Removal of Deleterious Glucosides from Safflower Meal[1]

C.K. LYON, M.R. GUMBMANN, A.A. BETSCHART, D.J. ROBBINS, and R.M. SAUNDERS,
Western Regional Research Laboratory, SEA, USDA, Albany, CA 94710

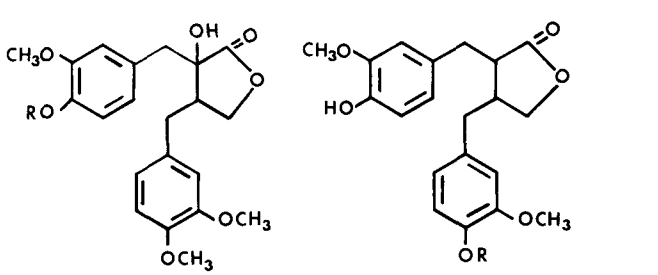
ABSTRACT

Safflower meal is a source of high quality protein for animal feeds but has not been used for human consumption because it is bitter and mildly cathartic. Deleterious glucosides in the meal were removed or modified by extraction with either water at the isoelectric point or with methanol, enzymatic treatment with β-glucosidase, dialysis, or by processing to prepare protein isolates. Analytical procedures were devised for quantitative measurement of two phenolic glucosides reported to be associated with bitterness and cathartic activity in safflower meal, and for estimating the cathartic activity of safflower meals and fractions.

INTRODUCTION

Safflower is grown primarily for its oil, which may be either the common high linoleic type or the more recently developed high oleic type. The seed contains ca. 40% oil, and the meal obtained after expression and extraction of the oil contains ca. 25-30% protein and 30-35% crude fiber. In the United States, safflower meal is commonly classified by screening (1,2) to yield a high protein fraction, containing ca. 42% protein and 16% crude fiber, and a high fiber fraction, containing ca. 20% protein and 38% fiber. These meals are used exclusively in animal feeds. Safflower meals and flours containing 50-60% protein and less than 5% crude fiber have been obtained by various laboratory processes (3,4). However, these meals are not suitable for human food since they are bitter and mildly cathartic (5).

Two phenolic glucosides have been isolated from safflower meal, identified and shown to be associated with the bitterness and cathartic activity (6-8); these are 2-hydroxyarctiin (cathartic) and matairsinol monoglucoside (bitter).



2-HYDROXYARCTIIN MATAIRESINOL MONOGLUCOSIDE

cathartic bitter

R = glucosyl

These β-glucosides and possibly other bitter or cathartic components that are soluble in alcohol or acetone have been extracted from safflower meal (3,4,9). The extracted meals were not bitter and produced little or no laxation when fed to rats. This paper describes further investigations of extraction and other methods of removing glucosides from safflower meal, and procedures for estimating the quantity of these constituents and their cathartic activity in safflower meal and fractions.

[1]Presented, in part, at the AOCS meeting, Cincinnati, OH, September, 1975.

EXPERIMENTAL PROCEDURES

Materials

Seed, expeller press cake and desolventized meal (42% protein, 16% crude fiber, 7% H_2O), all from commercial, high linoleic safflower, were obtained from PVO International Inc., Richmond, CA. Meal which was prepared from the seed by grinding in hexane, filtering, drying and sieving through a 40 mesh screen (10), contained 44% protein, 5.9% fat, 12.1% crude fiber, 7.2% ash and 6.9% H_2O. Meal prepared similarly (10,11) from the expeller press cake contained 51.4% protein, 0.7% fat, 8.4% crude fiber, 8.2% ash and 6.9% H_2O.

Hand dissection of safflower seeds yielded 61.9% kernel and 38.1% hull. Grinding and hexane-extracting of the kernel and hull yielded (based on dry seed) 22.2% kernel meal and 36.6% hull (oil free), or a total of 58.8% whole seed meal.

Safflower Meal Treatments

Extraction with alcohol. To achieve maximum extraction, safflower meal (51% protein) was extracted in a Soxhlet for 2 days with methanol to yield 19.0% extract solids and 75.9% extracted meal containing 65.1% protein, 12.1% crude fiber, 9.6% ash and 4.8% H_2O.

