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EFFECT OF MATURITY ON LIPID CLASSES AND FATTY ACID COMPOSITION OF *CASSIA* SEEDS

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The major lipid classes and their constituent fatty acids in maturing seeds of *Cassia absus; Cassia fistula* and *Cassia occidentalis* have been anlaysed. Generally in all the three seed oils a continuous increase in the contents of triacylglycerol was observed whereas the contents of all other neutral lipids decreased with maturity. The contents of polar lipids on the whole decreased with maturity. The major fatty acids in all the three species were palmitic, oleic and linoleic. Fatty acid composition of non-polar and polar lipids changed as the seeds matured, but the fatty acids of polar lipids were more saturated than those of the non-polar lipids throughout the sampling periods, 12 weeks for *C. absus* and *C. occidentalis* and 24 weeks for *C. fistula*.

Key words: Maturity, Lipid, *Cassia.*

INTRODUCTION

Formation of fatty acids takes place in the lipids of the developing seeds. This biosynthesis needs extensive studies for reaching any conclusions. However, it is necessary to know the period when the seed has the maximum lipid synthesising activity. One way of study is to quantitatively determine the lipid formation in the developing seeds right from the flowering stage till maturity. It is also known that the fatty acid composition of oils in seeds changes during the period of maturity. These changes as well as the lipid contents have been studied by various workers in the maturing seeds of soyabean [1], castor beans [2], rapeseed [3] and sunflower [4]. As a result of these studies it has been observed that there occurs a rapid synthesis of fatty acids during a very brief period early in the development of the seed oils.

The developing/maturing seeds utilise their fatty acids for the formation of different lipid fractions which can be determined by examining the changes in the lipid classes.. In fact many studies have been carried out wherein lipid classes of the developing seeds were separated and chemically examined [5-7]. The results of these studies confirmed that fatty acids in the developing seed oils were utilised in the formation of different classes of lipids.

In continuation of the studies related to the *Cassia* species the present paper provides information concerning the lipid synthesis in the developing seeds of *C. absus, C. fistula* and *C. occidentalis* seed oils. A literature survey revealed that no such study has so far been done. During the studies, it was observed that the seeds of *C. absus* and *C. occidentalis* matured in 12 weeks and those of *C. fistula* in 24 weeks after flowering. It was further observed that lipid synthesis was most active between 2nd to 6th week for *C. absus* and *C. occidentalis* and 4th to 12th weeks after flowering for *C. fistula* respectively. The lipids were extracted from the seeds with a mixture of chloroform and methanol (2:1 v/v) and separated into different classes by thin layer chromatograph on silicic acid. Generally it was noticed that triacylglycerol contents increased whereas neutral lipid classes decreased with maturity of the seeds, or the contents of polar lipids on the whole decreased with maturity.

The major fatty acids, in lipids of the three species, were palmitic, oleic and linoleic. The fatty acid composition of the non-polar and polar lipids also changed with the maturity of the seeds. The fatty acid compositions were usually determined by gas, column and thin layer chromatographic techniques.

Plant Material. Cassia absus; Cassia fistula and *Cassia occidentalis* seed samples were harvested in the fields of PCSIR Laboratories Lahore. Sampling commenced from 2nd to 12th week after flowering for *C. absus* and *C. occidentalis* and on 4th till 24th weeks after flowering for *C. fistula* seeds. These intervals were commenced as physiological maturity stage I-VI. The seeds from various maturity stages were combined and immediately frozen in plastic bags till used.

MATERIALS AND METHODS

Reagents and standards for thin layer chromatography were prepared by the method of Singleton, Thomas and Ralston [8,9].

Seeds were removed from storage, freeze dried and moisture contents were determined by heating the seeds in an airoven at 110^0 until a constant weight was obtained.

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Lipid extraction. Mature seeds were weighed, crushed and soaked in chloroform/methanol (2:1, v/v) for 18 hr and then extracted 3 times at 3 hr intervals. The pooled extracts were concentrated at 40^o in a rotary vacuum evaporator, washed with 0.9N sodium chloride solution to remove non lipid impurities and dried over anhydrous sodium sulphate [10]. The immature seeds were homogenized and the lipids extracted as in mature seeds. An aliquot of the total lipids extract was dried to a constant weight for the determination of the percentage of lipid [11].

