characterization of emulsion stability.," *Crit. Rev. Food Sci. Nutr.* 2007.

[12] P. Wierenga *et al.*, "Food emulsions," *Trends Food Sci. Technol.* 2017.

[13] E. Scholten, T. Moschakis, and C. G. Biliaderis, "Biopolymer composites for

engineering food structures to control product functionality," *Food Structure*. 2014.

[14] A. Tamayo Tenorio, J. Gieteling, C. V. Nikiforidis, R. M. Boom, and A. J. van der Goot, "Interfacial properties of green leaf cellulosic particles," *Food Hydrocoll.* 2017.

[15] D. J. McClements and S. M. Jafari, "Improving emulsion formation, stability and performance using mixed emulsifiers: A review," *Advances in Colloid and Interface Science*. 2018.

[16] D. J. McClements, *Food Emulsions: Principles, Practices, and Techniques, Second Edition*. CRC Press, Boca Raton, Florida, USA. 2004.

[17] C. C. Berton-Carabin, M. H. Ropers, and C. Genot, "Lipid Oxidation in Oil-in-Water Emulsions: Involvement of the Interfacial Layer," *Compr. Rev. Food Sci. Food Saf.* 2014.

[18] A. Schröder, C. Berton-Carabin, P. Venema, and L. Cornacchia, "Interfacial properties of whey protein and whey protein hydrolysates and their influence on O/W emulsion stability," *Food Hydrocoll.* 2017.

[19] J. T. Tzen and A. H. Huang, "Surface structure and properties of plant seed oil bodies.," *J. Cell Biol.* 1992.

[20] E. Dickinson, "Food colloids... How do interactions of ingredients control structure, stability and rheology?," *Current Opinion in Colloid and Interface Science*. 2007.

[21] F. Beisson, N. Ferté, S. Bruley, R. Voultoury, R. Verger, and V. Arondel, "Oil-bodies as substrates for lipolytic enzymes," *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids*. 2001.

[22] Z. Purkrtova, P. Jolivet, M. Miquel, and T. Chardot, "Structure and function of seed lipid body-associated proteins," *C. R. Biol.* 2008.

[23] P. Jolivet *et al.*, "Protein composition of oil bodies from mature Brassica napus seeds," *Proteomics*. 2009.

[24] T. L. Shimada, M. Hayashi, and I. Hara-Nishimura, "Membrane Dynamics and Multiple Functions of Oil Bodies in Seeds and Leaves," *Plant Physiol.* 2017.

[25] S. Maurer *et al.*, "The role of intact oleosin for stabilization and function of oleosomes.," *J. Phys. Chem. B*. 2013.

[26] D. Iwanaga *et al.*, "Soybean Oil Extraction and Characterization of Oil Bodies from Soy Beans : A Natural Source of Pre-Emulsified," *J. Agric. Food Chem.* 2007.

[27] C. V. Nikiforidis and V. Kiosseoglou, "Aqueous extraction of oil bodies from maize germ (Zea mays) and characterization of the resulting natural oil-in-water emulsion," *J. Agric. Food Chem.* 2009.

[28] G. Adams *et al.*, "Extraction, isolation and characterisation of oil bodies from pumpkin seeds for therapeutic use," *Food Chem.* 2012.

[29] W. Wang, C. Cui, Q. Wang, C. Sun, L. Jiang, and J. Hou, "Effect of pH on physicochemical properties of oil bodies from different oil crops," *J. Food Sci. Technol.* 2019.

[30] K. Campbell *et al.*, "Advances in aqueous extraction processing of soybeans," *JAOCS, J. Am. Oil Chem. Soc.* 2011.

[31] A. F. Torres, X. Xu, C. V. Nikiforidis, J. H. Bitter, and L. M. Trindade, "Exploring the Treasure of Plant Molecules With Integrated Biorefineries," *Front. Plant Sci.* 2019.

[32] Y. Ying, L. Zhao, L. Kong, X. Kong, Y. Hua, and Y. Chen, "Solubilization of proteins in extracted oil bodies by SDS: A simple and efficient protein sample preparation method for Tricine-SDS-PAGE," *Food Chem.* 2015.

[33] B. Chen, D. J. McClements, D. A. Gray, and E. A. Decker, "Physical and oxidative stability of pre-emulsified oil bodies extracted from soybeans," *Food Chem.* 2012.

[34] A. Matsakidou, F. T. Mantzouridou, and V. Kiosseoglou, "Optimization of water extraction of naturally emulsified oil from maize germ," *LWT*. 2015.

[35] C. V. Nikiforidis, A. Matsakidou, and V. Kiosseoglou, "Composition, properties and potential food applications of natural emulsions and cream materials based on oil bodies," *RSC Adv.* 2014.

[36] C. V. Nikiforidis, S. Donsouzi, and V. Kiosseoglou, "The interplay between diverse oil body extracts and exogenous biopolymers or surfactants," *Food Res. Int.* 2016.

[37] A. Matsakidou, C. G. Biliaderis, and V. Kiosseoglou, "Preparation and characterization of composite sodium caseinate edible films incorporating naturally emulsified oil bodies," *Food Hydrocoll.* 2013.

[38] I. D. Fisk, R. Linforth, G. Trophardy, and D. Gray, "Entrapment of a volatile lipophilic aroma compound (d-limonene) in spray dried water-washed oil bodies naturally derived from sunflower seeds (Helianthus annus)," *Food Res. Int.* 2013.

[39] Y. Yoshie-Stark, Y. Wada, and A. Wäsche, "Chemical composition, functional properties, and bioactivities of rapeseed protein isolates," *Food Chem.* 2008.

[40] I. Feussner, H. Kühn, and C. Wasternack, "Lipoxygenase-dependent degradation of storage lipids," *Trends Plant Sci.* 2001.

[41] C. Wijesundera and Z. Shen, "Mimicking natural oil bodies for stabilising oil-in-water food emulsions," *Lipid Technol.* 2014.

[42] S. De Chirico, V. di Bari, T. Foster, and D. Gray, "Enhancing the recovery of oilseed rape seed oil bodies (oleosomes) using bicarbonate-based soaking and grinding media," *Food Chem.* 2018.

[43] C. V. Nikiforidis, V. Kiosseoglou, and E. Scholten, "Oil bodies: An insight on their microstructure - maize germ vs sunflower seed," *Food Res. Int.* 2013.

[44] D. Karefyllakis, S. Salakou, J. H. Bitter, A. J. van der Goot, and C. V. Nikiforidis, "Covalent Bonding of Chlorogenic Acid Induces Structural Modifications on Sunflower Proteins," *ChemPhysChem.* 2018.

[45] K. Campbell and C. E. Glatz, "Mechanisms of aqueous extraction of soybean oil.," *J. Agric. Food Chem.* 2009.

[46] Y. Chen and T. Ono, "Simple Extraction Method of Non-allergenic Intact Soybean Oil Bodies That Are Thermally Stable in an Aqueous Medium," *J. Agric. Food Chem.* 2010.

[47] V. N. Kapchie, C. C. Hauck, H. Wang, and P. a. Murphy, "Process Improvement for Semipurified Oleosomes on a Pilot-Plant Scale," *J. Food Sci.* 2011.

2

The effect of monovalent (Na^+, K^+) and divalent (Ca^{2+}, Mg^{2+}) cations on rapeseed oleosome (oil-body) extraction and stability at pH 7

This chapter has been published as:
M. J. Romero-Guzmán, V. Petris, S. De Chirico, V. di Bari, D. Gray, R.M. Boom, C.V. Nikiforidis. "The effect of monovalent (Na^+, K^+) and divalent (Ca^{2+}, Mg^{2+}) cations on rapeseed oleosome (oil body) extraction and stability at pH 7," *Food Chem*. 2020.

Highlights

- Oleosomes extraction yield at pH 7 is enhanced by the addition of cations.
- The highest oleosome extraction yield at pH 7 (64 wt.%) was achieved with K^+.
- Monovalent cations cause minor coalescence of oleosomes.
- Divalent cations cause extensive coalescence of oleosomes.

Abstract

Oleosomes are storage vehicles of TAGs in plant seeds. They are protected with a phospholipid-protein monolayer and extracted with alkaline aqueous media; however, pH adjustment intensifies the extraction process. Therefore, the aim of this work was to investigate the extraction mechanism of rapeseed oleosomes at pH 7 and at the presence of monovalent and divalent cations (Na^+, K+, Mg^{2+}, and Ca^{+2}). The oleosome yield at pH 9.5 was 64 wt.%, while the yield at pH 7 with H_2O was just 43 wt.%. The presence of cations at pH 7, significantly enhanced the yield, with K^+ giving the highest yield (64 wt.%). The cations affected the oleosome interface and their interactions. The presence of monovalent cations resulted in aggregation and minor coalescence, while divalent cations resulted in extensive coalescence. These results help to understand the interactions of oleosomes in their native matrix and design simple extraction processes at neutral conditions.

2.1 Introduction

Oleosomes or oil bodies, as they are widely known, are the triacylglycerols (TAGs) storage organelles in plants, serving as the main energy source during seed germination. To retain the chemical quality of the TAGs against extreme environmental stresses, plant cells are building an amphipathic phospholipid-protein membrane around them [1]. Besides the in situ functionality of oleosomes, plant oils (i.e. soybean oil, rapeseed oil, sunflower oil) are generally extracted and used for numerous applications in food, pharmaceutical products, and as biofuels [2]. However, plant oil extraction requires the disruption of the oleosome membrane by a pressing step, followed by toxic organic solvent extraction [3]. When plant oils are extracted, they are used as bulk oils or as dispersed phases in oil-in-water emulsions, which requires an emulsification step and the use of an emulsifier [4]. Nevertheless, looking back to the oleosome physiology, all these process steps seem unnecessary, as oleosomes, are naturally emulsified oil droplets that could readily serve as the dispersed phase of oil-in-water emulsions. Therefore, instead of focusing only on oil extraction, efforts should be made towards the optimization of the oleosome extraction. For this reason, we have to deeply understand the properties of oleosome membrane and the interactions at the molecular level.

The most abundant proteins on the oleosome membrane are oleosins, which represent up to 75-80% of the oleosome membrane protein content [5],[6]. Oleosins are a group of proteins with a low molecular weight (14-17 kDa) and are composed by a hydrophobic tail that is anchored in the oil core and two short fairly hydrophilic terminals that are on the oleosome surface [7]. The other group of proteins present on the oleosome membrane are caleosins (24-28 kDa) and steroleosins (35-60 kDa) [7]. Similar to oleosins, these proteins

have also a hydrophobic tail, which is smaller than the one of oleosins and a longer domain exposed to the bulk phase [8]. Even though the exact biological functions of the membrane proteins are still to be defined [9], [10], it is known that caleosins have a unique Ca^{2+} binding site on the N-terminal of the protein that can also bind Mg^{2+} [11], [12], while steroleosins have a hydrophilic sterol-binding dehydrogenase domain [13]. Regarding the phospholipids at the oleosome interface, the main type present is phosphatidylcholine representing 65 % (wt.%) of the total phospholipids, followed by phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine [14], [15].

The understanding of the architecture of the oleosome interface, the molecular combination and the forces that might occur, will help towards optimizing their extraction. Both proteins and phospholipids are charged molecules and electrostatic forces can occur between neighbouring oleosomes and also between oleosomes and surrounding charged material [16]. Besides electrostatic forces, hydrophobic attractive forces might take place as well. The domains of the oleosome proteins that are exposed to the bulk phase are fairly hydrophilic, however, they also contain hydrophobic patches that can attract each other and lead to aggregation of neighbouring oleosomes [16]–[18]. Furthermore, the hydrophobic domains of extrinsic proteins might interact with the oleosome proteins leading to bridging flocculation [19]. Hydrophobic attractive forces can be prevented by using surfactants, like Tween or SDS [16], [20]. Nevertheless, the addition of surfactants may affect the oleosome membrane, therefore this research was mostly focused on affect these interactions by electrostatic interactions.

Oleosomes have a zero charge point between pH values of 4 and 6, therefore, to increase electrostatic repulsion and to enhance the extraction kinetics it has been proposed to perform the extraction at pH values above 9.0, where the electrokinetic potential is below -40 mV [21], [22]. However, in order to reduce the number of steps and chemicals used during the oleosome extraction, efforts should be made towards understanding the oleosome extraction mechanism at neutral pH values. An alternative to pH adjustment for altering the electrostatic interactions between proteins is the addition of cations [23]–[26]. Ionic environments weaken or strengthen the protein-protein electrostatic interactions, which can cause protein unfoldment and affects its solubility. Therefore, the aim of this work was to investigate the effect of monovalent (Na^+, K^+) and divalent (Ca^{2+}, Mg^{2+}) cations on oleosome extraction at pH 7. The effect of the cations was evaluated by comparing the oleosome extraction yields and the effect on the physical stability of the obtained oleosomes.

2.2 Experimental

2.2.1 Materials

Untreated rapeseeds (Brassica napus), type Allize were kindly pursued by the Division of Food Sciences, University of Nottingham, Sutton Bonington, UK. Magnesium Chloride ($MgCl_2$) was obtained from Merck (Darmstadt, Germany). All other chemicals including the sodium chloride, potassium chloride and calcium chloride (NaCl, KCl, $CaCl_2$) were obtained in analytical grade from Sigma-Aldrich (St. Louis, MO, USA). Solutions and dispersions were made with ultrapure water (MilliQ) obtained with a Merck Millipore device (Darmstadt, Germany).

2.2.2 Oleosome aqueous extraction

Rapeseed oleosomes were isolated using the extraction method proposed by De Chirico et al. (2018), with some modifications based on the method proposed by Nikiforidis et al. (2009). The different aqueous media were prepared by dissolving the different salts (NaCl, KCl, $MgCl_2$, $CaCl_2$, 0.2 mol/L) in ultra-pure water (MilliQ) and adjusting their pH to 7.0 with a solution of NaOH (0.1 mol/L) or HCl (0.1 mol/L). The additional aqueous solution made by NaCl (0.3 mol/L) was elaborated in a similar way than the other salted-aqueous media. The alkaline aqueous media was prepared similarly, by dissolving $NaHCO_3$ 0.1 mol/L and adjusting the pH to pH 9.5 with NaOH (1.0 mol/L). A SevenMulti™ dual meter pH/conductivity (Mettler Toledo, Greifensee, Switzerland) was used to monitor the pH. The seeds were soaked (1:1 w/v) in the different aqueous media for 16 h at 4 °C. After soaking, the solid/solvent ratio was adjusted to 1:7 w/v and the dispersion was blended for 60 s at 7200 rpm (Thermomix TM31, Utrecht, The Netherlands). The mixture was then filtered through two layers of cheesecloth (GEFU®, Eslohe, Germany). The first extract (filtrate) was centrifuged at 3,000 g for 15 min at 4 °C. After the centrifugation step, three different layers were observed: the cream, the serum and the precipitate. The oleosome cream was manually collected, dispersed in ultra-pure water (MilliQ) (1:4 w/v) and centrifuged at 10,000 g for 30 min at 4 °C. This washing step was repeated twice. The oleosome extraction yield was calculated based on the difference between lipid content remaining in the cake and the initial lipid content in the seeds.

2.2.3 Compositional analysis of all streams

The moisture content of the retentate and oleosome cream was determined using a Moisture Analyzer (MA35M, Sartorius Göttingen, Germany). Oil

quantification was performed on dry samples that where placed in a Soxhlet device (Buchi extractor, Büchi, Flawil, Switzerland) for 9 h, while the oil was extracted using petroleum ether. The protein content of the defatted samples was calculated by determining the amount of Nitrogen in the samples using the Dumas method and using a conversion factor of 5.5 as suggested by Lindeboom (2007) [27] (Nitrogen analyser, FlashEA 112 series, Thermo Scientific, Interscience, The Netherlands).

2.2.4 Determination of oleosome particle size distribution

The droplet size distribution of oleosome emulsions was determined by laser light scattering (Malvern Mastersizer 3000, Malvern Instruments Ltd, UK). The refractive index used was 1.47 for the dispersed phase (oleosomes) and 1.33 for the continuous phase (water). Average droplet sizes are reported using the surface weighted ($d_{3,2}$) mean diameter. All measurements were conducted on fresh oleosome creams diluted in ultrapure water (1:100 w/v).

2.2.5 Determination of oleosome zeta potential

A dynamic light scattering apparatus (DLS ZetasizerNanoZS, Malvern Instruments Ltd, UK) was used to analyse the ζ-potential of the emulsions. The creams were diluted 1000 w/v with ultra-pure water. After the dilution, the pH of the dispersions was adjusted manually. The refractive indexes used were 1.47 for the dispersed phase and 1.33 for the continuous phase.

2.2.6 Optical microscopy analysis of oleosome emulsions

Images of the oleosome emulsions were taken with the microscope Univision V 4.8.3.0 (Carl Zeiss MicroImaging, GmbH) equipped with a digital camera (Axiocam MRc 5). The oleosome cream for each treatment was diluted with

ultrapure water (1:100 w/v) and one drop of the emulsion was added on a glass slide and placed onto the microscope. The magnification used was 100x.

2.2.7 Statistical analysis

All the measurements and extractions were performed at least in triplicates. One-way analysis of variance (ANOVA) test was applied to detect differences among the extraction yields as function of the aqueous extraction media. Analyses were performed with the IBM SPSS statistics 23 software. Differences were considered to be significant at $p<0.05$.

2.3 Results & Discussion

2.3.1 Effect of cations on oleosome extraction yield and stability

To achieve high oleosome extraction yields, pH values above 9.0 are necessary, where proteins and oleosomes are soluble due to the high electro kinetic potential [21],[28]. For example, maize oleosomes have a zero charge point at around pH 4.5. Their extraction at pH 6.0 has a yield about 15 wt.% while at pH 9.0 it reaches a yield of up to 90 wt.% [28]. As an effort towards an alternative path to increase oleosome solubility without adjusting pH, we decided to investigate oleosome extraction and stability at neutral pH (7.0) and in the presence of monovalent or divalent cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}).

The extraction yields of rapeseed oleosomes in the presence of cations are shown in Table 2.1. When only ultra-pure water was used the lowest extraction yield was achieved, which was 42.7 wt.%. At the presence of K^+ (0.2 mol/L), the extraction yield was significantly enhanced and reached the highest value, of 64.2 wt.%. In contrast, the extraction performed with Na^+ (0.2 mol/L) reached a yield of 50.2 wt.%. When divalent cations were present,

the yield was 52.5 wt.% after the extraction with Mg^{2+} (0.2 mol/L) and 55.0 wt.% with Ca^{2+} (0.2 mol/L). The minimum amount of extracted rapeseed oleosomes was achieved when only ultra-pure water was used (42.7 wt.%), indicating that the cations interacted with the oleosome membrane, enhancing oleosome solubility and subsequently their extraction.

Table 2.1 Extraction yield of oleosomes recovered with different aqueous solvents.

Aqueous solvent	Oleosome extraction yield (wt.%)	Standard Deviation
H_2O (pH 7.0)	42.7[a]	± 1.9
Na^+ (0.2 mol/L, pH 7.0)	50.2[b]	± 2.0
Na^+ (0.3 mol/L, pH 7.0)	55.3[c]	± 1.8
K^+ (0.2 mol/L, pH 7.0)	64.2[d]	± 0.6
Mg^{2+} (0.2 mol/L, pH 7.0)	52.5[c]	± 4.9
Ca^{2+} (0.2 mol/L, pH 7.0)	55.0[c]	± 2.3
$NaHCO_3$ (0.1 mol/L, pH 9.5)	63.6[d]	± 0.5

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

According to Hofmeister series [29], a small difference between the effect of the two monovalent cations (Na^+ and K^+) was expected. More precisely a slightly stronger solubilization effect from Na^+ than K^+ was expected, due to the order of these cations in the series, being K^+ exactly to the left of Na^+ on the series; however, the expected difference was not of this significant extent as extraction yield at the presence of K^+ was higher than at the presence of Na^+. Besides the interaction with the membrane proteins, this phenomenon could be attributed to the interaction of the cations with the other membrane component, like the phospholipids and more specifically, phosphatidylcholine [30], [31]. It has been reported that in comparison to K^+, the binding capacity of Na^+ to phosphatidylcholine is 2.2 folds higher, most likely due to its larger surface charge [30]. This would mean that maybe a significant amount of Na^+ binds to phosphatidylcholine and is not available for the oleosome extraction but interacting with the phospholipid oleosome

membrane. To understand whether the available concentration of Na^+ had an effect to oleosome extraction yield, a solution with higher Na^+ concentration (0.3 mol/L) was also used. The oleosome extraction yield with higher concentration of Na^+ (0.3 mol/L) slightly increased and resulted significantly different from the obtained with Na^+ at 0.2 mol/L, reaching 55.3 wt.%, these difference could mean that when increasing the excess of cations not interacting with the phospholipid membrane could aid the extraction; however, still this higher concentration of Na^+ did not reach the extraction yield obtained when K^+ (0.2 mol/L) was present. Therefore, besides the interactions with other components of the interface and the effect on concentration, K^+ leaded to higher extraction yields. Furthermore, it is important to state that the yield in the presence of K^+ (0.2 mol/L) at pH 7 did not significantly differed from the yield obtained when $NaHCO_3$ buffer (0.1 mol/L) at pH 9.5 was used.

With regards to the divalent cations, they interacted as expected with oleosome interfacial proteins and significantly enhanced their extraction yield in comparison to pure water at the same pH. Divalent cations can affect salt bridges in proteins causing hydration and subsequent extraction [32]. This mechanism explains the fact that divalent cations had a positive effect on oleosome extraction in comparison to pure water, however, the formation of new bridges resulted in a lower extraction yield in comparison to K^+. Between the effect of the two divalent cations, no significantly differences were measured. According to Hofmeister series, this should be expected, since their effect on protein unfolding and solubility is similar [29]. The increase of the oleosome extraction yield with the aid of cations at neutral pH values is an important finding proving that high extraction yields of oleosomes cannot only be achieved in strongly alkaline environments.

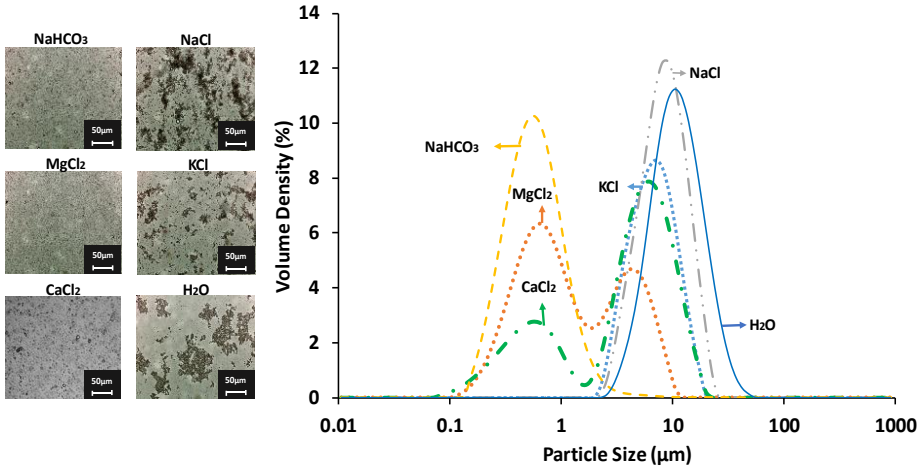

Fig. 2.1 Particle size distribution and microscopy images of the initial extracts obtained with (—) H_2O (pH 7) (-···-) Na^+ (0.2 mol/L, pH 7), (■) K^+ (0.2 mol/L, pH 7), (●) Mg^{2+} (0.2 mol/L, pH 7), (-·-·) Ca^{2+} (0.2 mol/L, pH 7) and (---) $NaHCO_3$ (0.1 mol/L, pH 9.5). The scale bar is 50 µm.

Besides the effect of the cations on extraction yield, their effect on the stability of the extracted oleosomes was also investigated. Fig. 2.1, shows the particle size distribution and the optical micrographs of the initially obtained oleosome extracts. Two types of peaks are observed, the first one observed from 0.1 to 2.0 µm, corresponding to individual oleosomes and the second one from 5 to 50 µm, corresponding to aggregates of oleosomes. The emulsions extracted at pH 9.5 ($NaHCO_3$, 0.1 mol/L) yielded oleosomes of around 1 µm, evident of native individual oleosomes [21]. The extracts with H_2O or the monovalent cations at pH 7 exhibited extensive aggregation, showing a broad peak between 10 and 50 µm. The oleosome aggregation when Na^+ and K^+ were present at pH 7 has been previously reported [1], [33]. This behaviour was expected due to the low electrokinetic potential (<21.5 mV) (Table 2.2) and resulting from low electrostatic repulsion.

Table 2.2 Zeta potential of oleosomes final recovered creams.

	Treatment	Zeta potential (mV)	Standard Deviation
Na^+	(0.2 mol/L, pH 7.0)	-21.5[a]	±0.4
K^+	(0.2 mol/L, pH 7.0)	-9.8[b]	±0.5
Mg^{2+}	(0.2 mol/L, pH 7.0)	-9.7[b]	±0.4
Ca^{2+}	(0.2 mol/L, pH 7.0)	-21.8[a]	±0.4
H_2O	(pH 7.0)	-20.24[c]	±0.4
$NaHCO_3$	(0.1 mol/L, pH 9.5)	-56.7[d]	±0.3

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

The aggregates were probably formed due to hydrophobic forces between oleosomes and also between oleosomes and co-extracted extraneous proteins that can bridge neighbouring oleosomes [28]. On the other hand, the emulsions extracted with divalent cations showed bimodal distributions as some of the oleosomes extracted with these cations were recovered as individual droplets with a similar distribution to those extracted at pH 9.5; however, aggregation was also observed. According to Table 2.2, the electrokinetic potentials of the divalent cations were in the same range (between -9.7 and -21.5) as when the monovalent cations were present and copious protein-protein hydrophobic interactions should be expected. However, the presence of individual oleosomes indicates interactions of the divalent cations with the membrane proteins and also with the extraneous proteins inhibiting hydrophobic attractive forces. As caleosins' N-terminal containing the calcium binding site [12], is exposed to the bulk phase, it has been reported that both Ca^{2+} and Mg^{2+} interact with this site affecting the protein configuration and overall hydrophobicity [11], however, more research is necessary to support this hypothesis.

2.3.2 Effect of cations on the physical stability of dense oleosome creams

To investigate further the effect of the cations on oleosome stability, high-speed centrifugation (10,000 g for 30 min) was applied to obtain densely packed oleosome creams. The ratio of oil and proteins obtained relates to the interactions of oleosomes with extraneous proteins [34], while possible physical destabilization indicates conformational changes on the membrane [35]. As it is presented in Table 2.3, the oleosome creams with K^+, Na^+ or Mg^{2+} had a lower oil to protein ratio compared to those that were extracted in the presence of Ca^{2+}. On one hand the higher protein content with K^+ and Na^+ could explain the observed aggregates (Fig. 2.1), where extraneous proteins bridge oleosomes through hydrophobic forces and hence they are difficult to remove [36]. On the other hand, the lower protein content observed when Ca^{2+} was present indicates that there is less extraneous protein entrapped in the cream [37].

Table 2.3 Protein and lipid content of the recovered oleosome creams extracted with different aqueous solvents.