Extraction with water. Safflower meal (51% protein) was extracted 3 times with 10 times its weight of water maintained with dilute HCl at pH 5, the isoelectric point of safflower protein. Meal and water were stirred for 1 hr at room temperature and then separated by centrifugation. Based on the original meal, 25.3% combined extract solids and 72.6% extracted meal (64.2% protein, 11.6% crude fiber, 3.5% ash and 7% H_2O) were obtained.

Another lot of safflower meal (44% protein) was also extracted 3 times at pH 5 as above to yield 25.7% combined extract solids and 68.6% extracted meal (54.9% protein, 16.8% crude fiber, 2.1% ash and 3.7% H_2O). The water extracts were analyzed to determine the loss of meal constituents and the efficiency of extraction of 2-hydroxyarctiin and matairesinol monoglucoside.

Protein isolate preparation. A protein isolate was prepared as previously described (11) with a yield of 24.5% by extracting safflower meal (51% protein) with dilute NaOH at pH 9 and precipitating the extracted protein at pH 6. This isolate contained 91.3% protein (N x 5.3), 0.3% fat, 0.2% crude fiber and 0.6% ash (moisture-free basis).

Enzymatic hydrolysis of glucosides. A mixture of 300 mg safflower meal (44% protein), 10 mg β-glucosidase (Emulsin, Sigma Chem. Co.) and 3 ml pH 5 buffer (0.1 M sodium acetate) was shaken at 37 C for 2 days; then freeze-dried.

Safflower protein isolate (50 g) was shaken 1 hr at 37 C with 50 mg β-glucosidase in pH 5 buffer, separated in a centrifuge and freeze-dried.

Samples of 2-hydroxyarctiin and matairesinol monoglucoside were shaken 1 day at 37 C with an equal weight of β-glucosidase and 0.5 ml pH 5 buffer per mg sample. These solutions were examined directly by thin layer chromatography.

Dialysis. Three grams of safflower meal (44% protein) in 20 ml water was dialyzed in a cellulose acetate casing (Visking Co.) against water at pH 5 for 2 days at 4 C; then freeze-dried to yield 2.10 g meal containing 56% protein,

4.3% ash and 4% H_2O. A sample of safflower protein isolate was dialyzed similarly against water at pH 6.

Analytical Methods

AOAC procedures (12) were used for proximate analyses. Unless indicated otherwise, protein is expressed as N x 6.25. Nonprotein N is defined as N not precipitated from an aqueous extract of safflower meal by 10% trichloroacetic acid. Sugars were measured by the phenol-sulfuric acid method (13). Phenols were determined using the Folin-Ciocalteu reagent without added cupric ion (14).

2-Hydroxyarctiin and matairesinol monoglucoside were determined by thin layer chromatography (TLC) of safflower extracts on 5 X 10 cm plates (0.25 mm silica gel F254, E. Merck). These extracts were prepared by shaking 0.25-0.50 g samples overnight at room temperature in 5 ml of 80% methanol. For qualitative investigations, the plates were spotted with 5 µl of these extracts along with reference standards of 2-hydroxyarctiin and matairesinol monoglucoside, 3-5 µl (2 mg/ml). Plates were developed with $CHCl_3/MeOH/H_2O$ (65:25:4) and visualized under ultraviolet light (254 nm). Free phenolic compounds were then located by the blue color obtained when the plates were sprayed with a fresh 1:1 mixture of 0.2 M $FeCl_3$/0.2 M $K_3Fe(CN)_6$. The spots of 2-hydroxyarctiin and matairesinol monoglucoside overlap slightly at about Rf 0.5 (Fig. 1a and b). Since matairesinol monoglucoside has a free phenolic hydroxyl group, its spot is blue.

