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Effect of tannins on the in vitro digestibility of jojoba meal proteins

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proteins**

Fuller, Sally Joyce, M.S.

San Jose State University, 1989

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EFFECT OF TANNINS ON THE IN VITRO
DIGESTIBILITY OF JOJOBA MEAL PROTEINS

A Thesis
Presented to
The Faculty of the Department of Nutrition and Food
Science
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in Nutritional Science

By
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May 1989

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DEDICATION

This thesis is dedicated to my parents, Don and Primetta Patterson, whose faith in me gave me an incentive for reaching this academic goal, and whose courage and optimism have brightened my life.

PREFACE

The following is a publication style thesis. The second chapter is written in journal format according to January, 1988 guidelines and will be submitted to the Journal of Agricultural and Food Chemistry. Chapters I and III are written according to guidelines outlined in the Publication Manual of the American Psychological Association, 3rd. edition, 1986.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Jojoba (Simmondsia chinensis), a desert shrub, is being commercially propagated in arid areas worldwide for the valuable oil that comprises 50% by weight of its seeds (National Academy of Sciences (NAS), 1985). Jojoba meal, a by-product of the jojoba oil industry, is, at present, of little economic value. Despite its relatively high protein content (Verbiscar & Banigan, 1978), attempts to feed jojoba meal to laboratory animals and livestock have resulted in poor feed efficiency, low weight gain, and even death (Booth, Elliger, & Waiss, 1974; Manos et al., 1986; Verbiscar et al., 1980, 1981). These undesirable effects have been attributed to the presence of toxic cyanoglycosides, called simmondsins, in jojoba seeds (Booth et al., 1974). While detoxification studies have shown improvement in feed intake and growth of poultry, sheep, and cattle (Verbiscar et al., 1980, 1981), there are little data on the effect of other anti-nutritional factors such as tannins on the nutritional value of jojoba meal.

Tannins are plant polyphenolic compounds which have been shown to adversely affect the digestibility of

proteins of several varieties of legumes (Barroga, Laurena, & Mendoza, 1984; De Lumen & Salamat, 1980; Elias, De Fernandez, & Bressani, 1979; Laurena, Van Den, & Mendoza, 1984; Ritter, Morr, & Thomas, 1987)) and grains (Chavan et al., 1979; Chibber, Mertz, & Axtell, 1980; Ikeda et al., 1986; Ramachandra, Virupaksha, & Shadaksharaswamy, 1977). Although jojoba seeds and meal have been shown to contain considerable amounts of tannins (Carduso & Price, 1980; Oldmark, 1986; Young, 1987), their effect on protein digestibility has not been studied.

Objectives

The objectives of this study are: 1) to determine the tannin content and in vitro protein digestibility (IVPD) of defatted jojoba meals from two sources, 2) to compare the IVPD of jojoba meal and jojoba protein concentrate prepared from dehulled seeds to the IVPD of commercial soy protein isolate, and 3) to determine the relationship between tannin content and IVPD for jojoba meal.

Significance of the Study

Since jojoba meal constitutes 50% of the whole seed, handling and disposal of this product presents a major problem. The jojoba oil industry is currently studying ways to utilize this by-product as an animal feed supplement and as a protein source for human application.

However, nutritional data are limited. The results of this study will contribute to the nutritional data base needed for the efficient utilization of jojoba meal.

Review of Literature

Jojoba

Jojoba Oil

Jojoba is a shrub which grows wild in the Sonoran desert areas of California, Arizona, and Mexico. The bush produces seeds which contain 50% by weight of an oil that is unique in the plant kingdom (NAS, 1985). The oil consists of esters of long chain mono-ethylenic alcohols and fatty acids and is classified as a liquid wax. It is chemically similar to sperm whale oil, a product now unavailable in the United States (U.S.) (NAS, 1985). This similarity aroused commercial interest in jojoba, and the industry has expanded from the harvest of wild plants to extensive commercial propagation. Approximately 40,000 acres are under cultivation worldwide, representing a 200 million dollar investment. Since jojoba grows well with little irrigation and since the oil and the seeds are not perishable, it is an attractive cash crop for developing countries (NAS, 1985).

Originally used mainly in cosmetics, the oil is now being considered for other uses both as a replacement for

spermwhale oil and for functions suited to its unique properties. Its extreme heat stability and high flash point make it valuable as a high temperature lubricant (NAS, 1985). The purity and molecular uniformity of jojoba oil make it attractive as a basic "feedstock" of the chemical industry. Hydrogenation of this oil creates a solid wax similar to beeswax and carnauba (NAS, 1985). Uses for jojoba oil appear to be only limited by the supply, but supplies are increasing as plantations come into production (NAS, 1985).

Jojoba Meal

Along with increasing oil production comes increasing amounts of jojoba meal. Comprising 50% by weight of the seed, jojoba meal is the residue remaining after oil extraction. Relatively high in protein and crude fiber but low in total sugars, jojoba meal also contains approximately 5% cyanoglycosides (Verbiscar et al., 1980).

Proteins. Jojoba meal contains 30-33% protein (Yermanos & Duncan, 1976). Total jojoba proteins are composed of 65% albumins, 21% globulins, 8% prolamines, and 6% glutelins (Carduso & Price, 1980). This relatively high protein content makes jojoba meal a potential source of protein for animals and/or humans (NAS, 1985). However, protein quality is not high. Compared to whole egg protein (FAO pattern), the albumin fraction of jojoba protein

concentrate showed lower levels of the essential amino acids: lysine, leucine, methionine, phenylalanine, and isoleucine. The globulins and glutelins showed lower levels of lysine, methionine, and isoleucine, and the prolamine was lower in methionine and isoleucine. The first limiting amino acid in total jojoba protein is methionine (0.6-1.2%) with an amino acid score of 77 (Carduso & Price, 1980).

