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Assessing functional properties of rapeseed protein concentrate versus isolate for food applications

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ABSTRACT

Rapeseed protein concentrate (RPC) and isolate (RPI) were fractionated from rapeseed meal and their techno-functional properties were characterized. RPC prepared by aqueous ethanolic washing process yielded less refined and insoluble fractions with largely denatured protein. Hydrated insoluble RPC particles held 6.7 g water without swelling. RPI fractionated from aqueous extraction and ultrafiltration still contained certain phenolic compounds but protein nativity was preserved including a high protein solubility of 78%. RPC dispersion with 40 *w/w* % showed the highest apparent complex modulus G* among all rapeseed materials. In contrast, the G* for RPI dispersion increased upon heating, suggesting a thermal induced denaturation and gelation capacity of the proteins. Thus, a largely denatured RPC free of phenolic compounds or a native but phenolic containing RPI were obtained by the applied processing conditions, which differ in their techno-functional properties and thus have different promising application potential in food applications.

Industrial relevance: The growing demand for plant as opposed to animal protein has sparked the interest of exploring currently underutilized plant protein sources in food industry. Rapeseed by-products obtained after oil extraction are promising but the presence of anti-nutritional components in rapeseed meal presents a barrier for its usage in food products. Aqueous ethanolic washing or aqueous extraction combined with membrane filtration were applied to remove the antinutritional factors from rapeseed meal to yield rapeseed protein concentrates and even more refined isolates. In this work, the functional properties of the fractionated rapeseed materials were characterized for its relevance in food applications. The similarities of the rheological properties between rapeseed protein concentrates and soy materials, as well as presence of native proteins in RPI might suggest their potential for diverse in food industry applications.

1. Introduction

Plant proteins from oilseeds and legumes can become an alternative to animal proteins. Rapeseed is one of the major crops cultivated for oil production next to soybean (USDA, 2020). Recently, the utilization of rapeseed oil for biodiesel generation has increased, leading to a higher production of rapeseed byproducts (meal) worldwide (Ivanova, Chalova, Uzunova, Koleva, & Manolov, 2016). The total production of rapeseed meal was 38 metric tons in 2018 worldwide (Laguna et al., 2018).

Rapeseed meal contains about 40% proteins, which are mainly composed of salt-soluble 12S globulins (cruciferin) and water-soluble 2S albumins (napin) (Bérot, Compoint, Larré, Malabat, & Guéguen, 2005). Both cruciferin and napin have high thermal stability, with a denaturation temperature (T_d) of 91 °C and 110 °C, respectively (Wu & Muir, 2008). Rapeseed proteins have a well-balanced amino acid profile with high quantities of indispensable amino acids (> 400 mg/ g protein), particularly sulphur-containing amino acids (such as methionine and

Abbreviations: CCR, closed cavity rheometer; DM, dry matter content; dRSM, defatted rapeseed meal; DSC, Differential Scanning Calorimetry; mRPC, milled rapeseed protein concentrate; NSI, nitrogen solubility index; PC, phenolic compounds; RPC, rapeseed protein concentrate; RPI, rapeseed protein isolate; RSM, rapeseed meal; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; SEM, scanning electron microscope; SPC, soy protein concentrate; SPI, soy protein isolate; sRPC, sieved rapeseed protein concentrate; T_d, denaturation temperature; WG, wheat gluten; WHC, water holding capacity.

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cysteine) in 1:1 ratios (40–49 mg/ g protein) (Bos et al., 2007; Citeau, Regis, Carré, & Fine, 2016). However, the nutritional value is counter balanced by the presence of glucosinolates, phenolic compounds (PC), and phytates (Tan, Mailer, Blanchard, & Agboola, 2011a). The main PC are sinapic acids of up to 1.8 g /g defatted meal and condensed tannins (500–800 mg catechin equivalents/ g defatted meal) (Tan, Mailer, Blanchard, & Agboola, 2011b). The presence of these antinutritional factors limits the usage of the rapeseed protein in food applications due to its association with bitter taste and formation of complexes with protein (Aider & Barbana, 2011; Khattab & Arntfield, 2009; Laguna et al., 2019; Naczk, Amarowicz, Sullivan, & Shahidi, 1998). Besides antinutritional factors, the hulls present in the meal also reduces its digestibility. Therefore, fractionation is often proposed to improve the applicability of rapeseed protein for human consumption (Das Purkayastha et al., 2014).

Rapeseed protein concentrates are often reported to be obtained by aqueous ethanolic washing process, which is as an effective method to remove oil, soluble sugars, PC, and other anti-nutrients from oilseeds (Chemat, Vian, & Cravotto, 2012; Kozlowska, Zadernowski, & Sosulski, 1983). Also aqueous ethanol mixture is a preferred green solvent due to low toxicity (Citeau et al., 2016; González-Pérez et al., 2002). However, rapeseed hulls are not effectively removed by the washing process. Thus, the large hulls can either be removed by sieving or be milled into smaller fractions (Tranchino, Costantino, & Sodini, 1983; Wanasundara, 2011). In addition, the aqueous ethanolic washing process in combination with high temperature was found to affect proteins. Aggregation and/or denaturation was reported and thus a reduced solubility, which hinders further protein purification (Kayser, Arnold, Steffen-Heins, Schwarz, & Keppler, 2020; Zuorro, Iannone, & Lavecchia, 2019). In contrast to protein concentrates, the fractionation process of rapeseed protein isolate usually starts with defatting of rapeseed meal (Wu & Muir, 2008; Yoshie-Stark, Wada, & Wäsche, 2008). Proteins are thought to retain their native structure in hydrophobic organic solvents, such as hexane (Mattos & Ringe, 2001). Alkaline treatment followed by acid precipitation or ultrafiltration are typical methods to remove antinutrients and enrich the protein from the defatted meal (Yoshie-Stark et al., 2008). Insoluble fibres and hulls are removed by a centrifugation step after alkaline treatment. However, direct alkaline purification with sodium chloride followed by acid precipitation was found to lead to low protein yield due to the wide range of isoelectric points of the constituent proteins (Tan et al., 2011a). Membrane filtration has proven to be suitable for the production of highly purified and functional rapeseed protein isolates (Yoshie-Stark et al., 2008). Membrane filtration can also be directly applied onto the supernatant after the alkaline treatment, which resulted in an isolate with potential gelling capacity (Yang, Wang, Vasanthan, & Chen, 2014).

Up to now, most research focused on the aqueous extraction of rapeseed protein isolate (RPI), while less attention has been paid on the functional properties of less refined rapeseed protein concentrate (RPC). In this work, we compared the functional properties of aqueous ethanolic washed RPC with or without hull removal, as well as ultrafiltered RPI, and discussed their potential applications as food ingredient. Physico-chemical properties (protein thermostability, nitrogen solubility index (NSI)), and techno-functional properties in regard to structured gel formation (water binding capacity (WHC), particle hydration and rheological properties during heating by oscillation in closed cavity rheometer (CCR)) are tested. The rheological properties of the rapeseed materials are further compared to soy protein concentrate (SPC), soy protein isolate (SPI), and wheat gluten (WG), since these ingredients are often used in meat analogue applications. In the end, the potential food applications will be discussed for the rapeseed materials, in terms of meat analogue or other food applications.

2. Materials and methods

2.1. Materials

Rapeseed cake (92% DM with 33.8% protein, 15.4% oil, 7.1% ash, 16.3% total fibres, 2.7% PC, and 2.6% tannins), rapeseed protein concentrate (RPC) and sieved rapeseed protein concentrate (sRPC) were provided by Avril group, France. Petroleum ether was purchased from Avantor company, and NaCl and NaOH were purchased from Sigma Aldrich (Missouri, USA). Soy protein isolate (SPI) (SUPRO 500E IP, 94% DM with 90% protein, 1% fat, and 5% ash) and soy protein concentrate (SPC, Alpha 8, 96% DM with 70% protein in dry base, 2% fat, 7 ash, 18% dietary fiber, and total carbohydrates of 19.6%) were purchased from Solae (St Louis, MO). Vital wheat gluten (WG, 92% DM with 83% protein in dry base) was purchased from Barentz (Hoofddorp, Netherlands).