For quantitative measurement of 2-hydroxyarctiin and matairesinol monoglucoside, the plates were streaked with 50 µl of extract, developed and visualized under 254 nm light as above. The band containing 2-hydroxyarctiin and matairesinol monoglucoside was scraped off and eluted with 4 ml of 80% methanol. The combined absorbance of 2-hydroxyarctiin and matairesinol monoglucoside in the eluate was measured in a 1 cm cell at the maximum at 279 nm and corrected for the slight absorbance of eluates from a blank plate. The concentration of matairesinol monoglucoside was then measured separately in a 2 ml sample of the eluate which was evaporated under N_2, dissolved in 2 ml water, mixed with 10 ml of 2% Na_2CO_3 in N/10 NaOH; then with 1 ml of 1 N Folin-Ciocalteu reagent (14). The absorbance of this solution was measured in a 5 cm cell at 750 nm and the concentration of matairesinol monoglucoside calculated from a standard curve. From a standard curve, the absorbance at 279 nm of this concentration of matairesinol monoglucoside was calculated. This absorbance was subtracted from the combined absorbance of 2-hydroxyarctiin and matairesinol monoglucoside at 279 nm to give the absorbance of 2-hydroxyarctiin alone. Using a standard curve, the concentration of 2-hydroxyarctiin was calculated. Below are linear regression equations for the standard curves used in this analysis. Concentrations are expressed as mg/4 ml.

$$[\text{2-hydroxyarctiin}] = 0.4112\ \text{Abs}_{279} - 0.0036$$

$$[\text{matairesinol monoglucoside}] = 0.4045\ \text{Abs}_{279} - 0.0076$$

$$[\text{matairesinol monoglucoside}] = 0.2058\ \text{Abs}_{750} - 0.0018$$

Assay for Cathartic Activity

This assay was patterned after the work of Masri (15) and Booth (8, Palter, et al.) but includes a study of dose-response effects. Weanling, male Sprague-Dawley rats, caged separately, were conditioned 3 days on a semipurified corn meal-casein diet and then fasted overnight. Ten grams of test diet were presented on each of the following 2 days. Test diets consisted of corn meal-casein diet plus test substance added at the expense of the former. Powdered cellulose was added so that each diet contained the same amount of nondigestible fiber.

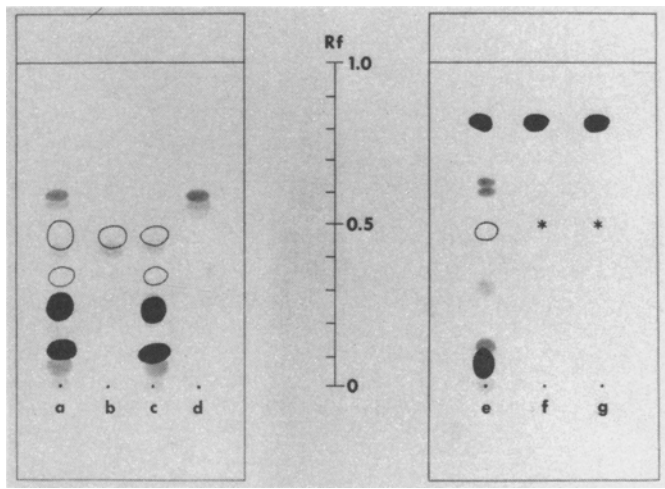


FIG. 1. TLC chromatograms on 5 x 10 cm plates (0.25 mm silica gel F254, E. Merck). Developed with $CHCl_3/MeOH/H_2O$(65:25:4), visualized under 254 nm light, then sprayed with $FeCl_3$-$K_3Fe(CN)_6$. *Untreated safflower fractions:* (a) meal (44% protein); (b) 2-hydroxyarctiin + matairesinol monoglucoside; (c) kernel meal; (d) oil-free hull. *Safflower fractions after treatment with β-glucosidase:* (e) meal (44% protein); (f) 2-hydroxyarctiin; (g) matairesinol monoglucoside; * location of component before glucosidase treatment.

Cathartic activity was estimated from the number of wet fecal pellets that stuck to and stained a sheet of white filter paper placed under the cage each day. Best results were obtained during the 24-48 hr period.