		H_2O		Na^+		K^+		Mg^{2+}		Ca^{2+}		$NaHCO_3$	
		wt. %		wt. %		wt. %		wt. %		wt. %		wt. %	
Wet basis	Lipids	42.8^{a}	±2.8	56.3^{b}	±3.5	52.2^{c}	±0.4	69.2^{d}	±0.4	66.6^{e}	±0.5	70.9^{d}	±1.2
	Protein	7.5^{i}	±1.2	7.1^{i}	±0.5	8.2^{i,ii}	±1.3	9.4^{ii}	±0.5	5.1^{iii}	±0.5	3.9^{iii}	±0.1
	Ratio lipids:proteins	5.7	-	7.8	-	6.3	-	7.3	-	12.8	-	17.5	-
Dry basis	Lipids	60.6^{a}	±2.8	81.1^{b,c}	±3.5	73.6^{d}	±0.4	79.1^{b}	±0.4	85.1^{e}	±0.5	84.2^{c,e}	±1.2
	Protein	10.6	±1.2	10.3^{i}	±0.5	12.6^{i}	±1.3	11.6^{i}	±0.5	6.6^{ii}	±0.5	4.7^{iii}	±0.1
	Ratio lipids:proteins	5.7	-	7.6	-	6.4	-	7.4	-	12.4	-	17.0	-

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

As it is shown in Fig. 2.2 and as it has been previously reported, extraneous proteins had a significant impact on oleosome stability against coalescence [16], [38]. The oleosome creams obtained with H_2O were the most stable against coalescence. Their size distribution showed a bimodal distribution

with a peak corresponding to small individual oleosomes from 0.05 to 0.7 μm and another peak corresponding to aggregates with a size between 0.3 to 20 μm, but no coalesced droplets were observed. The oleosome creams obtained with K^+ or Na^+, show similar distributions, where slight coalescence was observed. The case of Ca^{2+} and Mg^{2+} was different since there was minor aggregation after the oleosome extraction in comparison with the extracts recovered with monovalent cations, however, the applied centrifugal forces lead to extensive coalescence and subsequent oil separation.

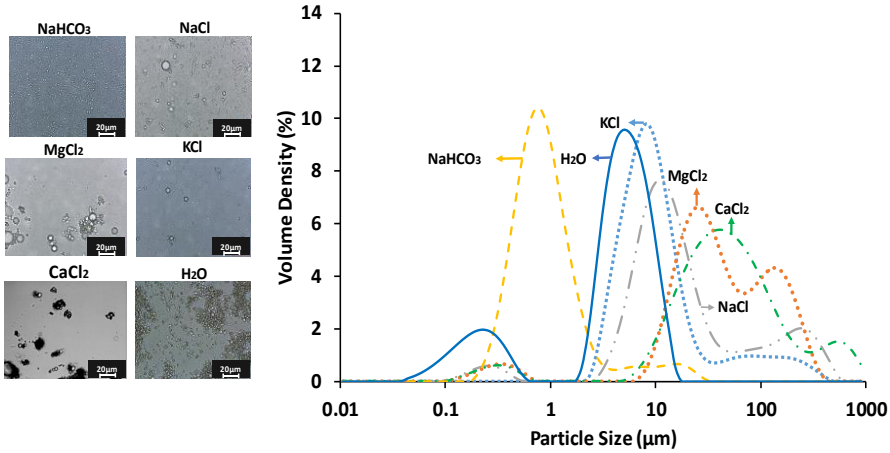

Fig. 2.2 Particle size distribution and microscopy images of the final oleosome creams obtained with(—) H_2O (pH 7) (-··-) Na^+ (0.2 mol/L, pH 7), (■) K^+ (0.2 mol/L, pH 7), (●) Mg^{2+} (0.2 mol/L, pH 7), (-·-·) Ca^{2+} (0.2 mol/L, pH 7) and (---) $NaHCO_3$ (0.1 mol/L, pH 9.5). The scale bar is 20 μm.

Finally, regarding the presence of $NaHCO_3$ (pH 9.5) the mechanism is completely different. The electrokinetic potential of the oleosomes at this pH is very high, -57 mV (Table 2.2), which creates strong repulsive electrostatic forces and preventing both aggregation and coalescence. This performance

has reported for most cases where pH values between 9.0 and 9.5 were used [21], [39].

2.4 Conclusions

The presence of monovalent (K^+ or Na^+) and divalent (Ca^{2+} or Mg^{2+}) cations significantly enhanced the extraction of oleosomes at pH 7. All extraction yields achieved in the presence of cations were significantly different than the one with H_2O at pH 7, which was about 43 wt.%. More specifically, the presence of K^+ at pH 7, reached a yield of 64 wt.% that was no significantly different that the one obtained when pH 9.5 was used. Cations at specific concentrations can break the salt bridges in proteins, interrupt their interactions and lead to an increase of their extraction yield. These results show that the interactions between oleosomes and between oleosomes and co-extracted proteins can be inhibited either by pH adjustment to strong alkaline environments or at the presence of cations. Moreover, the interactions of the cations with the oleosome membrane had an effect on the stability of oleosome extracts. In the absence of cations at pH 7, extensive aggregation was observed, which can be attributed to hydrophobic forces and the low electrokinetic potential of the system. The addition of monovalent cations caused extensive aggregation as well, while the divalent cations partly reduced the formation of aggregates. Divalent cations probably interacted with the oleosome membrane proteins, altering their re-configuration and inhibited the protein-protein hydrophobic interactions. However, when a dense oleosome cream was created, the oleosomes obtained with H_2O retained their integrity, while those obtained with monovalent cations showed slightly coalescence and those obtained with divalent cations where extensively coalesced. These results suggest that, membrane protein re-

configuration due to the presence of divalent cations has a significant negative impact on oleosome stability.

References

[1] J. T. Tzen, G. C. Lie, and A. H. C. C. Huang, "Characterization of the charged components and their topology on the surface of plant seed oil bodies," *J. Biol. Chem.* 1992.

[2] E. G. Hammond, L. A. Johnson, C. Su, T. Wang, and P. J. White, "Soybean Oil," in *Bailey's Industrial Oil and Fat Products*, 6th edition., edited by F. Shahidi, John Wiley & Sons, New York. 2005.

[3] U. Thiyam-Hollaender, N. Eskin, and N. Michael, "*Canola and rapeseed: production, processing, food quality, and nutrition*," CRC Press, Boca Raton, Florida, USA. 2012.

[4] D. J. McClements, *Food Emulsions: Principles, Practices, and Techniques, Second Edition*. CRC Press, Boca Raton, Florida, USA. 2004.

[5] P. Jolivet *et al.*, "Oil body proteins sequentially accumulate throughout seed development in Brassica napus," *J. Plant Physiol.* 2011.

[6] J. T. Tzen, "Integral Proteins in Plant Oil Bodies," *ISRN Bot.* 2012.

[7] L. J. Lin, P. C. Liao, H. H. Yang, and J. T. C. Tzen, "Determination and analyses of the N-termini of oil-body proteins, steroleosin, caleosin and oleosin," *Plant Physiol. Biochem.* 2005.

[8] T. L. Shimada and I. Hara-Nishimura, "Oil-body-membrane proteins and their physiological functions in plants.," *Biol. Pharm. Bull.* 2010.

[9] Z. Purkrtova, P. Jolivet, M. Miquel, and T. Chardot, "Structure and function of seed lipid body-associated proteins," *C. R. Biol.* 2008.

[10] W. Song *et al.*, "Delineation of plant caleosin residues critical for functional divergence, positive selection and coevolution," *BMC Evol. Biol.* 2014.

[11] D. Allouche, J. Parello, and Y. H. Sanejouand, "Ca2+/Mg2+ exchange in parvalbumin and other EF-hand proteins. A theoretical study," *J. Mol. Biol.* 1999.

[12] J. C. Chen, C. C. Tsai, and J. T. Tzen, "Cloning and secondary structure analysis of caleosin, a unique calcium-binding protein in oil bodies of plant seeds.," *Plant Cell Physiol.* 1999.

[13] Z. Purkrtova, C. Le Bon, B. Kralova, M. H. Ropers, M. Anton, and T. Chardot, "Caleosin of Arabidopsis thaliana: Effect of calcium on functional

and structural properties," *J. Agric. Food Chem.* 2008.

[14] M. Deleu, G. Vaca-Medina, J.-F. Fabre, J. Roïz, R. Valentin, and Z. Mouloungui, "Interfacial properties of oleosins and phospholipids from rapeseed for the stability of oil bodies in aqueous medium.," *Colloids Surf. B. Biointerfaces*. 2010.

[15] J. Tzen, Y. Cao, P. Laurent, C. Ratnayake, and A. Huang, "Lipids, Proteins, and Structure of Seed Oil Bodies from Diverse Species.," *Plant Physiol.* 1993.

[16] C. V. Nikiforidis and V. Kiosseoglou, "Competitive displacement of oil body surface proteins by Tween 80 – Effect on physical stability," *Food Hydrocoll.* 2011.

[17] P. Jolivet, L. Aymé, A. Giuliani, F. Wien, T. Chardot, and Y. Gohon, "Structural proteomics: Topology and relative accessibility of plant lipid droplet associated proteins," *J. Proteomics*. 2017.

[18] C. V. Nikiforidis, S. Donsouzi, and V. Kiosseoglou, "The interplay between diverse oil body extracts and exogenous biopolymers or surfactants," *Food Res. Int.* 2016.

[19] N. M. Eren, G. Narsimhan, and O. H. Campanella, "Protein adsorption induced bridging flocculation: The dominant entropic pathway for nano-bio complexation," *Nanoscale*. 2016.

[20] C. V. Nikiforidis, O. A. Karkani, and V. Kiosseoglou, "Exploitation of maize germ for the preparation of a stable oil-body nanoemulsion using a combined aqueous extraction–ultrafiltration method," *Food Hydrocoll.* 2011.

[21] S. De Chirico, V. di Bari, T. Foster, and D. Gray, "Enhancing the recovery of oilseed rape seed oil bodies (oleosomes) using bicarbonate-based soaking and grinding media," *Food Chem.* 2018.

[22] A. Matsakidou, F. T. Mantzouridou, and V. Kiosseoglou, "Optimization of water extraction of naturally emulsified oil from maize germ," *LWT - Food Sci. Technol.* 2015.

[23] K. D. Collins, "Ions from the Hofmeister series and osmolytes: Effects on proteins in solution and in the crystallization process," *Methods*. 2004.

[24] A. C. Dumetz, A. M. Snellinger-O'Brien, E. W. Kaler, and A. M. Lenhoff, "Patterns of protein – protein interactions in salt solutions and implications for protein crystallization," *Protein Sci.* 2007.

[25] Y. Levy and J. N. Onuchic, "Water and proteins: a love-hate relationship.," *Proc. Natl. Acad. Sci. U. S. A.* 2004.

[26] Y. Zhang and P. S. Cremer, "Interactions between macromolecules and ions: the Hofmeister series," *Curr. Opin. Chem. Biol.* 2006.

[27] N. Lindeboom and P. K. J. P. D. Wanasundara, "Interference of phenolic compounds in Brassica napus, Brassica rapa and Sinapis alba seed extracts with the Lowry protein assay," *Food Chem.* 2007.

[28] C. V. Nikiforidis and V. Kiosseoglou, "Aqueous extraction of oil bodies from maize germ (Zea mays) and characterization of the resulting natural oil-in-water emulsion," *J. Agric. Food Chem.* 2009.

[29] D. Roberts *et al.*, "Specific ion and buffer effects on protein-protein interactions of a monoclonal antibody," *Mol. Pharm.* 2015.

[30] A. A. Gurtovenko and I. Vattulainen, "Effect of NaCl and KCl on phosphatidylcholine and phosphatidylethanolamine lipid Membranes: insight from atomic-Scale simulations for understanding salt-induced effects in the plasma membrane," *J. Phys. Chem. B.* 2008.

[31] Y. Mao *et al.*, "Binding competition to the POPG lipid bilayer of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} in different ion mixtures and biological implication," *J. Phys. Chem. B.* 2013.

[32] T. Arakawa and S. N. Timasheff, "Mechanism of Protein Salting In and Salting Out by Divalent Cation Salts: Balance between Hydration and Salt Binding," *Biochemistry*. 1984.

[33] D. Iwanaga, D. A. Gray, I. D. Fisk, E. Andrew, J. Weiss, and D. J. Mcclements, "Extraction and Characterization of Oil Bodies from Soy Beans : A Natural Source of Pre-Emulsified Soybean Oil Extraction and Characterization of Oil Bodies from Soy Beans : A Natural Source of Pre-Emulsified," *J. Agric. Food Chem.* 2007.

[34] C. V. Nikiforidis, V. Kiosseoglou, and E. Scholten, "Oil bodies: An insight on their microstructure - maize germ vs sunflower seed," *Food Res. Int.* 2013.

[35] C. V. Nikiforidis and V. Kiosseoglou, "Physicochemical stability of maize germ oil body emulsions as influenced by oil body surface-xanthan gum interactions," *J. Agric. Food Chem.* 2010.

[36] B. Qi *et al.*, "Deciphering the characteristics of soybean oleosome-associated

protein in maintaining the stability of oleosomes as affected by pH," *Food Res. Int.* 2017.

[37] C. V. Nikiforidis, A. Matsakidou, and V. Kiosseoglou, "Composition, properties and potential food applications of natural emulsions and cream materials based on oil bodies," *RSC Adv.* 2014.

[38] L. Zhao, Y. Chen, Y. Chen, X. Kong, and Y. Hua, "Effects of pH on protein components of extracted oil bodies from diverse plant seeds and endogenous protease-induced oleosin hydrolysis," *Food Chem.* 2016.

[39] W. Wang, C. Cui, Q. Wang, C. Sun, L. Jiang, and J. Hou, "Effect of pH on physicochemical properties of oil bodies from different oil crops," *J. Food Sci. Technol.* 2019.

3

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

This chapter has been published as:
M. J. Romero-Guzmán, L. Jung, K. Kyriakopoulou, R. M. Boom, and C. V. Nikiforidis, "Efficient single-step rapeseed oleosome extraction using twin-screw press," J. Food Eng. 2020.

Highlights

- Twin-screw press recovers intact oleosomes at 1:1 solid to medium ratio.
- Twin-screw press can replace conventional blender extraction.
- Twin-screw press recovers oleosomes in a continuous and highly efficient step.
- Mechanical forces in the twin-screw press surpass the solubilizing effect of media.
- Water recovers competitive oleosome yields when using twin-screw press.

Abstract

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 can 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 the 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.%.

3.1 Introduction

Vegetable oil is stored in intracellular structures named oleosomes. Oleosomes consist of a core of triglycerides surrounded by a monolayer of phospholipids and proteins [1]. This structure provides oleosomes with great stability against physical and chemical stresses [2], [3]. 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 [4]. This, in turn, influences the oleosome solubility and hence their extractability [5], [6]. 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 [6]–[8]. This extraction procedure requires nevertheless a large amount of water, which makes upscaling difficult [9].

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 [10], [11]. While it has been successfully used for aqueous oil extraction [11], [12], 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 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.

3.2 Materials and methods

3.2.1 Materials

Rapeseed seeds (Alizze) were purchased from a seed producer. The used seeds are food grade as they do not contain erucic acid and have a low glucosinolate content (13mg/100g). 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).

3.2.2 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 [13], 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 [13]–[15]. 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 (3.2.2.1) or with a twin-screw press (3.2.2.2).

3.2.2.1 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.

3.2.2.2 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. 3.1 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.

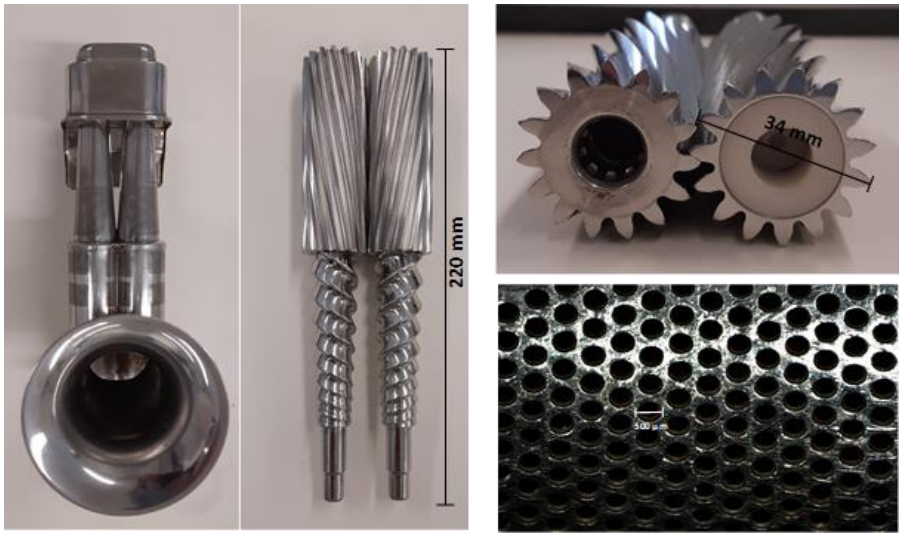

Fig. 3.1 Twin-screw case and filter (left), screws profile (centre), screws diameter (top right), zoom-in on the sieve attached to the case (bottom right) used for the oleosome extraction.

3.2.3 Isolation of oleosomes

Isolation of the oleosomes from the first extract was performed by centrifugation at 3,000 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.

3.2.4 Characterization of the streams

3.2.4.1 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 in the sample was determined as the weight difference between the initial and the dehydrated sample, divided by the initial mass of the sample.

3.2.4.2 Lipid content

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

3.2.4.3 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).

3.2.5 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 [6]. 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).

3.2.6 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 1.47. 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 as differential particle size distributions.

3.2.7 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 to 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.

3.2.8 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).

3.2.9 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 significant at $p < 0.05$.

3.3 Results & Discussion

3.3.1 Oleosome and protein extraction yields

Oleosome aqueous extraction differs from other known oil extraction procedures, such as dry-pressing [16], aqueous oil extraction [17] and aqueous enzymatic oil extraction [18]. 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 [19]–[22]. On the other hand, for the retrieval of intact oleosomes mild conditions (soaking blending, filtering) are used [5], [6], [13].

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 [23], [24]. In example, the charge profile with pH changes follows the same s-shaped pattern like seed storage

proteins [6], [13], [25], 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 [4], [6], [13]. 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 [4]. 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 [4]. The extraction yields are given in Fig. 3.2.

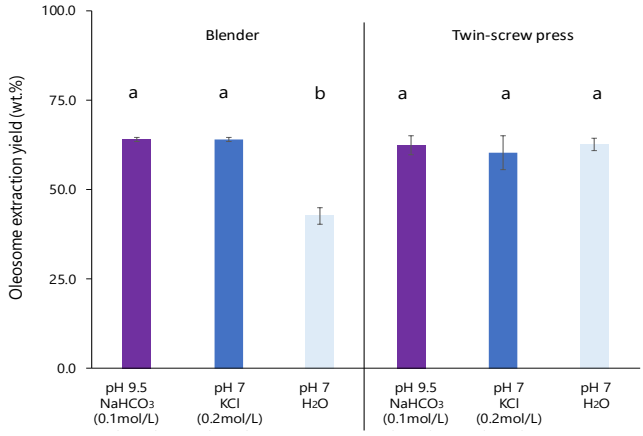

Fig. 3.2 Comparison of the yield (%) of oleosomes obtained from the extraction performed either with the twin-screw press of with the blender at either alkaline conditions (pH 9.5 $NaHCO_3$ 0.1M) or neutral conditions (pH 7 KCl 0.2 M and H_2O). An ANOVA statistical analysis was performed with a $p < 0.05$.

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 solubilisation due to the ionic environments created by pH or increased ionic strength [4],[6]. 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 [4].

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 [11]. 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).

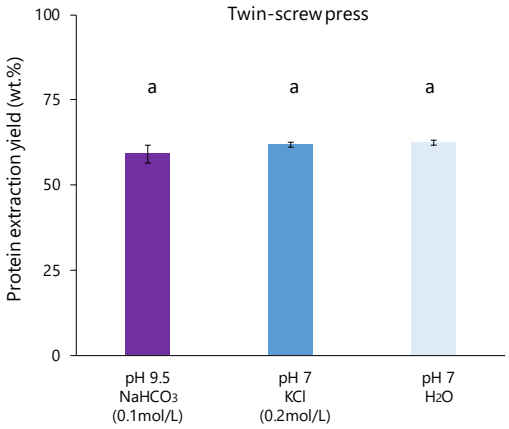

Fig. 3.3 Protein extraction yield (%) obtained with twin-screw press with each different extraction media either at alkaline (pH 9.5 $NaHCO_3$ 0.1 mol/L) or neutral conditions (pH 7 KCl 0.2 mol/L and H_2O). An ANOVA statistical analysis was performed with a $p < 0.05$.

Furthermore, to investigate the mass transfer of other components during the pressing step, the amount of extracted proteins was analysed as well (Fig. 3.3). It would be expected that the media extraction conditions (pH and ionic strength) can influence the solubilization and hence the extraction of storage proteins[26]. However, Fig. 3.3 shows that the mass transfer of the extraction is not affected by the media. The extraction of proteins is mostly mastered by the mechanical forces in the twin-screw press.

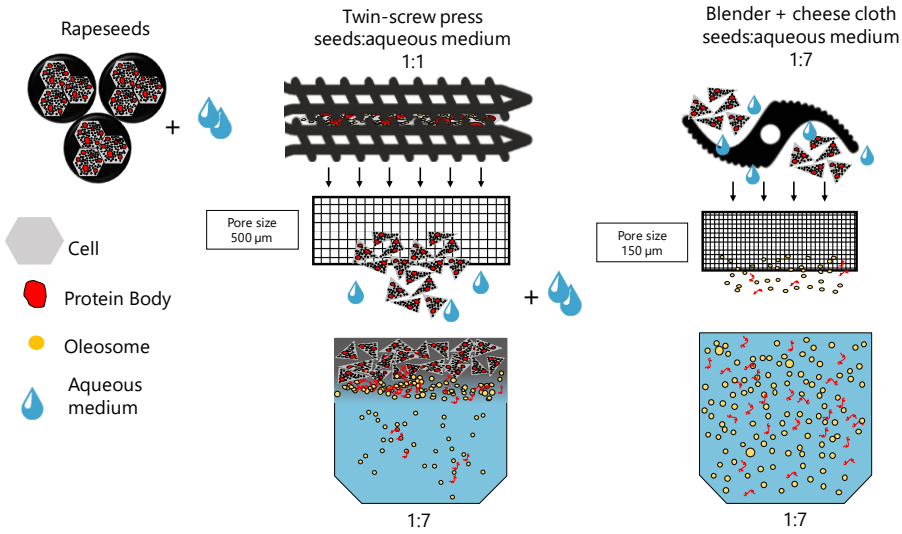

Fig. 3.4 Proposed mechanisms of extraction of oleosomes and proteins when using twin-screw press and blender extraction. A. During twin-screw press extraction, cell-lysis occurs, and cellular material is released; however, due to the limited amount of aqueous media, the solubilization of the material 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.

The similar yields obtained for all the extraction media suggested that similar cell wall breakage [7] and diffusion of cellular components [27] 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 the twin-screw press mechanism can lead to significantly lower water and chemicals use. In Fig. 3.4 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, to analyse in depth, the effect of the extraction methods on oleosome properties, the oleosomes where further isolated.

3.3.2 Effect of processing on oleosome protein interactions

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 [28]. 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.

3.3.2.1 Oleosome cream composition

Table 3.1 summarises the composition of the obtained oleosome creams, regarding oil, protein and moisture compositions.

Table 3.1 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_2O).

	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_2O	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_2O	45.0 ± 3.5^{c}	8.5 ± 2.0^{iii}	30.5± 3.3^{y}

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 pure water leads to more interactions between oleosomes and co-extracted proteins [4]. While the mechanical pressure in the twin-screw press allowed a similar extraction yield by opening the cells, it did not influence 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.

3.3.2.2 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. 3.5. Extraction at alkaline conditions using the blender gave a single strong band at ~18 kDa, indicative of oleosin[1], [29], plus some minor bands around 9 kDa. The twin-screw press at the same alkaline conditions exhibited much more diverse proteins, quite like 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 [30], [31]. 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 [32]. Similarly, napin was reduced from 14 kDa to 4 kDa and 9 kDa [33]. 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 [34].

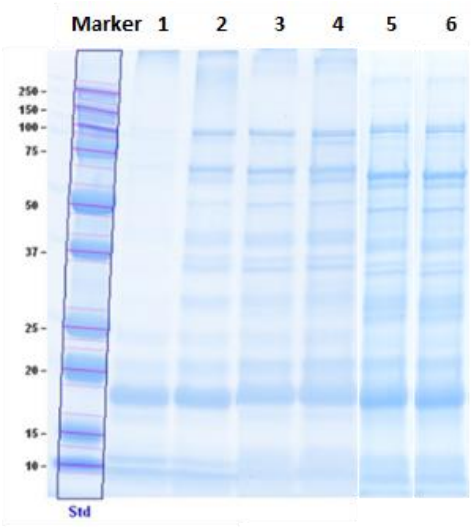

Fig 3.5 SDS-PAGE of protein extracts from isolated oleosome creams extracted at pH 9.5 with $NaHCO_3$ (0.1 mol/L) using the blender (1) or the twin screw press (2), extracted at pH 7 with KCl (0.2 mol/L) using the blender (3) or the twin-screw press (4), and at pH 7 with H_2O using the blender (5) or the twin screw press (6).

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. 3.4). For the extractions at neutral conditions (Fig. 3.5 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. 3.6). 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 [35].

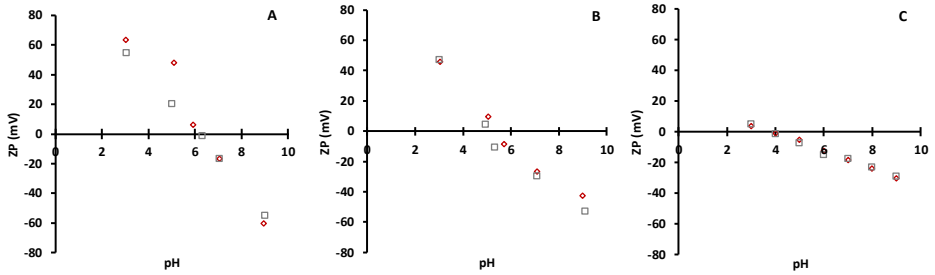

Fig. 3.6 Z-Potential of oleosome dispersions (1:100 wt./vol.) (A) extracted at pH 9.5 with $NaHCO_3$ (0.1 mol/L), (B) extracted at pH 7 with KCl (0.2 mol/L), and (C) extracted at pH 7 with H_2O with (◊) the blender or (□) the twin screw press in each case.

For the oleosomes extracted at neutral conditions with KCl (0.2 mol/L) or with H_2O, 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 [36], which can interact with the oleosome membrane [37].

3.3.2.3 Oleosome size distribution

The physical stability of the oleosome creams was investigated by analysing the particle size distribution and by microscopy (Fig. 3.7).

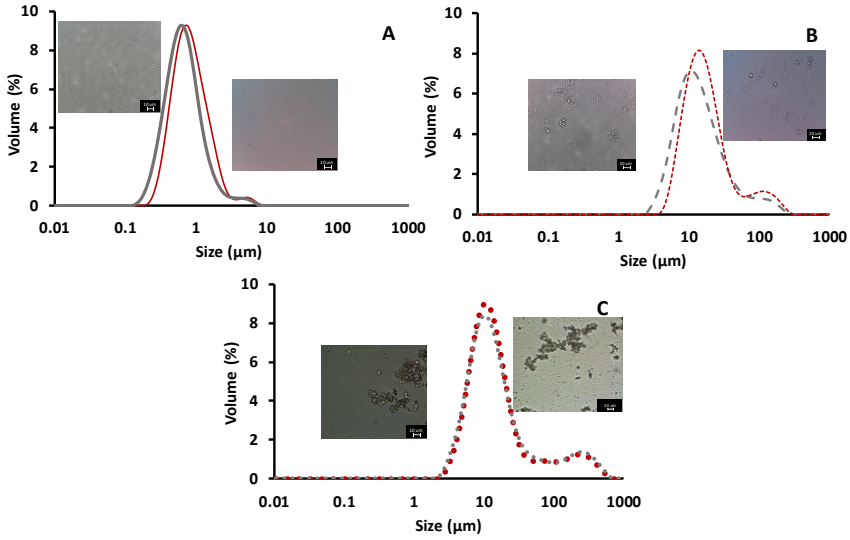

Fig. 3.7 Particle size distribution and corresponding microscopy images of washed oleosomes extracted (A) at pH 9.5 with $NaHCO_3$ (0.1 mol/L) using blender (—) or twin-screw press (—), (B) at pH 7 with KCl (0.2 mol/L) using blender (---) or twin-screw press (---) and (C) at pH 7 with H_2O using the blender (···) or the twin-screw press (···).

