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Rapid Communication

Antioxidant activity and water-holding capacity of canola protein hydrolysates

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Abstract

Canola protein hydrolysates were prepared using commercial enzymes, namely Alcalase, an *endo*-peptidase and Flavourzyme with both *endo*- and *exo*-peptidase activities. The hydrolysates so prepared were effective as antioxidants in model systems, mainly by scavenging of free radicals and acting as reducing agents. This effect was concentration-dependent and also influenced by the type of enzyme employed in the process. The hydrolysate prepared using flavourzyme showed the highest antioxidant activity among all samples, whereas the hydrolysates prepared by combination of Alcalase and Flavourzyme did not differ significantly ($P > 0.05$) in antioxidant effectiveness from that produced by Alcalase alone. The hydrolysates were also found to be effective in enhancing water-holding capacity and cooking yield in a meat model system. Their capability in improving the cooking yield of meat was in the order of Flavourzyme hydrolysates > combination hydrolysates > Alcalase hydrolysates. These results suggest that canola protein hydrolysates can be useful in terms of their functionality and as functional food ingredients and that their composition determines their functional properties and thus their potential application in the food and feed industries.
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Keywords: Canola protein hydrolysate; Alcalase; Flavourzyme; Antioxidant activity; Water-holding capacity

1. Introduction

Rapeseed is placed as one of the top five oilseed crops in the world and thus is of great importance to the global agricultural industry. Canola, the Canadian variety of rapeseed, is in its own right a major resource for North American agriculture and it is low in both glucosinolate and erucic acid, thus also known as "double zero" variety. Its seeds and oils are exported to many parts of the world, including the United States, Mexico, Japan, China and Europe. The global demand for canola is mainly for its edible oil. However, once the oil is removed from the seed, a protein-rich meal is left behind. Considering the large amount of byproduct produced by the extraction process, it would be greatly beneficial to the agricultural industry to develop techniques to use this protein source for developing value-added products. In order to do so, efforts in practice may be hampered by many factors, such as the presence of phytates and glucosinolates, as well as the fact that the commercial extraction process denatures the protein and, as a result, decreases its solubility (Vioque, Sánchez-Vioque, Clemente, Pedroche, & Millan, 2000). These factors make it almost impractical to use canola/rapeseed protein in any meaningful way for human food and hence the current use of rapeseed meals is generally restricted to animal feed and fertilizer.

Many investigations have focussed on utilization of canola protein for production of valuable food supplement. Some studies have focussed on removing the undesirable components from the meal, a process that has proven to be complicated (Diosday, Rubin, Philips, &

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Naczk, 1985; Dunford & Temelli, 1996; Shahidi, Gabon, Rubin, & Naczk, 1990; Shahidi, Naczk M., Rubin, & Diosady, 1988; Tzeng, Diosady, & Rubin, 1990). Others have focussed on the solubility issues (Mahjan, Dua, & Bhardwaj, 2002; Radwan & Lu, 1976). Thus, a method was needed to modify the physical and chemical characteristics of the protein meal without altering its overall amino acid composition. Further investigations demonstrated that simple hydrolysis, either by chemical or enzymatic means, was a potential solution to the solubility problem (Adler-Nissen,1986). Hydrolysis of a protein is a simple and inexpensive method to convert a protein into free amino acids and short chain polypeptides. Such products are far more soluble in water than the original protein yet their amino acid composition remains essentially unchanged.

