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THE DEVELOPMENT OF RAPID METHODS FOR THE ESTIMATION OF THE OIL-CONTENT OF SINGLE COTTON SEEDS

Part II.—A Preliminary Examination of some Non-destructive methods

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For carrying out genetic experiments on cotton seeds, the rapid estimation of the oil content of individual seeds is of considerable importance, and in an earlier communication[1] some experiments were described leading to the development of a simple technique, in which however the kernel had to be crushed. The present paper describes experiments directed towards developing techniques that do not impair the germinating power of the seeds. The specific gravity of the seeds and their electrical properties have been studied for this purpose and significant results have been obtained in both cases.

Study of the specific gravity of the seeds

For these measurements, the dehulled seeds (of the L. S. S. variety) were used because it was feared that the air enclosed in the shell and the linters would produce large variations not connected with the oil content of the kernel. A preliminary examination showed that some of the kernels floated on water, while others sank in it. Accurate measurements of the specific gravity were then made for ten seeds with the help of Archimedes's principle, using a metal sinker to ensure the full immersion of each seed. The specific gravity was then calculated as the ratio of weight of kernel to the loss of weight when immersed in water. These measurements (upper half of Table 1) confirmed the previous observation and indicated the presence of two groups of kernels with specific gravities in the neighbourhood of 0.95 and 1.10, respectively.

In order to examine this phenomenon further, a small specific gravity bottle with a capacity of 1 ml. was used to make accurate measurements of the specific gravity of individual kernels, which weigh on the average about 40 mgm each. Another fifteen kernels were examined with this specific gravity bottle, and five of the measurements were then repeated

Table 1.—Measured Specific Gravities of two lots of Kernels from L. S. S. Cotton Seeds

Serial No.	1	2	3	4	5	6	7	8	9	10
Wt. in air (mgm) ..	58.8	56.0	55.8	49.0	60.5	40.0	46.4	64.0	58.3	72.6
Loss in Wt. in water (mgm).	62.2	57.8	62.9	51.8	62.7	44.0	46.4	60.4	54.2	67.8
Specific gravity ..	0.945	0.969	0.887	0.946	0.965	0.909	1.000	1.060	1.076	1.071

Serial No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Wt. in air (mgm)·	43.4	39.0	46.7	48.6	37.6	43.8	41.7	50.0	46.0	31.7	34.7	45.8	69.2	93.2	38.6
Wt. of equal vol. of watelr (mgm).	45.2	36.0	43.7	48.8	34.8	39.1	40.5	45.1	43.1	29.9	35.1	44.2	65.2	90.2	40.7
Specific gravity	0.958	1.082	1.067	0.996	1.080	1.120	1.030	1.108	1.067	1.060	0.989	1.036	1.061	1.036	0.498

with the direct method based on Archimedes principle. The latter measurements were found to be consistently higher by 0.04±0.02, which is reasonable enough because the kernels had been wetted and would therefore have absorbed some water into their air cavities during the measurements with the specific gravity bottle. The results of measurements on all the twenty five kernels are collected in Table 1 and frequency distribution graph is plotted in Fig. 1 (solid circles and full line), using intervals of 0.05 for the specific gravity. This frequency curve brings out clearly the existence of the two groups of kernels with specific gravities centred about 0.95 and 1.07, respectively, the frequency of the latter group being the higher of the two.

Statistical Reliability of the Frequency Distribution

In order to establish the statistical significance of these observations, the measurements were repeated on another twenty five seeds, and the frequency distribution of these new readings is shown by the hollow circles and broken line in Fig. 1. Although the peak at the lower specific gravity (about 0.95) is less prominent than before, the general shape of the two curves in Fig. 1 is very similar, and therefore, the overall mean frequency distribution for the 50 seeds taken together has been calculated and plotted in Fig. 2. This mean distribution can be seen to be made up of two symmetrical distributions, represented by broken lines in Fig. 2, each with a half-value width of 0.08 units and centred about the values 0.97 and 1.09 for the specific gravity. The peak frequencies for the component distributions are 17% and 35% respectively, and these distributions account for 35% and 65%, respectively, of all the cotton seeds measured.

The variations noted above are probably attributable to genetic differences within a single variety, and, due to their large magnitude it appears unlikely that the specific gravity measurements can be used for estimation of the oil content, until the cause and nature of the variations are fully determined. Accordingly, further work in this direction has been undertaken on several other varieties of cotton seed and will be reported separately.

