Effects of Processing on the Functional Properties of Canola/Rapeseed Protein[1]

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Canola rapeseed is a major oilseed in Canada, Europe and Japan. Recently, Generally Recognized As Safe (GRAS) status was granted to low erucic acid rapeseed oil for use in the U.S. market. Commercial oil extraction of the seed results in a meal that contains 44% protein and which has been subjected to considerable heat. The meal is presently utilized as livestock feed supplement. A number of processes for the preparation of protein concentrates and isolates from canola/rapeseeds and meal have been proposed, although none have proven commercially viable. In addition to protein concentration, a successful process must reduce the levels of glucosinolates, phenolics, phytates and fiber. These antinutrients present a barrier to the use of canola/rapeseed protein materials in foods. Processes to produce protein concentrates have included water extraction of undesirable compounds from heat denatured, dehulled seed followed by solvent extraction for oil recovery and the isopropanol washing of dehulled, defatted flours. Isolates have been prepared by traditional alkaline extraction, and by acid or water extractions followed by isoelectric, heat or polyelectrolyte precipitation of the protein. Isolates have been chemically and enzymatically modified to improve food-use properties. In this paper, the effects of various processing methods on the functional properties of solubility, color and flavor of canola protein products are reviewed.

Rapeseed is an important oilseed worldwide, currently ranking fourth (after soybean, palm and sunflower) in terms of oil production (1). Canola is the name adopted by the rapeseed industry in Canada in 1978 to identify rapeseed cultivars which are genetically low in both erucic acid and glucosinolates (2). Canola oil accounts for approximately 57% of the domestic production of deodorized vegetable oils used in Canada (2). In January 1985, the U.S. Food and Drug Administration granted GRAS (Generally Recognized As Safe) status to low erucic acid rapeseed oil and presently it is being marketed in the U.S.

Processing. Canola/rapeseed is processed for oil recovery by flaking the seed to fracture the seed coat and rupture the oil cells. The flakes are cooked to rupture any remaining intact cells, to enhance coalescence of oil droplets by increasing fluidity and to inactivate enzymes, particularly myrosinase, the enzyme which hydrolyzes glucosinolates. The cooking temperature used is 77-100°C, depending on seed variety, for 15-20 min. In most cases, the flaked and cooked seed is screw-pressed to reduce the oil content from 42% to 16-20%; this operation also compresses the tiny flakes into large cake fragments. This cake is solvent-extracted with hexane to remove most of the remaining oil. Flaked, cooked canola seed can also be directly solvent-extracted, omitting the prepress step, but this is not common. The oil extracted seed meal is finally processed in a desolventizer-toaster with steam sparging at 100-130°C for 30 min to remove hexane and to improve the nutritional quality of the meal by removing volatile glucosinolates (2-4). It can be noted from this description of canola seed processing that protein denaturation can occur during both the initial cooking stage and the desolventizer-toaster stage. The crude protein content of the canola meal is about 40% on a dry basis.

Canola/rapeseed meal is used in animal feeds as a source of high quality protein to replace soybean meal, but in some feeding situations its use is restricted to less than full replacement of soybean meal due to the presence of antinutritional components.

The production of protein concentrates and isolates for food use from canola/rapeseed has been investigated for about 20 years. However, canola/rapeseed protein ingredients are not produced commercially because an economically feasible production process has not been identified. A number of research articles and several patents have described various processes. The seeds and meal contain glucosinolates, phenolics, phytate and fiber that require removal in order to produce food-grade material.

Antinutrients. The hydrolysis of glucosinolates, effected by the myrosinase found in canola/rapeseed, produces toxic products which interfere with the function of the thyroid gland and adversely affect growth (5). Plant breeders have reduced the level of glucosinolates in some cultivars of rapeseed to improve its value as a feed. By Canadian definition, canola meal must contain less than 30 μmoles of glucosinolates per g. Cultivars grown in Canada and Denmark tend to meet this definition, but for most of the remaining European crop, the levels are typically above 70 μmoles per g of meal (6). Glucosinolates also decrease the palatability of rapeseed meal as a feedstuff and are involved with egg taint in strains of poultry laying brown eggs (1). The presence of glucosinolates, even at levels around 20-30 μmoles per g, is considered the greatest stumbling block to the use of canola or rapeseed protein in foods and must be reduced by at least an order of magnitude (7,8).

