Table I. Oligosaccharide Content of Soybean Seeds Cooked in Water for Different Periods of Time and at Different Soybean to Water Ratios

cooking time, min	residual oligosaccharides, g/100 g of seeds, on a dry weight basis					
	sucrose		raffinose		stachyose	
	1:3 ratio	1:10 ratio	1:3 ratio	1:10 ratio	1:3 ratio	1:10 ratio
0	4.35 a[a]	4.35 a	0.74 a	0.74 a	3.53 a	3.53 a
30	2.34 b	1.27 b	0.37 b	0.24 c	2.03 b	1.05 d
60	2.09 b	1.09 d	0.31 b	0.22 c	1.82 b	1.04 d
90	1.89 c	0.64 e	0.30 b	0.10 d	1.68 c	0.47 e

[a] For each oligosaccharide, means followed by different letters in the column or in the row were statistically different at the 5% level of significance.

of whole soybean seeds in a 1:10 soybean to water ratio removed variable percentages of raffinose and stachyose depending on cooking time. According to these investigators, the solubility, molecular weight, location, and natural binding form of the sugars within the cell all play an important part in the extent to which saccharides are extracted from soybean seeds. The alternative treatments of soaking in association with cooking of coated and uncoated seeds showed no significant difference at the 5% level. On a dry weight basis, 1.46% sucrose, 0.32% raffinose, and 1.28% stachyose were detected in seeds soaked for 12 h and then cooked for 90 min with or without coats in fresh water at the 1:3 soy to water ratio, whereas 0.76% sucrose, 0.11% raffinose, and 0.67% stachyose were detected when the 1:10 soy to water ratio was used.

The reduction in oligosaccharide content, on a percentage and dry weight basis, produced by cooking the seeds at the most effective soy to water ratio was 83.0% for sucrose, 86.5% for raffinose, and 86.7% for stachyose, which are statistically identical values ($P = 0.05$).

In view of these data, we may conclude that soybean soaking before cooking is superfluous for the reduction of saccharides as long as cooking is carried out for 90 min using a 1:10 soybean to water ratio. Coat removal from soaked seeds before cooking is also ineffective.

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Registry No. Sucrose, 57-50-1; raffinose, 512-69-6; stachyose, 470-55-3.

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Chemical and Biochemical Nature of Fodder Tree Leaf Tannins

The chemical nature and biochemical nature of tannins such as the relative degree of polymerization (estimated from the ratio of total flavon-3-ol residues to terminal flavon-3-ol residues), protein precipitating capacity as tannic acid equivalents, and relative tannin specific activity (estimated from the ratio of absorbance in the protein precipitation assay to absorbance in assay of total phenols), in relation to crude protein digestibility of fodder tree leaves, are determined. The results suggest that the tannin content and their protein precipitating capacity, not the polyphenolic with a high relative degree of polymerization, reflect on the crude protein digestibility in a negative relationship.

Fodder obtained from tree/shrub leaves (top feed) has been classified as emergency fodder for livestock, but it forms an integral part of feed for ruminants in arid and semiarid regions. In general, top feeds are a good source of protein for grazing animals (Jain and Beniwal, 1982). However, in some cases not only their crude protein digestibility has been observed to be low (Nath et al., 1969; Lohan et al., 1980; Diagayete, 1981) but also several episodes of loss of livestocks have been ascribed to the presence of a high quantity of tannins in them (Wolter,

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Table I. Crude Protein Digestibility vis-à-vis Chemical and Biochemical Nature of Tannins of Fodder Tree Leaves

	A. nilotica	*Z. nummularia*	*P. cineraria*
% crude protein	14.65	16.07	13.66
% crude protein digestibility	74.68	53.52	32.83
polyphenolics, catechin equiv, mg/g	325.00	90.00	125.00
tannins, catechin equiv, mg/g	64.00	100.00	160.00
% protein precipitating capacity (TAE)	3.0	5.4	10.6
relative degree of polymerization	2.0	0.44	0.65
relative tannin specific activity	0.014	0.101	0.12
relative protein precipitating capacity per terminal flavon-3-ol residue	0.28	0.909	1.10

1974; Sandusky et al., 1977; Naser et al., 1982). The most relevant property of tannins for such effects is its strong affinity for the enzyme and feed protein. Therefore, this communication presents the study on tannins and polyphenolics of common top feeds of arid and semiarid areas to explain their low protein digestibility.