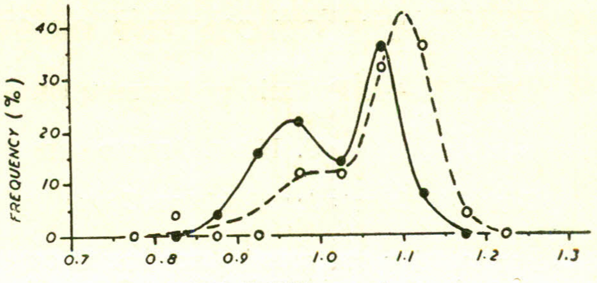

FIG. 2.—Full line shows the mean distribtuion of specific gravities for the fifty kernels of L. S. S. cotton, while the broken lines indicate the resolution into the two component distributions centred at 0.97 and 1.09 .

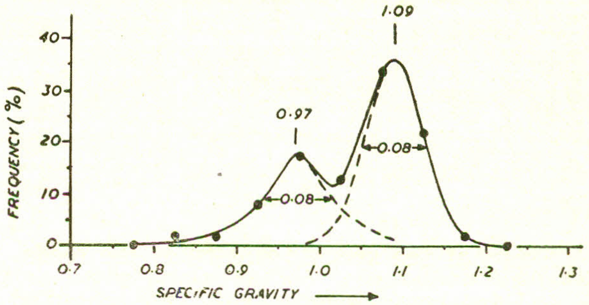

FIG. 1.—Frequency distribution of the specific gravities of two lots of kernels of the L. S. S. varity. Full line for first lot of 25 seeds, and broken line for the second lot.

Examination of the electrical method

The measurement of the dielectric capacity has been used with considerable success by several workers as a means of estimating the quantity of oil obtained by solvent extraction from various oil-bearing seeds. [2,3]. The quantities of oil measured have however been of the order of several grammes as against the 15 mgm or so present in a single cotton seed. Therefore, for the non-destructive application of this method to cotton seeds, the following points need to be studied :

(*a*) whether the sensitivity of the measurement can be improved so as to measure so small a quantity as 1 mgm of oil, which corresponds to about 1% in the oil content when measuring a single seed, and

(*b*) whether the whole seed, the delinted seed, or the dehulled kernel will give the proper capacitance response for estimation of the oil.

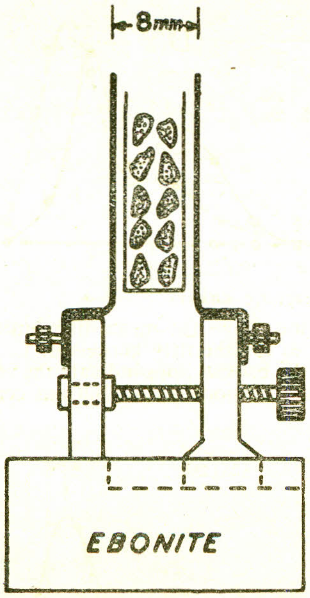

FIG. 3.—Sketch of the container and condenser used for the preliminary capacitance and conductance mesaurements on 10 to 40 seeds of M 4 variety.

The capacitance measurements were made by placing the seeds between the plates of a small parallel-plate condenser, which was constructed from two square aluminium plates of 3 cm. side mounted on an ebonite base in such a way that the distance between the plates could be varied from 2mm. upto 12 mm. (Fig. 3). The seeds were held in rectangular box about 6 mm. wide made out of used 35 mm. photographic film, the box being supported by means of an insulated wooden lath (attached to the top of the box) in such a way that the box was entirely within the electric field between the condenser plates, but did not touch either of them (Fig. 3). This last precaution was important because otherwise the conduction current often became much greater than the capacitance current. The electrical measurements were made with a Marconi Universal Bridge (type T F. 868A), working with a 1000 c.p.s. oscillator as the input to the bridge circuit. Stray capacitance variations were avoided by supporting the condenser on an insulating base placed on top of the bridge and by using short stiff copper strips for connecting it to the bridge terminals. For the preliminary mesurements, a distance of 8 mm. between the condenser plates was found suitable, as indicated by the following simple theoretical considerations.