Rapeseed contains about 10 times the quantity of phenolic compounds found in soybean (9). The main phenolic compound in canola/rapeseed is sinapine, an ester of sinapic acid and choline. Sinapine content of Canadian rapeseed meals is 1.0-2.5% (10). Sinapine has a bitterness intensity similar to caffeine (11), may cause feed palatability problems (12) and can cause a fishy taint in brown-shelled eggs when included in the feed of laying hens (13). Phenolic compounds in rapeseed are also responsible for the green and brown colors of alkali-treated rapeseed products (14).

Phytate is present in canola meal at levels as high

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as 5-7% (15). Its ability to complex cations can lead to a decrease in bioavailability of some essential minerals (16). Canola meal is fairly high in fiber; it contains about 30% hulls by weight (17) and has a crude fiber content of about 14% (18).

Thus, in the development of methods to produce food protein materials from canola and rapeseed, the reduction of these antinutritional components is a goal.

Flours. Oilseed flours are produced by dehulling and defatting the seed. Front-end dehulling of canola/rapeseed is not economical because the hulls contain about 10% of the seed oil (19) and because dehulled seed is difficult to process in a solvent extractor (20). Air-classification of defatted meal into fine and coarse fractions can increase the protein content of the fine fraction by 6% and reduce its crude fiber by 7-10%, but the small improvement is not worth the cost of processing (21). A process for separating a slurry of finely ground defatted meal and hexane by liquid cyclone processing into hulls (cyclone unders) and flour (cyclone overflow) has been patented by Sosulski and Zadernowski (21). This process is claimed to provide a practically hull-free flour. Canola or rapeseed flours are not suitable for human consumption unless the myrosinase has been inactivated and the glucosinolates removed.

Concentrates. In the production of canola/rapeseed protein concentrates, several approaches have been utilized to deal with the various antinutrients. Myrosinase has been inactivated most commonly by heat treatment (22), and also by alcohol (23). Glucosinolates and the hydrolysis products have been removed by aqueous or dilute alkali extraction. Sosulski *et al.* (24) have described a diffusion extraction method for whole rapeseed in which the seed coat acts as a semipermeable membrane, allowing low molecular weight compounds out of the seed while retaining protein. In the 1970's, two similar processes were developed at Agriculture Canada's Food Research Institute (25) and by the Karlshamns Company in cooperation with Alfa-Laval in Sweden (26). The myrosinase in cracked, dehulled (Karlshamns) rapeseed is inactivated with boiling water (FRI) or dry heat (Karlshamns). Glucosinolates are removed by water extraction, then the detoxified cotyledons are dried, solvent extracted and ground to obtain a pale yellow concentrate. In the FRI process, hulls are removed by air-classification at the end of the process. In another process developed at the Food Research Institute, dehulled, defatted seed is ground and detoxified by repetitive washing with isopropanol-water mixtures (27). Researchers at the University of Toronto patented a method in which canola/rapeseed is wet-ground with 10% ammonia in methanol, then extracted with hexane (28). Phase separation allows recovery of the oil-containing hexane miscella, the ammonia-methanol extractant and the nearly glucosinolate-free defatted meal. The myrosinase is inactivated in the process.

Isolates. In this paper, isolates are classified by the use of protein extraction processing rather than as containing 90%+ protein. Extraction and recovery of canola/rapeseed protein isolates has been conducted by various methods on seed, meal, flour and concentrate receiving a variety of pre-processing treatments. Extraction of protein has been accomplished using primarily dilute alkali (18,29), but also using water (30), dilute acid (31), sodium chloride (32-34), and/or sodium hexametaphosphate (35). In some cases, successive extractions have been carried out using more than one extractant (31). Recovery of protein from the extractant solution has most commonly been accomplished via isoelectric precipitation, but also by heat (36), acidic polymers (37) and ultrafiltration (38,39). To remove undesirable compounds, additional steps may be incorporated into the procedure. Treatments of the extracted and recovered protein have included activated carbon to remove glucosinolates (40), acylation or dialysis of protein to reduce phytate (15,41) and glucosinolate contents (39), and alcohol washing of precipitates to remove phenolic compounds (31,35).

Functional properties. Functional properties of canola/rapeseed protein materials measured most commonly include solubility, water absorption and adsorption, emulsifying properties, oil adsorption, foaming characteristics, color and sometimes flavor. The focus of this discussion is on solubility, color and flavor attributes.