EXPERIMENTAL SECTION

Leaves of *Acacia nilotica*, *Prospis cineraria*, and *Zizyphus nummularia* were collected in December 1982 from the Central Sheep & Wool Research Institute Farm. The leaves were dried, finely ground, and used for further analysis. Representative 0.1-g samples were extracted with 10.0 mL of methanol for 20 h in a 250-mL stoppered conical flask. After low-speed centrifugation the methanol extract was made to 10.0 mL by addition of a few drops of methanol.

Total polyphenolic content was measured by generally following the Prussian Blue assay of Price and Butler (1977). Extracts 0.1 mL were diluted with 60.00 mL of H_2O, and 3.0 mL of 0.05 M $FeCl_3$ in 0.1 N HCl was added. at t_0, 3.0 mL of 0.008 M $K_3Fe(CN)_6$ was added; 20 min later the absorbance was recorded. The protein (bovine serum albumin) precipitating capacity of 0.5 mL of extract was determined as described by Hagerman and Butler (1978) and modified by Bullard et al. (1981). The results were expressed as tannic acid equivalents (TAE) by using the standard curve prepared from 0.2 to 1.0 mg of tannic acid. The vanillin assay in methanol (for tannin determination) of 0.5-mL extracts was effected as described by Price et al. (1978) at 30 °C with a reaction time of 20 min. The vanillin reagent contained 4.0% concentrated HCl and 0.5% vanillin in methanol. For the measurement of anthocyanidin formation and vanillin assay in glacial acetic acid, 0.5 mL of extract was used according to the method of Butler (1982). The relative degree of polymerization, relative tannin specific activity, and relative protein precipitating capacity were calculated as suggested by Butler (1982). All the assays were carried out with the same extracts within 8 h of extraction.

Crude protein digestibility using pepsin and crude protein of the tree leaves (2.0 g) was estimated according to method described by Shirlaw (1967).

RESULTS AND DISCUSSION

Leaves of *A. nilotica*, *Z. nummularia* and *P. cineraria* are fairly rich in crude protein (Table I). However, the in vitro crude protein digestibilities of *Z. nummularia* (53.52%) and of *P. cineraria* (32.83%) are lower than that of *A. nilotica* (74.68%). Poor crude protein digestibility of *Z. nummularia* (39.0%) and *P. cineraria* (22.0%) in sheep has also been reported by Malik and Nath (1970) and Bohra (1980), respectively. The depressed crude protein digestibility may be ascribed to the presence of a higher quantity of condensed tannins in *Z. nummularia* (100.00 mg/g) and *P. cineraria* (160.00 mg/g) in comparison to that in *A. nilotica* (64.00 mg/g) assayed by vanillin. Plant tannins interact with leaf protein as well as with the enzyme (here pepsin) to form insoluble protein–tannin complexes (McLeod, 1974). Leaf protein, in the protein–tannin complex, may become resistant to pepsin action, whereas pepsin in the protein–tannin complex may loose its activity, resulting in the reduction in the crude protein digestibility. It is also observed that protein precipitating capacity of *Z. nummularia* and *P. cineraria* leaf extracts are high, i.e., 5.4% TAE and 10.6% TAE, respectively. Although *A. nilotica* contains 6.4% condensed tannins (polymerized flavon-3-ols) and 32.5% polyphenols (assayed by the Prussian blue method), it has lower protein precipiting capacity, i.e., total tannin (condensed and hydrolyzable), in comparison to other leaves (Table I).

McManus et al. (1981), by using purified protein and hydrolyzable tannin, demonstrated that polyphenols at low protein concentration associate at one or more sites on the protein surface to give a monolayer that is less hydrophobic than the protein itself. Aggregation and precipitation of proteins then ensue. When the protein concentration is high, a relatively hydrophobic surface layer is formed by complexation of the polyphenols on the protein and by the cross-linking of different molecules by the multidentate polyphenols. Therefore, an extract of *A. nilotica* leaves, although having a mixture of tannins, should have a higher protein precipitating capacity, as they contain highest content of polyphenolics (32.5%). On the contrary, their protein precipitating capacity is the lowest (Table I). This may be due to the higher relative degree of polymerization (2.0) of the polyphenols in *A. nilotica*. It has been shown that highly polymerized polyphenols have little protein precipitating capacity or astringency (Goldstein and Swain, 1965; Butler, 1982) due to their decrease in solubility. Therefore, the relative tannin specific activity and the relative protein precipitating capacity per terminal flavon-3-ol residue is lowest in *A. nilotica* (Table I). Further, the extremely low tannin specific activities in all the cases suggest that the Prussian blue assay is detecting a large amount of non-tannin material but that almost all the material detected by the vanillin assay is tannin rather than unpolymerized flavon-3-ols.

