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Influence of processing on composition and antinutritional factors of chickpea protein concentrates produced by isoelectric precipitation and ultrafiltration

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ABSTRACT

The effect of chickpea processing (i.e. defatting, isoelectric precipitation vs ultrafiltration/diafiltration) on the composition, protein recovery and antinutritional factors of protein concentrates was studied for two varieties (Mylese and Xena). Defatting did not affect significantly the content of antinutritional factors in the flours. However, production of concentrates from defatted flours by isoelectric precipitation resulted in higher phosphorous and phenolic contents compared to the concentrates produced by the same process using the full fat flours as starting material, while trypsin inhibitor content was not affected. When processed by ultrafiltration/diafiltration, protein concentrates produced from defatted flour showed a slightly lower trypsin inhibitor content than the ones produced from full fat flours in most cases, while the inverse was true for the phosphorous content, and for the phenolic content; this effect was a function of chickpea variety. Overall, UF pH 9/DF pH 6 resulted in concentrates with the lowest phosphorous content, while isoelectric precipitation and UF pH 9/DF pH 9 resulted in concentrates with lower phenolic content compared to the ones produced by UF pH 9/DF pH 6; for both processes the trypsin inhibitor content of the concentrates remained high.

Industrial relevance: Chickpea production is one of the major agricultural sectors of significant importance to Canada. Although chickpeas are grown in Canada for export, very little is exported in the value-added or processed form. Development of new extraction technologies and value-added products such as the ones presented in this paper is of interest for Canada since it would have a significant impact on the growth of the industry domestically, thus, creating opportunities to strengthen rural development in Canada. Successful implementation of these technologies would make interested Canadian companies viable competitors in the global plant protein production industry and would put Canada in a good position to enjoy a large share of this market both locally and internationally.

More specifically, we are the first research group to compare the use of isoelectric precipitation and ultrafiltration/diafiltration for the production of chickpea protein concentrates from full fat and defatted flours of Kabuli and Desi chickpea variety, and to quantify the effect of these processes on the composition and on the antinutritional factors (i.e. phytic acid, total phenolics and trypsin inhibitors) of the resulting concentrates. Overall, it was observed that isoelectric precipitation was effective to an extent in producing concentrates with low phosphorous and phenolic contents. UF pH 9/DF pH 9 was also effective to an extent in producing concentrates with low phenolic content, while UF pH 9/DF pH 6 was more efficient in producing concentrates with low phosphorous content. High-quality chickpea protein concentrates with improved nutritional properties and good functional properties could beneficially be combined with other protein sources, such as soy protein, or be used in the formulation of foods, such as meat analogues, dairy, and bakery products.

1. Introduction

Chickpea is an important grain legume which is widely grown in the world. A valuable part of the chickpea is the protein fraction (15–30%) (Chavan, Kadam, & Salunkhe, 1986; Fernandez & Berry, 1988), which can be extracted and further purified into a protein concentrate (at least 70% protein on a dry weight basis). In addition to being an important source of protein, chickpeas are also reported to be a good source of minerals (Nestares, Urbano, Lopez-Frias, & Barrionuevo, 1997). Although chickpeas are considered to be one of the most nutritious pulses, they contain several antinutritional factors such as phytic acid, polyphenols, and trypsin inhibitors that could limit their consumption and the nutritive utilization of their proteins (Chavan, Kadam, & Salunkhe, 1989).

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Phytic acid is the principal storage form of phosphorous in plants. Nestares, Barrionuevo, Urbano and Lopez-Frias (1999) reported that phytic acid occurs in raw chickpeas at a level of 5.9 mg/g. Phytic acid is recognized to reduce the bioavailability of divalent cations, such as Ca^{++} and Zn^{++} (Cheryan, 1980; Selle, Ravindran, Caldwell, & Bryden, 2000), and of proteins in the intestine. Some recent literatures have, however, suggested that phytic acid may have some health benefits such as anti-cancer function (Shamsuddin, 2002) and hypolipidemic action (Lee et al., 2007). However, for soy protein isolates, it was also observed that isolates with lower phytic acid content had higher solubility especially for the pH range of 2–4 (Cheryan, 1980; Selle et al., 2000; Mondor, Ippersiel, Lamarche, & Boye, 2004), which could make its removal of interest for food formulation. Polyphenols or tannins are also present in chickpeas. One of the major disadvantages of the presence of phenolic compounds is that they bind to proteins through non covalent interactions (electrostatic and hydrophobic interactions, and hydrogen bonding), which reduces their nutritional availability (Hahn, Rooney, & Earp, 1984). A second disadvantage is the browning which arises due to polymerization of low molecular weight phenolic precursors to brown colored high molecular weight condensed polyphenols. Other important antinutritional factors present in chickpeas are trypsin inhibitors, which are capable of binding trypsin, thus inhibiting its activity, and interfering with the digestion of proteins (Birk, 1989). Different techniques have been used to decrease trypsin inhibitor activity (e.g., heating the seeds with steam, boiling the seeds in water, etc.). In the case of soybean, processing using a combination of these techniques decreased values in the seeds from 171 TIU/mg to about 8–32 TIU/mg in the soy protein isolates (Anderson, Rackis, & Tallent, 1979; Liu & Markakis, 1989). It is, therefore, of great importance to develop a purification process for making protein concentrates with low content of phytic acid, polyphenols and trypsin inhibitors in order to improve the nutritional and/or functional properties of the protein concentrates. In terms of target values, the objective would be to simulate the amounts found in commercially available soy protein isolate that is to say a phytic acid content $\leq$15 mg/g of sample (Maga, 1982) and a trypsin inhibitor content $\leq$32 TIU/mg of sample (Liu & Markakis, 1989). High-quality chickpea protein concentrates could beneficially be combined with other protein sources, such as soy protein, or be used in the formulation of foods, such as meat analogues, dairy, and bakery products.

