COLOR OF COTTONSEED FLOUR AND ISOLATES AS AFFECTED BY MIXED SOLVENT EXTRACTION[1]

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ABSTRACT

Filter cake produced by the liquid cyclone process from glanded cottonseed was extracted with azeotropic mixtures of hexane and eight other solvents such as acetone, acetic acid, ethanol, benzene, and chloroform. Portions of the resulting flours were evaluated as such, and the other portions were used to prepare single-step isolates. Color of extracted flours and corresponding lyophilized isolates prepared from them was determined with a colorimeter and also by incorporating them into a biscuit mix. Some of the solvent mixtures improved the color of extracted flours, but neither the color of isolates made from these flours nor the resulting biscuits were improved. Most of the biscuits prepared from the isolates were darker than those made from the corresponding flours. The isolates also retained almost all of the original gossypol in the extracted flours, suggesting that gossypol has an effect on color. Gossypol cannot be removed by aqueous washes during isolate preparation.

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Cottonseed protein is an important potential source of high-quality proteins for human use (1). The presence of pigment glands containing gossypol and other colored pigments has been the major obstacle in using cottonseed flour in food products (1–3). Development of glandless varieties and the liquid cyclone process (LCP) for removing cottonseed glands has made it possible to incorporate nutritious proteins from this source in human foods (2–4). Research in our laboratory to develop spray-dried cottonseed protein isolates has shown that the greenish tan color of the isolate tends to be darker than the starting flour. Also, the isolates from LCP flour are slightly darker than those from glandless flours. Others (4,5) have also shown that both LCP and glandless flours impart a yellowish color to some of the food products when these flours are incorporated at levels of 10% or higher. Color and appearance are the primary initial considerations in accepting or rejecting foods.

Berardi et al (6) reported that lyophilized cottonseed isolates were a pale cream color, but became medium tan on spray drying. Kim et al (7) concluded that in glandless cottonseed isolates, free and total gossypol did not contribute to the undesirable color, and suggested that the color-imparting components were not polyphenolic in structure. No gossypol values were given to substantiate their hypothesis, however, and their work was done on glandless flour and isolates.

The exact chemical nature of the discoloration imparted by cottonseed protein products remains largely unknown, even though several classes of color-causing components have been isolated from cottonseed (8–10). These are gossypol and gossypol-related compounds, phenolic acids, and flavonoids. Most of the gossypol is present in the free form in the native (undamaged) seed and is reduced to less than 0.045% free and about 0.2% total gossypol during LCP (3).

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Maga et al (11) reported that cottonseed flours have a much higher free phenolic-acid content than do the isolates, suggesting that these acids are removed during isolate preparations. Pratt and Wender (9,10) isolated several flavonoids from cottonseed. Judging from the methods of extraction and isolation they used, flavonoids are probably present in relatively small amounts in the flour and in lesser amounts in the isolates. Therefore, they do not appear to be the major cause of discoloration in the cottonseed products. Gossypol in both free and bound form remains the important potential color-causing components in cottonseed products.

It was theorized that a second extraction of solvent-wet LCP filter cake with a mixture of hexane and some other solvents might remove the color-causing components. Some of the solvents used, either alone or in combination, have been evaluated previously (12–16), but only for their ability to extract total lipids and gossypol from glanded cottonseed to improve the meal for animal feed. None of these solvent mixtures, however, was evaluated as capable of improving the color characteristics of low gossypol-containing cottonseed protein products such as LCP flour.

The present study was made to help elucidate the nature of the color problem, to study the role of gossypol, and to develop methods to remove the color-causing components from LCP flours and the isolates made from them.

MATERIALS AND METHODS

LCP filter cake was used in this study. A careful review of the LCP process showed that filter cake containing hexane would be the ideal starting material for removing objectionable color. Hexane, a nonpolar solvent, apparently does not remove the color-causing components of glanded cottonseed during LCP. A desolventized flour was not used, because the heat applied would tend to fix the color-causing components, which would be difficult to remove in subsequent extractions.

