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Influence of process conditions during aqueous protein extraction upon yield from pre-pressed and cold-pressed rapeseed press cake

Andreas Fetzer[a,b,*], Thomas Herfellner[b], Andreas Stäbler[b], Michael Menner[b], Peter Eisner[a,b]

[a] Technical University of Munich (TUM), TUM School of Life Sciences Weihenstephan, Alte Akademie 8, 85354 Freising, Germany
[b] Fraunhofer Institute for Process Engineering and Packaging IVV, Giggenhauser Str. 35, 85354 Freising, Germany

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ABSTRACT

As rapeseed is the third most important plant oil source worldwide (after palm and soya), vast amounts of residual press cake containing high amounts of valuable protein are generated during industrial de-oiling processes. Because the utilization of rapeseed press cake in feed and food is limited due to antinutritional factors, such as glucosinolates, alternative applications of rapeseed proteins in the non-food area are being investigated to add value to the rapeseed industry. However, a major problem remaining in protein extraction from de-oiled rapeseed residues is low protein solubility, resulting from thermal protein denaturation during industrial pressing. The aim of this study was to enhance protein yields from aqueous protein extraction by investigating the influence of various extraction conditions. Two different rapeseed raw materials were examined: cold-pressed meal (CPM) and pre-pressed meal (PPM). Factors examined were the solid to liquid (s:l) ratio, extraction time, temperature, pH value, concentration of sodium chloride, number of extraction cycles and the employment of protease. Best yields at mild ambient conditions were 52.3% for CPM and 36.7% for PPM, with the NaCl concentration being the most critical factor among the studied parameters. Interestingly, a simple extraction at native pH (5.7–5.8) gave comparable yields to extractions at pH 7–9. Improved yields were obtained under strong-alkaline conditions and by the employment of Protease A-01 with the limitation of protein hydrolysis occurring under these conditions. The best protein-extraction yields obtained from enzyme-assisted processes for CPM and PPM were 59.5% and 60.6%, respectively, for one-step processes and 80.7% and 78.3%, respectively, for three-step processes. The results obtained contribute to improving the sustainability of protein utilization from industrial waste streams. Thus, they support the ongoing effort to add value to the rapeseed industry within a biobased economy.

1. Introduction

Rapeseed is the third most abundant oil plant worldwide (after palm and soya) and the most abundant oil plant in the European Union (EU). In 2014, the global annual production of rapeseed accounted for 73.8 Mt, with a share 24.3 Mt in the EU (Food and Agriculture Organization of the United Nations (FAO), 2017). In recent years, rapeseed oil has gained vast interest as a renewable source for the production of biodiesel (rapeseed oil methyl ester, RME), amounting to 70% of the total rapeseed-oil production in the EU in 2014/2015 (USDA Foreign Agricultural Service, 2016).

Rapeseed press cake (RPC) is the residual material left after defatting rapeseed by mechanical-extraction methods such as screw pressing. Conventionally, two different types of RPC are produced, depending on the residual fat content left after screw pressing. Full-pressed press cake (FPC) is obtained by complete mechanical oil extraction to a fat content of 5%–10% (Kemper, 2005). Pre-pressed press cake (PPC) is obtained by mechanical extraction to a lesser extent (15%–18% fat content). Pre-pressing is usually a pretreatment to prepare the seed material for subsequent solvent extraction (Kemper, 2005). In addition to these conventional processes, cold-pressing is sometimes applied for the production of niche-market native rapeseed oils (Leming and Lember, 2005). The residual material, cold-pressed press cake (CPC) has a residual fat content of approximately 15%–18%. If solvent extraction is applied to further extract oil from the press cake, rapeseed meal (RM) is obtained containing approximately 35%–40% protein (based on nitrogen content using a conversion factor of 5.7) and 1%–2% fat (Mosenthin et al., 2016). In 2014/2015, 14.6 Mt of RM were produced in the EU (USDA Foreign Agricultural Service, 2016).