The oleosomes extracted at pH 9.5 (Fig. 3.7A) 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. 3.7B) 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 [4]. 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 3.1). Water is probably trapped within the aggregates due to a stronger network formation between co-extracted material and oleosomes [38].

Our overall conclusion is therefore that the oleosomes remained intact under all extraction conditions, despite 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 [39]. 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.

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

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 [40]. 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 successfully been used [41], [42]. 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 [43], [44] 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.

3.4 Conclusions

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 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_2O) 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 solubilisation 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 to further investigate the oleosome extraction mechanism in bigger scales and with different seeds.

References

[1] J. Tzen, "Integral Proteins in Plant Oil Bodies," *ISRN Bot.* 2012.

[2] J. T. Tzen and A. H. Huang, "Surface structure and properties of plant seed oil bodies.," *J. Cell Biol.* 1992.

[3] Z. Purkrtova, P. Jolivet, M. Miquel, and T. Chardot, "Structure and function of seed lipid body-associated proteins," *C. R. Biol.* 2008.

[4] M. J. Romero-Guzmán *et al.*, "The effect of monovalent (Na^+, K^+) and divalent (Ca^{2+}, Mg^{2+}) cations on rapeseed oleosome (oil body) extraction and stability at pH 7," *Food Chem.* 2020.

[5] D. Iwanaga *et al.*, "Extraction and characterization of oil bodies from soy beans: A natural source of pre-emulsified soybean oil," *J. Agric. Food Chem.* 2007.

[6] C. V. Nikiforidis and V. Kiosseoglou, "Aqueous extraction of oil bodies from maize germ (Zea mays) and characterization of the resulting natural oil-in-water emulsion," *J. Agric. Food Chem.* 2009.

[7] A. Rosenthal, D. L. L. Pyle, and K. Niranjan, "Simultaneous aqueous extraction of oil and protein from soybean: Mechanisms for process design," *Food Bioprod. Process. Trans. Inst. Chem. Eng.* 1998.

[8] A. Rosenthal, D. L. Pyle, and K. Niranjan, "Aqueous and enzymatic processes for edible oil extraction," *Enzyme Microb. Technol.* 1996.

[9] L. T. Towa, V. N. Kapchie, C. Hauck, H. Wang, and P. A. Murphy, "Pilot plant recovery of soybean oleosome fractions by an enzyme-assisted aqueous process," *JAOCS, J. Am. Oil Chem. Soc.* 2011.

[10] U. Thiyam-Hollaender, N. Eskin, and N. Michael, "*Canola and rapeseed: production, processing, food quality, and nutrition*," CRC Press, Boca Raton, Florida, USA. 2012.

[11] E. Uitterhaegen and P. Evon, Twin-screw extrusion technology for vegetable oil extraction: A review. *Elsevier.* 2017.

[12] P. Evon, V. Vandenbossche, P. Y. Pontalier, and L. Rigal, "The twin-screw extrusion technology, an original and high-performance solution for sunflower whole plant biorefinery," *OCL - Ol. Corps Gras Lipides*. 2010.

[13] S. De Chirico, V. di Bari, T. Foster, and D. Gray, "Enhancing the recovery

of oilseed rape seed oil bodies (oleosomes) using bicarbonate-based soaking and grinding media," *Food Chem.* 2018.

[14] E. W. Simon, A. Minchin, M. M. McMenamin, and J. M. Smith, "The low temperature limit for seed germination" *New Phytol.*1976.

[15] A. Valero, F. Pérez-Rodríguez, E. Carrasco, J. M. Fuentes-Alventosa, R. M. García-Gimeno, and G. Zurera, "Modelling the growth boundaries of Staphylococcus aureus: Effect of temperature, pH and water activity," *Int. J. Food Microbiol.* 2009.

[16] B. Matthäus, "Possibilities of Sustainable Oil Processing," in *Breeding Oilseed Crops for Sustainable Production: Opportunities and Constraints*. 2015.

[17] K. Campbell *et al.*, "Advances in aqueous extraction processing of soybeans," *JAOCS, J. Am. Oil Chem. Soc.* 2011.

[18] M. Mat Yusoff, M. H. Gordon, and K. Niranjan, "Aqueous enzyme assisted oil extraction from oilseeds andemulsion de-emulsifying methods: A review," *Trends in Food Science and Technology*. 2015.

[19] J. M. L. N. De Moura, K. Campbell, A. Mahfuz, S. Jung, C. E. Glatz, and L. Johnson, "Enzyme-assisted aqueous extraction of oil and protein from soybeans and cream de-emulsification," *JAOCS, J. Am. Oil Chem. Soc.* 2008.

[20] L. C. Dickey, M. J. Kurantz, and N. Parris, "Oil separation from wet-milled corn germ dispersions by aqueous oil extraction and aqueous enzymatic oil extraction," *Ind. Crops Prod.* 2008.

[21] P. Evon, V. Vandenbossche, P. Yves Pontalier, L. Rigal, P. Y. Pontalier, and L. Rigal, "Direct extraction of oil from sunflower seeds by twin-screw extruder according to an aqueous extraction process: Feasibility study and influence of operating conditions," *Ind. Crops Prod.* 2007.

[22] R. A. Moreau, D. B. Johnston, M. J. Powell, and K. B. Hicks, "A comparison of commercial enzymes for the aqueous enzymatic extraction of corn oil from corn germ," *JAOCS, J. Am. Oil Chem. Soc.* 2004.

[23] S. Maurer *et al.*, "The role of intact oleosin for stabilization and function of oleosomes.," *J. Phys. Chem. B.* 2013.

[24] B. I. Zielbauer *et al.*, "Soybean oleosomes studied by small angle neutron scattering (SANS)," *J. Colloid Interface Sci.* 2018.

[25] G. Adams *et al.*, "Extraction, isolation and characterisation of oil bodies from

pumpkin seeds for therapeutic use," *Food Chem.* 2012.

[26] R. M. Kramer, V. R. Shende, N. Motl, C. N. Pace, and J. M. Scholtz, "Toward a molecular understanding of protein solubility: Increased negative surface charge correlates with increased solubility," *Biophys. J.* 2012.

[27] C. P. Passos, S. Yilmaz, C. M. Silva, and M. A. Coimbra, "Enhancement of grape seed oil extraction using a cell wall degrading enzyme cocktail," *Food Chem.* 2009.

[28] C. V. Nikiforidis, A. Matsakidou, and V. Kiosseoglou, "Composition, properties and potential food applications of natural emulsions and cream materials based on oil bodies," *RSC Adv.* 2014.

[29] P. Jolivet *et al.*, "Oil body proteins sequentially accumulate throughout seed development in Brassica napus," *J. Plant Physiol.* 2011.

[30] H. Næsted *et al.*, "Caleosins: Ca^{2+}-binding proteins associated with lipid bodies," *Plant Mol. Biol.* 2000.

[31] L.J. Lin, S. S. K. Tai, C.-C. Peng, and J. T. C. Tzen, "Steroleosin, a sterol-binding dehydrogenase in seed oil bodies.," *Plant Physiol.* 2002.

[32] L. Zhao, Y. Chen, Y. Chen, X. Kong, and Y. Hua, "Effects of pH on protein components of extracted oil bodies from diverse plant seeds and endogenous protease-induced oleosin hydrolysis," *Food Chem.* 2016.

[33] D. J. Murphy and I. Cummins, "Biosynthesis of Seed Storage Products during Embryogenesis in Rapeseed, Brassica napus," *J. Plant Physiol.* 1989.

[34] S. Perera, T. McIntosh, and J. Wanasundara, "Structural Properties of Cruciferin and Napin of Brassica napus (Canola) Show Distinct Responses to Changes in pH and Temperature," *Plants.* 2016.

[35] J. Tzen, Y. Cao, P. Laurent, C. Ratnayake, and A. Huang, "Lipids, Proteins, and Structure of Seed Oil Bodies from Diverse Species.," *Plant Physiol.* 1993.

[36] I. Eriksson, E. Westerlund, and P. Åman, "Chemical composition in varieties of rapeseed and turnip rapeseed, including several samples of hull and dehulled seed," *J. Sci. Food Agric.* 1994.

[37] A. Tamayo Tenorio, J. Gieteling, C. V. Nikiforidis, R. M. Boom, and A. J. van der Goot, "Interfacial properties of green leaf cellulosic particles," *Food Hydrocoll.* 2017.

[38] C. V. Nikiforidis and E. Scholten, "High internal phase emulsion gels (HIPE-gels) created through assembly of natural oil bodies," *Food Hydrocoll.* 2015.

[39] D. J. McClements, *Food Emulsions: Principles, Practices, and Techniques, Second Edition*. CRC Press, Boca Raton, Florida, USA. 2004.

[40] A. Gautam and G. S. Choudhury, "Screw configuration effects on residence time distribution and mixing in twin-screw extruders during extrusion of rice flour," *J. Food Process Eng.* 1999.

[41] H. Lian, K. Luo, Y. Gong, S. Zhang, and L. Serventi, "Okara flours from chickpea and soy are thickeners: increased dough viscosity and moisture content in gluten-free bread," *Int. J. Food Sci. Technol.* 2019.

[42] G. Montemayor-Mora, K. E. Hernández-Reyes, E. Heredia-Olea, E. Pérez-Carrillo, A. A. Chew-Guevara, and S. O. Serna-Saldívar, "Rheology, acceptability and texture of wheat flour tortillas supplemented with soybean residue," *J. Food Sci. Technol.* 2018.

[43] A. C. Dave, A. Ye, and H. Singh, "Structural and interfacial characteristics of oil bodies in coconuts (Cocos nucifera L.)," *Food Chem.* 2019.

[44] J. T. C. Tzen, G. C. Lie, and A. H. C. C. Huang, "Characterization of the charged components and their topology on the surface of plant seed oil bodies," *J. Biol. Chem.* 1992.

4

Influence of soaking time on the mechanical properties of rapeseed and their effect on oleosome extraction

This chapter has been submitted as:
M. J. Romero-Guzmán, E. Vardaka, Boom, C.V. Nikiforidis. "Influence of soaking time on the mechanical properties of rapeseed and their effect on oleosome extraction" 2020.

Highlights

- Rapeseed water absorption reaches a plateau at 8 h.
- Water diffusion is negatively associated to the mechanical strength of the seeds.
- The mechanical properties of rapeseeds after 8 h soaking time are ideal for oleosome extraction.
- The extraction yields are not affected by the soaking time when using a twin-screw press.

Abstract

Oleosomes (or oil-bodies) are the oil storage structures of plant seeds. We typically extract oil by disrupting the oleosomes through mechanical-pressing of dry seeds and by using organic solvents. Nevertheless, it is possible to extract whole oleosomes, by breaking pre-soaked seeds. A key point to avoid oleosome damage seems to be the soaking step. Hence the aim of this work was to understand the effect of soaking time on the mechanical properties of rapeseed and its effect on oleosome extraction. The results showed that the diffusion of water through the seeds was negatively associated to the mechanical strength of the seeds. This effect occurs in 3 stages and reaches a plateau at 8 h. The extraction of oleosomes and proteins kept constant for all different treatments. However, the duration of the soaking step influences oleosome integrity, as oleosomes extracted after shorter soaking times (<8 h) yielded bigger oleosomes with extraneous proteins bounded to them. After 8 h soaking time, the mechanical properties of the seeds kept constant and oleosomes of native size and with oleosome endogenous proteins were recovered. Therefore, a minimum soaking time of 8 h is required to achieve the extraction of intact oleosomes.

4.1 Introduction

Oleosomes, also known as oil bodies, are the oil storage structures present in seeds such as rapeseed seeds [1]. They are composed of a core of triglycerides surrounded by a monolayer of phospholipids and proteins that render their outer surface hydrophilic [2]. Their hydrophilic interface allows their extraction using water instead of an organic solvent [3]. Moreover, due to their particular structure, oleosomes have the potential to replace the synthetic oil droplets in food emulsions, pharma and cosmetics [4].

To extract oleosomes it is necessary to soak the seeds, which it is known as the step for conditioning the seeds before the extraction. More specifically, it is believed that the mechanical properties of the material are being softened and prepared for the cell-lysis. It is expected that soaking the seeds aids the aqueous medium to solubilize the cellular material [5] and to weaken the cellular structure to reduce the stresses experienced by the oleosomes during their extraction [6]–[8].

Recently it was reported that when using a twin-screw press to perform the aqueous extraction, solubilization of cellular material played a less important role during the extraction. Even when extractions were performed with less favourable conditions (i.e. low liquid to solids ratio and water at neutral pH), the oleosome and protein extraction yields were like those obtained using optimal conditions (i.e. high liquid to solid ratio and alkaline conditions) [9]. This could mean that soaking time plays a less important role during aqueous extraction, when using twin-screw press. However, despite its relevancy, no mechanistical study has been performed before to understand the exact influence of soaking time on the extraction yield and on the characteristics of the extracted oleosome. This lack of information hinders the feasibility of the

process as the proposed soaking times for the extraction are arbitrary, lasting between 16 to 24 h, and no rules have been proposed to objectively select a soaking time [5], [6], [10]–[12].

As soaking of the seeds is such an important step for the oleosome extraction [7], [13]–[15], the aim of this work was to obtain a better understanding on the effect of soaking time on the overall performance of oleosome extraction process using twin-screw press.

4.2 Materials and Methods

4.2.1 Materials

Rapeseed seeds were kindly provided by a seed producing company. Deionized water was obtained from a Milli-Q purification system (Merck Millipore, USA). KCl was bought from Merck (Merck, Germany). Petroleum ether p.a. was obtained from Fisher Scientific (Baker Fisher Scientific, USA). All other chemicals were obtained in analytical grade from Sigma-Aldrich (St. Louis, MO, USA).

4.2.2 Water soaking experimental design

Batches of 20 rapeseeds were soaked in 20 ml of water (pH~7) for 0, 1, 4, 6, 8, 16 and 24 h. This step was conducted at 4 °C to prevent seed germination [15], [16]. The rapeseeds were taken out of the water. The adhering water was gently removed with a piece of paper before their weight was determined. Immediately after weighing, a compression test was done on the seeds.

4.2.3 Seed compression test

A compression test was performed with the TA.XT plus C Texture Analyzer (Stable Micro Systems, UK), operating with the Exponent Connect software, using a 4 mm DIA cylinder stainless probe. After having been weighed, each

seed was placed under the probe, to obtain the force (N) necessary to break the seeds. For each soaking time, 10 different seeds were used.

4.2.4 Cryo-SEM sample preparation and imaging

Three to four soaked seeds were placed together into the slit of a copper stub and fixated with carbon glue (Leit-C, Neubauer Chemikalien, Germany). The stub was attached to a cryo-sample holder, put into the cryo-sample loading system (VCT 100, Leica, Vienna, Austria) and simultaneously frozen in liquid nitrogen. The frozen sample holder was transferred to the cryo-preparation system (MED 020/VCT 100, Leica, Vienna, Austria) onto the sample stage at -92 °C. The frozen seeds were frozen fractured longitudinally and freeze-dried for about 7 min at -92°C and 5.10-6 torr. After sputter coating with 20 nm tungsten at the same temperature, the sample holder was cryo-shielded and transferred into the field emission scanning electron microscope (Magellan 400, FEI, Eindhoven, The Netherlands) onto the sample stage at -120°C and analysed with SE detection at 2 kV and 6.3 pA.

4.2.5 Extraction of oleosomes and recovery of oleosome-rich creams

All the extractions were performed in batches of 100 g of seeds. Prior to the extraction, the seeds were soaked in different aqueous media (1:1 wt./wt.). The media used were 0.1 M $NaHCO_3$ adjusted to pH 9.5, 0.2 M KCl adjusted to pH 7 or H_2O at pH 7. The soaking times were 1, 4, 8, 16 or 24 hours at 4°C. The pH was adjusted with a solution of 1.0 M NaOH for the alkaline solution and a solution of 0.1 M NaOH for the neutral solution. The pre-soaked seeds were processed with an Angel 7500 extractor (lab-scale twin-screw press). From this extraction, two streams were recovered: (1) the press cake, which was collected at the end of the press and (2) a concentrated slurry (first extract), which exuded from small holes along the device. The first

extract was diluted with $NaHCO_3$ 0.1 mol/L solution at pH 9.5, KCl 0.2 mol/L solution at pH 7, or H_2O at pH 7, to a solid to liquid ration of 1:4. The oleosomes were recovered from this dilute via centrifugation at 3,000 g and 4°C for 15 min. An oleosome rich cream layer was recovered and drained from the rest of the fluid using filter paper. The collected oleosome cream was subsequently dispersed in the same solution as was used for the dilution after the primary extraction (to 1:4 wt./v). A second centrifugation followed at 10,000 g, 4°C for 30 min (Sorval Lynx 4000 Centrifuge, Thermo Scientific USA). This cycle of recovery and dilution was performed twice. The oleosome rich cream obtained after the third centrifugation was then again drained from the fluid using filter paper and dispersed in ultra-pure water at 1:4 wt./v and centrifuged at 10,000 g, 4°C for 30 min. This obtained cream was then collected and analysed for its composition and physical properties.

4.2.6 Characterization of the solid residue after the extraction (cake) and the creams (concentrated oleosomes)

4.2.6.1 Moisture content

One gram of sample was dried with a Moisture Analyzer (Leicester, UK) at 90°C until constant weight. The drying time varied from 10 to 40 min, depending on the sample. The moisture content was determined by the weight difference between the initial weight and the weight of the dehydrated sample, relative to the weight of the original sample.

4.2.6.2 Lipid content

The lipid content was determined in duplicates by Soxhlet extraction method with petroleum ether (B-811 Buchi Extractor, Switzerland). Oleosomes contain 98% lipids, and therefore, the oleosome extraction yield was

calculated based on the difference between lipid content remaining in the cake and the initial oil (36 wt.%) content in the seeds.

4.2.6.3 Protein Content

The protein content of dry and defatted samples was calculated by determining the amount of Nitrogen in the samples using the Dumas method and using a conversion factor of 5.5 as suggested in literature [17] (Nitrogen analyser, FlashEA 112 series, Thermo Scientific, Interscience, The Netherlands). The protein extraction yield was calculated based on the difference between protein content remaining in the cake and the initial protein (18 wt. %) content in the seeds. The measurements were done in triplicates.

4.2.7 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 oil refractive index used was 1.47. To perform the analysis the oleosome cream was first dissolved in ultra-pure water at a 1:10 (wt./v.). Each measurement was done in triplicate and expressed with volumetric particle size distributions.

4.2.8 Protein profile characterization

The protein profile was analysed qualitatively with 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 [5]. 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 were combined with Buffer 1 (1:1 v./v.) 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. Afterward, 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. An amount of 20 µl of each sample was then 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). 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), and analysed with software from Bio-Rad (Bio-Rad Laboratories Inc., Hercules, USA) to quantitatively determine the optical density (%) of the different proteins present in the samples.

4.2.9 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-basis) in ultra-pure water. After the dilution, the pH of the dispersions was adjusted manually in a range of 3 to 9 with either HCl (1.0 mol/L) or NaOH (1.0 mol/L) solution. The refractive indices used were 1.43 for the dispersed phase and 1.33 for the continuous phase.

4.2.10 Statistical analysis

A one-way analysis of variance (ANOVA) and a LSD post-hoc significance test were applied to find differences in the extraction yields between different soaking times. The analyses were performed with the IBM SPSS statistics 23 software. Differences were significant when $p < 0.05$.

4.3 Results and discussion

4.3.1 Seed mechanical properties and seed changes during soaking

Fig. 4.1 shows the weight increase and mechanical properties of the individual rapeseeds upon soaking. The mechanical properties were evaluated during a compression test as the maximum force that could be exerted on a seed without breaking it.

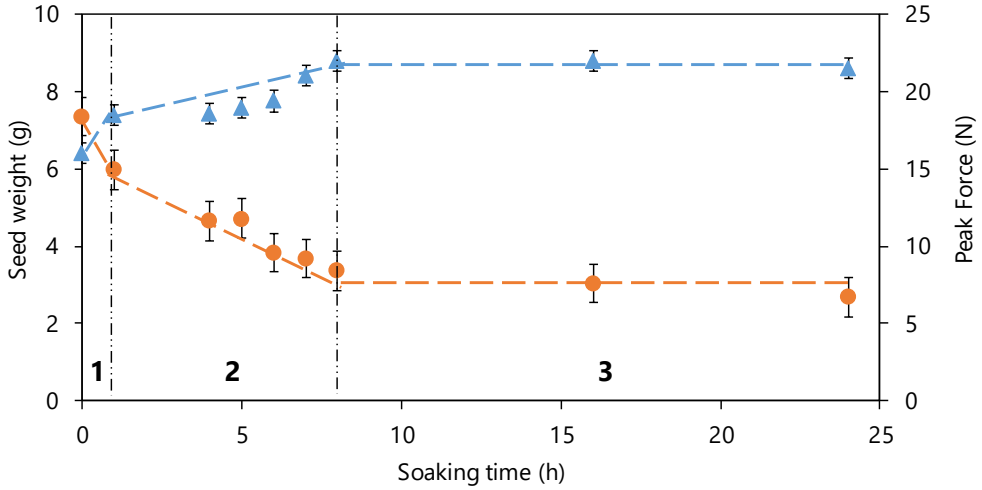

Fig. 4.1 Effect of the different soaking times on the rapeseed weight (g) (▲) and peak force (N) (●) or necessary force to break a rapeseed. Three different stages of water absorption and modifications of the strength of the material are observed. (1) A steep increase in water content and loss of strength. (2) A less steep increase of water content and loss of strength. (3) A plateau region with no variation in seed weight or seed mechanical properties.

The weight of individual dry rapeseeds started at 6 g ±1.3 g and increased to 7.4 g ±1.7 g during the first hour. The slope of the weight increase was less pronounced between 1 h and 8 h. At 8 h, a plateau at 8.8 ±1.4 g was reached. Similar patterns were previously reported for chickpeas and amaranth [18], [19]. The slow diffusion of water is due to the occurrence of complex mass transfer phenomena. The influx of water into the cells leads to the

solubilization of cell components. This reduces the concentration gradients of solutes between the cytosol and the media, slowing down the diffusion of water into the cells [18]. In the reported cases, similar as in Fig. 4.1, three stages in the water absorption were identified and related with the following phenomena: (1) A steep increase in weight (from 0 to 1 h) was associated with the diffusion of water through larger capillaries between different parts of the seeds. (2) A less pronounced slope of weight increase (from 1 to 8 h), which was associated with the diffusion of water between the capillaries and inter-cellular spaces on the seed coat (i.e. hilum) [18]. Finally, (3) a plateau (from 8 h onwards), which starts when the water absorption matched the counter-diffusion of solutes from the seeds into the water [8]. In other words, when solutes from the cells diffused into the water at a similar rate than water diffuses inside the seeds.

Interestingly the results of the compression test perfectly followed the same trend of the seed weight increase (Fig. 4.2). As it has been reported, the hardness of the cellulose-rich materials is strongly related to their moisture content [20][21]. At low moisture content a significant part of the matrix components may be in glassy state, thus exhibiting high rigidity. The absorption of water, on the other hand, may bring the amorphous parts between the (cellulose) crystals in the rubbery state, and ensure higher flexibility of the matrix. At the same time, cellulosic parts, still retain their coherence, due to the local crosslinking of the crystalline domains [22]. The gradual solubilization and extraction of other components such as pectin, proteins, and ions may also lead to softening of the structure. Especially as most of these soluble components are constituents of the middle lamella [14], which is identified as the cohesive material between cell-walls [23].

Recently it was reported that the softening of other plant material, such as beans during cooking, is related to the weakening of the middle lamella due to the solubilization of its polysaccharides [24], [25]. Hence, to correlate the effect of water absorption and mechanical properties with the microstructure of the rapeseeds, the physiological changes of the rapeseed microstructure were monitored using cryo-SEM. The micrographs of the seeds are depicted in Fig. 4.2.

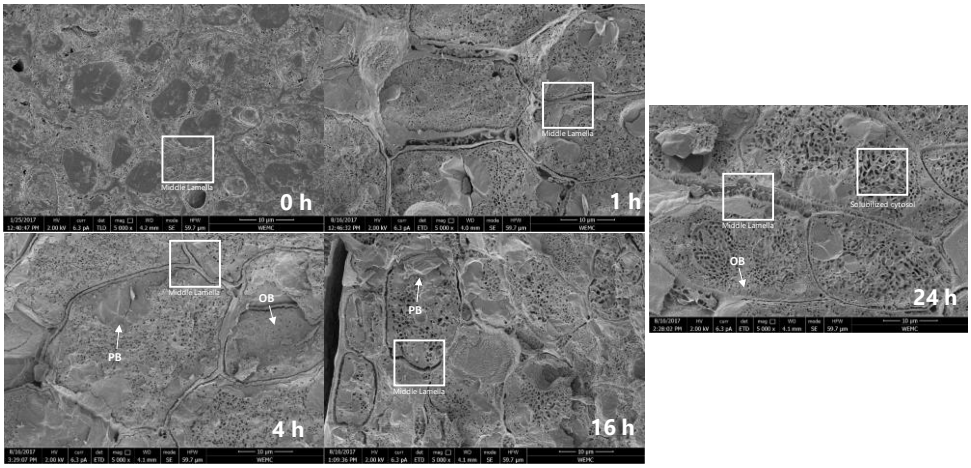

Fig. 4.2 Cryo-SEM images of the rapeseeds soaked for different amounts of time. The abbreviations OB and PB stand for oil bodies (or oleosomes) and protein bodies. The hollow squares indicate the middle lamella.

From the micrographs we can see that separation between cells occurred after the first hour of soaking time, and kept increasing over time. Moreover, the threads of a viscous material, which we believe correspond to the middle lamella, became more evident over time. Additionally, the density of the cellular structure also seems to increase as more gaps are observed in the cells. All these changes are positively related to soaking time. We believe that the observed gaps between cellular components corresponded to water crystals that were formed during the pre-preparation of the samples for cryo-SEM.

These gaps are more abundant as the amount of water inside the cells increases, even when the weight of the seeds reaches a plateau. Hence we believe that these gaps are also related with the solubilization of cellular material [26]. Similar results, where the soaking time is strongly related with the solubilization of cellular material, have been reported for other materials such as chickpeas [18].

Overall, the images are in line with the proposed mechanism for water diffusion and solubilization of components, previously described in Fig. 4.1. The images confirm that the migration of water inside the cells firstly affects the middle lamella and secondly it solubilizes the cellular material.

4.3.2 Effect of soaking time on the extraction yields

To determine the effect of soaking media and the seed softening on the release of intracellular oleosomes and proteins, the oleosome and protein extraction yields were determined for 3 different extraction media (0.1 mol/L $NaHCO_3$ at pH 9.5, 0.2 mol/L KCl at pH 7 and ultra-pure water at pH 7). Previously it was reported that when using twin-screw press, no significant difference was found on oleosome and protein yields between different extraction media [34]. However, in that work the effect of the soaking time was not depicted. Hence, similar aqueous media in combination with different soaking times were used to perform the extraction employing a twin-screw press. The results are shown in Fig. 4.3

Fig. 4.3 shows that there is no statistically significant difference between the oleosome and protein yields recovered after different soaking times for any of the soaking media. This indicates that, unlike the extraction using a blender, the moisture content of the seeds (and seed hardness) nor pH lead the

oleosome extraction, when using a twin-screw press. In our previous work, it was concluded that the shear forces inside the press mastered the extraction and that the solubilization effect of the different soaking media was superfluous. The same seems to hold for the different soaking times and states of the material. The similar yields obtained for all the treatments could be explained by the fact that friction inside the twin-screw press is favoured by the presence of dry material [27]. The increase in friction is desired as it increases the shear forces created in the screws and as consequence, it enhances the extraction of material. Even though the solubilization of cellular material enhances oleosome and protein extraction, it is negatively related to mechanical seed strength [28].