Functional properties of protein concentrates prepared from jojoba meal have been examined by Carduso and Price (1980) and Wiseman and Price (1987b). Oil and water absorption capacities were equivalent to soy protein concentrates, and foam capacity and stability were similar to those of egg albumin (Wiseman & Price, 1987b). Nitrogen solubility of the albumin and globulin fractions of jojoba protein concentrates was superior to that of soy protein concentrate (Carduso & Price, 1980). Based on these results, Carduso and Price (1980) concluded that "the application of jojoba seed proteins in the food industry has excellent potential" (p. 314).

Simmondsins. Simmondsins are a group of cyclohexcyanomethylene glycosides isolated from jojoba seeds and meal (Booth et al., 1974). Simmondsin and simmondsin 2'-ferulate are the most prevalent cyanoglycosides, comprising 2.0-3.0% and 0.75% of the

meal, respectively (Verbiscar & Banigan, 1978).

Toxicity studies involving both purified simmondsin and defatted jojoba meal have shown that toxicity is species specific. Dietary simmondsin levels of 0.3% or greater caused appetite depression and weight loss in rats. Large single doses of 4.0 g/kg orally or 3.6 g/kg intraperitoneally produced no toxic symptoms in rats, but all rats given doses of 0.75 g/kg daily for 5 days died within 10 days of the first dose (Booth et al., 1974). Rats fed diets containing up to 15% defatted jojoba meal exhibited the same pattern of decreased food intake, weight loss, and death as the rats fed diets containing up to 0.75% purified simmondsin (Booth et al., 1974).

No mortality or weight loss was observed in mice given purified simmondsin intraperitoneally at either acute (12.95 g/kg) or subacute (0.75 g/kg/day) levels (Williams & Price, 1980). Verbiscar et al. (1980) also found that intraperitoneal administration of simmondsin (0.75 g/kg/day) did not affect mice, but oral administration resulted in weight loss and 75% mortality after 14 days. Mortality occurred in mice fed jojoba meal as a sole source of protein when the toxicant level exceeded 0.1% of the diet (Verbiscar et al., 1980).

Manos et al. (1986) incorporated hexane extracted jojoba meal into lamb rations at levels of 5 and 10%.

Residues of simmondsin and simmondsin 2'-ferulate were not found in the blood, kidney, liver, or muscle of the experimental animals, and it was hypothesized that microorganisms in the gut of ruminants detoxify the meal. However, weight gain and feed intake and efficiency were lower for test animals.

Detoxification. Removal of simmondsins from jojoba meal has been attempted in several studies (Oldmark, 1986; Verbiscar et al., 1980, 1981). Solvent extraction (methanol, acetone, or methylene chloride), water washing, heat, chemical treatment (ammonia or ammoniacal hydrogen peroxide), and microbial methods resulted in reduced simmondsin levels (Verbiscar et al., 1980, 1981). However, data from feeding studies with detoxified jojoba meal reported varied results. Mice did not survive when methanol extracted jojoba meal was the sole source of protein although simmondsins were reduced to 0.014% of the total diet (Verbiscar et al., 1980). Mice, fed diets containing 10% ammonia treated jojoba meal (w/w) supplemented with whole egg to equal 7% dietary protein, showed similar food intake as controls, but weight gain was 67% less than controls over a three-week test period. Poultry feed efficiency (g feed consumed/g weight gained) equaled controls when ammoniacal hydrogen peroxide treated jojoba meal was included in the diet at the 5% level

(w/w), but efficiency fell by 25% when this detoxified meal was fed at the 10% level. Mice and lambs grew well on diets containing 10% water extracted jojoba meal; however, chicks did not (Verbiscar et al., 1980).

Verbiscar et al. (1981) found that, when jojoba meal was treated with Lactobacillus acidophilus, simmondsin levels decreased by 95-98%. This treatment resembles an ensilage process and is the most promising detoxification technique available to date. Lambs fed microbially detoxified meal as 10% (w/w) of the diet equaled control animals in growth and feed efficiency. However, this was not true for mice, chicks, or steers (Verbiscar et al., 1981).

Weight gain for mice whose diets contained microbially detoxified jojoba meal at the 10% level was 70% less than controls. Verbiscar et al. (1981) theorized that the quality of jojoba protein for mice is not good. Broiler chicks grew better on the test diet than mice, but growth was depressed by 12-32%. Microbial treatment appears to increase the palatability of jojoba meal for steers since voluntary feed intake was greater than for nondetoxified meal. Despite feed intake equal to controls, efficiency of feed conversion was about 16% less for steers on the treated meal diet. Data for cattle are limited, however (Verbiscar et al., 1981). Since the

simmondsin levels in the test diets were all below 0.05%, these results cannot be explained by the presence of cyanoglycosides. Other anti-nutritional factors such as tannins may contribute to the substandard weight gain and feed efficiency which occurs when jojoba meal is included in the diet.

Tannins

Tannins are defined as polyphenolic compounds having molecular weights ranging from 500-3000 and exhibiting the ability to form cross-links between protein molecules (Haslam, 1966). Tannins are generally divided into two groups: hydrolyzable and condensed. Hydrolyzable tannins are readily hydrolyzed by acids to gallic or ellagic acid plus sugars and polyhydric alcohols (Haslam, 1966) and are found in galls, acorns, fruit pods (Haslam, 1966), and oilseeds (Sosulski, 1970). Hydrolyzable tannins are responsible for the bitter flavors of these plant products (Sosulski, 1970).