Rapeseed protein is mainly composed of two globular storage

* Corresponding author at: Technical University of Munich (TUM), TUM School of Life Sciences Weihenstephan, Alte Akademie 8, 85354 Freising, Germany. Tel.: +49 8161 491423; fax: +49 8161 491444.
E-mail address: andreas.fetzer@ivv.fraunhofer.de (A. Fetzer).

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proteins: napin (basic 1.7–2S albumin) and cruciferin (neutral 12S globulin) (Dalgalarrondo et al., 1986; Ericson et al., 1986; Höglund et al., 1992). The more stable napin (12–15 kDa) is composed by two subunits linked through a disulfide bond (Nioi et al., 2012). Cruciferin (~300 kDa) show distinct dissociation characteristics with six subunits (~50 kDa), each composed of heavy α-chain (~30 kDa) and a light β-chain (~20 kDa) that are linked by a disulfide bond (Dalgalarrondo et al., 1986; Wanasundara, 2011). Minor proteins found in rapeseed include oleosins, lipid transfer proteins and protease inhibitors (Wanasundara, 2011). Although RPC and RM are rich in physiologically valuable protein, their utilization as feed can be limited due to their high fiber content and residual antinutrients, in particular phytic acid, glucosinolates and phenolic compounds (Von Der Haar et al., 2014). Utilization of rapeseed protein as an ingredient in human food is of growing interest due to its techno-functional properties, such as foaming and emulsification capacity (Wanasundara, 2011). Consequently, rapeseed protein was authorized as a novel food ingredient in the EU in 2014 (EU, 2014). However, the application of rapeseed protein for human nutrition continues to be problematic. This is mainly due to the remaining glucosinolate content of and the association of phenolic acids and condensed tannins with proteins, which causes dark colors and a bitter taste in protein products (Linnemann and Dijkstra, 2002; Naczk et al., 1998).

The utilization of proteins in technical applications, such as paints and glues, has been a common practice in the early 20th century (Audic et al., 2003), but was displaced by the triumphant advance of petrochemistry. However, with increasing concerns regarding resource scarcity and the environmental burden associated with fossil raw materials, the concept of using renewables in technical applications is regaining attention (Barone and Schmidt, 2006). Therefore, the development of bio-based or biodegradable products is gaining increasing interest on both the company and consumer sides. With their great variety in functional groups, plant proteins and in particular rapeseed proteins offer a multitude of functionalities, such as foaming, emulsification and film-forming properties (Wanasundara, 2011). Film formation of rapeseed proteins by wet casting was studied by Chang and Nickerson (2015) for the production of biodegradable edible packaging. Li et al. (2017) reported the preparation of cross-linked rapeseed protein films with increased thermostability using bisepoxide and a combination of wet casting and heat compression. The preparation of rapeseed protein plastics by extrusion was demonstrated by Manamperi et al. (2015), who reported good elongation and toughness properties. Moreover, adhesive properties of rapeseed proteins were studied recently. In this regard, the potential use of nanomaterials, in particular graphite oxide and nanocrystalline cellulose, to improve adhesive and water resistance of rapeseed protein based wood-adhesive was demonstrated (Bandara et al., 2017a, 2017b). These recent studies emphasize the potential of rapeseed proteins as ingredients in the non-food sector. Exploiting the techno-functional properties of rapeseed proteins in non-food applications such as adhesives, coatings and polymers particularly poses a promising alternative to feed and food utilization, as minor non-protein impurities may be negligible in the final product.

One general limitation remaining for both food and non-food utilization of rapeseed protein from processing by-products is the limited protein solubility in RPC and RM (Mosenthin et al., 2016; Salazar-Villanea et al., 2016). Many oil extraction technologies such as conventional screw pressing, and subsequent solvent defatting and solvent removal involve high temperatures (Kemper, 2005). This causes protein denaturation and thus reduced quality, in particular reduced protein solubility, of the residual material for further applications (Mosenthin et al., 2016; Salazar-Villanea et al., 2016).

