BIOCHEMICAL CHANGES IN COTTONSEED DURING GERMINATION

By

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INTRODUCTION

Changes in lipid and dry matter content have been observed in germinating seeds of *Brassica* (1) , *Cucurbita* (2) , Flax (3-5), peanuts (6-7), *Ricinus* (8), *soybean* (9-14), and wheat (15,16). Very little information is available on the changes taking place in cottonseed during germination. Similar to what happens in other oleaginous seeds, lipids are broken down during cottonseed germination (17-21), the total sugar contents decrease (17,22), while the nitrogen content remains about the same (17,22).

The changes in the oil characteristics and the fatty acid proportions during germination differ with different seeds. White (21) reported that during germination the rate of depletion of linoleic, oleic and saturated acids in cottonseed was proportional to their amounts in the reserve oil.

No precise information appeared in the literature concerning the changes in cottonseed phospholipids during germination. Weiss (23) indicated that although phosphatide phosphorous showed a little change in cereals and legumes, yet in oilseeds it increases.

The present study was designed to investigate the changes in cottonseed constituents during germination. Changes in oil characteristics including : unsaturation, free acidity, and fatty acid constitution are also reported. Total and individual phospholipid contents of the oils as well as the fatty acids of the mixed phospholipids were studied.

MATERIALS AND METHODS

The cottonseed (*Gossypium barbadense,* variety Bahteem-111) used in the present investigation was kindly supplied by Ministry of Agriculture Experimental Stations. Chester method (24) was used to separate sound seeds. The germination technique is basically the standared technique proposed by Simpson (25). Fifty gram samples of sound resting seeds were germinated for 2 to 18 days in dark at 28°C. At the end of each germination period the seed samples were heated in an air oven at 80°C for 4 hours. The partially dried seeds were decorticated. Care was taken to separate the germinated kernels, which were then completely dried at 105°C for 12 hours, placed in a desiccator and weighed.

Dry kernels were subjected to analysis in order to find the changes in the constituents during germination. The oil and ash contents were determined according to the A.O.C.S. procedures (26). The percentage nitrogen was determined by micro-Kjeldhal procedure (27) and the protein content was then calculated as « % nitrogen x 6.25 ». The crude fiber was determined according to A.O.A.C. procedure (28). The total gossypol content was determined according to Pons et al. procedure (29). The total carbohydrate content was obtained as proposed by Grindley (30) as the difference between the kernel weight and the summation of the above constituents of the dry kernel.

The acid value (A.V.) and the iodine value (I.V.) of the oil were determined according the A.O.C.S methods (26). The phospholipid fractions were isolated according to Hanahan procedure (31). T.L.C. of phospholipids was done according to El-Nockrashy and Osman technique (32), and quantitative determination according to El-Nockrashy et al. procedures (33,34). The component fatty acids of samples under investigation were converted to their methyl esters by esterification (35), and the mixed methyl esters were subjected to gas liquid chromatographic analysis using a Pye Argon Chromatograph.

RESULTS AND DISCUSSION

Changes in Kernel Constituents :

Table 1 shows the gram contents and Table 2 shows the percentage composition of the kernels during germination. Figure 1 illustrates the percentages consumption of oil, protein and carbohydrates.

TABLE 1

The Gram Contents of Kernels During Germination

Days	Kernel	Oil	Protein	Fiber	Ash	Gossypol	Carbohydrate
0	31.52	6.45	12.80	0.63	2.33	0.46	8.84
2	28.85	6.35	12.20	0.54	2.02	0.43	7.31
4	27.64	6.04	11.82	0.37	1.86	0.43	7.12
6	27.16	5.83	12.20	0.33	1.81	0.44	6.55
8	26.64	5.72	12.06	0.21	1.71	0.43	6.51
10	23.05	4.89	10.33	0.21	1.65	0.39	5.58
12	17.95	3.38	9.05	0.36	1.42	0.44	3.30
14	16.65	3.03	8.69	0.31	1.51	0.38	2.73
16	12.57	1.91	7.01	0.20	1.14	0.30	2.01
18	12.45	1.83	7.17	0.31	1.04	0.28	1.80