2.2. Preparation of rapeseed materials

2.2.1. Rapeseed meal (RSM)

The rapeseed cake (obtained through a de-hulling step and a cold-pressing step) was milled in an impact mill (ZPS50, Hosokawa-Alpine, Germany), which led to 80% de-hulled rapeseed meal (RPM). The conditions were ZPS speed of 6000 rpm, classifier wheel speed of 2500 rpm, a gas flow rate of 75 m^3/h and feed rate of 6 rpm (circa 1.5 kg/h).

2.2.2. Defatted rapeseed meal (dRSM)

Defatted rapeseed meal was prepared by further defatting the RSM with petroleum ether in a custom-built Soxhlet extractor. The extraction was carried out at a sample/solvent ratio of 1:4 for 6 h, and the solvent in the sample was evaporated in the vacuum fume overnight.

2.2.3. Rapeseed protein concentrate (RPC)

RPC was obtained by defatting RSM with hexane and consecutive aqueous ethanolic washing with 70% ethanol concentration and temperature of 75 °C, performed by Improved, Amiens, France. Milled rapeseed protein concentrate (mRPC) was prepared by milling RPC with an impact mill (ZPS50, Hosokawa-Alpine, Germany). The conditions were ZPS speed of 6000 rpm, classifier wheel speed of 2500 rpm, a gas flow rate of 75 m^3/h and feed rate of 6 rpm (circa 1.5 kg/h). Sieved rapeseed concentrate (sRPC) was obtained from RPC by using a 400 μm-sized sieve, performed by Improve, Amiens, France.

2.2.4. Rapeseed protein isolate (RPI)

RPI was produced in two steps: First, rapeseed protein was extracted by dispersing the dRSM in a 0.2 M NaCl solution at a solid/solvent ratio of 1:8. The pH of the dispersion was adjusted to 8 using 1 M NaOH. The dispersions were stirred for 1 h at 25 °C, followed by centrifugation at 8000 rpm for 10 min. The supernatant was collected after centrifugation. In the second step, ultrafiltration and dialysis were carried out to reduce the presence of small molecular weight components, such as glucosinolates, phytates, some PC, ash and soluble sugars. Therefore, the supernatant of step 1 was subjected to ultrafiltration in an Amicon stirred cell of 400 mL (Millipore Co. Bedford, USA) through a disc membrane (Synder membrane filters, VT, PES, Sterlitech Corporation, USA). The membrane had a thickness of 76 mm and a molecular weight cut-off of 3 kDa. A final volume concentration factor of 4.4 was reached. The retentate was further dialyzed with a 3.5 kDa standard regenerated cellulose membrane tube (Spectra/Por 3, USA) in demineralized water for 72 h to further remove salts and other small molecular weight compounds. Afterwards, the retentate was freeze dried and saved in 4 °C fridge for further analysis. Protein recovery yield for RPI was calculated as follows:

$$Protein\ yield = \frac{M_{RPI} * X_{RPI}}{M_{RSM} * X_{RSM}} \cdot 100\%[\%] \quad (1)$$

where M_{RPI} indicates the weight of freeze-dried RPI, and M_{RSM} indicates the weight of starting RSM, X_{RPI} and X_{RSM} indicates the protein fraction in the RPI and RSM. In total, two batches were made with the RPI.

2.3. Composition analysis

The composition of all rapeseed materials of RSM, dRSM, mRPC, sRPC and RPI was analyzed in terms of protein, ash, moisture, oil, total phenol content, and carbohydrate content. The nitrogen content was determined with the Dumas combustion method by using a Nitrogen Analyzer (Flash EA 1112 Series, Thermo scientific, Netherlands). The protein content was calculated as the nitrogen content (N) multiplied by a conversion factor of 5.53 for rapeseed (Sari, Bruins, & Sanders, 2013). Ash content was determined with the AACC 08–01 method, moisture with the AACC 44–15.02 method and oil content with the AACC 30–25.01 method. Oil content for RPI was not measured due to insufficient product obtained after the process and the oil was supposed to be removed by the defatting and fractionation process. Therefore, oil content in RPI was assumed to be zero. The total phenol content was measured with the Folin-Ciocalteu (FC) method (Drosou, Kyriakopoulou, Bimpilas, Tsimogiannis, & Krokida, 2015). The absorbance of the sample was measured at 750 nm using a spectrophotometer (DR3900, Laboratory VIS Spectrophotometer, Hach, USA). The total carbohydrates were calculated as the difference in DM and the known contents of constituents (protein, oil, total PC, ash). All measurements were performed in duplicates.

2.4. Soluble protein molecule profile with SDS-PAGE

The protein molecular size distribution of all rapeseed materials was characterized using SDS-PAGE. For this, mini-protein gels (low range PROTEAN TGXTM. 30 µl, Bio-rad, Netherlands) were used. The samples were diluted in Milli-Q water to reach a concentration of 2 mg/mL in a falcon tube. Afterwards, the samples were centrifuged to remove all the insoluble fractions, and the supernatant was used for the SDS-PAGE. In addition to this approach, the uncentrifuged sample (containing all material) was used for the SDS-PAGE analysis as a benchmark. The samples were prepared with Tris buffer containing 2% *w*/w SDS, 10% glycerol, 0.5% *w/v* bromophenol blue and 5% b-mercaptoethanol. The sample was mixed with a vortexer and heated at 95 °C for 10 min, afterwards the sample was centrifuged at 10000 rpm for 1 min. The electrophoresis was performed at 200 V for approximately 40 min in a Mini-Protean II electrophoresis cell (Bio-rad, Veenendaal, Netherlands). 5 µL of SDS-PAGE marker and 15 µL of the sample was loaded onto the gel wheels. The gels obtained after electrophoresis were stained using Bio-safe Coomassie Blue (Bio-rad, Netherlands). After 1 h, the gels were washed with Millipore water and scanned using a Gel scanner (Biorad - GS900, Netherlands). Triplicates were performed for the SDS-PAGE, and only one single lane was selected to present the result in Fig. 1. The benchmark samples containing the whole sample material are presented in the Fig. S1.

2.5. Nitrogen solubility index (NSI)

NSI is defined as the amount of solubilized nitrogen from the original sample. Rapeseed material of 1 g and 49 g of Milli-Q water was mixed in a 50 mL falcon tube and rotated for 24 h in a rotator (Bibby Scientific™ Stuart™ Rotator Disk SB3, Thermo Fisher Scientific, USA) for hydration at a speed of 20 rpm. Afterwards, the dispersion was centrifuged at a speed of 15,000 ×g, at 25 °C for 10 min. The supernatant was removed with a pipette and the wet pellet was transferred into an aluminum tray and dried in the oven at 105 °C for 24 h. The nitrogen content in the dry pellet ($N_{dry\ pellet}$) and in the original sample ($N_{original}$) were measured with the Dumas combustion method by using a Nitrogen Analyzer (Flash EA 1112 Series, Thermo scientific, Netherlands). The mass of the original sample and dry pellet were weighed, showed as $M_{original}$ and $M_{dry\ pellet}$[%]. The NSI was calculated as follows:

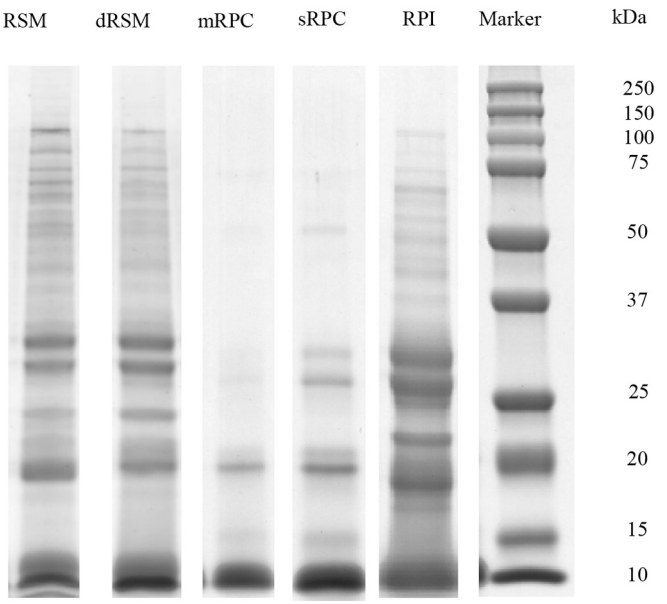


Fig. 1. SDS-PAGE for the soluble protein fractions of RSM, dRSM, mRPC, sRPC and RPI by adding the 2-mercaptoethanol.

$$NSI = \frac{N_{original} * M_{original} - N_{dry\ pellet} * M_{dry\ pellet}}{N_{original} * M_{original}}\ [\%] \quad (2)$$

Duplicates were performed for each rapeseed material and dumas measurement was performed in duplicates for each dry pellet.

