ORIGINAL PAPER

Effect of Acylation of Rapeseed Proteins with Lauroyl and Oleoyl Chloride on Solubility and Film-Forming Properties

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Abstract

Rapeseed proteins show good film-forming properties, giving them a promising potential as bio-based ingredients for the technical industry, e. g. for films and coatings. However, their hydrophilicity often poses problems in regard to water-stability of coatings. Protein modification using fatty acids is known to reduce hydrophilicity, however, it has not been tested to improve film-forming properties of rapeseed proteins before. In the present study, a rapeseed protein concentrate (RPC) was acylated at low and high modification degree using lauroyl chloride and oleyol chloride. The protein solubility was determined and the modified RPCs were used for the preparation of cast-films to measure the changes of mechanical properties (tensile strength, elongation at break), surface energy, oxygen permeability and light transmission. The protein solubility in water was lowered from 100% for the non-modified RPC to < 15% for highly modified RPCs at pH 7. The tensile strength of films increased by factors of 3.5 and 4 for highly modified samples, respectively. Surface energy and oxygen permeability revealed an increase of hydrophobicity that correlated with the modification degree. The light transmission was reduced by modification. The results confirm the increased hydrophobicity of acylated RPCs and demonstrate the potential of modified rapeseed proteins as an ingredient for technical products, such as packaging layers, coatings and adhesives.

Graphic Abstract
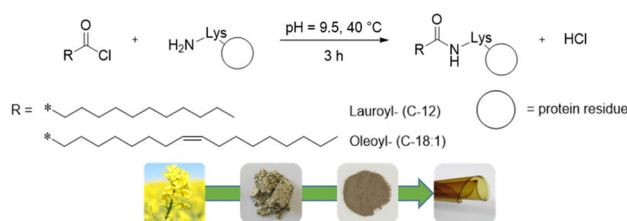
Statement of Novelty

Rapeseed proteins can be isolated from side-stream products of the rapeseed oil industry and could potentially be valorized as bio-based additives in the technical industry. Despite their great functional properties, rapeseed proteins are not well studied for utilization in the non-food industry. In the present study, acylation of rapeseed proteins was carried out in order to increase their hydrophobicity. This is the first time acylation of rapeseed proteins with long hydrocarbon chains was carried out with the aim to enhance the film-forming properties, which are of high relevance to a

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range of technical applications, such as adhesives, coatings or polymers. The results clearly demonstrate the increase of hydrophobicity, thereby improving the application potential of rapeseed proteins as a renewable alternative to conventional additives.

Introduction

Plant proteins from oilseed by-products are a promising alternative for petrol-based ingredients in technical applications. In particular, rapeseed proteins with their great functionalities such as foaming, emulsification and film-forming properties are an interesting functional ingredient, e.g. for paints, varnishes, glues and lubricants. Specifically, film-forming properties are of high relevance for such applications. However, as these products are often based on hydrophobic media, the polar nature of proteins can be problematic in regard to product formulation or water-stability [1]. Hence, methods to render rapeseed proteins more hydrophobic and thus to stabilize these formulations are needed.

Application of proteins in detergents, paints, glues and polymers is gaining increasing interest in the proclaimed bio-economy of the twenty-first century. Historically, proteins—predominantly from animal sources—have been an important raw material for adhesives, fire extinguishing foams or plastics [2, 3]. However, with the advent of the petroleum era, most of these products have been replaced by easier and cheaper methods to produce synthetic materials.

The main storage proteins in rapeseed are the 12S globulin cruciferin and the 1.7–2S albumin napin [4]. Due to their flexible structure comprising polar and non-polar residues, they are able to attach on interfaces, such as air/water or oil/water interfaces, to form and stabilize foams and emulsions [5]. Additionally, great film-forming properties of rapeseed proteins were demonstrated [6–8]. However, methods to increase the hydrophobicity of rapeseed proteins while preserving their functional properties are demanded to enhance the applicability in the non-food sector. A promising technique to achieve this is chemical derivatization of free functional groups in the protein with reagents bearing long hydrocarbon chains [9]. Thereby, polar residues, such as hydroxyl, sulfhydryl or amino groups can be converted to non-polar residues to increase the overall hydrophobicity.

Film-forming properties of rapeseed proteins were described in a number of studies. Several studies describe rapeseed protein-based film-formation by the solvent-cast method, using different protein concentrations, plasticizer types and concentrations [6, 8, 10, 11]. Mechanical and barrier properties of these films were reported to be low and comparable to other plant proteins [12]. Some improvements of physical properties were achieved using blends of rapeseed protein and *gelidium corneum* (a red algae with high

agar content) or gelatin [11]. In another study, denaturation by sodium dodecyl sulfate (SDS) prior to film formation also showed some improvement in tensile strength of films [8]. Moreover, the addition of genipin as a natural cross-linker increased tensile strength of sorbitol-plasticized films [10].

Chemical acylation of rapeseed proteins has been reported in a number of previous studies, predominantly in the form of acetylation and succinylation [13–18]. Film properties were investigated only in one study using succinylated rapeseed proteins [13]. The authors reported enhanced barrier and mechanical properties as well as increased hydrophobicity at the lowest modification degree. To the best of the author's knowledge, no study was carried out using rapeseed proteins modified with long hydrocarbon chains for film-preparation.