Portions of LCP filter cake containing 36% hexane, 0.6% residual lipid, 2.7% moisture, and free and total gossypol contents of 0.03% and 0.13% (moisture- and solvent-free basis), respectively, were batch-extracted with azeotropic mixtures of hexane and other solvents such as acetone, acetic acid, ethanol, ethyl acetate, isopropyl alcohol, benzene, and chloroform, as well as an azeotropic mixture of chloroform and methanol. Solvent/meal ratio was 3:1 by weight.

Extractions were conducted at both room temperature and at boiling points of the azeotropic mixture. For room temperature extractions, the mixture was stirred for 1 hr and then filtered through an Ametek cloth filter (WNH-V2mB-OD5). Boiling temperature extractions were conducted overnight in Soxhlet-type equipment. The extracted filter cake was desolventized at a temperature below 75° C in a vacuum oven and milled in a Waring Blendor to a particle size of less than 150 μ. Some of the extracted flours were used to prepare single-step isolates (6), which were adjusted to pH 7.0 and freeze-dried. The extracted flours and the lyophilized isolates were made into wafers (17), and their color was evaluated with a Hunter Colorimeter (HC). Two readings were taken on each of the wafers, and the values were averaged. Extracted flours and their isolates were also incorporated into a bleached wheat flour commercial biscuit mix at weight

ratios of 10% and 7.2%, respectively. The biscuits contained the same amounts of cottonseed protein. Tap water (23 g) was added to 20 g of the test biscuit mix, which was baked at 204° C for 25 min. An informal panel evaluated the color of the baked biscuits on a scale of 1 to 5. For comparison, crumbs of five progressively darker biscuits ranging from 1, a yellowish white biscuit, to 5, a greenish tan biscuit, were selected as standards. This procedure was used, because the open texture of the biscuit crumbs prevented repeatable values on the HC.

The solubility of 0.015% gossypol-acetic acid aqueous solution, representing the approximate total gossypol concentration of LCP flour in aqueous slurry during isolate preparation, was determined at various pHs. The solubility was measured as follows: A 0.015% reference gossypol acetic acid solution was prepared in deionized water at pH 12.0. The pH of the aliquots was adjusted to specific values by adding dilute H_3PO_4. The solutions were centrifuged under conditions comparable to those used for isolate preparation. The pH of the supernatant was adjusted back to 10.0 by adding dilute NaOH. Acetone was added to make a 70% acetone solution. Free gossypol was determined by official AOCS methods (18).

RESULTS AND DISCUSSION

The Hunter color values and the gossypol content of flours extracted with hexane and the various azeotropes, as well as the visual color of the biscuits containing 10% extracted flour, are presented in Table I. The hexane-extracted flour served as a control. The color of all azeotrope-extracted flours was improved. In general, the color of flours extracted at boiling temperatures was slightly lighter than that extracted at room temperature. Lighter colored flours tended to have lower free or total gossypol or both, suggesting that gossypol affected the color. This general observation had some exceptions. In the case of chloroform/hexane azeotrope extraction, the flour extracted at room temperature had a lighter color than did the flour extracted at the boiling temperature.

Improvement in color of extracted flours did not improve the color of biscuits significantly. Except for three flours extracted at room temperature, the color of biscuits in which the flours were incorporated did not improve. The exceptions were flours extracted by isopropyl alcohol/hexane, chloroform/hexane, and chloroform/methanol azeotropic mixtures. Also, improvement in the three flours was only minor compared with the control.