Solubility. The solubility of proteins varies with the processing treatments used to produce the protein material and applied to the recovered protein. Commercially processed canola/rapeseed meal has been subjected to two heating steps, each of which depresses protein solubility. Finnigan and Lewis (3) have demonstrated that nitrogen in meal commercially heated to inactivate myrosinase (cooker-prepress) is less soluble than nitrogen in unheated rapeseed meal. Nitrogen in meal which has undergone the entire commercial oil extraction process (including desolventizer-toaster) is less soluble than cooker prepress meal nitrogen. Gillberg and Törnell (42) have similarly noted that rapeseed meal prepared in the laboratory under no heat conditions has a greater quantity of soluble protein than meal from seed heated in a rotating drum at 90°C for 18 min to inactivate myrosinase. Rapeseed proteins have points of minimum solubility around pHs 4 and 8, although the effect is somewhat dampened in commercial meal (3). Low nitrogen solubilities are observed in rapeseed protein concentrates prepared from seed that has been heated to inactivate myrosinase and/or to enhance diffusion of glucosinolates by use of the FRI process, the diffusion extraction process (18), or the Karlshamns process (43). Extractability of nitrogen compounds in rapeseed meal is also a function of temperature. Korolczuk and Rutkowski (44) have noted that at pHs below 7, the temperature of maximum nitrogen extractability of a low-heat meal was around 65°C, but as pH was increased above 7, the temperature of maximum extractability was reduced to around 40°C. Most of the studies of functional properties of canola/rapeseed proteins have been conducted on protein materials prepared in the laboratory or pilot plant from seeds or meals which have received either no heat or low heat treatments.

The nitrogen solubility characteristics of the canola protein (cv. Tower) in a low heat meal, a low heat flour (i.e., a dehulled meal) and an isolate prepared from the flour by alkaline extraction and acid precipitation have been compared (18). These materials showed similar

nitrogen solubilities at pHs 2 and 4, but at pH 6–8, the nitrogen in the flour was more soluble, probably due to the removal of the less soluble hull nitrogen components. The isolate was poorly soluble at pH 6–8, possibly due to the presence of high amounts of phytate. The isoelectric precipitation process is probably also important in the low solubility of rapeseed protein isolates (45). Rapeseed protein concentrate recovered after acid extraction of a heated flour was found to be about twice as soluble as the concentrates recovered via water extraction or alkaline extraction (46).

Use of sodium hexametaphosphate (SHMP) to extract proteins in rapeseed flour (low heat) followed by precipitation at pH 2.5 results in a protein concentrate of low solubility at pHs 2 and 4 (47). The low solubility was attributed to interactions between protein and the SHMP. At pHs above 6, the nitrogen solubility of the SHMP concentrate was similar to the flour from which it was prepared. Extraction with 0.25% SHMP produced concentrates that were only two-thirds as soluble as pH 7 as those extracted with 2% SHMP (35). Dev and Mukherjee (48) have prepared rapeseed protein products to contain varying degrees of phytate; they generally found that nitrogen solubility (pH 7.0) increased with decreasing levels of phytic acid in the product. Use of the ammonia-methanol-hexane process to simultaneously extract glucosinolates and oil from canola/rapeseed also lowers the nitrogen solubilty of the meal by over 50% as compared to that of low-heat, hexane-extracted meal (7). The decreased solubility was attributed to protein aggregation resulting when methanol removed the water surrounding the protein molecules. Use of ethanol to precipitate extracted protein (in addition to pH adjustment) or to wash protein precipitates to improve color has been found to decrease nitrogen solubility (35). Heating a dispersion of rapeseed protein isolate (produced by alkaline extraction and acid precipitation) at temperatures of 105°C or higher for 20 min causes a decrease in nitrogen solubility from 24% to 12% (49).

Nakai *et al.* (45) have investigated methods of increasing the dispersibility of protein isolates prepared from commercially processed rapeseed meal by alkaline extraction and acid precipitation. Of the many surfactants tested, only anionic surfactants—such as the sodium and potassium salts of fatty acids—were effective. The effect was attributed to interaction of protein and surfactant hydrophobic groups, thus increasing the negative charge of the protein. Enzyme treatments of rapeseed concentrate (50) and isolate (45), are also effective in increasing dispersibility. Acylation of rapeseed flour and subsequent preparation of protein isolates by pH 8.5 extraction and acid precipitation increases the nitrogen solubility (pH 7) of the isolates considerably as compared to isolate prepared from unmodified flour or compared to unmodified flour itself (41). Increased solubility is often observed in acylated proteins due to increased protein-water interaction as net negative charge is increased.

