Canola protein hydrolyzates

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Abstract

Canola protein hydrolyzates were prepared using commercial enzymes, namely Alcalase and Flavrouzyme. While Alcalase is an endopeptidase, Flavourzyme acts as both endo- and exopeptidase. The hydrolyzate production was carried out under pre-selected conditions to reach a desired degree of hydrolysis (DH). The hydrolyzates so prepared were effective as antioxidants in inhibiting oxidation in model systems, mainly by scavenging of free radicals, presumably via participation of hydroxyl groups of aromatic amino acids as hydrogen donors. This effect was concentration-dependent and was also influenced by the type of enzyme employed in the process. The hydrolyzates were found effective in enhancing water-holding capacity and cooking yield in a meat model system. Canola hydrolyzates are therefore useful in terms of their functionality and as functional food ingredients.

Key words: Canola, protein hydrolyzates, Alcalase, Flavourzyme, antioxidant, water-holding capacity

Introduction

Canola is a trademarked cultivar of the rapeseed family, one of the top five oilseed crops grown around the world. It has a low content of both glucosinolates and erucic acid. Canola plant is one of the major crops in the agricultural industry of North America, especially in Canada. Considerable quantities of canola are cultivated in Canada and the seed and oil are exported to different parts of the world, including the United States, Mexico, Japan, China and Europe. Canola oil, as a widely consumed edible oil, is produced from canola seeds. The oil is extracted from seeds in large scale leaving behind a bulk of protein-rich meal as the byproduct. Utilization of canola protein following oil extraction has been a subject of continued interest.

The defatted canola meal is composed mainly of protein and is considered a potential source of protein for animal feed and fertilizer as well as for human consumption. However, the insolubility of both crude canola meal after oil extraction and its protein isolates has always been a challenge for their application in the food and animal feed industries. Investigations have been carried out in order to modify the physical and chemical characteristics of canola proteins without altering their biological and functional properties. Hydrolyzing the peptide bonds in the proteins by chemical or enzymatic means yields free amino acids and short-chain polypeptides and has been found to be an efficient and cost effective way to enhance the solubility and thus improving the utilization of proteins from canola meal. Protein hydrolyzates as a value-added product from different sources have been studied. These include capelin protein hydrolyzates (Shahidi *et al.,* 1995), seal protein hydrolyzates (Shahidi *et al.,* 1994), casein protein hydrolyzates (Mahmoud *et al.,* 1992), whey protein hydrolyzates (Turgeon *et al.,* 1992), rice bran protein hydrolyzates (Hamada, 2000) and sunflower seed protein hydrolyzates (Conde *et al.,* 2005), among others. The process of hydrolysis is able to increase the solubility of proteins to various extents, depending on the protein composition and the degree of hydrolysis. Moreover, the protein hydrolyzates produced may possess some physicochemical characteristics and bioactivities that are not found in the original proteins, such as water-holding capacity and antioxidant activity. Shahidi *et al.* (1995) reported that capelin protein hydrolyzates at a level of 0.5-3.0% inhibited the formation of thiobarbituric acid reactive substances (TBARS) by 17.7-60.4%. A similar effect was documented for potato protein hydrolyzates (Wang and Xiong, 2005). However, canola protein hydrolyzates as a potential antoxidant has not been studied. This work evaluated the antioxidant activity of canola protein hydrolyzates prepared by two different proteases, namely, Alcalase, an endopeptidase and Flavourzyme, a mixture of endopeptidase and exopeptidase. Their water-holding capacity in a meat model system during cooking was also examined.

Material and Methods

Preparation of canola protein hydrolyzates: Whole canola seeds were ground and defatted using hexane as the extraction solvent. The defatted canola meal samples were vacuum-packed and stored at -20ºC prior to hydrolysis. The crude protein content in the meal was determined by Kjeldahl analysis according to the AOAC (1990) in order to calculate the enzyme to protein ratio for the hydrolysis. The meal samples were divided into 3 groups and hydrolyzed under pre-selected conditions. Sample 1 was hydrolyzed at 50 ºC and pH 8 for 1 h using Alcalase and sample 2 at 50ºC and pH 7 for 2 h using Flavourzyme; sample 3 used the combination of the two enzymes, i.e. hydrolyzing with Alcalase for 1 h followed by Flavourzyme for an additional 2 h. Conditions were constantly monitored and maintained throughout the process. The pH value, which changes as a result of hydrolysis, was kept constant by addition of a known amount of sodium hydroxide. Upon completion of the hydrolysis, the enzymes were deactivated by dropping the pH to 5. The reaction mixtures were then filtered and hydrolyzates collected. The protein hydrolyzates obtained were freeze-dried and stored at -20 ºC for subsequent analyses.