Therefore we hypothesized that the effect of solubilization of cellular material on yield is partly offset by this loss of shear forces associated with the mechanical strength of the seeds. The counterproductivity of both phenomena results in the extraction being unaffected past 8h soaking time.

Due to the reduction of the friction inside the screws, resulting in the reduction of the dry mass, the overall extraction seems to be unaffected as both phenomena are counterproductive.

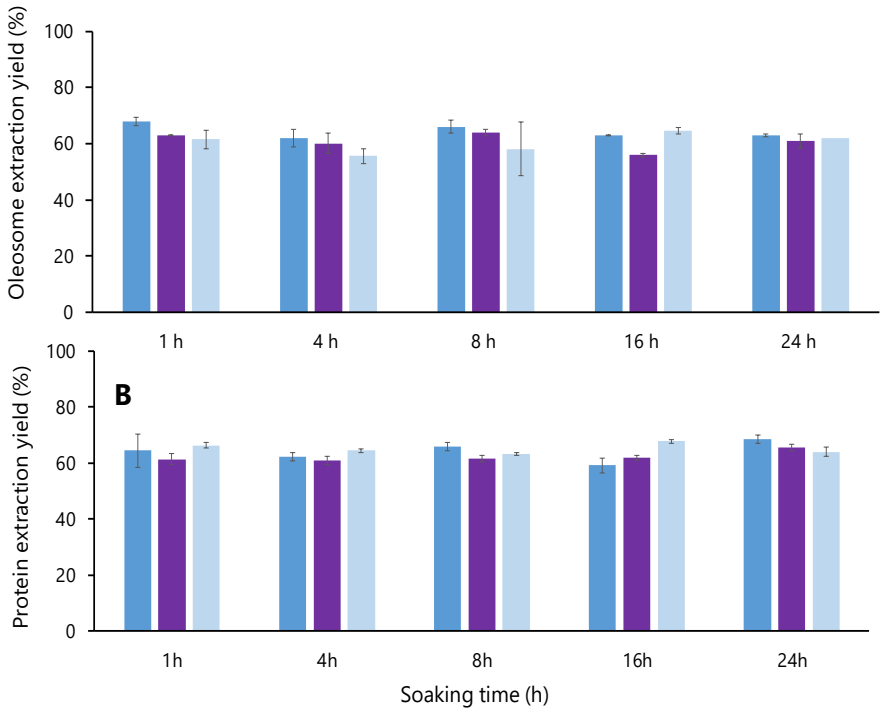

Fig. 4.3 Oleosome extraction yield (A) and protein extraction yield (B). The extractions were performed with 0.1 M $NaHCO_3$ at pH 9.5 (■), with 0.2 M KCl at pH 7 (■) and with ultra-pure water at pH 7 (■).

4.3.3 Oleosome characterization

To assess the quality of the extracted oleosomes at different soaking times, the oleosomes were recovered and their particle size distribution was characterized (Fig. 4.4).

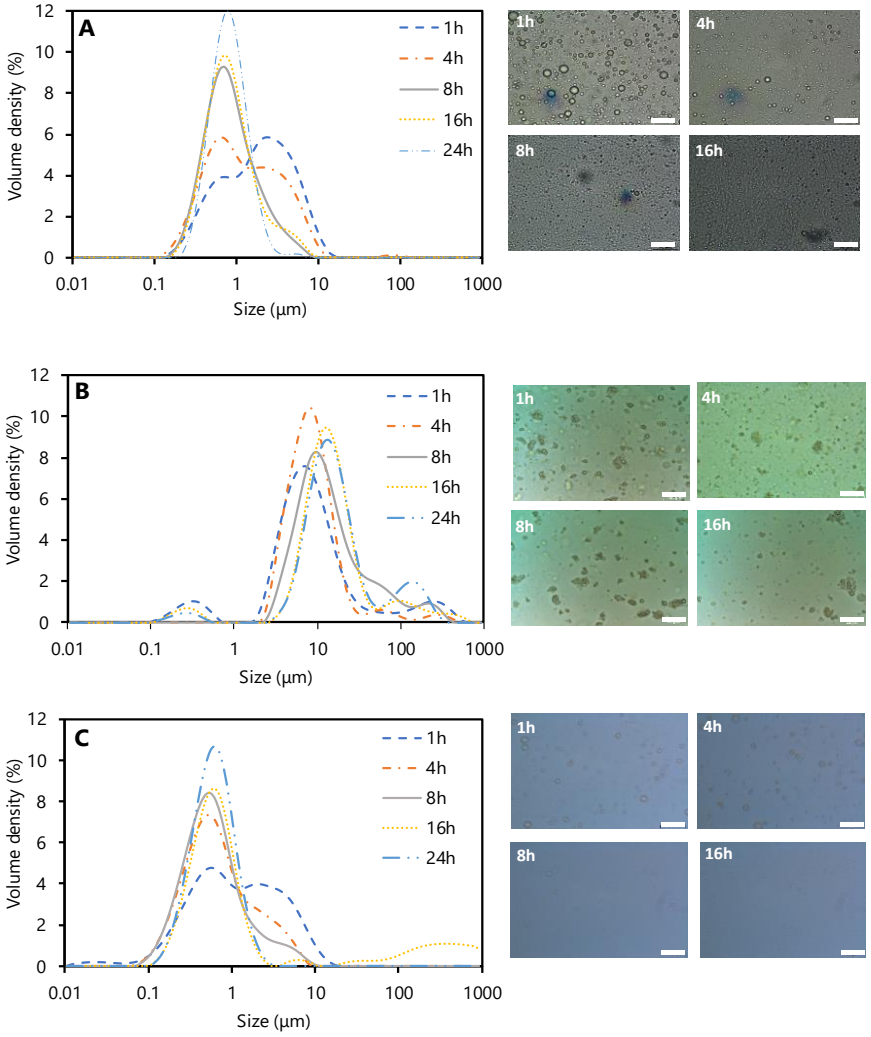

Fig. 4.4 Particle size distribution and micrograph of oleosome diluted creams recovered at (A) pH 9.5 with $NaHCO_3$ 0.1 mol/L, (B) at pH 7 with H_2O and (C) at pH 7 with H_2O and 1 wt.% SDS. Scale bar 20 µm.

The oleosomes recovered at alkaline conditions were extracted as individual droplets while those obtained with water at pH 7 were aggregated. This effect was reported before as an effect of the charge of the proteins that are associated with the surface of the oleosomes [6], [29], [30]. The oleosomes extracted at alkaline conditions (Fig. 4.4A) after short soaking times (1 h and

4 h) showed evidence of significant aggregation and coalescence, while those extracted after longer soaking times (at 8 h, 16 h and 24 h) mostly consisted of individual oleosomes. In the case of oleosomes extracted with water at neutral pH, the different soaking times did not affect the aggregation behaviour, as they remained aggregated, even when soaked for 24 h. To break the electrostatic interactions holding these aggregated oleosomes extracted at neutral pH, 1 wt.% of SDS was added during the dilution of the primary extract. In Fig. 4.4C, we can see that the addition of SDS efficiently broke the aggregates yielding individual oleosomes [29]. These oleosomes were like those obtained at alkaline conditions (0.1 mol/L $NaHCO_3$ at pH 9.5). Moreover, we see that the oleosomes recovered at neutral conditions after 1 and 4 h soaking was also enlarged in comparison with those recovered after 8 h or longer soaking times. Previously it was reported that the co-extracted material interacting with the oleosomes could act as a shield and protect oleosomes against coalescence during centrifugation [5], [31]. Hence, we concluded that the enlarged oleosomes recovered at shorter soaking times at either alkaline or neutral conditions were enlarged during the extraction and not during the centrifugation step. We hypothesized that probably as the cellular material was still in glassy state during the extraction (making it more brittle), the shear forces produced during the extraction may damage the oleosomes.

Overall, while the yields of oleosomes and proteins were as high at all soaking times, the seeds soaked for less than 8h, recovered damaged oleosomes. The incomplete hydration during short soaking times let the protein-oleosome cohesion in the cells intact, leading to brittle oleosomes, that may lose part of their native surrounding mono-layer during the extraction. At longer soaking times, all the cell material is hydrated and hence more flexible, leading to the

release of intact oleosomes. To proof this hypothesis, we characterized the protein profile of pure oleosomes (those extracted at alkaline conditions) as it can be seen in Fig. 4.5.

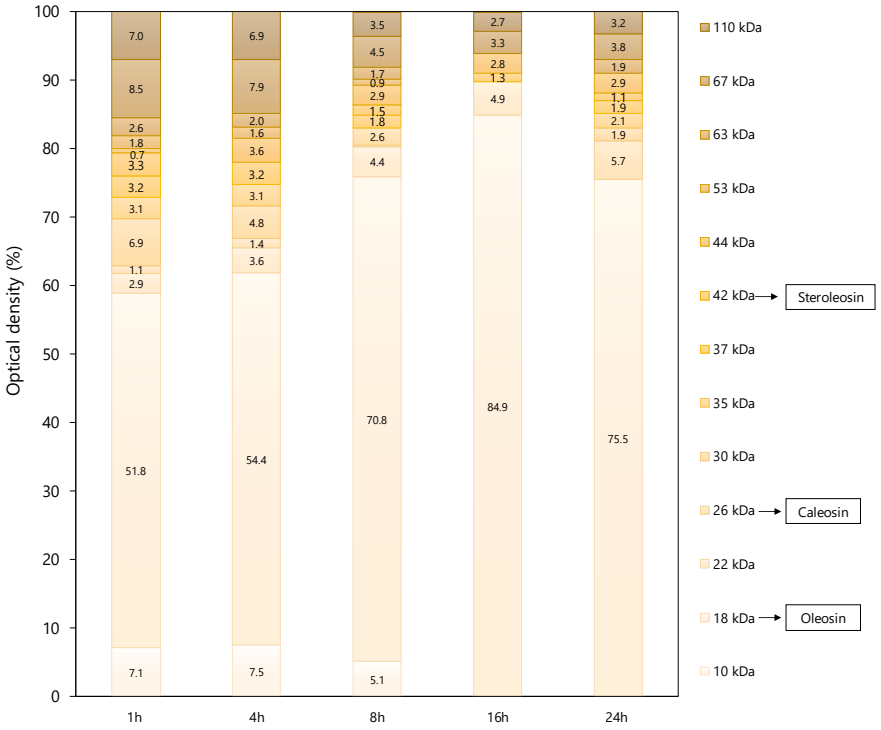

Fig. 4.5 Interfacial composition of the oleosomes obtained after different soaking times, determined with SDS-PAGE densitometry.

Mostly the three oleosome associated proteins [32]: oleosin (18 kDa), caleosin (26 kDa) and steroleosin (42 kDa) are attached to the oleosome interface. However, at 1h and 4 h there are more non-oleosome associated proteins present. These extraneous proteins are those very abundant rapeseed storage proteins, such as napin (10 kDa) [33]. Therefore, these results support the hypothesis that at short soaking times, oleosomes are damage and surface

active extraneous material attaches to their interface, creating hybrid oleosomes [6].

4.4. Conclusion

We can conclude that the migration of the water molecules inside the seeds affects the mechanical properties of the seeds. This effect is due to the interactions of the cellulosic materials with water, in combination with the solubilization and leaching of cellular material into the soaking fluid. Water diffusion, leads to solubilization of the middle lamella and extraction of solutes from the cytosol. These effects take place gradually and reach a plateau after about 8 h.

The changes in the mechanical properties and solubilization of the material are counter-effective when operating a twin-screw press. Dry material aids the friction generated inside the screws, and hence the extraction yields. Based on that statement, the short solubilization times, would favour the oleosome extraction; however, the solubilization of material happens at longer soaking times, which also favours the extraction of cellular material. Therefore, we concluded that there is a counteracting effect of these two phenomena, which results in similar oleosome and protein extraction yields at all soaking times.

The use of pH 9.5 aqueous media releases individual, non-aggregated oleosomes, while the use of water (pH 7) results on the extraction of aggregated oleosomes, even after 24h soaking time. These aggregates are broken up when using a 1wt% SDS solution, showing that the aggregates are relatively easily dissociated as they are hold by electrostatic interactions. Regarding the effect of soaking time on the quality of oleosomes, it was concluded that shorter soaking times than 8h lead to larger aggregates and

damage oleosomes, with significantly more non-associated oleosome proteins bounded to their interface. These results are independent from the aqueous media used during the extraction.

Overall, we can conclude that when using twin-screw press short soaking times, such as 1 h, can be used without affecting the extraction yield. Nevertheless, at least 8h soaking time are necessary to achieve the right mechanical properties of the seeds, which are vital to the recovery of intact oleosomes.

References

[1] P. Jolivet *et al.*, "Protein composition of oil bodies from mature Brassica napus seeds," *Proteomics*. 2009.

[2] S. Maurer *et al.*, "The role of intact oleosin for stabilization and function of oleosomes.," *J. Phys. Chem. B*. 2013.

[3] C. V. Nikiforidis, A. Matsakidou, and V. Kiosseoglou, "Composition, properties and potential food applications of natural emulsions and cream materials based on oil bodies," *RSC Adv.* 2014.

[4] C. V. Nikiforidis, "Structure and functions of oleosomes (oil bodies)," *Adv. Colloid Interface Sci.* 2019.

[5] C. V. Nikiforidis and V. Kiosseoglou, "Aqueous extraction of oil bodies from maize germ (Zea mays) and characterization of the resulting natural oil-in-water emulsion," *J. Agric. Food Chem.* 2009.

[6] S. De Chirico, V. di Bari, T. Foster, and D. Gray, "Enhancing the recovery of oilseed rape seed oil bodies (oleosomes) using bicarbonate-based soaking and grinding media," *Food Chem.* 2018.

[7] C. M. Chigwedere, D. M. Njoroge, A. M. Van Loey, and M. E. Hendrickx, "Understanding the Relations Among the Storage, Soaking, and Cooking Behavior of Pulses: A Scientific Basis for Innovations in Sustainable Foods for the Future," *Comprehensive Reviews in Food Science and Food Safety*. 2019.

[8] S. Sayar, M. Turhan, and S. Gunasekaran, "Analysis of chickpea soaking by simultaneous water transfer and water-starch reaction," *J. Food Eng.* 2001.

[9] M. J. Romero-Guzmán, L. Jung, K. Kyriakopoulou, R. M. Boom, and C. V. Nikiforidis, "Efficient single-step rapeseed oleosome extraction using twin-screw press," *J. Food Eng.* 2020.

[10] K. a Campbell and C. E. Glatz, "Mechanisms of aqueous extraction of soybean oil.," *J. Agric. Food Chem.* 2009.

[11] D. Iwanaga *et al.*, "Extraction and characterization of oil bodies from soy beans: A natural source of pre-emulsified soybean oil," *J. Agric. Food Chem.* 2007.

[12] Y. Yoshie-Stark, Y. Wada, and A. Wäsche, "Chemical composition, functional properties, and bioactivities of rapeseed protein isolates," *Food Chem.* 2008.

[13] G. Waschatko *et al.*, "Label-free in situ imaging of oil body dynamics and chemistry in germination.," *J. R. Soc. Interface*. 2016.

[14] S. H. Yiu, H. Poon, R. G. Fulcher, and I. Altosaar, "The Microscopic Structure and Chemistry of Rapeseed and its Products," *J. Food Struct. J. Food Struct. Food Microstructure*. 1982.

[15] C. E. Carter and F. A. Manthey, "Seed treatments affect milling properties and flour quality of black beans (Phaseolus vulgaris L.)," *Cereal Chem.* 2019.

[16] F. Beisson, N. Ferté, S. Bruley, R. Voultoury, R. Verger, and V. Arondel, "Oil-bodies as substrates for lipolytic enzymes," *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids*. 2001.

[17] E. W. Simon, A. Minchin, M. M. McMenamin, and J. M. Smith, "The low temperature limit for seed germination" *New Phytol.* 1976.

[18] N. Lindeboom and P. K. J. P. D. Wanasundara, "Interference of phenolic compounds in Brassica napus, Brassica rapa and Sinapis alba seed extracts with the Lowry protein assay," *Food Chem.* 2007.

[19] S. Johnny, S. M. A. Razavi, and D. Khodaei, "Hydration kinetics and physical properties of split chickpea as affected by soaking temperature and time," *J. Food Sci. Technol.* 2015.

[20] A. N. C. Resio, R. J. Aguerre, and C. Suarez, "Analysis of simultaneous water absorption and water-starch reaction during soaking of amaranth grain," *J. Food Eng.* 2005.

[21] S. Sun *et al.*, "Comparison of the mechanical properties of cellulose and starch films," *Biomacromolecules*. 2010.

[22] A. Chami Khazraji and S. Robert, "Interaction effects between cellulose and water in nanocrystalline and amorphous regions: A novel approach using molecular modeling," *J. Nanomater.* 2013.

[23] H. O'Neill *et al.*, "Dynamics of water bound to crystalline cellulose," *Sci. Rep.*, 2017.

[24] F. B. Daher and S. A. Braybrook, "How to let go: pectin and plant cell adhesion," *Front. Plant Sci.* 2015.

[25] C. M. Chigwedere *et al.*, "Mechanistic insight into softening of Canadian wonder common beans (Phaseolus vulgaris) during cooking," *Food Res. Int.* 2018.

[26] D. N. Sila, C. Smout, S. T. Vu, A. Van Loey, and M. Hendrickx, "Influence of pretreatment conditions on the texture and cell wall components of carrots during thermal processing," *J. Food Sci.* 2005.

[27] E. R. Budiaman and O. Fennema, "Linear Rate of Water Crystallization as Influenced by Viscosity of Hydrocolloid Suspensions," *J. Dairy Sci.* 1987.

[28] P. Evon, V. Vandenbossche, P. Yves Pontalier, L. Rigal, P. Y. Pontalier, and L. Rigal, "Direct extraction of oil from sunflower seeds by twin-screw extruder according to an aqueous extraction process: Feasibility study and influence of operating conditions," *Ind. Crops Prod.* 2007.

[29] A. Matsakidou, F. T. Mantzouridou, and V. Kiosseoglou, "Optimization of water extraction of naturally emulsified oil from maize germ," *LWT - Food Sci. Technol.* 2015.

[30] M. J. Romero-Guzmán *et al.*, "The effect of monovalent (Na^+, K^+) and divalent (Ca^{2+}, Mg^{2+}) cations on rapeseed oleosome (oil body) extraction and stability at pH 7," *Food Chem.* 2020.

[31] J. T. Tzen and A. H. Huang, "Surface structure and properties of plant seed oil bodies.," *J. Cell Biol.* 1992.

[32] C. V. Nikiforidis, S. Donsouzi, and V. Kiosseoglou, "The interplay between diverse oil body extracts and exogenous biopolymers or surfactants," *Food Res. Int.* 2016.

[33] Z. Purkrtova, P. Jolivet, M. Miquel, and T. Chardot, "Structure and function of seed lipid body-associated proteins," *C. R. Biol.* 2008.

[34] J. P. D. Wanasundara, T. C. McIntosh, S. P. Perera, T. S. Withana-Gamage, and P. Mitra, "Canola/rapeseed protein-functionality and nutrition," *OCL - Oilseeds fats, Crop. Lipids*. 2016.

5

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

This chapter has been published as:
M. J. Romero-Guzmán, N. Köllmann, L. Zhang, R. M. Boom, and C. V. Nikiforidis, "Controlled oleosome extraction to produce a plant-based mayonnaise-like emulsion using solely rapeseed seeds, " LWT. 2020.

Highlights

- An innovative production process for vegan mayonnaise is presented.
- Plant-based mayonnaise is extracted solely from rapeseeds.
- No emulsification steps were needed.
- The number of purification steps affects the oleosome aggregation behaviour.
- The oil content on the oleosome cream could be controlled by the pH of extraction.

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 fibres. 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 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.

5.1 Introduction

Oleosomes (also known as oil bodies) are micron-sized (0.3 to 4.0 μm) lipid storage structures found abundantly in oleaginous seeds such as rapeseed seeds [1]. These lipid structures also present in nuts in which sizes up to 20 μm have been reported [2]. Oleosomes consist of a triglycerides (TAGs) core, surrounded by a mixed monolayer of phospholipids and proteins such as oleosin, caleosin and steroleosin [3]–[6]. The arrangement of these proteins at oleosomes interface renders them hydrophilic, and therefore they can be extracted using water as extraction medium [7], [8]. Due to their structure, oleosomes can potentially replace any kind of manufactured oil droplets broadly used [9]. For example the use of intact oleosomes was already suggested for dressings, sauces, dips, beverages, and desserts [10] and specially as emulsifiers [11]–[13].

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 [14]. This extraction yields an emulsion that still contains exogenous storage proteins and cell wall components such as fibres 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 [15]–[17].

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 [18]–[20], 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 [19]. Hence, those parameters can be used to adapt the properties of the natural emulsion towards a specific application, instead of adapting the properties by 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 [21], [22]. Recently, it has been discovered one can adapt the properties of these natural emulsions by tuning their interactions with the co-extracted material [19]. 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 [23], [24]. Mayonnaise shows a semisolid and viscoelastic behaviour, also known as plastic [23]. Many emulsion characteristics such as droplet size and the interactions between droplets play an important role in its viscoelastic behaviour [24]. Therefore rheological characterization is a common quality control parameter of mayonnaise [25].

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.

5.2 Materials and Methods

5.2.1 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 ± 1.2 g /100 g, oil 36.0 ± 1.3 g/100 g, and protein 18.0 ± 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.

5.2.2 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 $NaHCO_3$ (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 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 $NaHCO_3$ 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.

5.2.2.1 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 was subsequently dispersed in water (1:3 wt./v) and the pH was adjusted to 3.8 with CH_3COOH 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.

5.2.2.2 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 wt./v, the pH of this dispersion was also adjusted to 3.8 with CH_3COOH 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 $NaHCO_3$ 0.1 mol/L is shown in Fig. 5.1.

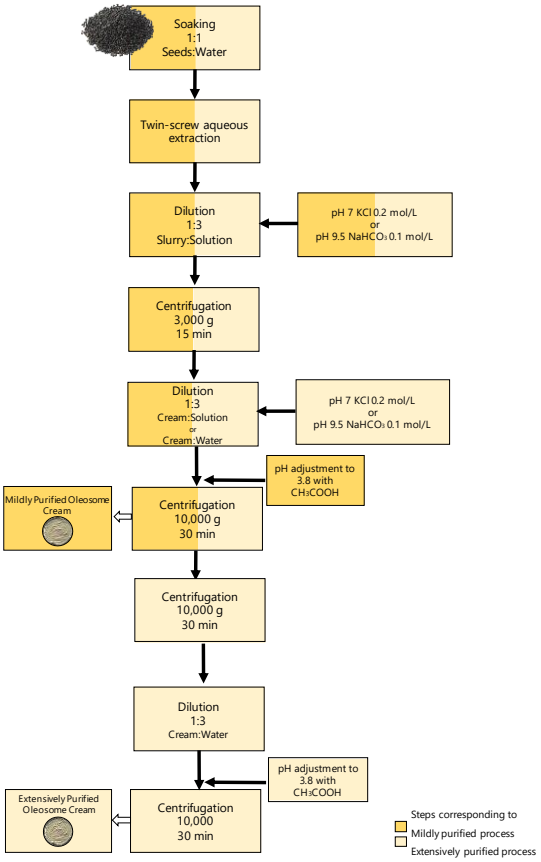

Fig. 5.1 Scheme for the recovery of concentrated oleosome creams either mildly purified (■) or extensively purified (□), extracted at pH 7 with KCl 0.2 mol/L or pH 9.5 with $NaHCO_3$ 0.1 mol/L.

5.2.3 Characterization of the streams

5.2.3.1 Moisture content

To determine the moisture content of the oleosome creams, 1 g of sample was dried in a Moisture Analyzer (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.

5.2.3.2 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.

5.2.3.3 Protein Content

The protein content of dry and defatted samples was determined using the Dumas method. The % N_2 was quantified; a conversion factor of 5.5 [26] was used to convert it to protein %. All analyses were performed in duplicate.

5.2.4 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 [8]. 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.), were combined with Buffer 1 (1:1 v./v.) 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).

5.2.5 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 to 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.

5.2.6 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 (wt./v). Each sample was measured in triplicate. The distributions were expressed in volumetric particle size distributions.

5.2.7 CLSM microscopy

The creams were diluted in ultra-pure water at 1:100 (v/v). 1% v/v 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.

5.2.8 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.

5.2.9 Statistical 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 $p < 0.05$.

5.3 Results and discussion

5.3.1 Oleosome cream composition

The oleosomes were extracted with two different aqueous media: (1) at pH 9.5 $NaHCO_3$ 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. 5.1. Abbreviations were assigned for all the treatments as follows: 9.5MP (extracted at pH 9.5 $NaHCO_3$ 0.1 mol/L with mild purification), 9.5EP (extracted at pH 9.5 $NaHCO_3$ 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. 5.2.

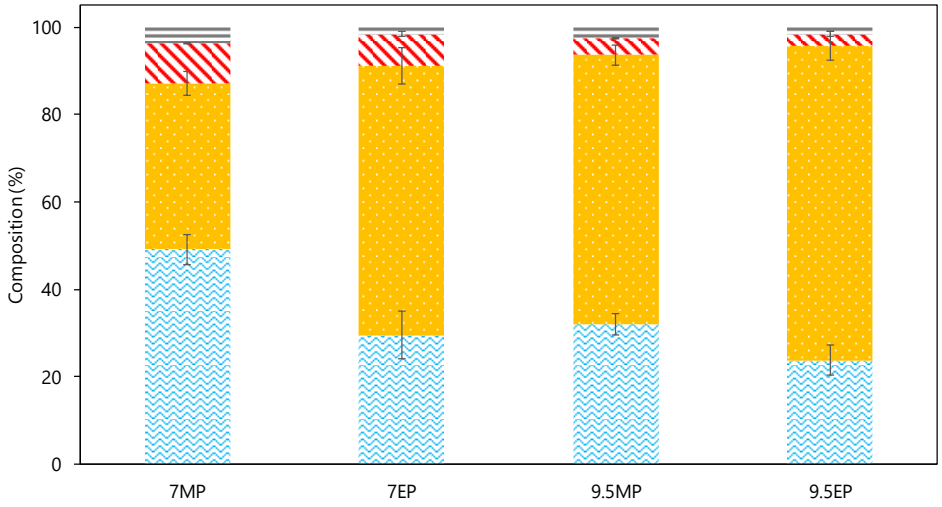

Fig. 5.2 Composition of the oleosome creams obtained with KCl 0.2 mol/L at pH 7 mildly purified (7MP) and extensively purified (7EP) and with $NaHCO_3$ 0.1 mol/L at pH 9.5 mildly purified (9.5 MP) and extensively purified (9.5EP). Each of the components is indicated as follows: Water ( ), Oil ( ), Protein ( ) and Others ( ).

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 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 [27], [28]. 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 [29]. 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 [30] and hence, the repulsion between oleosomes and co-extracted materials is enhanced. This repulsion favours their separation, increasing the oleosome purity [15]. 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 [19]. 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.

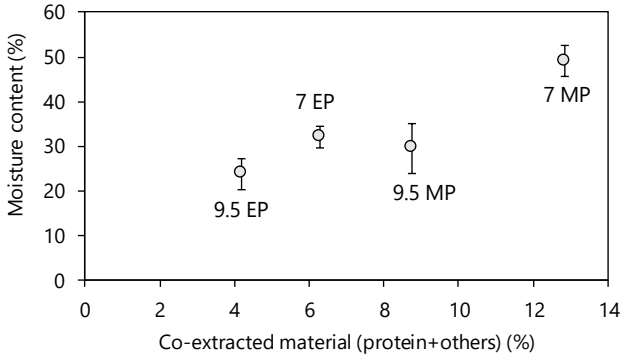

Fig 5.3 Relation between the percentage of co-extracted material and the moisture content of the different oleosome creams.

Furthermore, a clear relation between the moisture content and co-extracted material was observed (Fig. 5.3). The water holding capacity of plant proteins is typically between 2 to 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 [31]. 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 [32]. 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 [33].