Condensed tannins undergo progressive polymerization under acidic conditions and are responsible for "tannin reds" which are formed in plants from flavonol precursors similar to the pigment, anthocyanin (Haslam, 1966). They are found most abundantly in bark and have been detected in various legumes such as common beans (Phaseolus vulgaris) (Bressani et al., 1983), cowpeas (Laurena et

al., 1984), and mung beans (Barroga et al., 1985) and in grains such as sorghum, buckwheat (Price, Hagerman, & Butler, 1980), and millet (Ramachandra et al., 1977).

Tannin-Protein Interactions

Multiple bonding occurs when either condensed or hydrolyzable tannins react with protein. Four types of bonds have been suggested to contribute to the formation of tannin-protein complexes: covalent bonds between the oxidized hydroxyl groups of tannins and nucleophilic R-groups in the protein (Haslam, 1966), hydrogen bonds between the amide groups of the protein and the phenolic groups of the tannin, ionic linkages between phenolate ions and cationic sites on the protein molecule, and hydrophobic interactions between the aromatic ring structures of tannins and hydrophobic regions of protein (Kumar & Singh, 1984). Hydrolyzable tannins are more susceptible to oxidation than condensed tannins, and the oxidized derivatives form covalent linkages with the R-groups of lysine and methionine (Sosulski, (1979). Proteins high in proline and hydroxyproline have a high affinity for condensed tannins due to the open conformation of these proteins, their capacity to form hydrogen bonds (Kumar & Singh, 1984), and their numerous hydrophobic regions (Hagerman & Butler, 1980b).

Multiple bonding between tannins and collagen

proteins, the basis of the tanning process in leather manufacture, makes leather resistant to water, bacteria, heat, and abrasion (Haslam, 1966). In foods, however, tannin-protein interactions are undesirable. Insoluble complexes form which are resistant to the digestive enzymes of monogastric and ruminant animals (Sosulski, 1979). According to Sosulski (1979), a small degree of oxidation of a food containing hydrolyzable tannins and already limiting in lysine or methionine, such as soy flour, can result in a substantial loss of the nutrient value of the protein. Tannins can also react with and inactivate digestive enzymes (Kumar & Singh, 1984).

Tannins in Foods

Condensed tannins are found primarily in the seed coats of some legumes including common beans (Bressani et al., 1983; Elias et al., 1979), mung beans (Barroga et al., 1985), winged beans (De Lumen & Salamat, 1980), and cowpeas (Laurena et al., 1984) and in the pericarp of grains such as sorghum (Chavan et al., 1979, Chibber et al., 1980), millet (Ramachandra et al., 1977), and buckwheat (Ikeda et al., 1986). Tannin content varies among cultivars of a crop with the darker colored strains having higher tannin contents (Elias et al., 1979; Laurena et al., 1984; Ramachandra et al., 1977). During cooking of legumes, seed coat tannins leach into the cooking broth

which then contains the majority of tannins (Elias et al., 1979). During commercial milling of sorghum, tannins remain in the flour (Chavan et al., 1979).

Protein digestibility. Since tannins are present in crops that are important sources of protein in developing countries, their effect on protein digestibility has been extensively studied. Tannin content was negatively correlated to IVPD (pepsin hydrolysis) in finger millet with a 8.6% decrease in IVPD for a 1.0% increase in tannins. Low tannin strains of millet had an IVPD of 85%, while high tannin varieties had an IVPD of 55% (Ramachandra et al., 1977). Dehulling (Chibber et al., 1980) and alkali extraction (Chavan et al., 1979) decreased the tannin content of high tannin sorghum and increased IVPD dramatically. Addition of tannic acid to buckwheat globulin produced an inhibitory effect on IVPD when either peptic or pancreatic enzymes were used (Ikeda et al., 1986).

Elias et al. (1979) found that, since tannins are leached from beans into the cooking broth, IVPD was lower for red and black strains of common beans with broth added back than for the same beans without broth. This was not true for white common beans which contain much lower tannin levels. Protein efficiency ratios (PER) fell from 3.26 for black common beans without broth to 1.69 for

black common beans with broth (Elias et al., 1979). Similar results were obtained for cowpeas (Laurena et al., 1984). Adding isolated condensed tannins to white cowpeas (Laurena et al., 1984) and mung beans (Barroga et al., 1985) decreased IVPD by 3.6 and 3.1%, respectively.

Trypsin inhibitor activity. Protein quality in legumes is reduced by the presence of trypsin inhibitors. The primary trypsin inhibitor present is proteinaceous and heat labile. Some trypsin inhibitor activity remains after beans are cooked, however, and several studies (Barroga et al., 1985; De Lumen & Salamat, 1980; Elias et al., 1979) have attributed this heat resistant inhibition to tannins. De Lumen and Salamat (1980) found that heat resistant trypsin inhibitor is concentrated in the seed coat of winged beans. Heat treatment of tannin extracted from winged beans, commercial tannin, and proteinaceous soy trypsin inhibitor showed that, although tannins have 30% lower trypsin inhibitor activity than proteinaceous trypsin inhibitor, tannins are not affected by boiling. However, the proteinaceous trypsin inhibitor was completely inactivated by heat.

Heat resistant trypsin inhibitor is high in the seed coat of common beans, whereas heat labile trypsin inhibitor is highest in the cotyledons (Elias et al.,

1979). These researchers also reported that heat resistant trypsin inhibition is directly related to the color of the bean and to tannin content (Elias et al; 1979). Parallel results were obtained for mung beans (Barroga et al., 1985). According to DeLumen and Salamat (1980), it is possible that tannins inhibit digestive enzymes other than trypsin since the ability of tannins to bind to proteins is nonspecific.