TABLE 2

The Percentage Composition of Kernels during Germination

Days	Oil	Protein	Fiber	Ash	Gossypol	Carbohydrate
0	20.5	40.6	2.00	7.39	1.46	28.1
2	22.0	42.3	1.87	7.00	1.49	25.3
4	21.9	42.8	1.34	6.73	1.56	25.8
6	21.5	44.9	1.22	6.66	1.62	24.1
8	21.5	45.3	0.79	6.42	1.61	24.4
10	21.2	44.8	0.91	7.16	1.69	24.2
12	18.8	50.4	2.01	7.91	2.45	18.4
14	18.2	52.2	1.89	9.07	2.28	16.4
16	15.2	55.8	1.59	9.07	2.39	16.0
18	14.7	57.6	2.49	8.35	2.25	14.6

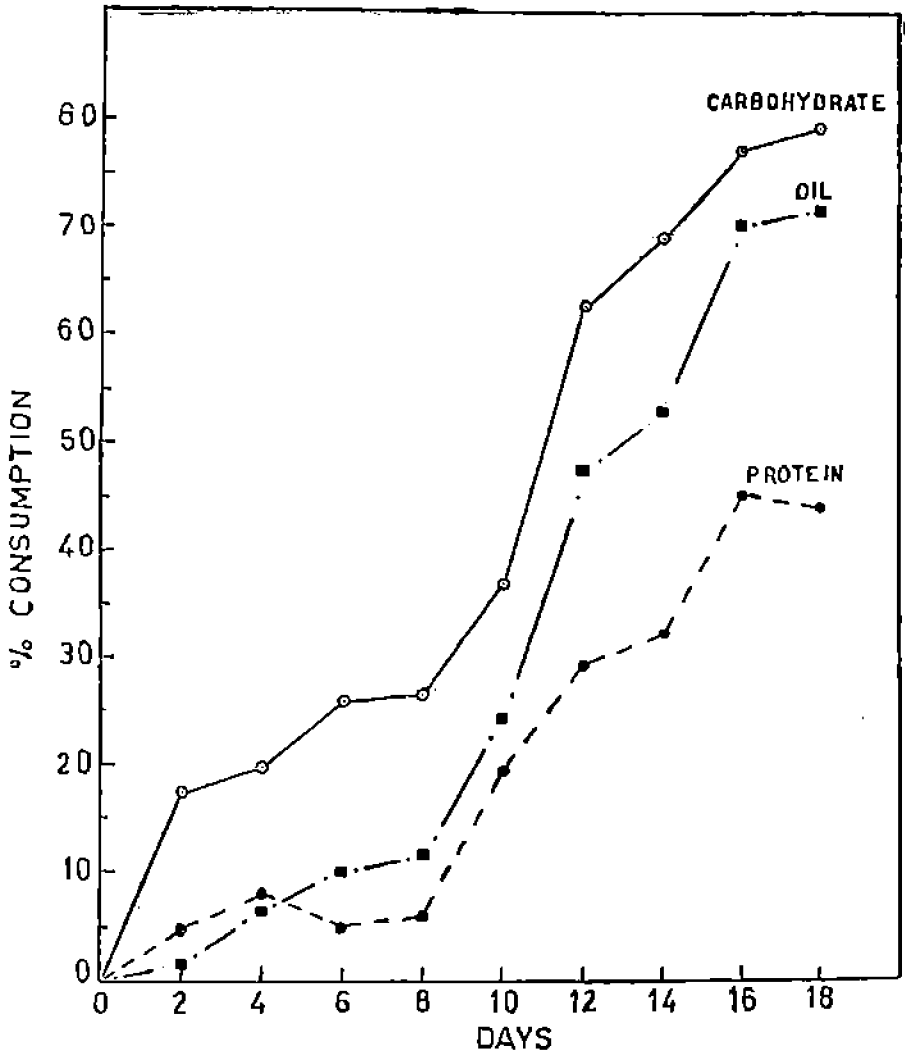

Fig. 1 — Percentage Consumption of Kernel Constituents during Germination.

