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The effect of deamidation and lipids on the interfacial and foaming properties of ultrafiltered oat protein concentrates

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ABSTRACT

The aim of this study was to investigate the air-water interfacial and foaming properties of oat protein concentrates produced by an enzyme-aided ultrafiltration method with and without deamidation. A further aim was to determine the role of polar and non-polar lipids at the air-water interface and in foams. The deamidated and ultrafiltered oat protein concentrate (DE-UF-OPC) exhibited higher surface tension compared to the ultrafiltered oat protein concentrate (UF-OPC). DE-UF-OPC had a significantly higher negative zeta potential value (-50 mV) compared to the UF-OPC (-38 mV) at pH 7.0. The higher net charge of the DE-UF-OPC may have decreased the equilibrium concentration of oat proteins at the interfacial layer due to higher repulsion between them. Both of the ethanol extracted OPCs exhibited higher surface tension values most likely due to the partial denaturation of albumins and/or globulins. Removal of the majority of non-polar lipids had no effect on the equilibrium surface tension of OPCs. DE-UF-OPC and UF-OPC exhibited some, but limited foaming ability. The removal of non-polar lipids significantly improved the foamability and stability of DE-UF-OPC and UF-OPC, but the removal of polar lipids only improved the foamability of DE-UF-OPC.

1. Introduction

Oats are unique cereals due to their high protein (15–20% of the groats) and lipid content (3–15% of the groats) compared to other cereals, such as wheat (9–16% protein) and barley (9–12% protein) (Békés & Wrigley, 2016; Hartunian & White, 1992; Lásztity, 1998). Additional benefits are the gluten-free and mildly allergenic protein composition of oats compared to soy protein and wheat gluten and this makes oats an interesting alternative protein ingredient (Breiteneder & Radauer, 2004; Fric, Gabrovska & Nevoral., 2011). Oat proteins are distributed throughout the oat groats and the concentration of proteins increases from the innermost parts towards the periphery (Miller & Fulcher, 2011). Oat proteins mostly consist of globulins (70–80% of the total protein content based on the Osborne classification) while the rest of the protein fractions are avenins (that represent prolamins), albumins, and glutelins (4–15%, 1–12%, and <10% of the total protein content, respectively) (Klose & Arendt, 2012). Most of the oat lipids are stored in the endosperm and bran fractions (Price & Parsons, 1979). In oats, about

20–40% of the total lipids are non-polar triacylglycerols and, polar phospho- and glycolipids account for about 10% and 11–16% of the total lipid content, respectively (Doehlert, Moreau, Welti, Roth, & McMullen, 2010; Lehtinen & Kaukovirta-Norja, 2011; Zhou, Robards, Glennie-Holmes, & Helliwell, 1999).

Currently (to the best of our knowledge), there is only one commercial oat protein concentrate (OPC) available, with a protein concentration of 60 g/100 g, that is produced by enzyme-aided wet fractionation (Mel & Malalgoda, 2022). Wet extraction of oat proteins is performed by dispersing the oat flour into an alkaline solution followed by separation with a decanter centrifuge to separate the protein from the rest of the material. Finally performing iso-electric precipitation to precipitate and further concentrate the proteins (Cluskey, Wu, & Inglett, 1973; Mel & Malalgoda, 2022). Furthermore, wet extraction processes utilize harsh extraction conditions, such as high pH (above 9.0) and isoelectric precipitation that might have an impact on the solubility of plant-based proteins, which consequently alter their techno-functional properties (Momen, Alavi, & Aider, 2021; Tanger, Engel, & Kulozik,

2020). Especially, the isoelectric precipitation has been shown to alter the interfacial and emulsification properties of globular pea proteins (Geerts, Nikiforidis, van der Goot, & van der Padt, 2017; Kornet et al., 2021, 2022). Additionally, water-soluble albumins are lost during the isoelectric precipitation where globulins are usually precipitated (Yang, Kornet, et al., 2022).

Oat proteins have limited techno-functional properties in the pH range from neutral to slightly acidic (Loponen, Laine, Sontag-Strohm, & Salovaara, 2007). This has been attributed to the glutamine-rich regions on the surface of oat globulins that makes them less hydrophilic than other globular plant-based proteins (Shotwell, Afonso, Davies, Chesnut, & Larkins, 1988). Many studies have investigated the alteration of techno-functional properties such as foaming and emulsification of oat proteins by using enzymatic (Jiang et al., 2015; Wang et al., 2021) or chemical modifications (Ma & Wood, 1987; Mirmoghtadaie, Kadivar, & Shahedi, 2009; Mohamed, Biresaw, Xu, Hojilla-Evangelista, & Rayas-Duarte, 2009). Despite these previous works, only a couple of studies have focused on the characterization of the interfacial properties of oat proteins (Brückner-Gühmann, Heiden-Hecht, Sözer & Drusch., 2018; Ercili-Cura et al., 2015; Nivala, Mäkinen, Kruus, Nordlund, & Ercili-Cura, 2017). Furthermore, all of these studies focused on the characterization of highly purified oat protein isolates, while the industrial importance relies on defining the extent of purification required for ingredient functionality. Moreover, oat protein concentrates (OPC) produced from non-defatted oat flour will likely contain lipids, which can negatively impact the techno-functional properties of OPC. Oat lipids decrease the foamability of oat protein concentrates (Ma, 1983). Kaukonen et al. (2011) and Konak et al. (2014) showed that the foaming properties of oat flour were significantly improved by removing non-polar lipids with supercritical carbon dioxide and thus, indicating that non-polar lipids were responsible for the poor foaming properties of oat proteins.

In our previous work, a mild fractionation process based on enzyme-aided slightly alkaline (pH 8.0) extraction with ultrafiltration and subsequent diafiltration, i.e. further rinsing, to produce a highly soluble OPC, was presented (Immonen, Myllyviita, Sontag-Strohm, & Myllärinen, 2021). Furthermore, it was demonstrated that the deamidation reaction catalyzed by protein-glutaminase (PG) enhanced the extractability of oat proteins. More specifically, the protein content of the produced OPC was increased from 40 to 50 g/100 g. Additionally, both deamidated and ultrafiltered OPC and only ultrafiltered OPC produced equally strong heat-induced gel structures at a protein concentration of 10 g/100 g. One of the main criteria for protein ingredients to work as a foaming agent, is high solubility in water, and it is well defined that proteins that exhibit poor solubility have limited functionality in aqueous food products (Amagliani, Silva, Saffon, & Dombrowski, 2021). Furthermore, interfacial properties play an important role in the structure formation and stability of multiphasic foods (i.e., foams and emulsions). The OPCs presented in our previous study exhibited high solubility in water at pH 7.0 (Immonen, Myllyviita, Sontag-Strohm, & Myllärinen, 2021). It was hypothesized that deamidation and surface-active lipids might have a significant effect on the behavior of OPCs in multiphasic food systems. Deamidation by PG is known to alter the surface charge of globular proteins, which increases the electrostatic repulsion under neutral conditions. This effect may be an important factor for the interfacial properties of globular proteins. Therefore, the aim of this study was to determine the effect of deamidation and lipids on the interfacial and foaming properties of OPCs. The OPCs were characterized by their composition focusing on their surface-active components (i.e., proteins and lipids) and physico-chemical properties were determined by zeta potential, particle size, and gel electrophoresis. Interfacial properties were evaluated by surface tension measurements and preliminary foaming experiments. To the best of our knowledge, the air-water interfacial properties of OPCs produced with and without enzymatic deamidation combined with ultra/diafiltration have not been studied.

