CCIV. NOTES ON OIL DEVELOPMENT IN THE SEED OF A GROWING PLANT.

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SOME years ago, in connection with problems arising in the Linen Industry, an examination was made of certain biological changes which take place in the flax plant during the processes of growth and of ripening. These had to do with the study of so-called "quality" of fibre for spinning and with "quality" of seed for special purposes. In the course of these investigations, results were obtained of considerably wider interest than those in view at the time, and although reference has been made to these investigations [Armstrong, 1924] the results have not hitherto been published. Through the courtesy of the Linen Industry Research Association it is now possible to give an account of this work.

The only previous work bearing on this question was that described by Ivanoff [1912] who examined the biological aspect of oil accumulation. This work was repeated under far more exactly controlled conditions by Eyre and Fisher [1915] who confirmed the general conclusions of Ivanoff and showed clearly that oil accumulates rapidly during the early stages of seed development, after which further increase is comparatively slight. During that year, in collaboration with Morrell [1915], who drew attention to the commercial significance of this work, attempts were made to trace changes in the chemical characteristics of this oil as the seed developed and matured, but the difficulty of obtaining, at any particular stage of development or ripeness, sufficiently large and uniform quantities of seed was not overcome and the results, although of considerable interest, could only be regarded as tentative. The flax plant continues to flower day by day over a long period; the individual flowers last but a few hours; they are self-fertilised, after which, generally about noon, the petals fall and from then onwards the seed develops and ripens.

On resuming this work with Barker during 1921 and 1922 special precautions were taken to raise a perfectly uniform crop of flax and each season more than 8000 flowers were individually marked at stated intervals to provide the requisite quantity of seed for a fuller examination. The variety of flax grown in 1921 was that known commercially as Blue Flowering Dutch. Warm fine weather prevailed during the whole period of the trials. In 1922 two varieties were grown, namely the tall fibre-bearing blue-flowering *Linum usitatissimum* from Riga and *Linum cribrosum*, a short seed-bearing variety

from Madrid. Cool, generally wet, conditions prevailed and the season was fully two weeks later than in 1921. The plots were maintained in such good order as to ensure uniform development and ripening over the whole area.

In order to avoid oxidation all samples of seed collected were at once removed from the capsule and dried at 100° in a current of dry carbon dioxide, a procedure which avoided the possibility of after-ripening of the seed and the undesirable accumulation of water in the light petroleum used for the oil extraction. The following method was employed for determining the amount of oil present in the dried samples of seed taken from time to time. The weighed sample was ground with purified sand, transferred to a Soxhlet thimble and extracted with light petroleum (B.P. 50° to 70°) for 8 hours, taking care that the whole process was conducted in a current of dry carbon dioxide. The iodine values of these extracted oils were determined under the usual standard conditions by means of Wijs's solution, comparisons being made with a linseed oil of known iodine value.

In 1921 the first sample was picked 6 days after marking (flowering) and was found to contain only a trace of oil¹. Subsequently 26 further samples were taken at short intervals over a period of 53 days after flowering. The seeds themselves when examined within the capsule at a very early stage of development appeared white and almost translucent. About the fifth day, as chlorophyll made its appearance in the young seeds, they assumed a greenish hue which increased to deep green by about the eighteenth or twentieth day. After this stage had been reached, when the seeds were fully plump and well grown, the green colour gradually disappeared and a browning set in, until, by about the fortieth day after flowering the seeds were a full brown colour, becoming darker as the seeds ripened. The analytical results of examining the seeds and the oil extracted therefrom are shown graphically as curves *A*, *B* and *C* in

Fig. 1.

Fig. 1. On reference to these it will be seen that the synthesis of oleaginous extractable matter begins almost immediately after flowering and proceeds

¹ *I.e.* matter extractable by light petroleum.

comparatively slowly during the first 11 days. At the end of that time a rapid accumulation of oil sets in, amounting to about 3 % per day for some 10 days, when, after about 21 days from the time of flowering, the maximum oil content is reached and ceases with the disappearance of chlorophyll. As will be seen, observations were extended over a further period of 32 days, but during that period the variations in oil content were practically negligible when allowance is made for experimental errors due to sampling. Similar results were obtained with the two varieties grown in 1922, as will be seen on reference to the full line graphs shown in Fig. 2.

Fig. 2.

Changes in the character of linseed oil during seed development and ripening.

