H_2O_2 on polyunsaturated fatty acids contained in membrane phospholipids. Such lipid peroxidation is associated with formation of "holes" in the membrane permitting the efflux of hemoglobin (Barker and Brin, 1975). Another function of phenolic compounds acting against hemolysis may be a specific interaction of these inhibitors with membranous phospholipids and proteins leading to physicochemical stabilization of the membranes and reduction of their permeability (Lucy, 1972). This concept is supported by the experimental evidence provided by Leibowitz and Johnson (1971) for the incorporation of the phenolic antioxidant BHT in membranes, which results in their stabilization.

Species differences in the composition of erythrocyte membranes, particularly the higher content of polyunsaturated fatty acids in the phospholipid fraction of erythrocyte membranes in ruminant animals (Nelson, 1967), may contribute to the different susceptibility of erythrocytes of sheep, rats, and rabbits to the antihemolytic action of isoflavones.

The hemolysis-enhancing action of small amounts of isoflavones on rabbit erythrocytes is not entirely unexpected. As outlined above the antihemolytic action of isoflavones on sheep erythrocytes is at least partly due to the antioxidative property of the isoflavones. According to the scheme generally accepted (Sherwin, 1972) for the mode of action of antioxidants, peroxide radicals of lipids (RO_2) are trapped by the antioxidant (AH):

$$ROO\cdot + AH \rightleftarrows ROOH + [A\cdot]$$

This equilibrium depends on the structure of the lipid (R) and can even be shifted to the left (Witting, 1974). This may occur also in the herewith examined system, namely H_2O_2-oxidized rabbit erythrocyte membranes + isoflavones. Thereby, the hemolysis-enhancing activity of isoflavones exerted on rabbit blood cells in the presence of small amounts of H_2O_2 could be correlated to the particular structure of membrane phospholipids present in rabbit erythrocytes (Horn et al., 1974).

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Coumestrol Content of Fractions Obtained during Wet Processing of Alfalfa

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The concentration of coumestrol was determined in alfalfa, alfalfa fractions, and 16 selected food products of plant origin. More than 80% of the initial coumestrol of alfalfa remained in the fibrous residue (pressed alfalfa) that resulted from a juicing operation. Compared to fresh alfalfa, whole leaf protein concentrate (LPC) pressed alfalfa had higher coumestrol contents, and both green LPC and white LPC had lower contents. At alkaline pH levels, coumestrol was extracted more readily from fresh alfalfa and also resisted co-precipitation with white LPC during its heat coagulation. The coumestrol content of white LPC prepared under mildly alkaline conditions or by diafiltration was within the range found in other vegetable products.

Alfalfa is a promising source for the production of leaf protein concentrates (LPC) which can help meet the protein needs of the world. Our laboratory has developed two processes for the production of LPC. (1) The Pro-Xan process produces a dehydrated alfalfa meal for ruminants, LPC for nonruminants, and alfalfa solubles for possible use in single cell protein production (Kohler et al., 1968). (2) The Pro-Xan II process yields, in addition to the above-mentioned products, white LPC which is designed for human consumption (Edwards et al., 1975).

A large number of plant species, including a number of those consumed by humans, contain many compounds which exhibit biological activity in laboratory and farm animals (Bennetts et al., 1946; Bradbury and White, 1954; Ershoff, 1954). Some of the active substances in these

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plants have been identified as phenolic compounds such as isoflavones (clover and soybeans) and coumestrol (alfalfa and soybeans) (Bickoff et al., 1960; Bradbury and White, 1951; Cheng et al., 1953; Curnow, 1954; Guggolz et al., 1961; Wada and Yuhara, 1964; Wong and Flux, 1962). These compounds have the capacity to cause uterine enlargement in immature or ovariectomized mice, increased teat length in wether lambs, and other biological effects (Bickoff et al., 1962; Bradbury and White, 1954; Braden et al., 1964; Magee, 1963; Story et al., 1957). Since white LPC from alfalfa is being considered for human food purposes, it is desirable to process the alfalfa in such a manner that the coumestrol content is minimized. This paper reports the effects of some processing variables on the coumestrol content of alfalfa fractions and, also, compares the coumestrol content of some food products with that of white LPC.

EXPERIMENTAL SECTION

Preparation of Products. Freshly chopped alfalfa was treated with gaseous ammonia to a pH of 8.5 and pressed in a twin-screw press to yield a pressed alfalfa and a juice containing chlorophyll and protein (Knuckles et al., 1972). Pro-Xan (whole LPC) was prepared by heating 1000-ml batches of this juice with steam injection to 80–85 °C, separating the green protein curd from the solubles, pressing the curd to approximately 40% solids, and freeze drying.

