NONPIGMENT CONSTITUTION OF COTTONSEED PIGMENT GLANDS

By

M. H. ABBASY

Univ. Coll. for Women, Ain Shams University

Y. EL-SHATTORY and A. S. NOCKRASHY

Fats and Oils Laboratory, National Research Centre, Dokki, Cairo, Egypt.

INTRODUCTION

Most of the pigments of cottonseed are contained in distincit morphological structures, which are large ovoid or spherical bodies (100-400 microns), designated as pigment glands. Investigations carried on the structure of these pigment glands showed the presence of a gland wall and an internal membraneous mesh like-network (1-3). Gossypol is found as discrete particles (0.2 - 1.0) microns, within the mesh, while it was suggested that gossypurpurin may be concentrated in the outer wall of the glands (3). Besides these two pigments, gossyverdurin was reported as a natural pigment in the pigment glands (4).

The pigment glands are extremely sensetive to polar organic solvents, where rapid swelling of the network takes place with subsequent release of the pigment particles. The A.O.C.S. procedure (5) recommends the use of 80% acetone for the quantitative recovery of gossypol from the pigment glands. El-Nockrashy (6) reported values of gossypol when using acetone-hexane-water azeotrope (53 : 44 : 3, v/v) to extract the pigment glands, where it was found that all above three pigments were recovered.

No work has been reported on the nonpigment constituents of cottonseed pigment glands. The aim of the present investigation is to study the amino acid constituents of cottonseed (*G. barbadense*) meal, pigment glands and gland wall, the lipoidal constituence of the pigment glands are also studied using column chromatographic fractionation.

EXPERIMENTAL

Isolation of pigment glands and fractionation of glands constituents :

Hexane defatted cottonseed meal (Sample I) was subjected to flotation technique (7) to isolate cottonseed pigment glands (Sample II). Pigment glands (5 g) were extracted with acetone-hexane-water (53 : 44 : 3, v/v) in an Omni-mixer. A total of six extractions (150 ml. each) were used for complete recovery of pigment constituents. The extracts were discarded and the pigment glands residue (Sample III) was re-extracted six times with water (100 ml. each) in Omni-mixer. The water extracts were lyophilised (Sample IV) and kept in a closed container. The water-insoluble residue (Sample V) was dried in an oven at 100°C for 4 hours, then over phosphorous pentoxide under vacum.

Amino acid analysis :

Total nitrogen of the samples was determined by microkjeldahl procedure (8), and the protein content was expressed as % N X 6.25. Samples were hydrolyzed with 6 N-Hcl in sealed ampoules (20 mg. per ml. acid solution) at 100°C for 24 hours. The amino acids for each hydrolysate were then determined as described by Moore et al. (9) through the use of an automatic amino acid analyzer constructed essentially as described by Spackman et al. (10).

Column chromatography of lipids :

Isolation of pigment lipids : Cottonseed pigment glands (5 g) were extracted with acetone-hexane-water (53 : 44 : 3) in an Omni-mixer as previously described. The extract was evaporated to dryness, dissolved in 100 ml. diethyl ether, and then washed three times with 50 ml. portions of 1.5 N sodium hydroxide containing 5% sodium dithionite. The ethereal extract was then washed with water, dried over anhydrous sodium sulphate. and evaporated to dryness.

Fractionsation of pigment glands lipids : El-Nockrashy and El-Shattory technique (11) for column chdomatographic fractionation of lipids was used.

Chromatographic column (2 X 60 cm.) fitted with sintered glass disc and stopcock on one side and a ball-joint Q,F adaptor on the other side was packed with silicic acid (Malinckrodt, Chromatography grade) to give a column height of ca. 60 cm. A nitrogen pressure of 4 pounds per square inch was used for packing and elution. An

exact weight of above extracted oil (250 mg.) was put onto the column. The column was then eluted with the following solvents, successively.

Solvent	Composition	cc.collected
Hexane		200
Hexane-benzene	(85-15)	500
Hexane-ether	(95-5)	500
Hexane-	(85-15)	250
	(75-25)	200
	(50-50)	200
	(10-90)	200
Chloroform		200
Methanol		500

Samples, 25 ml. each were collected, transferred to previously weighed 50 ml. capacity Q.F. round bottom flasks, evaporated under vacum at 40°C, using in a rotary evaporator, then weighed to find the exact weight. Shifting from one solvent to another was done when no material was eluted from the column. Eluted fractions were transferred with the smallest volume of chloroform in screw capped vails.

Thinlayer chromatography of eluted fractions was done as described by El-Nockrashy and El-Shattory (11). The identity of the spots was achieved by using authentic standards (Supelco INC. Products, Bellefonte, Pa. U.S.A.).

