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Isolation and structural characterization of the major protein fraction from NorMan flaxseed (*Linum usitatissimum* L.)

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Abstract

Proteins extracted from dehulled and defatted flaxseed (NorMan cultivar) were fractionated by anion exchange chromatography to yield a major fraction with molecular weight of 365,000 Da, as determined by Sephacryl S-300 gel permeation chromatography. Reducing and non-reducing SDS–PAGE revealed three predominant bands (20, 23 and 31 kDa) and two predominant bands (40 and 48 kDa), respectively, as well as several minor bands. Isoelectric focusing separated three components having isoelectric points (p*I*) of 4.7, 5.1, and 5.6, with acidic (p*I* 4.5, 5.9, 6.1) and basic (p*I* 9.6) components being observed under reducing and denaturing conditions. The flaxseed major fraction had high disulfide but low sulfhydryl content, high contents of glutamate (or glutamine) and aspartate (or asparagine), and lower lysine/arginine ratio than soy or canola globulins. FT-Raman spectroscopy indicated high β-sheet content and a strong band near 1065 cm^{-1}, which is typical of intermolecular sheet interactions, supporting the oligomeric nature of the protein.

Keywords: Flaxseed; Protein; Subunits; Structural properties

1. Introduction

Flaxseed, also known as linseed, is enjoying an upsurge in popularity as a result of reports on its benefits to human health and its potential to reduce the risk of certain diseases (Oomah & Mazza, 2000). However, the current market for edible flaxseed, as well as recent research on its role in human health, has focussed on the whole intact flaxseed and its oil component (Oomah, 2001). The defatted meal, which is the residue after extraction of flaxseed oil, is presently being used as livestock feed, and limited attention has been given to the physicochemical and functional properties of the constituent proteins. This information is essential for increasing the utilization of flaxseed proteins, which have the potential to become important value-added products from the edible oil industry, as evidenced by the successful entry of soy proteins into the functional food ingredient market.

Flaxseed proteins were first isolated by Osborne in 1892, who reported the presence of a globulin with 18.6% nitrogen and an albumin-like protein with 17.7% nitrogen (Vassel & Nesbitt, 1945). Thereafter, research on flaxseed proteins has been concerned primarily with methods for the extraction of protein from the oil-crushed meal (Dev, Quensel, & Hansen, 1986a; Madhusudhan & Singh, 1983; Oomah, Mazza, & Cui, 1994; Painter & Nesbitt, 1946; Smith, Johnson, & Beckel, 1946; Sosulski & Bakal, 1969; Vassel & Nesbitt, 1945; Wanasundara & Shahidi, 1996, 1997).

Only a few studies have been conducted on the characteristics and functionality of the protein components fractionated from flaxseed. These studies reported that flaxseed consists of two major storage proteins, a predominant salt-soluble fraction with high molecular weight (11–12S), and a water-soluble basic component with low molecular weight (1.6–2S) (Dev, Sienkiewicz, Quensel, & Hansen, 1986b; Dev & Sienkiewicz, 1987; Madhusudhan & Singh, 1983, 1985a, 1985b; Marcone,

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Kakuda, & Yada, 1998a; Youle & Huang, 1981), and suggest similarity between the properties of the major storage protein of flaxseed and those of other important oilseeds (Madhusudhan & Singh, 1985c, 1985d; Marcone, Kakuda, & Yada, 1998b, 1998c; Oomah & Mazza, 1993). However, most of the properties of the flaxseed major protein fraction are still awaiting investigation (Marcone, 1999; Oomah, 2001).

The main objective of this study was to isolate and characterize the major protein fraction from flaxseed of the NorMan cultivar, with the long-term objective to obtain basic information for understanding the structure–function relationship and potential applications of flaxseed proteins in foods. The molecular characteristics and structural properties that were investigated include the molecular weight of the major protein fraction and its constituent polypeptides, isoelectric point, amino acid composition, content of sulfhydryl groups and disulfide bonds, and molecular structure as analysed by FT-Raman spectroscopy.