Separation of lipid classes by column and thin layer chromatography. The total lipid extracts were separated into neutral and polar fractions by silicic acid adsoption chromatography. The (35-70) mesh silicic acid (E. merck ASTM KorngröBe Germany) activated hy heating at 120^o for 24 hours and hexane/diethyl ether (7:3, v/v) were used as the eluting solvents. The solvents were evaporated under nitrogen at 50^o and lipid fractions were stored. The non polar and polar classes of lipids were isolated by thin layer chromatography on silica gel G (E. merck D. G. KorngröBe Germany) using 20 x 20 cm plates. For neutral lipids hexane/diethyl ether/acetic acid (80:20:2) and for polar lipids chloroform / methanol / ammonia (30%) / water (60:35:5:2.5) were used for developing plates. 5 μl of 10% solution of each of the lipid samples in chloroform were spotted on an activated plate. The various lipid fractions were identified by spraying the plates with the specific reagent [12]. Aliquots of the non-polar and polar lipids (75-50 mg) were streaked on glass plates (20 x 20 cm) and developed with the solvent system given above. The resulting bands were made visible under UV lamp by spraying with methanolic 2′, 7′, - dichlorofluorescein. Lipid classes were identified by comparison of their Rfs with those of the standard under identical condition.

Typical Rfs of the non-polar lipid classes were hydrocarbon-wax ester, 0.97-0.95; triacylglycerol, 0.90; free fatty acid, 0.42; Sn-1, 3-diacylglycerol, 0.40; Sn-1, 2-diacylglycerol, 0.39; monoacyl glycerol **1**, 0.30; monoacylglycerol **2**, 0.28.

The Rfs of the polar lipid classes were monoglactosyl glyceride, 0.13; phosphatidyl ethanolamine, 0.30; phosphatidyl choline, 0.46; digalactosyl glyceride, 0.58; lysophosphatidyl ethanolamine 0.72; lysophosphatidyl choline, 0.86.

Gas chromatography. The bands were marked and then scraped into 50 ml conical flasks containing 1 ml of internal standard (1% w/v, margaric acid dispersed in diethyl ether) and 25 ml of diethyl ether. The contents of the flasks and additional 25 ml washings were filtered through a fritted disc funnel and the filtrate evaporated to dryness in a rotary evaporator under nitrogen. The dried residue was dissolved in 1.5ml of chloroform/methanol and methylation was carried out by the method of Moorison and Smith [3].

The GC analysis was carried out on a PYE-Unicam 204 Series unit using a glass column, (1.5 x 4mm), packed with 20% PEGS on diatomite (80-100 mesh). Column temperature was maintained at 200^o and nitrogen was used as carrier gas at a flow rate of 40 ml/min. Detection was made by ionisation detection. The detector was maintained at 250^o. Peak areas and thus percentage of the fatty acids were obtained from the product of the retention times and respective peak heights. The amounts of total fatty acids were determined by comparing the total peak area of all the fatty acids in each fraction with that of the internal standard. The relative weight percent of each fatty acid was used to calculate the proportions of non polar and polar lipid fraction. The results of GC analysis are given in Table (3-16).

RESULTS AND DISCUSSION

Changes in lipid and lipid class contents. Percentages of lipids extraction from *C. absus*, *C. fistula* and *C. occidentalis* mature seeds as determined by AOCS official methods, were 4.8%, 4.0% and 3.55% respectively. The lipid contents of the seeds increased during the early maturity stages and the most rapid increase occurred after stage 3. This indicates that at stage 3 and beyond, lipid synthesis becomes the dominant reserve accumulation mechanism. The total lipids extracted and moisture contents are given in Table 1.

The distribution of lipid classes in the three seed oils presented in table 2 showed great similarity in the

Table 1. Moisture and lipid contents of six physiological stages.

Maturity stage	Moisture %	Total lipid %	Neutral lipid %	Polar lipid %	Neutral lipid/ Polar lipid
(a) *Cassia absus* seed oil					
1.	68.20	0.71	41.45	58.55	0.70
2.	57.30	2.10	42.32	57.88	0.73
3.	46.40	3.24	43.77	56.23	0.77
4.	39.10	3.70	46.71	53.29	0.87
5.	13.00	4.30	47.52	52.48	0.90
6.	6.40	4.80	49.89	50.11	0.99

(Continued ......)