Various rapeseed-protein-extraction studies have been reported in the literature with great variations in the utilized raw-material quality and process conditions. Klockeman et al. (1997) reported an extraction yield of 99% from conventionally defatted RM by using 0.4% (w/v) NaOH as a highly alkaline extraction medium. Ismond and Welsh (1992) applied a method using different buffers at mild acidic pH, based on a method previously reported by Murray et al. (1981). However, both studies failed to report yields. Another study by Tzeng et al. (1988b) reported rapeseed-protein extractability of more than 80% at pH ≥ 7 with the addition of sodium hexametaphosphate, using mildly defatted seeds as a non-denatured raw material. More recently, several authors have studied various methods in more detail to improve protein extractability from rapeseed-residual materials. Gerzhova et al. (2016) studied the influence of pH (10–12), s:l ratio, and salt addition on protein extractability from conventional RM and obtained an optimal yield of 58%. Das Purkayastha and Mahanta (2014) applied a response-surface-methodology approach to optimize conditions and reported yields in the range of 35%–46% at pH 11. Furthermore, the employment of various types of enzymes to increase protein yield was studied. Employment of carbohydrate-hydrolyzing enzymes was reported to obtain protein yields for rapeseed materials of various qualities of 50%–80% (Rommi et al., 2014; Sari et al., 2013). Combined utilization of carbohydrate-hydrolyzing enzyme and protease afforded yields of 80% and 80%–83% for cold-pressed rapeseed material, and directly extracted dehulled seeds, respectively (Niu et al., 2012; Zhang et al., 2006). Protease treatment was also studied in combination with the application of ultrasound and was found to improve protein yield by 64% compared to experiments without ultrasonic treatment (absolute yields not reported; Wang et al. (2016)). Lastly, utilization of phytase was tested, resulting in a protein-extraction yield of 72% at pH 12.5 (Rodrigues et al., 2016).

In summary, there are some limitations when comparing methods for rapeseed-protein extraction. First, the raw materials employed differ greatly in quality from conventionally defatted materials to laboratory-scale, mildly treated materials. Second, strongly alkaline extraction is often reported. This leads to improved yields but can lead to protein hydrolysis and thus alter functionality. Finally, some authors failed to report protein yields, preventing an economic evaluation of their extraction methods.

The goal of this study was to overcome these limitations by comparing various process parameters for protein extraction from rapeseed raw materials of different qualities. In particular, the potential of different rapeseed-residual materials (CPC, PPC, FPC and conventional RM) for protein extraction was investigated. Two different RM qualities, cold-pressed meal (CPM) and pre-pressed meal (PPM), were obtained from the appropriate press cakes by mild defatting and compared in detail in terms of protein extractability. Protein-extraction yields were investigated in dependence of solid to liquid (s:l) ratio, extraction time, temperature, pH value, concentration of sodium chloride and number of extraction cycles. Moreover, employment of protease was studied as a tool for enhancing protein extractability.

2. Materials and methods

2.1. Raw materials and chemicals

Rapeseed (Brassica napus L.*) raw materials*

All rapeseed raw materials were of 00 type. Full-fat hulled seeds and hulled, cold-pressed press cake (CPC), both reduced in seed coat content, were provided by Teutoburger Ölmühle GmbH (Ibbenbüren, Germany). Pre-pressed press cake (PPC) and solvent-defatted, desolventized/toasted meal (TM) were provided by Bunge Deutschland GmbH (Mannheim, Germany). Full-pressed press cake (FPC) was provided by Saria A/S GmbH & Co. KG (Selm, Germany). Temperatures during pressing typically did not exceed 40 °C (cold-pressed) and were in the range of 105 °C–140 °C (pre-pressed) or 100 °C–125 °C (full-pressed). For the production of TM, temperatures during solvent extraction typically were in the range of 55 °C–60 °C and temperatures during desolventizing/toasting typically did not exceed 107 °C– 110 °C. All press cakes were further defatted as stated in the section “Preparation of Rapeseed Raw Material” to obtain the corresponding

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