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# Physicochemical and Functional Properties of Rapeseed Protein Isolate: Influence of Antinutrient Removal with Acidified Organic Solvents from Rapeseed Meal

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Supporting Information

**ABSTRACT:** The presence of antinutritional constituents in rapeseed protein products (RPI), such as polyphenols, phytates, allyl isothiocyanates, and glucosinolates, is a formidable constraint. The effect of antinutrient removal from rapeseed meal with an organic solvent mixture (methanol/acetone, 1:1 v/v, combined with an acid (hydrochloric, acetic, perchloric, trichloroacetic, phosphoric)) on the physicochemical and functional properties of RPI was investigated. The extraction resulted in a substantial reduction of antinutrients from RPI, especially polyphenols and phytates, with concomitant decreases in protein yield and solubility. Treatment harbored significant improvement in the degree of whiteness, which was highest in the perchloric acid case. Surface hydrophobicity and free sulfhydryl group of RPI changed considerably, with perchloric acid-treated samples showing higher values, whereas the disulfide content remarkably increased in trichloroacetic acid- and phosphoric acid-treated samples, signifying aggregation. Intrinsic emission fluorescence and FTIR spectra showed significant changes in proteins' tertiary and secondary conformations, and the changes were more pronounced in samples treated with higher concentrations of acids. No appreciable alteration appeared among the electrophoretic profiles of proteins from pristine meal and those treated with lower levels of acids. Interfacial surface properties of proteins were variably improved by the solvent extraction, whereas the converse was true for their extent of denaturation. The results suggest that the physicochemical and conformational properties of RPI are closely related to its functional properties.

**KEYWORDS:** *rapeseed press-cake, antinutrients, protein*

## INTRODUCTION

The usefulness of rapeseed protein is mainly restricted by the presence of undesirable components such as polyphenols, phytates, allyl isothiocyanates (AITC), and glucosinolates. Despite the introduction of rapeseed varieties with very low glucosinolate content (i.e., canola), the use of rapeseed meal as a source of food protein is still thwarted by the presence of high amounts of polyphenols and phytates. These antinutritional compounds must be removed from the meal and its protein products as much as possible prior to human consumption. To date, several works in the literature addressing the removal of antinutrients from rapeseed meal or its protein preparations have been available, but all reported problems with incomplete removal, loss of protein, or excessive cost.[1] Moreover, knowledge of the influence of varied extraction techniques and purification methods on structural and functional properties of rapeseed/canola proteins has either not been fully understood or is inconsistent on the basis of different works.

The choice of extraction solvent(s) usually comes from a systematic study of several mixtures because the solvent employed must allow extraction of the principal compound(s) of interest, concurrently avoiding chemical changes in the resulting protein and/or solubilizing proteins to reduce the losses. However, appropriate processing treatments that lead to structure modification can often improve functional properties and enhance utilization of proteins as ingredients in food industries.[2] Rapeseed press-cake valorization has been the focus of our ongoing research. To complete our previous work regarding antinutrient extraction from rapeseed press-cake using various solvent systems,[3] this work focuses on the investigation of the influence of the removal of antinutrients from rapeseed flour on the physicochemical and functional properties of rapeseed protein.

## MATERIALS AND METHODS

**Chemicals and Sample Preparation.** Rapeseed press-cake (from a mixture of local cultivars called "Lai behar" and "full behar") was obtained from Assam Khadi and Village Industries Board, Guwahati, India. Ground press-cake was passed through a 60 mesh size sieve and then stored at −20 °C until use. All solvents and reagents were obtained from E. Merck (India), of either high-performance liquid chromatography (HPLC) grade or analytical reagent grade, and were used without further purification.