In this work, we applied the acylation of rapeseed protein preparations by Schotten-Baumann-reaction with fatty acid chlorides (FACs) [19, 20]. Type and degree of modification were investigated and resulting functional properties were compared with the non-modified standard. In particular, the influence on water solubility was studied. Additionally, cast films of rapeseed protein preparations were characterized regarding their mechanical and barrier properties. The overall aim was to show the application potential of rapeseed proteins for technical products requiring film-forming properties such as glues, lubricants, paints and varnishes.

Materials and Methods

Raw Materials and Chemicals

Prepressed rapeseed press cake was provided by Bunge Deutschland GmbH, Mannheim, Germany. Rapeseed press cake was defatted with isohexane in a percolator (volume 1.5 m^3, e&e Verfahrenstechnik GmbH, Warendorf, Germany) and flash desolventized with isohexane (400–500 mbar) prior to steam desolventation to obtain defatted rapeseed meal. Isohexane was obtained from Biesterfeld AG (Hamburg, Germany). Lauroyl chloride and olyeol chloride were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany). Phytase Quantum Blue 5G was obtained from AB Enzymes GmbH (Darmstadt, Germany). All other chemicals were of analytical grade and obtained from Th. Geyer GmbH & Co. KG (Renningen, Germany), if not stated otherwise.

Preparation of Rapeseed Protein Concentrate

The RPC was prepared on a pilot scale as follows: Rapeseed meal was added to a 0.25 M aqueous NaCl-solution at 30 °C with an s:l-ratio of 1:13. The mixture was adjusted to pH 9 using 3 M NaOH and stirred for 60 min, before separation

was carried out using a decanter (GEA Westfalia Separator Group GmbH, Oelde, Germany). The supernatant was adjusted to pH 4 with 3 M HCl at 30 °C and stirred for 30 min. Separation of the mixture was carried out using a disc separator (GEA Westfalia Separator Group GmbH). The sediment was not of further use in this study. The supernatant was adjusted to pH 5.5 with 3 M NaOH and 1.1 g of phytase (Quantum Blue 5G) were added. Ultrafiltration was carried out at 50 °C on a Microza polysulfone ultrafiltration module SLP-3053 (Pall Corporation, Port Washington, NY, USA) with a molecular weight cutoff of 10 kDa and a membrane area of 4.5 m². The neutralized retentate was spray-dried at a product inlet temperature of 180 °C and a product outlet temperature of 75–80 °C to obtain an RPC with high protein solubility. This was mandatory to ensure proper reactivity during further protein modification.

Modification of RPC

100 g of RPC were dispersed in 900 g of demineralized water and stirred at 40 °C. The pH was adjusted to pH 9.5 using 1 M NaOH and lauroyl chloride or oleoyl chloride (15 or 120 mmol) were added dropwise. The pH was controlled during addition of fatty acid chloride (FAC) and maintained at pH 9.5. After 3 h, a stable pH indicated the end-point of the reaction (Fig. 1). The dispersion was cooled to 30 °C, neutralized using 1 M HCl and dialyzed against demineralized water at 1 °C for 48 h with a molecular weight cutoff of 3.5 kDa (Spectra/Por® 3, Spectrum LifeSciences, LLC, Rancho Dominguez, CA, USA). The demineralized water was changed twice a day. The dialyzed samples were lyophilized, homogenized by mortar and pestle and washed six times with ethanol (3 mL/g) for removal of non-reacted free fatty acids. After air-drying under a fume hood, the modified RPCs were used for further analysis. A control

sample was prepared and treated the same way without the addition of FAC to the protein dispersion. All samples were prepared in duplicate.

Chemical Composition of Rapeseed Samples

Chemical composition (dry matter, protein) of rapeseed protein samples was analyzed in duplicate. Dry matter was analyzed using a thermo-gravimetrical system (TGA 601, Leco Corporation, St. Joseph, MI, USA) at 105 °C and 950 °C, respectively. Protein content was measured by Dumas combustion method on a TruMac N system (Leco Corporation), using 5.7 as the conversion factor [21]. Free fatty acids in the ethanol washing fractions of modified samples were determined by gas chromatography with flame ionization detector (GC-FID) as described by Mahmoud et al. [22].

Properties of Rapeseed Protein Samples

Protein Solubility

The protein solubility (PS) was determined in duplicate following the method of Morr et al. [23], as previously described [24]. For each measurement 1.5 g of rapeseed protein concentrate were suspended in a total volume of 50 mL of a 0.1 M NaCl solution. The pH was adjusted using 0.1 M NaOH or 0.1 M HCl, respectively. After stirring for 1 h at room temperature, the samples were centrifuged ($20,000 \times g$, 15 min, 15 °C) and the supernatant was filtrated (Whatman No. 1 filter paper). Protein content of the supernatant was determined according to Dumas following the method given in section "Analysis of Chemical Composition of Rapeseed Preparations". Protein solubility was calculated using Eq. 1 and reported giving the mean value ± absolute deviation.

$$\text{Protein solubility } [\%] = \frac{\text{initial volume [mL]} \times \text{protein content in supernatant } [\text{mg/mL}]}{\text{sample mass [mg]} \times \text{protein content [\%dm]} \times \text{dry matter[\%]}} \times 100 \qquad (1)$$