The flours extracted at boiling temperatures darkened the biscuits more than did those extracted at room temperature. The exact cause of this darkening effect is not clear, because the flours extracted at boiling temperatures usually had lower free and bound gossypol. Free gossypol, and perhaps other color-causing components such as phenolic acids and flavonoids, appear to mask the dark color that the bound gossypol effects. The fact that flours extracted at boiling tempreature contained low amounts of free gossypol but still imparted a darker color to the biscuits compared with flours extracted at room temperature tends to confirm this hypothesis. More gossypol is probably oxidized during extraction and desolventization at boiling temperature of azeotropes than at

room temperature. Thus, the darker color of the biscuits may be due to water-soluble gossypol breakdown components. More work is needed to confirm either of these two hypotheses.

Simiar data for freeze-dried isolates are presented in Table II. The Hunter color values of isolates prepared from flours extracted at room temperature was either comparable to or darker than that of the control isolate, whereas isolates from flours extracted at boiling temperature were lighter. Most of the isolates from flours extracted at room temperature retained nearly all of the original gossypol, even though the isolates contained only about 63% of the original nitrogen of the cottonseed flour. Isolates prepared from flours extracted at boiling tempreatures and two isolates from flours extracted at room temperature, ie, flours extracted with acetic acid/hexane and methanol/hexane, had improved color and low bound gossypol. Apparent improvement in color of these isolates is probably due to excessive protein denaturation of extracted flours during extraction and desolventization, as noted from the substantial decrease in the yield of isolates. Gossypol complexes with protein by heat (19). The amount of nitrogen extracted from the flour decreased during preparation of isolates, and the denatured protein is therefore discharged into the residue together with the bound gossypol.

TABLE I
Color Values and Gossypol Contents of Extracted Flours and Visual Color of Biscuits

	Extracted Flour						
	Hunter Color Values			Gossypol Content (%)			
Solvent[a]	L	a	b	Free	Bound	Total	Color[b] of Biscuit[c]
Hexane (control)	76.1	−1.8	17.1	0.04	0.09	0.13	1.5
Acetone/hexane	85.8[d]	−3.4	15.5	0.04	0.08	0.12	1.7
	(87.3)[e]	(−2.5)	(13.4)	(0.02)	(0.04)	(0.06)	(2.0)
Acetic acid/hexane	85.2	−3.1	15.2	0.03	0.10	0.13	1.7
	(84.1)	(−1.5)	(13.9)	(0.01)	(0.05)	(0.06)	(2.0)
Ethanol/hexane	85.6	−3.3	15.9	0.04	0.10	0.14	1.7
	(87.1)	(−3.1)	(14.5)	(0.01)	(0.11)	(0.12)	(2.0)
Methanol/hexane	84.1	−3.4	17.4	0.03	0.06	0.09	1.8
	(84.2)	(−2.0)	(16.3)	(0.01)	(0.08)	(0.09)	(2.0)
Ethyl acetate/hexane	85.3	−3.4	15.2	0.04	0.08	0.12	1.7
	(87.5)	(−2.7)	(13.4)	(0.02)	(0.07)	(0.09)	(2.0)
Isopropyl alcohol/ hexane	85.9	−3.2	15.2	0.04	0.08	0.12	1.3
	(86.8)	(−2.8)	(13.9)	(0.01)	(0.10)	(0.11)	(3.0)
Benzene/hexane	84.3	−2.2	15.1	0.05	0.07	0.12	2.0
	(87.2)	(−2.8)	(13.9)	(0.01)	(0.08)	(0.09)	(2.0)
Chloroform/hexane	86.1	−3.3	15.0	0.05	0.03	0.08	1.3
	(73.1)	(−1.0)	(19.6)	(0.03)	(0.08)	(0.11)	(2.0)
Chloroform/methanol	84.2	−2.7	16.5	0.04	0.03	0.07	1.3
	(85.7)	(−3.2)	(16.3)	(0.01)	(0.13)	(0.14)	(2.0)

[a]Used to extract LCP cottonseed flour.
[b]Average of ten individual scores based on range of 1 to 5.
[c]Contained 10% extracted flour with 65% protein.
[d]Data without parentheses are for extraction at room temperature.
[e]Data in parentheses are for extraction at boiling point of azeotrope.