5.3.2 Oleosome interfacial proteins and surface charge

The electrophoretic patterns of the 4 different creams are presented in Fig. 5.4. Besides the oleosome surface proteins, co-extracted storage proteins were present as well. As it has been previously reported [15] 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 [19]. 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 [19]. The storage proteins are cruciferin (globulin) and napin (albumin) [34]. These proteins are in situ in structures named protein bodies and they represent around 70 wt.% of the total amount of proteins present in the rapeseed [35].

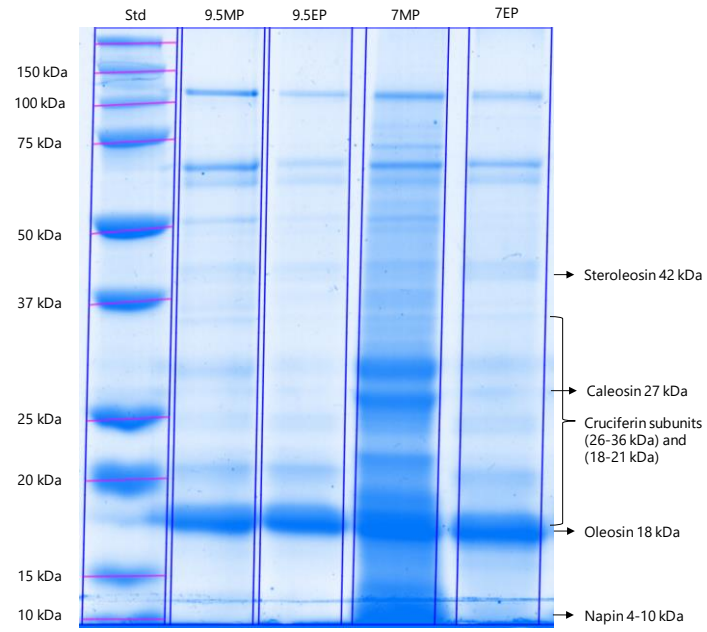

Fig. 5.4 SDS-PAGE of the different oleosome creams obtained with $NaHCO_3$ 0.1 mol/L at pH 9.5 after a mild purification 9.5MP (1) or an extensive purification 9.5EP (2) and with KCl 0.2 mol/L at pH 7 after a mild purification 7MP (3) or an extensive purification 7EP (4).

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 [36], [37].

Regarding the effect of the different treatments, in case of the creams extracted at alkaline conditions (9.5MP and 9.5EP) (Fig. 5.4 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. 5.4 bands 3 and 4). In the extensively purified cream 7EP (Fig. 5.4 band 4) mostly oleosins are observed (~18 kDa),

while storage proteins can be observed in the mildly purified cream 7MP (Fig. 5.4 band 3). Interestingly, the steroleosin and caleosin bands are stronger in the mildly purified creams 9.5MP and 7MP (Fig. 5.4 bands 1 and 3), which suggests that also these mixed monolayer-bound proteins were partially removed during the purification process [38]. This, could be explained due to the effect of cations on the oleosome mixed monolayer, which was previously studied [19]. 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.

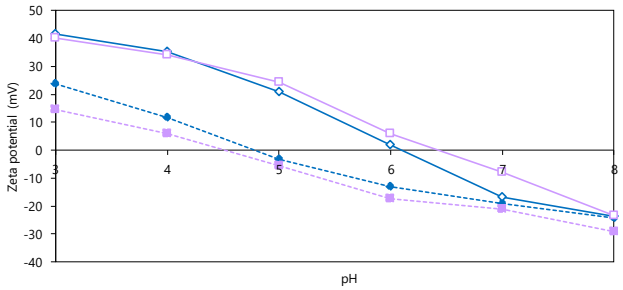

Fig. 5.5 Dependence upon pH of the ζ-potential of the oleosome creams obtained with KCl 0.2 mol/L at pH 7 extensively purified 7EP (-◊-) and mildly purified 7MP (--●--) and with $NaHCO_3$ 0.1 mol/L at pH 9.5 extensively purified 9.5EP (-□-) and mildly purified 9.5MP (--■--).

The zeta-potential measurements (Fig. 5.5) 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 [39]. 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) [15], [30], 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.

5.3.3 Oleosome size distribution and microstructure

The oil droplet size distribution (Fig. 5.6) has a big impact on the rheological properties of the final product [23]. 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 [40], [41]. 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 to 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 [19]; hence, both mildly purified samples had most of their oleosomes aggregated. Remarkably is that the amount of extraneous material (Fig. 5.2) was not relevant for their aggregation behaviour (Fig. 5.6).

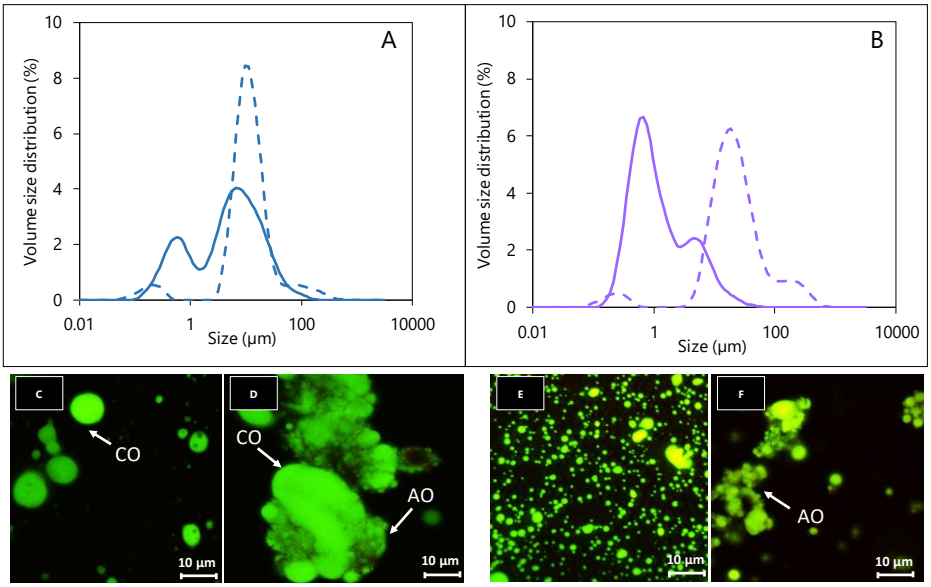

Fig. 5.6 Particle size distribution of the oleosome creams obtained with (A) KCl 0.2 mol/L at pH 7 mildly purified 7MP (---) and extensively purified 7EP (—) and with (B) $NaHCO_3$ 0.1 mol/L at pH 9.5 mildly purified 9.5MP (---) and extensively purified 9.5EP (—). Confocal images of oleosome creams are: (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 which represent the oil phase. Scale bar 10 µm.

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 [42].

5.3.4 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 5.1) (Fig. 5.7).

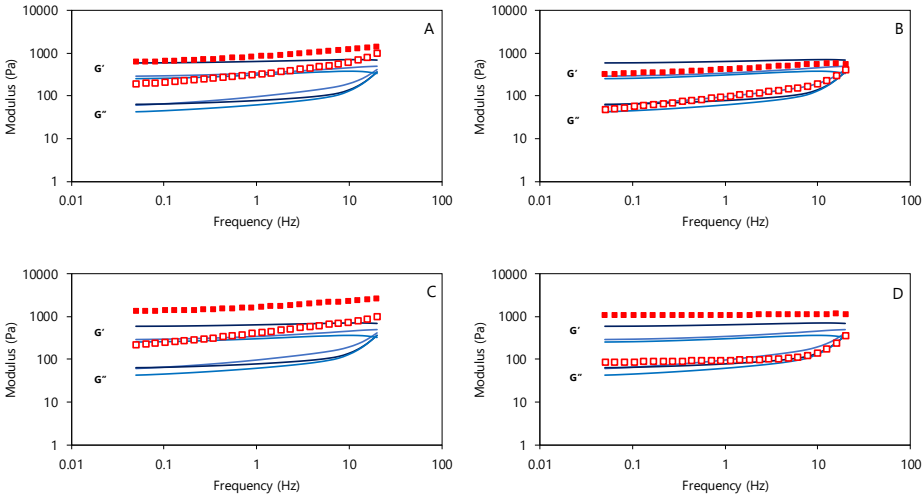

Fig. 5.7 Storage modulus-G' (■) and Loss modulus-G" (□) of the creams obtained with KCl 0.2 mol/L at pH 7 mildly purified 7MP (A) and extensively purified 7EP (B) and with $NaHCO_3$ 0.1 mol/L at pH 9.5 mildly purified 9.5MP (C) and purified 9.5EP (D). The blue lines represent 3 different benchmark mayonnaises.

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 [43]. 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 [18]. 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 [44].

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 [45]. 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. 5.6). This increase in droplet size may reduce the G' which positively weakened the structure [46]. In general, the extensively purified cream extracted at pH 7 had a perfect match of G' and G" with commercial mayonnaises.

5.4 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 threatens 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.

Appendix 5.1

Table A5.1 Composition of the commercial mayonnaises and mayonnaise-like products used in this work as reported on their package.

	Remia Mayolijn	AH Halvanaise	Hellmann's with olive oil
Fat (g/100g)	31	41	62
Saturated fat (g/100g)	2.1	4	5.4
Monounsaturated (g/100g)		32	39
Polyunsaturated (g/100g)		5	17
Carbohydrates (g/100g)	10	11	2.3
Sugars (g/100g)	6.6	7.5	1.5
Protein (g/100g)	0.7	0.6	0.7
Salt (g/100g)	1.2	1.5	1.1

Table A5.2 Ingredients list of the commercial mayonnaise and mayonnaise like products used in this work as reported on their package.

Remia Mayolijn	AH Halvanaise	Hellmann's with olive oil
Water	Sunflower oil (39 wt.%)	Rapeseed oil
Rapeseed oil (30%)	Water	Water
Glucose-fructose syrup	Vinegar	Vinegar
Vinegar	Sugar	Olive oil (5 wt.%)
Modified starch	Egg yolk (3.5 wt. %)	Egg yolk (4.6 wt.%)
Mustard (water, mustard seeds, vinegar, spices)	Starch (gluten free wheat, tapioca)	Sugar
Salt	Salt	Salt
Potato starch	Mustard flour	Modified corn starch
Aroma	Lactic acid	Concentrated lemon juice
Lactic acid	Thickeners (guar gum, xanthan gum)	Antioxidant E385
Potassium sorbate	Natural mustard aroma	Aroma's
Calcium dipotassium-EDTA	Rosemary extract	Paprika extract
Beta carotene	Carrot extract	

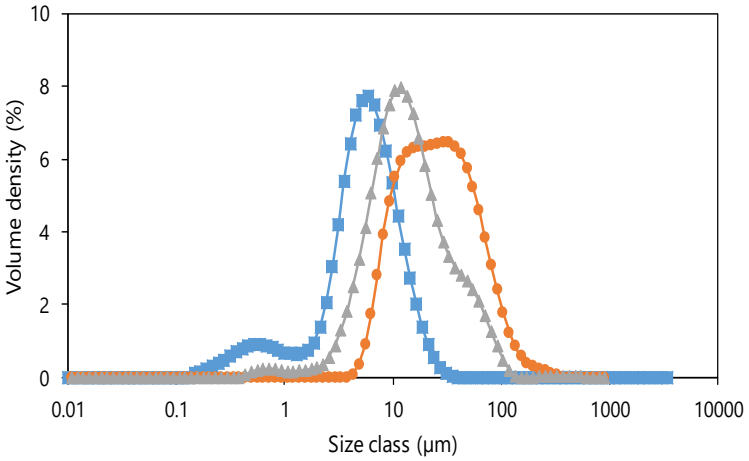

Fig. A5.1 Particle size distribution of the commercial mayonnaises used as benchmarks in this work. (■) Hellmann's with olive oil, (●) Remia Mayolijn, (▲) AH Halvanaise.

References

1] A. Barre, M. Simplicien, G. Cassan, H. Benoist, and P. Rougé, "Oil bodies (oleosomes): Occurrence, structure, allergenicity," *Rev. Fr. Allergol.*. 2018.

[2] A. C. Dave, A. Ye, and H. Singh, "Structural and interfacial characteristics of oil bodies in coconuts (Cocos nucifera L.)," *Food Chem.* 2019.

[3] P. Jolivet *et al.*, "Protein composition of oil bodies from mature Brassica napus seeds," *Proteomics*. 2009.

[4] Z. Purkrtova, C. Le Bon, B. Kralova, M. H. Ropers, M. Anton, and T. Chardot, "Caleosin of Arabidopsis thaliana: Effect of calcium on functional and structural properties," *J. Agric. Food Chem.*. 2008.

[5] T. L. Shimada and I. Hara-Nishimura, "Oil-body-membrane proteins and their physiological functions in plants.," *Biol. Pharm. Bull.*. 2010.

[6] J. Tzen, "Integral Proteins in Plant Oil Bodies," *ISRN Bot.*. 2012.

[7] D. Iwanaga *et al.*, "Soybean Oil Extraction and Characterization of Oil Bodies from Soy Beans: A Natural Source of Pre-Emulsified," *J. Agric. Food Chem.* 2007.

[8] C. V. Nikiforidis and V. Kiosseoglou, "Aqueous extraction of oil bodies from maize germ (Zea mays) and characterization of the resulting natural oil-in-water emulsion," *J. Agric. Food Chem.* 2009.

[9] B. I. Zielbauer *et al.*, "Soybean oleosomes studied by small angle neutron scattering (SANS)," *J. Colloid Interface Sci.* 2018.

[10] C. V. Nikiforidis, A. Matsakidou, and V. Kiosseoglou, "Composition, properties and potential food applications of natural emulsions and cream materials based on oil bodies," *RSC Adv.* 2014.

[11] T. Ishii, K. Matsumiya, Y. Nambu, M. Samoto, M. Yanagisawa, and Y. Matsumura, "Interfacial and emulsifying properties of crude and purified soybean oil bodies," *Food Struct.* 2017.

[12] D. Karefyllakis, A. Jan Van Der Goot, and C. V. Nikiforidis, "The behaviour of sunflower oleosomes at the interfaces," *Soft Matter*. 2019.

[13] S. Maurer *et al.*, "The role of intact oleosin for stabilization and function of oleosomes.," *J. Phys. Chem. B*. 2013.

[14] M. J. Romero-Guzmán, L. Jung, K. Kyriakopoulou, R. M. Boom, and C. V. Nikiforidis, "Efficient single-step rapeseed oleosome extraction using twin-screw press," *J. Food Eng.* 2020.

[15] S. De Chirico, V. di Bari, T. Foster, and D. Gray, "Enhancing the recovery of oilseed rape seed oil bodies (oleosomes) using bicarbonate-based soaking and grinding media," *Food Chem.* 2018.

[16] M. Millichip, A. S. Tatham, F. Jackson, G. Griffiths, P. R. Shewry, and a K. Stobart, "Purification and characterization of oil-bodies (oleosomes) and oil-body boundary proteins (oleosins) from the developing cotyledons of sunflower (Helianthus annuus L.)," *Biochem. J.* 1996.

[17] C. V. Nikiforidis and E. Scholten, "High internal phase emulsion gels (HIPE-gels) created through assembly of natural oil bodies," *Food Hydrocoll.* 2015.

[18] C. V. Nikiforidis, C. G. Biliaderis, and V. Kiosseoglou, "Rheological characteristics and physicochemical stability of dressing-type emulsions made of oil bodies–egg yolk blends," *Food Chem.* 2012.

[19] M. J. Romero-Guzmán *et al.*, "The effect of monovalent (Na^+, K^+) and divalent (Ca^{2+}, Mg^{2+}) cations on rapeseed oleosome (oil body) extraction and stability at pH 7," *Food Chem.* 2020.

[20] D. A. White, I. D. Fisk, J. R. Mitchell, B. Wolf, S. E. Hill, and D. A. Gray, "Sunflower-seed oil body emulsions: Rheology and stability assessment of a natural emulsion," *Food Hydrocoll.* 2008.

[21] V. N. Kapchie, L. Yao, C. C. Hauck, T. Wang, and P. A. Murphy, "Oxidative stability of soybean oil in oleosomes as affected by pH and iron," *Food Chem.* 2013.

[22] C. Wijesundera and Z. Shen, "Mimicking natural oil bodies for stabilising oil-in-water food emulsions," *Lipid Technol.* 2014.

[23] D. J. McClements, *Food Emulsions: Principles, Practices, and Techniques, Second Edition.* CRC Press, Boca Raton, Florida, USA. 2004.

[24] E. Scholten, "Composite foods: from structure to sensory perception," *Food and Function.* 2017.

[25] H. Liu, X. M. Xu, and S. D. Guo, "Rheological, texture and sensory properties of low-fat mayonnaise with different fat mimetics," *LWT.* 2007.

[26] N. Lindeboom and P. K. J. P. D. Wanasundara, "Interference of phenolic

compounds in Brassica napus, Brassica rapa and Sinapis alba seed extracts with the Lowry protein assay," *Food Chem.* 2007.

[27] A. G. J. Voragen, G. J. Coenen, R. P. Verhoef, and H. A. Schols, "Pectin, a versatile polysaccharide present in plant cell walls," *Struct. Chem.* 2009.

[28] I. Eriksson, E. Westerlund, and P. Åman, "Chemical composition in varieties of rapeseed and turnip rapeseed, including several samples of hull and dehulled seed," *J. Sci. Food Agric.* 1994.

[29] M. Naczk and F. Shahidi, "Carbohydrates of Canola and Rapeseed," in *Canola and Rapeseed*, 1990.

[30] J. T. C. Tzen, G. C. Lie, and A. H. C. C. Huang, "Characterization of the charged components and their topology on the surface of plant seed oil bodies," *J. Biol. Chem.* 1992.

[31] R. Ohlson and K. Anjou, "Rapeseed protein products," *J. Am. Oil Chem. Soc.*, 1979.

[32] J. F. Zayas and J. F. Zayas, "Water Holding Capacity of Proteins," in *Functionality of Proteins in Food.* 1997.

[33] C. Chang *et al.*, "Effect of protein microparticle and pectin on properties of light mayonnaise," *LWT.* 2017.

[34] J. P. D. Wanasundara, "Proteins of Brassicaceae oilseeds and their potential as a plant protein source.," *Crit. Rev. Food Sci. Nutr.* 2011.

[35] S. Perera, T. McIntosh, and J. Wanasundara, "Structural Properties of Cruciferin and Napin of Brassica napus (Canola) Show Distinct Responses to Changes in pH and Temperature," *Plants.* 2016.

[36] L.-J. Lin, S. S. K. Tai, C.-C. Peng, and J. T. C. Tzen, "Steroleosin, a sterol-binding dehydrogenase in seed oil bodies.," *Plant Physiol.* 2002.

[37] H. Næsted *et al.*, "Caleosins: Ca2+-binding proteins associated with lipid bodies," *Plant Mol. Biol.* 2000.

[38] Y. Cao, L. Zhao, Y. Ying, X. Kong, Y. Hua, and Y. Chen, "The characterization of soybean oil body integral oleosin isoforms and the effects of alkaline pH on them," *Food Chem.* 2015.

[39] L. Flutto, "PECTIN | Food Use," in *Encyclopedia of Food Sciences and Nutrition*, 2004.

[40] J. T. C. Tzen, Y.-Z. Cao, P. Laurent, C. Ratnayake, and A. H. C. Huang, "Lipids, Proteins, and Structure of Seed Oil Bodies from Diverse Species'," *Plant Physiol.* 1993.

[41] Y. Ying, L. Zhao, L. Kong, X. Kong, Y. Hua, and Y. Chen, "Solubilization of proteins in extracted oil bodies by SDS: A simple and efficient protein sample preparation method for Tricine-SDS-PAGE," *Food Chem.* 2015.

[42] M. E. J. Geerts, E. Mienis, C. V. Nikiforidis, A. van der Padt, and A. J. van der Goot, "Mildly refined fractions of yellow peas show rich behaviour in thickened oil-in-water emulsions," *Innov. Food Sci. Emerg. Technol.* 2017.

[43] L. J. Harrison and F. E. Cunningham, "Factors Influencing the Quality of Mayonnaise: a Review," *J. Food Qual.* 1985.

[44] B. Muhoza, S. Xia, and X. Zhang, "Gelatin and high methyl pectin coacervates crosslinked with tannic acid: The characterization, rheological properties, and application for peppermint oil microencapsulation," *Food Hydrocoll.* 2019.

[45] J. A. Depree and G. P. Savage, "Physical and flavour stability of mayonnaise," *Trends Food Sci. Technol.* 2001.

[46] R. Pal, "Effect of Droplet Size on the Rheology of Emulsions," *AIChE J.*, 1996.

6

Exergetic resource use efficiency of mayonnaise production

-Oleosome extraction vs Conventional processing-

This chapter has been submitted as:
M. J. Romero-Guzmán, G. Gousias, C. V. Nikiforidis, R. M. Boom, "Exergetic resource use efficiency of mayonnaise production, oleosome extraction vs conventional processing, " 2020.

Abstract

Oleosomes are plant storage structures, which resemble synthetic emulsions. Their structure renders them hydrophilic and hence their extraction in aqueous media is possible. This extraction yields a natural emulsion, whose properties can be tailored for a specific application by adjusting the extraction conditions. It is believed that the use of these naturally emulsified structures could be an environmental-friendly alternative to emulsion-based products. To test this hypothesis, we performed a thermodynamic assessment, known as exergy analysis, of the production process of a mayonnaise. In this assessment the conventional process combining oil extraction and mayonnaise production was compared against a conceptual oleosome aqueous extraction process. This process recently has shown to yield a mayonnaise-like product. The results show that despite its complexity, the conventional process has low physical exergy loss. However, the limited usability of the obtained cake after solvent extraction, negatively influences the overall exergetic performance of this process. On the other hand, oleosome aqueous extraction is clearly a simpler process, which allows co-recovery of native protein and fibres and not just oil. However, the use of water in the process increases the size of streams and dehydration is intensive in energy and exergy. Nevertheless, the use of the side-streams in more diluted systems could positively impact oleosome aqueous extraction. Therefore, oleosome aqueous extraction seems a good alternative to the conventional process, as long as the solids are used for high value applications.

6.1 Introduction

The demand for food, which is predicted to double in the coming 50 years, in combination with the stress that global warming puts on the production capacity of raw agricultural materials, requires us to find new and more efficient ways to convert raw materials into consumer-ready food products [1], [2]. In current processes, the yield of one particular product is often optimized at the cost of the other fractions from the same raw material. While this is efficient for the single primary product, it leads to an overall inefficiency, as the rest of the raw material has to be allocated to lower-value applications such as animal feed [2].

Mild refining processes yield less pure ingredients or " intermediate" fractions, which may still retain better functionality due to the milder processing conditions used for their recovery [3]. These intermediate fractions have been proposed as more sustainable alternatives to conventional ingredients [4]–[6]. A major advantage of these mild extraction methods is that they do not require the use of organic solvents or other toxic auxiliary chemicals and that they limit the creation of large waste streams and energy consumption [7].

An interesting example of these intermediate fractions are oleosomes (oil-bodies), which are the intracellular structures that store oil in oleaginous seeds [8]. They consist of a core of triacylglicerides surrounded by a mixed-monolayer of phospholipids and proteins [9]. As their structure resembles that of manufactured emulsion droplets, it was suggested that these structures can be extracted without damage and used as such in emulsion-based products [4].

The extraction of oleosomes can be done via aqueous extraction [10]. This extraction broadly requires 3 steps: softening of the cellular material, disruption of the cell walls and extraction of the cellular material [11]. Although the process is quite simple, it has not yet been realized on industrial scale due to 4 main problems: (1) the necessity of large volumes of water, (2) the use of alkaline conditions, (3) the required long soaking times and (4) the lack of a mature and scalable process. Recently these challenges have been under investigation [12]–[14] and some approaches have been proposed, bringing oleosome aqueous extraction closer to feasibility for larger scale.

The properties of the emulsion that results after extraction can be adapted by changing the conditions of the extraction. This can be used to direct the properties of final products that are assembled from these emulsions without the addition of other materials such as carbohydrates or proteins [13]. As proof of concept, a vegan mayonnaise-like product was proposed using just rapeseeds, water, salts, and vinegar [14]. Emulsifiers nor other stabilizers were used, possibly allowing a 'clean' label. However, it is not yet clear whether this new route is more resource efficient than the conventional way to produce food emulsions.

Therefore, in this work we assess the resource efficiency of the production of a mayonnaise-like emulsion product from rapeseed through this recently proposed process [14] and compare this to the conventional route to mayonnaise production, which is divided in the creation of refined ingredients (e.g. oil extraction) [15] and the structuring of the final product [16].

We calculate the overall resource use of both mayonnaise production processes, using the overall cumulative use of exergy. Since the proposed

aqueous extraction process includes both the extraction step and the final emulsion product creation, we will use the production of mayonnaise as a model product for both systems. Exergy analysis was chosen as method to perform the analysis, as it requires the least possible assumptions and is objective, being directly based on the first and second law of thermodynamics [17]. Furthermore, exergy analysis allows us to translate all resources into one unit: the potential work by exchange with our environment [17]. This does not just allow the comparison of different systems, but also gives an indication of the theoretical minimum use of resources [18].

6.2 Materials & Methods

6.2.1 Materials

Rapeseed seeds (Alizze) were purchased from a seed producer. The used seeds are food grade as they do not contain erucic acid and have a low glucosinolate content (13mg/100g). Their composition is 9.0% ± 1.2% moisture, 36.0 wt.% ± 1.3% of oil and 18.0 wt.% ± 0.7% of protein, on wet basis. All of 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).

6.2.2 Experimental

6.2.2.1 Extraction

Pre-soaked seeds (1:1 seed:solution by weight and soaked for 1h) were directly processed with a lab-scale twin-screw press (Angel 7500, Naarden The Netherlands). The velocity of the rotation of the screws 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.

6.2.2.2 Characterization of the streams

6.2.2.2.1 Moisture content

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

6.2.2.2.2 Lipid content

The lipid content of dried samples was determined by Soxhlet extraction with petroleum ether (B-811 Buchi Extractor, Switzerland). 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 (36.0 ±1.3 wt.%) and the amount of oil in the cake. In this calculation we assumed that all the extracted oil was expected to be either as native oleosomes or as emulsified oil.

6.2.2.2.3 Protein content

The protein content was determined using the Dumas method. The protein content was quantified using a conversion factor of 5.5 for the nitrogen content [19]. 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 (18.0 ± 0.7 wt.%).

6.2.3 Scenarios description

Two scenarios were evaluated. Both scenarios include the steps necessary to convert rapeseeds into mayonnaise or a mayonnaise-like emulsion food product. The scenarios are depicted in Fig. 6.1:

Scenario A. Conventional oil extraction [20], [21] followed by a conventional mayonnaise production process [16] [22].
Scenario B. Oleosome aqueous extraction from rapeseed seeds directly into a mayonnaise-like product [14].

To limit the complexity of the comparison, some steps of the process were not included, either because they were out of the scope of our primary assessment, or because they were present in both processes, and thus their addition would not make a difference in the comparison of the processes. The steps that are not included in this analysis were harvesting and transportation of the seeds, cleaning of the seeds, and pasteurization of the final product. An important difference between both mayonnaises, was the use of 2 % of egg yok as emulsifier [23]. The complete effect of using animal-derived ingredients cannot be fully observed in this work as egg was just added as an additional ingredient and its production on the farm was not considered.

The sizes and the compositions of the generated streams were obtained from literature or from experiments as described in section 6.2.2. The yields and changes in composition are summarized in Appendix 6.1 .