2. Materials and methods

2.1. Materials

Commercial organic heat-treated whole grain oat flour was obtained from Fazer Mills (Lahti, Finland). Alpha-amylase BAN480L activity 480 KNU-b/g) was obtained from Novozymes (Rotterdam, Netherlands). Amyloglucosidase Amigase Mega L (activity 36 000 AGI/g), and β-glucanase Filtrase NL Fast (activity 40 000 BFG/g) were obtained from DSM (Delft, Netherlands). Protein-glutaminase PG500 (activity 500 U/g) was obtained from Ajinomoto (Tokyo, Japan).

2.2. Methods

2.2.1. The batchwise enzyme-aided extraction of oat proteins in a pilot-scale

The preparation of the OPCs was based on the extraction method that was reported by Immonen, Myllyviita, et al. (2021) and is briefly described here. The deamidation was optionally performed with PG enzyme (dosage of 11 U/g protein) and it was added at the start of the extraction process together with other enzymes. First, the enzymes (PG, β-glucanase, α-amylase, and amyloglucosidase) were dispersed in water followed by mixing the oat flour (20% w/w) in the enzyme-water mixture (total amount of oat-water dispersion was 40 kg). This oat flour-water dispersion was then incubated at pH 6.5 at 60 °C for 1 h under constant stirring. After this, the pH was adjusted to 8.0 with 10 g/100 g NaOH and the mixture was further incubated for 3 h at 60 °C. The dispersions were centrifuged at 4000×*g* (Beckman Model J-6M Induction Drive Centrifuge, Beckman Instruments Inc, UK) for 10 min at 25 °C. The soluble protein was collected and incubated at 75 °C for 5 min to inactivate the enzymes. However, α-amylase was not inactivated, due to its heat stability at 75 °C.

The supernatants were concentrated batchwise using a pilot-scale ultrafiltration plant with a 50 L feed vessel that was equipped with 2.5″polymeric polyethersulfone spiral wound membrane element with a 10 kDa molecular weight (MW) cut-off and surface area of 2.044 m² (Synder Filtration model ST3B, California, USA). During the filtration process the temperature at 50 °C, transmembrane pressure at 1.6 bar, and the pressure difference across the element at 0.8 bar were kept constant. The supernatants (retentate) was concentrated 2.7 times and this was measured by constantly weighing the accumulated permeate. After this step, the feed was diluted to its initial volume by adding tap water (>40 °C) and subsequently re-concentrating the diluted feed back to the original concentration (diawater to initial feed ratio of 3.2:1). This was performed to remove excess sugars and low MW molecules. Finally, the concentrated oat protein was spray-dried (Buchi, mini spray dryer B-290, Flawil, Switzerland) at an inlet temperature of 165 °C and outlet temperature of 100 °C. The dried OPCs were stored in a double-sealed plastic bag until further use. This extraction process was performed with and without deamidation to produce DE-UF-OPC [(with a deamidation degree of about 45% as presented in our previous study Immonen, Myllyviita, et al. (2021)] and UF-OPC, respectively.

2.2.2. Defatting of the oat protein concentrates

The OPCs were defatted to remove non-polar and polar lipids. This was performed to investigate the role of non-polar and polar lipids for the interfacial properties of OPCs (described in section 2.2.1). The non-polar lipids were extracted according to the method by Yang et al. (2021) with slight modifications. The DE-UF-OPC and UF-OPC were dispersed in hexane at a ratio of 1:10 (w/v), followed by 2 h mixing at room temperature under constant stirring using a magnetic stirrer. The OPC-hexane mixtures were filtered using Whatman No. 4 filtration paper and the OPCs were dried under a fume hood overnight at room temperature. The hexane extracted OPCs were named Hx DE-UF-OPC and Hx UF-OPC, respectively.

The polar lipids were extracted according to the method by

Myllymäki, Mälkki, and Autio (1988) with slight modifications. The OPCs were dispersed in a mixture of ethanol (purity of 99.5%)-water (85:15% v/v) in a ratio of 1:10 (w/v), followed by 2 h mixing at room temperature. The dispersions of aqueous ethanol and OPC were centrifuged at 4000×g for 10 min. Finally, the supernatants were discarded and the insoluble fractions containing the defatted OPCs were dried under a fume hood overnight at room temperature. The ethanol-water extracted oat protein concentrates were named EtOH DE-UF-OPC and EtOH UF-OPC. The protein (nitrogen conversion factor of 6.25, Kjehdahl) and fat content of Hx DE-UF-OPC, EtOH DE-UF-OPC, Hx UF-OPC, and EtOH UF-OPC were determined in duplicate according to the methods ISO 8968–1:2014 and ISO 1735:2004, respectively.

2.2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The proteins in Hx DE-UF-OPC, Hx UF-OPC, EtOH DE-UF-OPC, and EtOH UF-OPC were determined using a method that was presented by Immonen, Chandrakusuma, Sibakov, Poikelispää, and Sontag-Strohm (2021). In brief, the proteins were characterized with SDS-PAGE, using 10% Bis-Tris gel (Criterion™ XT, BIO-RAD, Hercules, CA, USA) under both non-reduced and reduced conditions. The used running buffer in the SDS-PAGE analysis was 10% 3-(N-morpholino) propane sulfonic acid (MOPS), and the reducing agent was mercaptoethanol. SeeBlue Plus 2 Pre-stained protein standard (marker proteins appeared in the areas of 250, 150, 100, 75, 50, 37, 25, 20, and 15 kDa) (Thermo Fischer Scientific, Invitrogen, Carlsbad, CA, USA) was used as an MW marker, and protein bands were stained with Coomassie brilliant blue.