Fig. 1 Reaction scheme for the modification of rapeseed proteins via Schotten-Baumann-reaction
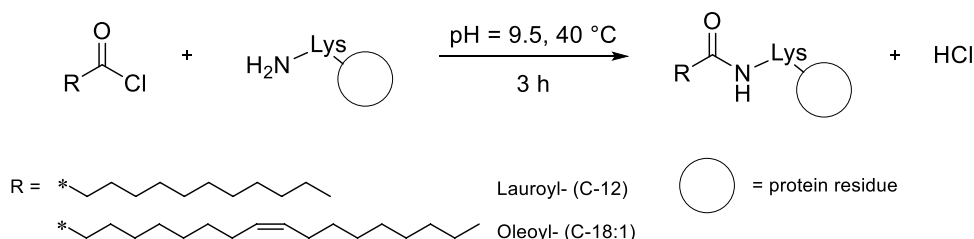
Film-Forming Properties

Preparation of Cast Films

Cast films of RPCs were prepared as described by Chang and Nickerson [6] with minor modifications. 11.25 g of modified or non-modified RPC were mixed with 120 mL of demineralized water in a glass bottle and the pH was adjusted to pH 3 using 1 M HCl. The mixture was stirred at room temperature for 60 min at 300 rpm on a MIX 15 magnetic stirrer (2mag AG, Munich, Germany), before 2.81 g of glycerol were added. The total weight was adjusted to 150 g at pH 3 using demineralized water and 1 M HCl and stirring was continued for 10 min. The mixture was degassed in an ultrasound bath at 35 kHz and 50 °C for 15 min, stirred at 50 °C and 300 rpm and 33 g were cast in square petri dishes (120 mm × 120 mm). The cast film was dried at 23 °C and 50% RH for 14 days to equilibrium moisture content.

Film Thickness

The rapeseed protein films were peeled off the petri dishes and the film thickness was determined using a Precision Thickness Gauge (Hanatek Instruments, St. Leonards-on-Sea, UK) as previously described by Schmid [25]. The measurements were performed at 23 °C and 50% RH with a five-fold determination at five different positions. The arithmetic average of the film thickness was used to calculate standardized oxygen permeability (OP) and mechanical film properties.

Surface Energy

The surface energy of RPC-based films was measured by the sessile drop method as previously described [25], using the contact angle measuring system G2 (Krüss GmbH, Stephanskirchen/Rosenheim, Germany). The contact angle of three testing liquids (water, diiodomethane, ethylene glycol) was measured at 22.4 °C and 42% RH at five different positions on the film surface of five replicates. The results are given as the arithmetic average ± standard deviation. According to Young's equation, the surface energy was calculated as follows:

$$\sigma_s = \gamma_{sl} + \sigma_l \times \cos\theta \qquad (2)$$

where σ_s is the surface tension of the solid (film) in mN/m, σ_l is the surface tension of the liquid in mN/m, γ_{sl} is the interfacial tension between the solid and the liquid in mN/m and cos θ is the contact angle between the surface tension of the liquid and the interfacial tension between the liquid and solid in angular degree. The disperse and polar fractions of the surface energy were calculated according to the method

by Owens et al. [26–28]. As the rapeseed films were water-soluble, the measurement had to be performed directly after drop application rather than at equilibrium. The calculated values can thus not be classified as absolute surface energy values and are only used for comparison of the samples in this study.

Tensile Testing

Tensile properties of RPC films were measured on a universal compression-tension testing machine Zwick 1445 (ZwickRoell GmbH & Co. KG, Ulm, Germany) at 23 °C and 50% RH in accordance to the standard DIN 527-1. The cast films were cut into strips (15 mm × 70 mm) and the thicknesses of the film strips were measured. The film strip was clamped in the loading frame using pneumatic grips and an initial gauge length of 50 mm. The sample was subjected to an applied force using a load cell of 50 N and was stretched with a testing speed of 100 mm/min. For each sample, a five-fold determination was performed and the tensile strength (TS; MPa) and elongation at break (%) were reported as the arithmetic average ± standard deviation.

Oxygen Permeability

The oxygen permeability (OP) was measured according to the standard DIN 53380-3 at 23 °C and 50% RH on a Mocon Twin instrument (Mocon Inc., Minneapolis, USA) as previously described [25, 29]. The OP values (Q) were converted to a thickness (d) of 100 μm (Q_{100}), according to Eq. 3, and are given in the unit [$cm^3\ m^{-2}\ d^{-1}\ bar^{-1}$].

$$Q_{100} = Q \times \frac{d}{100} \qquad (3)$$

Light Transmission

Light transmission of rapeseed protein films was measured with a spectrophotometer TMQ (Carl Zeiss GmbH, Oberkochen, Germany) in duplicate at wavelengths of 350 nm, 430 nm, 450–750 nm (intervals of 25 nm), and 800–1000 nm (intervals of 50 nm).