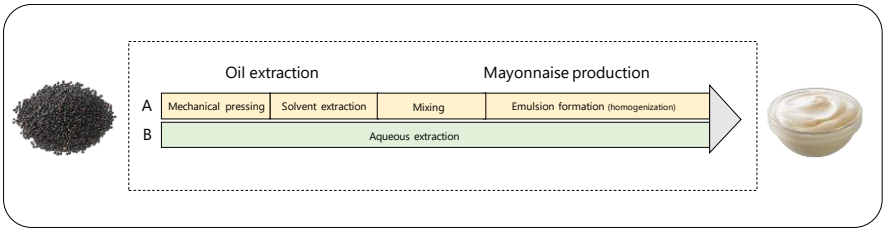

Fig. 6.1 Overview of the compared scenarios to produce mayonnaise from rapeseeds

Fig. 6.2 schematically depicts scenario A. The first step in this scenario is the oil extraction and refining, which includes drying, dehulling, seed pressing and

hexane extraction, followed by degumming, neutralization, bleaching, dewaxing and deodorization to obtain a stable and clear oil [15]. Even though in some cases the oil from the mechanical extraction has more value than the oil obtained during the solvent extraction step [20], in this work it was assumed that the two streams of extracted oil were mixed and refined together, since refined oil is necessary for the production of emulsion-based products [24]. The presence of surface active components could lead to physicochemical instabilities in the created emulsion [24], and thus the phospholipids and proteins are removed in the refining steps [25]. This refined oil is then used to prepare mayonnaise. This process consists of the combination of the refined oil with the other constituents of mayonnaise, followed by high-pressure homogenization [26].

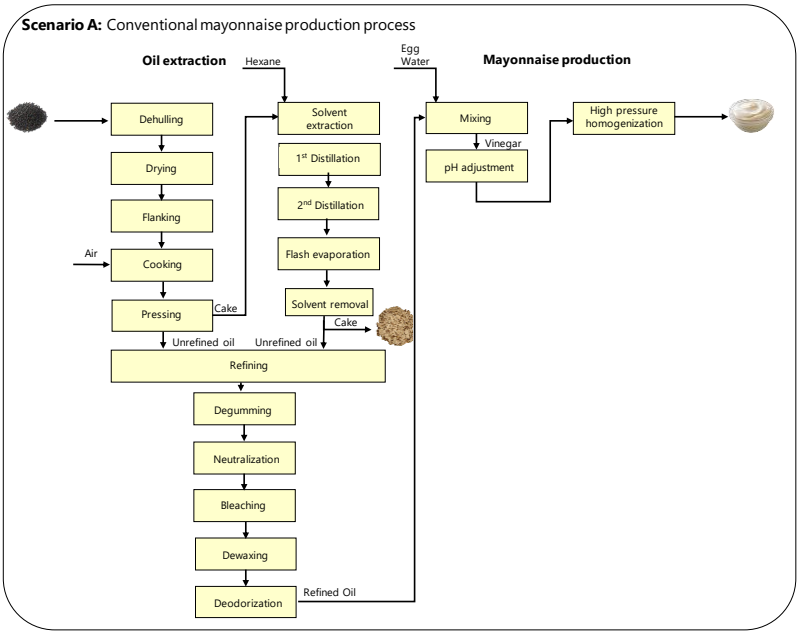

Fig. 6.2 Conventional mayonnaise production process from seeds to mayonnaise

Scenario B entails the oleosome aqueous extraction and it is depicted in Fig. 6.3. This process begins with the soaking of the rapeseed seeds in an

electrolyte solution, followed by the extraction of oleosomes with a twin-screw press. This pressing yields a slurry, which is centrifuged to remove the solids that were entrained by the juice, and to separate the oil-rich fraction containing the oleosomes in combination with co-extracted material, such as proteins [14]. The interaction between the oleosomes and the proteins can be adjusted by the pH and the ionic composition of the aqueous extraction medium, and by the number of centrifugation steps [13]. The conceptual process was based on the yields and compositions of the raw material and the desired mayonnaise composition as reported in our previous work [14].

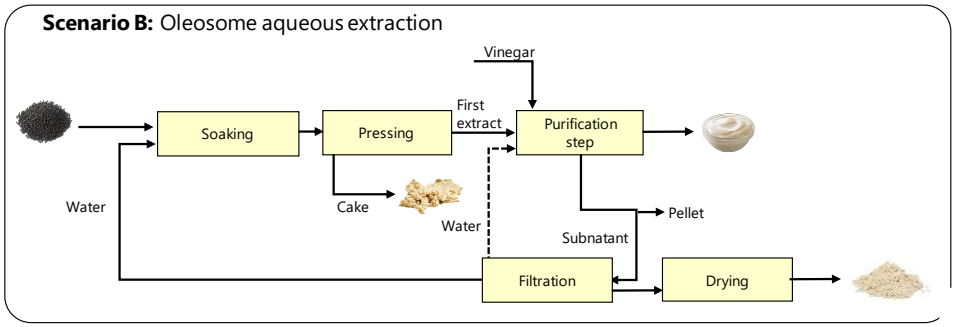

Fig. 6.3 Oleosome aqueous extraction process from seeds to mayonnaise-like product

6.2.4 Mass and exergy assessment

6.2.4.1 Mass balance

The mass balances were calculated assuming steady state and the conservation of mass (Eq. 1), where m_{in} and m_{out} are the masses entering and exiting, respectively.

$$\sum m_{in} = \sum m_{out} \quad (1)$$

The inputs and outputs of the mass balances were estimated with yields reported in literature for conventional oil extraction [20], [21], [27] and the

oleosome aqueous extraction [13][14]. Tables with all flows and compositions can be found in Appendix 6.1.

6.2.4.2 Exergy balance

Both chemical and physical exergy (thermal and pressure) were considered. The chemical exergy of each stream was calculated using Eq. 2,

$$B_{ch} = \sum m_i b_{ich} \tag{2}$$

where B_{ch} is the chemical exergy of a stream, m_i is the mass of component i and b_{ich} is the specific chemical exergy of component i. As most components are macromolecular, any mixing exergies are very small and could be neglected in the analysis. The compositions of all the streams were obtained from the mass balances. The thermal exergy of each stream was calculated using Eq. 3,

$$B_{th} = \sum m_i c_{pi} \left(T - T_R - T_R ln \left(\frac{T}{T_R} \right) \right) \tag{3}$$

where B_{th} is the thermal exergy of a stream, m_i is the mass of component i in this stream, c_{pi} is the specific heat capacity of component i, T is the temperature and T_R here is the reference temperature which was taken equal to 293 K [17]. Finally, the exergy resulting from non-standard pressures was calculated with Eq. 4.

$$B_{pr} = \sum m_i \frac{RT_R}{Mw_i} ln \left(\frac{p_i}{p_R} \right) \tag{4}$$

In this equation, B_{pr} is the pressure exergy of a stream, and m_i is the mass of component i of this stream, Mw_i is the molecular weight of component i, R is the gas constant, p_i is the partial pressure of component i and p_R is the reference pressure equal to 1 bar. The sum of all contributions gives the total

exergy, defined as the work that a stream could potentially perform by exchange with the reference environment [17]. The exergetic comparison was done by using different exergy indicators [17]: the cumulative exergy cost (CExC), the specific exergy losses (SEL) and the process efficiency (η). The definitions of these indicators are given in Eq. 5 to 7, respectively, where B_{in} is the total exergy content of all the natural resources introduced in the process, B_{out} is the total exergy content of all the natural resources that are coming out of the process, m_{useful} is the total mass of the useful output of the overall process and η is the exergetic efficiency of the process.

$$SEL = \frac{B_{in} - B_{out}}{m_{useful}} \quad (5)$$

$$CExC = \frac{B_{in}}{m_{useful}} \quad (6)$$

$$\eta = \frac{B_{out}}{B_{in}} \cdot 100\% \quad (7)$$

6.2.5 The mass and exergy flow visualization

To visualize the flows of mass and exergy of the compared scenarios Sankey diagrams (mass balances) and Grassmann diagrams (exergy analysis) were used. These diagrams were created with the software e!Sankey Pro, (v3.2.2.558, ifu Hamburg GmbH).

6.2.6 Assumptions

- The reference environment has a T_R of 293.15 K and P_R of 101.325 kPa.
- Water used as input has a temperature of 293.15 K.
- Steady state is assumed everywhere.

- The water content of the hulls and the de-hulled seeds was assumed the same.
- During conventional extraction, the only component separated from the seeds is oil.

6.3 Results and discussion

6.3.1 Conventional process

The mass and exergy streams for the conventional process to produce 1 metric ton of mayonnaise, are shown in a Sankey and a Grassmann diagram in Fig. 6.5 and Fig. 6.6; please note that exergy is not preserved but it is necessarily lost in every step.

In the conventional process (Fig. 6.5), the press step yields 2 main streams. The first stream is the oil directly obtained from the pressing step, which is up to 50% of the total oil [20]. The second stream is a cake which still contains the rest of the seed oil and other seed components such as proteins and fibres. This cake is subjected to solvent extraction that increases the overall oil extraction yield to up to 95% [27]. After the solvent extraction two streams are obtained: the solvent-extracted oil and the cake. The solvent-extracted oil passes through a series of distillation and evaporation steps to remove the solvent and is then combined with the pressed oil for further refining [20]. The cake which still contains some solvent passes through a heating or desolventization step, in which the cake is heated up to 100 °C. These conditions help to remove nearly all the residual solvent [27]. Although the cake contains valuable components, such as proteins rich in lysine and sulphur containing amino acids [28] and it is free of solvent, it is generally used as animal feed [29]. The extraction and heating steps denature the proteins [30], [31] and create covalent bonds between the proteins and the phenolic

constituents, which reduces their usability. Therefore, it is indicated as waste in the mass balance. For every 2647 kg of seeds that are required to produce 1 ton of mayonnaise, 1193 kg of press cake is obtained, which represents 45 wt.% of the initial raw material.

The mayonnaise formulation requires egg yolk, here assumed to be dried egg yolk. The yolk represents just the 8 wt.% of the whole egg and the rest of the components (21% of egg whites and 71% of water) [32] are not used in this process, so they could be considered a side stream. However, this side stream can and will be utilized in many other food applications, and hence it is not considered as waste.

The conventional oil extraction process is very efficient, as almost all of the oil is extracted from the seeds. However, some of the oil is lost in the refining process. From the 950 kg of oil initially present in the seeds, 897 kg is recovered from the seeds as unrefined oil and from this, 800 kg is recovered as refined oil. Therefore, although this process has extraction efficiencies of up to 95 wt. %, the refinement of the material leads to a reduction of 11 wt. %, leading to an overall recovery of 84 wt.% of the initial oil. The major losses of oil originate from the dehulling step (air classification) and the centrifugation after the neutralization step.

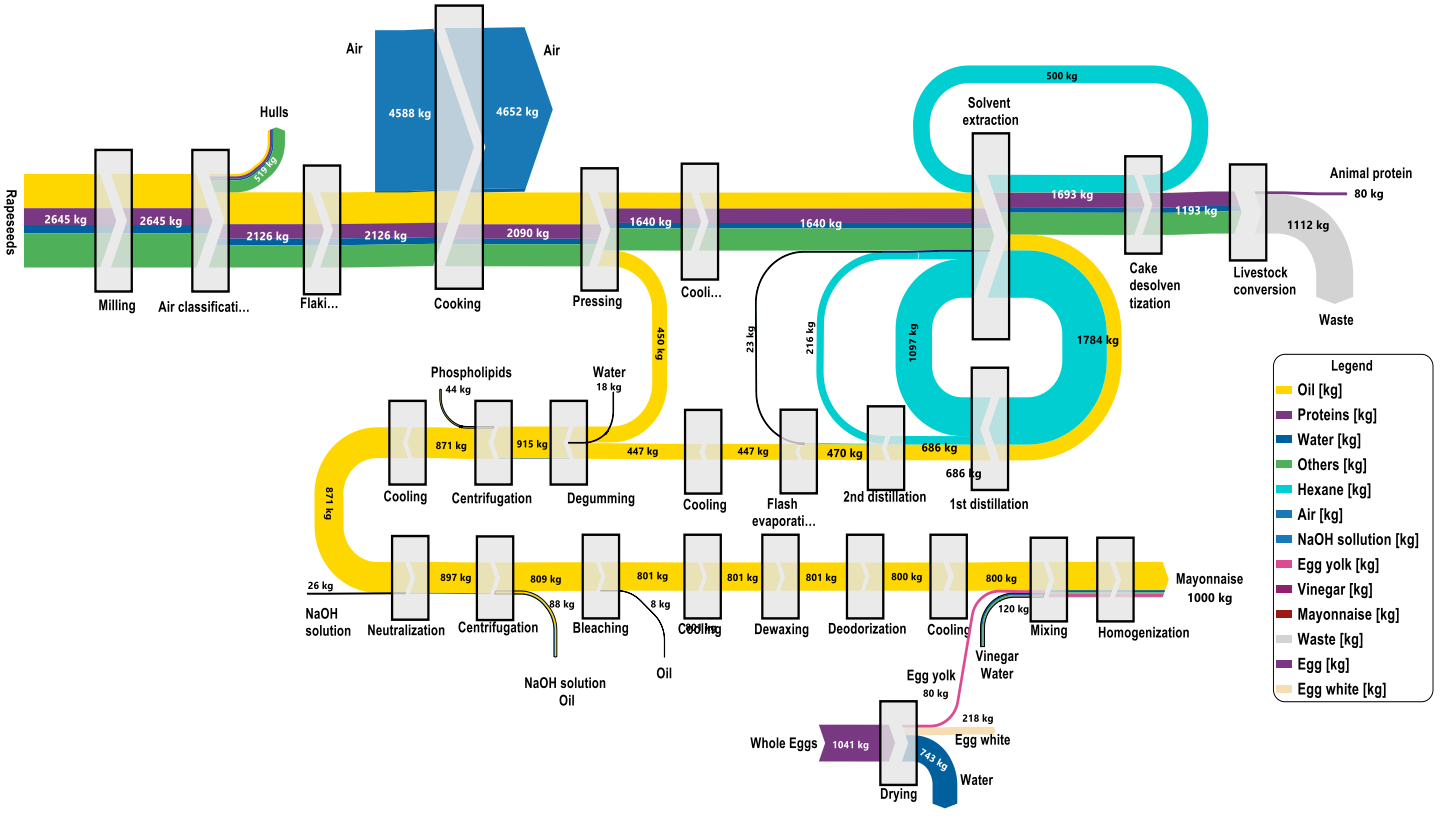

Fig. 6.5 Sankey diagram of the conventional mayonnaise production process.

The Grassmann diagram of the process (Fig. 6.6) shows that the exergy analysis is dominated by the chemical exergy. It is difficult to distinguish in this figure the contribution of the physical exergy inputs such as electricity and natural gas. Therefore, the physical exergy requirements were condensed in Fig. 6.7 to facilitate their discussion.

In Fig. 6.6 one can observe that the biggest exergy losses occur in steps during which valuable material is lost. This can also be seen in figure 6.5, where the lost (wasted) materials are shown in outbound arrows. The steps in which this occurs are dehulling (after air classification), neutralization (after centrifugation), and by far the largest loss of chemical exergy is the press cake. Despite its high density of potentially valuable components, its limited functionality due to the denaturation and complexation of the proteins and the fact that it was in contact with organic solvents makes the cake suitable for animal feed only. The global average dry mass conversion in livestock production is just 6% [34], meaning that on average 94% of the exergetic value of the cake is lost by using it as livestock feed. With an estimated chemical exergy content of the solvent-free cake of 20.86 MJ/kg, the net value of the cake after use as livestock feed is around 1.25 GJ for every ton of mayonnaise produced. In comparison, the net value of the cake could have been 23.712 GJ/ton if it could have been used directly for human consumption. Therefore, the heating step leads to an extra exergy loss of 22.289 GJ/ton mayonnaise, due to the denaturation and degradation of the cake.

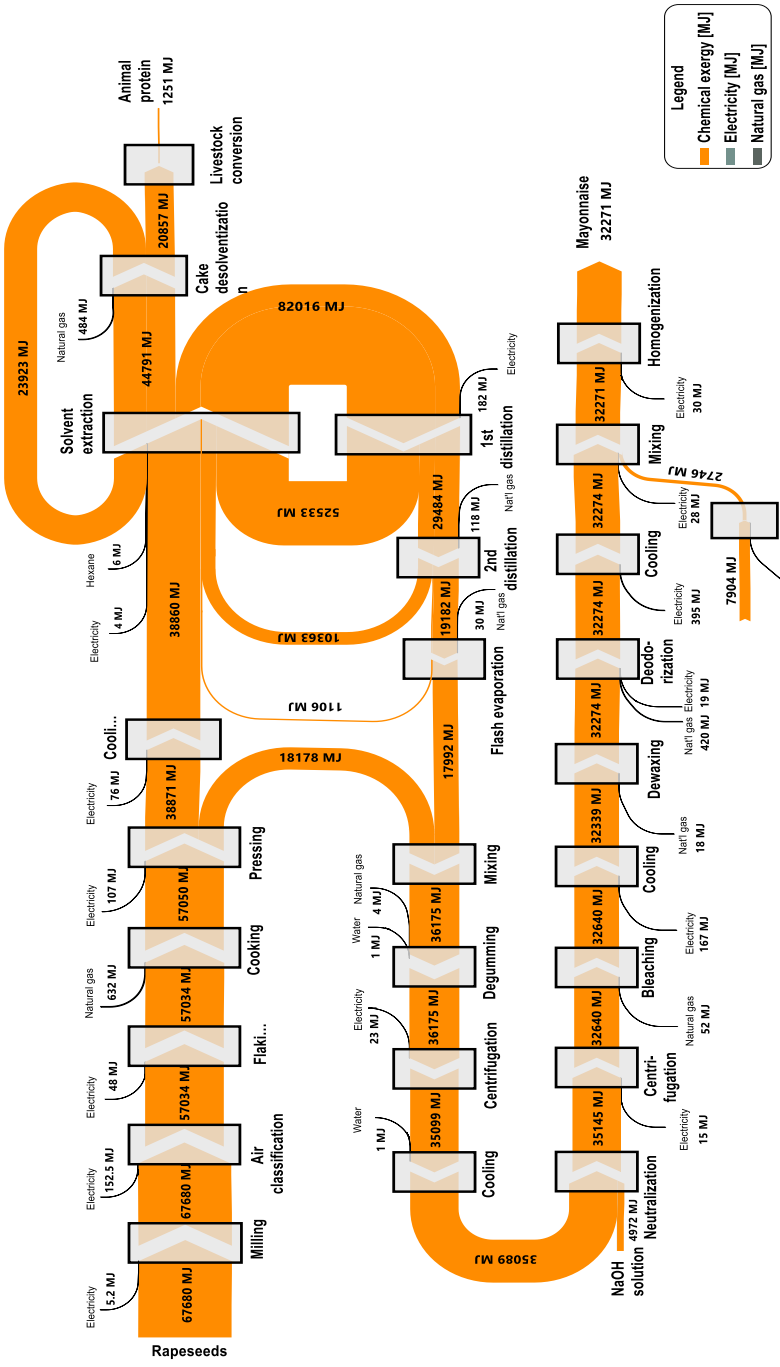

Fig. 6.6 Grassmann diagram of the conventional production of mayonnaise from seeds to mayonnaise.

In Fig. 6.7, one can observe that the steps in which natural gas is used as fuel, have the highest exergy requirements: seeds cooking, cake desolventization and deodorization.

The cooking of the seeds is a necessary step to ensure the right conditions for pressing [27]. The cake desolventization also is necessary to recycle most of the organic solvent which has a high chemical exergy (Fig. 6.6) and to utilize the cake. Finally, both oils (i.e. pressed and solvent-extracted) are deodorized at 220 °C [21]. The oil is then cooled using a mixture of water and ethylene glycol [15], [21]. This active cooling is necessary to avoid thermal degradation of the oil. Therefore, these three most exergetically inefficient steps in the conventional oil extraction cannot be avoid or modified, as they are key steps to ensure the quality of the final product.

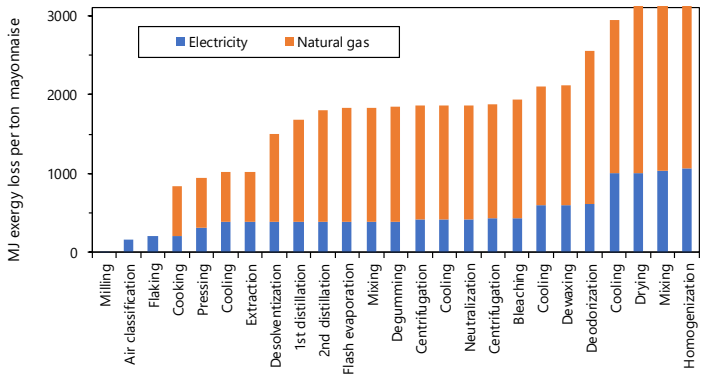

Fig. 6.7 Cumulative physical exergy costs (electric power, natural gas) in MJ per ton of mayonnaise produced.

6.3.2 Aqueous extraction

The aqueous extraction is less complex than the conventional mayonnaise production process. In the Sankey diagram depicted in Fig. 6.8, one can see that the main downside is that aqueous extraction requires big quantities of

water. Although efforts were made to reduce the consumption of water with good results (six fold reduction when using twin-screw press) [12], there is still a large amount of water needed since a 1:1 seeds to water ratio is required at least. Moreover, the co-extracted protein fraction retains much of this water, which hinders water recycling.

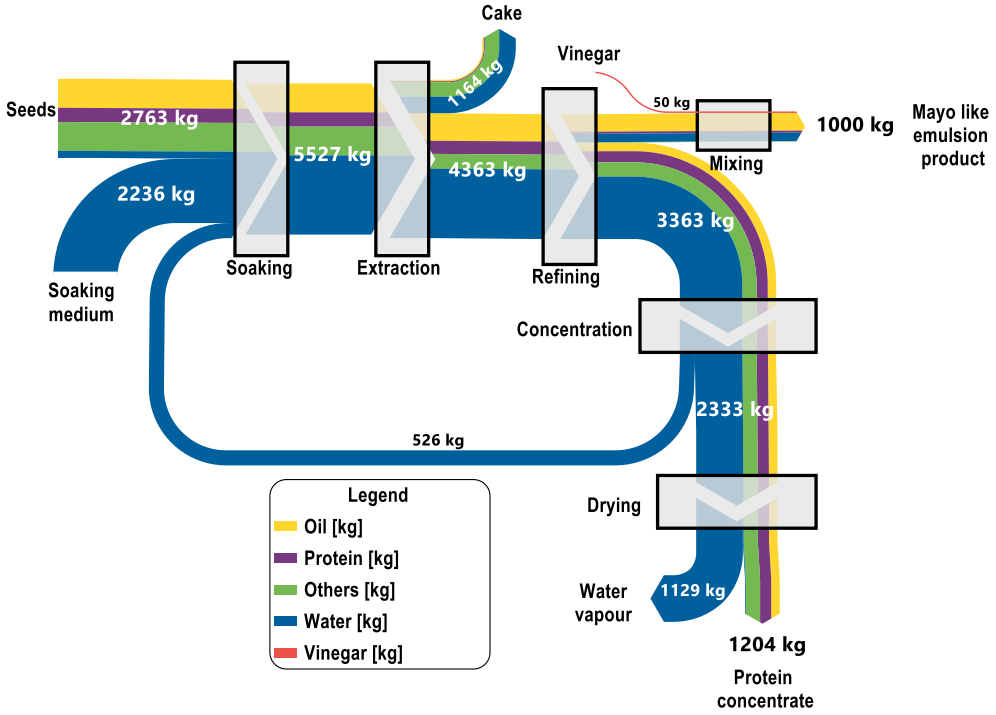

Fig. 6.8 Sankey diagram of the mayonnaise-like product production process via aqueous extraction.

Fig. 6.8 shows that for the production of 1000 kg mayonnaise-like emulsion, 2763 kg rapeseeds are necessary. These seeds are mixed in a 1:1 weight ratio with water, after which they are pressed into a fibre-rich cake (1164 kg), and a slurry from which later two other fractions can be recovered. The separation of these two fractions can be done using centrifugation (indicated as 'refining' in figure 6.8) and the streams obtained from this are an emulsion (1000 kg)

and a protein rich mass (3363 kg). This protein-rich fraction can be dried and concentrated to 1204 kg (98% of solid mass).

It is interesting and relevant to note that the overall oil extraction yield of this oleosome aqueous extraction process is similar to that obtained with the conventional process. Nevertheless an important difference between these processes comes from the fact that all of the oil that does not end-up in the emulsion product is not lost, but recovered as part of the protein concentrate stream. Moreover, the aqueous extraction cake is mostly composed of insoluble fibres and water and hardly any proteins. This stands in contrast to the cake from the conventional process, which comprises the proteins. Based on its composition and characteristics, we assumed that the cake obtained from the aqueous extraction is similar to okara, the solid residue obtained from the production of tofu or soy milk [35], [36]. Okara is also rich in fibres and although is generally treated as waste, it can be successfully used in foods as dietary fibre [37]. Some examples of this are the supplementation of tortillas [38] and of bread [36]. Therefore in our analysis this side-stream was not considered waste.

An important part of the oleosome aqueous extraction is the separation of the oleosomes from the first extract that contains both proteins and carbohydrates, to create a mayonnaise-like emulsion [14]. Soluble carbohydrates like pectin need to be removed from the oleosomes, since the addition of vinegar and the resulting pH shift to values down to 3.8, would lead to the formation of coacervates [39]. Such a separation can be done using a series of centrifugation or decanting steps. Fig. 6.9 illustrates this with 3 decanters in counter-current mode, as described in previous work [14].

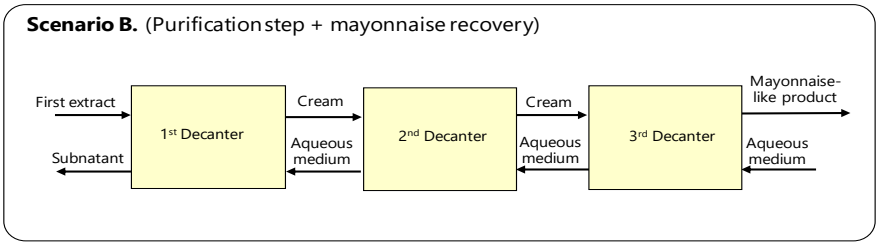

Fig. 6.9 Theoretical purification step belonging to the aqueous extraction process from first extract to mayonnaise-like product.

The protein-rich fraction contains 41 wt.% of soluble carbohydrates, 32 wt.% of protein and, as mentioned, the oil that was removed during the purification step, corresponding to 24 wt.% of the fraction. One may either further purify this mass, since the proteins, the soluble carbohydrates and the oil are valuable for human food production, or one may use it directly for the creation of foods, for example in the formulation of dairy and meat alternatives [40], [41]. However, this stream contains most of the water added for the extraction, and thus may require drying before its utilization, increasing the exergy consumption [9].

Fig. 6.10 shows the exergy flows in the system. As with the conventional process, the chemical exergy flows dominate the system, indicating the importance of utilizing as much of the raw material as is possible.

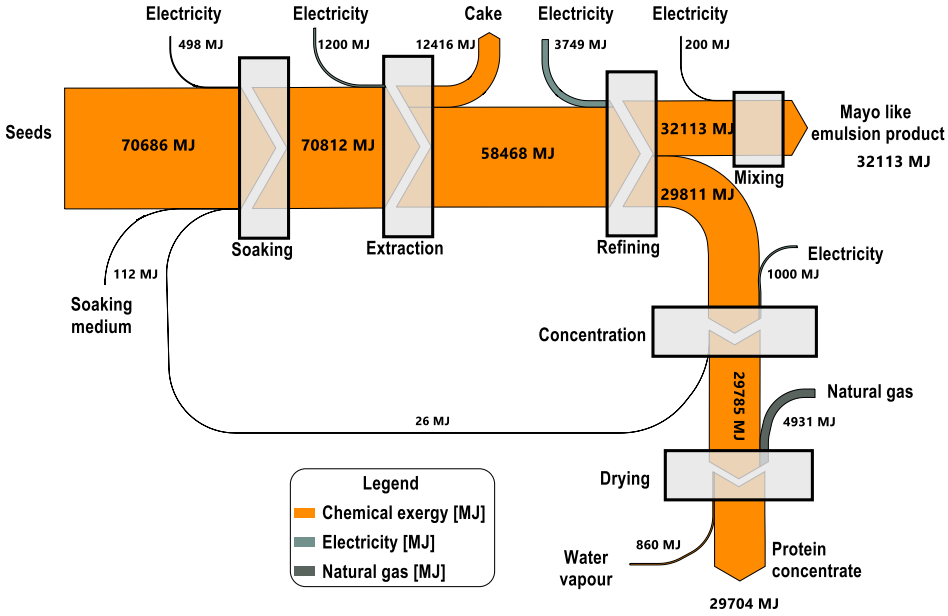

Fig. 6.10 Grassmann diagram of the aqueous extraction production of mayonnaise-like emulsion from seeds to mayonnaise.