Isoelectric precipitation (IEP) is the most widely used procedure to prepare legume concentrates (Swanson, 1990; Paredes-Lopez, Ordorica-Falomir, & Olivares-Vazquez, 1991; Fuhrmeister & Meuser, 2003; Makri, Papalamprou, & Doxastakis, 2005). After alkaline (pH 8–10) solubilization of proteins and removal of insoluble matter by centrifugation, proteins are precipitated by adding acid until the isoelectric point (~pH 4.5) is reached. The curd is recuperated by centrifugation, washed to remove non-protein soluble matter, neutralized (pH 7) and spray dried to obtain a protein concentrate. In this process, the pH is often adjusted with a food grade acid such as sulphuric acid, phosphoric acid or hydrochloric acid. Another process for recovering the proteins from the alkaline extract involves the use of ultrafiltration (UF) membrane technologies (Mondor et al., 2004; Fuhrmeister & Meuser, 2003; Makri et al., 2005). The chief advantages of UF are its mild operating conditions and relatively high selectivity. It was demonstrated that by proper selection of membrane characteristics and operating parameters, combining UF and diafiltration (DF) steps can be an effective method for removing oligosaccharides which are relatively small in molecular size compared to the proteins. In addition, when membranes with a molecular weight cut off of 50 kDa are used, the most favorable combination of permeate flux and protein yield in economic terms were achieved (Fuhrmeister & Meuser, 2003).

Most studies have focused on the effect of soaking and germination on the nutritional quality of legumes, however information on the effect of processes such as defatting, alkaline extraction, IEP and UF/DF on the nutritional quality of protein extracted from legumes is scarce. The objective of this work, therefore, was to evaluate the effect of processing (full fat versus defatted; IEP versus UF/DF) on the content of protein, on the yield of protein recovery, and on selected antinutritional factors of chickpea protein concentrates (i.e. phytic acid, phenolics and trypsin inhibitors) produced from two chickpea varieties (Mylese and Xena).

2. Material and Methods

The two varieties of certified pulses under study were mature seeds of Desi chickpea (Mylese desi chickpea) and Kabuli chickpea (Xena kabuli chickpea) grown in Canada. They were purchased from R. Young Seeds, Inc. (Mortlach, Sk., Canada).

2.1. Preparation of chickpea full fat and defatted flour

Whole chickpea seeds were frozen in liquid nitrogen and ground using a Brinkmann centrifugal mill (Brinkmann Instruments Canada, Mississauga, Ont., Canada) equipped with a 5-mesh sieve with 1.5 mm pore size. To obtain the defatted flours, an additional defatting step using hexane was carried out as follows: the full fat flours were suspended in hexane (ratio 1:4 w/v) with continuous stirring for 2 h at room temperature, and the mixtures were allowed to decant overnight at 4 °C, after which the hexane layer was siphoned off. The defatted flours were then recuperated and dried under a vacuum in an oven at 35–40 °C for two days, and then stored at 4 °C until used.

2.2. Preparation of chickpea protein extracts and isoelectric concentrates

Preliminary studies were done at different solid/liquid ratios, pHs, extraction times and temperatures. The optimal conditions in terms of yield, productivity and protein nativity were retained. One part by weight of each of the four chickpea flours (i.e. Desi or Kabuli, full fat or defatted) was suspended with vigorous stirring in 9 parts of water. The pH was adjusted to 9.5 with 2N NaOH. After extracting for 60 min at 25 °C, the insoluble material was removed by centrifugation at 9000 *g* using a Beckman J2-21 centrifuge (Beckman Instruments, Inc., Mississauga, Ont., Canada) for 30 min at 4 °C. The insoluble material was resuspended in 4 parts of water and a second extraction was carried out under the same conditions as the first extraction. The two extracts were pooled together and the resulting solution was separated into two batches; one half for IEP and the other half for the UF/DF. Samples labelled as Desi full fat extract, Desi defatted extract, Kabuli full fat extract and Kabuli defatted extract were also kept and analysed for their protein and antinutritional factors content. For the IEP work, the pH of the extracts was adjusted to 4.5 to precipitate the proteins, followed by centrifugation at 9000 *g* for 30 min at 4 °C to recuperate the precipitated proteins, washing and freeze-drying. Samples were labelled as IEP full fat Desi, IEP defatted Desi, IEP full fat Kabuli and IEP defatted Kabuli concentrates, respectively, and stored at 4 °C until analyzed for protein and content of antinutritional factors. For the UF/DF work, the extracts were rapidly frozen before being lyophilized. (As there is no scientific evidence to suggest that freeze drying of extracts would influence the level of antinutritional factors, the samples were lyophilized in order to preserve them for subsequent tests which were impossible to carry out on the same day (i.e., isoelectric precipitation or UF/DF).) Dry extracts were stored at 4 °C until processed by UF/DF. Preparation of chickpea protein extracts for UF/DF and isoelectric concentrates was conducted in triplicate.

2.3. Membrane ultrafiltration of chickpea protein extracts

Tangential flow UF experiments were carried out with a laboratory scale Quixstand module (GE Healthcare, Ste-Anne de Bellevue, QC, Canada) with a capacity of approximately 1 L. The filtration experiments employed a 50 kDa hollow fiber membrane module