Degree of Modification Using the *o*-Phtaldialdehyde Method

Calculation of the degree of modification (DM) was performed via determination of free α-amino groups with *o*-phtaldialdehyde (OPA) according to Nielsen et al. using *N,N*-dimethyl-2-mercaptoethylammonium chloride as the thiol component [30, 31]. Protein samples were analyzed in

triplicate using serine as a standard. Initially, the degree of hydrolysis (DH) was calculated using the Eqs. 4–6.

$$DH = \frac{h}{h_{tot}} \times 100\%$$ (4)

where h_{tot} is the total number of peptide bonds per protein equivalent (adopted from soy protein with $h_{tot} = 7.8$ according to Adler-Nissen [32]) and h is the number of hydrolyzed bonds.

$$h = \frac{Serine - NH_2 - \beta}{\alpha} \frac{meqv}{g_{Protein}}$$ (5)

where Serine-NH_2 = meqv serine-NH_2/$g_{protein}$, $\alpha = 0.970$ and $\beta = 0.342$ (constants adopted from soy protein according to Adler-Nissen [32]).

$$Serine - NH_2 = \frac{OD_{sample} - OD_{blank}}{OD_{standard} - OD_{blank}} \times 0.9516 \frac{meqv}{L} \times \frac{0.1L \times 100}{Xg \times P}$$ (6)

where 0.1 L is the sample volume, X is the sample mass (in grams) and P is the protein content of the sample (in percent).

The DM was calculated from the DH using Eqs. 7 and 8.

$$Percentage\ of\ free\ amino\ groups = \frac{DH_{modified\ RPC}}{DH_{RPC}} \times 100\%$$ (7)

where $DH_{modified\ RPC}$ is the DH of the RPC after modification and DH_{RPC} is the DH of the RPC.

$$DM = 100\% - percentage\ of\ free\ amino\ groups$$ (8)

Due to the inaccuracy of the method at low DH [30], no absolute values were used for the comparison of modified RPCs. Instead, the relative degree of modification (RDM) was used, which was calculated in relation to the sample with the highest DM (RPC-LH) according to Eq. 9:

$$RDM = \frac{DM_{modified\ RPC}}{DM_{RPC-LH}} \times 100\%$$ (9)

Statistical Analysis

All data are given as mean values ± standard deviation of at least two measurements (n = 2). Outliers were eliminated after applying Grubb's test and the data was tested for normal distribution according to Shapiro–Wilk. Significant differences were statistically analyzed by one-way analysis of variance using Tukey's test (p < 0.05). Statistical analysis was performed using the software OriginPro (OriginLab Corporation, Northampton, USA).

Results and Discussion

## Modification of Rapeseed Protein Concentrate

The RPC was chemically modified by the Schotten-Baumann-reaction using lauroyl chloride (LC) and oleoyl chloride (OC). A screening showed the relative degree of modification (RDM) in dependence of the amount of fatty acid chloride (FAC) used (Fig. 2). As expected, the RDM increased with increasing amounts of FAC. The RDM was in the range of 41.0–86.6% and 39.5–70.4% for LC and OC, respectively. Higher RDMs for lauroyl-modified samples were possibly due to lower sterical hindrance for the reaction with the protein residues, due to the smaller and less bulky chemical structure of LC in comparison to OC.
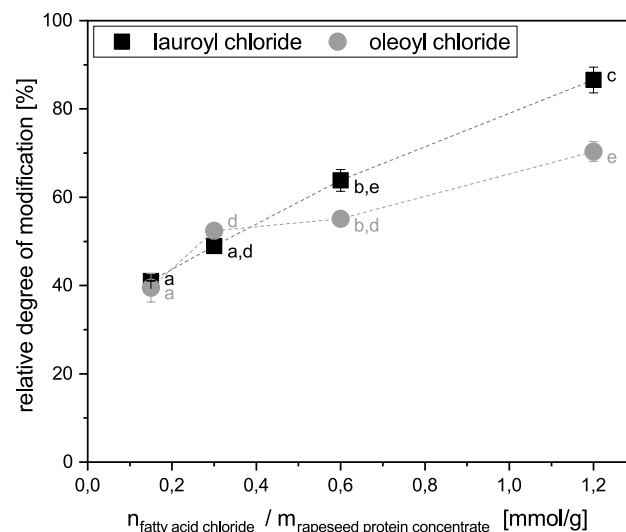
Fig. 2 Screening for the degree of modification in dependence of the amount of fatty acid chloride (FAC) used in the reaction (mean values ± absolute deviation); data points having the same superscript indicate no significant difference (p > 0.05)

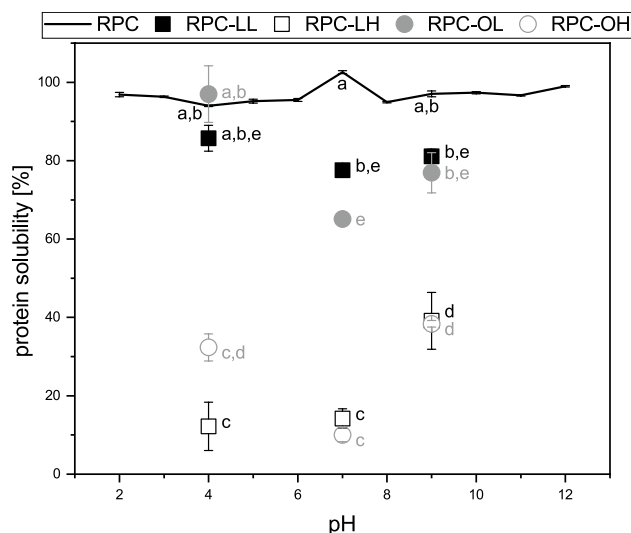**Table 1** Relative degree of modification and composition of rapeseed protein concentrates (mean values ± absolute deviation): *LL* Lauric-Low, *LH* Lauric-High, *OL* Oleic-Low, *OH* Oleic-High; protein contents were calculated with a nitrogen-to-protein conversion factor of 5.7; values within one row having the same superscript indicate no significant difference (p > 0.05)