The fibrous cake may be utilized or may be regarded as a waste stream. In the latter case, the extraction creates losses, due to this waste stream. Next to this step, the other main exergy loss is in the drying step, as significant amounts of water need to be evaporated. This is a loss of physical exergy. The requirement of water to suspend or solubilize the different components, and the fact that the press cake and the oleosome stream contain only a small part of the originally added water, imply that the protein stream is quite diluted. Mild drying will result in proteins with good functionality; obviously a spray drying process is more energy intensive than for example a drum drying process; however the latter will compromise the protein quality [42].

Similarly as for the conventional process, the chemical exergies of the streams overshadow the physical exergies. Therefore the physical exergy losses are visualized in Fig. 6.11. One observes that the physical exergy losses are about

three times larger than the physical exergy losses in the conventional production process (Fig. 6.7). These large physical exergy losses of the aqueous process are especially due to the requirement of refining and drying.

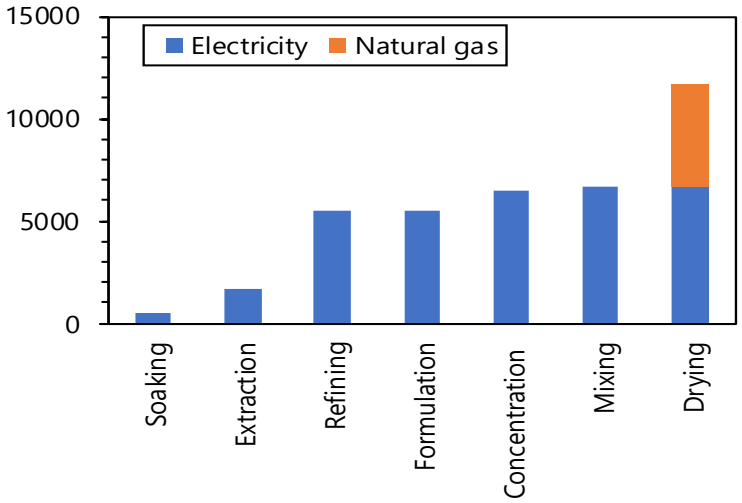

Fig. 6.11 Physical exergy losses during aqueous extraction of oleosomes from rapeseeds.

With the aid of Fig. 6.11 we see that around 50% of the overall exergy used to produce a kg of useful product comes from the removal of water. This is not surprising as it was mentioned that around 50% of the total dry mass entering the process ends up in the protein rich fraction, which needs to be dried.

6.3.3 Exergy indicators

Table 6.1 gives a numerical comparison between the conventional and the aqueous process regarding the overall inputs of exergy used during the process, the exergy outputs obtained per process and the mass of useful streams obtained per process. The exergy inputs were calculated by summing up all the chemical and physical exergies that were used in each process. The

exergy outputs were calculated by summing-up all of the chemical exergies of the recovered streams (e.g. mayonnaise and cake). Finally the useful mass was calculated by summing up the mass of the streams that could be utilized for human consumption (e.g. mayonnaise, protein concentrate). Although aqueous extraction is simpler than the conventional process and completely plant-based, it requires big exergy inputs. These exergy inputs are larger than those required by the conventional process and they stem mostly from the fact that the aqueous extraction is intensive in physical exergy. However, big differences are also observed in the obtained outputs, as aqueous extraction allows the recovery of more useful material.

Table 6.1 Exergy inputs, exergy outputs and useful mass obtained for conventional and aqueous extraction processes.

	Conventional	Aqueous extraction
Exergy input (MJ)	70680	82686
Exergy output (MJ)	33522	74233
Useful mass (kg)	1080	3368

To make this comparison simpler and more meaningful, 3 exergy indicators were chosen. In Table 6.2 the two processes are compared on these bases.

Table 6.2 Exergy indicators for the evaluated production processes from seeds to mayonnaise.

Exergy indicator	Conventional production process (Mayonnaise)	Aqueous extraction (Mayonnaise-like emulsion)
SEL (MJ/kg useful output)	34.4	2.7
CExC (MJ/kg useful output)	65.4	26.7
η (% efficiency)	47.4	89.7

The indicators show that the aqueous extraction utilizes the natural resources more efficiently. The specific exergy losses (SEL) show the exergy loss per kg of useful output. The lower the SEL, the smaller the amount of resources that are wasted during the process. On the other hand, the cumulative exergy loss indicator (CExC) shows the amount of raw materials or resources that is necessary per kg of useful output. The lower the CExC, the smaller the amount of resources needed to produce one kg of useful output. Finally, the efficiency of the process indicates the percentage of the input exergy that remains useful after processing. The results indicate that aqueous extraction is better with regards to all three indicators. This is due to the choice to utilize almost all streams in the aqueous process, including cake and proteins, as the cake obtained from the conventional oil extraction was denatured and degraded and not considered as a useful output. Therefore the utilization of the protein-rich stream is vital to the efficiency of the proposed aqueous extraction. Overall, once this utilization is ensured, the aqueous extraction process is twice as efficient as the conventional process.

6.4 Conclusion

We to compared a recently proposed way to produce a mayonnaise-like emulsion via aqueous extraction, to the conventional mayonnaise production process, which combines the isolation of refined oil and the subsequent formulation of mayonnaise.

The tailored aqueous extraction process that goes from seeds to final product can be more efficient than the conventional way in which emulsified products are produced. However, to achieve this efficiency, it is important to make good use of all the fractions that are produced. These are, next to the oil emulsion, a protein-rich fraction and a fibre-rich press cake. The fact that the

components are not denatured due to the mild process conditions, makes this possible and feasible.

Overall, oleosome aqueous extraction has the potential to be more efficient than the conventional emulsion production process and thus we conclude that this novel process is a promising path to produce emulsion-based foods.

Nomenclature

$\boldsymbol{m}$	Mass (kg)
$\boldsymbol{C}$	Capacity (kg)
$\boldsymbol{Q}$	Heat (kJ)
$\boldsymbol{C_p}$	Heat capacity (kJ/ kg·K)
$\boldsymbol{T}$	Temperature (K)
$\boldsymbol{a}$	Heat transfer coefficient (W/m2·K)
$\boldsymbol{t}$	Time (s)
$\boldsymbol{B}$	Exergy (kJ)
$\boldsymbol{p}$	Pressure (bar)
$\boldsymbol{R}$	Gas constant (kJ/ mol·K)
$\boldsymbol{Mw}$	Molecular weight (kg/mol)
$\boldsymbol{\eta}$	Exergetic efficiency (-)
$\boldsymbol{SEL}$	Specific exergy losses (kJ/kg)
$\boldsymbol{CExC}$	Cumulative exergy content (kJ/kg)

Appendix 6.1. Model inputs

Table A6.1 Composition of the streams involved in the aqueous extraction

Component	Seeds (%)	Cake (Solid Residue) (%)	First milk (%)	Subnatant (%)	Mayonnaise (%)
Oil	36.6	4.3	22.0	8.8	61.8
Proteins	18.0	3.0	10.5	11.5	7.2
Others	36.4	43.0	11.8	14.8	1.6
Water	9.0	49.6	55.7	65.9	29.4

Table A6.2 Yields and changes in composition of the streams considered for the mass balance of the conventional process.

Process	Mass changes	Reference
Air classification	Yield of 80.36% on total material Yield of 93.01% on oil Yield of 85.89% on protein	Experimental work
Cooking	Water content reduces from 9% to 6%	Unger (1990)
Pressing	50% of the oil removed	Matthäus (2016)
Hexane extraction	99% of the oil removed	Unger (1990)
1st evaporation	Initially in the miscella: 25% Finally in the miscella: 65%	Matthäus (2016)
2nd evaporation	Initially in the miscella: 65% Finally in the miscella: 95%	Matthäus (2016)
Oil stripping	Initially in the miscella: 95% Finally: 800 ppm solvent	Matthäus (2016)
Degumming	Initial phospholipid content: 3% Final phospholipid content: 0,2% Water: 2% of the oil stream	O'brien (2008)
Neutralization	Oil loss is 7%	Purwasasmita et al. (2015)
Bleaching	2% bleaching earth is used The bleaching earth absorbs 45% oil	O'brien (2008)
Dewaxing	Initial waxes content: 2000 ppm Final waxes content: 10 ppm	O'brien (2008)
Deodorization	No significant losses	-

Table A6.3 Temperatures used for the calculations of the energy and exergy analysis.

Process	Temperature (°C)	Reference
Milling	20	-
Air classification	20	-
Flaking	20	-
Cooking	70	Unger (1990)
Pressing	70	-
Solvent Extraction	50	Unger (1990)
Cake-Desolventization	100	Unger (1990)
1st evaporation	50	Matthäus (2016)
2nd evaporation	80	Matthäus (2016)
Oil stripper	100	Matthäus (2016)
Degumming	80	Matthäus (2016)
Cooling	35	O'brien (2008)
Neutralization	74	O'brien (2008)
Bleaching	100	O'brien (2008)
Cooling	8	O'brien (2008)
Dewaxing	18	O'brien (2008)
Deodorization	220	O'brien (2008)
Mayonnaise homogenization	20	-

Table A6.4 Chemical exergies of all the components for conventional and aqueous extraction process.

Substance	Chemical exergy (kJ/kg)	Reference
Rapeseed oil	40338	[43]
Water	50	[44]
Steam	528	[44]
$NaHCO_3$	257	[44]
Cellulose	18875	[45]
Hemicellulose	19177	[45]
KCl	338	[44]
Acetic acid	15120	[44]
Hexane liquid	47843	[46]
Hexane gas	47890	[46]
NaOH	1873	[44]
Lignin	37133	[45]
Rapeseed protein	21197	Calculated from the exergies of amino-acids reported in Mady & Oliveira, 2013.
Ash	1700	[48]
KCl solution 0.2M	54	Calculated from water and KCl exergies reported.
Ethylene glycol	19473	[46]
$NaHCO_3$ solution 0.1M	52	Calculated from water and $NaHCO_3$ exergies reported.

References

[1] D. Tilman, K. G. Cassman, P. A. Matson, R. Naylor, and S. Polasky, "Agricultural sustainability and intensive production practices," *Nature*. 2002.

[2] J. I. Boye and Y. Arcand, "Current Trends in Green Technologies in Food Production and Processing," *Food Engineering Reviews*. 2013.

[3] M. E. J. Geerts, M. Strijbos, A. van der Padt, and A. J. van der Goot, "Understanding functional properties of mildly refined starch fractions of yellow pea," *J. Cereal Sci.* 2017.

[4] C. V. Nikiforidis, A. Matsakidou, and V. Kiosseoglou, "Composition, properties and potential food applications of natural emulsions and cream materials based on oil bodies," *RSC Adv.* 2014.

[5] A. J. Van Der Goot *et al.*, "Concepts for further sustainable production of foods," *J. Food Eng.* 2016.

[6] M. E. J. Geerts, B. L. Dekkers, A. van der Padt, and A. J. van der Goot, "Aqueous fractionation processes of soy protein for fibrous structure formation," *Innov. Food Sci. Emerg. Technol.* 2018.

[7] F. Chemat, M. A. Vian, and G. Cravotto, "Green extraction of natural products: Concept and principles," *International Journal of Molecular Sciences*. 2012.

[8] C. Boulard *et al.*, "The structural organization of seed oil bodies could explain the contrasted oil extractability observed in two rapeseed genotypes," *Planta*. 2015.

[9] J. Tzen, "Integral Proteins in Plant Oil Bodies," *ISRN Bot.* 2012.

[10] D. Iwanaga *et al.*, "Extraction and characterization of oil bodies from soy beans: A natural source of pre-emulsified soybean oil," *J Agric Food Chem.* 2007.

[11] K. a. Campbell *et al.*, "Advances in aqueous extraction processing of soybeans," *JAOCS, J. Am. Oil Chem. Soc.* 2011.

[12] M. J. Romero-Guzmán, L. Jung, K. Kyriakopoulou, R. M. Boom, and C. V. Nikiforidis, "Efficient single-step rapeseed oleosome extraction using twin-screw press," *J. Food Eng.* 2020.

[13] M. J. Romero-Guzmán *et al.*, "The effect of monovalent (Na^+, K^+) and

divalent (Ca^{2+}, Mg^{2+}) cations on rapeseed oleosome (oil body) extraction and stability at pH 7," *Food Chem.* 2020.

[14] M. J. Romero-Guzmán, N. Köllmann, L. Zhang, R. M. Boom, and C. V. Nikiforidis, "Controlled oleosome extraction to produce a plant-based mayonnaise-like emulsion using solely rapeseed seeds, " *LWT*. 2020.

[15] U. Thiyam-Hollaender, N. Eskin, and N. Michael, "*Canola and rapeseed: production, processing, food quality, and nutrition*," CRC Press, Boca Raton, Florida, USA. 2012.

[16] L. J. Harrison and F. E. Cunningham, "Factors Influencing the Quality of Mayonnaise: a Review," *J. Food Qual.* 1985.

[17] F. K. Zisopoulos, L. Overmars, and A. J. van der Goot, "A conceptual exergy-based framework for assessing, monitoring, and designing a resource efficient agri-food sector," *J. Clean. Prod.*, 2017.

[18] L. Jankowiak, J. Jonkman, F. J. Rossier-Miranda, A. J. van der Goot, and R. M. Boom, "Exergy driven process synthesis for isoflavone recovery from okara," *Energy*, 2014.

[19] N. Lindeboom and P. K. J. P. D. Wanasundara, "Interference of phenolic compounds in Brassica napus, Brassica rapa and Sinapis alba seed extracts with the Lowry protein assay," *Food Chem.* 2007.

[20] B. Matthäus, "Possibilities of Sustainable Oil Processing," in *Breeding Oilseed Crops for Sustainable Production: Opportunities and Constraints*, 2015.

[21] R. D. O'Brien, "Fats and oils: formulating and processing for applications," *Book*. 2004.

[22] S. Kerkhofs, H. Lipkens, F. Velghe, P. Verlooy, and J. A. Martens, "Mayonnaise production in batch and continuous process exploiting magnetohydrodynamic force," *J. Food Eng.* 2011.

[23] J. A. Depree and G. P. Savage, "Physical and flavour stability of mayonnaise," *Trends Food Sci. Technol.* 2001.

[24] D. J. McClements, *Food Emulsions: Principles, Practices, and Techniques, Second Edition*. CRC Press, Boca Raton, Florida, USA. 2004.

[25] E. G. Hammond, L. A. Johnson, C. Su, T. Wang, and P. J. White, "Soybean Oil," in *Bailey's Industrial Oil and Fat Products*, 6th edition., edited by F. Shahidi, John Wiley & Sons, New York. 2005.

[26] R. Pichot, R. L. Watson, and I. T. Norton, "Phospholipids at the interface: current trends and challenges," *International journal of molecular sciences*. 2013.

[27] E. H. Unger, "Commercial Processing of Canola and Rapeseed: Crushing and Oil Extraction," in Canola and Rapeseed. *Book*. 1990.

[28] A. Rutkowski, S. A. Kaczmarek, M. Hejdysz, S. Nowaczewski, and D. Jamroz, "Concentrates made from legume seeds (lupinus angustifolius, lupinus luteus and pisum sativum) and rapeseed meal as protein sources in laying hen diets," *Ann. Anim. Sci.*, 2015.

[29] A. Martin, R. Osen, A. Greiling, H. P. Karbstein, and A. Emin, "Effect of rapeseed press cake and peel on the extruder response and physical pellet quality in extruded fish feed," *Aquaculture*, 2019.

[30] S. Ren *et al.*, "Pectin plays a role in restricting the utilization of rapeseed meal by Chinese mitten crab, Eriocheir sinensis," *Aquac. Res.*, 2019.

[31] A. Fetzer, T. Herfellner, A. Stäbler, M. Menner, and P. Eisner, "Influence of process conditions during aqueous protein extraction upon yield from pre-pressed and cold-pressed rapeseed press cake," *Ind. Crops Prod.*, 2018.

[32] F. Van Immerseel, Y. Nys, and M. Bain, Improving the safety and quality of eggs and egg products. *Book*. 2011.

[33] D. Karefyllakis, S. Salakou, J. H. Bitter, A. J. van der Goot, and C. V. Nikiforidis, "Covalent Bonding of Chlorogenic Acid Induces Structural Modifications on Sunflower Proteins," *ChemPhysChem*. 2018.

[34] P. Alexander, C. Brown, A. Arneth, J. Finnigan, and M. D. A. Rounsevell, "Human appropriation of land for food: The role of diet," *Glob. Environ. Chang.*, 2016.

[35] D. K. O'Toole, "Characteristics and use of okara, the soybean residue from soy milk production - A review," *Journal of Agricultural and Food Chemistry*. 1999.

[36] H. Lian, K. Luo, Y. Gong, S. Zhang, and L. Serventi, "Okara flours from chickpea and soy are thickeners: increased dough viscosity and moisture content in gluten-free bread," *Int. J. Food Sci. Technol.*, 2019.

[37] J. W. Anderson *et al.*, "Health benefits of dietary fiber," *Nutrition Reviews*. 2009.

[38] G. Montemayor-Mora, K. E. Hernández-Reyes, E. Heredia-Olea, E. Pérez-

Carrillo, A. A. Chew-Guevara, and S. O. Serna-Saldívar, "Rheology, acceptability and texture of wheat flour tortillas supplemented with soybean residue," *J. Food Sci. Technol.*, 2018.

[39] L. Flutto, "PECTIN | Food Use," in *Encyclopedia of Food Sciences and Nutrition*, 2004.

[40] M. N. Riaz, "Texturized vegetable proteins," in *Handbook of Food Proteins*, 2011.

[41] K. Kyriakopoulou, B. Dekkers, and A. J. van der Goot, "Plant-Based Meat Analogues," in *Sustainable Meat Production and Processing*, 2019.

[42] A. Lomascolo, E. Uzan-Boukhris, J. C. Sigoillot, and F. Fine, "Rapeseed and sunflower meal: A review on biotechnology status and challenges," *Appl. Microbiol. Biotechnol.* 2012.

[43] K. J. Ptasinski, M. J. Prins, and A. Pierik, "Exergetic evaluation of biomass gasification," in *ECOS 2005 - Proceedings of the 18th International Conference on Efficiency, Cost, Optimization, Simulation, and Environmental Impact of Energy Systems*, 2005.

[44] B. R. Bakshi, T. G. Gutowski, and D. P. Sekulić, *Thermodynamics and the destruction of resources*. 2011.

[45] P. Silva Ortiz and S. de Oliveira, "Exergy analysis of pretreatment processes of bioethanol production based on sugarcane bagasse," *Energy*, 2014.

[46] D. R. Morris and J. Szargut, "Standard chemical exergy of some elements and compounds on the planet earth," *Energy*, 1986.

[47] C. E. K. Mady and S. Oliveira, "Human body exergy metabolism," *Int. J. Thermodyn.*, 2013.

[48] G. Song, L. Shen, J. Xiao, and L. Chen, "Estimation of specific enthalpy and exergy of biomass and coal ash," *Energy Sources, Part A Recover. Util. Environ. Eff.*, 2013.

7

General Discussion

7.1 Introduction

The aim of this thesis was to investigate the phenomena that are important in the aqueous extraction of oleosomes from rapeseeds and from this, propose an extraction process that is more efficient in time, resources and leads to emulsions that are stable and suitable for food products. The central hypothesis was that the choice of the correct process conditions, in combination with a good process design, will enable just this. This chapter will summarize the main findings from the previous chapters, compile these into an overall conclusion and relate this to the aim of the thesis. Finally, an outlook towards the future will be given.

7.2 Main findings

Most of the proposed protocols for oleosome extraction are focusing on the extraction of pure oleosomes and high yields [1]–[3]. The reported protocols follow the rules of those who proposed the extraction for the first time, for the isolation of oleosomes for an extensive analysis [4]. Therefore, little attention was paid to the amount of resources used, and the state in which the other streams end up. In this thesis, the attention has therefore been not only on the yield and purity of the oleosomes, but also on the amount of water that is needed, the use of chemicals (e.g., for adjustment of the pH) and on the mildness of the processes, which is important for retaining the functionality of the co-extracted proteins.

In **Chapter 2** the effect of cations on the solubilization of oleosomes during aqueous extraction was investigated. One typically uses alkaline conditions to obtain high oleosome yield [2], [5]; however, the use of alkaline conditions to achieve this, intensifies the process and affects the co-extracted streams [6]. We hypothesized that the use of different cations (K^+, Na^+, Mg^{2+}, Ca^{2+}) could

affect the molecular interactions of the oleosomes and the co-extracted material, and hence influence their extractability. Indeed, it was found that the use of cations positively affects the extraction of oleosomes. The effect of the cations on oleosome extraction did not follow the well-known Hofmeister series [7]–[9]. Instead, the cations interacted with the charged molecules or with specific sites of the proteins, affecting oleosome extractability and stability. Nevertheless, the use of potassium ions resulted in an overall extraction that was as good as when using alkaline conditions.

In **Chapter 3** we explored the potential of twin-screw press technology to perform the oleosome extraction. Most of the proposed extraction methods for oleosome extraction are at a lab scale and are proposing the use of devices such a blender or a coffee mill [10], [11]. The problem for all of these technologies is that they are difficult to scale-up and required the use of big quantities of water [11], [12]. Therefore, in this chapter it was investigated whether it is possible to use a twin-screw press, preceded by a soaking step in a limited amount of water. The results showed that it is possible to extract intact oleosomes with this method, which basically separates the fibrous parts, such as the hulls and pericarp, from the deformable, water-swollen endosperm, which mostly contains oleosomes, protein and other soluble components. The solubilization of the cellular material, that is generally facilitated by using alkaline conditions, was less important using twin-screw press, since the mechanical forces expel any deformable matter, whether completely dissolved or not. Therefore, the use of electrolytes or alkaline conditions was unnecessary for good extraction. Next to this, a six-fold reduction in water use could be obtained.

In **Chapter 4** the influence of the water soaking before the expression was investigated. It is generally assumed that long soaking times are required for good extraction The discrepancy between the proposed times in literature, which range between 16h up to 24h [5], [13], suggests that those long soaking times are an arbitrary choice that impacts the feasibility of the process. However, it was unclear whether this was also the case when using a pressure-driven process. Therefore, we decided to link the effect of soaking time with the mechanical properties of the seeds and their effect on the oleosome extraction and characteristics. Indeed, the optimal soaking time was found to be linked to the mechanical properties of the seeds. We showed that mechanical properties of the seeds from 8h are optimal to extract intact oleosomes. For shorter soaking times, the oleosomes were not extracted intact, but enlarged with co-extracted material attached to their interface.

Extraction of oleosomes yields a suspension of oleosomes that also contains other components, such as proteins and some carbohydrates. The exact composition and amount of these components depend on the conditions during the extraction. **Chapter 5** discusses the possibility to match the extraction conditions such, that the composition of the extract would already match as much as possible to that of an emulsion-based food product. The results of this chapter show that it is possible to adapt the rheological properties to those that are expected of a commercial mayonnaise, by adapting the exaction conditions. No additional biopolymers were necessary for the formulation of the final product, as the customization of the rheological properties was done by adapting the interactions between oleosomes and between oleosomes and co-extracted material.

Finally, in **Chapter 6**, an assessment was made of the resource usage that is required to produce a mayonnaise-like emulsions food, using olesome aqueous extraction. This alternative-process was assessed in comparison to the existing route that involves complete refining of the oils, and then reformulation with animal-derived ingredients (egg yolk). It was shown that the oleosome route was overall more resource efficient. This efficiency was primarily due to the creation of a protein fraction that is still in its native state, allowing a complete use of the raw material for human foods. On the other hand, the protein that results after conventional oil extraction is denatured during the oil extraction, due to extensive heating and exposure to chemicals, and therefore is only suited to be used as livestock feed. However, the aqueous process for extracting oleosomes is energy intensive, as a large amount of water needs to be evaporated. However, the exergy loss because of this is small compared to the reduction of exergy loss due to the use of the proteins for human consumption.

7.2 The remaining challenges and considerations

The main benefit of the use of intact oleosomes for the production emulsion-based foods is that it retains their natural structure. This protects the oil against oxidation and coalescence. Therefore, no stabilizers, such as antioxidants, sequestrants and emulsion stabilizers must be added. The aqueous process that is proposed in this thesis has as main benefit that it preserves the rapeseed proteins in their native state, enabling direct use in products for human consumption. Disadvantages however are that (1) the protein is not recovered as pure protein, but as a protein rich stream; and (2) that it uses a relatively large quantity of water, which necessitates an energy consuming drying step. The first disadvantage could be amended by further

refining the protein rich stream, for example by using ultrafiltration, or possibly by size exclusion chromatography, depending on the purity that would be required. This will however require more water and may further increase the amount of water necessary.

An alternative route is to not refine the proteins down to purity, but to find optimal applications to the fractions that are created. As Jonkman already stated [14], consumer foods are generally not pure materials, but complex mixtures by themselves. Therefore, it is reasonable to see whether relatively "impure" fractions could be used directly, or after some minor adjustment of the composition, into formulated foods.

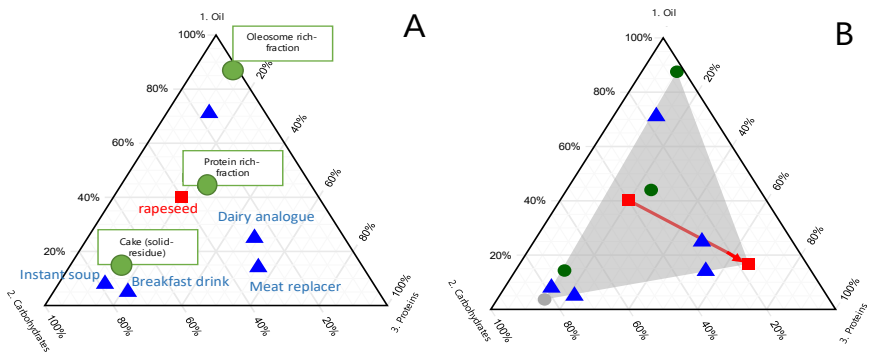

Fig 7.1. A) Compositions of the three main fractions created during production of the oleosomes, plus compositions of some typical food products. B) Some purification of the protein, and combination with a carbohydrate source, would enable the preparation of many food products.

Following the approach of Jonkman, Fig. 7.1 shows the compositions of the three fractions created during the production of the oleosomes. As one can see, the protein fraction does not have a concentration that is too dissimilar from that of the original rapeseeds. As such, it would not be sufficient to create foods, but some further purification of the protein stream would be

necessary. This can for example be done using microfiltration to separate the residual oil and undissolved components, and ultrafiltration to isolate the proteins from the low molecular weight carbohydrates [15].

Another approach would be to adapt the extraction conditions. Alkaline conditions are useful for removing material interacting with oleosomes, as at these conditions oleosomes are charged, repelling co-extracted material such as protein [5]. However, other process aspects as application of dehulling also affect the overall separation obtained. In Fig. 7.2 it is possible to compare the effect of dehulling and pH on the composition and size of oleosomes (oil bodies) and the subnatant (protein-rich fraction).

