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A comparative study between the electro-activation technique and conventional extraction method on the extractability, composition and physicochemical properties of canola protein concentrates and isolates



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ABSTRACT

A novel technology of electro-activation was used for protein extraction from canola meal. An alkaline solution was generated in the cathodic compartment under the influence of electric field. It has been reported to have improved extractive properties when compared to chemically alkalinized solutions. The study aims to verify the efficiency of electro-activated solutions for protein extraction from canola oil cake by analyzing the effect of extraction method on the extractability rates, composition, and secondary structure of extracted proteins.

The tested parameters included NaCl concentration (0.01–1 M), duration of electro-activation (10–60 min), and current intensity (0.2, 0.3 A). The electro-activation was performed in a three-compartment cell separated by ion exchange membranes, after which the obtained solutions were used for 1-h extraction. Maximal protein extractability was $34.32 \pm 1.21\%$ obtained with the electro-activated solution generated under 0.3 A irrespective of the activation time. The conventional extraction under the same conditions (pH 7–10) yielded $31.18 \pm 1.89\%$ of proteins. Electrophoretic profiles of electro-activated protein concentrates and isolates analyzed by SDS-PAGE were clearly more distinguishable compared to those obtained by conventional method. FTIR study revealed considerable difference in proteins' secondary structures between different treatment conditions (pH and salt concentration) as well as between conventional and electro-activated samples, showing less denatured spectra for the latter.

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1. Introduction

The interest towards new protein sources has grown dramatically in the past few years. Increasing malnutrition in developing countries, high cost of proteins from animal sources, health concerns such as intolerance to animal proteins and a conscious choice of many to refrain consuming animal proteins has led to a substantial search for alternative sources of proteins which could replace conventional ones. Alternative sources have been thoroughly studied in recent years with proteins derived from plants, bacteria and yeasts being the most promising ones. Among them oilseeds are interesting options as the protein rich oil cake left after oil extraction is a byproduct which can be valorized.

Canola has become an important agricultural crop in Canada and around the world. It was developed in Canada primarily as a source of edible oil. Canola is also used for the production of biodiesel and its byproduct, the protein rich canola oilcake also finds use as forage for livestock. Other possible applications of canola meal proteins include adhesives, plastics, and biocomposites (Gillberg & Tornell, 1976). The protein content of an oilcake left after oil extraction accounts for 20–50% on a dry weight basis, similar to soybean which is extensively used in food industry (Tan, Mailer, Blanchard, & Agboola, 2011a). However, at present its utilization is limited to the production of animal feed. Numerous studies done on canola proteins' physicochemical, functional and bioactive properties indicate potential for it to be used in the food industry (Aider & Barbana, 2011; Fleddermann et al., 2012; Ghodsvali, Khodaparast, Vosoughi, & Diosady, 2005; Khatlab & Arntfield, 2009; Moure, Sineiro, Domínguez, & Parajó, 2006; Rodrigues, Coelho, & Carvalho, 2012; Wanasundara, 2011; Yoshie-Stark, Wada, Schott, & Wäsche, 2006; Yoshie-Stark, Wada, & Wäsche, 2008). Currently the most common extraction technique is a direct alkaline extraction method which comprises protein solubilization at a highly alkaline pH ≥ 10 with subsequent precipitation either at its isoelectric point or by the use of membrane technologies. Processing in a highly alkaline medium allows to extract up to 60% (Ghodsvali et al., 2005) or even up to 80% of total proteins (Gillberg & Tornell, 1976; Pedroche et al., 2004) depending on the canola variety but at the same time causes undesirable modifications such as protein denaturation, reduction of digestibility and loss of essential amino acids (Rodrigues et al., 2012; Sari, Bruins, & Sanders, 2013). Furthermore, the presence of antinutritive factors is another limiting factor. However, the amount of antinutritive factors can be significantly reduced by the use of ultrafiltration and diafiltration (Ali, Mondor, Ippersiel, & Lamarche, 2011; Xu & Diosady, 2002).