For each assay, a dose-response relationship was determined by feeding a control safflower meal at levels from 0 to 25% of the diet (Table I, bottom). Dose-response correlations were calculated for log (wet fecal count + 1) vs. log percent safflower meal. It was necessary to determine this dose-response relationship for each assay since each group of rats responded differently. The amount of safflower meal required to produce the same effect as each test diet was calculated from the linear portion of the dose-response curve. Relative potency was calculated as the safflower meal equivalent of the test diet divided by the percent sample in the diet (Table I). Although the large variation inherent in this bioassay must be considered, this assay was useful for estimating relative cathartic activities of samples.

RESULTS AND DISCUSSION

Phenolic Glucoside Content of Safflower Seed Fractions, Meals and Isolates

The amounts of 2-hydroxyarctiin and matairesinol monoglucoside found in safflower meals and seed fractions are listed in Table II. Included are the values for oil-free, hand-dissected kernel and hull from commercial seed. All of the 2-hydroxyarctiin and matairesinol monoglucoside appear to be contained in the kernel. This can be seen in Fig. 1 c & d. In the chromatogram of the hull, no spot was visible at the Rf for 2-hydroxyarctiin and matairesinol monoglucoside. In an eluate from that location (Rf 0.47), a small amount of a free phenolic compound was found, which was probably caused by tailing from the preceding spot. Other analyses were corrected for this tailing. The TLC fraction (Rf 0.47) containing the glucosides 2-hydroxyarctiin and matairesinol monoglucoside from pure kernel meal was found by phenol-sulfuric acid analysis (13) to contain 0.35 g glucose/ g (2-hydroxyarctiin + matairesinol monoglucoside); (theory 0.33 g glucose/g). The amounts of 2-hydroxyarctiin and matairesinol monogluco-

TABLE I

Cathartic Assay of Extracted Meals, Extracts and Isolates Prepared from Safflower Meal (51% Protein)

Safflower sample	Percent of diet[a] %	Wet fecal count (mean + S.E.)		Safflower meal equivalent, mean[b]	Relative potency[c]
Run 1					
MeOH-extracted meal	19.0	5.0 ± 1.0		4.6	0.24
MeOH extract solids	4.95	47.4 ± 9.0		13.0	2.63
				17.6	
pH5 Water-extracted meal	18.2	5.8 ± 2.0		4.4	0.24
Water extract solids	6.60	66.0 ± 7.3		16.1	2.45
				20.5	
Run 2					
Protein isolate (91% protein)					
untreated	25.0	15.4 ± 1.3		4.4	0.18
dialyzed	25.0	16.6 ± 1.8		4.7	0.19
β-glucosidase treated	25.0	16.1 ± 2.7		4.2	0.17
		Run 1	*Run 2*		
Safflower meal (51% protein)	0	1.1 ± 0.4	11.4 ± 2.0		
	5.00	7.5 ± 2.1	18.4 ± 2.4	dose	
	8.55	21.5 ± 4.5	24.8 ± 2.5	response	1.00
	14.6	54.0 ± 7.0	43.1 ± 6.5	curves	
	25.0	70.9 ± 8.5	53.8 ± 9.3		

[a]Replaced indicated percent of corn meal/casein basal diet. Equal amounts of nondigestible fiber attained in all diets by addition of powdered cellulose. Fed 8 rats per diet.

[b]Calc. from dose-response curve (linear regression) of log percent safflower meal vs. log (wet fecal count + 1), omitting 0 and 25% safflower meal diets.

[c]Equals safflower meal equivalent/percent of diet.

side in the meals increased in the order: whole seed, commercial, laboratory, kernel; approximately in proportion to the amount of kernel material in the meal.

Substances removed from safflower meal by three successive extractions with pH 5 water are shown in Table III. Twenty-seven percent of the meal solids were extracted and the largest component of the extract was the sugars. Extraction removed 7.5% crude protein, but 80% of this was found to be nonprotein N compounds. Total amounts of 2-hydroxyarctiin and matairesinol monoglucoside in the extracts were 84 and 49%, respectively, of that in the original meal.