2.2.4. Total concentration of polar lipids

Oat lipids were extracted from 50 mg of the OPCs as previously described by Heiniö, Lehtinen, Oksman-Caldentey, and Poutanen (2002). The samples in screw-capped 10 mL kimax tubes were supplemented with 5.7 mL of dichloromethane-methanol (2:1) and shaken vigorously (350 rpm) at room temperature for 2 h, after which the tubes were centrifuged (1500×g) for 15 min. The supernatant was transferred into a clean kimax tube and evaporated to dryness under a nitrogen stream at 30 °C. The pellet from the centrifugation was re-extracted as described above, and the solvent was combined with the first extract and dried under a nitrogen stream. The lipid samples were stored frozen under a nitrogen atmosphere until further analysis.

The polar and neutral lipids were separated as previously described by Avalli and Contarini (2005) with minor modifications. The extracted lipid samples were supplemented with 10 μL of 1,2-dipentadecanoyl-sn--glycero-3-phosphatidylcholine standard (Larodan, Sweden) that was dissolved in chloroform at the concentration of 10 mg/mL. After this 0.25 mL of dichloromethane-methanol (2:1) was added to dissolve the lipids. The solid-phase extraction (SPE) cartridges (Supelclean LC-SI, 3 mL, bed weight 500 mg; Supelco, Bellefonte, USA) were conditioned with 4 mL of hexane after, which the lipid sample was applied to the cartridge. The neutral lipids were eluted with 2 mL of hexane-diethylether (4:1) and 2 mL of hexane-diethylether (1:1). The polar lipids were eluted with 2 mL of methanol and 2 mL of dichloromethane-methanol-H$_2$O (3:5:2), collected in clean kimax tubes, and evaporated to dry the collected sample at 37 °C.

The polar lipids were methylated with 0.5 mL of borontrifluoride (14% in methanol; Sigma-Aldrich, MO, USA) by incubating the sample containing tubes for 90 min in a water bath with boiling water. After cooling to room temperature, the samples were supplemented with 1 mL of hexane-metyl-tert-butylether (1:1) and 1.5 mL of distilled water. The tubes were shaken vigorously (350 rpm) for 10 min at room temperature. The water phase (lower) was removed by Pasteur-pipette and the upper layer was washed with 2 mL of 10% NaOH. The tubes were shaken for 5 min (350 rpm) and centrifuged (1500×g) for 20 min at room temperature. The upper layer was collected and dried with anhydrous sodium sulfate and transferred to the 1.5 mL gas chromatography vial. The sample was concentrated by evaporating it to dry under a nitrogen

stream and redissolved in hexane (0.1 mL). Agilent 7890 A GC-system equipped with flame ionization detector and Zebron ZB-FAME (60 m × 250 μm x 0.2 μm) column was used to analyze the fatty acid content and composition. A split ratio of 20:1 was used. The temperature program in the oven was as follows: hold at 70 °C for 4 min; 70–110 °C at 8 °C/min; 110–190 °C at 3 °C/min; hold at 190 °C for 5 min; 190–280 °C at 6 °C/min; hold at 280 °C for 3 min. The gas flow conditions were as follows: H$_2$ in detector 30 mL/min, air 350 mL/min, and N$_2$ (carrier gas) 35 mL/min. The concentration of fatty acids was calculated by comparing the peak area of the fatty acid to the area of standard fatty acid (C15:0). All measurements were performed in triplicate.

2.2.5. Zeta potential and particle size

The zeta potential of DE-UF-OPC and UF-OPC were determined according to the method by Vogelsang-O'Dwyer et al. (2020) with slight modifications. Zeta potential measurement was performed to determine the effect of deamidation on the surface charge of oat proteins. The samples were prepared by dispersing the OPCs in MilliQ-water (0.1 g/100 g protein w/w) and mixed for 1 h at room temperature with a magnetic stirrer. After this, the samples were divided into smaller portions, and the pH was adjusted between pH 2.0–9.0 in 1.0 increments utilizing 1 M HCl or 0.1 M NaOH. The samples were then centrifuged at 2000×g for 10 min to remove the insoluble material. The zeta potential was determined utilizing a ZetaSizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK). The measurements were performed with an automatic voltage selection and the zeta potential was calculated utilizing the Smoluchowski model. A refractive index and absorption of 1.45 and 0.001, respectively, were used. The particle size distributions were determined from the samples at pH 7.0. All measurements were performed in triplicate at room temperature.

2.2.6. Differential scanning calorimetry (DSC)

The denaturation temperature and denaturation enthalpies of OPCs were determined by differential scanning calorimetry (DSC 3+ Thermal analysis system, Mettler Toledo GmbH, Greifensee, Switzerland) using 30 μL high-pressure gold-plated stainless-steel crucibles. The OPCs were weighed with water in a ratio of 1:5 (w/w) into the high-pressure crucibles. An empty gold-plated stainless-steel high-pressure crucible was used as a reference and nitrogen was used as a carrier gas during the measurements. The sealed samples were stored at 6 °C for 24 h. The sealed samples were taken out of cold storage 1 h before the measurement. All measurements were performed using the following parameters: heating rate of 5 °C/min from 30 to 150 °C. All samples were measured in triplicate.

2.2.7. Surface tension

The surface tension (σ) of OPCs at the air-water interface was measured using the straight pendant drop method with Theta flex tensiometer (Biolin Scientific Oy, Espoo, Finland). OPC dispersions were prepared by mixing 20 mg of oat protein powder (DE-UF-OPC, UF-OPC, Hx DE-UF-OPC, Hx UF-OPC, EtOH DE-UF-OPC, and EtOH UF-OPC) in 10 mL phosphate buffer (10 mM, pH 7.0) for 1 h at room temperature using a magnetic stirrer followed by centrifugation (4200× g, 15 min) to remove the insoluble particles. The centrifugation was performed due to the sedimentation of insoluble particles can alter the shape of dispensed droplet, which can have impact on the measured results (Delahaije, Sagis, & Yang, 2022). The supernatants were diluted to the target protein concentration (0.005, 0.01, and 0.04 g/100 g). The protein content of the supernatants was determined before dilution by the Bio-Rad DC protein assay (Bio-Rad, Hercules, California, USA) according to the manufacturer's instructions. The supernatants were diluted to fit the bovine serum albumin protein standard curve (0.2–1.5 mg/mL protein) and then the diluted supernatants were analyzed with a UV–Vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Kyoto, Japan) at 750 nm.

