recorded up to 95 ppm, with psoralen at 24 ppm. Austad and Kavli (1983) reported dermatitis in celery handlers with total furocoumarins (5-MOP, 8-MOP, and sphondin, no psoralen was observed) of about 18 ppm.

Low levels for total furocoumarins in fresh, nonstressed celery were not considered to be hazardous. Under normal circumstances, furocoumarins in fresh celery can be as low as 0.1 ppm and perhaps as high as 3–4 ppm, depending on the variety and growing conditions (Ashwood-Smith et al., 1985; Beier et al., 1983b).

Risks from furocoumarins to man would appear to rest in the utilization of diseased celery for the production of soups and sauces. Several batches of less than palatable and clearly diseased celery have been seen displayed by supermarkets and grocery stores labeled "for soup preparation". Ivie et al. (1981) reported psoralen, 5-MOP, and 8-MOP to be present in fresh parsnips at levels of about 40 ppm, which were unaffected by standard cooking procedures. It is very probable that furocoumarins in celery are also stable to cooking, although this particular point has not been ascertained in these present studies. Ingestion of 200 g of infected celery, in soup for example, would result in the equivalent of a 10-mg dose of mixed furocoumarins. The normal dosage of 8-MOP administered in the photochemotherapy (PUVA treatment) of psoriasis is about 20 mg. Diseased celery contains about 30% of total furocoumarins as the very active psoralen (Ashwood-Smith et al., 1982), and thus the biological action of ingesting 200 g of diseased celery might well result in similar effects to the standard chemotherapeutic regime. The comments of the World Health Organization (IARC, 1983) are apposite in this discussion.

It follows from these observations and comments that certain recommendations, no doubt open to some modification depending on different varieties of celery and growing conditions, are self-evident. Only fresh celery or celery stored at low temperatures (4 °C) for periods no longer than about 2–3 weeks should be sold, and the use of "less than fresh" celery should be discouraged.

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**Registry No.** 5-MOP, 484-20-8; 8-MOP, 298-81-7; psoralen, 66-97-7.

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# Effect of Condensed Tannins on the in Vitro Protein Digestibility of Mung Bean (*Vigna radiata* (L.) Wilczek)

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Condensed tannins isolated from mung bean and tannic acid decreased the in vitro protein digestibility (IVPD) of polyvinylpolypyrrolidone- (PVP-) treated and untreated boiled seeds without broth of two mung bean varieties by 3–6%. Addition of PVP to boiled seeds without broth increased IVPD significantly by 2%. However, when PVP was added to boiled seeds with broth, the slight increase in IVPD was not significant.

## INTRODUCTION

Among legumes, mung bean (*Vigna radiata* (L.) Wilczek) is the most popular and widely consumed in the Philippines and in other Asian countries (Engel, 1977; Payumo, 1977; Tsou and Hsu, 1977; Vignarajah, 1977). Mung bean that contains 20–25% protein either is utilized as whole or germinated seeds or can be processed into flour or noodles. Whole mung bean seeds are utilized in native delicacies such as "butse-butse" and "hopia", as a soup, or combined with sugar as snacks or dessert (PCARR, 1977). Germinated seeds or sprouts that are more digestible, with higher vitamin C, riboflavin, thiamine, and protein contents (Kylen and McCready, 1975; Purdente and Mabesa,

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1981; Beltran et al., 1983) are mixed or combined with shrimp or meat and used as a vegetable dish. A portion of wheat flour is sometimes substituted with mung bean flour to obtain a high-protein bread. Mung bean–cereal combinations are the basis of "nutripak", which are nutritious food packets for children and as weaning foods (Payumo, 1977).

Ordinary cooking by boiling has been found effective in destroying some of the antinutritional and toxic factors, especially the heat-labile trypsin inhibitors and hemagglutinins, thus improving the nutrititional value of legume proteins (Liener, 1976; Elias et al., 1979; Phillips et al., 1981; Beltran et al., 1983; Bressani et al., 1983; Eusebio and Eusebio, 1984; Laurena et al., 1984a; Tan et al., 1984). Unlike other legumes, mung bean has low or nondetectible levels of trypsin inhibitors (Elkowicz and Sosulski, 1982), hemagglutinating activity (Beltran et al., 1983), tannins (Price et al., 1980), and flatulence factors (Murphy, 1972). However, others (Narasinga Rao and Prabhavathi, 1982; Beltran et al., 1983; Laurena et al., 1984b; Barroga et al., 1985) have reported the presence of tannins in mung bean. Barroga et al. (1985) further observed that mung bean tannins are concentrated in the seed coat and that soaking, germination, roasting, and boiling reduced tannin content.