The reduced contents of phenolic monoglucosides in safflower meal after extraction with water at pH 5 or with methanol are shown in Table II. The 2-hydroxyarctiin and matairesinol monoglucoside contents of the methanol and water-extracted meals were 0 and 15%, respectively, of those in the starting meal.

The reduced phenolic monoglucoside contents obtained by processes other than extraction are also shown in Table II. A safflower protein isolate, prepared by a process that would be expected to eliminate water soluble impurities, contained only 11% as much 2-hydroxyarctiin and 15% as much matairesinol monoglucoside as the original meal. Expressed as a percent of protein, the contents of these phenolic glucosides were reduced 91-94%.

Since dialysis removed most of the 2-hydroxyarctiin and matairesinol monoglucoside from safflower meal (Table II), membrane filtration processes, such as ultrafiltration, should be suitable for purifying the meal.

β-Glucosidase treatment of safflower meal appeared to have hydrolyzed all the 2-hydroxyarctiin and most of the matairesinol monoglucoside. Thin layer chromatographic studies of this hydrolysis are shown in Fig. 1a & e. After enzyme treatment, most of the 2-hydroxyarctiin plus matairesinol monoglucoside spot at about Rf 0.5 disappeared and a new free phenol spot appeared at about Rf 0.8. After enzyme treatment of the pure glucosides (Fig. 1f & g), the original spots at about Rf 0.5 disappeared and the free phenol spots of 2-hydroxyarctigenin and matairesinol appeared at Rf 0.8. It was also found that autolysis of safflower meal for 2 days at 37 C in water at pH 5 reduced the 2-hydroxyarctiin content by 53% and the matairesinol monoglucoside content by 65%. Hydrolysis of glycosides often causes changes in their physiological activity. This has been reported for matairesinol monoglucoside which loses its bitterness when hydrolyzed to matairesinol (6). Similarly, the cathartic activity of 2-hydroxyarctiin may be lost on hydrolysis to 2-hydroxyarctigenin, but this needs to be investigated further.

Cathartic Activity of Processed Meals and Protein Isolates

Cathartic activities of extracted safflower meals, extracts and protein isolates are listed in Table I. Activities are calculated as relative potencies compared to that of untreated safflower meal as 1.00. The cathartic activity of both methanol and water-extracted meals was 24% of that of the original meal even though the methanol-extracted meal contained no 2-hydroxyarctiin, while the 2-hydroxyarctiin content of the water extracted meal was 15% of that of the original meal. The extracts, with their high 2-hydroxyarctiin contents, had high cathartic activities. In another assay, pure 2-hydroxyarctiin was found to have a relative potency of 17. The percent of extracted meal or extract fed was that which could be obtained from the unextracted safflower meal as 25% of the diet. The sums of the safflower meal equivalent values calculated from the bioassay for each extracted meal plus its extract, 17.6 and 20.5%, are close to the 25% meal equivalent actually used.

The cathartic activity of the safflower protein isolate was 18% of that of the meal from which it was prepared. Dialysis and β-glucosidase treatment reduced slightly the phenolic glucoside contents of this isolate (Table II) but did not further decrease its low cathartic activity.

These studies have demonstrated that several methods are available for removing deleterious glucosides from safflower meal. Choice of a method would depend on costs and the functional properties desired. It has already been demonstrated (3,10,11,16,17) that properly processed

TABLE II

Phenolic Glucosides in Safflower Seed Fractions, Meals and Isolates

Safflower sample	2-Hydroxyarctiin, %[a]	Matairesinol monoglucoside %[a]
Seed fractions		
kernel meal	2.19	0.48
hull	0	0
whole seed meal[b]	0.83	0.18
whole dry seed[b]	0.49	0.11
Meals		
commercial (42% protein)	1.62	0.39
prep. in laboratory:		
from seed (44% protein)	1.93	0.57
from expeller cake (51% protein)	1.93	0.41
Extracted meals and extracts[c]		
MeOH-extracted meal	0	0
MeOH extract solids	10.3	2.27
pH5 water-extracted meal	0.28	0.06
water extract solids	6.47	1.06
Protein isolate (91% protein)[d]	0.21	0.06
Dialyzed and enzyme-treated samples		
protein isolate (91% protein)-		
dialyzed	0.05	0.04
β-glucosidase treated	0.13	0.08
meal (44% protein)-		
dialyzed	0.15	0
β-glucosidase treated	0	0.10

[a]Concentrations on moisture-free basis.