2.6. Thermal properties with DSC

The thermal properties of all the rapeseed materials were analyzed using DSC (TA instrument 250, USA). Approximately 8 mg of 20% (*w/v*) sample slurry was weighed in a pan volume of 60 µL. The pan was hermetically sealed and placed into the DSC cell. The pan was heated from 25 °C to 140 °C with a heating rate of 5 °C/min. Both the peak temperature and the integrated enthalpy values of the endothermic peaks were collected by the Trios data analysis software. The enthalpy was expressed based on total amount of protein in the dispersion to allow for the different protein contents in the samples. The measurements were performed in triplicates.

2.7. Particle properties

Scanning Electron Microscope (SEM) (JEOL JCM-7000, the Netherlands) was firstly used to observe the morphology of all the rapeseed materials. The dry samples were added on the double-side adhesive conductive carbon tabs and the samples were sputter-coated with gold. Compressed air was used to distribute the sample evenly on the surface of carbon tabs. The accelerating voltage was 10 kV. SEM pictures were taken at a scale of 30–80 µm.

2.7.1. Particle hydration properties

Particle hydration of all the rapeseed materials was performed by making a 2 *w/v* % dispersions in a 100 mL beaker with Milli-Q water and continuously stirred at 300 rpm with a magnet. The dispersions were stirred for 24 h, and samples were taken at time intervals of 0, 1 and 24 h. Particle size distribution was measured using a Mastersizer-3000 device (Malvern Instrument Ltd., UK). The pH of the dispersions was measured with the pH meter at fresh with RSM, sRPC and RPI as 6.24, 6.42 and 6.22 which was changed into 5.65, 6.58 and 6.23 after 24 h hydration.

Particle size distribution of the hydrated samples at different time intervals was measured using a Mastersizer-3000 device (Malvern Instrument Ltd., UK). For each sample, five replicate measurements were performed and the average particle size was expressed in volume density and surface area density.

Besides, a Microscope Axioscope (Carl Zeiss Microscopy, LLC, United States) with camera was used to inspect the hydrated samples. The samples were prepared on a glass slide at 25 °C and covered with a cover slip. Snapshots of 100 μm were taken.

2.7.2. Water holding capacity (WHC)

In this work, the WHC is defined as the water that the insoluble part of the rapeseed material absorbed and retained after 24 h of hydration and subsequent centrifugation.

WHC was determined by adding 1 g of material and 49 g of Milli-Q water in a 50 mL falcon tube, and similar process was followed as the NSI measurement (section 2.5) to obtain the wet pellet and dry pellet. Duplicates were performed for each rapeseed material. The weight of the wet pellet ($M_{wet\ pellet}$) and after drying ($M_{dry\ pellet}$) was measured. The WHC was calculated as:

$$WHC = \frac{M_{wet\ pellet} - M_{dry\ pellet}}{M_{dry\ pellet}}\ [\text{g water/g dry pellet}] \quad (3)$$

2.8. Viscoelastic properties

Viscoelastic properties were measured with a Closed Cavity Rheometer device (CCR) (RPA elite, TA instruments, USA). The complex modulus (G*) was calculated by the software based on the measured storage modulus (G') and loss modulus (G").

Rapeseed materials, SPC, SPI and WG were mixed with Milli-Q water, at 40% DM with a total mass of approximately 5 g, for a hydration time of 30 min. The samples were vacuum-sealed in a plastic bag before and the sample was taken out of the bag shortly before transferring into CCR. The samples were then placed in between two plastic foils in the closed cavity (disk geometry). A pressure of 4 bars was applied to prevent water evaporation.

Pretests of frequency and strain sweep were performed to determine the linear viscoelastic region (LVR) at 120, 130 and 140 °C, which revealed that measurements using a constant frequency of 1 Hz and a strain of 1% were in the LVR up to a temperature of 120 °C. Above 120 °C, measurements were outside the LVR and therefore apparent complex modulus G* was reported instead of complex modulus.

Temperature sweeps were performed by heating the samples from 40 °C to 150 °C using a heating rate of 10°/min, a constant strain of 1% and a frequency of 1 Hz. After reaching 150 °C, the sample was cooled until 40 °C by blowing dry air over the disk. Time sweep experiments were performed at temperatures of 120 °C and 140 °C for 15 min with a constant strain of 1% and a frequency of 1 Hz. Then, samples were cooled to 40 °C. Duplicates were performed under each condition.

2.9. Statistics analysis

The data of DSC was analyzed using SPSS software (IBM statistical analysis Version 25.0). Univariate general linear model with LSD test was performed to investigate significant differences in between the rapeseed samples of RSM, dRSM, mRPC, sRPC and RPI. Differences were considered significant if $P < 0.05$.

3. Results and discussion

3.1. Characterization

3.1.1. Composition

The composition of all rapeseed materials is shown in Table 1. The protein content in the RPI reached 94%, and the yield was 36%. This is in the same range as previously reported by Yoshie-Stark et al. (2008) for ultrafiltrated rapeseed protein isolate (98% protein content and 36% protein recovery yield). sRPC showed higher protein content compared to the mRPC: protein enrichment is because sieving removes macroscopic hulls (larger than 400 μm) that contain low amount of protein.

Table 1
Composition analysis of RSM, dRSM, mRPC, sRPC and RPI in dry basis. Protein recovery yield of dRSM and RPI were 98% and 36% respectively.

Composition (%)	RSM	dRSM	mRPC	sRPC	RPI
Protein	32.82 ± 0.26	38.68 ± 0.65	58.39 ± 0.26	61.89 ± 0.33	94.39 ± 1.70
Oil	19.39 ± 0.60	1.45 ± 0.04	0.80 ± 0.01	0.82 ± 0.02	ND
TPC	2.15 ± 0.03	2.60 ± 0.03	0.20 ± 0.002	0.19 ± 0.002	3.60 ± 0.01
Ash	6.98 ± 0.01	8.06 ± 0.25	8.49 ± 0.02	8.82 ± 0.004	1.36 ± 0.06
Total carbohydrates[a]	38.66	50.66	32.13	28.29	0.65

[a] Total carbohydrates were calculated by difference.

The concentrates possessed the lowest content of PC (less than 0.2%), which indicates that PC was effectively removed by the aqueous ethanolic washing treatment. A high total content of PC (i.e., 3.6%) was measured in the RPI-sample. Nevertheless, 78% of the PC present in the original sample were removed during the ultrafiltration process. The remaining PC either have a high molecular weight (large condensed tannins that could not pass the filter membrane) or interacted with the proteins and were thus retained. The ash content within RPI was much lower compared to the other rapeseed materials, indicating an effective ash removal by ultrafiltration and dialysis. The carbohydrate content of concentrates was lower than that of RSM and dRSM due to the removal of soluble components, such as sugars. sRPC presents a slightly lower carbohydrate content than mRPC, which is caused by the removal of the macroscopic hulls by sieving.

3.1.2. Gel electrophoresis

SDS-PAGE analysis was performed to investigate the effect of processing on the soluble protein composition of all rapeseed materials. Fig. 1 shows the bands of proteins separated by the mass (kDa) of soluble rapeseed materials under reducing conditions by adding 2-mercaptoethanol. RSM, dRSM and RPI showed similar profiles. In total, 5 major bands (between 10, 18–25 and 26–37 kDa) were found. The results were in agreement with the findings of Akbari and Wu, 2015 that cruciferin dissociates into α-polypeptides (26.7–37.1 kDa) and β-polypeptides (18.3–22.9 kDa) chains in the presence of 2-mercaptoethanol. In addition, also the napin dissociated into two bands of 5 and 10 kDa. The high molecular weight bands between 37 and 100 kDa present in the meal and RPI are not described in literature yet. One hypothesis is that these bands correspond to covalently cross-linked complexes of proteins and PC. Since these complexes were present in RSM, dRSM and RPI, one can speculate that they were already formed before the isolate preparation in the defatting process.