	Relative degree of modification (RDM) (%)	Dry matter (dm) (%)	Protein content (% dm)
RPC	–	94.9 ± 0.0^a	83.7 ± 0.1^a
RPC-LL	32.6 ± 0.2^a	94.4 ± 0.1^a	83.5 ± 0.1^a
RPC-LH	100.0 ± 3.2^b	95.3 ± 0.0^a	76.7 ± 0.5^b
RPC-OL	27.8 ± 2.6^a	94.4 ± 0.0^a	82.7 ± 0.4^a
RPC-OH	72.3 ± 1.3^c	94.6 ± 0.4^a	77.1 ± 0.0^b
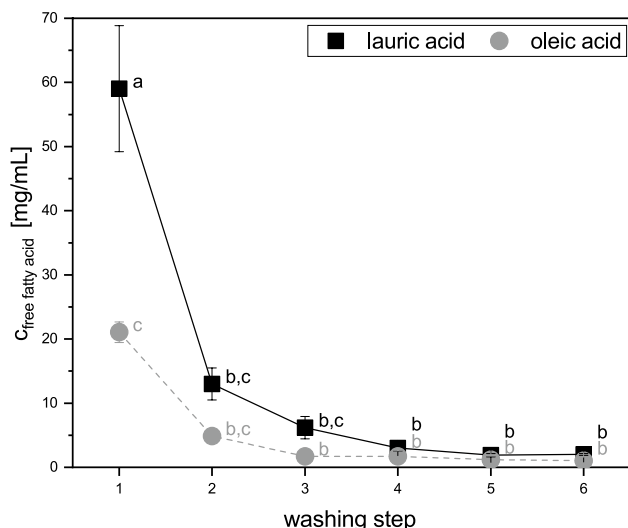
Fig. 3 Concentration of free fatty acids in the ethanol washing fractions (mean values ± absolute deviation); data points having the same superscript indicate no significant difference ($p > 0.05$)

Fig. 4 Protein solubility in 0.1 M NaCl of rapeseed protein concentrates (mean values ± absolute deviation); data points having the same superscript indicate no significant difference ($p > 0.05$)

Based upon the screening, the amount of FAC added to the reaction was chosen to yield one low-modified and one high-modified RPC for lauroyl and oleoyl chloride. Accordingly, an up-scale of the reaction was carried out using 0.15 and 1.2 mmol$_{FAC}$/g$_{RPC}$. The RDM for the low-modified species was determined to be 32.6% and 27.8% for LC and OC, respectively (Table 1). Lower RDMs compared to the screening could be explained by a higher degree of FAC that was hydrolyzed in the aqueous mixture due to a lower initial concentration upon dropwise addition of the reagent. In case of the high-modified species, the RDM of 72.3% was comparable to the screening for OC. However, for LC the RDM (100.0%) was higher compared to the screening.

To remove free fatty acids from the reaction product, dialysis of the reaction mixture was followed by ethanol washing of the lyophilized samples. The content of fatty acids in the washing fractions was analyzed for the high-modified samples RPC-LH and RPC-OH (Fig. 3). The concentration of fatty acids in the first fraction was 59.0 mg/mL and 21.1 mg/mL and dropped to 2.0 mg/mL and 1.0 mg/mL in the final fraction for RPC-LH and RPC-OH, respectively. In total, six washing steps were carried out for the purification of the modified RPCs. Statistical analysis revealed no significant differences in the concentration of free fatty acids after the third washing step ($p > 0.05$).