Pro-Xan II (green LPC) and white LPC were prepared from alfalfa treated with sodium metabisulfite (1000 ppm of SO_2 fresh weight basis) but without ammonia as described by Edwards et al. (1975). Briefly, the green juice at pH 6 was heated rapidly to 60 °C, held for 20 s, cooled rapidly to 45 °C, and then centrifuged. The chlorophyll-containing fraction which sedimented in the centrifuge was adjusted to pH 8.5, heated to 95 °C, pressed to approximately 40% solids, and freeze dried. The white LPC was precipitated from the liquid fraction from the centrifuge by steam injection to 80 °C, collected by centrifugation, washed by resuspending in water at pH 4.5, and then freeze dried. In some cases, the white LPC was precipitated and washed at pH 8.5. Soluble white LPC was prepared from the chloroplast-free plant serum by diafiltration using a hollow fiber ultrafilter unit (Bio-Rad) with membranes of 30 000 mol wt cut off (Knuckles et al., 1975a). Briefly, the plant serum was added to the unit where pressure difference (600 mm vacuum) across the membrane caused water and nonprotein materials to pass through. As the liquid passed through the membranes, water was added continuously to replace it. The total volume of water added was equal to eight times the volume of serum. In experiments designed to determine the effect of pH on the coumestrol content of white LPC, 400-ml batches of chloroplast-free plant serum, adjusted to selected pH levels, were heated in beakers to 80 °C, and the coagulated protein was separated by centrifugation and decantation. The protein fraction was washed twice at the selected pH levels in water at a 20 to 1 dilution, and freeze dried.

Selected food products were obtained from commercial sources and, if not already dry, were freeze dried. All samples were ground to pass a 20 mesh screen before analysis.

Analytical Methods. Two-dimensional paper chromatography was used to demonstrate the presence of coumestrol in food products (Bickoff et al., 1967). Extraction of the products was by soaking in 75% ethanol for 16 h. Each extract and pure coumestrol were applied near one corner of individual chromatograms. The chromatograms were first developed in acetic acid–water (1:1, v/v), then in isopropyl alcohol–ammonium hydroxide (2:1, v/v). The presence of coumestrol in products was determined by comparison of chromatograms of the extracts with the chromatogram of coumestrol under ultraviolet light. Coumestrol was determined quantitatively by the method of Knuckles et al. (1975b). Briefly, chlorophyll was removed from alcohol extracts of freeze-dried samples by phasic distribution between chloroform and borate buffer at pH 10. The extracts and coumestrol standards were applied to chromatographic paper. The chromatograms were developed in acetic acid–water (1:1, v/v). Intensity of the coumestrol spots was measured fluorometrically without elution from the paper. The coumestrol content of extracts was determined from the galvanometric response curve for the coumestrol standards. The lowest level of coumestrol that could be measured reliably was 0.1 μg/g. Amounts less than 0.1 μg/g are designated as trace.

Moisture was determined by measuring weight loss following drying at 110 °C for 2 h in a forced-draft oven.

Table I. Coumestrol Content of Products from Wet Processing of Alfalfa

Product	Coumestrol, μg/g,[a] for run no. 1	2	3	4
	Pro-Xan Process[b]			
Whole alfalfa	118	11	15	24
Pressed alfalfa	142	12	16	39
Pro-Xan (whole LPC)	121	18	16	33
Alfalfa solubles	13	4	6	6
	Run no.			
	5	6	7	8
	Pro-Xan II Process[c]			
Whole alfalfa	14	38	32	25
Pressed alfalfa	18	42	38	33
Pro-Xan II (green LPC)	8	37	30	23
White LPC (pH 6.0, pH 4.5)[d]	4	15	17	7
Alfalfa solubles	Trace	Trace	Trace	Trace

[a] Dry weight basis. [b] Alfalfa of 21.2 to 23.8% protein was harvested and processed at selected times over a 5-month period. [c] Alfalfa of 20.8 to 23.2% protein was harvested and processed at 1-week intervals during October, 1974. [d] Heat coagulated at pH 6.0 and washed at pH 4.5 as described by Edwards et al. (1975).