The surface tension of DE-UF-OPC and UF-OPC were measured at

protein concentrations of 0.005, 0.01, and 0.04 g/100 g for 7200 s, and the defatted OPCs were measured at a protein concentration of 0.04 g/100 g for 14 000 s with a picture taken every 9 s (0.11 fps) in both measurements. All surface tension measurements were performed in triplicate and the calculated averages were based on 6-point moving averages. The 14 000 s measurement was performed only once for the DE-UF-OPC and UF-OPC samples to investigate the change in the surface tension values. The longer duration was chosen to ensure that the equilibrium surface tension values were achieved. The used droplet volume was 8 μL. Furthermore, to form a stable droplet, approximately 150 μL of the sample was filled into the plastic pipette tip and about 100 μL of this sample was then dispensed out before starting the surface tension measurement. Furthermore, the surface tension measurements could not be performed above protein concentrations of 0.04 g/100 g as the dispensed droplet expanded during the measurement until it dropped from the tip of the pipette.

2.2.8. Foaming properties

The foaming properties of OPCs were determined according to the method by Konak et al. (2014) with slight modifications. The OPC dispersions (DE-UF-OPC, UF-OPC, Hx DE-UF-OPC, Hx UF-OPC, EtOH DE-UF-OPC, and EtOH UF-OPC) were prepared by mixing the OPC in phosphate buffer (10 mM, pH 7.0) for 15 min at room temperature (protein content of the samples were constant at 1 g/100 g w/w). The mixed dispersions (100 g in total) were then poured into a household milk frother (Melitta Cremio MEL21563, Shenzhen, Guangdong, China) and whipped for 2 min. The whipped dispersion was poured into a 250 mL glass measuring cylinder (inner diameter of 49 mm and height of 197 mm). Foam volume (mL) was recorded at 0, 5, 10, and 30 min. All foaming property measurements were performed in triplicate.

2.3. Statistical analysis

All results were expressed as an average of triplicate measurement, unless said otherwise, with error values according to the mean standard deviation, if not otherwise mentioned. One-way ANOVA was used for statistical analysis of the surface tension, denaturation enthalpies, and the foaming properties of oat proteins, and this was followed by Tukey's honestly significant difference (HSD) test. The limit of statistical significance was set at $p < 0.05$. Statistical analysis was performed using Minitab Statistical Software v.20.1.1 (Minitab, Inc., State College, PA, USA).

3. Results and discussion

3.1. Composition and thermal properties of oat protein concentrates

The defatting of OPCs with hexane reduced the total lipid content of DE-UF-OPC and UF-OPC from 22.3 to 27.6 g/100 g to 9.8 and 8.3 g/100 g, respectively. The EtOH (85% v/v) and hexane significantly ($p < 0.05$) reduced the concentration of polar lipids in both OPCs (Table 1). The total concentration of fat was significantly ($p < 0.05$) lower in DE-UF-OPC compared to UF-OPC, which was shown in our previous work that deamidation by PG had a significant effect on the lipid-protein ratio of OPCs (Immonen, Myllyviita, et al., 2021). In our previous study, it was observed that deamidation significantly increased the amount of soluble proteins, which could explain the reduction of the concentration of other constituents in the deamidated concentrate (DE-UF-OPC). In addition to lipids and proteins, the concentrates are expected to contain carbohydrates composed of various monosaccharides and molecular weights due to the enzymatic pre-treatment. Previously it has been shown that hexane extracts some of the polar lipids from oat flour (Doehlert et al., 2010). Surprisingly, non-polar hexane and polar EtOH had the same effect on the polar lipid content of UF-OPC but for DE-UF-OPC the solvent polarity affected the extraction efficiency. The relative proportion of non-polar lipids increased in EtOH extracted OPCs

Table 1

The protein, total fat, content of polar lipid, neutral lipids (calculated, total fat content – polar lipids), and polar lipid/protein ratio as well as combined protein denaturation enthalpies (90–110 °C) of deamidated and ultrafiltered OPC (DE-UF-OPC), ultrafiltered OPC (UF-OPC), hexane extracted deamidated and ultra-filtered OPC (Hx DE-UF-OPC), hexane extracted ultrafiltered OPC (Hx UF-OPC), EtOH extracted deamidated, and ultrafiltered OPC (EtOH DE-UF-OPC), and EtOH extracted ultrafiltered OPC (EtOH UF-OPC). Different letters ([a–i]) indicate the significant difference ($p < 0.05$) within the same row. The total concentration of protein and fat measurements were performed in duplicate. The total content of polar lipids and the denaturation enthalpy measurements were performed at least in triplicate.

	DE-UF-OPC	Hx DE-UF-OPC	EtOH DE-UF-OPC	UF-OPC	Hx UF-OPC	EtOH UF-OPC
Protein (g/100 g)	52.4 ± 0.6[a]	60.1 ± 0.1	57.9 ± 0.9	45.0 ± 0.1[a]	54.8 ± 0.0	49.5 ± 0.5
Fat (g/100 g)	22.3 ± 0.1[a]	9.8 ± 0.2	18.2 ± 0.1	27.6 ± 0.1[a]	8.3 ± 0.6	28.3 ± 0.0
Polar Lipids (g/100 g)	6.6 ± 0.4[a]	4.3 ± 0.1[b]	1.3 ± 0.0[c]	9.3 ± 0.4[d]	4.0 ± 0.5[b]	4.0 ± 0.1[b]
Neutral lipids calculated (g/100 g)	15.7 ± 0.5	5.5 ± 0.8	17.1 ± 0.0	18.3 ± 0.4	4.3 ± 0.7	24.3 ± 0.2
Polar lipid/protein ratio	0.13	0.07	0.02	0.21	0.07	0.08
Denaturation enthalpy (J/g protein)	15.2 ± 0.6[f]	12.7 ± 0.4[f,h]	8.3 ± 0.8[g]	13.5 ± 0.3[f,h]	11.5 ± 1.9[h,i]	10.1 ± 0.4[i]

[a] Results presented from a previous study by Immonen, Myllyviita, et al. (2021) due to same OPCs were utilized in this study.

due to a reduction in other components (Supplementary Fig. S1A).