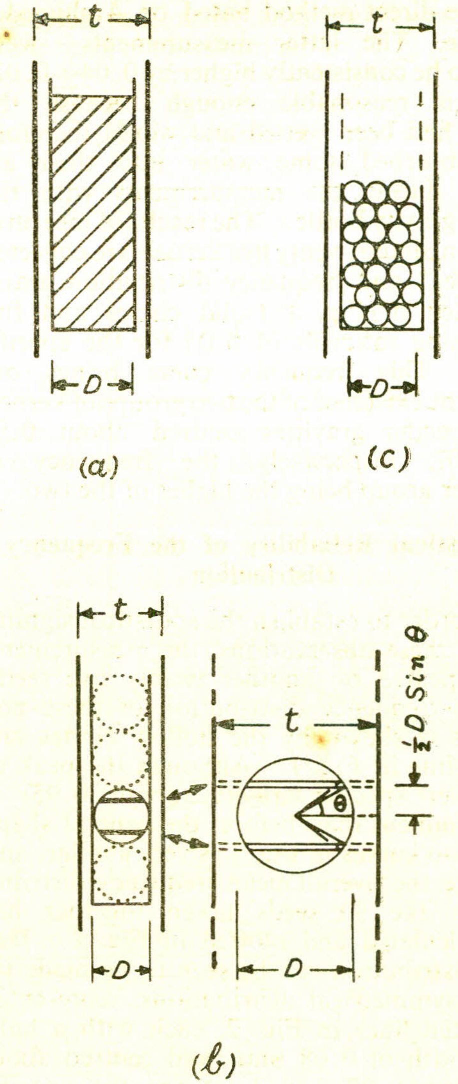

FIG. 4.—Diagrams showing the dispositionof three different forms of material between the plates of the condenser (of Fig. 3), namely

(*a*) slab of thickness D, e.g. of cotton seed cake,

(*b*) large spherical seeds of diameter D, just fitting in the container,

(*c*) a "slab" ofthickness D composed of a large number of small close-packed seeds (e. g. cottonseed kernels) in the container.

Consider first a slab of dielectric, such as cottonseed cake, of thickness D placed between and parallel to the plates of the condenser (Fig. 4(*a*)) of area 'A' and inter-plate spacing 't'. If K is the dielectric constant of the slab, then the increase in capacity, $\triangle$ C, of the condenser is known to be

$$(\triangle C)_{\mathrm{slab}} = \frac{A}{4\pi(\ (t - D) + D/k)} - \frac{A}{4\pi t}$$

$$= \frac{A}{4\pi t}\ (\frac{1}{1-(1-1/K)D/t} - 1)$$

$$= (1 - 1/K)\frac{A\,D}{4\pi t^2}$$

$$\Big/\{1-(1-1/K)\,D/t\}$$

$$= (1-1/K) \times \frac{\text{Volume of slab}}{4\pi t^2}$$

$$\Big/\{1-(1-1/K)D/t\} \qquad 1(a)$$

We can now calculate the effect of placing a single spherical seed of diameter D and dielectric constant K between the plates of the same condenser if we split up the condenser plates into annular strips of radius $\frac{1}{2}$ D Sin θ and width $\frac{1}{2}$ D Cos $\theta d\theta$, as indicated in Fig. 4(*b*). Putting D Cos $\theta = x$, the required increase in capacity is thus found to be

$$(\triangle C)_{\text{Single seed}} = \Sigma\Big(\frac{\delta A}{4\pi(t - x\ (1 - 1/K)}$$

$$- \frac{\delta A}{4\pi t}\Big)$$

$$= \int_0^{\pi/2} \Big(\frac{\pi D \sin\theta . \frac{1}{2} D \operatorname{Cos}\theta\, d\theta}{4\pi\ [t - x(1-1/K)]}$$

$$- \frac{\pi D \sin\theta . \frac{1}{2} D\cos\theta\, d\theta}{4\pi t}\Big)$$

$$= \frac{1}{8}\int_0^{d} \Big(\frac{x\,dx}{t - x(1-1/K)} - \frac{x\,dx}{t}\Big)$$

$$= \frac{1}{8}\int_0^{d} \Big(\frac{[t/(1-1/K)]dx}{t - x(1-/K)} - \frac{d\,x}{1-1/K}$$