Color of biscuits prepared from isolates varied considerably. Biscuits from all isolates were much darker than were the biscuits prepared from the control LCP flour, even though all biscuits had the same amount of cottonseed proteins. The isolates from flours extracted at boiling temperatures gave biscuits that were

TABLE II
Color and Gossypol Contents of Freeze-Dried Isolates and Visual Color of Biscuits

	Freeze-Dried Isolate						
	Hunter Color Value			Gossypol Content (%)			
Azeotrope[a]	L	a	b	Free	Bound	Total	Color[b] of Biscuit[c]
Hexane (control LCP flour)	75.6	−1.4	19.5	0.03	0.15	0.18	4.0
Acetone/hexane	73.7[d]	−1.5	19.9	0.00	0.14	0.14	4.3
	(79.8)[e]	(−2.3)	(16.6)	(0.01)	(0.13)	(0.14)	(3.0)
Acetic acid/hexane	75.6	−0.4	11.0	0.01	0.07	0.08	3.5
	(completely denatured the flour)						
Ethanol/hexane	75.0	−1.4	17.7	0.00	0.18	0.18	4.8
	(78.7)	(−1.9)	(15.5)	(0.00)	(0.04)	(0.04)	(2.0)
Methanol/hexane	73.2	−0.5	15.0	0.00	0.05	0.05	3.0
	(82.8)	(−2.7)	(13.7)	(0.00)	(0.02)	(0.02)	(2.0)
Ethyl acetate/hexane	74.0	−1.5	17.5	0.01	0.16	0.17	4.3
	(76.8)	(−1.4)	(15.6)	(0.01)	(0.12)	(0.13)	(3.0)
Isopropyl alcohol/hexane	65.4	−0.2	18.2	0.02	0.20	0.22	5.0
	(77.5)	(−1.6)	(16.4)	(0.01)	(0.10)	(0.11)	(3.0)
Benzene/hexane	64.9	0.9	18.8	0.00	0.19	0.19	4.3
	(77.5)	(−1.9)	(17.2)	(0.01)	(0.09)	(0.10)	(3.0)
Chloroform/hexane	70.1	−0.9	18.2	0.02	0.15	0.17	3.8
	(79.4)	(−2.4)	(15.6)	(0.00)	(0.07)	(0.07)	(3.0)
Chloroform/methanol	75.6	−0.8	19.5	0.02	0.15	0.17	4.3
	(82.2)	(−2.2)	(14.5)	(0.00)	(0.00)	(0.00)	(2.0)

[a]Used to extract flour.
[b]Average of 10 individual scores, based on a range of 1 to 5.
[c]Contained 7.2% isolate, having 90% protein.
[d]Data without parentheses are for extraction at room temperature.
[e]Data in parentheses are for extraction at boiling point of azeotrope.

TABLE III
Effect of pH on Gossypol Solubility in Aqueous Solution

pH	Gossypol Solubility (%)
12	75.5
11	95.0
10	97.3
9	100.0
8	91.5
7	76.5
6	65.7
5	0.1
4	0.1
3	0.1