DPPH radical scavenging assay: The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of enzymatically prepared canola protein hydrolyzates were determined following the procedure described by Shahidi *et al.*

(2006) with minor modifications. Hydrolyzate samples at different concentrations (1.25, 2.5, 5 and 10 mg/ml) were mixed with 50 μM ethanolic DPPH solution and the mixtures were allowed to stand at room temperature for 30 min. The absorbance was then read at 517 nm using a spectrophotometer and the scavenging of DPPH by protein hydrolyzates was calculated as follows:

$$\% \text{ scavenging} = 100 \text{ X } [Abs_{control}-(Abs_{sample}- Abs_{blank})]/Abs_{control,}$$

where, $Abs_{control}$ stands for absorbance of DPPH without protein hydrolyzates, while Abs_{blank} represents absorbance of protein hydrolyzates without DPPH.

Determination of reducing power: Reducing power of canola protein hydrolyzates was measured according to Duh *et al.* (2001) with some modifications. Briefly, canola protein hydrolyzates were dissolved in a 0.2M phosphate buffer solution (pH 6.6) at concentrations of 1.25, 2.5, 5 and 10 mg/ml. They were then added to a 10 mg/ml potassium ferricyanide solution and incubated at 50ºC for 20 min. To the mixtures after incubation, deionized water and a ferric chloride solution (1.0 mg/ml) were added. The absorbance was recorded immediately at 700 nm. A control with no hydrolyzates present and a blank containing only hydrolyzate samples were used because proteins also absorb at the same wavelength.

Determination of water-holding capacity: Water-holding capacity of canola protein hydrolyzates in a meat model system was determined according to Shahidi and Synowiecki (1997). To a mixture containing 8.5 g of ground pork and 1.5 g of distilled water, the canola protein hydrolyzate samples were added at concentrations of 0.5 and 1% (w/w) and mixed thoroughly. The mixture was allowed to stand in a cold room for 1 h and subsequently cooked at 95ºC in a water bath for 1 h followed by cooling under a stream of cold tap water. The drip water was removed with a filter paper and the weight of the meat was recorded. The drip volume was obtained by calculating the weight loss after cooking.

Results

Based on the amount of sodium hydroxide consumed over the course of the reaction, the sample hydrolyzed with Alcalase and with the combination of Alcalase and Flavourzyme had similar degrees of hydrolysis (DH), while the lowest DH was observed in Flavourzyme hydrolyzates (data not shown).

The results of the DPPH assay and the reducing power test show that all protein hydrolyzates possessed antioxidant properties. Figure 1 presents the DPPH radical scavenging capacity of canola protein hydrolyzates prepared by different enzymes. A concentration-dependent effect was observed for all protein hydrolyzates on scavenging of DPPH radicals. The hydrolyzates generated by Alcalase alone and those generated by the combination of Alcalase and Flavourzyme appeared to have similar scavenging capacity against DPPH radicals. The hydrolyzates prepared by Flavourzyme alone showed the highest antioxidant activity among all samples at all concentrations.

A similar trend was obtained for reducing power. The reducing power of the hydrolyzates increased with increasing concentration (Figure 2). The results strongly correlated with those of DPPH scavenging capacity. The Flavourzyme hydrolyzates had the highest reducing power among all samples, while hydrolyzates by Alcalase alone and by combination of Alcalase and Flavourzyme were not significantly different in their reduction potentials.

The canola protein hydrolyzates enhanced water-holding capacity of meat during cooking and thus improved the cooking yield. The drip volume decreased in meat treated with protein hydrolyzates in comparison with that devoid of protein hydrolyzates. This effect was concentration-dependent and influenced by the enzymes employed during hydrolysis (Figure 3). Flavourzyme hydrolyzates were most effective in decreasing the drip volume, followed by hydrolyzates prepared by combination of Alcalase and Flavourzyme and then those by Alcalase alone.