Gastrointestinal effects. Tannins suppress growth in laboratory animals and livestock (Kumar & Singh, 1984). This effect on growth is not reversed by protein supplementation, suggesting that reduced protein utilization is not the only consequence of tannins in the diet (Glick & Joslyn, 1970a). When tannic acid was incorporated at 4, 5, and 8% levels in rat diets, food intake depression and increased nitrogen excretion were observed. Radioactive tracer studies by Glick and Joslyn (1970b) showed that the excess nitrogen excreted was from endogenous sources rather than from dietary proteins.

According to Mitjavila et al. (1977), hydrolyzable tannins increased the secretion of sialic acid and glucosamine in the rat. Since glucosamine is necessary for the synthesis of mucous glycoproteins in the gastrointestinal tract, they concluded that reduced apparent digestibility of protein in the presence of

tannic acid is due to the excretion of these endogenous glycoproteins and not the indigestibility of tannin-protein complexes or the inhibition of digestive enzymes.

Palatability. Phenolic compounds contribute to sour, bitter, and astringent tastes (How & Morr, 1982). Since phenolic acids have a taste threshold of 40-90 ppm (Sosulski, 1979), use of oilseed flours is limited due, in part, to the bitter and beany tastes. Removal of phenolic compounds from soy protein extracts improves both flavor and color (How & Morr, 1982). The astringency of tannins, while valuable as a bird repellent, reduces the palatability of grains for livestock. Addition of some sorghum varieties which are high in tannin to livestock feed is restricted due to lower voluntary feed intake by animals (Kumar & Singh, 1984).

Tannins in Jojoba

Defatted jojoba meal prepared from the whole seed contained 2.5% tannins (Young, 1987) which is higher than the tannin concentration of legumes (Bressani et al., 1983; Price et al., 1980) and is lower than the 3.4% tannin recorded for high tannin sorghum varieties (Chavan et al., 1979). Wiseman and Price (1987a) reported 8.1% total polyphenolic content in jojoba meal, but separation and purification techniques yielded only trace amounts of

tannins.

Young (1987) showed that dehulling of jojoba seeds prior to oil extraction resulted in a significant reduction of tannins in jojoba meal. Carduso and Price (1980) indicated that both whole seed and dehulled jojoba meal contained high amounts of polyphenolic compounds which interfered with the preparation of protein isolate by alkaline extraction. Although this was attributed to tannins, no qualitative results were presented.

Although the presence of tannins in jojoba seeds has been established, their nutritonal significance has not been studied. The poor palatability, appetite depression, reduced feed efficiency, and low weight gain observed in animals fed jojoba meal are all symptoms that have been associated with dietary tannin intake (Glick & Joslyn, 1970a; Kumar & Singh, 1984). Reduced protein digestibility in the presence of tannins is well documented (Barroga, et al., 1985; Chavan et al., 1979; Ikeda et al., 1986; Laurena et al., 1984; Ramachandra et al., 1977). This study will attempt to determine the effect of tannins on the in vitro digestibility of jojoba meal proteins.

CHAPTER 2
JOURNAL ARTICLE

Authors Title Page

EFFECT OF TANNINS ON THE IN VITRO DIGESTIBILITY
OF JOJOBA MEAL PROTEINS

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ABSTRACT

Jojoba meals from whole seeds (WSJM), dehulled seeds (DJM), commercially processed defatted press-cake (DPC), and jojoba seed hulls (JSH) were prepared using batchwise hexane extraction. Jojoba protein concentrate (JPC) was prepared from DJM by isoelectric precipitation. Commercially solvent extracted jojoba meal (SEJM) and methanol extracted WSJM (MEJM) were also analyzed. Soy protein isolate (SPI) was used as a control. Protein content of the deoiled jojoba meals ranged from 26.1% for JSH to 35.5% for MEJM. JPC was 78.0% protein, and SPI was 93.2% protein. Dehulling and methanol extraction reduced tannin content to 0.7% from the 2.1% in WSJM. Both commercial meals (DPC and SEJM) contained about 1.0% tannin, JPC was 0.76% tannin, and SPI was 0.56% tannin. No significant difference was found between in vitro protein digestibility (IVPD) values for WSJM, DJM, JSH, and DPC (72.9%-73.7%). Improved IVPD was noted for MEJM (77.0%), SEJM (77.2%), and JPC (86.3%). Although adding back isolated tannins reduced the IVPD of MEJM, no reduction was noted for DJM or SPI.

INTRODUCTION

Jojoba (Simmondsia chinensis), a desert shrub, is being commercially propagated in arid areas worldwide for the valuable oil that comprises 50% by weight of its seeds (National Academy of Science (NAS), 1985). Jojoba meal, a by product of the growing jojoba oil industry, is, at present, of little economic value. Despite a 30% protein content (Yermanos and Duncan, 1976), attempts to utilize jojoba meal as an animal feed supplement have resulted in poor feed efficiency, reduced weight gain, and even death (Booth et al., 1974; Manos et al., 1986; Verbiscar et al., 1980, 1981). These undesirable effects have been attributed to the presence of toxic cyanoglycosides in jojoba seeds called simmondsins (Booth et al, 1974). While detoxification studies have shown improvement in feed intake and growth of poultry, sheep, and cattle (Verbiscar et al; 1980, 1981), there are little data on the effect of other antinutritional factors such as tannins on the nutritional value of jojoba meal.