(Table 1, continued)

(b) *Cassia fistula* seed oil

1.	63.60	0.48	34.85	65.15	0.54
2.	58.50	1.45	36.70	63.30	0.68
3.	50.00	2.62	37.10	62.90	0.59
4.	38.50	3.00	39.06	60.94	0.64
5.	15.00	3.53	40.14	59.85	0.67
6.	7.81	4.00	41.28	58.72	0.72

(c) *Cassia occidentalis* seed oil

1.	65.25	0.59	38.38	61.62	0.62
2.	54.00	1.73	39.48	60.52	0.65
3.	42.3	2.80	41.26	58.74	0.70
4.	30.01	2.95	43.04	56.00	0.76
5.	15.00	3.41	45.51	54.49	0.83
6.	5.00	3.55	46.44	53.56	0.86

development of lipid classes as the seeds matured. Triacylglycerol, the major component of the lipids present increased from 4.65% - 28.20% in *C. absus*; 3.21% - 19.65% in *C. fistula* and 2.35% - 22.37% in *C. occidentalis*. The increases were highest during the more immature stages examined. This increase in triacylglycerols results in increase of seed oil content. There was also a high content of free fatty acid in immature seeds and their relative weight percentages decreased from 15.09% - 3.82%, in *C. absus*; 8.20% - 1.43% in *C. fistula* and 10.20% - 2.76% in *C. occidentalis* as the seed matured.

Diacyglycerols were the dominant lipids alongwith small amounts of monoacylglycerols. The percentage of Sn-1, 2-diacylglycerols, Sn-1, 3-diacylglycerols; monoacylglycerols 1 and 2 and hydrocarbon-wax ester fractions were decreased with maturity and were almost similar throughout maturity stages. It appears that these classes take part in the earlier stages of development. The main components of polar lipids were monogalactosyl glyceride; phosphatidyl ethanol amine; phosphatidyl choline; digalactosylglyceride; lysophosphatidyl ethanolamine and lysophosphatidyl choline. Other polar lipids were present in quantities too small to be detected. Phosphatidyl ethanol amine was the main component in the early stages and decreased from 26.6% - 15.8% in *C. absus*; 27.32% - 18.21% in *C. fistula* and 25.91% - 17.20 in *C. occidentalis* as the seeds matured. Phosphatidyl choline relatively minor at the

Table 2. Percentage composition of lipid classes in six physiological stages.

Maturity stage	Hydro-carbon wax ester	Triacyl glyce-rol	Free fatty acid	1, 3-Di acyl glycerol	1,2-Di acylgly-cerol	Mono-acyl glycerol 1	Mono-acyl glycerol 2	Monoga-lacto-syl gly-ceride	Phospha-tidyl ethanol amine	Phospha-tidyl choline	Diagala-ctosyl glyceride	Lyso-phospha-tidyl ethanol amine	Lyso-phospha-tidyl choline
(a) *Cassia absus* seed oil													
1.	1.45	4.65	15.09	5.49	6.21	4.11	4.45	12.31	26.06	6.30	10.56	2.21	1.11
2.	1.43	6.11	14.48	5.46	6.08	4.01	4.25	11.29	25.31	8.60	9.98	1.78	0.92
3.	1.22	13.90	9.62	5.33	5.91	3.78	4.01	10.32	21.14	14.21	8.32	1.43	0.81
4.	1.20	20.41	7.01	5.25	5.42	3.32	3.90	8.89	19.43	16.14	6.66	1.40	0.77
5.	1.19	24.03	5.86	5.23	5.10	3.18	3.81	7.43	17.99	18.71	5.99	1.31	0.75
6.	1.17	28.20	3.82	5.20	4.88	3.01	3.61	7.21	15.81	19.31	5.76	1.30	0.72
(b) *Cassia fistula* seed oil													
1.	2.31	3.21	8.20	6.70	6.41	4.11	3.92	12.52	27.32	8.11	11.31	3.54	2.35
2.	2.21	7.64	6.22	6.50	6.24	4.02	3.89	10.71	24.77	12.36	10.24	3.01	2.21
3.	2.02	11.72	3.21	6.31	6.14	3.99	3.71	8.98	22.44	18.04	8.68	2.92	1.84
4.	1.98	15.27	2.68	5.99	5.91	3.81	3.42	8.72	20.94	19.38	7.78	2.66	1.46
5.	1.92	18.25	1.69	5.44	5.65	3.70	3.40	8.33	19.06	21.69	6.97	2.51	1.29
6.	1.86	19.65	1.43	5.10	5.30	3.56	3.38	8.11	18.21	22.60	6.30	2.40	1.10
(c) *Cassia occidentalis* seed oil													
1.	1.54	2.35	10.21	6.11	6.88	5.55	5.74	13.93	25.91	9.05	8.81	2.16	1.76
2.	1.46	5.30	8.75	6.01	6.82	5.43	5.71	11.44	24.64	12.37	8.51	1.87	1.69
3.	1.40	14.35	3.21	5.87	5.76	5.31	5.36	10.69	22.78	16.66	6.01	1.46	1.14
4.	1.36	17.41	2.85	5.56	5.63	4.99	5.24	8.27	20.60	18.31	5.50	1.35	0.97
5.	1.33	20.69	2.81	5.31	5.50	4.76	5.11	6.90	18.45	21.85	5.08	1.29	0.92
6.	1.23	22.37	2.76	5.21	5.48	4.48	4.91	6.51	17.20	23.31	4.46	1.22	0.86