On the basis of the results obtained in an earlier study,[3] solvents for detoxification of rapeseed meal were deemed "acceptable" when the simultaneous extractability of all the known antinutrients (i.e., total phenols, total tannins, protein-precipitating phenols, phytates, glucosinolates, and AITC) from the meal by the solvent is either

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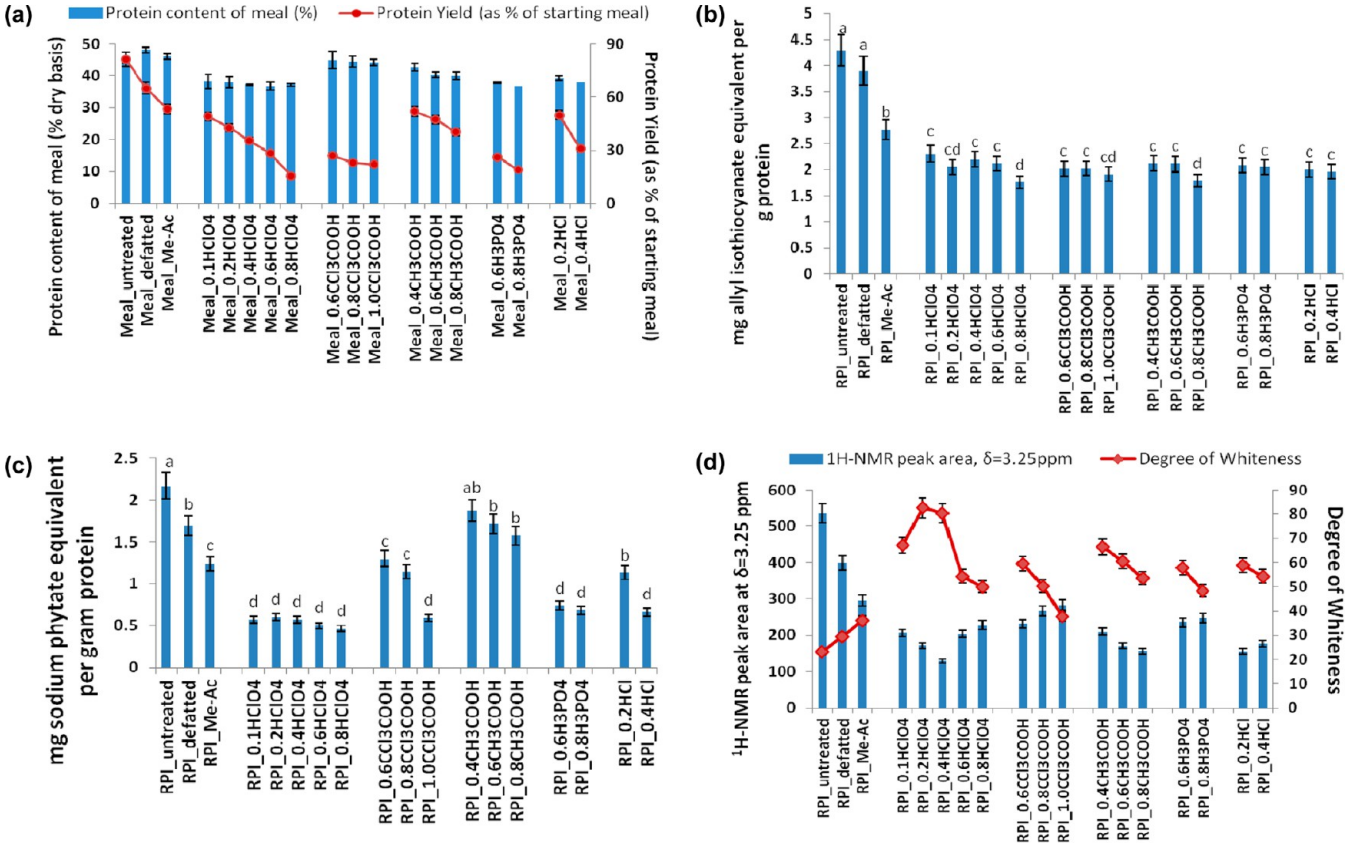


**Figure 1.** (a) Protein content of rapeseed meals treated with different solvents and their corresponding protein yield; impact of solvent treatment on (b) AITC, (c) phytates, and (d) sinapine content and degree of whiteness of the produced isolates.