In the case of concentrates (mRPC and sRPC), less bands were visible. The major band of 10 kDa indicates the presence of napin, whereas the other major band of 20 kDa for mRPC and four major bands for sRPC (molecular weights of 20–21, 29–33 kDa) are probably related to the dissociated cruciferin in the presence of the 2-mercaptoethanol. In contrast to RPI, RSM and dRSM, there were no high molecular weight bands above 37 kDa. It is speculated that the aggregates are probably removed by aqueous ethanolic washing as discussed in the previous section, or the concentrates were hardly soluble, therefore these high molecular weight proteins were removed with the pellet of insoluble proteins. In the Fig. S1, more bands in the molecular weight range between 18 and 37 kDa are shown for both concentrates when both soluble and insoluble fraction were used. This indicates that the cruciferin in the

concentrates is mainly insoluble.

3.1.3. Protein thermostability and solubility

Protein thermostability was measured with the DSC to understand the effect of process conditions on the nativity of protein. The protein denaturation temperature (T_d), onset of T_d and the protein denaturation enthalpy (ΔH) of the samples are shown in Table 2. Two denaturation peaks were detected for rapeseed materials at 86–89 °C and 107–109 °C. This result is consistent with the results previously reported of 91 °C and 110 °C for purified cruciferin and napin fraction (Wu & Muir, 2008). The onset temperature for the first and second denaturation peak is around 79–82 °C and 96–102 °C.

The increase of $T_{d,1}$ for dRSM compared to that of RSM indicates enhanced thermal stability, and the decrease of denaturation enthalpy for the second peak as ΔH_2 for dRSM suggests partial denaturation of napin by defatting with petroleum ether (Griebenow & Klibanov, 1996). Hardly any denaturation peak of cruciferin was detected for both mRPC and sRPC. The relatively high temperature of 75 °C used during the aqueous ethanolic treatment might result in nearly complete denaturation of cruciferin, as this temperature is very close to the onset T_d of cruciferin. Another explanation for the complete denaturation of cruciferin could be that the contact of ethanol with the protein leads to protein denaturation (Peng, Xu, Li, & Tang, 2020). The decrease in ΔH_2 was observed for both concentrates. Likewise, the exposure of napin to the aqueous ethanolic solvent is responsible for partial denaturation of napin. With respect to RPI, the denaturation enthalpy of cruciferin increased, whereas the denaturation enthalpy of napin decreased. A possible explanation is that fractionation leads to higher loss of napin than cruciferin, which increased the cruciferin concentration in the RPI, and thus its response in the DSC measurement. This is confirmed by the observation that the SDS-PAGE band of napin (10 kDa) was less intense in the case of RPI compared to other rapeseed materials (Fig. 1).

The NSI of rapeseed samples is shown in Fig. 2. Slightly higher solubility of dRSM was found compared to that of RSM. Both mRPC and sRPC displayed values below 20%, indicating a low protein solubility. This lower solubility was in line with the high protein denaturation in RPC observed in DSC results (Table 2). In contrast to RPC, RPI exhibited a high NSI (76%) and also a high protein nativity. The native rapeseed proteins cruciferin and napin are expected to be soluble at pH 6, also because their pI values are higher (7.2 and 11, respectively). The remaining 24% insoluble protein in RPI might be due to the low salt content, as any salt added during extraction was removed by the dialysis process. Salt is known to facilitate protein solubility. Wanasundara, Abeysekara, McIntosh, and Falk (2012) reported that the removal of salt by dialysis changes the soluble protein composition.

Table 2
The onset of denaturation temperature, denaturation peak temperature and denaturation enthalpy of rapeseed materials (ND = not determined). Significant differences ($P < 0.05$) within one column are indicated by superscript letters.

Rapeseed fractions	Onset $T_{d,1}$ (°C)	$T_{d,1}$ (°C)	ΔH_1 (J/g protein)	Onset $T_{d,2}$ (°C)	$T_{d,2}$ (°C)	ΔH_2 (J/g protein)
RSM	79.70 ± 0.64^{a}	86.49 ± 0.95^{a}	2.31 ± 0.28^{a}	98.50 ± 0.50^{a}	107.70 ± 1.12^{a}	3.17 ± 0.28^{a}
dRSM	81.88 ± 1.19^{b}	90.54 ± 1.05^{b}	2.89 ± 0.75^{c}	100.71 ± 0.57^{b}	108.01 ± 0.20^{a}	1.35 ± 0.13^{b}
mRPC	82.20 ± 0.09^{b}	89.43 ± 1.21^{b}	0.05 ± 0.01^{b}	97.22 ± 1.41^{a,c}	108.23 ± 1.56 ^{a}	1.18 ± 0.26^{b}
sRPC	ND	ND	ND	96.26 ± 0.26 ^{a,c}	108.20 ± 0.79 ^{a}	1.25 ± 0.36^{b}
RPI	79.22 ± 0.59^{a}	88.92 ± 0.56^{b}	3.45 ± 0.43^{c}	102.31 ± 1.85^{b}	108.67 ± 0.40 ^{a}	0.30 ± 0.03^{c}

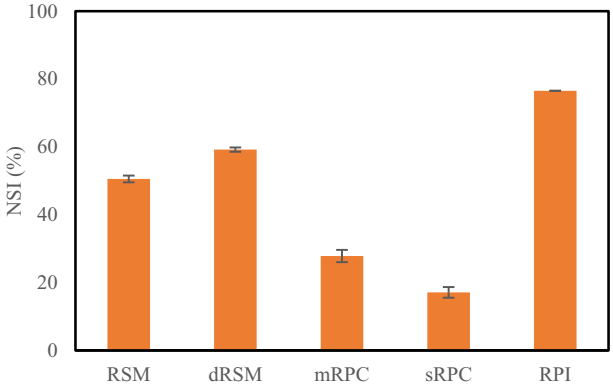


Fig. 2. Nitrogen solubility index (NSI) for the rapeseed materials of RSM, dRSM, mRPC, sRPC and RPI.

3.2. Particle properties

3.2.1. Morphology

The particles of the rapeseed fractions were visualized by representative SEM images (Fig. 3), to understand the morphology change of the cellular matrix induced by the different process conditions. Laguna et al. (2018) reported globular protein bodies to be stored inside the cellular matrix in RSM. There is no evidence of globular protein bodies in meal and concentrates in Fig. 3, since the surface of the RSM particles was more compact and less opened compared to the reported milled rapeseed kernels from a dry fractionation. In Laguna's work, the cell wall structure was opened by intensive milling process leading to an average particle size of less than 100 μm. Here, milling was less intensive, as evidenced by a larger average particle size of 100–400 μm. The less intensive milling could be a reason for observing more complete cell wall structure of rapeseed particles for the meal and concentrates in the present study. This could explain that the protein bodies and other components were entrapped inside the matrix. The SEM pictures of RPI showed disordered flake fragments and it is likely that the protein bodies in the cellular matrix were dissolved in to water during isolate fractionation and formed flakes during freeze drying. A similar structure of flakes was also found in the literature with respect to the freeze-dried soluble plant protein (Berghout, 2015; Jiang et al., 2014).

3.2.2. Particle hydration properties

The rapeseed materials were hydrated with water and the change of particle size during hydration was measured to understand the melting and swelling of the particles as a function of time. It is hypothesized that the insoluble part of the flakes and cellular matrix observed in SEM with the size larger than 100 μm will either decrease in size with increasing hydration time due to dissolvement or increase in size caused by swelling.