The presence of tannins in jojoba meal has been established (Carduso and Price, 1980; Oldmark, 1986; Wiseman and Price, 1987a; Young, 1987), as well as their effects on protein utilization, amino acid availability, and digestive enzymes (Kumar and Singh, 1984). Tannins have also been linked to appetite depression (Glick and

Joslyn, 1970a), excretion of endogenous proteins (Glick and Joslyn, 1970b; Mitjavila et al., 1982), and poor palatability (How and Morr, 1982; Sosulski, 1979). In vitro protein digestibility is lowered by tannins in legumes (Barroga et al., 1984; De Lumen and Salamat, 1980; Elias et al., 1979; Laurena et al., 1984) and grains (Chavan et al., 1979; Chibber et al., 1980; Ikeda et al., 1986; Ramachandra et al., 1977).

Jojoba meal proteins have been extracted, characterized, and evaluated for amino acid content (Carduso and Price, 1980) and functionality (Wiseman and Price, 1987b), but data on digestibility are not available. This study will determine the in vitro digestibility of jojoba meal proteins. The relationship between tannin levels and digestibility of protein will also be addressed.

MATERIALS AND METHODS

Sample Preparation. Jojoba seeds were obtained from Hyder Jojoba Inc., Pheonix, AZ. and divided into two batches. Whole seed jojoba meal (WSJM) was prepared from one batch by grinding whole seeds in a Straub Model 4E grinding mill and extracting 3 times with hexane at room temperature. The meal was air dried and reground using a Scienceware Micro-mill to pass a 60-mesh screen. The second batch of jojoba seeds was dehulled by soaking the

seeds in water for 24-48 hours and removing the hulls manually with a knife. Dehulled jojoba meal (DJM) was prepared from air dried dehulled seeds following the same procedure as for WSJM. Jojoba seed hulls (JSH), which contained some seed material, were air dried and processed as for WSJM. Methanol extracted jojoba meal (MEJM) was prepared from WSJM by batchwise extraction with methanol/acetone (3:1). Extraction was repeated 3 times at room temperature. The meal was then vacuum filtered and air dried.

Jojoba protein concentrate (JPC) was prepared from DJM to obtain a low tannin product (Young, 1987). Using an isoelectric precipitation method, proteins were extracted twice with distilled water (1:100 w/v) adjusted to pH 8.5 with 1M NaOH. The extracts were centrifuged for 10 minutes (IEC clinical centrifuge, maximum speed) each time. The combined supernatants were adjusted to pH 4.0 with 1 M HCl to precipitate proteins and centrifuged for 10 minutes. The pellets were air dried and ground with a mortar and pestle.

Commercially processed cold pressed jojoba meal and solvent extracted jojoba meal were obtained from Desert King JMC, Ltd., Chula Vista, CA. Defatted press-cake (DPC) was prepared by batchwise hexane extraction, air dried, and ground. Solvent extracted meal (SEJM) was passed

through a 60-mesh screen and analyzed.

Samples were analyzed for moisture content and crude protein (macro-Kjeldahl method; $N \times 6.25$). Crude oil content was determined by Soxhlet extraction with petroleum ether (AOAC, 1984). All other analyses were performed on totally defatted samples after Soxhlet extraction.

Tannin Analysis. Tannins were determined both by the protein precipitation method of Hagerman and Butler (1978) with bovine serum albumin (Sigma Fraction V, #A-7906) as the standard protein and tannic acid (Sigma) as the reference standard and by the vanillin method following the parameters recommended by Price et al. (1978) with catechin (Sigma) as the reference standard. Protein precipitation assays were performed in triplicate, and vanillin assays were performed in duplicate. Means and standard deviations were calculated.

Extraction and Addition of Tannins. Partially purified tannins were prepared from WSJM using the method of Hagerman and Butler (1978) with minor modifications. Three 1.0 g samples of WSJM were extracted with methanol (30ml/g meal) while stirring for two hours at room temperature. After centrifugation, the extracts were passed through a Sephadex LH-20 column which had been previously equilibrated with methanol. Tannins were eluted

from the column with 50% aqueous acetone. Acetone was removed under reduced pressure in a rotary evaporator, and the tannin containing residue was combined with 1.0 g samples of DJM, MEJM, and SPI. The samples were oven dried and reground.

In Vitro Protein Digestibility (IVPD). The multienzyme technique of Hsu et al. (1977) was used to estimate protein digestibility. The enzymes used were: porcine pancreatic trypsin (Sigma Type IX; 18,300 BAEE units/mg protein), bovine pancreatic chymotrypsin (Sigma Type II; 51 units/mg powder), and porcine intestinal peptidase (Sigma Grade III; 115 units/g powder).

Digestions in triplicate were performed in a 37 degree C water bath with constant stirring. The multienzyme solution (1.24 mg/ml trypsin, 3.65 mg/ml chymotrypsin, 0.45 mg/ml peptidase) was held in an ice water bath prior to use. Aqueous suspensions of samples (25 ml; 6.25 mg protein/ml) were equilibrated in a water bath to 37 degrees C and adjusted to pH 8.0 with 0.1 N NaOH and/or HCl. The cold enzyme solution (2.5 ml) was then added, and the pH was recorded at 30 second intervals for 10 minutes.

The extent of decrease in pH after 10 minutes (Fig. 1) is highly correlated to in vivo apparent digestibility (Hsu et al., 1977). Digestibility was calculated using the

regression equation of Hsu et al. (1977): $Y = 210.64 - 18.103X$, where Y = % digestibility, and X = pH of the sample suspension 10 minutes after addition of the enzyme solution.

Statistical analysis. Means and standard deviations were calculated for all assays. Significant differences between IVPD results were determined using the Students t test.