(i) *The colour of linseed oil.* During the course of this work wide variations were observed in the character of the linseed oil extracted at the various stages of development and ripeness of the seed. At first the oil was more than usually viscous and was of a greenish hue, but this gave place to a greater mobility and the characteristic full golden yellow colour as the age of the seed advanced. Further the oil became increasingly lighter in colour until, after 53 days from flowering, the seed yielded a very pale yellow oil and, when the harvested plants themselves, or merely the capsules containing the seeds, were stored under cover, indoors, the oil extracted from the seed was practically colourless.

(ii) *The unsaturated character of linseed oil.* In the case of a crop raised from *L. usitatissimum*, Dutch seed, the young seeds taken from the capsules when only 10 days old were found to yield an oleaginous residue having an iodine value of 114. Difficulties which attend the isolation of a sufficient quantity of this extract from seeds less than 10 days old prevent great reliance being placed upon analytical results obtained with such material, but it appears that the iodine value may well be as low as 100.

The iodine value of a good linseed oil varies between 180 and 200. On reference to Graph *B* in Fig. 1 it will be seen that the lower limit was reached after about 35 days, whilst the oil from seed 53 days old gave an iodine value of 191.9.

From a comparison of the graphs *A* and *B* in the same figure there appears to be no evidence of a change in the oil content parallel with the change in the iodine value of the oil. The interesting fact is revealed that the greatest rate of increase in the iodine value takes place after the oil content has attained its maximum value. The iodine value does not reach a maximum, but rises throughout the whole period during which observations were made, namely for 53 days after flowering, although the rate of increase diminishes somewhat towards the end of the period. The unsaturated character of the oil develops to a marked degree after the oil has been synthesised and the oil does not, as was generally supposed, become more and more saturated in character as the seed develops. Indeed, evidence points to the probability that changes in the degree of saturation are independent of those processes conditioning oil formation. Rapid oil formation occurs during those early days, 0 to about 20, when the colour both of the seed and of the extracted oil denotes the presence of chlorophyll; after that period, when the seed assumes a yellowish brown and full brown colour and the oil a full deep yellow colour, the iodine value of the oil rises rapidly, although no further increase in the oil content takes place. This view is supported by the following observed changes in the oil derived from seed when allowed to ripen under different conditions. It appears probable that these changes in the colour and iodine value of the oil are connected in some way either with residual life processes which persist in the plant, or are a separate set of changes controlled possibly by the carotene which persists throughout this period. Whether carotene is able to act so vigorously as an oxygen carrier as to remove hydrogen from the glyceride is not known, but from evidence available this does not seem improbable. The carotene itself may become oxidised during the final stages and thus account for the gradual loss of colour of the oil. In Table I will be found data relating to:

Material (a). Seed derived from detached capsules freshly gathered.

Material (b). Material as in (*a*) but set aside in the laboratory for varying periods.

Material (c). Seed derived from capsules not detached, but allowed to remain on the living plant while material (*b*) was after-ripening in the laboratory.

(iii) *The acidity of linseed oil.* The oils and fats which a plant produces from simple carbohydrates and stores as a reserve food material in its seed generally make their appearance as the seed approaches maturity, and, on germination, such reserve food is again converted into carbohydrates to support the young plant. In flax, however, it appears that oil formation sets in at an early stage, that the nature of the oil progressively changes, becoming more and more unsaturated in character, and contrary to expectations oil from fully mature seed is of greater value as a drying oil than is oil from less mature seed.

Table I.

TABLE I.													
Material (a) Seed from detached capsules freshly gathered			Material (b) Seed from detached capsules as (a) but after remaining at room temperatures for various periods						Material (c) Seed from capsules not detached from plant but allowed to remain on living plant while material (b) was at room temperatures				
Days after flowering	Oil %	Iodine value of oil	Total days after flowering				Increase of iodine value	No. of days after flowering	Oil %	Increase of oil content %	Iodine value	Increase of iodine value	
			No. of days after flowering (a)										
15	27.85	124.7	5	20	33.25	5.40	128.5	3.8	20	35.98	8.13	129.8	5
15	27.85	124.7	15	30	34.59	6.74	145.6	20.9	30	36.9	9.1	172	47
20	35.98	129.8	5	25	36.12	0.14	149.5	19.7	25	37.0	1.0	162	32
20	35.98	129.8	15	35	36.08	0.10	154.6	24.8	35	36.7	0.7	179	49
29	36.56	171.9	4	33	36.86	0.30	173.7	1.8	33	36.8	0.3	176	4

Glycerol, a substance closely related to the simple carbohydrates (sugars) from which presumably these compounds are formed, has been thought by some investigators to be the first product in the natural formation of linseed oil, the fatty acids being produced at a much later stage. Ivanoff, on the other hand, has suggested that in the synthesis of fats the higher saturated fatty acids are the first to be formed from the carbohydrates and that they are in turn converted into unsaturated acids which then combine with the glycerol present to form the fat.

