Study on the valorization of rapeseed press-cake with special emphasis on meal protein

ABSTRACT

The thesis includes a methodical study to remove the anti-nutritional factors from rapeseed meal, and their subsequent effect on physicochemical and functional properties of protein in the treated meal. Optimal condition for protein extraction from treated meal was derived by Response Surface Methodology and the recuperated protein isolate was chemically treated with maleic anhydride for improving its functional properties. The fibrous meal residue left after protein extraction, was then treated by hydrothermal carbonization for the formation of carbon nanoparticles (≈10 nm), the applicability of which was assessed in making rapeseed protein-based nanocomposite film.

The thesis is divided into six chapters which are briefly discussed below:

Chapter 1 presents the general introduction about rapeseed press-cake and its under-utilized role as an important industrial waste. Different techno-functional properties of the meal and its protein have been detailed. It reviews the important works or techniques proposed for removing the anti-nutritional components from rapeseed meal. It also appraises the chemical modifications performed on rapeseed protein and their useful outcomes in terms of functional features. A brief note on processing and usability of rapeseed protein film has also been added. It highlights the gap of study and background behind the selection and planning of current investigation. The scope and objectives of the thesis are included at the end.

Chapter 2 deals with the testing of different solvent mixtures containing acetone or methanol pure or combined with water or an acid (hydrochloric, acetic, perchloric, trichloroacetic, phosphoric) for their efficiency in extraction of the antinutritive compounds from rapeseed press-cake. Acidified extraction mixtures (non-aqueous) were found to be superior to the non-acidified ones (p<0.05). The characteristic differences in the efficacy of these wide varieties of solvents were studied by principal components analysis, based on which the mixture of 0.2% perchloric acid in methanol-acetone (1:1 v/v) was deemed as ‘the best’ for detoxification of rapeseed meal. Despite its high reductive (antioxidant) potential, hemolytic activity of the extract from this
solvent mixture clearly indicated the toxicity of the above-mentioned compounds on mammalian erythrocytes.

In Chapter 3, effect of anti-nutrients removal from rapeseed meal with organic solvent mixture (methanol-acetone, 1:1 v/v, combined with an acid (hydrochloric, acetic, perchloric, trichloroacetic, phosphoric)) on the physico-chemical and functional properties of rapeseed protein isolate (RPI) was investigated. The extraction resulted in substantial reduction of anti-nutrients from RPI, especially polyphenols and phytates, with concomitant decrease in protein yield and solubility. Treatment harbored significant improvement in their Degree of Whiteness, which was highest in the perchloric acid case. Surface hydrophobicity and free sulphydryl group of RPI changed considerably, with perchloric acid-treated samples showing higher values; while the disulfide content remarkably increased in trichloroacetic 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 concentration of acids. No appreciable alteration appeared among the electrophoretic profile of proteins from pristine meal and those treated with lower level 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 close relation between the physicochemical and functional properties of rapeseed protein isolate.

In Chapter 4, optimization using Response Surface Methodology (RSM), for producing high yield of protein, having superior whiteness and emulsion properties, and reduced level of residual phytate content, has been presented.

In Chapter 5, influence of maleylation on the physicochemical and functional properties of rapeseed protein isolate was studied. Acylation increased whiteness value and dissociation of proteins, but reduced free sulphydryl and disulfide content (p<0.05). Intrinsic fluorescence emission and FTIR spectra revealed distinct perturbations in maleylated proteins’ tertiary and secondary conformations. Increase in surface hydrophobicity, foaming capacity, emulsion stability, protein surface load at oil-water interface and decrease in surface tension at air-water interface, occurred till moderate level of modification. While maleylation impaired foam stability, protein solubility and emulsion capacity were markedly ameliorated (p<0.05), which
are concomitant with decreased droplet size distribution \(d_{32}\). \textit{In-vitro} digestibility and cytotoxicity tests suggested no severe ill-effects of modified proteins, especially up to low degrees of maleylation. The study shows good potential of maleylated proteins as functional food ingredient.

\textbf{Chapter 6} describes the hydrothermal carbonization of oil-and-protein spent rapeseed meal to form antioxidative, hemocompatible, fluorescent carbonaceous nanoparticles (FCD). In the present investigation, an interesting application of FCD in fabricating low-cost rapeseed protein-based fluorescent film, with improved antioxidant potential (17.5-19.3 fold) and thermal stability has been demonstrated. The nano-composite film could also be used as forgery-proof packaging due to its photoluminescence property. For assessing the feasibility of antioxidative FCD in real food system, a comparative investigation was further undertaken to examine the effect of such nano-carbon loaded composite film on the oxidative shelf-life of rapeseed oil. Oil samples packed in nano-composite film sachets showed significant delay in oxidative rancidity compared to that packed in pristine protein-film sachet (free fatty acids, peroxide value and thiobarbituric acid-reactive substances reduced upto 1.4, 2 and 1.2 fold, respectively). The work presents a new concept of bio-based fluorescent packaging and avenues for harnessing this potent waste.

\textbf{Chapter 7} presents the salient findings and future scope of the current investigation. It concludes that the major anti-nutritive compounds of rapeseed press-cake can be reduced substantially using acidified organic solvents (0.2% perchloric acid in methanol:acetone (1:1 v/v)). Maleylation can be a key to improve the functional properties of rapeseed protein. These treatments in-turn result in amelioration of colour and surface activities of the protein, together with marked changes in its secondary and tertiary conformations. Oil-and-protein spent meal has unequivocally been shown as a raw material for synthesis of beneficial CNP. Further studies should be undertaken to analyze their effect on organoleptic properties, gastro-intestinal tract, and toxicological assessment using \textit{in-vitro} and \textit{in-vivo} models.