RESULTS AND DISCUSSION

Values for crude protein, moisture, and crude oil contents are presented in Table I. Protein values for the meals of 28-33% are in agreement with values obtained in other studies (Verbiscar and Bannigan, 1978; Verbiscar et al., 1981; Yermanos and Duncan, 1976). The hull fraction was higher in protein than previously reported (Young, 1987) possibly due to varying percentages of seed cotyledon residue since the hulls were removed manually. The 75.6% protein content of JPC was higher than values reported by Wiseman and Price (1987a) for water extracted and spray-dried protein concentrates. It is theorized that further purification steps are necessary to equal the 85.5% protein content of SPI.

Since WSJM, DJM, and JSH were higher in residual oils than either MEJM, DPC, or SEJM, all tannin analyses and all digestions were performed on samples completely

defatted by Soxhlet extractions to eliminate oil content as a variable. Data in Table II contain the protein and tannin contents and IVPD of dry, oil free samples. These protein values were used to calculate the amount of sample used in each digestion.

Tannins. The two tannin assays used yield different information regarding the tannin content. The protein precipitation method assays only those polyphenolics which precipitate bovine serum albumin and does not distinguish between hydrolyzable and condensed tannins. Low molecular weight tannins do not precipitate protein and are therefore not measured (Price et al., 1980). This method is intended to provide information about the biological activity of tannins and the nutritional value of tannin containing foods (Hagerman and Butler, 1978). The vanillin assay is specific for the flavan-3-ol and flavan-3,4-diol ring structures found in condensed tannins, but this method does not distinguish between tannin and its monomeric components (Price et al., 1980).

Agreement between the two assays varies since both the degree of polymerization of the phenolics and the presence of low molecular weight phenolics affect the results in different ways (Hagerman and Butler, 1978). Although Price et al. (1980) found close agreement between the two assays when examining several varieties of pulses, Hagerman and

Butler (1978) found large quantitative differences in results when sorghum was assayed. The vanillin assay gave higher tannin values for 50% of the sorghum samples tested (Hagerman and Butler, 1978).

Since both assays are colorimetric methods, results were calculated as percent tannic acid or catechin equivalents by means of standard curves. The curve (absorbance vs. amount tannic acid) used for the protein precipitation method was linear ($r=0.9836$). Although results are interpreted as percent tannins, the conversion from tannic acid equivalents may not be direct since the method is 50% more sensitive for tannic acid than for purified sorghum tannins (Hagerman and Butler, 1978). The parameters set for the vanillin assay by Price et al. (1980) were designed to reduce the variability previously noted between laboratories. Following their recommendations, a linear standard curve ($r=0.9915$) was constructed using catechin concentrations up to 0.3 mg/ml and extrapolating to higher concentrations. Price et al. (1980) found that the catechin curve deviated considerably from linearity at higher concentrations while a purified sorghum condensed tannin curve did not. Catechin equivalents are also interpreted as percent tannins, but the values are not equivalent. For sorghum, the true percent by weight of extractable tannin was 42% of the

catechin equivalent values (Price et al., 1980).

Comparison of the results in this study of the two tannin assays showed that the majority of tannins present in jojoba are condensed tannins from the hulls, but there are also hydrolyzable tannins present in the seeds. Whole seed jojoba meal contains 2.44% tannins as measured by the vanillin assay and 2.10% as measured by the protein precipitation method. This indicates that the tannins are primarily condensed and that low molecular weight flavanols may also be present. This concentration exceeds the tannin content of all varieties of pulses analyzed by Price et al. (1980) and of common beans (Bressani et al., 1983) but is less than the amount in high tannin strains of sorghum (Chavan et al., 1979) and finger millet (Ramachandra et al., 1977) which have been reported to contain over 3.4% tannins.

The hull fraction contained only a slightly greater percentage of tannins than WSJM. In contrast, Young (1987) found that the hulls contained 7.4-9.6% tannins. The jojoba seeds in this study were soaked in water for a longer period of time, and Chavan et al. (1979) reported that soaking for 24 hours reduced tannin levels in sorghum. Dehulling greatly reduces tannins in jojoba meal. DJM retains 0.74% tannins as measured by protein precipitation, but only a trace of condensed tannins.

Methanol extraction of jojoba meal had a tannin reduction effect similar to dehulling.

Commercially obtained jojoba meals were lower in tannin than those prepared in the laboratory. This was especially true for SEJM which contained no condensed tannins that could be determined by the vanillin assay. It is hypothesized that commercial solvent extraction has an effect similar to methanol extraction under laboratory conditions. Defatted press-cake contained 2.03% tannins as measured by the vanillin method but only 1.10% tannins measured by the protein precipitation method. It is theorized that the vanillin value is more accurate since this is a whole seed meal that was not subjected to commercial solvent extraction and was expected to have a tannin level similar to WSJM. The results from the protein precipitation assay for this sample also had a high standard deviation.

The jojoba protein concentrate contained the same amount of protein precipitable tannins as the meal from which it was made. This was expected since tannin precipitation is maximum at pH values close to the isoelectric point of protein (Hagerman and Butler, 1980a). Soy protein isolate contained the lowest level of total tannins (0.56%). This made it a good control protein for IVPD since the tannins present were of the same type as in

the reduced tannin jojoba meals and JPC. Jojoba protein concentrate and SPI, like SEJM, contained no compounds measurable by the vanillin assay.