equal to or higher than that of the solvent system having only methanol and acetone mixture (Me-Ac, 1:1 v/v). This criterion was fulfilled by 15 different solvent systems containing Me-Ac (1:1 v/v) in combination with different acids: 0.1, 0.2, 0.4, 0.6, or 0.8% perchloric acid ($HClO_4$); 0.6, 0.8, or 1.0% trichloroacetic acid ($CCl_3COOH$); 0.4, 0.6, or 0.8% acetic acid ($CH_3COOH$); 0.6 or 0.8% phosphoric acid ($H_3PO_4$); and 0.2 or 0.4% hydrochloric acid (HCl). As such, samples used for the preparation of protein isolates included (i) meal without any solvent treatment as control (untreated), (ii) meal treated with diethyl ether for removing lipids (defatted), (iii) defatted meal treated with Me-Ac (1:1 v/v) without any added acid, and (iv) defatted meal treated with Me-Ac (1:1 v/v) added with various concentrations of different acids as stated above.

**Preparation of Solvent-Treated Meals.** Meal (1 g) was treated with 10 mL of different solvents (mentioned above) according to earlier protocol.[3]

**Preparation of Protein Isolate from Untreated or Solvent-Treated Meals.** Meal (20 g) was extracted with water (meal/solvent ratio = 1:20 (w/v)) at room temperature for 1 h under constant agitation (200 rpm) in an orbital shaker (Sartorius Stedin Biotech, CERTOMAT IS) and then centrifuged at 4 °C (Sigma 3-18K centrifuge) to get aqueous extract. The pellet was re-extracted as before with NaOH solution at pH 11. Both extracts were combined, to which ammonium sulfate was added up to 85% saturation.[4] The resulting precipitate was redispered in water, adjusted to pH 7, dialyzed against Milli-Q water, and finally freeze-dried to obtain rapeseed protein isolate (RPI).

The respective meal and its corresponding isolate are denoted on the basis of the solvent used for their processing (mentioned in Figure 1a,b). Protein purity of all the isolates was >80% (dry basis), based on Kjeldahl nitrogen (N × 6.25). The yield was calculated as follows:

$$\%\ \text{protein yield} = \frac{\text{wt of protein isolate} \times \%\ \text{protein in isolate}}{\text{wt of starting meal} \times \%\ \text{protein in starting meal}} \times 100 \quad (1)$$

**Quantification of Antinutritional Compounds.** Sinapine content was determined according to the method developed by Wanasundara et al.[5] Freeze-dried protein was extracted with 99.8% methanol (1:20, w/v) in a round-bottom flask for 48 h with stirring. The extract was filtered through cotton wool, concentrated under vacuum, to which internal standard (*N*,*N*-dimethylformamide) was added, and then resuspended in deuterium oxide (1 mL). Proton NMR signals were recorded at 400 MHz using a JEOL $^1$H NMR system. Singlet peak at $\delta$ 3.25 ppm was identified as phenylpropanoid ester (sinapic acid ester or sinapine).

Protocols for estimating phytates, glucosinolates, and AITC are the same as those reported earlier.[3]

**Analytical Methods.** Color was determined with a Hunter Lab colorimeter (Ultrascan, VIS-Hunter Associates Laboratory, Reston, VA, USA). The majority of the isolates displayed a similar off-white color (Supporting Information Figure S1). However, upon dissolution in water, their solution showed a light brown color of different intensities, an observation that was consistent with a previous paper.[6] Therefore, following the protocol of Xu and Diosady,[6] colorimetric evaluation was performed by scanning their aqueous solution (7 mg/mL). Measured values were expressed as $L$ (lightness), $a$ (redness/greenness), and $b$ (yellowness/blueness) color units. Whiteness was calculated as follows:[7]

$$\text{degree of whiteness} = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2} \quad (2)$$

Ultraviolet (UV)−visible (vis) spectroscopy was performed on a UV−vis CECIL 7400 spectrophotometer (Aquarius, 7000 series) by scanning an aqueous protein solution (2 mg/mL).


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