Characterization of Rapeseed Protein Concentrates

Chemical Composition

The chemical composition was determined for the non-modified and all modified RPCs (Table 1). The protein content of
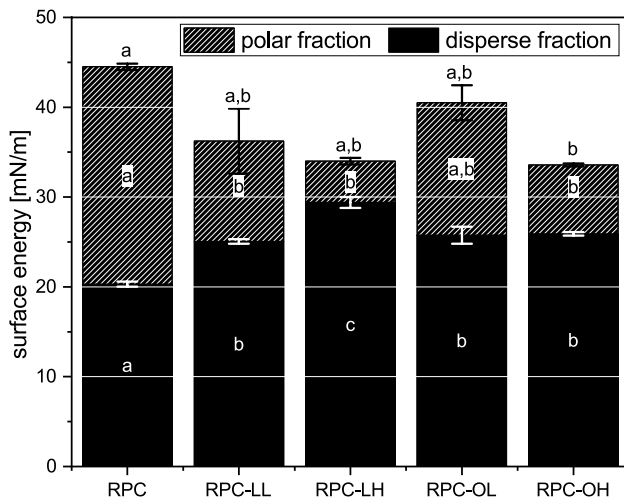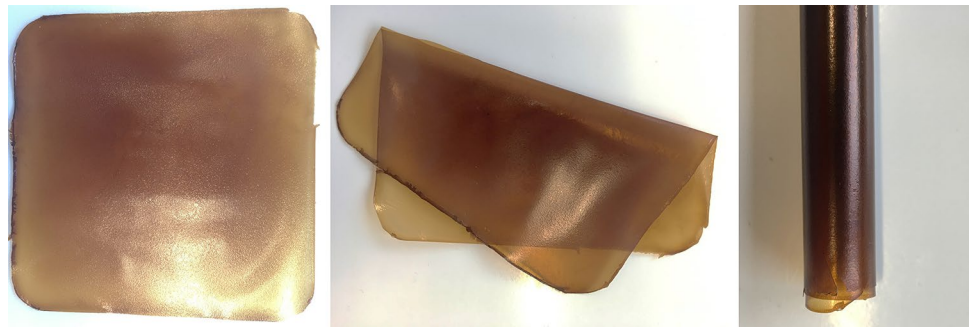
the non-modified RPC was 83.7% dm (N × 5.7). As expected, the protein content determined using the same conversion factor was reduced with increasing modification degree. The protein content was reduced marginally (0.2–1.0 percentage points) for the low-modified species RPC-Lauric-Low (-LL) and RPC-Oleic-Low (-OL). For the high-modified species, the protein content dropped to 76.7% and 77.1% for RPC-Lauric-High (-LH) and RPC-Oleic-High (-OH), respectively.

Properties of Rapeseed Protein Concentrates

Protein Solubility To obtain good reactivity during the reaction, an RPC with good water solubility was chosen. The protein solubility of the RPC was > 90% in the range of pH 2–12 (Fig. 4), which was achieved by removal of the low-soluble protein fraction by a preceding precipitation step. For the modified RPCs the solubility was determined at pH 4, 7 and 9 to analyze the effects of acylation on water solubility in acidic, neutral and alkaline environment. The solubility was reduced for all modified RPCs with one exception. The effect was less pronounced for the low-modified species RPC-LL and RPC-OL, with reductions of 8–25% points and 0–37% points, respectively. For RPC-LH and RPC-OH, the protein solubility dropped to 12.2–39.1% and 10.0–38.4%, respectively. Reduction of solubility can be explained by fatty acid amidation of free amino groups (primarily the ε-amino group of exposed lysyl residues), and to some degree of free hydroxyl and sulfhydryl groups. Water solubility is reduced, as these groups are limited in the ability to form hydrogen bonds in aqueous environment after acylation. Additionally, refolding of the protein structure has to be considered, which can lead to the liberation

Fig. 5 Photographs of rapeseed protein films after drying and removal of the petri dish

Fig. 6 Surface energy of cast-films made from rapeseed protein concentrates (mean values ± absolute deviation); columns having the same superscript indicate no significant difference ($p > 0.05$); significance indexes are given for disperse fraction, polar fraction and surface energy separately

Surface Properties Measurement of the surface energy showed the increased hydrophobicity of films prepared from acylated RPCs (Fig. 6). Films from the unmodified RPC showed a surface energy of 44.5 mN/m, with a polar fraction of 24.2 mN/m. For the modified RPCs, the surface energy decreased with increasing modification degree. This was caused by a decrease of the polar fraction, which was higher than the increase of the disperse fraction. The surface energy decreased to 36.2 mN/m (LL) and 34.0 mN/m (LH) for lauroyl-modifications and to 40.5 mN/m (OL) and 33.6 mN/m (OH) for oleoyl-modifications. The findings and the increase of disperse interactions caused by the introduction of hydrophobic side chains into the protein.

Barrier Properties All films (non-modified and modified) showed a low water-stability and thus water vapor permeability (WVP) could not be measured. WVP values were reported previously for cast films of rapeseed proteins prepared by a similar method as the one used in this study [6, 10]. This indicates that the nature of the rapeseed protein sample may be crucial for the resulting water stability of the obtained film. High water solubility of rapeseed protein films suggests possible applications such as a water-soluble packaging for dish-washer tabs. For the application as a packaging material or coating, improvement of water resistance must be achieved, e. g. by means of protein denaturation, such as through temperature treatment, or the inclusion of hydrophobic materials in the film. However, the introduction of lauroyl or oleoyl side chains in this study did not sufficiently improve the water stability of the films.

The oxygen permeability (OP) increased with increasing modification degree of the samples (Fig. 7), demonstrating the increase in hydrophobicity within the protein film layer, as oxygen is a nonpolar molecule. In addition, the covalently bound acyl residues can act as an internal plasticizer [33], thereby increasing gas diffusivity. For low-modified RPCs, the OP increased only marginally by 9–26% without significance ($p > 0.05$). The effect was greatly higher with significance ($p < 0.05$) for high-modified samples, with an increase of the OP by 143–159%. No studies were found reporting OP values for films made from rapeseed proteins such that