The total dry matter, the oil, protein, carbohydrate, fiber, ash gram weight contents of cottonseed kernels shows continuous reduction during germination. The percentages of these constituents also decrease, with the exception of the percentage protein in the germinated kernels which increases.

The highest reduction in kernel dry weight and the highest oil, protein and carbohydrate consumptions occurred after the 8th. day, when active metabolism starts. The percentage carbohydrate consumption was always higher than the oil or protein consumptions throughout the 18 days germination. With the exception of the first 4 days, the percentage oil consumption was higher than the percentage protein consumption. By the end of the 18 days germination 79.4% of the original carbohydrate present in the kernels was consumed, compared to 71.7% and 44.0% oil and protein, respectively. Although the percentage protein consumption was usually lower than the percentage oil consumption, yet the amounts of protein consumed were usually higher than the oil.

Olcott and Fontaine (18) reported ca. 50% reduction in the oil content of cottonseed after 139 hours germination. Malowan (22) found that Ca. 45% of the total sugars were consumed in less than 4 days, while the nitrogen content remains about the same.

Changes in Oil Characteristics :

Figure 2 illustrates the changes in the iodine value and the acid value of the oil during germination. The I.V. shows gradual continuous increase from 101.4 to 107.5 during the 2nd to 8th days of germination, after which the I.V. was reduced to a more or less constant value (ranging from 105.1 to 105.9). Increase in the I.V. during the germination of soybean (9), peanuts (6) *Ricinus* seeds (8) is also reported.

The A.V. showed gradual continuous increase from 0.95, before germination, to 5.79, after 16 days germination. There is a more or less general agreement that the A.V. of oleaginous seeds increases during germination. Studies on flaxseed (4) peanuts (6) *Ricinus* (8) revealed similar behaviour, Rabadie et al. (6) attributed such increase to the hydrolysis of glycerides by water.

Table 3 gives the fatty acid composition of oil samples. Surprisingly, only little changes occurred in the fatty acid composition of the oil even during their most rapid loss from the kernels. This general observation has been also noted by other investigators in their studies