The denaturation enthalpies of oat proteins were investigated with DSC to determine if there were any changes in the degree of protein denaturation caused by solvent-based defatting. In the thermograms of the OPC samples, two regions were identified with peak denaturation temperatures of about 90 and 110 °C (Supplementary Figs. S2 and S3). These temperature regions have been previously linked to denaturation temperatures of oat protein fractions: albumins and globulins (Ma & Harwalkar, 1984). The reported protein denaturation endotherms were first integrated as a single area (Table 1). Further on, the protein denaturation enthalpies of albumins were acquired with partial integration of the peaks. The denaturation enthalpy was unchanged indicating no significant changes ($p < 0.05$) in the degree of denaturation of oat proteins when defatting was performed with hexane. However, EtOH extraction decreased the globulin denaturation enthalpy of UF-OPC and albumin as well as globulin denaturation enthalpies of DE-UF-OPC. This indicated that EtOH extraction altered the degree of denaturation of globulins or albumins, but in the case of DE-UF-OPC, both albumins and globulins were altered (Supplementary Fig. S4). Previous studies have shown that the extraction of lipids from soy flour with ethanol can alter the structure of globular proteins, thus reducing the proteins' denaturation enthalpy (Peng et al., 2021). Additionally, it has been shown previously by Vatansever, Ohm, Simsek, and Hall (2022) that supercritical carbon dioxide extraction combined with ethanol extraction led to the unfolding of globular proteins, which resulted in more stretched and disordered structure compared to the highly ordered structure of native proteins.

3.2. Molecular weight distributions of oat proteins by SDS-PAGE

SDS-PAGE was performed to investigate whether there were any noticeable alterations (i.e. hydrolysis) in the oat proteins during the defatting processes (Fig. 1). Before loading the proteins into the wells of SDS-PAGE the concentration of extracted proteins was determined with the DC-protein assay method and based on the results all the samples were diluted to the protein concentration of 2 mg/mL prior to loading
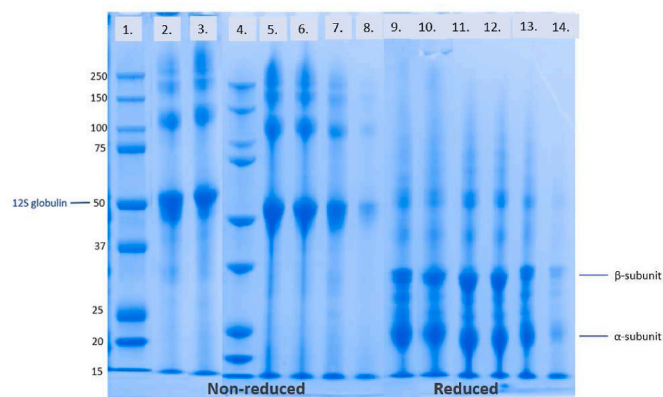
Fig. 1. Non-reduced samples were 1. molecular weight (Standard), 2. deamidated and ultrafiltered OPC (DE-UF-OPC), 3. ultrafiltered OPC (UF-OPC), 4. molecular weight (Standard), 5. hexane extracted deamidated and ultrafiltered OPC (Hx DE-UF-OPC), 6. EtOH extracted deamidated ultrafiltered OPC (EtOH DE-UF-OPC), 7. hexane extracted ultrafiltered OPC (Hx UF-OPC), 8. EtOH extracted ultrafiltered OPC (EtOH UF-OPC) and mercaptoethanol-reduced samples were 9. ultrafiltered OPC (UF-OPC), 10. deamidated and ultrafiltered OPC (DE-UF-OPC), 11. hexane extracted deamidated and ultrafiltered OPC (Hx DE-UF-OPC), 12. EtOH extracted deamidated ultrafiltered OPC (EtOH DE-UF-OPC), 13. hexane extracted ultrafiltered OPC (Hx UF-OPC), and 14. EtOH extracted ultrafiltered OPC (EtOH UF-OPC) investigated with 10% Bis-Tris gel.

them into the gel. The protein bands of hexane-treated OPCs were comparable to the UF-OPC and DE-UF-OPC and thus, defatting with hexane did not seem to cause degradation of proteins. All samples had the main oat globulin band (50 kDa) present in the non-reduced SDS-PAGE that breaks down into 30 and 20 kDa subunits in reducing conditions (Walburg & Larkins, 1983). Major oat albumin bands have been shown to have MW of 14–17, 20–27, and 36–47 kDa under reducing conditions (Lapveteläinen & Aro, 1994; Ma, 1983). This may suggest that the protein bands of albumins overlapped with the protein bands of oat globulins in certain areas of the SDS-PAGE, but the protein bands in the 15 kDa area could be associated with the lowest MW of albumins. The electrophoretic patterns of EtOH-treated OPCs were fainter than the UF-OPC and DE-UF-OPC OPCs. In the present work, there was a noticeable slight shift in the protein bands of deamidated OPCs. Similar results have been reported by Yong, Yamaguchi, Gu, Mori, and Matsamura (2004) and Yong, Yamaguchi, and Matsumura (2006) for deamidated gluten and α-zein. The authors explained that this shift was caused by the increased negative charge of proteins that limited the interaction with SDS, which essentially determines the migration of proteins during SDS-PAGE.

3.3. Particle size distribution and zeta potential

The particle size distributions of UF-OPC and DE-UF-OPC were measured at pH 7.0 and in both of OPCs particle size distributions two peaks were noticed. The volume-based mean diameters of the peaks for UF-OPC were 1345 ± 200 and 192 ± 30 nm and for DE-UF-OPC were 430 ± 110 and 100 ± 20 nm (Fig. 2A). The peaks of the volume-based particle size distribution of DE-UF-OPC were smaller but also wider compared to the UF-OPC. All the detected particles were larger than any single macromolecules in the system indicating that they were aggregates mostly likely consisting of solubilized constituents from centrifuged OPCs. The effect of deamidation on the particle size may be due to increased charge density, which led to more extensive dissociation of aggregates. Furthermore, the reduced particle sizes of DE-UF-OPC can be one factor why the lipids were extracted more easily compared to UF-OPC. Reduction of the particle size has been shown to increase the extraction yield to a certain extent due to increased interaction between the surface of the solute and the solvent (Putra et al., 2018).
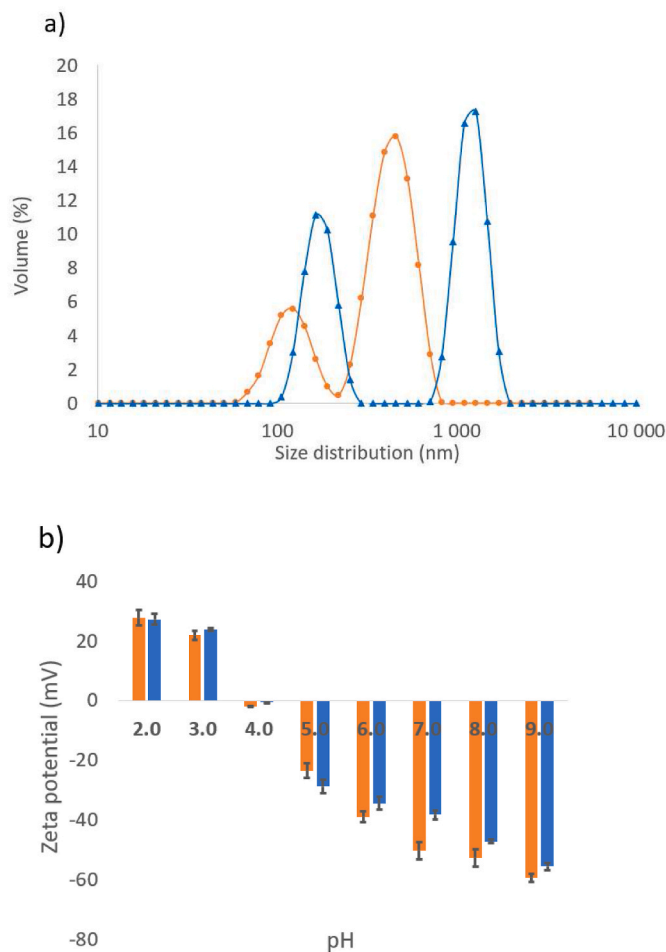