[b]Calculated from kernel meal + hull.

[c]From 51% protein meal.

[d]Protein = N x 5.3.

TABLE III

Substances Extracted from Safflower Meal (44% protein) by Water at pH 5

Extract No.[a]	1	2	3	Total
Amounts extracted, as percent of original meal solids	%	%	%	%
Solids	19.9	4.94	1.83	26.7
Sugars[b]	6.97	0.97	0.24	8.18
Ash	4.40	0.89	0.27	5.56
Protein[c]	1.10	0.27	0.12	1.49
Nonprotein N x 6.25[d]	3.68	1.42	0.90	6.00
Total free phenols[e]	2.61	1.08	0.57	4.26
2-Hydroxyarctiin	1.07	0.42	0.13	1.62
Matairesinol monoglucoside	0.16	0.09	0.03	0.28

[a]Extracted each time with 10 x wt. of orig. meal.

[b]Calc. as sucrose.

[c]6.25 x N precipitated from extract with 10% trichloroacetic acid.

[d]6.25 x N not precipitated from extract with 10% trichloroacetic acid.

[e]Calc. as matairesinol monoglucoside even though this makes up only 17% of the free phenols extracted.

safflower proteins possess a variety of useful nutritional and functional properties and may be effectively incorporated into select foods. Analytical methods developed should also assist plant breeders in trying to produce safflower plants with reduced quantities of bitter and cathartic components.

ACKNOWLEDGMENTS

We thank R.H. Purdy, PVO International, Inc. for supplying safflower seed, meal and expeller cake, K.V. Smith and coworkers for assistance with pilot plant experiments, L.V. Richards for the proximate analyses and R. Palter for authentic samples of 2-hydroxyarctiin and matairesinol monoglucoside.

REFERENCES

1. Kopas, G.A., and J.A. Kneeland, U.S. Pat. 3,271,160, Sept. 6, 1966.
2. Huff, R.F., and K. Robe, Food Proc. 34(5):46 (1973).
3. Kohler, G.O., in "World Protein Resources," Edited by R.F. Gould, Advances in Chemistry Series, No. 57. American Chemical Society, Washington, D.C., 1966.
4. Goodban, A.E., and G.O. Kohler, U.S. Pat. 3,542,559, Nov. 24, 1970.
5. Kohler, G.O., and C.K. Lyon, in "Food Proteins," Edited by J.R. Whitaker and S.R. Tannenbaum, Avi Publishing Co., Westport, CT, 1977.
6. Palter, R., and R.E. Lundin, Phytochemistry 9:2407 (1970).
7. Palter, R., W.F. Haddon, and R.E. Lundin, Ibid. 10:1587 (1971).
8. Palter, R., R.E. Lundin, and W.F. Haddon, Ibid. 11:2871 (1972).
9. Kohler, G.O., D.D. Kuzmicky, R. Palter, J. Guggolz, and V.V. Herring, JAOCS 43:413 (1966).
10. Betschart, A.A., J. Food Sci. 40:1010 (1975).
11. Betschart, A.A., and R.M. Saunders, Ibid. 43:713 (1978).

12. AOAC, "Official Methods of Analysis," 12th ed., Association of Official Analytical Chemists, Washington, DC, 1975.
13. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, Anal. Chem. 28:350 (1956).
14. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall, J. Biol. Chem. 193:265 (1951).
15. Masri, M.S., L.A. Goldblatt, F. DeEds, and G.O. Kohler, J. Pharm. Sci. 51:999 (1962).
16. Betschart, A.A., R.Y. Fong, and M.M. Hanamoto, J. Food Sci. (In press), (1979).
17. Betschart, A.A., U.S. Pat. 4,072,669, Feb. 7, 1978.

[Received May 18, 1978]