early stage increased at stage 3 and at maturity was one of the major polar lipids. The increase in this lipid class was 6.30% - 19.31% in *C. absus*; 8.11% - 22.60% in *C. fistula* and 9.05% - 23.31% in *C. occidentalis.* The relative percentage of polar lipids such as monogalactosyl glyceride, digalactosyl glyceride, phosphatidyl ethanolamine, lysophosphatidyl ethanolamine and lysophosphatidyl choline decreased with maturity. The active period of lipid synthesis is between 2nd to 6th week for *C. absus* and *C. occidentalis* and 4th to 12th week after flowering for *C. fistula.* As maturation and lipid accumulation proceeded, the contents of polar lipids and diacylglycerols, the precursors and intermediates in triacylglycerol biosynthesis, decreased while triacylglycerol increased.

Changes in fatty acid composition of the lipid classes. The fatty acid composition of hydrocarbon-wax-ester; triacylglycerol; free fatty acids; Sn-1, 3-diacylglycerol; Sn-1, 2-diacyl glycerol; monoacylglycerols **1** and **2**, polar lipids and whole, chloroform/methanol extracted oils of *C. absus; C. fistula* and *C. occidentalis* seed oils are shown in tables (3-16).

Table 3. Effect of maturity on fatty acid composition of hydrocarbon-wax-ester.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	0.85	55.85	9.11	7.31	20.77	6.11
2.	0.84	51.81	8.65	8.52	24.53	5.65
3.	0.63	47.21	6.35	11.31	29.52	4.98
4.	0.41	40.65	5.85	11.61	36.87	4.61
5.	0.39	35.75	5.35	12.53	42.33	3.75
6.	0.21	30.58	4.85	14.91	48.65	2.80
(b) *Cassia fistula* seed oil						
1.	0.80	68.70	2.66	10.02	14.07	1.84
2.	1.40	65.70	2.29	12.07	16.26	1.79
3.	1.32	59.08	1.82	16.40	19.00	1.69
4.	1.20	54.07	1.79	17.00	24.30	1.60
5.	1.11	50.09	1.70	19.32	26.19	1.59
6.	1.03	46.12	1.60	21.06	28.66	1.53

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	0.22	1.87	66.81	5.0	12.85	9.69	3.55
2.	0.21	1.50	64.71	3.99	14.00	12.33	3.26
3.	0.21	1.10	59.96	3.82	15.37	16.53	3.01
4.	0.20	0.98	55.15	3.70	16.70	20.33	2.94
5.	0.19	0.96	51.03	3.61	17.07	24.31	2.83
6.	0.14	0.70	46.35	3.00	18.25	29.06	2.40

Table 4. Effect of maturity onfatty acid composition of triacyl glycerol.

Maturity stage.	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	1.98	51.76	7.52	8.13	24.33	6.10
2.	1.90	49.78	6.78	9.75	26.89	4.90
3.	1.73	46.50	6.11	11.56	30.71	3.39
4.	1.35	41.15	5.76	12.03	36.62	3.09
5.	1.01	35.90	5.20	14.33	40.63	2.93
6.	0.38	25.50	4.28	16.62	49.83	2.83
(b) *Cassia fistula* seed oil						
1.	2.30	67.75	2.95	12.80	10.10	1.80
2.	2.11	65.94	2.28	17.00	11.00	1.87
3.	1.92	61.00	2.01	21.25	12.17	1.63
4.	1.43	57.01	1.85	24.08	16.03	1.60
5.	1.21	48.26	1.58	20.90	18.53	1.52
6.	0.52	44.86	1.23	30.00	22.20	0.70

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	0.20	2.57	75.20	7.85	2.20	10.33	2.65
2.	0.18	2.12	70.93	6.53	5.40	13.50	2.07
3.	0.10	1.80	67.10	5.90	6.30	16.84	1.96
4.	0.06	1.55	65.00	4.60	8.71	18.11	1.77
5.	0.01	1.34	63.15	3.17	10.41	20.30	1.62
6.	traces	1.27	55.56	2.95	13.18	25.52	1.52

Table 5. Effect of maturity on fatty acid composition of free fatty acid.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	1.76	55.11	7.00	8.20	23.02	4.91
2.	1.55	52.32	6.91	9.00	26.11	4.11
3.	1.37	46.85	5.87	10.99	31.21	3.71
4.	1.11	38.41	5.50	13.51	38.26	3.21
5.	0.98	34.37	5.01	14.57	42.46	2.81
6.	0.31	29.66	4.75	15.73	47.05	2.50
(b) *Cassia fistula* seed oil						
1.	1.53	67.82	2.20	10.20	16.30	1.95
2.	1.38	62.75	2.85	13.00	19.00	1.82
3.	1.35	59.21	1.80	14.50	21.42	1.72
4.	1.30	55.72	1.66	16.14	23.58	1.60
5.	1.25	52.86	1.47	18.20	24.71	1.51
6.	1.20	49.74	1.30	20.60	25.68	1.48

(continued . . . . . .)