The percentages of individual lipid classes eluted from the silicic acid column were determined after the identification on T.L.C. plates. Eluted fractions containing similar constituents were collected together and the summation of residue for each class was calculated.

RESULTS AND DISCUSSION

Fractionation of pigment glands constituents : (Table 1).

The exhaustive extraction of cottonseed pigment glands (CSPG) with acetons-hexane-water results in a residue (Sample III) which makes 53.8% of the CSPG weight. This residue contains negligible amount of gossypol (0.03%) when compared with the high gossypol content of CSPG (44.96%). The extraction of sample II, with water

followed by the lyophilisation of the water-soluble resulted in a fluffy purple coloured residue (Sample IV) which makes 8.6% of the original CSPG weight. The water insoluble residue (Sample V) is greyish-brown and makes 44.0% of the CSPG weight. No gossypol was detected in these last two fractions.

TABLE 1

Fractions of cottonseed pigment glands.

	Sample	* Fraction	% Gossypol	% Nitrogen	% Protein
I	Meal	—	1.63	9.80	61.25
II	Glands	—	44.96	4.04	25.25
III	A-H-W Insol. of II	53.8	0.03	6.80	42.50
IV	Water-Sol of III	8.6	0.00	1.89	11.81
V	Water Insol. of III	44.0	0.00	10.01	62.56

* of the pigment glands weight.

The nitrogen content of CSPG is less than half the amount detected in cottonseed meal (CSM) they were isolated from. However, the extraction of the pigments from the CSPG results in considerable increase in the nitrogen content of the glands residue (Sample III). The water-insoluhle of this later fraction contains a total nitrogen which is slightly higher than the CSM, while the water-soluble contains ca. 1.9% nitrogen.

Amino acid patterns : (Table 2).

Comparing the amino acid pattern of the CSPG with that of CSM it is clear that the most pronounced feature is the presence of glucoseamine in the CSPG-hydrolysate (6.67%), while the CSM-hydrolysate is devoid of it. Proline in the CSPG is almost three times the amount present in CSM. Aspartic acid, threonine, serine and leucine are also present in higher amounts in the CSPG. The total basic amino acids (Lys, His, and Arg) in CSM is considerably higher than that found in CSPG (ca. 20.4 and 12.1%, respectively).

The A-H-W-insoluble residue of the CSPG (Sample III) differs from the intact CSPG in being considerably higher in the glucosamine content. Other amino acids are more or less found in almost identical amounts in both samples.

TABLE 2

Amino acid patterns of cottonseed meal, cottonseed pigment glands

Amino Acid.	Sample Number 1				
	I	II	III	V	IV
Lys	4.55	3.92	3.82	2.21	5.03
His	2.94	1.00	1.06	—	2.59
Amm.	2.16	0.82	0.41	—	2.07
Arg. Glu-	12.87	7.18	6.00	6.28	9.43
NH_2	—	6.67	9.09	9.36	4.53
Asp	9.77	12.39	12.38	13.67	9.16
Thr.	3.30	4.32	3.95	8.25	3.63
Ser	4.53	5.26	5.07	6.53	4.45
Glu	22.52	21.33	21.42	6.98	18.18
Pro	4.07	12.47	12.42	17.27	3.72
Gly	4.42	3.62	3.61	2.52	4.30
Ala	4.05	3.74	3.55	5.23	4.49
Gys	—	—	—	—	2.85
Val	4.82	2.52	2.27	1.08	4.88
Met	1.43	0.68	0.79	0.66	1.62
Ileu	3.36	3.02	2.81	3.96	3.91
Leu	6.07	6.47	6.68	9.72	6.84
Tyr	3.34	1.44	1.43	—	2.97
Phe	5.78	3.17	3.31	—	5.36

Sample Number 1

I : Cottonseed meal, II : Cottonseed pigment glands, III : CMN - insoluble residue of pigment glands, IV : Water-soluble of III, V : Water-insoluble of II.

TABLE 3

Percentage constitution of oil constituents of cottonseed and cottonseed pigment glands

Fraction Number	Cottonseed Oil	Pigment glands Oil
1	0.3	4.2
2	—	48.5
3	90.3	19.3
4	2.4	27.9

Fraction 1 : Sterol ester, Fraction 2 : Unknown.
Fraction 3 : Triglyceride, Free Fatty acids and sterols.
Fraction 4 : Phospholipids.

The amino acid pattern of the water-soluble fraction (Sample IV) of the CSPG — residue differs markedly from the rest of the analysed samples. It contains the highest glucosamine content. Aspartic acid, threonine, serine, proline, alanine, isoleucine and leucine are also found in higher amounts in this sample. Glutamic acid, glycine, valine, methionine are detected in lower amounts. Histidine, tyrosine, phelylalanine, cysteine and ammonia are missing.