A vast number of studies have been conducted on the preparation of hydrolysates from different protein sources in an effort to better understand their properties. These include capelin protein hydrolysates (Amarowicz & Shahidi, 1997; Shahidi & Amarowicz, 1996; Shahidi, Han, & Synowiechi, 1995), seal protein hydrolysates (Shahidi, Synowiecki, & Balejko, 1994), casein protein hydrolysates (Mahmoud, Malone, & Cordle, 1992), whey protein hydrolysates (Turgeon, Gauthier, & Paquin, 1992) and sunflower seed protein hydrolysates (Conde, Escobar, Jiménez, Rodríguez, & Patino, 2005). In general, the results tend to show that hydrolysis increases solubility of proteins to varying extents, depending on protein composition and the degree of hydrolysis. Moreover, protein hydrolysates produced may possess some physicochemical characteristics and bioactivities not found in the original proteins, such as antioxidant activity and water-holding capacity. For instance, Shahidi et al. (1995) reported that capelin protein, at a level of 0.5–3.0%, inhibited the formation of thiobarbituric acid-reactive substances (TBARS) by 17.7–60.4% in a cooked pork model system. A similar effect was documented for potato protein hydrolysates (Wang & Xiong, 2005). Protein hydrolysates from seal meat were found to improve water-holding capacity in meat products (Shahidi & Synowiecki, 1997). In addition to antioxidant activity and water-holding capacity, angiotensin I converting enzyme (ACE) inhibitory activity was also reported for bovine skin gelatin hydrolysate (Kim, Byun, Park, & Shahidi, 2001).

Hydrolysis has also been proposed for better use of the rapeseed meal and studies have been carried out to evaluate the hydrolysis products. Vioque et al. (1999) and Vioque et al. (2000) have investigated rapeseed protein hydrolysates produced under different conditions. In this study, the antioxidant activity of canola protein hydrolysates prepared by two different proteases, namely, Alcalase, an *endo*-peptidase and Flavourzyme, a mixture of *endo*-peptidase and *exo*-peptidase, was evaluated. The water-holding capacity in a meat model system, as affected by each hydrolysate, was also examined.

2. Materials and methods

2.1. Materials

Seeds of Cyclone canola used in this study were grown in several locations in western Canada and were procured from Limagrain Canada Seeds Inc., Saskatoon, SK. The seeds were bulked, sub-sampled and then stored at −18 °C until used. Alcalase and Flavourzyme were purchased from Novozymes, Bagsvaerd, Denmark. All chemicals used were obtained from Fisher Scientific Ltd. (Ottawa, ON, Canada) or Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). The solvents used were of ACS grade, pesticide grade or HPLC grade and were used without any further purification.

2.2. Preparation of canola protein hydrolysates

Whole canola seeds were ground and defatted with hexane, following a small-scale hexane extraction method described by Tzeng et al. (1990). The defatted canola meal samples were vacuum-packed and stored at −20 °C prior to hydrolysis. The crude protein content in the meal was determined by Kjeldahl analysis according to the AOAC (1990) method in order to calculate the amount of sample required for the hydrolysis process, based on enzyme/protein ratio. The meal samples were divided into three groups (each containing 29 g of canola meal and 200 ml of water) and hydrolyzed under pre-selected conditions according to Vioque et al. (1999) with some modifications. Sample 1 was hydrolyzed at 50 °C and pH 8 for 1 h using Alcalase (1.05 ml) and sample 2 at 50 °C and pH 7 for 2 h using Flavourzyme (0.40 ml). Finally, sample 3 was produced, using a combination of the two enzymes, i.e. hydrolyzing first with Alcalase for 1 h, followed by Flavourzyme for an additional 2 h (Table 1). Conditions were constantly monitored and maintained throughout the process. The pH value, which changes as a result of hydrolysis, was kept constant by the addition of a known amount of 4N NaOH. Upon completion of the hydrolysis, the enzymes were deactivated by dropping the pH to 5. The reaction mixtures were then filtered and the hydrolysates collected. The protein hydrolysates obtained were freeze-dried and stored at −20 °C for subsequent analysis.

2.3. DPPH radical-scavenging assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity of enzymatically prepared canola protein hydrolysates was determined, following the procedure described by Shahidi, Liyana-Pathirana and Wall (2006) with minor modifications. Freeze-dried hydrolysate samples were dissolved in 95% ethanol at a series of concentrations (1.25, 2.5, 5 and 10 mg/ml). An aliquot (0.1 ml) of the sample solution was mixed with 1.9 ml of ethanolic DPPH$^{\cdot}$ solution (50 μM) and the mixtures were allowed to stand at room temperature for 30 min. The absorbance was then

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