Color. The color of canola/rapeseed protein products is frequently poor and limits the use of these products in foods. The colors of aqueous slurries of rapeseed meal, flour, concentrate and isolate were described by Sosulski *et al.* (18) as greenish-brown to brown. The color of a slurry of rapeseed protein concentrate prepared by the FRI water extraction process (25) was described as light brown, similar to the color of soy flour.

Blaicher *et al.* (29) have studied methods for improving the color of protein isolates produced by alkaline extraction and acid precipitation processing of a low-heat rapeseed meal. Addition of insoluble polyvinylpyrrolidone to rapeseed meal to remove phenolic substances prior to protein extraction results in lighter colored protein isolates than in its absence. Lower extraction pH also improves color, but decreases protein yield. The use of a reducing agent, 1% sodium sulfite in the extracting solvent, to inhibit the oxidation of phenolic substances was also found effective in improving isolate color, but it decreased protein yield rather drastically. Keshavarz *et al.* (31) also noted that sulfite improves protein isolate color significantly.

Protein isolates prepared from acylated rapeseed flour have been found to be darker than protein isolates prepared from unmodified flour and darker than the starting material flour (41). Ethanol washing of the acylated isolates improved color lightness, but the washed isolates were still darker than the flour starting material or than soy isolate.

Flavor. The flavor of rapeseed protein products has not been measured to a great extent. The flavor of rapeseed protein concentrate produced by the Karlshamns process has been found to be stronger than that of textured soy flour and soy protein concentrate. The flavor of the concentrate was characterized as sulfurous, bitter and a little musty. However, when tested as an extender in meat patties, a consumer panel preferred meat patties with rapeseed protein concentrate to those containing more meat (51). Other research has indicated that in meat systems, rapeseed protein concentrate flavor is better masked than is the flavor of soy products. It was suggested that the flavor attributes of rapeseed protein products are more similar to the familiar flavors of other *Brassica* foods such as mustard and cabbage (52).

The ammonia-methanol process has been reported to produce rapeseed meals that are almost bland in flavor. This was attributed to removal of glucosinolates, polyphenols and some non-protein nitrogen compounds by the process (7).

Other properties. With regard to other functional properties, canola proteins have been reported as better or poorer than soy proteins, depending on the processing treatment, the soy product used for comparison and the methodology employed. The water absorption capacity of canola/rapeseed protein materials, measured as the quantity of water held by a protein pellet after mixing with excess water and centrifugation, has been reported at between about 2 and 4 g water/g protein material (18,48). Water absorption is reported to be increased by enzyme treatment (53), decreased by use of a sodium hexametaphosphate extraction (47), and variably affected by heating (49,54).

Rapeseed protein materials absorb between 2.4 and 4.1 ml of oil per g. Flour absorbs more oil than meal, isolate absorbs more oil than flour or meal, and concentrate absorbs the most oil (18). However, values reported by other authors for meal and isolate have shown

some differences (54,55). Oil absorption by canola protein is increased by methanol-ammonia-hexane extraction of the seed (7) and by acylation of the protein (41). Canola protein materials have been reported to be superior in fat absorption as compared to corresponding soy products (47). Dev and Mukherjee (48) have reported that rapeseed products generally have lower emulsifying capacities, but higher emulsifying stabilities than soy products, although processing treatment can alter this result. Isolates tend to have improved emulsifying properties as compared to concentrates (35,46,47).

Rapeseed proteins are generally reported as having better foaming properties than soy proteins (18,47,48). Solubilizing treatments of canola protein have been found to increase foam volume attained, although stability was poor (56), as does use of a sodium hexametaphosphate extraction to prepare canola concentrate (47). Use of ammonia-methanol-hexane extraction of seed to produce low glucosinolate meal (7) or acylation of protein (41) decreases protein whipping capacity.

Thus, various processing methods have been applied in an endeavor to produce canola flour, concentrates and isolates with acceptable levels of glucosinolates, phenolics, phytate and fiber, and desirable functional properties. Poor color and flavor and the presence of glucosinolates appear to be the biggest obstacles to producing food use materials. When these are solved, canola protein will be processed into useful protein ingredients. However, the extensive processing required to recover acceptable concentrates and isolates has thus far proved uneconomical.

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