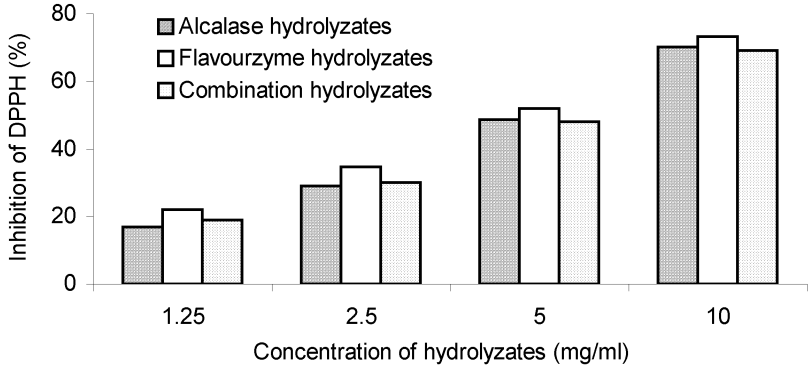

Fig. 1. DPPH radical scavenging capacity of canola protein hydrolyzates

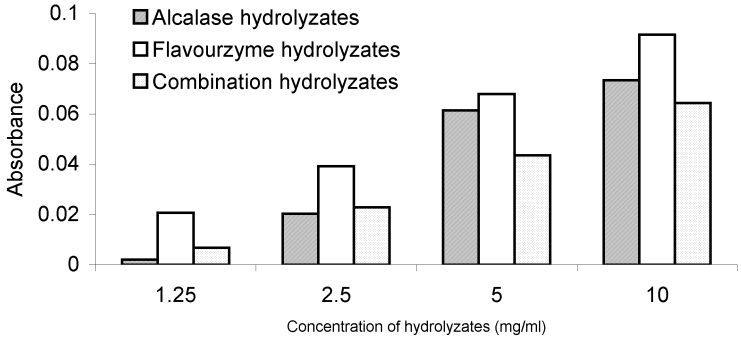

Fig. 2. Reducing power of canola protein hydrolyzates

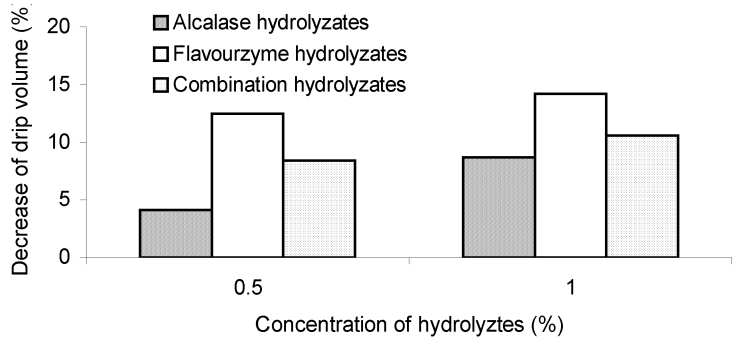

Fig. 3. Decrease of drip volume (%) in meat treated with canola protein hydrolyzates

Discussion

The enzymes employed in this work hydrolyzed the canola meal proteins to different degrees. The lowest DH was found in Flavourzyme hydrolyzates, while unexpectedly, Alcalase and the combination of Alcalase and Flavourzyme resulted in similar DH. It is possible that in the combination, the Flavourzyme did not hydrolyze or hydrolyzed only to a limited extent the proteins and therefore most of the hydrolysis was carried out by Alcalase. In addition to varied DH, the enzymes hydrolyze proteins in different manners, and as a result generate protein hydrolyzates with varied compositions. Alcalase as an endopeptidase cleaves peptide bonds in the interior of the polypeptide chain, producing mainly small portions of proteins or large polypeptides and great portions of small- and medium-size peptide fractions (Adler-Nissen, 1986). The Flavourzyme hydrolyzates generally contain more low-molecular-weight components than Alcalase hydrolyzates, such as small/medium peptides or amino acids, as Flavourzyme acts as both endo- and exopeptidase (Hamada, 2000). The composition of the resultant protein hydrolyzates determines their functional properties and thus their potential applications in the food and animal feed industries.

The canola protein hydrolyzates prepared by both enzymes exhibited antioxidant effectiveness in radical scavenging, presumably via participation of hydroxyl groups of aromatic amino acids as hydrogen donors. Like most other antioxidants, they also showed a reducing power in redox reactions. Their antioxidant activity was concentration-dependent and varied among samples. Flavourzyme hydrolyzates had the highest radical scavenging capacity and reducing power, indicating that the polypeptide fractions obtained by Flavourzyme possessed a higher antioxidant activity than those prepared by Alcalase and the combination of Alcalase and Flavourzyme. The hydrolyzates produced by the combination of two enzymes did not differ significantly from those by Alcalase alone in their antioxidant activity, possibly because Flavourzyme did not contribute to the hydrolysis in combination, as discussed earlier. In addition to their antioxidant activity, all hydrolyzates had a dose-dependent effect in enhancing the water-holding capacity of a meat model system. Their capability in improving the cooking yield of meat was in the order of Flavourzyme hydrolyzates > combination hydrolyzates > Alcalase hydrolyzates, suggesting that smaller-size polypeptides and/or amino acids may be more effective in enhancing water-holding capacity of meat than larger-size polypeptides. Further studies are needed to identify the peptide fractions responsible for antioxidant potential and water-holding capacity of the hydrolyzates.

Conclusions

Canola protein hydrolyzates prepared by commercial proteases Alcalase and Flavourzyme exhibited antioxidant efficacy in terms of radical scavenging capacity and reducing power, with Flavourzyme hydrolyzates possessing the highest antioxidant activity. Alcalase and the combination of Alcalase and Flavourzyme resulted in similar antioxidant activity of the hydrolyzates. The canola protein hydrolyzates were also able to enhance the water-holding capacity of a meat model system and therefore improve the cooking yield.

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