RESULTS AND DISCUSSION

Coumestrol contents of alfalfa and alfalfa products are given in Table I. The coumestrol content in the whole alfalfa ranged from 11 to 118 μg/g. This wide range of values is attributed to climatic conditions and biological attack (foliar disease and insect damage) which are known to affect the production of coumestrol in the plant (Hanson et al., 1965; Loper and Hanson, 1964; Loper, 1968; Stuthman et al., 1966). These variations were reflected in the coumestrol content in the products obtained from the alfalfa. Pressed alfalfa and the whole LPC contained a higher level of coumestrol than did the whole alfalfa. However, both LPC fractions from the Pro-Xan II process contained less coumestrol than did whole alfalfa.

The pressed residue from the Pro-Xan II process contains more of the total coumestrol than the pressed residue from the Pro-Xan process (Table II). The difference in the amount of coumestrol extracted is due to processing under mildly acid conditions in the Pro-Xan II process rather than mildly alkaline conditions. This difference in the extractability of coumestrol in acid and

Table II. Distribution of Solids and Coumestrol in Products from Wet Processing of Alfalfa[a]

Product	Pro-Xan process, %		Pro-Xan II process, %	
	Solids	Coumestrol	Solids	Coumestrol
Pressed alfalfa	76.2 ± 2.09	84.6 ± 1.24	74.6 ± 1.14	93.0 ± 2.37
Pro-Xan	8.3 ± 0.38	10.8 ± 0.33		
Pro-Xan II			6.9 ± 0.61	6.2 ± 1.50
White LPC			2.1 ± 0.08	0.8 ± 0.14
Alfalfa solubles	15.4 ± 0.79	4.4 ± 0.92	16.4 ± 0.57	0.1

[a] Mean and standard deviation of the percent of the total for four samples; solids distribution data supplied by R. H. Edwards.

Table III. Effect of pH on the Coumestrol Content of White LPC's

Method and pH of coagulation	pH of wash	Coumestrol,[a] μg/g
Heat coagulation (80 °C)		
pH 6.0	4.5	14 (2)
	6.0	9 (3)
	8.5	5 (3)
	9.5	5 (2)
pH 8.5	4.5	9 (2)
	6.0	6 (2)
	8.5	3 (3)
	9.5	3 (2)
pH 9.5	6.0	7 (2)
	8.5	6 (2)
	9.5	2 (2)
Acid coagulation		
pH 3.5	3.5	24 (3)
pH 4.5	4.5	13 (3)

[a] Dry weight basis. Numbers in parentheses refer to number of samples.

Table IV. Coumestrol Contents of White LPC's and Other Plant Products

Product[a]	Coumestrol,[b] μg/g
White LPC	
Heat coagulated[c]	1.1 (2)
Diafiltered, freeze dried[d]	0.4 (2)
Alfalfa sprouts (fresh)	5.0 (2)
Soybean sprouts (fresh)	71.1 (3)
Soybean (dry)	1.2
Soybean meal, defatted (dry)	0.4 (2)
Soybean concentrate (dry)	0.2
Soybean isolate[e] (dry)	0.6 (3)
Green beans (frozen)	1.0 (2)
Snow peas (frozen)	0.6
Green peas (frozen)	0.4
Brussell sprouts (frozen)	0.4
Red beans (dry)	0.4
Split peas (dry)	0.3
Spinach leaf (frozen)	0.1
Corn protein (dry)	<0.1[f]
Safflower meal (dry)	<0.1[f]
Wheat germ (dry)	<0.1[f]

[a] Fresh or frozen samples were freeze dried prior to analysis. [b] Dry weight basis. Numbers in parentheses refer to number of samples. [c] Coagulated and washed at pH 8.5 in a pilot plant as described by Edwards et al. (1975). [d] Prepared as described by Knuckles et al. (1975a). [e] Precipitated by acid from a mildly alkaline extract of defatted meal. [f] Limit of analytical method.

base has been utilized in earlier work on its isolation and analysis (Bickoff et al., 1958; Knuckles et al., 1975b).

Heat coagulation and washing at pH 8.5 and 9.5 resulted in white LPC with relatively low coumestrol content because of the increased solubility of coumestrol at alkaline pH (Table III). The data show that the wash solution should be at or above the pH of the juice from which the protein is coagulated. Laboratory preparations of white LPC contained more coumestrol than pilot plant preparations when the pH of coagulation and washing were the same. The white LPC's coagulated and washed at pH 8.5 in the laboratory and pilot plant were 3 and 1 μg/g, respectively. The difference between the coumestrol content of laboratory and pilot plant preparations may result from differences in techniques for dispersing the coagula in the wash solutions and/or differences in the coumestrol contents of the original alfalfas.