$$- \frac{x\,dx}{t}\Big)$$

$$= \frac{-t}{8(1-1/K)^2}\ln\Big(\frac{t - D(1-1/K}{t}\Big)$$

$$- \frac{D}{8\,(1-1/K)} - \frac{D^2}{16\,t}$$

$$= \frac{t}{8\,(1-1/K)^2}(D(1 - 1/K)/t + \tfrac{1}{2}D^2$$

$$(1-1/K)^2/t^2 + \tfrac{1}{3}D^3(1-1/K)^3/t^3 + \ldots .)$$

$$- \frac{D}{8\,(1-1/K)} - \frac{D^2}{16t}$$

$$= (1-1/K)\frac{D^3}{24t^2} + (1-1/K)^2\frac{D^4}{32t^3}$$

$$+ (1-1/K)^3\,\frac{D^5}{40t^4} + \ldots\ldots$$

$$= (1-1/K)\frac{\pi D^3/6}{4\pi t^2}(1 + \tfrac{3}{4}(1 - 1/K)\,D/t$$

$$+ 3/5(1-1/K)^2\,D^2/t^2 + \ldots .)$$

$$\simeq (1-1/K) \times \frac{\text{Volume of seed}}{4\pi t^2}\Big/\{1 - 0.8$$

$$(1-1/K)\,D/t\}, \qquad 1(b)$$

which has the identical form as equation 1(*a*) for the case of a slab, and has a small difference in the factor within curly brackets.

When the spherical seeds have a diameter nearly equal to the width of the (insulating) container, they will rest one above the other (or alongside each other) as shown by the dotted circles in Fig. 4(*b*), so that the lines of electric force at any point from one plate to the other will only cut one of the spheres. The capacity effect of a large number of such seeds will therefore be similar to that for a single seed, and formula 1(*b*) will hold unaltered. If, however, the spherical seeds are smaller and have diameters much less than the width of the container, they will pack together in some sort of close-packed arrangment (like the atoms in metals and many alloys), as shown in Fig. 4(*c*), so that in the regions where the spheres interlock with each other, the lines of force at any point from one plate to the other will cross twice as many spheres as elsewhere, and the proportion of dielectric encountered by the lines will become considerably greater (than in Fig. 4(*b*), and will always lie between D/t and 0.7 D/t. The capacity change will therefore be greater than the corresponding value for single spheres (equation 1(*b*)), but less than that for a solid slab (equation 1(*a*)). By comparing equations 1(*a*) and 1(*b*) in this context, we conclude that, in this general case of small close-packed seeds,

$$(\triangle C)_{\text{Close-pakced seeds}} = (1 - 1/K) \times \frac{\text{Volume of sample}}{4\pi t^2} \Big/ \{1 - \alpha(1 - 1/K)D/t\}, \quad 1(c)$$

where $0.8 < \alpha < 1$, and D is the mean thickness of the slab of seeds.

For quantitative measurements, it is desirable that the effect of this variability in α be minimized, which is possible if we keep D/t small. Thus, if $D/t = 3/4$, then for $K = 3$, the factor within curly brackets in equation 1(*c*) has a value of

$$\{1 - \alpha \times \tfrac{2}{3} \times \tfrac{3}{4}\} = \{1 - \alpha/2\} = \{1 - (0.9 \pm 0.1)/2\} = 0.55 \pm 0.05$$

showing a *maximum possible* variation of $\pm 9\%$ which is acceptable for the present preliminary investigation. Since the diameter D of the cottonseeds is of the order of 6 mm or less, the above condition gives $t \sim 4/3D = 8$ mm. The variation can be made smaller still by increasing 't' further, but this tends to reduce the sensitivity rapidly (in the ratio of $1/t^2$), and is therefore not desirable.

Experimental Results

With this setting for t, the capacity of the whole arrangement (without the box between the plates) was read as 1.75 micro-micro farads on the bridge dial, of which about 0.5 represents the effect of connecting strips, etc. When the box was introduced between the plates, this reading rose to 2.00 micro-micro farads.

Using both the capacity and tan δ dials on the bridge, several measurements were made of the change in capacity and phase angle, produced by filling the box (of nearly 3 ml. capacity) with weighed quantities of (*i*) seeds (of the M4 variety), (*ii*) oil cake, and (*iii*) oil. and the results are presented in Table 2 along-with their estimated standard deviations. From the values of tan δ , the conductance has been calculated as $1/R = \omega C \tan \delta$. The changes in capacity and conductance per gm of material* are given in the last two columns of the table, and these are the important quantities deduced from the measurements. It is seen that (*a*) the capacity increase per gm. decreases progressively as the seeds are first delinted and then dehulled, and (*b*) the conductance measurements give values of <0.1, 0.3, and 0.4 respectively for the oil, the dehulled kernel and the oil cake, (*i.e.* the proteinous residue). These values of conductance are in the expected sequence and it appears probable that an accurate measurement of the conductance can provide an estimate of the proteinous residue, because the pure oil has practically zero conductance, cf. its high insulating property.