of buried hydrophobic amino acid residues. For the low-modified samples, the protein solubility was reduced only to a limited extent, with significant differences only at pH 7 ($p < 0.05$). However, the solubility was greatly reduced for high-modified samples. The solubility reduction was most pronounced at pH 7. This might be explained by a shift of the isoelectric point from the alkaline into the neutral region upon amidation of basic lysyl groups.
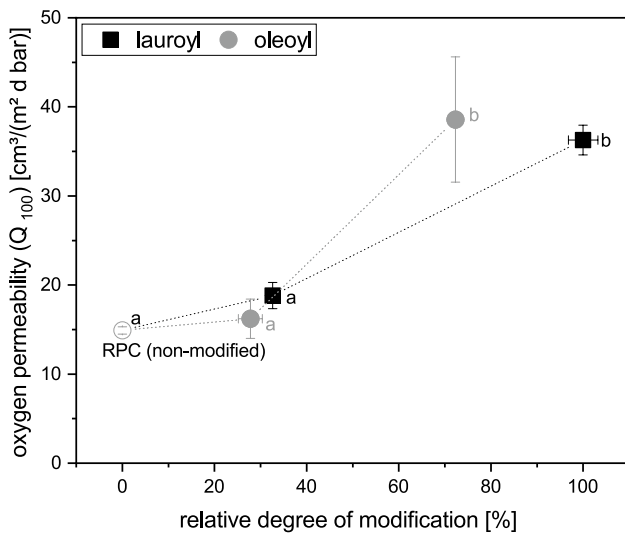
Properties of Rapeseed Protein Concentrate Films

All RPCs showed good film-forming properties in cast-film experiments using glycerol as a plasticizer (25% on the RPC sample basis). After removal of the dried film from the petri-dish, all films appeared homogenous, without defects, flexible, and were of brown color. The appearance as well as foldability and rollability of films is displayed in Fig. 5.

Fig. 7 Oxygen permeability (Q_{100}) at 23 °C and 50% RH of films prepared from modified and non-modified rapeseed protein concentrates (mean values ± absolute deviation); data points having the same superscript indicate no significant difference ($p > 0.05$)

a comparison was not possible. However, the OP obtained for the non-modified RPC and low-modified RPCs was comparable for the value of 18.4 cm^3 m^{-2} d^{-1} bar^{-1} reported by Schmid et al. [34] for a whey protein based cast film.

Mechanical Properties Compared to other studies using rapeseed proteins, the tensile strength (TS) for films prepared from unmodified RPC was low with 0.6 MPa (Fig. 8). Modification of the RPC showed an increase of the TS with increasing modification degree, presumably due to an
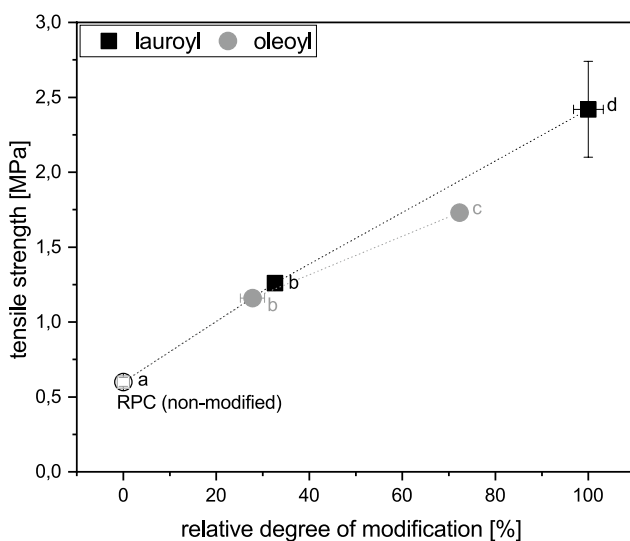
increase in non-polar interactions (van der Waals forces) within the film matrix. Low-modified samples showed values of 1.3 MPa (lauroyl) and 1.2 MPa (oleoyl). The TS of high-modified samples increased to 2.4 MPa (lauroyl) and 1.7 MPa (oleoyl). In accordance, the elongation at break was reduced with increasing degree of modification from 151% (non-modified RPC) to 104%/121% and 9.5%/43.3% for RPC-LL/-OL and RPC-LH/-OH, respectively (Fig. 8).

The modification of RPC showed an increase in the TS, however, all values determined were low in comparison to literature studies. Chang and Nickerson [6] reported a TS of 6.6 MPa for cast-films prepared with a similar method as in the present study using 7.5% rapeseed protein sample (based on the sample mass) and 30% glycerol. In a previous study by the authors, formulations of 5% protein and 50% glycerol, sorbitol or PEG-400 as a plasticizer were tested [10]. While glycerol showed the lowest TS (1.2 MPa), improvements were achieved using PEG-400 (5.2 MPa) or sorbitol (10.0 MPa) as a plasticizer. With the addition of genipin as a cross-linker, TS values increased to 2.6 MPa (glycerol), 9.6 MPa (PEG-400) and 12.6 MPa (sorbitol). Using a casting process at pH 11 and 70 °C, Shi and Dumont [8] measured a TS of 0.7–1.7 MPa for films prepared from 5% protein sample and 25–40% glycerol. Denaturation with SDS (5–15%) prior to film formation increased TS to 2.3–3.8 MPa, while the use of stearic acid (5–15%) had a lesser effect (1.5–2.2 MPa). In another study, the TS of non-modified rapeseed protein was ~5.3 MPa for films prepared using 2.6% protein sample and 42.5% glycerol by solvent-casting followed by heat-compression [35].