It is of importance to have definite information on these points because for most technical purposes, as in the case of paint and varnish making, linseed oils of low acidity are highly esteemed. Should acid formation take place in the main during a definite period of seed development or ripening the character of the resulting oil may be in this respect influenced materially by harvesting the seed at a particular time. With the object of gaining information on these points in addition to following the course of oil formation, the acidity was measured of the oleaginous product obtained by extracting with light petroleum seeds of different degrees of ripeness. This was done by titrating with standard NaOH a solution of the oleaginous extract in neutral 90 % alcohol free from carbon dioxide, using phenolphthalein as indicator. The substances which are extracted from very young seeds by light petroleum are obtained as a solid or semi-solid mass when the solvent is evaporated. This extracted material is greenish-yellow in colour, readily becomes fluid when slightly warmed and is decidedly acidic.

On reference to Table II, columns 11, 12 and 13, it will be observed that as the seed of *L. usitatissimum* (Riga) ages the acidity of the oil diminishes rapidly, finally becoming almost constant at the low value of approximately 0.6. Commercial Baltic oil has an acidity of approximately 3 to 4 and Calcutta oil approximately 1 to 2. In the case of *L. cribrosum* (Madrid) similar results are recorded in Table III, columns 11, 12 and 13, from which it will be seen that in this variety of flax the oleaginous extract from the very young seeds is far more acidic than is the case with the *L. usitatissimum*, although it finally reaches a somewhat lower acid value. In this variety also the light petroleum extract from seeds 10 to 14 days old was obtained as a solid mass at ordinary

Table II. *Linum usitatissimum (Riga).*

Freshly gathered seeds		Calculated for 1000 seeds										Oil from dry seeds		
		Volatile					Dry seeds					Oil from dry seeds		
		Wt. of seeds g.	at 100° %	Oil %	Vol. at 100° g.	Oil g.	Carbo- hydrates and protein g.	Wt. of seeds g.	Oil %	Colour	Acid value of oil*	Acid (calculated) as oleic acid†	Remarks	
Age days														
1	2		3	4	5	6	7	8	9	10	11	12	13	
12	6.3637	82.57	0.383	0.553	5.2545	0.0244	1.0848	1.1092	2.20	Greenish	—	—	Oil semi-solid at	
14	7.3264	80.88			5.9256	0.0405	1.3603	1.4008	2.89	yellow	8.6	43.428	ordinary tem-	
16	7.2771	78.17	1.207	0.5885	5.6885	0.0878	1.5008	1.5885	5.53		8.4	41.454	peratures; oil	
18	7.6685	76.99	2.078	0.789	5.8963	0.1594	1.6128	1.7722	8.99		7.8	38.493	fluid but of high	
19	7.8838	74.63	2.935	0.935	5.6598	0.2226	1.7014	1.9240	11.57	Changing	4.8	24.083	viscosity; becom-	
20	7.9179	74.92	4.015	1.015	5.9321	0.3179	1.6679	1.9568	16.01	gradually	4.2	20.727	ing less viscous	
23	7.7178	68.89	7.267	1.267	5.3168	0.5609	1.8401	2.4010	23.36	from green	2.2	10.857	as green colour	
25	7.6743	63.22	10.751	1.751	4.8517	0.8251	1.9975	2.8226	29.23	to deep	1.0	4.935	disappears; oil	
27	7.5928	59.63	13.140	2.140	4.5276	0.9977	2.0675	3.0652	32.55	yellow	0.7	3.553	much less vis-	
30	7.7642	57.69	14.707	2.707	4.4688	1.1392	2.1382	3.2774	34.76		0.6	3.158	cous; marked	
32	8.1220	55.42	16.062	3.062	4.5012	1.3046	2.3162	3.6208	36.03	Green	0.6	2.961	decrease in vis-	
38	8.0422	47.92	20.254	3.8538	3.8538	1.6289	2.5595	4.1884	38.89	disappeared	0.56	2.763	cosity and oil	
46	7.6621	38.32	23.550	1.9361	1.7891	1.7891	2.9369	4.7260	38.18	Pale yellow	0.60	2.961	more readily ox-	
66	6.7209	28.70	26.673	1.9289	1.7927	2.9993	2.9993	4.7920	37.41	Very pale	0.52	2.566	idised	
76	6.3032	24.03	28.443	1.5147	1.7928	1.7928	2.9957	4.7885	37.44	yellow	0.48	2.369		