The measured values of capacity per gm. are apparently in the reverse order, because the oil was expected to give the highest figure. The precise reason for this is still being investigated, although it is believed that moisture is the main cause.) It is to be noted that the figure for the kernels is intermediate between those for the cake and the oil. We may for the arrangement of Fig. 3 use a figure of 0.4 $\mu\mu$ F/gm for the oil and 0.8 $\mu\mu$ F/gm for the proteinous residue, and calculate the % oil (on the weight of kernel) from eq. (2) below, assuming (cf. eq. 1(c)) that the capacity change, $\triangle C$, is proportional to the quantity of kernels in the container,

$$100\frac{\triangle C}{\text{mass}} = (0.4 \times \text{oil}\% + 0.8 \times \text{Residue}\%) = (80 - 0.4 \times \text{Oil}\%) \qquad (2)$$

By combining the estimate from the conductance measurement with the capacity measurement, an alternative technique for estimating the oil content can perhaps be obtained. Thus we have

$$1/R = \text{mass} \times (0.3 \times \text{Residue}\%)/100,$$

whence by using equation (2), $0.4 \times \text{oil}\%$

$$= \left(\frac{\triangle C}{\text{mass}} - \frac{0.8}{0.3} \times \frac{1/R}{\text{mass}}\right) \times 100 \qquad (3)$$

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*Because of the variable packing of some of the materials, like cotton-seed cake it is better to work in terms of unit mass, although equations (1) indicate proportionality with volume.

TABLE 2.—ELECTRICAL MEASUREMENTS WITH MACRO QUANTITIES OF M4 COTTON SEEDS.

Nature of material	Specific Gravity	Weight (gms)	No. of seeds	Capacity dial Reading (*uu*F)	tan δ	Conductance $1/R = \omega C \tan \delta$ (m *u* Mho)	Increase per gm of material	
							Capacity (*uu*F/gm)	Conductance (m*u*Mho/gm)
Seeds with linter	..	0.801	13	3.75±0.05	0.11	2.6	2.18±0.07	3.2
Delinted seeds	..	1.137	20	3.20±0.05	0.05	1.0	1.06±0.05	0.9
Dehulled kernels	1.0	1.584	39	3.00±0.05	0.02_5	0.4_5	0.63±0.05	0.3_0
Oil cake	0.9	1.110	..	2.90±0.05	0.02_5	0.4_5	0.81±0.05	0.4_1
Cotton seed oil	0.96	1.24	..	2.50±0.05	<0.01	$<0.1_5$	0.40±0.05	<0.1

The above equations may need to be modified to take account of the different response for the same material at different humidities, and when in the form of ellipsoidal pellets (like the actual kernels) rather than spheres.

Discussion

The relations (2) or (3) can be used to estimate *non-destructively* the oil content of gram quantities of kernel and it remains to be seen whether they are suitable experimentally. This is linked up with the accuracy attainable in the two measurements, namely $\triangle C$ and (1/R). With the Marconi Bridge as supplied, an absolute accuracy of $\pm$ 0.3 $\mu\mu$ F is claimed in the measurement of capacity 'C' when it is of the order of 2$\mu\mu$ F, but in practice it has been found to be reliable to $\pm$0.05 $\mu\mu$ F for measurements of $\triangle$ C i.e. changes in capacity. At the small values of 'C' necessary in the apparatus of Fig. 3, the accuracy attainable in the measurement of the phase angle δ is also small, being of the order of $\pm$ 0.005 so that 1/R will be in error by $\pm$ 0.05m μ Mho. Substitution of these values in equation (3) shows that for an accurancy of $\pm$ 1% in the oil content, the total mass, m, of kernels should be about three grammes. Although this is an improvement on previous rapid methods, which are destructive and use several ounces of seeds, it is nevertheless a far cry from our aim of working with a single seed.

It appears, therefore, that, for the non-destructive estimation of the oil content of a single cotton seed, it will be necessary (*a*) to increase the accuracy of the electrical measurements nearly a hundred-fold and (*b*) to investigate in detail the effect of humidity and (*c*) to study the relation between the number of seeds and the capacitance and conductance changes produced by introducing them one by one into the condenser. All these factors are presently being studied, and the results, which have a bearing on the design of a suitable measuring apparatus, will be reported separately.

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