(Table 5, continued)

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:2}$
1.	0.21	2.88	70.95	5.72	5.03	11.37	3.84
2.	0.17	2.40	68.03	4.65	7.00	15.35	3.40
3.	0.14	1.93	61.19	3.89	10.01	18.60	3.44
4.	0.13	1.65	53.19	2.88	12.90	22.20	3.21
5.	0.09	1.35	50.49	2.56	14.46	28.03	3.11
6.	0.07	1.15	44.36	2.38	16.04	33.02	2.98

Table 6. Effect of maturity on fatty acid composition of 1,3, diacyl-glycerol.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	2.93	50.69	6.90	6.83	26.85	5.80
2.	2.41	43.56	6.25	8.14	29.22	5.21
3.	1.99	41.12	5.60	12.33	34.02	4.85
4.	1.63	39.11	4.28	13.29	37.68	4.01
5.	1.41	37.06	3.98	13.91	40.66	2.98
6.	1.23	35.11	3.65	14.77	42.98	2.26
(b) *Cassia fistula* seed oil						
1.	1.35	68.87	2.20	10.02	15.40	1.23
2.	1.28	64.00	2.03	12.20	18.20	1.90
3.	1.25	61.32	1.95	13.50	20.10	1.88
4.	1.12	59.00	1.86	15.20	21.02	1.80
5.	1.05	52.73	1.84	20.40	22.29	1.69
6.	0.97	45.22	1.73	23.93	26.50	1.65

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	0.10	3.11	69.71	5.12	7.94	11.30	2.72
2.	0.10	2.66	65.65	4.60	9.60	14.89	2.50
3.	0.09	1.87	60.28	4.00	12.40	19.05	2.31
4.	0.07	1.62	55.78	3.31	14.86	22.25	2.11
5.	0.05	1.12	51.81	3.10	15.70	26.32	2.00
6.	0.01	0.72	45.20	2.83	19.10	30.15	1.99

Table 7. Effect of maturity on fatty acid composition of 1,2 diacyl-glyceral.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	1.79	53.21	6.52	8.21	25.16	5.11
2.	1.71	51.01	5.98	9.11	27.68	4.51
3.	1.53	42.24	5.43	12.51	34.51	3.78
4.	1.35	34.23	4.91	14.63	41.77	3.11
5.	0.99	30.67	4.73	16.11	44.42	2.88
6.	0.36	26.51	4.60	17.34	48.40	2.79
(b) *Cassia fistula* seed oil						
1.	1.38	65.07	2.23	13.60	15.10	2.80
2.	1.18	59.04	1.83	16.01	19.25	2.69
3.	0.98	55.98	1.72	18.00	21.09	2.23
4.	0.86	52.30	1.60	19.40	24.00	1.85
5.	0.82	47.41	1.40	20.18	28.30	1.79
6.	0.79	43.02	1.28	22.55	30.65	1.71

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C18:_{1}$	$C_{18:2}$	$C_{18:3}$
1.	0.16	1.21	68.51	5.91	8.66	13.11	2.44
2.	0.12	1.20	64.50	4.70	10.66	16.49	2.23
3.	0.11	1.01	60.68	3.32	12.45	20.25	2.00
4.	0.09	0.71	54.58	3.19	14.50	25.14	1.89
5.	0.07	0.51	48.01	2.82	17.47	29.29	1.83
6.	0.06	0.44	42.70	2.43	20.24	32.34	1.79

Table 8. Effect of maturity on fatty acid composition of monoacyl glyceral 1.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	1.45	57.31	5.85	6.40	24.88	4.11
2.	1.39	52.11	5.30	8.19	29.10	3.91
3.	1.31	48.04	4.78	9.39	32.61	3.00
4.	1.00	40.10	4.18	9.59	39.29	2.89
5.	0.78	33.31	4.10	10.78	48.61	2.51
6.	0.29	27.47	4.01	12.91	53.11	2.21
(b) *Cassia fistula* seed oil						
1.	1.13	65.25	2.34	14.05	15.2	2.03
2.	1.04	61.89	2.08	16.00	17.00	1.99
3.	1.00	57.05	1.99	19.20	18.85	1.91
4.	1.05	53.58	1.81	19.40	22.46	1.70
5.	0.93	51.78	1.63	20.06	24.09	1.60
6.	0.89	45.03	1.48	23.47	27.58	1.55