2. Materials and methods

2.1. Materials

Flaxseed of the NorMan cultivar was obtained from the Agriculture and Agri-Food (AAFC) Diversification Research Centre, Morden, Manitoba. The seeds were dehulled at the AAFC Pacific Agri-Food Research Centre in Summerland, BC, and ground delipidated powder was prepared as previously described (Li-Chan & Ma, 2002; Li-Chan, Sultanbawa, Losso, Oomah, & Mazza, 2002b).

All chemicals used were of analytical reagent grade, and Tris buffers were prepared from ultra-pure grade Tris buffer salt (ICN Biochemical Inc., Costa Mesa, CA).

2.2. Extraction of flaxseed proteins

Flaxseed proteins were extracted from the dehulled, delipidated powder using 0.10 M NaCl in 0.10 M Tris buffer at pH 8.6, with a 1:16 (w/v) seeds to buffer ratio and magnetic stirring at 4 °C for 16 h (Li-Chan & Ma, 2002). The extract was then passed through a double layer of cheesecloth and centrifuged at 20,400*g* for 30 min at 8–10 °C. The protein extract thus obtained is hereinafter referred to in this study as the "whole extract".

2.3. Isolation of the major fraction of flaxseed proteins

The whole extract was loaded onto a 5×20 cm chromatographic column (Bio-Rad Laboratories, Hercules, CA) packed with 225 ml of DEAE-Sephacel (Amersham Pharmacia Biotech Inc., Quebec) that was pre-equilibrated with 0.10 M NaCl in 0.10 M Tris at pH 8.6 (conductivity of 10–11 mS/cm). The equilibrating buffer was used to wash out the unbound fraction (termed "flow through" fraction in this study). Preliminary experiments indicated that protein compositions were similar for fractions eluted by 0.15, 0.20 and 0.25 M NaCl and therefore, a single step elution of 0.25 M NaCl in 0.10 M Tris at pH 8.6 (conductivity of 19–22 mS/cm) was employed to elute the fraction which comprised 63.7% of the total proteins in flaxseed (termed the "major protein fraction" or simply "major fraction" in this study). Absorbance at 280 nm (Unicam UV2 Spectrophotometer, Analytical Technology Inc, Cambridge, UK) and conductivity (Yellow Springs Instrument Conductivity Bridge YSI Model 31, Yellow Springs, Ohio) were measured to monitor the progress of elution.

The major fraction was lyophilized (LABCONCO® model 75018, Labconco Corporation, Kansas City, MO), reconstituted with distilled–deionized water, and desalted by dialyzing against 5 mM Tris buffer at pH 8.6 using Spectra/Por® Molecularporous dialysis membrane (Spectrum®, Laguna Hills, CA) with molecular weight cut off of 6–8 kDa. The major fraction prepared according to the above-mentioned protocol in six replicate processes were pooled for subsequent analyses, after SDS–PAGE analysis confirmed the similarity of protein composition in the six preparations.

2.4. Analyses

Protein contents of solid samples were determined by the Leco nitrogen combustion method using a LECO FP-428 (LECO Cooperation, Joseph, MI) calibrated with ethylenediaminetetraacetic acid. Protein contents of liquid samples were analysed by the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL) with bovine serum albumin as the protein standard.

The molecular weight (MW) of the major fraction of flaxseed protein was determined by size exclusion chromatography on Sephacryl S-300 (Amersham Pharmacia Biotech Inc., Quebec) according to Marcone et al. (1998a). The pooled 0.25 M fraction was dialysed against phosphate buffer (32.5 mM K_2HPO_4, 2.6 mM KH_2PO_4 and 0.4 M NaCl, pH 7.5); 40 mg protein was loaded to the S-300 gel filtration column which was pre-equilibrated with the same buffer, and eluted at a flowrate of 0.5 ml/min. Blue dextran and sodium azide were used to evaluate the void volume (V_o) and total elution (V_t) volume, respectively, and the elution volumes (V_e) of thyroglobulin, ferritin, catalase, alkaline phosphatase and α-lactalbumin with MW of 669, 440, 232, 140 and 14 kDa, respectively, were used to establish the calibration curve ($K_{av} = -0.3715 \log \mathrm{MW} + 1.3182$, $R^2 = 0.9966$). The available