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Studies on the Lotus-Seed

I. On the Water Content of the Old but Still Viable Seed

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大沢義信・斎藤千代・中条勝：蓮の種子に関する研究

I. 古いが尙生きている種子の含水量について

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The lotus-seed retains the vital force for some thousand years if it is buried in the peart-bed**.

It has been well known that the seed could be stored very much longer under the dried condition.

The water content of the lotus-seed is very interesting in this point of view.

This report is mainly concerned with the moisture content of the lotus-seed.

Materials and Methods

The lotus-(*Nelumbo mucifera* Gartnr.) seed used in this study was collected from the bottommud of Shinobazu-pond in Tokyo, Japan, during the period from April to May 1946, and it was considered that the seed ripened more than ten years before the time collected. After collecting, that was placed in the atomospheric air at the room temperature until the measurements were performed (from Sep. to Dec. 1954), but it seems that the water content of the seed might not undergo the rapid and noteworthy change, for the seed is hard and impermeably coated.

The weight of seed was 0.6—0.9 gram at the time determined.

The morphological characteristics did not differ from the seed harvested at present, and the ability of germination was fully maintained.

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** Ohga, I. A study of the ancient but still viable fruit of the indian lotus found in the peat bed near Pulantien, South Manchuria. (1927)

The measurements of moisture content were made by the vacuum-oven method* (heating in a vacuum-oven with P_4O_{10} at about $60^\circ C$ and 5 mm Hg for 3 hours), and Karl Fischer's back titration method¹⁾. In the latter case, the apparatus modified by the writers was used (see Fig. 1).

Fig. 1. The apparatus used in this study. The right half is omitted.

a; burette, *b*; titration flask, *c*; mixer, *d*; electrode, *e*; mercuric stopper, *f*; to the amplifier for electric titration, *g*; reagent, *h*; $CaCl_2$, *i*; cock, *j*; micro-syringe, *k*; magic eye.

Karl Fischer reagent used in this study was prepared by Nipponkasei Chemical Industries, Limited, Japan, and its potency was 1.83 mg./cc. The methanol containing 2 mg./cc of water, which was prepared by Kishida Chemical Co. Ltd. Japan, was used for the back titration.

The adequate number drawn from 83 seeds by the random sampling was used for testing.

After the removing of seed-coat in the dry chamber (moisture: less than 30%), the seed was divided, then the embryo and the endosperm were crushed separately using the cutter made of the bundle of the blades of safety-razor.

The weighing of the powdered sample for Karl Fischer's method was carried out in the dried place (moisture: less than 30%) on the hanging drop slide of which both wings had been cut off by one-third, and brought into the titration flask together with that glass.

It seems that the reagent may be diffused into the crushed sample within sixty minutes, for the green pigment** contained in the smashed embryo were decolorized within that time, so the titration was made at ninety minutes after the addition of samples to titration flask.

Results and Conclusions

The results obtained are shown in Tables 1 and 2.

The mean values gained by these two methods might be said to differ significantly from each other in one group of the endosperm in the fiducial limits at the 5% level, but not at the 1% level. There was no significant difference between both values in the other group of the endosperm and the embryo at the 5% level (see Tables 1 and 2).

The values of water content of the embryo were almost same as that of the albumen in Karl Fischer's method.

* [The standards of biological medicaments] issued in 1953 by the Ministry of Health and Welfare, Japanese Government.

** This pigment was identified with chlorophyll a and b by these writers (unpublished).

Tab. 1. The water content of lotus-embryo determined by two methods.

Exp. No.	Weight of sample in mg.	Moisture content %	Average	Method
1	25.19	4.36	4.07 ±0.72	vacuum-oven method
2	17.57	4.82		
3	17.51	3.94		
4	18.12	3.25		
5	17.59	3.98		
6	19.79	4.09	3.99 ±0.23	
7	17.83	3.98		
8	19.91	3.91		
1	181.15	4.20	4.66 ±0.72	Karl Fischer's titration
2	101.02	5.18		
3	161.41	4.89		
4	183.60	4.38		

Note: Two groups having the average value of 4.07 and 3.99 were measured on the other day in the entirely same manner.

Table. 2. The water content of lotus-endosperm determined by two methods.

Exp. No.	Weight of sample in mg.	Moisture content %	Average	Method
1	73.81	7.96	6.78 ±1.60	vacuum-oven method
2	57.51	5.27		
3	89.77	6.07		
4	94.14	8.29		
5	78.84	6.31		
6	88.29	4.68	5.97 ±1.56	
7	74.96	7.40		
8	72.23	6.86		
9	110.01	6.24		
10	72.04	4.67		
1	106.12	4.85	4.86 ±0.61	Karl Fischer's titration
2	340.58	4.42		
3	250.77	5.36		
4	292.10	4.81		

Note: Two groups having the average value of 6.78 and 5.97 were measured on the other day in the entirely same manner.

On the other hand, the one mean value obtained by the vacuum-oven method with the endosperm was observed to differ significantly from that of the embryo at the 1% level and the other one differ noteworthy from that of embryo at the 5% level, but not at the 1% level.

The constant value was seldom given by the vacuum-oven method with the endosperm, i.e. its deviation was somewhat larger than in other cases, and it was probably introduced from the crushing treatment for a rather long time in this case, so the value generally showed a larger numeral than what it was.

we may believe that the values of moisture content of embryo and endosperm obtained by the vacuum-oven method are also equal, if we consider this circumstance.

Now, M. Izawa^{1,2)}, ascertained that the value obtained by the gravimetry was obviously smaller than that of Karl Fischer's method with penicillin in oil and wax and some other biological products, and deduced that the difference may be explicable as three alternatives:

- 1) Karl Fischer reagent may react with some compounds in the sample other than the water.
- 2) By the ordinary method, the water cannot be eliminated completely.
- 3) The nature of water detectable by the two methods may be different, that is, as Karl Fischer's method is carried out under dissolved or finely dispersed states, the water such as crystalline water should also be detectable, which cannot be removed by heating.

Then, he concluded that the water cannot be removed completely by the conditions described in his paper.

While, he demonstrated that the results of two methods in the cases of solid penicillins showed nearly the same order and the consumption of the reagent became negligible when samples were dried in the vacuum-oven for an appropriate time.

So, we may say that the moisture content of lotus-seed is determined at about the same order by the two methods as in the cases of solid penicillins and that the embryo contains the same amount of water as the endosperm.

At any rate, the content of water is not extremely small in the lotus-seed examined. In addition, I. Ohga reported in 1927 on the problem in question by using the ordinary gravimetry as follows: old fruit; 12.38%, new fruit; 12.5%.

Thus, the reason why the lotus-seed retain the vitality for a long time may not be in the smallness of water content.

Summary

- 1) The water content of lotus-seed collected from the bottom-mud of pond was determined by the use of two methods.
- 2) The apparatus for Karl Fischer's back titration method was modified by the writers, and used in this study.
- 3) The results obtained from the different regions of seed by these two methods were compared, and then discussed.
- 4) Because the moisture content of the seed is not extremely small, the reason why the lotus-seed retain the vitality for a long time may not be in the smallness of water content.

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