In order to increase the protein yield and improve the qualitative characteristics of the protein extracted from different vegetable sources, the effect of various conditions and reagents such as salt (Eromosele, Arogundade, Eromosele, & Ademuyiwa, 2008; Karaca, Low, & Nickerson, 2011), temperature (Gillberg & Tornell, 1976), time of extraction, and meal to solvent ratio (Nioi, Kapel, Rondags, & Marc, 2012) has been studied. Alternative methods to direct alkaline extraction has also been investigated such as protein micellar mass (Ismond & Welsh, 1992), the use of enzymes (Sari et al., 2013), and Osborne

scheme (Manamperi, Chang, Wiesenborn, & Pryor, 2012; Tan et al., 2011a; Tan, Mailer, Blanchard, and Agboola, 2011b).

One of the most promising methods for the protein extraction is the use of direct electric current. Water subjected to an electric field known as electro activated solution (EAS) was claimed to possess improved extracting, cleaning and disinfecting properties (Aider, Gnatko, Benali, Plutakhin, & Kastyuchik, 2012b). The energy supplied by electricity transforms water to a metastable state, characterized by abnormal physicochemical properties (Leonov, Prilytskiy, & Bakhir, 1999; Plutakhin, Aider, Koshchaeve, & Gnatko, 2013). Electro-activation (EA) may be explained by the phenomenon of water electrolysis and oxido-reduction reactions which take place on the electrodes under the influence of electric field. This leads to drastic changes in physicochemical properties in the near electrode layer, resulting in the formation of an acid solution in the anodic side and an alkaline solution in the cathodic side. The products of oxido-reduction reactions responsible for physicochemical activity of the solutions have been identified: (1) stable products of electro-chemical reactions responsible for pH changes; (2) non-stable over reactive substances such as OH^- , H_3O_2^- , H_2 , HO_2^\bullet , HO_2^- , O_2^- ; (3) other products formed near the electrode surface in the form of free structural complexes or hydrated shells of ions, molecules, radicals and atoms (Sprinchan, Bologa, Stepurina, & Polikarpov, 2011). The simultaneous formation of aqua complexes which can also increase the reactivity of the medium has also been documented (Leonov et al., 1999). The abnormal properties of EAS were studied by analyzing the Raman spectra and fluorescence and comparing them with that of chemically alkalinized water (Belovolova, Glushkov, Vinogradova, 2006; Pastukhov and Morozov, 2000). The authors concluded that the metastable state of the near-electrode solutions was the cause of EAS activity (Aider, Gnatko, Benali, Plutakhin, & Kastyuchik, 2012a). It was also reported that the physicochemical properties of EAS could be manipulated by using various configurations of the reactor, membranes, and the time of treatment (Liato, Labrie, Benali, & Aider, 2015). The EAS as mentioned before in addition to their acid and alkaline properties contain other substances which increase their reactivity (Prilutskii & Bakhir, 1997; Tomilov, 2002). These substances are very unstable, yet they might contribute to the extraction processes. In the food industry electro-activation EA has been used for protein extraction from sunflower seed; protein solution was pumped through the cathodic chamber to extract and subsequently through the anodic chamber to precipitate the extracted proteins (Plutakhin, 2005). Comparing the yield, the authors found that chemical extraction at (10.2%), was not significantly higher than electro-chemical extraction (8.9%) showing the sufficiently high efficiency of extraction of sunflower proteins by means of EA.

The aim of the current study was to use the catholyte from cathodic compartment for protein extraction from canola oilcake and to compare them with conventional extraction in terms of protein and total dry matter extractability, subunit composition and secondary structure of extracted proteins, amount of total phosphorus (as an estimate of phytic acid content), and the amount of free amino-acids. In order to find the optimal conditions the effect of the type of configuration, salt concentration, time of treatment, and current intensity was studied.

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