Table III. *Linum cribrosum (Madrid).*

Freshly gathered seeds		Calculated for 1000 seeds										Oil from dry seeds		
		Volatile					Dry seeds					Oil from dry seeds		
		Wt. of seeds g.	at 100° %	Oil %	Vol. at 100° g.	Oil g.	Carbo- hydrates and protein g.	Wt. of seeds g.	Oil %	Colour	Acid value of oil*	Acid (calculated) as oleic acid†	Remarks	
Age days														
10	4.8426	84.43	0.11	0.086	0.0053	0.7487	0.7540	0.68	0.68	Deep	—	—	Oil solid or semi-	
14	7.0844	81.76	0.56	0.56	5.7922	0.0397	1.2545	1.2922	3.08	greenish	83.0	420.462	solid at ordinary	
15	7.5610	79.77	0.83	0.83	6.0314	0.0628	1.4668	1.5296	4.09	yellow	—	—	temp.; oil fluid	
17	7.6892	77.35	1.46	1.46	5.9476	0.1123	1.6293	1.7416	6.45		23.3	118.045	but of high vis-	
20	7.8856	75.88	2.44	2.44	5.9836	0.1924	1.7096	1.9020	10.11	More	14.2	72.248	cosity becoming	
23	8.0137	72.19	4.62	4.62	5.7851	0.3702	1.8584	2.2286	16.62	intensely	7.0	35.532	less viscous as	
28	8.3667	67.71	8.97	8.97	5.6651	0.7505	1.9511	2.7016	27.79	green	3.2	16.582	green colour dis-	
32	7.9594	60.60	13.07	13.07	4.8234	1.0403	2.0957	3.1360	33.18	Faintly	—	—	appears	
36	7.6480	53.07	16.83	16.83	4.0588	1.2872	2.3020	3.5892	35.87	green	1.4	6.909	Oil much less	
44	8.6093	45.23	19.76	19.76	3.8940	1.7012	3.0141	4.7153	36.07	Green	1.1	5.525	viscous; decrease	
52	7.9823	41.16	21.14	21.14	3.2855	1.6875	3.0093	4.6968	35.93	absent	0.8	4.343	in viscosity. Oil	
66	7.3620	35.27	22.74	22.74	2.5866	1.6741	3.0913	4.7654	35.13	Very pale	0.7	3.553	more readily ox-	
73	6.9107	31.90	24.01	24.01	2.2045	1.6593	3.0469	4.7062	35.26	yellow	0.18	0.928	idised	
84	5.6634	16.81	29.32	29.32	0.9520	1.6605	3.0509	4.7114	35.24	oil	0.12	0.592		

* KOH per 1000 g. of oil.

† Calculated as oleic acid per 1000 g. of oil.

temperatures. It is noteworthy also that this material was found to consist entirely of free acids, no indication being found of the presence of glycerides or other hydrolysable substances. It seems clear therefore that fatty acids are stored in the seed in the first instance, from which, presumably, the glycerides constituting the linseed oil are formed at a later stage. In Fig. 2 will be seen recorded graphically as full line curves the rate of development of the oleaginous extract or oil for the two varieties referred to, and as broken curves the corresponding acid development expressed in terms of oleic acid, as a convenient unit of acidity. The trend of these curves shows clearly how widely different the rates of acid formation in the seed of different varieties of flax may be, although the rate at which the oleaginous extract is stored in the seed is approximately the same in both varieties.

Through lack of material the lipase activity of the seeds at different stages of growth was not followed; it should certainly be done as this will probably throw interesting light on the mechanism of natural oil synthesis. If one may judge, however, from observations made regarding the activity of linase and the incidence of linamarin in the foliage and seeds of the same genus [Armstrong and Eyre, 1912] one may expect lipase activity to be coincident with glyceride formation.

SUMMARY.

A study has been made of changes which take place in the rate of formation and in the character of the oil in the seed of flax—*Linum usitatissimum* and *L. cribrosum*—throughout its development and ripening period.

Observations show that in oil formation the acidic constituents are formed first. Whether the glycerol is formed at the same time, only combining with the fatty acid at a later stage, or whether the formation of glycerol itself is delayed is not clear. The observations reveal a remarkably rapid oil formation over a period of some 15 days, during which a maximum of about 36 % of oil, calculated on the dry weight of the seeds, is reached.

Changes in the nature of the oil continue after oil formation has ceased; its unsaturated character, as measured by its iodine absorption, continues to increase. It is pointed out that this is apparently an entirely separate change, independent of oil formation.

The interesting point is brought out that whereas during the early stages a reducing system must prevail in order to account for the formation of acids from carbohydrates, this must change to an oxidising system leading to the formation of unsaturated acids.

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