Fig. 2. a) The particle size distribution of DE-UF-OPC (orange line) and UF-OPC (blue line) measured at pH 7.0. b) The zeta-potential measurements of UF-OPC (blue bar) and DE-UF-OPC (orange bar) as a function of pH from 2.0 to 9.0. The black bars in the zeta-potential graph represents the standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The zeta potential was determined to investigate how the surface charge of oat proteins changed as a function of pH due to deamidation by PG. The zeta potential was significantly ($p < 0.05$) changed at pH ranges from 4.0 to 8.0 (Fig. 2B). An increase in the absolute value of zeta potential was noticed at pH 7.0 where it was changed from -38 to -50 mV due to the deamidation by PG, which explains the increased solubility in water reported in our previous work (Immonen, Myllyviita, et al., 2021). Furthermore, Ercili-Cura et al. (2015) reported that oat proteins treated with transglutaminase formed electrostatically stable dispersions while the zeta potential was -45 mV at pH 7.2. The increase in the net charge by deamidation was to be expected, as deamidation increases the negative surface charge by converting glutamine and asparagine to glutamic and aspartic acid, respectively (Jiang et al., 2015). The absolute value of zeta potential shifted at pH 4.0 from -0.6 to -1.9 mV, which was consistent with previous literature (Fu, Chen, Cheng, & Liang, 2022). This shifts the isoelectric point of the DE-UF-OPC towards more acidic compared to the UF-OPC.

3.4. Air-water surface properties

The adsorption of oat proteins at the air-water interface was studied by measuring surface tension with the pendant drop method at protein concentrations of 0.005, 0.01, and 0.04 g/100 g for 7200 s (Fig. 3). The air-water surface properties were investigated at pH 7.0 as it is generally
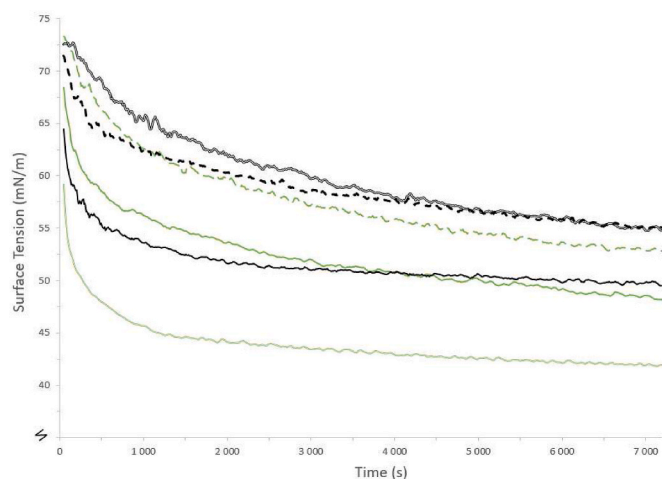
Fig. 3. The surface tension of deamidated and ultrafiltered OPC at 0.005 (DE-UF-OPC, 0.005%, hollow black line), 0.01% (DE-UF-OPC, 0.01%, dashed black line), 0.04% protein (DE-UF-OPC, 0.04% black line) and ultrafiltered OPC at 0.005% (UF-OPC, 0.005%, dashed green line), 0.01% (UF-OPC, 0.01%, green line) and 0.04% (UF-OPC, 0.04%, hollow green line) as a function of time at pH 7.0. The graphs represent the averages of three replicate measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the pH of milk alternatives and it was demonstrated in our previous study that DE-UF-OPC and UF-OPC exhibited high water-solubility at neutral pH (Immonen, Myllyviita, et al., 2021). The surface tension values after 2 h were from the lowest concentration to the highest as follows: 53, 48, and 42 mN/m for UF-OPC and 55, 55, and 50 mN/m for DE-UF-OPC. The surface tension of UF-OPC was significantly (p < 0.05) lower at concentrations of 0.01 and 0.04 g/100 g compared to the DE-UF-OPC. Additionally, the initial surface tension of UF-OPC was lower than that of DE-UF-OPC, which indicated that the surface components of UC-OPC occupied the air-water interface more rapidly that may affect the formation of foam bubbles. Ercili-Cura et al. (2015) observed that oat protein isolate dispersion reduced surface tension at the air-water interface to about a similar value than reported in this study for UF-OPC. The differences between UF-OPC and DE-UF-OPC. One explanation for this could be the increased negative surface charge of DE-UF-OPC due to the deamidation by PG. This can be seen from the zeta potential, which increases the repulsive forces between the oat proteins (Jiang et al., 2015). It has been previously shown that increased negative surface charge raised the energy barrier that proteins need to overcome before they can adsorb to the air-water interface and this slows the proteins' rate of adsorption (Wierenga, Meinders, Egmond, Voragen, & de Jongh, 2005).

Extensive deamidation by PG has been shown to dissociate highly ordered protein structures into smaller-sized clusters, which mainly consisted of monomers (Fu et al., 2022). Ercili-Cura et al. (2015) observed with AFM that oat protein isolate (OPI) formed a protein film at the air-water interface that was composed of large protein aggregates at neutral pH. Additionally, they showed that transglutaminase (TG) treatment reduced the size of these oat protein aggregates at the air-water interface. Untreated oat proteins, on the other hand, have been shown to associate with neighboring proteins by self-assembling to form large protein aggregates in an aqueous solution at pH 7.0 (Liu et al., 2009). Deamidation of soy protein isolate by PG has been recently shown to reduce the surface tension compared to the non-deamidated soy protein (Jiang et al., 2022). There are several factors, such as the chosen method to extract and deamidate the proteins, protein source, deamidation degree, and protein concentration used in the surface tension measurement that can explain the differences between the reported results. The deamidation degree of soy protein isolate that was

reported by Jiang et al. (2022) was similar to the deamidation degree of DE-UF-OPC (above 40%), which was reported in our previous study (Immonen, Myllyviita, et al., 2021). However, they used a considerably higher protein concentration (2 g/100 g) in the surface tension measurements. Moreover, with increased protein concentration the presence of surface-active impurities (i.e., other surface active components that are not proteins) leads to higher interfacial concentration at the interface, which could cause a decrease in the equilibrium surface tension (Tamm, Sauer, Scampicchio, & Drusch, 2012).