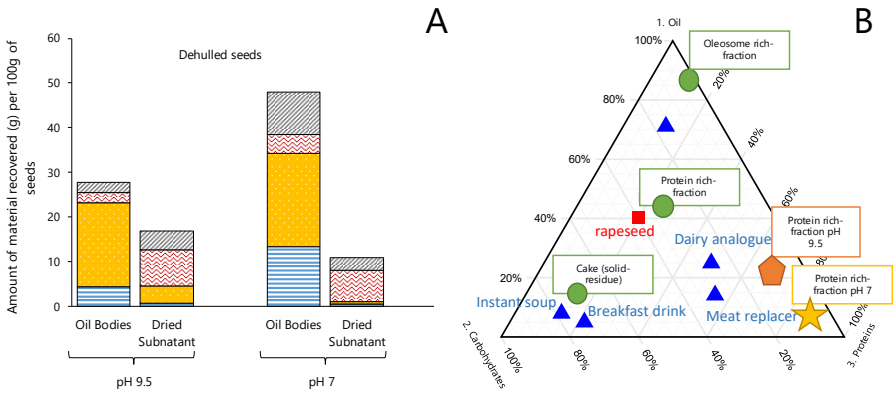

Fig 7.2. A) Compositions and yields of rapeseed oleosomes(oil-bodies) and the dried-subnatant obtained after aqueous extraction of de-hulled rapeseed at neutral or alkaline conditions. The different components of the stream are B) Identification of the protein rich-fractions obtained from the extractions on the ternary diagram. Water ( ), Oil ( ), Protein ( ) and Others ( ).

The process conditions would not just affect the ratio of the components obtained, but also the type of components. For example depending on the pH and ionic strength of the extraction media and the initial status of the

seeds, different proteins could be extracted from the seeds [19]. In Fig. 7.3 a comparison of the effect of these conditions on protein extraction is depicted.

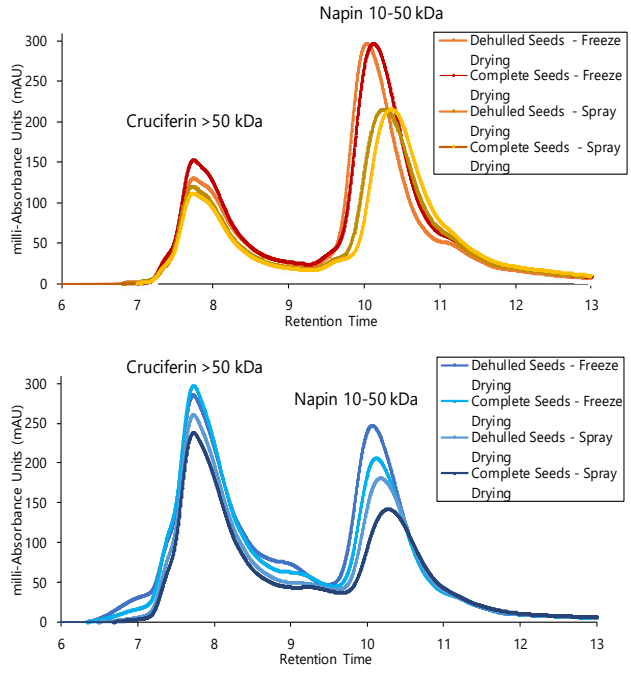

Fig 7.3 HP-Sec performed on the subnatant obtained after aqueous extractions performed with complete or dehulled seeds, at neutral (top graph-orange-yellow lines) or alkaline conditions (bottom graph-blue lines) and dried either by spray drying or freeze drying.

While this thesis was mainly oriented towards the extraction of oleosomes, it is important that in the future the functionality and properties of the protein extract will be investigated. Wanasundara et al. (2016) [20] discussed that rapeseed protein has good nutritional properties, containing all essential amino acids with a balanced amino acid profile, with the Sulphur-containing amino acids closer to the reference protein pattern established by the FAO/UNU/WHO requirements for humans, than legume sources. Cruciferin and napin are different with respect to their solubility with napin

being in general more soluble, even though both are soluble above pH 5.5. Cheung et al (2015) [18] found that cruciferin is a better emulsifier than napin, Nitecka et al. (1986) [19] showed that both proteins have excellent foaming properties. In terms of gelation, napin seems to not form a gel between pH 4 and 8 [20] but globular rapeseed proteins form stronger gels, with maximum gel strength around pH 7 [21]. Mixtures of rapeseed proteins make strong gels, primarily at alkaline conditions [21].

Apart from the positive functionalities of ingredients, also negative aspects have to be considered, such as the presence of anti-nutritional components [22], [23], and the allergenicity of components. The most common antinutritional component for rapeseed is phytic acid, which is strong chelator of metals and it is abundant in seeds and nuts. [24], [25]. However, relatively benign methods like fermentation have shown their use in reducing the level of these types of components [26], [27].

7.3 Outlook to future research

This thesis was focused on the design of a sustainable and feasible oleosome recovery, while considering the extraction of oleosome characteristics and extraction yield. A new process was proposed that can extract an oleosome-rich fraction in a form of an emulsion-based food, and that has the scope to be more resource efficient than the current route depending on full refining of all ingredients.

Chapter 2 and 5 illustrated that the precise conditions during the extraction determine the exact properties of the emulsions, and thus the process should be adapted to the final products that it aims to serve. There are however many more parameters to investigate, next to the type of electrolyte present, and the

pH. In addition, the equipment used for the cell-lysis has not been optimized yet; it is reasonable to expect that further gains can be found here as well (chapters 3 and 4). The adaptation of the extraction process to the ultimate foods in which the extracts will be applied raises the question which are the exact properties that are required from an intermediate product, such as a precursor emulsion coming from the extraction process. Here, research into the nature of the techno-functionalities would be important.

Overall, this thesis contributed to the integration of the preparation of ingredients or intermediates from raw materials, and the design of consumer foods from these components, and it has shown that the intact extraction of oleosomes from oilseeds may well be an excellent route towards this, combining better resource use with the possibility of reducing the use of additives, and the preparation of fully plant-based foods.

References

[1] P. Li *et al.*, "Effects of roasting temperatures and grinding type on the yields of oil and protein obtained by aqueous extraction processing," *J. Food Eng.* 2016.

[2] C. V. Nikiforidis and V. Kiosseoglou, "Aqueous extraction of oil bodies from maize germ (Zea mays) and characterization of the resulting natural oil-in-water emulsion," *J. Agric. Food Chem.* 2009.

[3] A. Matsakidou, F. T. Mantzouridou, and V. Kiosseoglou, "Optimization of water extraction of naturally emulsified oil from maize germ," *LWT*. 2015.

[4] J. T. Tzen and A. H. Huang, "Surface structure and properties of plant seed oil bodies.," *J. Cell Biol.* 1992.

[5] S. De Chirico, V. di Bari, T. Foster, and D. Gray, "Enhancing the recovery of oilseed rape seed oil bodies (oleosomes) using bicarbonate-based soaking and grinding media," *Food Chem.* 2018.

[6] D. Karefyllakis, S. Salakou, J. H. Bitter, A. J. van der Goot, and C. V. Nikiforidis, "Covalent Bonding of Chlorogenic Acid Induces Structural Modifications on Sunflower Proteins," *ChemPhysChem*. 2018.

[7] D. Bastos-González, L. Pérez-Fuentes, C. Drummond, and J. Faraudo, "Ions at interfaces: The central role of hydration and hydrophobicity," *Curr. Opin. Colloid Interface Sci.* 2016.

[8] A. C. Dumetz, A. M. Snellinger-O'Brien, E. W. Kaler, and A. M. Lenhoff, "Patterns of protein – protein interactions in salt solutions and implications for protein crystallization," *Protein Sci.* 2007.

[9] D. Roberts *et al.*, "Specific ion and buffer effects on protein-protein interactions of a monoclonal antibody," *Mol. Pharm.* 2015.

[10] L. C. Dickey, M. J. Kurantz, and N. Parris, "Oil separation from wet-milled corn germ dispersions by aqueous oil extraction and aqueous enzymatic oil extraction," *Ind. Crops Prod.* 2008.

[11] L. T. Towa, V. N. Kapchie, C. Hauck, H. Wang, and P. a. Murphy, "Pilot plant recovery of soybean oleosome fractions by an enzyme-assisted aqueous process," *JAOCS, J. Am. Oil Chem. Soc.* 2011.

[12] A. Rosenthal, D. L. L. Pyle, and K. Niranjan, "Simultaneous aqueous extraction of oil and protein from soybean: Mechanisms for process design,"

Food Bioprod. Process. Trans. Inst. Chem. Eng. Part C. 1998.

[13] W. Lili, C. Yeming, and L. Zaigui, "The effects of freezing on soybean microstructure and qualities of soymilk," *J. Food Eng.* 2013.

[14] A. Castiglioni, J. Jonkman, R. Akkerman, and A. van der Padt, "Selection of fractionation pathways and intermediates for mixed consumer products," in *Computer Aided Chemical Engineering*. 2018.

[15] M. Cheryan, *Ultrafiltration and Microfiltration Handbook*. 1998.

[16] J. P. D. Wanasundara, "Proteins of *Brassicaceae* Oilseeds and their Potential as a Plant Protein Source," *Crit. Rev. Food Sci. Nutr.* 2011.

[17] J. P. D. Wanasundara, T. C. McIntosh, S. P. Perera, T. S. Withana-Gamage, and P. Mitra, "Canola/rapeseed protein-functionality and nutrition," *OCL - Oilseeds fats, Crop. Lipids*. 2016.

[18] L. Cheung, J. Wanasundara, and M. T. Nickerson, "The Effect of pH and NaCl Levels on the Physicochemical and Emulsifying Properties of a Cruciferin Protein Isolate," *Food Biophys.* 2014.

[19] E. Nitecka and K. D. Schwenke, "Functional properties of plant proteins. Part 8. Effect of succinylation on some functional properties of the main globulin fraction from rapeseed (Brassica napus L.)," *Food / Nahrung*, 1986.

[20] Y. L. Folawiyo and R. K. O. Apenten, "The effect of heat- and acid-treatment on the structure of rapeseed albumin (napin)," *Food Chem.*1997.

[21] J. H. J. Kim, N. V. Varankovich, and M. T. Nickerson, "The effect of pH on the gelling behaviour of canola and soy protein isolates," *Food Res. Int.* 2016.

[22] A. Fetzer, T. Herfellner, A. Stäbler, M. Menner, and P. Eisner, "Influence of process conditions during aqueous protein extraction upon yield from pre-pressed and cold-pressed rapeseed press cake," *Ind. Crops Prod.* 2018.

[23] F. Dingyuan and Z. Jianjun, "Nutritional and anti-nutritional composition of rapeseed meal and its utilization as a feed ingredient for animal," *Int. Consult. Gr. Res. Rapeseed*, 2007.

[24] S. Astley and P. Finglas, "Nutrition and Health," *Ref. Modul. Food Sci.* 2016.

[25] G. D. Hill, "Plant Antinutritional Factors | Characteristics," *Encycl. Food Sci. Nutr.* 2003.

[26] A. M. Sokrab, I. A. Mohamed Ahmed, and E. E. Babiker, "Effect of fermentation on antinutrients, and total and extractable minerals of high and low phytate corn genotypes," *J. Food Sci. Technol.* 2014.

[27] R. K. Gupta, S. S. Gangoliya, and N. K. Singh, "Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains," *Journal of Food Science and Technology*. 2013.

Summary

Summary

Many food products are emulsion-based. These are generally produced from refined oils that have been extracted using hexane and have been refined to yield a very pure oil. The oils are then emulsified using emulsifiers and stabilizers. In this work we proposed a different route, where the oil-storing structures in oil-bearing seeds, oleosomes, are extracted intact into natural emulsion. While the phospholipid-protein monolayer provides physical and chemical stability to these natural droplets, it also makes aqueous extraction possible. Existing procedures to extract oleosomes exist, however they are not sufficient scalable. Therefore, the work discussed in this thesis considered process re-design to enable larger-scale production but did also investigate the relation between the process conditions, and the properties of the resulting emulsions.

Chapter 1 introduces the reader to the topic, giving the context, aim and scope of the investigation. In **Chapter 2** we investigated the effect of cations on the solubilization of oleosomes during aqueous extraction. We hypothesized that the presence of different cations (K^+, Na^+, Mg^{2+}, Ca^{2+}) affect the association of the oleosomes and co-extracted material and hence their extractability. Indeed, the presence of these ions increases the extraction yield, and influences the aggregation of the oleosomes, the effect of cations on oleosome extraction did not follow the well-known Hofmeister series but may exhibit specific interaction with the phospholipid-protein monolayer.

Chapter 3 explores the use of a twin-screw press to extract the oleosomes. It was found possible to extract intact oleosomes using this technology. The solubilization of the cellular material is less important during the extraction, probably due to the shear forces applied on the seeds and the pore-size of the device. Therefore, the use of ions or alkaline conditions were unnecessary. The use of water could also be reduced from 1:7 to 1:1 using this screw press.

As the soaking step has long residence times, in **Chapter 4** we investigated the real requirements of this step. The variation between the proposed times in literature ranging from 16h up to 24h showed that long soaking times may be an arbitrary choice that impacts the feasibility of the process. Soaking time is indeed a key parameter to ensure the extraction of native oleosomes and moreover that this nativity is strongly related to the mechanical properties of the seeds. In this chapter we concluded that 8 hours soaking times ensures nativity of the oleosomes, representing a significant shortening of the soaking time.

Chapter 5 focuses on the emulsions that are obtained from the extraction process and relates it to the properties that are desired in an emulsion-based consumer food. A mayonnaise-like product as an example. In this chapter, we showed that the extraction conditions affect the composition of the co-extracted material in the oleosome-rich fraction and that this affects the rheological properties of the emulsion. This allows the adaptation of the extraction conditions, such that the resulting emulsion shows similar (rheological) behaviour to typical foods. This chapter is the first one in scientific literature that bridges process conditions with the properties of final oleosome-based product. Therefore, this chapter is the proof of concept showing it is possible to tailor the interactions of the seed components (i.e. oleosomes, proteins and soluble carbohydrates) during the extraction and by picking the right process conditions emulsion-based food products can be formulated using solely biopolymers contained in seeds.

In **Chapter 6,** the resource usage of the proposed process is compared with the conventional way to produce emulsion-based products. The process described in Chapter 5 was used as basis for this analysis. The results showed that even though the aqueous route requires more energy (i.e. water removal) than the conventional process, it is overall more resource efficient, since it allows much better use of all fractions, due to the milder process conditions, since no elevated temperatures or organic solvents are used.

Finally, in **Chapter 7** the conclusions from the previous chapters were compiled into guidelines for the extraction process and the resulting emulsions. An important aspect is the full use of all fractions resulting from the extraction process; hence some thoughts are spent on the properties of the protein containing fraction, that results after most of the oleosomes have been extracted. Finally an outlook towards future investigations is given.

Extras

Acknowledgements, about the author, publications and overview of completed training activities

Acknowledgements

First, I would like to thank my supervisors **Costas** and **Remko**, for giving me the opportunity to do this PhD and for believing in me. Thank you, **Costas**, for introducing me to the world of oil-bodies and share with me your vast knowledge about the topic. Thank you for being very critical to improve always the quality of my work, but especially thank you for allowing me to add my perspective and insights to the project. Thank you **Remko** for your critical comments and for you insights and thank you for always have a very open attitude for discussion. Thank you for letting me be part of FPE, I strongly believe that the success of this group is related with the harmonious and friendly environment that you and the other staff members have created and embraced.

Thank you to **David** for welcoming me in your group during our collaboration.. Also a special thank you to **Simone** and **Vincenzo** for helping me there and of course for the valuable discussions that we had and resulted in valuable research pieces.

I would also like to thank you **Atze-Jan** (Dankewol!), thank you for adopting me in the food structuring group. Thank you for keeping me involved in many activities and meetings. Thank you for keeping an eye on my progress and always keeping me active the discussion of the group and of course thank you for your insights.

Thank you to all FPE secretaries, but a special thanks to **Marjan**, for being so helpful, caring and nice. Thank you for all your so valuable help and your warm smile. Thank you also to all the technicians: **Jarno**, **Maurice**, **Jos**, **Martin** and **Wouter**. Thank you for helping me during trouble-shutting, for the cakes, the gardening, the cakes, the experiments, the coffee breaks, the PhD-trip organization, the accounting and more cakes! There were just too many cakes :). I really enjoyed having lunch with you and sharing the coffee breaks. Thank you **Jarno** for the organization of the FPE garden, the garden dinners and all the sport activities. Thank you, **Maurice**, for always laugh and helping me to find chocolates :). Thank you, **Jos**, for your nice energy and your help trouble-shooting. Thank you **Wouter** for all the talks during the breaks and your nice attitude. Thank you, **Martin**, for the helping me with the accounting and for always pushing me to practice my Dutch.

Thank you to all my officemates. My first-generation officemates: **Kacie**, **Patty**, **Zhao Jun**, **Pina** and **Anja**. Thank you, **Kacie** and **Patty**, for being always so kind and caring and trying always to organize trips and dinners. Thank you, **Zhao Jun**, for always smile and have a positive attitude. Thank you **Pina** for all your support and talks, I really appreciate that you were always there to hear me and thank you for all those nice moments during our courses and trips. Thank you all of you for all the nice talks and many adventures that we shared together. My second-generation officemates: **Luo Qi** and **Dimitri** and 2.1 **Alime.** Thank you, **Luo Qi**, for all the dinners, board game nights and nice moments. Thank you, **Dimitri**, for the food conference at your house and your funny remarks. Thank you **Alime** for the introduction to Turkish cuisine and for your kind and sweet attitude. My third generation of officemates: **Boxin** and **Katharina**. Thank you to both of you for the dinners, the scientific and non- scientific talks, the coffees, the sweets and all your support in the last stage of my PhD. I enjoyed a lot our walks to get coffee and our lunches and dinners together. Thank you again to all of you, it was a pleasure to share this ride and the office with you. To my adoptive officemates for a month **Anna**, **Murat** and **Zulhaj**, you are amazing! :) thank you for all the nice moments, discussions, talks and dinners!! Thank your **Zulhaj** for always sharing your snacks with me :).

Thank you to my favorite FPE-Mexican great cook: **Andrea.** Thank you, **Andie**, for your delicious food and nice talks. Thank you for being so kind and for sharing with me a little piece of the most delicious part of our common heritage: Mexican Food.

Thank you to all PhDs colleagues and friends for your kindness and friendliness to all. Thank you **Qinhui** for the dinners and the talks, I really appreciate your point of view and your love for good food. Thank you to **Anton,** for the coffee break visits, especially those with **Cleme** :). **Jaapie** thank you for all the pranks and fun. I was so happy to have you around. Thank you, **Victor A.**, for the dinners and the nice moments and the nice songs. Thank you, **Birgit**, for all the board game nights and intense sport afternoons. Thank you also to the Nederlandse-lunch Jongens: **Anna, Steven, Jan** en **Bijoy**. Bedankt, zonder jullie heb ik geen inburgeren diploma gekregen. Bedankt ook voor de lunches en "avondetenjes", en Sinterklaas. Thank you, **Joanna**, for organizing the Velueweloop and thank you to all the team members, it was a lot of fun. Thank you **Konstantina** for all the

lunches, coffees, nice talks and nice moments we have shared together. Thank you **Lu** for invited me to the painting club and all the nice moments we spent together. To **all FPE people (Emma, Nynke, Sten, Yu, Wanqing, Floor, Anouk, Sirinan, Evrila, Eric, Ivanna, Isabel** & **Evelien)** thank you very much for making FPE an amazing place to work!

I would also like to thank to my students: **Kaylee**, **Simha**, **Vassilis**, **Orestis**, **Irene**, **Louis**, **Nienke**, **George** and **Eirini**. Thank you very much for your commitment and effort during your thesis, thank you for your questions and for teaching me so much. Thank you for the coffees, dinners, sweets and of course all the scientific and non-scientific talks, I really enjoyed working with all of you.

Thank you **Arnoud** for the nice discussions and for always help me to brainstorm, your insights helped me a lot to come out with some new solutions to my common problems :).

Thank you to my friends outside FPE that were always supporting me and filling me with love and nice moments to relax and re-load to keep going. Thank you to my Rijnveste 34 roommates and to **Vannesa** who introduced me to them, thank you girls (**Cristina, Iskra, Leah, Laura, Chiara, Amelie,** and **Marcela**) for all the dinners, parties, brunches and all those nice moments that we shared together and all your support during the beginning of my PhD. **Macecito** gracias por todas las cenas mexicanas, tus bailes, por todo tu cariño y apoyo, gracias por consentirme, cuidarme, y por tu ayuda invaluable con la startup-up. Thank you, **Matthi** for all the Saturday morning coffees, our sporty moments, the delicious healthy dinners and our nice talks. Gracias **Fierrito** por nuestras sesiones de boot-camp de alta intensidad, las cenas con y sin tequila, los aguachiles, las bbqs, y las sangrías en verano, las charlas, la música y por todo los demás. **Everardo**, **Pancho, Yafte**, **Nancy**, **Edgar**, **Ale** y **Angelito,** gracias por el Simposio CONACyT y nuestras aventuras antes, durante y después de Francia. Thank you to **Cami** and **Raqui** my strong independent women for all the coffees, dinners, brunches and talks. Grazie **Enri** per la playlist e la cultura generale da Bologna e L'Emilia-Romagna, grazie anche per le cene italiane e anche per i colloqui su tutto. Gracias **Gerardo, Karim** y **Cristy** por estar siempre ahí para mí, porque la distancia no significa nada para nuestra amistad ♡.

Muchas gracias a mis dos familias la mexicana y la frisoncita. Gracias por todo el amor y muestras de cariño que me dieron durante estos 4 años, ese cariño y esos cuidados me llenaron de energía cada vez que más lo necesitaba. En especial quiero agradecer a mi **mamá** por todo lo que ha hecho por mi, espero que te sientas orgullosa y sientas que todo ha valido la pena. ¡Te amo Mamá! Gracias **Sofí** por siempre contagiarme de tu amor desmedido por la ciencia, y por inspirarme con tus ganas por siempre querer aprender. Gracias **papá** por nuestras pláticas filosóficas y por hacerme ver que la vida sin filosofía simplemente no es. Gracias a mis **abuelas** por su amor incondicional y sus muestras de cariño, gracias por siempre consentirme. Gracias **familia** ♡ este logro también es de ustedes. Bedankt of Dankewol naar **heitie, memi, Harmien, Theun, Wieger, Lois, Sanna** e **beppe** voor jullie steun en liefde. Ik halden fan jullie!

Durk, muchas gracias por todo, estos cuatro años no serían nada sin tu apoyo, tu comprensión y tu cariño. Gracias por estar siempre ahí, paciente para escucharme y aconsejarme. Gracias por alentarme, animarme, inspirarme, leer mi tesis, cocinarme, consentirme... Gracias por todo absolutamente todo lo que has hecho por mí ¡Te amo ♡! Ik hâld fan dei!

Juliana
Wageningen, March 2020

About the Author

María Juliana Romero Guzmán was born in Orizaba Veracruz, Mexico on August 30th, 1990. In 2008, she moved to Monterrey, Mexico where she followed a four and a half year Food Engineering program at The Institute of Technology of Monterrey (ITESM). During her studies she participated in a student exchange program to the University of North Carolina A&T (USA) were she obtained a minor in nutrition and food trends. She obtained her BSc degree with honours on December 2012. Parallel to her studies she worked as a Food Auditor after receiving her HACCP certificate from the International HACCP Alliance in Texas (USA). She additionally worked at The Hershey's Company (Mexico) as an intern in the department of Operation Technologies. In 2013 she moved to the Netherlands to start her studies at Wageningen University where she followed a Master in Food Technology with specialization in Sustainable Food Processing. In the first year of her master she followed several courses on Food Engineering and Biotechnology. On her second year she did her master thesis at the department of Biobased Chemistry and Technology in collaboration with the Netherlands Organisation for Applied Scientific Research (TNO) in Wageningen and Zeist (The Netherlands), respectively. During her thesis she studied the potential of membrane distillation to replace reverse osmosis and evaporation to produce concentrated milk. After completing her thesis, she did her internship at CO_2DRY a spin-off company of FeyeCon Carbon Dioxide Technologies in Weesp (The Netherlands) where she developed and optimized the drying processes for strawberries and mushrooms, using supercritical CO_2. She obtained her master diploma in August 2015. In October 2015 she started her PhD at the Laboratory of Food Process Engineering at Wageningen University (The Netherlands). The results of her PhD work are described in this thesis.

Contact: romero.guzman@hotmail.com

Publications

- M. J. Romero-Guzmán, V. Petris, S. De Chirico, V. di Bari, D. Gray, R.M. Boom, C.V. Nikiforidis. "The effect of monovalent (Na^+, K^+) and divalent (Ca^{2+}, Mg^{2+}) cations on rapeseed oleosome (oil body) extraction and stability at pH 7," *Food Chem.* 2020.

- M. J. Romero-Guzmán, L. Jung, K. Kyriakopoulou, R. M. Boom, and C. V. Nikiforidis, "Efficient single-step rapeseed oleosome extraction using twin-screw press," J. *Food Eng.* 2020.

- M. J. Romero-Guzmán, E. Vardaka, Boom, C.V. Nikiforidis. "Influence of soaking time on the mechanical properties of rapeseed and their effect on oleosome extraction," 2020. *Submitted.*

- M. J. Romero-Guzmán, N. Köllmann, L. Zhang, R. M. Boom, and C. V. Nikiforidis, "Controlled oleosome extraction to produce a plant-based mayonnaise-like emulsion using solely rapeseed seeds, " *LWT.* 2020.

- S. De Chirico, V. di Bari, M. J. Romero-Guzmán, C.V. Nikiforidis, T. Foster, D. Gray, "Assessment of rapeseed oil body (oleosome) lipolytic activity as an effective predictor of emulsion purity and stability," *Food Chem.* 2020.

- M.J. Romero-Guzmán, G. Gousias, C.V. Nikiforidis, R.M. Boom, "Exergetic resource use efficiency of mayonnaise production, oleosome extraction vs conventional processing, " 2020. *Submitted.*

Overview of completed training activities

Discipline specific activities			
Molecular Affinity Separations	OSPT	Amersfoort, NL	2016
Numerical Methods for Chemical Engineers	OSPT	Eindhoven, NL	2016
International School on Modelling and Simulation in Food and Bio Processing	ISEKI	Capri, IT	2016
Food Proteins: Significance, Reactions and Modifications	VLAG	Copenhagen, DK	2016
Microscopy and Spectroscopy in Food and Plant Sciences	VLAG	Wageningen, NL	2017
Han-Sur-Lesse Winter School Physical Chemistry and Soft Matter	Wageningen University	Han-Sur-Lesse, BE	2018
Conferences			
Food Colloids Conference	Wageningen University	Wageningen, NL	2016
Food Colloids Conference [b]	Leeds University	Leeds, UK	2018
1st International Conference on Oil Bodies [a]	Wageningen University	Wageningen, NL	2018
8º Symposium CONACyT [a]	CONACyT	Strasbourg, FR	2019
8º International Symposium on Food Rheology and Structure [a]	ETH	Zurich, CH	2019
General courses			
VLAG PhD week	VLAG	Apeldoorn, NL	2015
Competence Assessment	WGS	Wageningen, NL	2015
Scientific Writing	WGS	Wageningen, NL	2017
Scientific Publishing	WGS	Wageningen, NL	2017
PhD Carrousel	WGS	Wageningen, NL	2017
Philosophy and Ethics of Food Science and Technology	VLAG	Wageningen, NL	2018
PhD Carrousel	WGS	Wageningen, NL	2019
Career Assessment	WGS	Wageningen, NL	2019
Summer School Entrepreneurship	EIT	Madrid, ES	2019
Optional			
Preparation of research proposal	FPE	Wageningen, NL	2015
PhD-Trip 2016	FPE	Switzerland & Germany	2016
PhD-Trip 2018	FPE	Canada	2018
Weekly group meetings	FPE	Wageningen, NL	2015-2019

[an] oral presentation

[b] poster presentation

Teaching obligations

FPE-31806 Sustainability in the food chain-Assistant (2016, 2017, 2018)
2 BSc students, 7 MSc students

The research described in this thesis was financially supported by The Mexican Council of Science and Technology – Consejo Nacional de Ciencia y Tecnología (CONACyT). Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover design by Maria Juliana Romero Guzman.
Printed by GVO drukkers- Proefschriftmaken.nl 80 copies