RSM and dRSM dispersions showed similar particle sizes in Fig. 3. For RSM, the particle distribution of the large particles above 100 μm changed towards a larger size during hydration, while particle size of dRSM was hardly changed. The difference might be associated with the oil removal from RSM: defatting could result in a more hydrophobic nature of the dRSM, reducing its water uptake and thus its swelling capacity, or it could have affected the cellular matrix leading to a more closed and compact structure. The volume of small particles with the size of approximately 7–10 μm increased over time for RSM. One explanation might be associated with structural changes of the fibres during hydration towards smaller structures. On the other hand, a similar increase of small particles (between 7 and 10 μm) was found with RPI as well. Therefore, the increasing of small particles might also be explained by the liberating of remaining insoluble protein particles from RSM and RPI, which disentangled into smaller particles further upon prolonged hydration. This is confirmed by the presence of around 24%

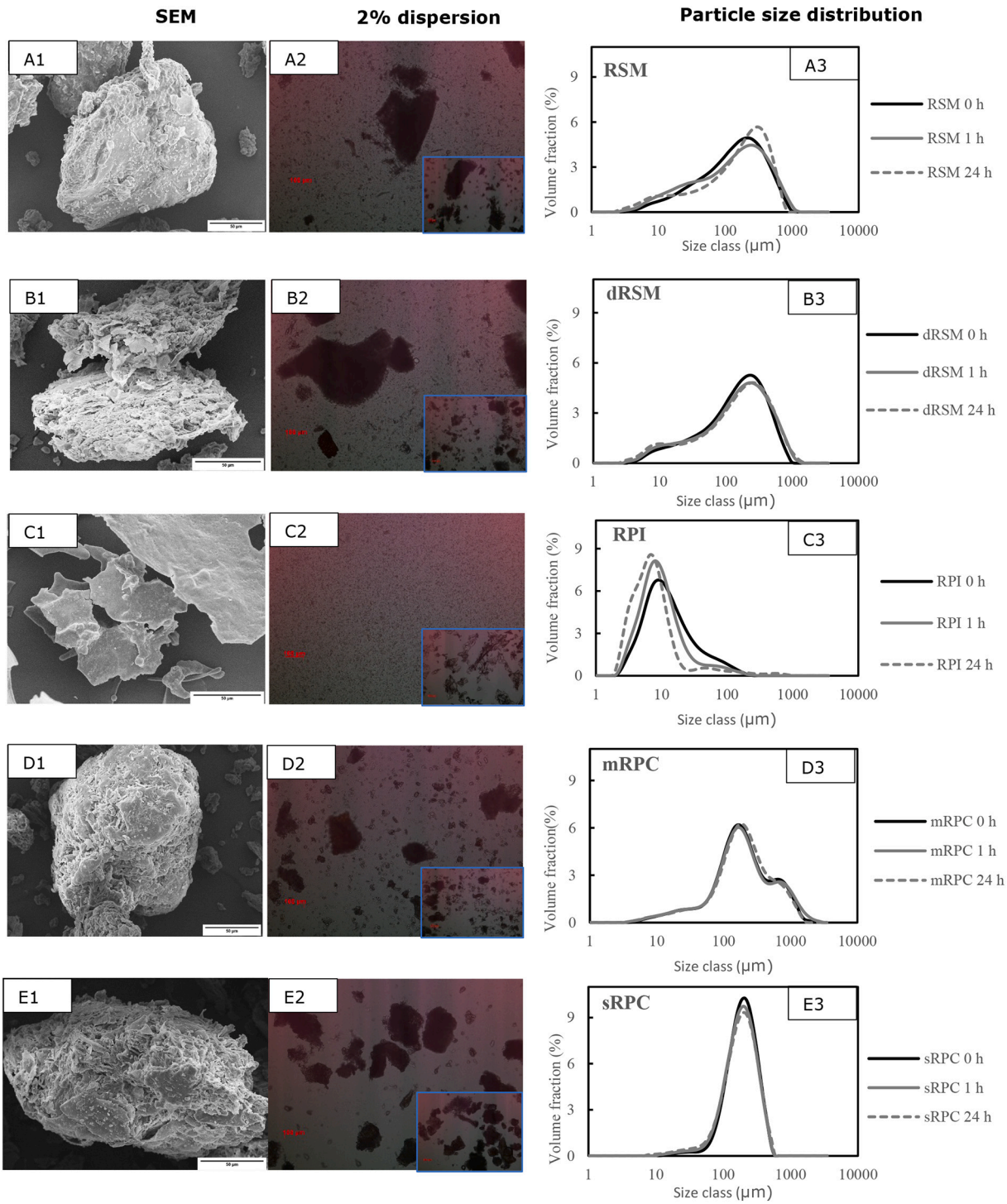


Fig. 3. SEM images of RSM, dRSM, mRPC, sRPC and RPI (A1-E1), and light microscopy of particle hydration in a 2% dispersion, snapshots were taken fresh (small picture on the right bottom corner) and after 24 h (A2-E2). Particle size distribution of RSM, dRSM, mRPC, sRPC and RPI (A3-E3) during hydration in water at the time interval of 0 min, 30 min, 1 h and 24 h. The scale of the light microscopy is 100 μm and the scale of SEM is 50 μm.

insoluble protein fraction in RPI, according to difference calculations based on the 76% soluble protein determined in NSI (section 3.1.3). Unlike these rapeseed materials, hardly any small particles were produced during hydration for mRPC and sRPC and the particles seem to retain in their original state. The large particles of concentrates remained even more stable in size distribution compared to RSM and dRSM. This result indicates that both RPCs were hardly hydrated. It is possible that the aqueous ethanolic washing treatment led to dense and even glassy particles, which do not allow water to penetrate the particles. Similar observations have been found for soy okara (Jankowiak, Trifunovic, Boom, & Van Der Goot, 2014),

3.2.3. *Water holding capacity (WHC)*

WHC is an important property since it can provide information about juiciness and tenderness of food products such as meat or meat analogues (Cornet et al., 2021; Dijkstra, Linnemann, & Van Boekel, 2003). The WHC of the insoluble fractions from rapeseed materials is shown in Fig. 4. WHC of RSM was found to be 6.2 g water/g dry pellet and that of

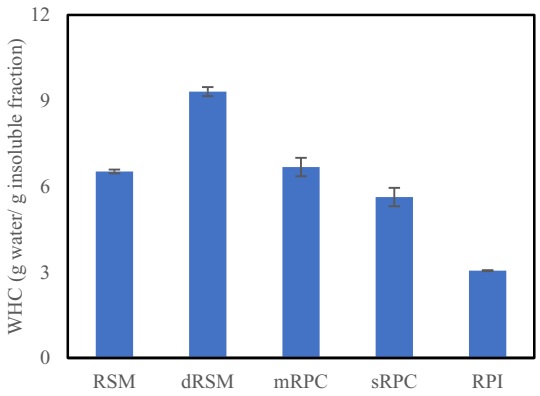
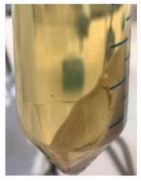
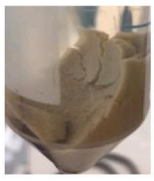
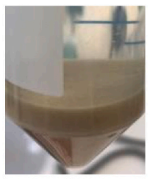
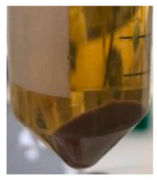
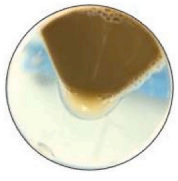


Fig. 4. Water holding capacity (WHC) for RSM, dRSM, mRPC, sRPC and RPI.

dRSM showed highest value of 9.3 g water/g dry pellet. The concentrates of mRPC showed slightly higher WHC values of 6.7 g water/g dry pellet compared to 5.6 g water/g dry pellet in case of sRPC. The lowest WHC was found with RPI.

The differences in the WHC among rapeseed materials could to be the result of the following two reasons. Firstly, different components within the rapeseed fractions might hold different amount of water, such as protein and carbohydrates, and the composition between the fractions was different. The WHC of rapeseed protein is likely to be similar low as the WHC of RPI, since RPI was mainly composed of native protein. In contrast to protein, for carbohydrates it has been reported that the water-insoluble dietary fiber can lead to high WHC (Yamazaki, Murakami, & Kurita, 2005), which explains the slightly higher WHC in the concentrates. Slightly lower WHC with the sRPC compared to mRPC also indicate that macroscopic hulls removed by sieving contribute to the WHC. However, the impact of PC on the WHC is still unclear yet. Alu'datt et al. (2014) reported higher WHC after PC removal from soybean and flaxseed protein isolate, while other authors reported that PC did not modify the WHC of sunflower protein concentrates (Alu'datt, Rababah, & Alli, 2014; Salgado, Ortiz, Petruccelli, & Mauri, 2012).

Table 3
Pictures of dry powder, particle hydration samples with 2% *w/w* dispersion after centrifugation, wet pellet after removal of supernatant with respect to the RSM, dRSM, mRPC, sRPC and RPI. CCR samples of temperature sweep from 40 to 150 °C for 40% rapeseed materials, WG, SPC and SPI of 40% DM.