The removal of non-polar and polar lipids was performed to determine the effect of lipids on the surface properties of OPCs (Fig. 4). The protein concentration in the surface tension measurement was 0.04 g/100 g and an elongated equilibration of 14 000 s was used. The reduction of non-polar lipids did not have any significant changes to the equilibrium surface tension of Hx UF-OPC (39 mN/m) or Hx DE-UF-OPC (46 mN/m), but these samples are comparable in terms of polar lipids to protein ratio of 0.07 (Table 1) and thus, confirm the difference in the equilibrium surface tension values between Hx DE-UF-OPC and Hx UF-OPC. On the other hand, the reduction of polar lipids due to EtOH extraction significantly (p < 0.05) increased the equilibrium surface tension values of EtOH UF-OPC (45 mN/m) and EtOH DE-UF-OPC (49 mN/m). The role of polar lipids competing for the interface could not be concluded: The equilibrium surface tension values of hexane and EtOH extracted UF-OPCs were distinctly different even though both the concentration of polar lipids and its share of surface-active components were similar. Globular proteins can reduce the surface tension of the air-water interface to low as 47 mM/m compared to 22 mM/m for low molecular weight surfactants (i.e., lipids) (Bos, M, & van Vliet, 2001). Thus, the lower surface tension value of UF-OPC would point to the presence of polar lipids at the air-water interface in addition to proteins. Interestingly, the higher surface tension value of DE-UF-OPC point towards a more protein-dominated interface. Additionally, the denaturation of proteins due to the EtOH extraction might have an impact on the surface activity of OPCs, but it remains to be clarified. Karefyllakis, van der Goot & Nikiforidi (2019) reported that a mix of phospholipids and
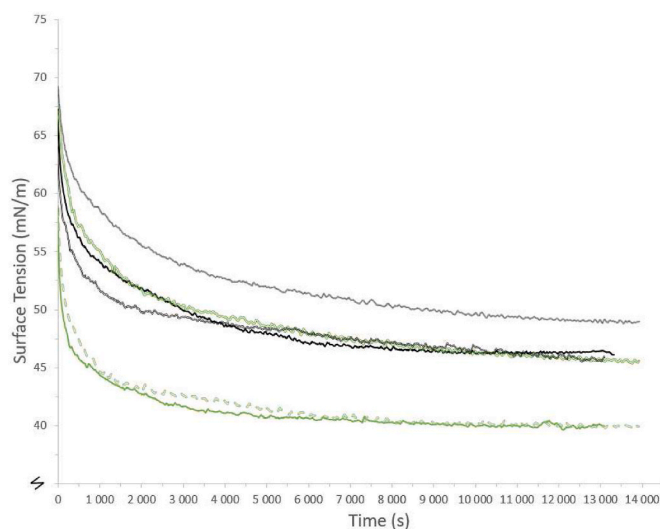

Fig. 4. The surface tension of deamidated and ultrafiltered OPC (DE-UF-OPC, hollow black line), ultrafiltered OPC (UF-OPC, hollow dotted green line), hexane extracted deamidated and ultrafiltered OPC (Hx DE-UF-OPC, black line), hexane extracted ultrafiltered OPC (Hx UF-OPC, dark green line), EtOH extracted deamidated and ultrafiltered OPC (EtOH DE-UF-OPC, thin black line), and EtOH extracted ultrafiltered OPC (EtOH UF-OPC, hollow green line) as a function of time measured with a protein concentration of 0.04% and at pH 7.0. The graphs represent the averages of three replicate measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

globular proteins had a higher ability to reduce surface tension than pure globular proteins at the same protein concentrations (0.001 and 0.01 g/100 g). Additionally, Yang et al. (2021) showed that defatted rapeseed protein concentrate (RPC) had lower surface activity compared to the non-defatted RPC. This might suggest that the surface-active lipids were adsorbed into the air-water interface together with the proteins.

3.5. Foaming properties

The foaming properties of proteins relate to their ability to stabilize gas-liquid interfaces by forming a thin protein layer at the interface (Li et al., 2022). In all of the foaming measurements, the foam columns produced by mechanical stirring utilizing a milk frother were formed on top of the liquid column (part of the dispersion, which did not produce foam). Thus, the foaming ability of OPCs and their defatted counterparts were determined by measuring the initial foam volume (mL) and the foam volume (mL) at selected time points (5, 10, and 30 min) at pH 7.0 and at a protein concentration of 1.0 g/100 g. The foaming experiments were performed using higher protein concentration compared to the drop tensiometer to ensure sufficient foam formation.

DE-UF-OPC produced a slightly higher amount of foam (below 20 mL) and more stable foam when compared to UF-OPC foam (10 mL), which dissipated after 10 min (Fig. 5). Makri, Papalamprou, and Doxastakis (2005) stated that in order to achieve good foam volume proteins were required to adsorb rapidly at the air-water interface continued by rapid conformational change and rearrangement at the interface while the formation of the cohesive viscoelastic film supports foam stability. However, in the present study, the adsorption of deamidated proteins was slower as compared to their non-treated counterparts. Thus, the rate of adsorption might not be critical for foaming in a system where the concentration of protein was higher compared to the interfacial studies. Moreover, similar contradicting results between the drop tensiometer data and the recorded foaming results were observed by Yang, de Wit, et al. (2022b). They concluded that these contradicting results were explained by the different mechanisms of interface formation, which in the drop tensiometer was based on the diffusion of proteins compared to the mechanism in the formation of foams, which was based on diffusion combination with the convectional flow that was developed by whipping. This may have altered the composition of proteins that stabilized the interfaces of droplets and the air bubbles in foams. Furthermore, other factors such as, increased water-solubility and increased structural flexibility of oat proteins due to the deamidation by PG could be

associated with the increase in foam volume (Immonen, Myllyviita, et al., 2021; Jiang et al., 2015). This slight improvement in foam volume was due to the deamidation, which aligned with results reported by Mirmoghtadaie et al. (2009) and Ma and Khanzada (1987). Mirmoghtadaie et al. (2009) showed that chemically deamidated oat proteins had a slight increase in the foaming capacity due to the reduction in the molecular weight of deamidated proteins while Ma and Khanzada (1987) attributed the increased foam volume of chemically deamidated oat proteins to the improvement in water-solubility.