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	0.18	2.00	65.50	4.35	9.33	13.93	4.76
2.	0.10	1.64	63.57	3.17	11.40	15.51	4.61
3.	0.09	1.41	60.52	3.11	12.55	18.77	3.55
4.	0.08	1.38	56.11	2.71	14.35	22.14	3.23
5.	0.08	1.35	50.00	2.56	15.33	27.57	3.11
6.	0.05	1.30	43.61	2.52	18.77	30.67	3.08

Table 9. Effect of maturity on fatty acid composition of monoacyl glycerol 2.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	1.70	56.11	5.54	5.61	24.73	5.31
2.	1.64	53.34	4.98	7.75	28.14	4.15
3.	1.48	49.89	4.83	8.01	32.16	3.63
4.	1.13	38.38	4.55	10.06	34.65	2.79
5.	0.78	35.87	4.01	10.89	46.56	2.49
6.	0.33	28.63	3.99	11.79	52.85	2.41
(b) *Cassia fistula* seed oil						
1.	2.18	66.14	2.75	10.01	17.00	1.92
2.	1.94	61.69	2.35	12.11	20.15	1.76
3.	1.83	54.89	2.06	15.16	24.56	1.50
4.	1.79	51.36	1.89	16.20	27.32	1.44
5.	1.61	46.96	1.74	17.00	31.01	1.32
6.	1.20	43.06	1.40	19.90	33.20	1.24

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	1.28	2.14	65.16	3.11	10.21	12.33	5.77
2.	1.25	1.90	61.33	3.01	12.74	14.79	4.98
3.	1.20	1.63	59.22	2.89	14.33	16.40	4.33
4.	1.10	1.49	54.32	2.77	15.41	18.33	3.58
5.	1.10	1.21	48.72	2.61	16.33	20.81	3.04
6.	0.09	1.19	46.67	2.50	17.19	21.03	3.35

Table 10. Effect of maturity on fatty acid composition of phosphatidyl ehtanol amine.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	2.1	55.25	11.11	7.77	18.46	5.31
2.	1.89	51.31	10.85	8.73	22.31	4.91
3.	1.65	49.24	10.01	9.65	25.45	4.10
4.	1.43	46.01	9.62	10.31	29.21	3.42
5.	1.22	43.70	9.25	10.85	32.33	2.74
6.	1.10	41.70	8.91	11.37	34.50	7.42
(b) *Cassia fistula* seed oil						
1.	2.33	74.12	3.43	8.38	10.21	1.53
2.	1.99	69.02	2.91	10.21	14.45	1.42
3.	1.63	62.93	2.01	13.41	18.64	1.38
4.	1.51	58.01	1.85	15.21	22.21	1.21
5.	1.42	55.21	1.55	16.23	24.44	1.15
6.	1.33	51.71	1.30	17.70	26.91	1.05

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	1.2	4.3	72.50	8.3	4.8	6.8	2.10
2.	1.09	4.01	70.00	7.9	6.3	8.7	2.00
3.	0.98	3.75	65.85	7.2	7.53	12.94	1.75
4.	0.91	3.25	64.11	6.8	9.12	14.36	1.45
5.	0.88	2.90	62.30	6.1	10.90	16.00	1.21
6.	0.80	2.50	61.02	5.6	11.45	17.75	1.05

Table 11. Effect of maturity on fatty acid composition of phosphatidyl choline.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	2.31	56.16	9.91	8.22	18.75	4.65
2.	1.94	52.98	9.01	8.91	22.91	4.25
3.	1.61	48.35	8.75	10.61	26.67	4.01
4.	1.49	45.91	7.67	12.36	28.79	3.78
5.	1.24	42.23	7.01	13.12	33.31	3.09
6.	1.19	40.31	6.51	14.33	35.25	2.41
(b) *Cassia fistula* seed oil						
1.	1.73	73.61	2.55	7.71	12.44	1.96
2.	1.62	70.67	2.21	8.82	15.02	1.66
3.	1.58	65.68	2.09	10.40	18.75	1.50
4.	1.51	61.20	1.89	13.30	20.66	1.44
5.	1.44	58.41	1.73	14.99	22.11	1.32
6.	1.41	55.79	1.60	16.50	23.41	1.29