IVPD. The IVPD of jojoba meal proteins ranged from 72.9% for WSJM to 86.3% for JPC. There were no significant differences (Students t test, $p=0.01$) in IVPD for WSJM, DJM, JSH, or DPC. The average IVPD value for the four treatments was 73.4%. This value is similar to that of corn-milo grain which had the lowest IVPD (73.6%) of 23 protein sources reported by Hsu et al. (1977). A small but significant (4%) improvement in IVPD was observed for both MEJM and SEJM. These meals have a digestibility equivalent to that of whey protein (Hsu et al., 1977). The IVPD of JPC was 12.6% higher than that of WSJM, making it as digestible as wheat flour or bean protein (Hsu et al., 1977). The IVPD of SPI was 5% higher (91.4%) than JPC.

There was close agreement between replicate samples for all treatments. The IVPD results for JSH had the highest standard deviation (± 1.18).

Tannins and IVPD. The relationship between tannin content and IVPD of jojoba is not consistent. Methanol extraction reduced tannins and increased IVPD by 4%. This result was reversed when isolated WSJM tannins were added back (Table III). The 72.7% IVPD value for MEJM plus tannins is not significantly different from WSJM. Laurena

et al. (1984) observed a 3.6-5.1% reduction in IVPD for white cowpeas when isolated tannins from darker cowpea strains were added. Barroga et al. (1984) reported similar results for mung beans.

In contrast to these findings, dehulling reduced tannins in jojoba meal to the same levels as methanol extraction but did not increase IVPD. Adding isolated tannins to DJM actually increased IVPD. Dehulling of sorghum (Chavan et al., 1979) and finger millet (Ramachandra et al., 1977) greatly improved IVPD only for cultivars of these grains that had tannin levels over 3.4%. Since the tannin content of the WSJM used in this study was 2.1% and since the tannin content of the commercial meals was much lower, it is possible that this concentration falls below a threshold level required to affect the IVPD (Chavan et al., 1979) and that other factors are involved.

Many variables are involved in studying the effect of tannins on IVPD. Tannins do not have an equal affinity for all proteins. Affinity is highest for proline-rich proteins (Hagerman and Butler, 1980b). Jojoba proteins contain about 1% proline (Verbiscar and Bannigan, 1978); therefore, tannin-protein complex formation may be less extensive than for other plant proteins. Conditions employed in various IVPD methods may also affect results.

Acidic pH increases tannin-protein complex formation (Hagerman and Butler, 1980a). Tannin has the greatest effect on the IVPD of sorghum when acid hydrolysis with pepsin is the method used (Chibber et al., 1980). The method used in this study utilized pancreatic enzymes at pH 8.0.

CONCLUSIONS

Tannins in jojoba meal are high enough to warrant further research on anti-nutritional effects other than reduced IVPD. Animal feeding studies with jojoba meal could reveal poor palatability, reduced feed efficiency, and lowered weight gain (Manos et al., 1986; Verbiscar et al., 1980, 1981). Phenolic acids have taste thresholds of 40-90 ppm and contribute significantly to bitter and astringent flavors in oilseed flours (Sosulski, 1979). Tannic acid caused food intake depression and decreased growth in rats (Glick and Joslyn, 1970a). Dietary tannins also resulted in increased fecal nitrogen excretion from endogenous sources (Glick and Joslyn, 1970b) and intestinal mucus hypersecretion (Matjavila et al., 1977).

Methionine and lysine are the limiting amino acids in jojoba proteins (Carduso and Price, 1980). Tannins increase methionine deficiency in rats (Bressani et al., 1983). Hydrolyzable tannins are susceptible to oxidation and their oxidized derivatives form covalent bond with

lysine and methionine, rendering these amino acids unavailable (Sosulski, 1979). Thus, tannins could result in a substantial loss of the nutritive value of jojoba proteins.

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Figure 1. pH changes over time during IVPD. Values are the average of triplicate samples.

Table I. Crude Protein, Moisture, and Crude Oil Contents of Jojoba Meals, Jojoba Protein Concentrate, and Soy Protein Isolate.

Treatment	Crude protein (%)	Moisture (%)	Crude oil (%)
WSJM	28.2±0.70 ^a	6.97±0.27	11.20±0.47
DJM	29.2±1.07 ^a	9.97±0.21	9.47±0.56
JSH	22.7±0.23	2.96±0.01	10.08±0.09
MEJM	33.2±0.04	5.79±0.38	1.52±0.06
DPC	28.6±0.45	7.10±0.17	1.20±0.07
SEJM	29.8±0.99	5.91±0.06	1.85±0.13
JPC	75.6±0.95	3.04±0.05	---- ^b
SPI	85.5±1.90	7.86±0.12	0.50±0.06

^a Value is the average of triplicate samples + standard deviation. All other values are the average of duplicate samples ± standard deviation.

^b Not available due to limited supply of sample.

Table II: Protein, Tannins, and In Vitro Protein Digestibility (IVPD) of Oil-Free Jojoba Meals, Jojoba Protein Concentrate, and Soy Protein Isolate.

Treatment	Protein (%)	Tannins		IVPD ^a (%)
		Protein precipitation ^{a,c}	Vanillin ^{b,d}	
WSJM	34.1±0.70 ^a	2.10±0.28	2.44±0.05	72.9±0.00
DJM	35.8±1.07 ^a	0.72±0.16	0.09±0.02	73.7±0.82
JSH	26.1±0.23 ^b	2.48±0.08	3.32±0.13	73.2±1.18
DPC	31.2±0.45 ^b	1.10±0.32	2.03±0.02	73.7±0.44
MEJM	35.5±0.04 ^b	0.74±0.10	0.14±0.04	77.0±0.09
SEJM	32.3±0.99 ^b	1.00±0.22	---- ^e	77.2±0.00
JPC	78.0±0.95 ^b	0.76±0.14	---- ^e	86.3±0.18
SPI	93.2±1.90 ^b	0.56±0.04	---- ^e	91.4±0.72

^a Values are the average of triplicate samples ± standard deviation.