Rapeseed fractions	RSM	dRSM	mRPC	sRPC	RPI
Powder					
Particle hydration (2% w/w concentration)					
Wet insoluble pellet					
CCR rapeseed samples (40% DM) Tempearture sweep (40-150 °C)					
CCR samples (40% DM) WG/SPC/SPI Temperature sweep (40-150 °C) From left to right)					

Secondly, the WHC was also reported to be influenced by the structure of the pellet and hydration of the particles. Since the proteins within rapeseed meal and concentrates were likely trapped inside the cellular matrix, the water held by the cellular matrix and in between the particles might have a big impact on the WHC. This was further visualized with the wet pellets shown in Table 3. The pellets of the different rapeseed fractions were found to be composed of sand-like particles, except for the consistent gel-like pellet of RPI. The high WHC of rapeseed concentrates is unexpected, considering that these sand-like particles do not seem to swell (section 3.2.2) and have a low protein solubility (section 3.1.3). This confirms the assumption that aqueous ethanolic washing treatment lead to a glassy matrix in RPC which probably hindered hydration and water uptake. The hypothesis is that after hydration, the wet pellet formed a viscous gel which was held together by interstitial water between the hardly swollen particles.

3.2.4. Hydration effect on color change

The color of the RPI solution and insoluble pellet was much darker compared to the rest of the materials in Table 3. The color formation could be due to covalent interaction between proteins and PC as indicated in the discussion about the composition of the fractions (section 3.1.1) and in the SDS-PAGE (section 3.1.2). Further, oxidized PC condensation products are also of dark color (Keppler, Schwarz, & Jan van der Goot, 2020). The light color of the concentrates solution also indicates that most of the PC were removed by the aqueous ethanolic washing.

3.3. Rheological properties

Rheological properties with temperature sweep and time sweep can be used to assess the potential structural properties of the rapeseed materials. The comparison was made with soy protein concentrate (SPC), soy protein isolate (SPI) and wheat gluten (WG), which are commonly used for the structuring purpose in meat analogue application.

The apparent complex modulus G* was measured by oscillation while heating at 1% strain and 1 Hz frequency with the rapeseed materials of 40% DM. In Fig. $5A_1$, three regions can be identified for RSM, a region with a constant apparent G* value (50 kPa from 40 to 80 °C), a region where the apparent G* slightly increased (70 kPa at 118 °C), and a region where the apparent G* decreased (17 kPa at 150 °C). A similar pattern was found with dRSM with approximately three times higher apparent G* values. This result indicates that the fat removal of RSM has a positive impact on viscoelastic properties. Both concentrates mRPC and sRPC show a decrease in apparent G* starting from 580 kPa for mRPC and 643 kPa for sRPC at 40 °C, which decreased to around 110 kPa at 150 °C for both concentrates. The apparent G* of both concentrates was found to be higher than the rest of the rapeseed materials during heating, which might be linked to their glassy and hardly hydrated particle properties as shown in section 3.2.2. For RPI two peaks were observed. The first peak was found to be around 8 kPa at 95 °C. After a slight decreasing, the apparent G* was further increased to the second peak of 15 kPa at 125 °C and finally decreased to 3 kPa at 150 °C. The two peaks were corresponded to the two denaturation peaks of cruciferin and napin obtained with DSC in Table 2.

The apparent G* was also measured with WG, SPI and SPC under the same conditions as rapeseed materials as a comparison (Fig. $5A_2$). WG showed stable apparent G* from 40 to 98 °C and an increase was found from 4 kPa at 98 °C to 30 kPa at 130 °C, followed by a decrease to 22 kPa at 150 °C. Heat-induced polymerization of WG was reported to be caused by the formation of disulphide bonds (Domenek, Morel, Bonicel, & Guilbert, 2002; M. Azad Emin & Schuchmann, 2017). Similar to WG, RPI is also rich in sulphur-containing amino acid, which can contribute to crosslinking during heating. Besides, the decrease of apparent G* for WG above 130 °C was explained by the degradation of the crosslinked structure (M. Azad. Emin, Quevedo, Wilhelm, & Karbstein, 2017), and this effect is likely to occur in RPI as well. A decreasing trend was found with the apparent G* of SPC and SPI when heated from 25 °C to 150 °C, and this is an expected behavior as increased temperature normally leads to lower viscosity. Above 120 °C, the decrease was more pronounced and suggested some kind of structure breakdown. Remarkably, the G* of sRPC and mRPC hardly decreased when heated above 120 °C and the value was stable for both RPC materials in the whole temperature range. The results showed a stronger material of rapeseed concentrates during heating even compared to the soy protein materials and WG. One might speculate the result to be linked with the aqueous ethanolic washing treatment which make the RPCs glassy and more compacted. Also the presence of high fiber content in the RPCs is likely to cause the strong rheological properties.

Fig. $5B_1$ and Fig. $5B_2$ displays the effect of processing time on the apparent G* of sRPC, dRSM, RPI, SPC and WG through isothermal time sweep at 120 °C and 140 °C. The samples of sRPC and dRSM showed a prompt initial increase which was a result of the sample heating up to the set temperature. After the initial increase, the G* for sRPC and dRSM was stable at 240 kPa and 140 kPa when heated at 120 °C, respectively. The initial increase of apparent G* for RPI and WG was a bit slower, but then the apparent G*-value remained more or less constant. Unlike rapeseed materials and WG, no initial increase was found for SPC. The G*-value was more or less constant during the full 15 min when heated at 120 °C. This might indicate the structure formation prior to experiment by the vacuum of the material and the structure was stable upon heating at 120 °C.

For all the materials studied, the rheological properties of apparent G* seems to be hardly affected by the time sweep at 120 °C for 15 min. It is also reported stable complex viscosity of WG with 16 kPa during time sweep with the same conditions at 120 °C (M. Azad Emin & Schuchmann, 2017), and this value was found to be similar as the apparent G* of WG (between 20 and 30 kPa) at 120 °C. Therefore, the structure degradation hardly occurred at this temperature. It is interesting to observe that the degradation started when heating at 140 °C for all the materials studied. sRPC and SPC showed a fast decrease of the G* at the first 5 min and the decrease became slower afterwards at 140 °C. However, a plateau was reached after a fast decreasing of the apparent of G* in case of dRSM around 5 min heating. The trend of decreasing with G* for RPI and WG was similar, shown in a slow pattern and continuously upon heating at 140 °C. This is in accordance with the temperature sweep results shown above, where lowering of the apparent G* took place at elevated temperatures. Also the complex viscosity of the WG was reported to be decreased from approximately 12 kPa to 8 kPa after 15 min of time sweep at 140 °C, and this value is similar to the WG (22–11 kPa) presents in Fig. 5. The apparent G* of the rapeseed materials at different temperatures were in agreement with the temperature sweep results comparing the same temperatures.

3.4. Rapeseed materials for food application

An outlook for the potential application of the rapeseed materials is discussed in this section, concerning the different process conditions of defatting, aqueous ethanolic washing and aqueous extraction combined with ultrafiltration. As can be seen in Fig. 4 and Table 2, dRSM combines high WHC and interesting rheological behavior which is promising from a functional point of view. Both RPCs contained hardly any PC and similar functional and structural properties were found with both RPCs. The presence of hulls with small particle size in the mRPC might require further investigation on digestibility and sensory properties. Sieving turned out to be effective for the removal of the hull fractions of sRPC, which is a potential ingredient for food application. High apparent G* were found with RPCs compared to the rest of the rapeseed fractions, SPI, SPC and WG, indicating the formation of the structures with strong mechanical strength (Fig. 5), as needed for example for meat analogue production (Dekkers, Emin, Boom, & van der Goot, 2018; M. Azad Emin & Schuchmann, 2017).

