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Study of the functional properties of canola protein concentrates and isolates extracted by electro-activated solutions as non-invasive extraction method

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ABSTRACT

This study compared the functional properties of canola protein isolates and concentrates produced by electro-activated solutions and conventional alkaline extraction. In the case of the isolates, the results showed no significant difference in terms of protein solubility, surface hydrophobicity, water absorption and fat absorption capacity. However, for the protein concentrates, higher fat absorption capacity was observed for the proteins extracted by electro-activated solutions. Moreover, some surface active properties were also enhanced, notably higher emulsion activity index and smaller droplet size were observed for the proteins extracted by the electro-activation method. In all cases, the functional properties were strongly dependent on the pH of the medium and the behavior of the isolates and concentrates was quite different in the pH range close to isoelectric point, in neutral and alkaline regions. For all the functionalities, generally the results showed that the concentrates were more effective at pH 4, whereas isolates performed better at pH 7 and pH 9.

1. Introduction

Proteins in the form of isolates or concentrates are important ingredients in many food products, where they perform specific functions (Kinsella & Melachouris, 1976). The quality of a food protein is established by its nutritional and functional properties (Schwenke, 2001). Nutritional properties include nutritive value, which is the amount of essential amino acids, their bioavailability, or in other words "properties affecting the body after passage of food into the alimentary canal" (Akiva, 1981). Functional properties could be defined as "physical and chemical properties which affect the behavior of proteins in food systems during processing, storage, preparation and consumption" (Kinsella & Melachouris, 1976) or "properties influencing foods prior to entering the body" (Akiva, 1981). This term has been widely used in close relationship to the industrial utilization of proteins in various food components. The range of functional properties is very wide and shows whether the protein can be incorporated in a food matrix so as to impart some specific property; e.g. organoleptic (color, flavor, mouthfeel), textural (viscosity, adhesion), rheological (gelation, dough formation, elasticity), surface active (emulsification, foaming), etc. (Kinsella & Melachouris, 1976). These properties may be more important than nutritional properties when the protein is applied not as a main component but as an ingredient in a complex food system (Schwenke, 2001). Nutritive value is of little importance if the protein or the product in which it is incorporated is not acceptable for eating due to inappropriate texture, mouthfeel, appearance, or flavor (Kinsella & Melachouris, 1976). Functional properties of a protein are governed by the qualitative/quantitative content and the sequence of amino acids, building long polypeptide chains of different conformations. Molecular size, shape, and net surface charge are no less important. Yet not only the intrinsic protein properties but also their interactions with other food ingredients will determine their further utilization. The majority of food systems are multicomponent and their functionality is the result of numerous interactions between the different components. Thus, emulsions include the interplay between proteins, water, and lipids, whereas foams are formed from water,

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protein and air. In addition, the environmental conditions such as temperature, pH and ionic strength play a significant role (Kinsella, 1981).

Among new and developing sources for food applications, proteins from oilseeds deserve particular consideration. Although canola is mostly regarded as a source of healthy oil, it has also been reported to be rich in balanced proteins, which could be found in the oil cake left after oil extraction (Khattab & Arntfield, 2009; Ohlson & Anjou, 1979). Many studies were devoted to various types of protein extraction and characterization and the results were quite inspiring (Ghodsvali, Khodaparast, Vosoughi, & Diosady, 2005; Tzeng, Diosady, & Rubin, 1988). Its nutritive value is similar to soybean proteins which are extensively used in food processing (Bell, 1993; Delisle et al., 1984). Amino acid composition is balanced and highly suitable for use in products for 10–12-year-olds and adults (Klockeman, Toledo, & Sims, 1997; Pedroche et al., 2004; Tan, Mailer, Blanchard, & Agboola, 2011). Apart from having high biological value, canola proteins were found to possess highly interesting functional properties which imparts their further utilization in the industry so as to potentially substitute animal proteins (Aluko & McIntosh, 2001; Ghodsvali et al., 2005).

The type of protein isolation technique directly influences its quality and composition as well as functional properties by acting directly on their conformation and on the types of proteins being extracted. A conventional direct alkaline extraction (DIR) which comprises extraction with highly alkaline medium (pH > 10) using diluted NaOH solutions or deionized water with pH adjustment with precipitation at the isoelectric point has been reported to have adverse effects on proteins. Harsh conditions used during extraction cause a set of undesirable reactions such as protein denaturation, dissociation and amino acid racemization (Moure, Sineiro, Domínguez, & Parajó, 2006; Pedroche et al., 2004). In addition, the use of chemicals generates large amounts of pollutants which need further waste management and which negatively affect the environment. Thus, a milder extraction method is required, which could solubilize proteins without damaging their native conformation, maintain their activity and at the same time would give high percentage of extracted proteins. In our previous work (Gerzhova, Mondor, Benali, & Aider, 2015b), a novel technology that uses Electro-activated solutions (EAS) was used for protein and total dry matter extraction from canola meal. This method uses an electric field to produce alkaline solutions on the basis of water electrolysis and which have been claimed to possess good extractive properties. To assess the quality of proteins extracted by EAS and DIR, the analysis of secondary structure and physicochemical properties were performed and revealed certain differences. Changes in protein secondary structure may cause changes in the hydrophobicity or hydrophilicity, and structural stability of protein which are in close connection with the functional properties such as water holding capacity, surface hydrophobicity and emulsifying activity.

