PROTEIN PRECIPITATION CAPACITY OF CANOLA TANNINS

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ABSTRACT

The protein precipitation capacity of low- and high-tannin canola hulls of Westar, Excel and Cyclone varieties were examined. The effect of pH on the affinities of canola tannins for dye-labelled protein was monitored. The affinity of canola tannins for dye labelled proteins in the presence of competitor proteins such as collagen, BSA, fetuin, lysozyme and pepsin is discussed.

INTRODUCTION

The available information on the biological activity of rapeseed tannins is still diverse and fragmentary. Mitaru *et al.* (1982) reported that condensed tannins isolated from rapeseed hulls were not capable of inhibiting the *in vitro* activity of α-amylase. On the other hand, Leung *et al.* (1979) found that tannins isolated from rapeseed hulls formed white precipitates after addition to a 1% gelatin solution. However, they did not attempt to quantify the biological activity of tannins.

Recently Naczk *et al.* (1994) reported that condensed tannins isolated from canola hulls exhibit significant biological activity. It was found that tannins isolated from low-tannin hulls exhibited greater affinity for proteins than those isolated from high-tannin canola hulls. The biological activity of tannins, expressed as precipitation index, correlated well with the content of tannins determined by the vanillin assay. The objective of this study was to determine the effect of pH and the presence of competitor on the biological activity of condensed tannins isolated from canola hulls.

EXPERIMENTAL

Material and methods

Hulls of Westar, Excel and Cyclone canola varieties were prepared according to the procedure described by Sosulski and Zadernowski (1981). Hulls were extracted with hexane for 12 h using a Soxhlet apparatus and then dried at 50°C in a forced air oven for 18 h.

The condensed tannins were isolated as follows: one gram of hulls was extracted twice with 10 mL 70% (v/v) aqueous acetone using a Polytron (Brinkman PT- 3000) (60 sec, 15,000 rpm) at room temperature. After each centrifugation (10 min, 5,000 rpm), the supernatants were collected, combined and evaporated to dryness at 30°C under vacuum. The extracted phenolics were dissolved in 10 mL methanol.

The condensed tannins were assayed colorimetrically by the modified vanillin method of Price *et al.* (1978). The content of tannins in the hulls was expressed as catechin equivalents (mg/100 g of hulls, on a dry weight basis).

The effect of pH on the formation of canola tannin-protein complexes was assayed using the precipitation method of Hagerman and Butler (1978). Standard protein solutions (1 mg/mL) of BSA, fetuin, lysozyme, collagen and pepsin were prepared by mixing a 0.01M phosphate buffer with solutions of proteins (2 mg/mL) in the ratio of 1:1 (v/v). The effect of pH was also assayed by the dye-labelled protein assay (Asquith and Butler, 1985). Standard protein solutions (2 mg/mL) of blue BSA were prepared by mixing the 0.20M phosphate buffer with a BSA solution (4 mg/mL) in the ratio of 1:1 (v/v).

Varying amounts of unlabelled BSA (500-6000 µg) were mixed with 1 mg blue BSA solution or added and mixed vigorously for 5 min with the pellet of tannin-protein complex to study the effect of competitor on the precipitation of blue labelled BSA before and after formation of tannin-protein complex. Conditions used in this study were adapted from Asquith and Butler (1985). All assays were conducted at room temperature (about 22°C) using appropriate samples and blanks. The results presented are mean values of at least six determinations.

Results and discussion

The total content of condensed tannins in hulls of Westar, Excel and Cyclone cultivars determined by vanillin assay are 1556, 144 and 994 mg catechin equivalents/100g, respectively. Figure 1 shows the effect of pH on the amount of canola tannins precipitating with selected proteins. BSA, fetuin, collagen and pepsin were precipitated markedly at pH values between 3.0 and 5.0. However, maximum precipitation of lysozyme occurred at a pH > 8.0. The results indicate that precipitation of proteins by canola tannins does not depend only on the availability of unionized phenolic groups for hydrogen bonding. The optimum pH for carrying out the protein precipitation assay of Hagerman and Butler (1978) for determination of biological activity of canola tannins was 4.0. The effect of pH on the amount of dye-labelled BSA

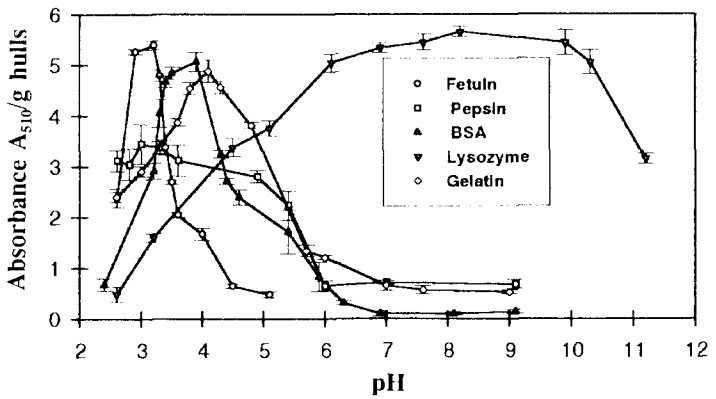

Figure 1. The pH dependence of complex formation between selected proteins and Westar tannins.

precipitated by tannins isolated from Excel (low-tannin) and Cyclone (high-tannin) indicated an optimum pH of 3.5 for carrying out the blue BSA assay of Asquith and Butler (1985). The results of blue BSA assay are less discriminatory than those obtained by the vanillin test; existing differences in the molecular weight of tannins isolated from high- and low-tannin samples of canola hulls may be contemplated.

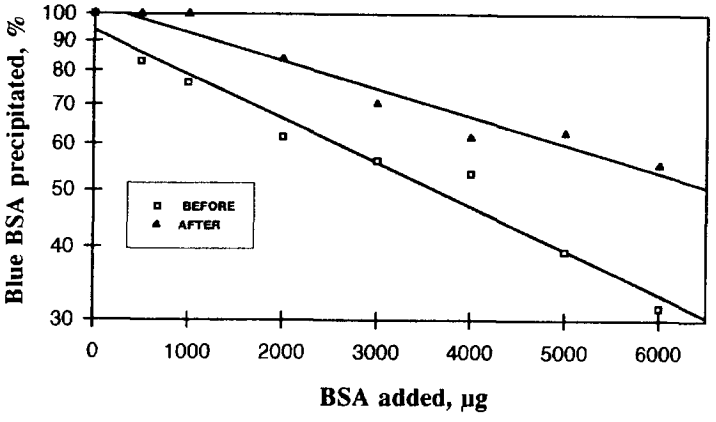

Figure 2. Competition between blue and unlabelled BSA for Cyclone tannins; unlabelled BSA was added before or after formation of blue BSA-tannin complex.

Figure 2 shows the competition between blue and unlabelled BSA for canola tannins. The relative affinity of BSA for canola tannins was 0.275 and was higher than that reported for sorghum tannins (Asquith and Butler, 1985). On the other hand, the relative affinity of BSA for canola tannin complexed with blue BSA was 0.15.

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