^b Values are the average of duplicate samples ± standard deviation.

^c % tannic acid equivalents.

^d % catechin equivalents.

^e No tannins detected by the assay.

Table III. IVPD of DJM, MEJM, and SPI Before and After Adding Isolated Tannins.

Treatment	IVPD ^a (%)	IVPD with tannins ^a added (%)
MEJM	77.0 $\pm$ 0.09	72.7 $\pm$ 0.90
DJM	73.7 $\pm$ 0.82	75.8 $\pm$ 0.28
SPI	91.4 $\pm$ 0.72	93.9 $\pm$ 0.18

^a Values are the average of triplicate samples $\pm$ standard deviation.

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CHAPTER 3

SUMMARY AND RECOMMENDATIONS

Summary

Defatted jojoba meals prepared from whole and dehulled seeds, as well as from jojoba seed hulls and commercially processed jojoba meals, were evaluated for protein and tannin contents and IVPD. Jojoba protein concentrate was prepared from dehulled jojoba meal and also analyzed. Commercial soy protein isolate was used as a control. Whole seed meal prepared in the laboratory contained 34.1% protein. Dehulled jojoba meal was slightly higher in protein at 35.8%, while the hull fraction was 26.1% protein. Whole seed meal, extracted with methanol, was 35.5% protein. Defatted jojoba press-cake and commercially solvent extracted jojoba meal contained 31.2% and 32.3% protein, respectively. Jojoba protein concentrate contained 78.0% protein, and SPI was 93.2% protein. All protein values computed on a dry, oil-free basis.

Condensed tannin levels in WSJM and DPC, as measured by the vanillin assay, are 2.44 and 2.03%, respectively. Although this concentration exceeds that found in legumes (Bressani et al., 1983; Price et al., 1980) and most grains (Chavan et al., 1979; Ikeda et al., 1986; Price et

al., 1980), it is lower than the 3.4% tannins reported for high tannin strains of sorghum (Chavan et al., 1979) and finger millet (Ramachandra et al., 1977). The hull fraction contained the highest amount of condensed tannins (3.32%). It is possible that extensive soaking prior to dehulling removed tannins from the hulls.

Dehulling and methanol and commercial solvent extractions all reduced condensed tannins to very low levels. Since tannins in these treatments were measured in the 0.72-1.00% range by the protein precipitation method, it is assumed that these are hydrolyzable tannins. Jojoba protein concentrate contained the same amount of tannin as the meal from which it was made.

The in vitro protein digestibility of the jojoba meals ranged from 72.9 to 77.2%. This compares favorably to some plant protein sources such as raw beans (Barroga et al., 1984) and corn-milo grain (Hsu et al., 1977); however, IVPD for jojoba meal is considerably lower than the digestibility of either wheat protein (Hsu et al., 1977) or SPI. Digestibility was improved to 86.3% for JPC.

The effect of tannins on the IVPD of jojoba meal proteins was not consistent. Dehulling did not improve IVPD. No significant difference was found between IVPD values for WSJM, DJM, JSH, and DPC which were between 72.9 and 73.7%. In contrast, dehulling of sorghum (Chavan et

al., 1979) and finger millet (Ramachandra et al., 1977) improved IVPD from 48% to 79% and from 55% to 93%, respectively. This improvement was reported only in strains of sorghum or millet in which the tannin concentration was above 3.4%, however.

Methanol and commercial solvent extractions improved IVPD about 4%. When tannins isolated from WSJM were added to MEJM, the improvement in IVPD was lost. This is comparable to results obtained for the effect of tannins on the IVPD of cowpeas (Laurena et al., 1984) and mung beans (Barroga et al., 1984).

Recommendatons

Although the in vitro digestibility of jojoba meal proteins is comparable to other plant protein sources currently in use, in vitro results need to be confirmed with animal feeding studies. Further research is also necessary to determine the effects of factors not analyzed in this study on the digestibility of jojoba meal proteins. Simmondsins are extracted by both methanol and water; therefore, a reduced simmonmdsin level may improve IVPD of MEJM and JPC. Proteins are denatured by both the organic solvents, hexane and methanol, used to prepare MEJM and SEJM and the acid precipitation used to prepare JPC. Denatured proteins are hypothesized to be more

readily digested by proteolytic enzymes (Belitz & Grosh, 1987), a factor which needs to be investigated.

Tannin levels in whole seed meal are high, but tannins do not appear to adversely affect IVPD. Both dehulling and methanol extraction reduced tannin levels, but neither process improved digestibility over the level found for untreated commercially solvent extracted meal. Dehulling is not economically feasible since hulls are difficult to remove and since the presence of hulls aids in the expression of jojoba oil (Verbiscar et al., 1980). Dehulling also did not improve the IVPD jojoba. Methanol extraction did improve digestibility. However, methanol is highly toxic, and methanol treated meal did not support mice as a sole protein source (Verbiscar et al., 1980). Continuing research on jojoba tannins should focus on gastrointestinal effects, bitterness, and reduced methionine availability.

Further research on jojoba protein concentrates is recommended. The in vitro protein digestibility of the jojoba protein concentrate prepared in this study compared favorably to that of soy protein isolate. Water extraction reduced simmondsin levels and improved the palatability of jojoba meal for livestock (Verbiscar et al., 1980). Other studies (Carduso & Price, 1980; Wiseman & Price, 1987b) indicate that the functional properties of jojoba protein

concentrates would make them valuable in food processing applications. The emphasis of future research might be to develop an acceptable water extracted protein concentrate from commercially processed jojoba meal.

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