The exhaustive extraction of the CSPG with A-H-W followed by water extraction results in a residue (Sample V) which is believed to be the CSPG walls. The amino acid pattern of the CSPG walls is more or less similar to the CSM from which the CSPG were isolated. However, this sample contains lower total basic amino acids, glutamic acid and proline, besides the fact that it contains glucosamine and cysteine, both of which were not detected in CSM.

Lipoidal Constituents :

Figure L is a thin layer chromatographic representation showing the lipoidal constituents of cottonseed oil compared to those of the oil extracted from the pigment glands. It is quite evident that the triglycerides make the bulk portion of the seed oil, on the other hand their amounts are very small in the pigment gland oil. However, the pigment gland oil contains a constituent, RF. value 0.67 above the triglyceride spot, which makes the major constituent.

Although all spots took a brown colour upon exposure to iodine vapour, this unknown took a permanent blue colour.

Column chromatographic fractionation indicated that the sterol esters which are eluted with hexane make 0.3% of the seedoil and 4.2% of the pigment glands oil. The unknown constituent which makes Ca. 48.5% of the pigment glands oil and which is not detected in the seed oil was eluted by hexane-benzene (85 : 15). Its exact nature will be the subject of further investigation, however, evidences indicate that it is not a gossypol or related pigment since it does not give the characteristic red colour of this group of pigments. The third fraction, which makes Ca. 19% of the pigment glands oil and more than 90% of the seed oil, was eluted with hexane-ether solvent mixtures given in the text. Although the triglycerides make about 86% of the seed oil, it was found that the sterols which make the bulk of this fraction in the pigment gland oil make 15% out of the 19.3%. The phospholipids eluted in fraction 4 with methanol makes Ca. 28% of the pigment glands oil and only 2.4% of the seed oil.

SUMMARY

The cottonseed pigment glands were fractionated with the aim of finding the nonpigment constituents The amino acid patterns of the glands and the gland wall are reported and compared to those of the cottonseed meal. The nature of lipoidal constituents of the pigment glands differs from the cottonseed lipids. Although triglycerides make the bulk portion of cottonseed oil, they only constitute a small percentage of the pigment gland lipids. An unknown lipiodal constituents present in the glands at about 49% is not detected in the cottonseed oil.

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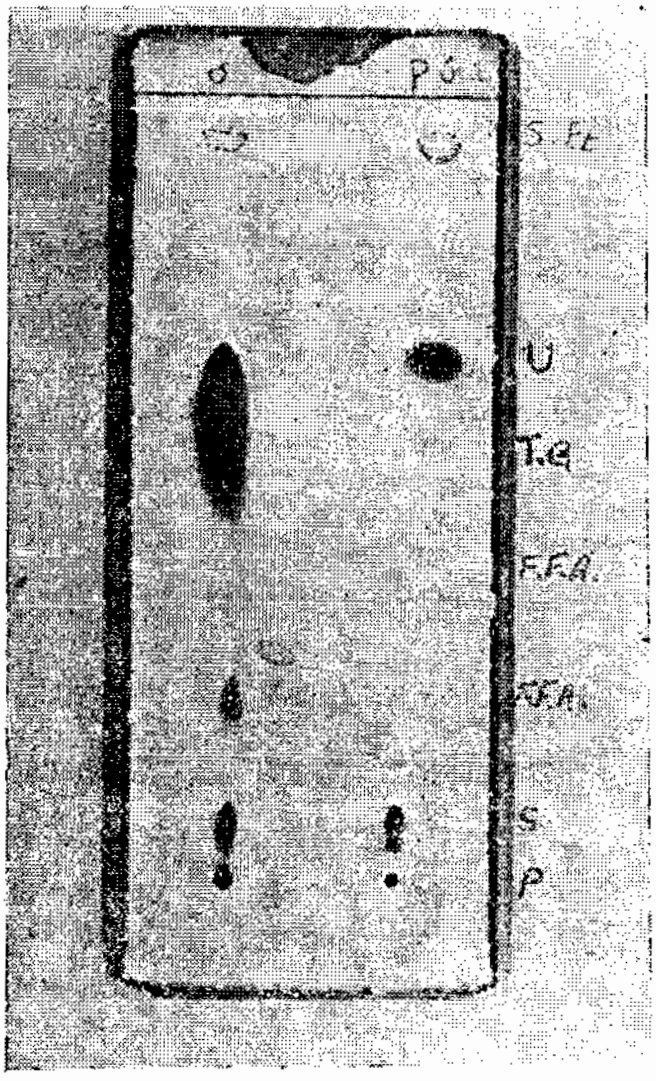

Fig. 1 — T.L.C. of Cottonseed Oil and cottonseed pigment glands oil.