By removing the majority of non-polar lipids the foam heights significantly (p < 0.05) increased for both OPCs (DE-UF-OPC and UF-OPC), but the stability of the foams was almost similar. The results were in accordance with earlier studies that showed non-polar lipids weakening the formation and the stability of foams produced from oat flours (Kaukonen et al., 2011; Konak et al., 2014). The non-polar lipids in aqueous systems might disrupt the interfacial films upon foam drainage by breaking down the foam lamellae and increasing the possibility of film rupture (Husband, Wilde, Clark, Rawel, & Muschiolik, 1994; Kaukonen et al., 2011). The EtOH DE-UF-OPC with a much-reduced amount of polar lipids formed a noticeable amount of foam (initially 30 mL) although with EtOH UF-OPC no foam was formed. Along with the reduction in polar lipids in the ethanol extraction, the share of non-polar lipids was increasing in the EtOH UF-OPC. This increase in the concentration of non-polar lipids and denaturation of globulins and albumins (Supplementary Fig. S4) during the EtOH extraction process can explain the reduction in the ability to produce foam (see section 3.1). Further on, comparing the polar lipids to protein ratio between EtOH DE-UF-OPC (0.02) and EtOH UF-OPC (0.08) supports the observation that in addition to non-polar lipids, also polar lipids in oat concentrate may interfere with foaming ability. Based on our data, it was not easy to link surface tension measurements with foaming ability, as the concentrates are complex in their composition. Yet, it is evident that even with a small share of surface active components, polar lipids competed for the air-water interface with proteins, but the presence of polar lipids for foaming was less detrimental than the presence of non-polar lipids. Interestingly, hexane-extracted samples with the best foaming ability had the most rapid initial decay of surface tension of all samples, which could indicate the importance of dynamic surface tension at the initial stages of foaming rather than the equilibrium value.

4. Conclusions

In this work, the air-water interfacial and foaming properties of OPCs produced by an enzyme-assisted extraction combined with ultrafiltration with and without deamidation and their defatted counterparts were investigated. The deamidation by PG decreased the particle size of OPCs. The DE-UF-OPC exhibited less ability to reduce surface tension at the air-water interface compared to UF-OPC. Reduction of non-polar lipids did not have significant changes in the surface tension of these OPCs, but in these samples, the ratio of polar lipids to protein was the same, and the effect of deamidation increased repulsion between the oat proteins and thus, a less dense interfacial layer of DE-UF-OPC could be established. EtOH extraction was shown to reduce surface tension in both OPCs possibly due to the partial denaturation of globulins of UF-OPC and globulins as well as albumins of DE-UF-OPC. Their presence may have an impact on the interfacial stability due to non-synergistic stabilization mechanisms, but this could not be shown in the preliminary foaming experiments of the present study. Instead, the removal of non-polar lipids improved the foaming properties (4–5 times higher and stable foam) of OPCs. Efficient reduction of the polar lipids increased the foamability and foam stability of DE-UF-OPC. However, interfacial measurements of this study do not fully explain the complex factors of stabilization of foams and this should be elaborated in the future for instance with dilational surface rheology. The concentration of non-polar lipids due to the ethanol extraction may have been responsible
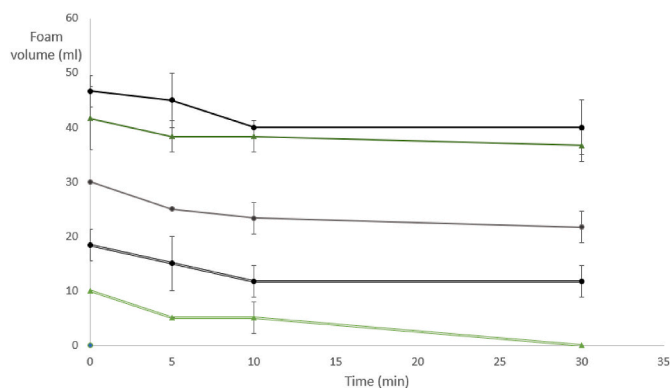

Fig. 5. Foam volume (ml) as a function of time of deamidated and ultrafiltered OPC (DE-UF-OPC, hollow black line), ultrafiltered OPC (UF-OPC, hollow green line), hexane extracted deamidated and ultrafiltered OPC (Hx DE-UF-OPC, dark black line), hexane extracted ultrafiltered OPC (Hx UF-OPC, dark green line), EtOH extracted deamidated and ultrafiltered OPC (EtOH DE-UF-OPC, light black line), and EtOH extracted ultrafiltered OPC (EtOH UF-OPC, no foam). The graphs represent averages and standard deviations of three replicate measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

for the loss of foaming ability of EtOH UF-OPC. The results presented in this study showed that OPC produced by an enzyme-assisted extraction method that utilized deamidation combined with ultrafiltration slightly improved the foamability of the concentrate, but a more critical role in foaming was associated with the removal of non-polar lipids. The surface rheological properties have an important role in the stabilization of multiphasic foods such as foams and emulsions. Therefore, the industrial significance of this study was insights into the interfacial and foaming properties of OPCs and the information about the effect of polar and non-polar lipids on these properties. These results can assist the food industry in the development of novel multiphasic food products from less refined OPCs.

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CRediT authorship contribution statement

Mika Immonen: Conceptualization, Methodology, (protein extraction, defatting, surface tension, thermal properties, foaming properties), Validation, Formal analysis (protein extraction, surface tension, thermal properties, foaming properties), Investigation, Writing – original draft, preparation, Visualization. **Angga Chandrakusuma:** Methodology, (Surface tension and surface hydrophobicity), analysis (surface tension, surface hydrophobicity). **Sanna Hokkanen:** Methodology, (polar lipids), Analysis (polar lipids). **Riitta Partanen:** Investigation, Writing – review & editing, Review, Editing. **Noora Mäkelä-Salmi:** Investigation, Supervision, Writing – review & editing, Review, Editing. **Päivi Myllärinen:** Supervision, Writing – review & editing, Review, Editing, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest. Mika Immonen, Angga Chandrakusuma, Sanna Hokkanen, Riitta Partanen, and Päivi Myllärinen are employees of Valio Ltd.

Data availability

All of the data has been presented in the research paper

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.114016.

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