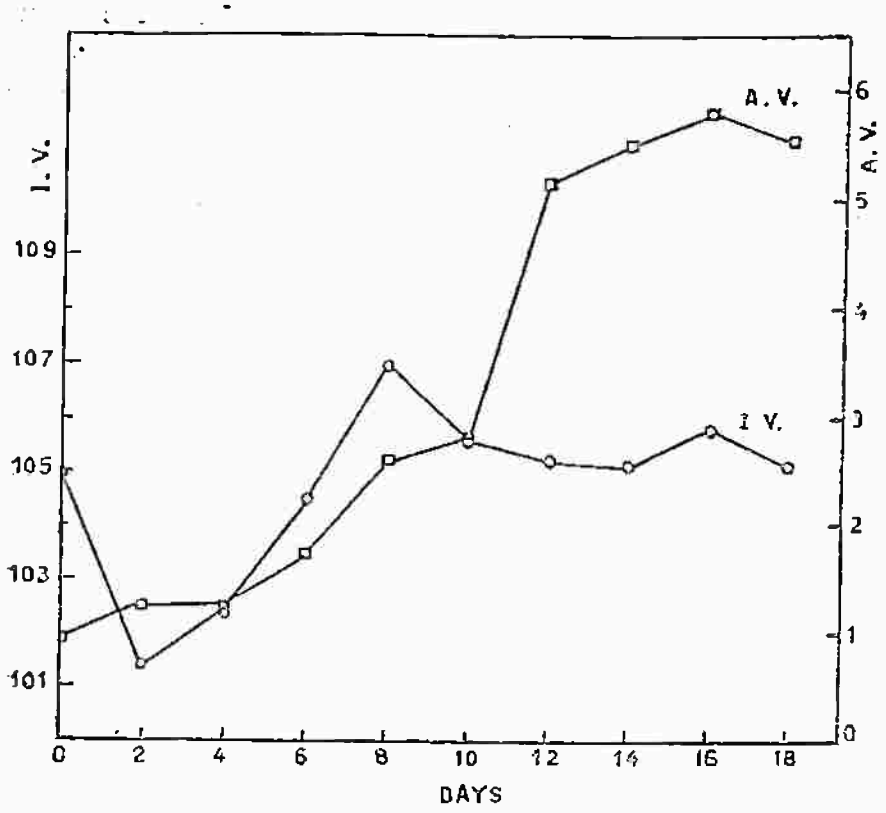

Fig. 2 — Changes in I. V. and A. V. during Germination.

TABLE 3

Fatty acid Composition of Oil *

Fatty Acid	Days				
	0	4	10	14	18
$C_{14:0}$	0.8	1.2	0.8	0.7	0.8
$C_{16:0}$	22.9	23.0	24.3	25.0	25.2
$C_{16:1}$	1.5	2.1	2.1	2.2	1.5
$C_{18:0}$	1.0	0.8	1.0	1.0	0.9
$C_{18:1}$	27.7	27.8	27.8	28.1	30.0
$C_{18:2}$	46.1	45.1	44.0	43.0	41.6

* Relative areas under the curves for the methyl esters of the component fatty acids as estimated from GLC tracing.

on the germination of some oleaginous seeds (9,10,36,37). The percentgae total saturated fatty acids increased from 24.7 to 26.9% in the 18 days germination. Oleic acid did not show any appreciable change up to 14 days after which it showed some increase. Linoleic acid showed continuous reduction, from 46.1 to 41.6% in the 18 days germination. Preferential utilization of linoleic during germination of peanuts was reported by Vyas et al. (7).

Figure 3 illusterates the losses in the three major fatty acids, namely, palmitic, oleic and linoleic acids from the kernels expressed as percent of the original. Generally, it can be seen that the rate of loss of fatty acids coincides with the rate of loss of oil, being lower at early stages of germination and increases continuously thereafter. However, the rate of loss of palmitic was the lowest and that of oleic was the closest to the rate of oil consumption.

Changes in Phospholipids :

Thinlayer chromatography of phospholipids isolated from oils revealed the presence of : phosphatidly inositol «PI», lysolecithin «LS», phosphatidyl serine «PS», lecithin «LE», phosphatidyl ethanolamine «PE» and an unknown phospholipid «U», with RF values 0.04, 0.16, 0.39, 0.45, 0.63, 0.85, respectively. Identification of these phospholipids was verified through the use of authentic standards (Supelco INC. Products, Bellefonte, Pa.) These six phospholipids were previously reported in cottonseed by El-Nockrashy and El-Shattory (38-40).

Analysis for the total phospholipid contents of the oil revealed continuous increase in the percentage from ca. 2.4% before germination to ca. 6.4% after 18 days germination (Table 4). The fact that highest rate of increase in the phospholipid content starts after 10 days germination, which is also the period of active metabolism, suggests that phospholipids might have a role in the metabolism of germinating seeds. Weiss (23,41) found that although phospholipid phosphorous shows little changes during the germination of cereals and legumes, yet it increases in case of oilseeds. Halden and Hinrich (11) reported increase in the phospholipid content during soybean germination. Weiss (41) indicated the participation of phospholipids in the utilization of lipid reserves.

In table 4 the individual phospholipid contents are expressed as percent in oil and as percent of total phospholipids. Lecithin which has been long recognised as the major plant phospholipid (32,38,42) was found to make ca, 28% of the total phospholipids of the non-