Other methods of preparing white LPC are acid coagulation (Miller et al., 1975) and ultrafiltration/diafiltration (Knuckles et al., 1975a). The acid coagulated product has a higher coumestrol content than LPC coagulated and washed at or above pH 6 (Table III). The diafiltered (soluble) LPC has a lower coumestrol content than either the heat or acid coagulated LPC (Table IV). This soluble LPC contains only 0.4 μg/g coumestrol.

We felt it desirable to compare the coumestrol content of white LPC with that of conventional vegetable food products listed in Table IV. By two-dimensional chromatography, 13 of 16 vegetable products were shown to contain coumestrol. We confirmed the literature reports of coumestrol in soybeans and soybean sprouts (Wada and Yuhara, 1964), and in commercial samples of frozen peas and beans (*Chem. Eng. News*, 1958). Coumestrol was not detected in corn protein, safflower meal, or wheat germ. The coumestrol contents of selected vegetable products are given in Table IV. The samples of alfalfa and soybean sprouts were markedly higher than the other vegetables tested. In general, the white LPC's had coumestrol values quite similar to most of the other plant products that were examined.

The experiments reported in this paper have demonstrated that the conditions used in the preparation of white LPC are very important in determining the coumestrol content of the final product. As currently proposed in the Pro-Xan II process, the juice is expressed at pH 6, a pH which inhibits the extraction of coumestrol from the alfalfa (see Table II). In order to minimize co-precipitation of coumestrol and protein, the pH should be made alkaline before heat coagulation and washing (see Tables I and IV). If these conditions are used, it is possible to prepare white LPC from alfalfa with a coumestrol content comparable to common vegetables.

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A Compendium of Inorganic Substances Used in European Pest Control before 1850

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A list of 24 inorganic chemicals used in European agriculture up to 1850 for pest control is given, together with descriptions of their recorded usage from classical times. Attempts have been made to assess their possible efficacy.

The use of pesticides is sometimes considered to have dated from the latter half of the nineteenth century. However, a survey of the classical literature (Smith and Secoy, 1975) has shown that there were frequent references to chemicals and natural products which appear to have been used for the control of plant disease and for killing unwanted plants and animals. As a result of further research into contemporary writings, a compendium of inorganic chemicals used for pest control in European agriculture from earliest recorded times until the middle of the nineteenth century is now presented with attempts to assess their possible success.

ALUM

Reference is made to the use of alum as a fly repellant in the "Geoponika" (**13**, 12), compiled by Cassianus Bassus in the sixth or seventh century A.D., and also by Hill (1586, p 68). The insects were reputedly driven away from places where the compound had been sprinkled. Hill further maintained that flies would not touch plants which had been sprinkled with a mixture of alum, origanum, and milk.

From the eighteenth century onward alum became a common additive for seed steeps used for the prevention of smut diseases and as such was recommended by Mortimer (1721, p 84), Hale (1756, p 364), Duhamel du Monceau (1762, p 94), and Somerville (1800). An unknown contributer signing himself P.H. (*The Farmer's Magazine (Edinburgh)*, 1801) held that alum dissolved in tobacco liquor would kill caterpillars on gooseberry bushes.

Alum is now defined as hydrated potassium aluminum sulfate, but in the past the term was also generally given to other double sulfates containing aluminum. Alum is an astringent and could have acted as an insect repellant by changing the flavor of plants. The high osmotic pressure of a concentrated solution of alum or alum in tobacco solution could have an effect on soft-bodied forms.

ANTIMONY

A recipe of unknown authorship in *The Farmer's Magazine* (1778) called for 1 oz of cantharides and 1 oz of crude antimony to be powdered together and added to 0.5 lb of currants and 1 pint of oatmeal. This poisoned bait was to be placed near rats' nests together with a supply of water for the rats to drink after eating the mixture.

According to Taylor (1957, p 147), before the nineteenth century the word "antimony" was correctly applied only to the black mineral stibnite (the trisulfide). As a rat poison the above concoction should have been effective although the cantharides may have proved more toxic than the antimony.

ARSENICAL COMPOUNDS

Sandarach (or realgar) and orpiment (or auripigmentum) are sulfides of arsenic and were known and used by classical agriculturists. Arsenical compounds and "arsenic" (the oxide of the metal) have been in continuous use from early times as a poison to kill pests and vermin. Thus, the burning of arsenical sulfides to kill scorpions was referred to in the "Geoponika" (**13**, 9). The twelfth century Arab writer Ibn-Al-Awam (1864, Vol. 2, p 338) and Speed (1659, p 176) wrote that birds could be killed using baits treated with arsenical poisons. Worlidge (1669, p 194) and Ellis

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