Tannins have been found to lower the protein digestibility of several legumes and cereals (Ramachandra et al., 1977; Elias et al., 1979; de Lumen and Salamat, 1980; Griffiths and Moseley, 1980; Griffiths 1981; Phillips et al., 1981; Satwadhar et al., 1981; Narasinga Rao and Prabhavathi, 1982; Bressani et al., 1983; Laurena et al., 1984a,b) presumably due to the ability of tannins to bind with proteins. This study was aimed at determining the effect of condensed tannins on the in vitro protein digestibility of mung bean.

## MATERIALS AND METHODS

Mature seeds of two mung bean varieties (Pag-asa 1, green, and Pag-asa 3, yellow) were obtained from the Seed Production Section of the Institute of Plant Breeding, University of the Philippines at Los Baños. All the chemicals used were of analytical grade.

**Preparation of Condensed Tannins.** Condensed tannins were isolated and purified from the two mung bean varieties according to the method of Hagerman and Butler (1980). The isolated condensed tannins, when subjected to acid hydrolysis, turned from an amber-colored liquid to scarlet red. Hydrolyzable tannins were apparently absent due to the absence of glucose or any sugar in the paper chromatography of the aqueous fraction.

**In Vitro Protein Digestibility (IVPD).** The digestibility of proteins of raw and boiled seeds of mung bean were determined with trypsin, chymotrypsin, and peptidase following the multienzyme technique of Hsu et al. (1977). The in vitro digestibility was calculated from the regression equation $Y = 210.64 - 18.103X$ where $Y$ = in vitro digestibility (%) and $X$ = pH of the sample suspension after 10-min digestion with the multienzyme solution.

For raw samples, ground seeds (100 mesh) were made into 25-mL aqueous suspensions (6.25 mg of protein/mL). For cooked samples, enough seeds were boiled for 30 min and 25-mL suspensions prepared containing 6.25 mg of protein/mL. Analysis was done on boiled seeds with broth and boiled seeds without broth. For the latter, the broth was decanted and an equal amount of boiled water was substituted. The aqueous protein suspension was adjusted to pH 8.0 with 0.1 N HCl and/or NaOH, while stirring in a 37 °C water bath. The multienzyme solution (2.5 mL) was added and the pH recorded immediately and every 2 min over a 10-min period.

**Table I. In Vitro Protein Digestibility of Raw and Boiled Mung Bean Seeds[a]**

| sample | IVPD, % Pag-asa 1 (green) | IVPD, % Pag-asa 3 (yellow) |
|---|---|---|
| raw whole seed | 77.34 c | 76.54 c |
| boiled seeds, with broth | 85.50 b | 83.31 b |
| boiled seeds, without broth | 90.44 a | 89.56 a |

[a] Means followed by a common letter in a column are not significantly different at the 5% level (Duncan's multiple-range test, DMRT).

The effects of tannic acid and condensed tannins isolated from the green and yellow mung bean varieties on the digestibility of boiled mung bean seed proteins were also investigated. A 10-mg portion of the tannins or tannic acid was added to the boiled seeds without broth and the digestibility determined as above. Polyvinylpolypyrrolidone (PVP) (50 mg), a tannin complexing agent, was added to the boiled seeds, prior to the addition of condensed tannins or tannic acid. PVP was also added in varying amounts (50, 100, 150 mg) to boiled seeds with and without broth. These components were added 30 min prior to pH adjustment.

## RESULTS AND DISCUSSION

**Effect of Boiling on IVPD.** Boiling increased significantly the IVPD of mung bean from 77.36 and 73.54% to 85.50 and 83.31% and 90.44 and 89.56% for the green and yellow varieites with and without broth, respectively (Table I). Cooked seeds without broth had higher IVPD values (90.44 and 89.56%). Using animal assay, Eusebio and Eusebio (1984) observed an increase in digestibility of mung bean from 71.46 to 89.48% after boiling and only up to 80.06% after roasting. A similar 6–8% increase in IVPD of cowpea after boiling had been reported (Laurena et al., 1984a) and up to 15% with winged bean (Tan et al., 1984). Boiling reduced the tannin content of mung bean seeds by 73% and resulted in increased tannin level in the cooking broth (Barroga et al., 1985).

Boiling also decreased trypsin inhibitor activity in mung bean from 71 to 89% (Beltran et al., 1983; IPB, 1983) and up to 79% in dehulled toasted mung bean (Eggum et al., 1984). Tannins might therefore play an important role in the reduction of protein digestibility of mung bean.