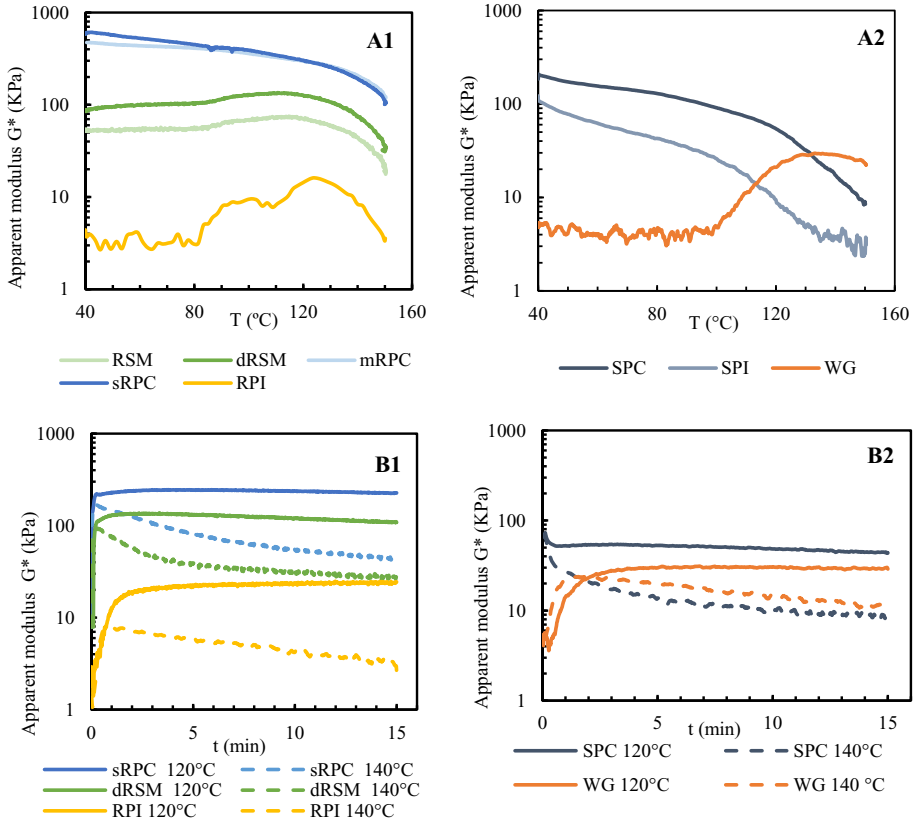


Fig. 5. Apparent complex modulus G* for anisothermal experiments from 40-150°C: A_1) for RSM, dRSM, mRPC, sRPC, RPI; $A_{2)}$ for SPC, SPI, WG; Apparent complex modulus for isothermal experiments at 120°C and 140°C: B_1) for dRSM, sRPC and RPI; B_2) for SPC and WG. An instability of the curve below 5kPa was observed that corresponds to the detection limit of the machine.

RPI is the most refined protein fractions of all tested samples, but still contained certain amount of PC (Table 1). Its high protein solubility (Fig. 2) can make the material suitable for food applications that require native protein with high solubility, i.e., emulsifying and foaming properties (Lawal, 2005). The rheological properties of RPI during heating showed certain similarities to those of WG. The sulphur-containing amino acids and PC present in the RPI have the potential to contribute to the polymerization, which can also be of interest in structure formation processes (for example for meat and dairy analogues) (Keppler et al., 2020). However, the sensory properties need to be tested since a considerable amount of PC are still in the material. If low PC is desired then further treatment is still required for the RPI.

From a processing and economic point of view, less treatment is generally preferred. Therefore, the less refined RPC is a potential plant protein source to be used in sustainable food products. RPC could be considered as an alternative for SPC and given its under usage as a waste stream or as feedstock after oil extraction, it is even more economically valuable. Further purified RPI that still contains some PC has the ability to polymerize, which is a key property of WG. Therefore, it is interesting to further investigate possible similarities in behavior between RPI and WG for structuring purposes. RPI might be suitable for other applications, such as emulsifying and foaming, in which high solubility and low gelling capacity is required. The low protein recovery yield of RPI led to a loss of protein during fractionation, also by-products were generated during fractionation which is not as economically favorable as RPC.

4. Conclusion

In this study, the effect of fractionation on the techno-functional properties of rapeseed materials was studied. The results clearly showed that the fractionation process strongly affects the obtained material: nearly dephenolized RPC was obtained from the aqueous ethanolic washing process, but the proteins were mostly denatured with reduced solubility under the process condition applied. In contrast, RPI obtained through aqueous extraction and ultrafiltration process still contained a high amount of PC, but the proteins were native. The changes in material composition and protein nativity is reflected in the techno-functional properties. The insoluble RPC particles hardly swelled during hydration, but held more water compared to the insoluble RPI particles. The RPC dispersion with 40% DM showed highest complex modulus G* with rheological measurement, compared to the other rapeseed/soy materials and WG. Therefore, the insoluble RPC produced through aqueous ethanolic washing process has a good potential for structuring purposes that require high WHC and certain mechanically strength, such as meat analogues structures. In addition, the less refined material also produces less waste-streams and caters to the need for obtaining sustainable produced raw materials.

In contrast to RPC, the measured G* of RPI dispersions showed an increase upon heating at 80 °C and 110 °C suggesting a thermal induced structure formation. The highly soluble RPI with preserved protein nativity has a different structuring potential than RPC and seems mostly suitable for food application that require high solubility (for example plant-drinks) and certain gelation capacities at different high temperatures (such as texturized products). However, this material is more strongly refined than RPC. In the future, the effect of different fractionation processes on functionality and composition needs to be further explored in order to obtain tailored functional fractions for food applications.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2021.102636.

References

Aider, M., & Barbana, C. (2011). Canola proteins: Composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity - a practical and critical review. *Trends in Food Science and Technology, 22*(1), 21–39. https://doi.org/10.1016/j.tifs.2010.11.002

Akbari, A., & Wu, J. (2015). An integrated method of isolating napin and cruciferin from defatted canola meal. *LWT - Food Science and Technology, 64*(1), 308–315. https://doi.org/10.1016/J.LWT.2015.05.046

Alu'datt, M. H., Rababah, T., & Alli, I. (2014). Effect of phenolic compound removal on rheological, thermal and physico-chemical properties of soybean and flaxseed proteins. *Food Chemistry, 146*, 608–613. https://doi.org/10.1016/J.FOODCHEM.2013.09.104

Berghout, J. A. M. (2015). *Functionality-driven fractionation of lupin seeds*.

Bérot, S., Compoint, J. P., Larré, C., Malabat, C., & Guéguen, J. (2005). Large scale purification of rapeseed proteins (Brassica napus L.). *Journal of Chromatography B, 818*(1), 35–42. https://doi.org/10.1016/J.JCHROMB.2004.08.001

Bos, C., Airinei, G., Mariotti, F., Benamouzig, R., Bérot, S., Evrard, J., … Gaudichon, C. (2007). The poor digestibility of rapeseed protein is balanced by its very high metabolic utilization in humans. *The Journal of Nutrition, 137*(3), 594–600. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17311946.

Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences, 13*(7), 8615–8627. https://doi.org/10.3390/ijms13078615

Citeau, M., Regis, J., Carré, P., & Fine, F. (2016). Value of hydroalcoholic treatment of rapeseed for oil extraction and protein enrichment. *OCL, 26*(1). https://doi.org/10.1051/ocl/2018035

Cornet, S. H. V., Snel, S. J. E., Lesschen, J., Jan, A., Der Goot, V., & Van Der Sman, R. G. M. (2021). Enhancing the water holding capacity of model meat analogues through marinade composition. *Journal of Food Engineering, 290*, Article 110283. https://doi.org/10.1016/j.jfoodeng.2020.110283

Das Purkayastha, M., Gogoi, J., Kalita, D., Chattopadhyay, P., Nakhuru, K. S., Goyary, D., & Mahanta, C. L. (2014). Physicochemical and functional properties of rapeseed protein isolate: Influence of Antinutrient removal with acidified organic solvents from rapeseed meal. *Journal of Agricultural and Food Chemistry, 62*(31), 7903–7914. https://doi.org/10.1021/jf5023803

Dekkers, B. L., Emin, M. A., Boom, R. M., & van der Goot, A. J. (2018). The phase properties of soy protein and wheat gluten in a blend for fibrous structure formation. *Food Hydrocolloids, 79*, 273–281. https://doi.org/10.1016/j.foodhyd.2017.12.033

Dijkstra, D. S., Linnemann, A. R., & Van Boekel, T. A. J. S. (2003). Towards sustainable production of protein-rich foods: Appraisal of eight crops for Western Europe. PART II: Analysis of the technological aspects of the production chain. *Critical Reviews in Food Science and Nutrition, 43*(5), 481–506. https://doi.org/10.1080/10408690390246332

Domenek, S., Morel, M.-H., Bonicel, J., & Guilbert, S. (2002). Polymerization kinetics of wheat gluten upon thermosetting. A mechanistic model. *Journal of Agricultural and Food Chemistry, 50*, 5947–5954. https://doi.org/10.1021/jf0256283

Drosou, C., Kyriakopoulou, K., Bimpilas, A., Tsimogiannis, D., & Krokida, M. (2015). A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification byproducts. *Industrial Crops and Products, 75*, 141–149. https://doi.org/10.1016/J.INDCROP.2015.05.063

Emin, M. A., & Schuchmann, H. P. (2017). A mechanistic approach to analyze extrusion processing of biopolymers by numerical, rheological, and optical methods. *Trends in Food Science & Technology, 60*, 88–95. https://doi.org/10.1016/j.tifs.2016.10.003

Emin, M. A., Quevedo, M., Wilhelm, M., & Karbstein, H. P. (2017). Analysis of the reaction behavior of highly concentrated plant proteins in extrusion-like conditions. *Innovative Food Science & Emerging Technologies*. https://doi.org/10.1016/j.ifset.2017.09.013

González-Pérez, S., Merck, K. B., Vereijken, J. M., Van Koningsveld, G. A., Gruppen, H., & Voragen, A. G. J. (2002). Isolation and characterization of undenatured chlorogenic acid free sunflower (Helianthus annuus) proteins. *Journal of Agricultural and Food Chemistry, 50*(6), 1713–1719. https://doi.org/10.1021/jf011245d

Griebenow, K., & Klibanov, A. M. (1996). On protein denaturation in aqueous-organic mixtures but not in pure organic solvents. Journal of the American Chemical Society Doi:https://doi.org/10.1021/ja961869d.