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	1.50	3.1	75.30	7.70	4.80	6.0	1.60
2.	1.43	2.98	70.81	7.10	6.60	9.53	1.55
3.	1.21	2.61	69.35	6.31	8.11	10.98	1.43
4.	1.10	2.11	68.01	5.83	10.50	11.23	1.22
5.	0.91	1.90	66.1	4.9	11.13	13.98	1.08
6.	0.75	1.70	64.80	4.4	13.71	15.15	0.99

Table 12. Effect of maturity on fatty acid composition of lyso phosphatidyl ethanol amine.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	3.83	50.21	8.21	8.31	24.31	5.13
2.	2.94	47.85	7.74	9.09	27.65	4.73
3.	2.73	45.23	6.88	10.31	30.84	4.01
4.	2.47	43.43	6.11	10.97	33.41	3.25
5.	2.01	42.33	5.25	12.01	35.27	3.11
6.	1.81	39.65	4.85	13.61	37.44	2.64

(Continued ......)

(Table 12, continued)

(b) *Cassia fistula* seed oil

1.	1.37	69.41	4.81	9.31	12.00	3.11
2.	1.31	65.04	4.11	11.45	15.31	2.79
3.	1.29	62.22	3.86	12.01	18.41	2.21
4.	1.21	59.72	2.89	12.89	20.21	1.87
5.	1.01	58.76	2.63	13.99	21.89	1.72
6.	0.91	54.91	2.40	15.19	24.99	1.60

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	1.5	3.37	73.71	6.1	5.50	7.41	2.41
2.	1.31	2.92	71.85	5.99	6.10	9.71	2.12
3.	1.27	2.21	69.31	4.91	8.81	11.60	1.89
4.	1.20	2.11	66.43	4.11	10.21	14.31	1.63
5.	1.19	2.01	64.81	3.45	11.65	15.22	1.41
6.	1.01	1.98	63.90	3.1	12.20	16.51	1.3

Table 13. Effect of maturity on fatty acid composition of lyso phosphatidyl choline.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	3.79	47.18	8.99	8.99	26.30	4.75
2.	3.38	45.31	8.24	10.01	28.81	4.25
3.	3.14	42.44	7.91	11.69	30.71	4.11
4.	2.93	39.91	7.11	12.71	33.31	3.85
5.	2.81	37.51	6.21	13.31	36.51	3.65
6.	2.41	36.41	4.34	15.44	38.40	3.00
(b) *Cassia fistula* seed oil						
1.	2.6	67.78	3.01	8.01	14.93	3.67
2.	2.1	65.31	2.59	10.63	16.26	3.11
3.	1.91	61.21	2.11	11.77	20.24	2.76
4.	1.88	57.66	1.79	12.24	24.01	2.42
5.	1.80	55.98	1.63	13.23	25.15	2.21
6.	1.78	53.55	1.54	14.73	27.9	2.10

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	2.69	3.4	71.90	9.11	4.10	5.6	3.2
2.	2.34	3.12	70.01	7.25	6.98	7.5	2.80
3.	1.85	2.77	68.17	6.99	8.31	9.60	2.30
4.	1.40	2.30	67.85	6.40	8.95	10.72	1.90
5.	1.40	2.30	67.10	5.99	9.9	11.75	1.56
6.	1.30	2.2	66.60	5.43	10.21	13.11	1.15

Table 14. Effect of maturity on fatty acid composition of whole oil.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
(a) *Cassia absus* seed oil						
1.	0.98	53.50	8.75	5.68	26.10	4.99
2.	0.65	52.76	8.35	8.47	25.12	4.65
3.	0.48	40.63	5.99	10.05	38.99	3.86
4.	0.45	37.75	4.78	11.95	42.06	3.01
5.	0.31	32.03	4.49	13.05	47.24	2.68
6.	0.29	28.10	4.35	13.44	51.50	2.30
(b) *Cassia fistula* seed oil						
1.	1.69	67.0	2.40	11.02	14.60	2.55
2.	1.50	62.36	2.01	14.11	16.21	2.00
3.	1.39	58.33	1.83	15.41	19.33	1.86
4.	1.24	54.91	1.62	17.30	23.07	1.73
5.	0.98	50.5	1.43	20.10	25.70	1.62
6.	0.90	45.30	1.20	22.90	28.20	1.50

(Continued . . . . . .)