**Effects of Addition of Tannic Acid, Mung Bean Condensed Tannins and PVP.** Tannic acid and isolated condensed tannins reduced significantly the IVPD values of boiled seeds (without broth) by 5–6% and 3–4% for the green and yellow varieties, respectively (Table II). The reduced digestibility values approximated those of boiled seeds with broth. Laurena et al. (1984a) recently reported a 4% decrease in IVPD of raw white cowpea after treatment with condensed tannins from red cowpea and tannic acid.

Addition of PVP to boiled seeds (without broth) increased IVPD significantly by 2% (Table II). However, when PVP was added to boiled seeds with broth, the slight increase in IVPD was not significant. PVP complexes with tannins, which results in higher digestibility values (average of 4%) as observed with field beans (Bond, 1976), bean and peas (Griffiths, 1981), and cowpea (Laurena et al., 1984a). Presumably, the broth contained large amounts of tannins that bound the PVP. Boiled seeds (without broth) with tannic acid or condensed tannins previously treated with 50 mg of PVP have low IVPD values comparable to the treatments without PVP and to the boiled seeds with broth. The results indicate that condensed

**Table II. Effect of Tannins and PVP on the in Vitro Protein Digestibility of Boiled Mung Bean Seeds[a]**

| sample | IVPD, % Pag-asa 1 (green) | IVPD, % Pag-asa 3 (yellow) |
|---|---|---|
| boiled seeds (without broth) | 90.44 a | 89.56 a |
| boiled seeds + tannic acid | 84.88 c | 84.39 d |
| boiled seeds + PVP + tannic acid[b] | 85.32 c | 85.64 c |
| boiled seeds + condensed tannins | 86.24 b | 86.49 b |
| boiled seeds + PVP + condensed tannins[b] | 86.76 b | 86.05 bc |
| boiled seeds, without broth | 90.44 b | 89.56 b |
| +50 mg PVP | 92.02 a | 89.51 b |
| +100 mg PVP | 92.19 a | 90.90a |
| +150 mg PVP | 91.82 a | 90.25 ab |
| boiled seeds with broth | 85.50 a | 83.31 a |
| +50 mg PVP | 86.08 a | 83.66 a |
| +100 mg PVP | 86.24 a | 83.16 a |
| +150 mg PVP | 86.22 a | 83.34 a |

[a] Means followed by a common letter within a treatment are not significantly different at the 5% level (DMRT). Tannic acid (10 mg) or isolated condensed tannins were added to 25 mL of aqueous suspensions of boiled seeds containing 6.25 mg of protein/mL. [b] PVP (50 mg) was added first to the boiled seeds followed by condensed tannins/tannic acid after about 5 min.

tannins or tannic acid overcomes the effect of the added PVP on the IVPD of boiled seeds without broth. It is possible that addition of higher amounts of PVP will eventually result in increased IVPD.

The above results indicate a small (3–6%) but definite lowering of in vitro protein digestibility of mung bean by condensed tannins. Boiling decreased tannin content and increased IVPD significantly especially when the broth that contained high levels of tannin was removed. However, local viands of mung bean usually contain the broth, which is tasty and is often sered as soup. Sweetened boiled mung bean with its broth is commonly eaten as dessert. Although roasting or toasting decreased tannin content (Barroga et., 1985; Eggum et al., 1984), it also resulted in lowering the lysine content (Eggum et al., 1984). Different heat treatments have been observed to reduce tannin content. However, the increase in IVPD was not proportional (Tan et al., 1984; Laurena et al., 1984c). Since the decrease in tannins is monitored by a chemical assay, this suggests that the products of heating tannins could still reduce protein digestibility particularly in cases of dry heating like roasting and microwave.

Processing of mung bean like dehulling results in higher energy and protein digestibility and lower levels of phenolics, neutral detergent fiber, and nonreducing sugars (Eggum et al., 1984). Raw dehulled and sprouted mung bean had higher PER than raw whole and boiled or roasted mung bean (Eusebio and Eusebio, 1984). Germination also reduced tannin content by 36% in mung bean (Barroga et al., 1985). Cowpea sprouts had up to 87% less tannins and 15% improvement in IVPD (Laurena et al., 1984c). Weaning food formulations based on milled rice and mung bean often use dehulled bean (Roxas et al., 1976), and thus the ill effects of condensed tannins on digestibility would be minimal or nil.

Thus, although results indicate that condensed tannins decrease in vitro protein digestibility, simple precooking and cooking treatments like mechanically dehulling seeds, boiling with minimal intake of broth and germination for sprouts, and the use of varieties containing low levels of tannins could minimize their adverse effects.

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