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# Effects of ball milling on the structural, thermal, and rheological properties of oat bran protein flour

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## ABSTRACT

Oat bran protein flour (OBPF), containing protein, starch, and lipid as major constituents, was ball milled and subsequently evaluated on structural conformation, thermal properties, particle size distributions, and rheological properties. Prior to ball milling, characterisation of OBPF were conducted by means of Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) showing the existence of aggregated protein and starch-lipid complexes as predominant constituents of OBPF. Ball milling altered structural conformations of both protein and starch. Moreover, increase of ball milling time gradually decreased the transition enthalpy changes of amylose-lipid complexes upon heating which can be related to disruption of amylose-lipid complexes helical structure. Ball milling at higher speed resulted to smaller average particle size distributions of OBPF. Dynamic mechanical spectra of concentrated dispersions containing ball milled OBPF exhibited lower storage ( $G'$ ) and loss ( $G''$ ) moduli compared to control sample due to reduced particles volume packing. Moduli-frequency sweep data satisfactory fitted the Power Law's model.

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## 1. Introduction

Interests in protein-rich foods are rising favoured by its health-promoting effects. The benefits of dietary protein intake has been associated with body weight management and maintenance of muscle mass and function (Deutz et al., 2014; Westerterp-Plantenga et al., 2017). The urge to search for alternative food protein sources have led to valorisation of underexploited plants and side stream agricultural products.

Oat bran is a by-product of oat flour milling and have been used as a fibre-enriching ingredient. Although oat bran contains abundance of protein, its full utilisation remains challenging. Enzymatic pre-treatment of oat bran thick cell walls is necessary to assist protein extraction, otherwise a highly alkaline pH is required to release protein that adversely affect the nutritional quality (Jodayree et al., 2012). Low solubility of oat protein in slightly acidic to neutral pH range also restrict its applicability for foods (Konak et al., 2014). In order to bridge the gap between the limitations and advantages of oat bran protein for utilisation in foods, a feasible

separation technique has been introduced to obtain protein-enriched flour with low degree of purity (Sozer et al., 2017). The presence of protein, starch and lipid, as constituents of composite flour, are known contributing to the physicochemical properties and functionality of flour (Puncha-Arnon and Uttapap, 2013; Saleh, 2017).

Physical modification of food materials by means of ball milling has gained attention due to ability in changing functionality. The modified functionality can be attributed to alteration in structural conformation as found in ball milled starch and starch-enriched food materials (Dhital et al., 2011; Liu et al., 2011; Roa et al., 2014a). However, there are limited studies reporting the effects of ball milling on structural conformation changes in food proteins including whey and soy protein (Liu et al., 2017; Sun et al., 2015).

This present study aims to evaluate the alteration on structural conformation of chemical constituents within ball milled oat bran protein flour, and subsequently to extrapolate the conformational changes into the thermal and rheological properties of its dispersion. This study would provide useful insights to extend the utilisation of oat bran protein flour in food formulations.

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## 2. Materials and methods

### 2.1. Materials

Oat bran protein flour (OBPF) was supplied by Tate & Lyle Oat Ingredients (Kimstad, Sweden) as a brand of PrOatein<sup>®</sup>. This material was in the form of coarse powder and declared to have ca. 94% dry matter containing 54% protein, 17% fat, 18% carbohydrate, and 2% fibre on a dry basis. This material was prepared from oat bran that have gone through enzymatic, thermal, and physical separation processes.

### 2.2. Elimination of lipid and starch

Elimination of lipid and reduction of starch were intended to provide a correct assignment of FTIR spectra bands and interpretation of thermal properties in the later analyses. Lipid were extracted using solvent in soxhlet apparatus as described by Pandey and Shrivastava (2017) with modifications. Two solvents with different polarity were used, i.e. hexane for the non-polar solvent, and isopropanol for the polar solvent. Defatted samples are addressed as NPDO for the hexane-defatted OBPF and PDO for the isopropanol-defatted OBPF. Protein extraction from defatted samples, i.e. NPDO and PDO, did not lead to reasonable protein yields for further analysis. Therefore, in this study, starch reduction was chosen to obtain a higher protein concentration. Starch reduction was conducted by dispersing and stirring the defatted samples into alkaline solution for 30 min as described by (De Souza et al. 2016). The sample was then lyophilised to obtain dry powder and addressed as APDO.