The higher protein precipitating capacity of *P. cineraria* tannin in comparison to that of *Z. nummularia* tannin, despite the lower relative degree of polymerization of the later, could be due to larger amounts of tannins in the former. Therefore, the present study indicates that the tannin content and their protein precipitating capacity, not the polyphenolic with a high relative degree of polymerization, reflect on the crude protein digestibility in a negative relationship.

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Electrophoretic Analyses of Selected Enzymes from Tomato Cultivars with Different Fruit Maturation

Acetone powders from Chico III and Homestead-24, two tomato (*Lycopersicon esculentum* M.) cultivars with different maturing characteristics, were prepared at the small green, mature green, turning, and ripe stages of fruit development, and their proteins were extracted. The electrophoretic patterns of six enzyme systems were investigated by disc gel electrophoresis. Malate dehydrogenase (EC 1.1.1.37), alcohol dehydrogenase (EC 1.1.1.1), acid phosphatase (EC 3.1.3.2), and leucine aminopeptidase (EC 3.4.11.2) patterns changed, and the number of their isozymes varied during maturation. The number of peroxidase (EC 1.11.1.7) isozymes increased from the mature green to the ripe stage in both cultivars. The number of esterase (EC 3.1.1.1) isozymes varied but was higher at the ripe stage in Chico III extracts than for Homestead-24. Total protein, on a fresh weight basis, decreased in both cultivars during maturation.

The softening that occurs during the ripening of the tomato fruit is the result of enzymatic breakdown of the cell wall polysaccharides. Hobson (1965) found a relation between firmness and polygalacturonase activity of tomatoes. Babbitt et al. (1973) associated the solubilization of the cell wall protopectin material by pectinase with fruit softening. The possibility that β-glycosidases may contribute to cell wall modification leading to softening has also been reported (Wallner and Walker, 1975). Recently, the loss of fruit firmness in the nonripening rin tomato mutant related to a decrease in cell wall components, notably galactose (Gross and Wallner, 1979). This decrease occurred in the absence of polygalacturonase activity and polyuronide solubilization, suggesting that other components could be involved in cell wall modification.

The enzymes involved during tomato ripening are not known. However, the presence of several enzyme systems in developing tomato fruit has been revealed by disc gel electrophoresis (Hobson, 1974). Several of these enzyme systems were reported to be present in dormant seeds and young tomato seedlings (Stein and Lime, 1978). Since many enzymes are in multiple molecular forms within a single cell, certain enzyme systems have been investigated in depth with respect to their physiological roles in fruit ripening (Hobson, 1974; Hulme, 1972). Pressey and Avants (1972) identified four forms of pectinesterase in extracts of green tomatoes and separated two forms of polygalacturonase forms from ripe tomato extracts using chromatographic techniques (Pressey and Avants, 1973a). Multiple polygalacturonase activity has also been found in ripening peaches (Pressey and Avants, 1973b) and cucumbers (Pressey and Avants, 1975).

The objectives of this study were to (1) confirm the presence of several selected enzymes at four stages of the tomato fruit ripening process and (2) determine the number of molecular forms of each enzyme at each development stage and ascertain if differences existed in enzymic forms between Homestead-24 (soft maturing) and Chico III (firm maturing) tomato cultivars.

MATERIALS AND METHODS

Plant Material. Field-grown tomato fruit from Chico III (C-III) and Homestead-24 (H-24) cultivars were used at the small green, mature green, turning, and ripe fruit stages (Besford and Hobson, 1973). Acetone powders were prepared by immersing 250 g of tomato slices in an acetone–dry ice mixture of −70 °C (Clements, 1965). This technique minimizes the action of proteinases on the enzymes present. The frozen powders were stored at −5 °C to delay denaturation.

Extraction of Proteins. Protein extracts were prepared from 2 g of acetone powder for each fruit stage, according to Hobson's method (1974). The protein content of the crude extract was measured by using the method of Lowry et al. (1951).

Polyacrylamide Disc Gel Electrophoresis. Polyacrylamide gels (7%) were prepared after Davis (1964). Four microliters of the crude protein extract was layered on the gel, and electrophoresis was carried out for 1 h using a current of 3 mA/tube. Electrophoretic separations were

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