TABLE IV
Correlation of Color Values With Gossypol Content

Treatment Description	Correlation Coefficient[a]	Coefficient of Variation	Standard Error of Regression	Standard Error Within the Experimental Measurement
Free gossypol vs L value of extracted flour	0.42	0.05	4.26	0.03
Free gossypol vs a value of extracted flour	−0.24	0.28	0.78	0.10
Free gossypol vs b value of extracted flour	−0.68*	0.06	1.13	0.03
Bound gossypol vs L value of extracted flour	−0.37	0.05	4.35	0.03
Bound gossypol vs a value of extracted flour	0.26	0.28	0.78	0.10
Bound gossypol vs b value of extracted flour	0.32	0.09	1.47	0.03
Total gossypol vs L value of extracted flour	−0.21	0.05	4.59	0.03
Total gossypol vs a value of extracted flour	0.22	0.28	0.79	0.10
Total gossypol vs b value of extracted flour	−0.03	0.09	1.55	0.03
Free gossypol vs L value of isolate	−0.01	5.73	4.17	0.04
Free gossypol vs a value of isolate	−0.23	1.00	0.80	0.11
Free gossypol vs b value of isolate	0.34	0.15	2.64	0.04
Bound gossypol vs L value of isolate	−0.51	0.04	3.57	0.04
Bound gossypol vs a value of isolate	−0.02	1.03	0.82	0.11
Bound gossypol vs b value of isolate	0.74**	0.10	1.86	0.04
Total gossypol vs L value of isolate	−0.47	3.29	3.66	0.04
Total gossypol vs a value of isolate	−0.06	1.03	0.82	0.11
Total gossypol vs b value of isolate	0.75**	0.10	1.84	0.04
Free gossypol vs visual color of biscuit with 10% flour	−0.34	0.13	0.22	0.16
Bound gossypol vs visual color of biscuit with 10% flour	0.35	0.13	0.22	0.16
Total gossypol vs visual color of biscuit with 10% flour	0.67	0.14	0.23	0.16
Free gossypol vs visual color of biscuit with isolate	0.32	0.17	0.69	0.23
Bound gossypol vs visual color of biscuit with isolate	0.93**	0.06	0.25	0.23
Total gossypol vs visual color of biscuit with isolate	0.92**	0.07	0.28	0.23

[a]*= Significant at 95% confidence level; ** = significant at 99% confidence level.

lighter in color and had lower bound gossypol than did the flours extracted at room temperature, indicating potential role of gossypol in color of baked products.

Relative solubility of gossypol at various pHs was studied; the results are presented in Table III. The solubility of 0.015% aqueous gossypol solution was maximum between pH 9 and 10, and decreased at lower pHs. At pH 7.0, the solubility was about 77%; at 5.0 and 4.0, it was less than 1.0%. These three pHs represent the isoelectric points of isolate II (storage proteins), single-step isolate, and isolate I (functional proteins), respectively (6). At these pHs, remaining gossypol precipitates with protein in isolates as a protein-gossypol complex. The gossypol values reported in Table II are from single-step isolates, and since most of the gossypol is insoluble at pH 5.0, it concentrates in isolates.

The correlation of various forms of gossypol with color values of flours, isolates, and biscuits is presented in Table IV. Highly significant correlation was observed between both bound and total gossypol and the b values of isolates, as well as between both gossypol forms and visual color of biscuits prepared with isolates. The poor correlation of Hunter L, a, and some b values of flours and isolates may be due to gossypol oxidation during processing and, to some extent, to removal of varying amounts of phenolic acids and flavonoids. Gossypol is known to be sensitive to oxidation, particularly in alkaline pHs (8). The fact that standard error of regression is greater than that of experimental measurements also confirms that the gossypol contents alone do not explain all of the color variation in flours, isolates, and biscuits.

Results of this study do not agree with the conclusion of Kim et al (7). No suitable explanation can be given for their different conclusion, since no gossypol values were given in their report. They evaluated a glandless flour that might have had a low gossypol content; in that case, other minor color-causing components may be the main cause of the color problem.

This study indicates that bound gossypol is the major cause of color in cottonseed protein isolates prepared from LCP flour. Presence of free gossypol and other minor color-causing components such as phenolic acids and flavonoids probably masks the grayish green color that bound gossypol introduces. During azeotrope extraction and isolate preparation, the amounts of minor constituents are greatly reduced, and the free gossypol is either converted to bound form or oxidized, which results in darker color. Results also show that light-colored, reextracted cottonseed flour does not necessarily give a light-colored biscuit. Heating of protein-containing cottonseed products during baking or food processing, especially in the presence of water, increases color problem. Based on results of this study, removing all bound gossypol and other minor color-causing components may be essential to improve the color of protein-containing cottonseed products.

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