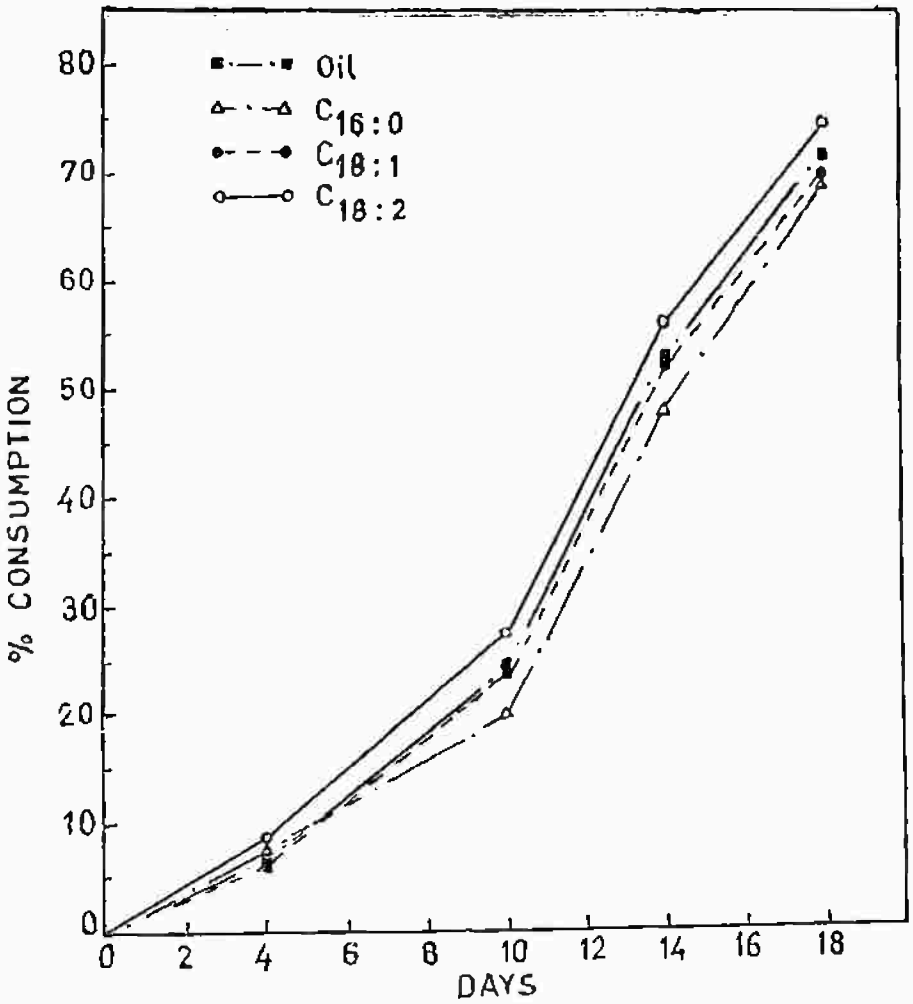

Fig. 3 — Oil and Majr Fatty Acids Consumptions during Germination.

TABLE 4

Changes in Phospholipid Constitution during Germination

Phospholipid ***	Day 0		2		4		6		8		10		12		14		16		18	
	% in * O.	in * P.	in O.	in P.	in O.	in P.	in O.	in P.	in O.	in P.	in O.	in P.	in O.	in P.	in O.	in P.	in O.	in P.	in O.	in P.
PI	0.28	11.7	0.40	12.4	0.43	12.6	0.52	14.0	0.49	12.8	0.58	14.7	0.72	15.7	0.84	16.3	0.99	16.3	1.06	16.4
LS	0.31	13.1	0.43	13.4	0.50	14.6	0.55	14.0	0.57	14.7	0.68	17.1	0.82	17.6	0.95	18.3	1.26	18.5	1.58	24.6
PS	0.34	14.2	0.49	15.3	0.58	16.9	0.64	17.3	0.81	20.9	0.65	16.4	0.70	15.2	0.73	14.0	0.78	12.8	0.65	10.2
LE	0.67	28.2	0.91	28.4	1.02	29.8	1.10	29.8	1.17	30.1	1.12	28.3	1.16	25.1	1.24	23.9	1.29	21.3	1.23	19.2
PE	0.43	17.9	0.52	16.0	0.45	13.1	0.47	12.7	0.44	11.5	0.48	12.2	0.53	11.4	0.59	11.5	0.66	10.9	0.64	10.0
U	0.36	14.9	0.47	14.5	0.45	13.1	0.42	11.3	0.38	9.9	0.44	11.2	0.70	15.2	0.83	16.0	1.10	16.2	1.26	19.7
Total	2.39		3.22		3.41		3.70		3.86		3.95		4.63		5.18		6.08		6.42	

* % Individual phospholipid in oil.