### 2.3. Ball milling procedure

Ball milling was carried out by using Planetary Micro Mill (Pulverisette 7 classic line, Fritsch GmbH, Germany) as described by Abbaszadeh et al. (2014) with modifications. Two and a half grams of OBPF was put into 12 ml grinding bowl containing 6 Zirconium dioxide grinding balls. High speed milling was run at 800 revolutions per minute (rpm) for 15 min. The low speed milling was carried out at 200 rpm for 240 min. The applied milling cycle configuration was 5 min milling followed by 5 min rest to dissipate heat. Reversed milling directions were set to prevent ball slippage at both milling speeds.

### 2.4. Preparation of OBPF dispersions

OBPF dispersions (5, 20, and 30%wt) were prepared by suspending the flour into distilled water as described by Roa et al.

(2015) with modifications. These concentrations were selected according to previous trials conducted with OBPF dispersions from 5 to 30%wt. A dilute system (5%wt) was selected to evaluate the particle size distributions, while the 20%wt dispersion was subjected to thermal analysis for its flow-ability reason, and the 30%wt dispersion was selected for rheological analysis to avoid sedimentation.

### 2.5. Attenuated total reflectance – fourier transform infrared (ATR-FTIR) spectroscopy

The infrared spectra were obtained by using a Bruker Tensor 27 System (Bruker Optik GmbH, Ettlingen, Germany) equipped with diamond Attenuated Total Reflection (ATR) crystal and operated using OPUS software version 7.2.139.1294. The tests were performed at ambient temperature. The open-air background test was run prior to sample measurement. Approximately 50 mg of dry sample was placed into contact with the diamond ATR crystal and sample holder. Each absorbance measurement was conducted under 128 scans with 4 cm<sup>-1</sup> resolutions. The presented infrared spectra were obtained as a mean from three measurements of duplicate samples that have undergone normalisation and baseline correction. Height of peaks were determined by calculating the absorption intensity differences between peak and its own baseline. Peak height ratio of selected absorption bands was used to quantify the infrared spectral changes. The amide I band, i.e. ~1630 cm<sup>-1</sup>, was selected as reference band in evaluating spectral changes due to elimination of lipids and starch. Calculation of absorbance intensity ratio ~997 to ~1022 cm<sup>-1</sup> was used to assess structural conformation changes on starch as described by Liu et al. (2011). The use of second derivative spectra was intended to resolve the overlapping bands both in amide and saccharide regions (Fig. 3). The assignment of protein secondary structure within amide I band referred to a study reported by Tang and Ma (2009). Further processing of infrared spectra was conducted using Spectragryph optical spectroscopy software version 1.0.2 (<https://www.efemm2.de/spectragryph/>).

### 2.6. Micro-differential scanning calorimetry

Scanning calorimetry was performed in a Micro-Differential Scanning Calorimeter III (Setaram Instrumentation, France). About 800 mg of dispersions containing 20%wt solid sample was transferred into hermetically sealed sample vial made from hastelloy, while similar weight of deionized water was used as reference. Heating-cooling processes were performed in two cycles from 20 to 120 °C at a rate of 1 °C min<sup>-1</sup>. Measurement was run in duplicate for each sample. The heat flow curves were processed using Calisto Processing software v1.43 (AKTS, Switzerland) to obtain the maximum heat absorption temperatures (T<sub>m</sub>) and the transition enthalpy changes (ΔH).

### 2.7. Particle size distributions

Particle size and size distributions were measured based on the principles of light scattering using LS13320 Laser Diffraction Particle Size Analyzer (Beckman Coulter, High Wycombe, UK). A dispersion of 5% OBPF in distilled water was pipetted into a liquid module sample cell. The measurement was run for 60 s under following conditions: Fraunhofer theory was used as optical model treating particles in spherical approximation, refractive indexes of dispersant and sample were 1.33 and 1.6 respectively. The particle size was determined by the volume-weighted mean diameter size. Each measurement was run in duplicate.

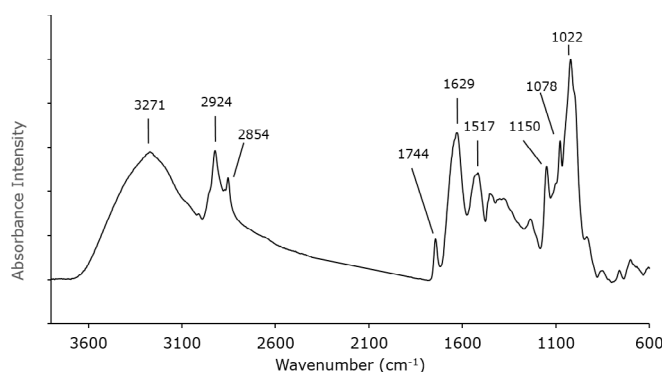


Fig. 1. Typical infrared spectra of oat bran protein flour.

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