The studies listed above demonstrate the various methods available to increase the TS of protein films. While the effect
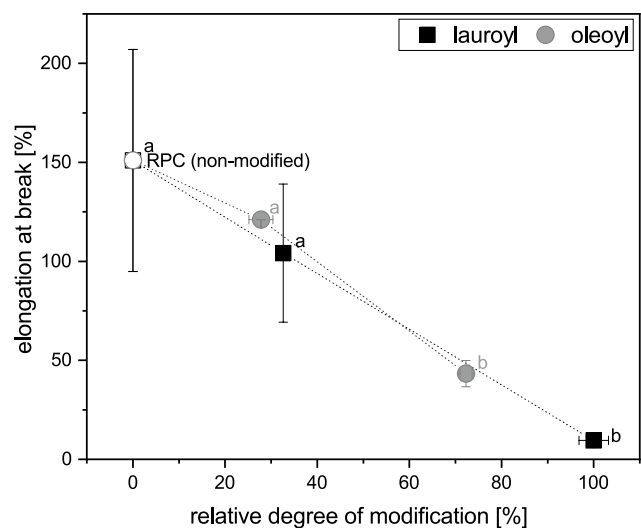
Fig. 8 Tensile strength and elongation at break of films prepared from rapeseed protein concentrates (mean values ± absolute deviation); data points having the same superscript indicate no significant difference ($p > 0.05$)
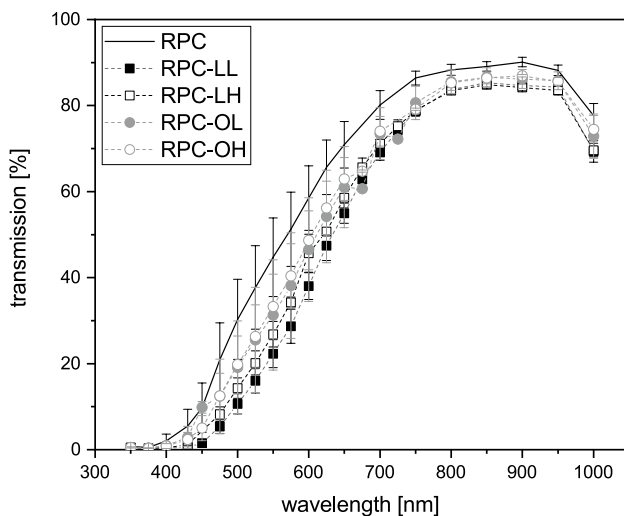
Fig. 9 Light transmission of films prepared from rapeseed protein concentrates in the range of 350–1000 nm (mean values ± absolute deviation)

of protein acylation prior to film formation applied in the present study showed only limited effect, a combination of methods, such as chemical modification and heat treatment, may be the subject of further studies.

Light Transmission All films were of brown color and showed only limited transparency. Light transmission was measured at wavelengths of 350–1000 nm. All films showed a transmittance of < 10% below 450 nm and very low transmittance of ≤ 2% in the range of 350–400 nm (Fig. 9). Modification of the RPC reduced the light transmittance of the prepared films. The effect was stronger for lauroyl modifications as compared to oleoyl modifications. The RDM also had a minor effect such that, interestingly, films with lower modification degree showed slightly lower transmittance. The introduction of lipids into protein films has been related to increased opacity in a number of studies [36–40]. In one study, the fatty acid saturation degree correlated with opacity, indicating that saturated stearic acid increased opacity, whereas the effect of oleic acid and linoleic acid was only marginal [40]. The observation may be explained by a lower melting temperature of oleic and linoleic acid compared to stearic acid. Similarly, Jiménez et al. [41] studied the opacity of hydroxypropyl-methylcellulose films formulated with various fatty acids. Saturated fatty acids showed an increased ability to form lipid aggregates, which was thought to be the reason for higher opacity.

The low light transmittance of rapeseed protein films in general, and of films from modified RPCs in particular, suggests the utilization as a packaging material with good light protection. However, as the films were highly water-soluble and showed low barrier properties against oxygen in this study, the rapeseed protein film needs to be used in combination with other materials, e. g. in a multi-layered material. Alternatively, the natural color of the obtained rapeseed protein films might serve well in an application such as a coating for cardboard or wood.

Conclusions

The reduction of hydrophilicity of rapeseed protein concentrates was successfully achieved by acylation with lauroyl and oleoyl chloride. The protein solubility in water at neutral pH was reduced from 100% to < 15% for highly modified samples. Contact angle measurement revealed the increased hydrophobicity of cast films from modified RPCs, however, the films still showed low water stability. The observed properties may serve well for the production of water-soluble foils, such as for dish-washer tabs. Films from modified protein samples showed an increase in tensile strength with increasing modification degree, while the elongation at break was reduced consequently. The light transmission of all films was < 10% below 450 nm and was further reduced by modification, suggesting a potential application as a light-protecting packaging layer. In summary, the application potential of rapeseed proteins—both modified and non-modified—as bio-based and biodegradable ingredients with great film-forming properties was demonstrated. Based on the results, suggested application fields are adhesives, coatings, paints, packaging layers, or detergents. Further studies need to be performed to test their applicability in more detail.

Compliance with Ethical Standards

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