** Individual phospholipid as percent of total phospholipids.

*** PI : phosphatidyl inositol, LS : lysolecithin, PS : phosphatidyl serine, LE : lecithin, PE : phosphatidyl ethanolamine, U : unknown phospholipid.

germinated seed. Combined cephalins « PI, PS and PE » constitute ca 43.8%, i.e. LE — cephalin ratio 1 : 1.55.

It is quite clear that, as germination proceeds, considerable changes in the individual phospholipid percentages take place. The individual phospholipid contents of the oil show a more or less continuous increase by germination. After 18 days germination, the LE content of the oil was only slightly less than double of amount before germination. The total cephalin content showed also continuous increase to become more than doubled.

The LE and total cephalin contents of the mixed total phospholipids reached their maximum after 8 days germination then gradually decrease. Although the LE — cephalin ratio did not show appreciable change up to 10 days (average 1 : 1.5), lower LE — cephalin ratios were found thereafter. In the nongerminated seed PE makes the major cephalin, followed by PS then PI. During germination the percentage PE in the total phospholipids decreases continuously, while PI increases to exceed the PE after 4 days germination. The percentage PS also increases to exceed PE after 2 days and reaches a maximum after 8 days germination. In the 18 days sample PI makes the highest percentage. The LS content shows continuous increase as germination proceeds. Its value in the 18 days germinated seed oil is more than five folds that originally present in the nongerminated seed oil.

Fatty Acid Composition of Mixed Phospholipids :

While the fatty acids of the oil showed only little changes during germination, those of the phospholipid showed significant changes (Table 5).

TABLE 5

Fatty Acid Composition of Mixed Phospholipids *

Fatty Acid	Days					
	0	4	10	14	16	18
$C_{14:0}$	0.7	0.4	0.6	1.2	2.8	1.2
$C_{16:0}$	28.3	30.6	32.5	33.8	29.8	24.8
$C_{16:1}$	2.1	1.0	1.4	2.9	2.3	1.0
$C_{18:0}$	0.8	0.9	1.3	1.1	1.0	0.5
$C_{18:1}$	28.0	28.1	29.5	27..5	24.9	17.2
$C_{18:2}$	40.0	39.0	34.8	33.6	39.2	55.3

* Relative areas under the curves for the methyl esters of the component fatty acids as estimated from GLC tracing.

The mixed phospholipid contains all the fatty acids persent in the oil, however their percentage total saturated fatty acids content is higher. Phospholipids of oleaginous seeds are characterized by higher saturated fatty acids compared to their oils (38,42,43).

Linoleic acid makes the major unsaturated fatty acid, and palmitic acid makes the major saturated acid as in the case of the oil.

The percentage total saturated acids increases continuously and regularly up to 14 days germination and recreases thereafter. Palmitic acid shows also a maximum in the 16 days germinated sample.

Linoleic acid content decreases as germination proceeds up to 14 days germination then shows great increase. Oleic acid shows some increase during the first 10 days, after which continuous reduction in its content was observed.

SUMMARY

Changes in oil, protein, carbohydrate, fiber, ash and gossypol contents of cottonseed during germination were studied. Highest reduction in kernel dry weight and highest oil, protein and carbohydrate consumptions occured after the 8th. day. Percentage carbohydrate consumption was always higher than oil or protein consupmtions. Only little changes in fatty acid composition of oil was detected even during most rapid loss from kernels. The rate of losses of the three major fatty acids from kernels were in the following order : linoleic oleic palmitic. There was a continuous increase in the percentage total phospholipids as germination proceeds. The lecithin-cephalin ratio was 1 : 1.5 up to 10 days germination, after which the ratio was lowered. The data indicate significant changes in the fatty acid constitution of the phospholipids throughout the germination period.

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