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	0.22	1.62	56.20	1.46	12.5	14.50	3.85
2.	0.11	1.42	55.22	1.55	12.90	25.00	3.80
3.	0.08	1.29	50.27	1.66	13.40	29.73	3.57
4.	0.06	1.10	47.66	1.89	14.90	30.99	3.40
5.	0.05	1.01	47.15	1.93	15.50	31.00	3.36
6.	0.04	0.92	46.50	2.02	15.80	31.58	3.14

Table 15. Effect of maturity on fatty acid composition of monoglactosyl glyceride.

Maturity stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	1.70	66.91	7.88	8.17	11.91	3.43
2.	1.66	64.22	7.80	9.69	13.21	3.42
3.	1.45	60.81	6.17	12.78	16.31	2.48
4.	1.32	58.34	5.77	13.99	18.19	2.39
5.	1.29	57.23	5.14	14.45	19.66	2.23
6.	1.27	56.25	4.21	15.39	20.76	2.14
(b) *Cassia fistula* seed oil						
1.	1.44	63.15	7.48	12.21	13.76	1.96
2.	1.31	60.31	7.41	14.10	15.14	1.73
3.	1.21	55.81	6.79	16.31	18.19	1.69
4.	1.1	52.71	6.41	18.10	20.04	1.64
5.	0.93	50.59	6.28	19.75	21.01	1.44
6.	0.82	48.81	6.11	20.71	22.34	1.21

(c) *Cassia occidentalis* seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	3.11	2.31	63.61	6.76	9.41	10.01	4.73
2.	2.82	2.28	61.71	6.21	10.21	12.24	4.53
3.	2.27	2.07	68.81	5.95	11.71	15.38	3.81
4.	2.19	1.98	57.81	5.25	13.19	16.41	3.17
5.	2.13	1.72	57.11	4.99	13.92	17.03	3.10
6.	2.11	1.63	56.79	4.84	14.51	17.37	2.75

Table 16. Effect of maturity on fatty acid composition of digalactosyl glyceride.

Maturity stage.	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Cassia absus seed oil						
1.	1.73	68.71	8.16	7.43	10.30	3.67
2.	1.68	66.31	7.79	8.62	12.39	3.21
3.	1.42	62.07	5.89	12.48	15.33	2.81
4.	1.38	60.21	5.65	13.61	16.42	2.73
5.	1.36	59.54	5.34	14.09	17.21	2.46
6.	1.31	57.98	5.13	14.54	18.79	2.25
Cassia fistula seed oil						
1.	1.35	66.73	7.33	9.54	12.29	2.76
2.	1.27	62.35	6.83	11.57	15.35	2.63
3.	1.16	59.53	5.79	13.20	18.41	1.91
4.	0.89	56.41	5.61	15.10	20.17	1.82
5.	0.73	54.32	5.33	16.29	21.85	1.48
6.	0.69	52.11	5.21	18.62	22.12	1.25

Cassia occidentalis seed oil

Maturity stage	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1.	2.89	2.71	60.21	7.11	10.21	12.56	4.31
2.	2.74	2.41	57.31	6.66	12.46	14.20	4.22
3.	2.31	2.11	55.61	6.42	13.49	16.10	3.96
4.	2.15	1.94	54.32	6.21	14.11	18.06	3.21
5.	2.01	1.91	53.56	6.11	14.99	18.28	3.14
6.	1.98	1.88	52.34	5.81	15.75	19.70	2.54

A comparison of the fatty acids of lipid classes in oils extracted with chloroform/methanol (2:1, v/v) is provided for the three *Cassia* seed oils. Lower amount of 16:0 (28:10%) was found in *C. absus* as compared to *C. fistula* (45.30%) and *C. occidentalis* (46.50%). The amount of 18:1 was high in *C. fistula* (22.90%) whereas *C. absus* and *C. occidentalis* contained low amounts (15.61% and 15.80%) of 18:1. Higher amounts of 18:2 were found in *C. absus* (56.52) as compared to *C. occidentalis* and *C. fistula* seed oils which contained (31.58% and 28.20%) of 18:2. Myristic and linolenic acids were present as the minor components in all the seed oils. Sn-1, 2-diacylglycerols contained slightly less 16:0 and more 18:2 as compared to 1,3-diacylglycerols which contained proportionally more 16:0 and less 18:2. The fatty acid composition of **1** and **2** monoacylglycerols were almost similar.

The polar lipid classes differed from non polar lipid classes in fatty acid composition and in general, were more saturated.

Changes in the fatty acid composition of non-polar and polar lipid classes, generally, followed trends reported in whole oils. The percentages of 18:1 and 18:2 found in all fractions were relatively low in immature seeds but increased as the seeds matured. Increases in 18:1 and 18:2 percentages and rduction in 16:0 percentages also followed a similar trend in all the lipid fractions examined.

The results of this investigation were found to be similar to those reported by others.

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