Considering these factors, data on the functional properties would be useful in terms of verifying the possibility of their incorporation in food matrix (Zhu et al., 2010). The aim of this study was therefore to evaluate and compare the functional properties of protein isolates and concentrates extracted by conventional technique and by means of alkaline electro-activated solutions generated in an electro-activation reactor without any alkali addition.

2. Materials and methods

2.1. Raw materials and extraction methods

Protein isolates and concentrates were produced from defatted canola meal (kindly provided by Bunge ETGO, Becancour, Québec, Canada). NaCl, HCl, NaOH were purchased from Laboratoires MAT Inc (Montreal, Canada). Two extraction methods were used: the conventional alkaline extraction at pH 10 in 0.01 M NaCl and the extraction by EAS (reactor's configuration I, 0.01 M NaCl solution in the cathodic compartment, current intensity 0.3 A, electro-activation time 60 min) as described in our previous study (Gerzhova et al., 2015b). For both methods, the extraction was conducted during 60 min. The protein concentrates were obtained by freeze drying of the supernatant straight after extraction and centrifugation at 10,000 × *g*, while the protein isolates were obtained by precipitation of the proteins from the supernatant with 1 M HCl followed by freeze drying. Protein isolates and concentrates extracted by EAS will be referred to as “electro-activated protein isolate“ (EAPI) and “electro-activated protein concentrate“ (EAPC) accordingly, and those extracted by the conventional alkaline extraction as conventional protein isolate (CPI) and conventional protein concentrate (CPC).

2.2. Nitrogen solubility index

Protein solubility was analyzed according to the method of Jarpa-Parra et al. (2014). Concentrates and isolates were dissolved in distilled water to obtain a 0.5% protein concentration and the pH of the suspension was adjusted to 2–10 by using 0.1 M HCl or NaOH solutions. Afterwards they were stirred for 1 h and centrifuged during 10 min at 10,000 × *g* at 23 °C with the help of Eppendorf centrifuge 5804R (Eppendorf AG, Hamburg, Germany). After that, the supernatant was carefully decanted and the protein content was analyzed by the bicinchoninic acid (BCA) protein assay (Fisher Scientific, Waltham, MA, USA) with bovine serum albumin (BSA) as the protein standard. All the analyses were performed in triplicate. Protein solubility was calculated as nitrogen solubility index (NSI) as follows:

$$NSI\,(\%) = \frac{\text{Supernatant protein concentration}\left(\frac{\text{mg}}{\text{mL}}\right) * V\,(\text{sample})}{\text{Sample weight}\,(\text{mg}) * \frac{\text{Sample protein concentration}(\%)}{100}} * 100 \quad (1)$$

2.3. Water absorption capacity (WAC)

Water absorption capacity of the canola protein concentrates and isolates was measured according to Ghodsvali et al. (2005). Sample of 1 g of each material was carefully mixed with 10 ml deionized water in 15 ml centrifuge tubes with the help of a glass rode during 30 s. This was done every 10 min, and after 30 min the tubes were centrifuged at 2000 × *g* in the Eppendorf centrifuge 5804 R for 15 min at ambient temperature (21 ± 1 °C). The supernatant was decanted and the tube was inverted and drained for 15 min before being weighed. The WAC was expressed as the amount of water absorbed per gram of sample.

2.4. Fat absorption capacity (FAC)

Fat absorption capacity was measured as described by Pedroche et al. (2004). Sample of 0.5 g of each material was mixed with 6 ml of canola oil during 30 s with a glass rode every 5 min and after 30 min, the tube was centrifuged in the Eppendorf centrifuge 5804 R at 1600 × *g* for 25 min at 23 °C. Afterwards, the free oil was decanted and the FAC was expressed as the amount of absorbed oil per gram of sample by weight difference.

2.5. Surface characteristics

Dispersions of 0.2% (w/w) protein content of isolates and concentrates were prepared by solubilizing them in deionized water at room temperature (21 ± 1 °C) for 1 h at pH 4, 7, 9 and left