Ivanova, P., Chalova, V., Uzunova, G., Koleva, L., & Manolov, I. (2016). Biochemical characterization of industrially produced rapeseed meal as a protein source in food industry. *Agriculture and Agricultural Science Procedia, 10*, 55–62. https://doi.org/10.1016/J.AASPRO.2016.09.009

Jankowiak, L., Trifunovic, O., Boom, R. M., & Van Der Goot, A. J. (2014). The potential of crude okara for isoflavone production. *Journal of Food Engineering, 124*, 166–172. https://doi.org/10.1016/j.jfoodeng.2013.10.011

Jiang, L., Wang, J., Li, Y., Wang, Z., Liang, J., Wang, R., … Zhang, M. (2014). Effects of ultrasound on the structure and physical properties of black bean protein isolates. *Food Research International, 62*, 595–601. https://doi.org/10.1016/J.FOODRES.2014.04.022

Kayser, J. J., Arnold, P., Steffen-Heins, A., Schwarz, K., & Keppler, J. K. (2020). Functional ethanol-induced fibrils: Influence of solvents and temperature on amyloid-like aggregation of beta-lactoglobulin. *Journal of Food Engineering, 270* (October 2019), Article 109764. https://doi.org/10.1016/j.jfoodeng.2019.109764

Keppler, J. K., Schwarz, K., & Jan van der Goot, A. (2020). Covalent modification of food proteins by plant-based ingredients (polyphenols and organosulphur compounds): A commonplace reaction with novel utilization potential. *Trends in Food Science & Technology, 101*(October 2019), 38–49. https://doi.org/10.1016/j.tifs.2020.04.023

Khattab, R. Y., & Arntfield, S. D. (2009). Functional properties of raw and processed canola meal. *LWT - Food Science and Technology, 42*(6), 1119–1124. https://doi.org/10.1016/J.LWT.2009.02.009

Kozlowska, H., Zadernowski, R., & Sosulski, F. W. (1983). Phenolic acids in oilseed flours. *Food / Nahrung, 27*(5), 449–453. https://doi.org/10.1002/food.19830270517

Laguna, O., Barakat, A., Alhamada, H., Durand, E., Baréa, B., Fine, F., … Lecomte, J. (2018). Production of proteins and phenolic compounds enriched fractions from rapeseed and sunflower meals by dry fractionation processes. *Industrial Crops and Products, 118*(December 2017), 160–172. https://doi.org/10.1016/j.indcrop.2018.03.045

Laguna, O., Odinot, E., Bisotto, A., Baréa, B., Villeneuve, P., Sigoillot, J.-C., … Lecomte, J. (2019). Release of phenolic acids from sunflower and rapeseed meals using different carboxylic esters hydrolases from Aspergillus Niger. *Industrial Crops and Products, 139*, 111579. https://doi.org/10.1016/J.INDCROP.2019.111579

Lawal, O. S. (2005). Functionality of native and succinylated Lablab bean (Lablab purpureus) protein concentrate. *Food Hydrocolloids, 19*(1), 63–72. https://doi.org/10.1016/j.foodhyd.2004.04.015

Mattos, C., & Ringe, D. (2001). Proteins in organic solvents. *Current Opinion in Structural Biology, 11*(6), 761–764. https://doi.org/10.1016/S0959-440X(01)00278-0

Naczk, M., Amarowicz, R., Sullivan, A., & Shahidi, F. (1998). Current research developments on polyphenolics of rapeseed/canola: A review. *Food Chemistry, 62*(4), 489–502. https://doi.org/10.1016/S0308-8146(97)00198-2

Peng, L. P., Xu, Y. T., Li, X. T., & Tang, C. H. (2020). Improving the emulsification of soy β-conglycinin by alcohol-induced aggregation. *Food Hydrocolloids, 98*(May 2019), 105307. https://doi.org/10.1016/j.foodhyd.2019.105307

Salgado, P. R., Ortiz, S. E. M., Petruccelli, S., & Mauri, A. N. (2012). Functional food ingredients based on sunflower protein concentrates naturally enriched with antioxidant phenolic compounds. *JAOCS, Journal of the American Oil Chemists' Society, 89*(5), 825–836. https://doi.org/10.1007/s11746-011-1982-x

Sari, Y. W., Bruins, M. E., & Sanders, J. P. M. (2013). Enzyme assisted protein extraction from rapeseed, soybean, and microalgae meals. *Industrial Crops and Products, 43*(1), 78–83. https://doi.org/10.1016/j.indcrop.2012.07.014

Tan, S. H., Mailer, R. J., Blanchard, C. L., & Agboola, S. O. (2011a). Canola proteins for human consumption: Extraction, profile, and functional properties. *Journal of Food Science*. https://doi.org/10.1111/j.1750-3841.2010.01930.x

Tan, S. H., Mailer, R. J., Blanchard, C. L., & Agboola, S. O. (2011b). Extraction and characterization of protein fractions from Australian canola meals. *Food Research International, 44*(4), 1075–1082. https://doi.org/10.1016/j.foodres.2011.03.023

Tranchino, L., Costantino, R., & Sodini, G. (1983). Food grade oilseed protein processing: Sunflower and rapeseed. *Qualitas Plantarum Plant Foods for Human Nutrition, 32* (3–4), 305–334. https://doi.org/10.1007/BF01091192

USDA. (2020). Oilseeds: World markets and trade. In *Circular series FOP 06-17. Global oilseed consumption continues to grow despite slowing trade and production* (pp. 1–50). https://doi.org/10.1016/S1097-8690(11)70006-3

Wanasundara, J. P. D. (2011). Proteins of *Brassicaceae* oilseeds and their potential as a plant protein source. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408391003749942

Wanasundara, J. P. D., Abeysekara, S. J., McIntosh, T. C., & Falk, K. C. (2012). Solubility differences of major storage proteins of brassicaceae oilseeds. *JAOCS, Journal of the American Oil Chemists' Society, 89*(5), 869–881. https://doi.org/10.1007/s11746-011-1975-9

Wu, J., & Muir, A. D. (2008). Comparative structural, emulsifying, and biological properties of 2 major canola proteins, Cruciferin and Napin. *Journal of Food Science, 73*(3), C210–C216. https://doi.org/10.1111/j.1750-3841.2008.00675.x

Yamazaki, E., Murakami, K., & Kurita, O. (2005). Easy preparation of dietary Fiber with the high water-holding capacity from food sources. *Plant Foods for Human Nutrition, 60*, 17–23. https://doi.org/10.1007/s11130-005-2537-9

Yang, C., Wang, Y., Vasanthan, T., & Chen, L. (2014). Impacts of pH and heating temperature on formation mechanisms and properties of thermally induced canola protein gels. *Food Hydrocolloids, 40*, 225–236. https://doi.org/10.1016/j.foodhyd.2014.03.011

Yoshie-Stark, Y., Wada, Y., & Wäsche, A. (2008). Chemical composition, functional properties, and bioactivities of rapeseed protein isolates. *Food Chemistry, 107*(1), 32–39. https://doi.org/10.1016/j.foodchem.2007.07.061

Zuorro, A., Iannone, A., & Lavecchia, R. (2019). Water-organic solvent extraction of phenolic antioxidants from brewers' spent grain. *Processes, 7*(3). https://doi.org/10.3390/pr7030126