

REMARKS ON ASSAY OF PEPSIN AND ITS PREPARATIONS.

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The following results were obtained with three various methods for testing the digestive power of pepsin. The amount of undigested egg albumen left after two and one-half hours digestion at 52° C. was practically the same in all three methods, viz: less than 1 cc.

Method No. 1—U. S. P. Method.

Method No. 2—U. S. P. Revised Method.

The latter method is the same as the U. S. P. Method with the following exceptions:

Instead of adding 20 cc. of diluted acid at once to the 10 gm. of white of egg, only 2 cc. at a time is added and the white of egg disintegrated with a glass rod tipped by a piece of pure rubber tubing.

This has an advantage over the U. S. P. Method, as it is much easier to disintegrate the white of egg which has become compressed to a certain extent by passing it through the sieve, in a small volume of the acid solution, than in a large volume, such as 20 cc. added at once.

In the revised U. S. P. Method, during the two and one-half hours digestion the bottles are rotated three times every ten minutes, while in the U. S. P. Method the bottles are inverted. We believe inverting to be superior to rotating.

Referring to that sentence in the U. S. P. Revised Method which states "then remove it from the water bath, add 50 cc. of cold distilled water, transfer the mixture to a conical measure having a diameter not exceeding 1 cm. at the bottom and let it stand for half an hour"; we would suggest that it be substituted by the following: "Remove it from the water bath, pour contents into a conical measure having a diameter not exceeding 1 cm. at the bottom and then wash the undigested egg albumen which adheres to the sides of the bottle, with small portions (about 10-15 cc. at a time) of distilled water until 50 cc. has been used."

We would also suggest that the conical measure be inverted or its contents be stirred after the 50 cc. of distilled water has been added, as this no doubt will accelerate the settlement of the undigested albumen.

Method No. 3—This method is the same as the U. S. P. Method with the following exception:

Instead of disintegrating the white of egg with a rubber-tipped glass rod, the mixture is shaken vigorously for about five times. Results were the same after about two and one-half hours digestion at 52° C., less than 1 cc. of undigested egg albumen was left. Absolutely fresh eggs, not older than five days and always kept in an ice chest, were used in all our experiments.

The same lot of pepsin was used in these tests.

Conclusion—As seen from this outline, all three methods give concordant

results, if carried out strictly according to directions. Personally, we give preference to Method No. 2.

Some samples of pepsin previously analyzed by us and found to be of required strength were submitted, on request, to a certain college for comparative tests.

The amounts of undigested egg albumen left on their assays varied considerably from the amounts we found in our laboratory. Second assays made by us verified our first findings, while at the college different findings were obtained on the second assays.

It is without doubt that in all cases fresh eggs were used, that the pepsin was correctly weighed and that the dilutions were made in standardized measures, etc.

Now, what is the reason for these discrepancies in assay by different parties?

So far as we could see, the only possible cause for the deviation was the different strength of the hydrochloric acid used in making the tests.

That our supposition was correct will be clearly shown by the following:

The percentage of absolute hydrochloric acid in U. S. P. hydrochloric acid made by different manufacturers varies very greatly. The U. S. P. requires 31.9% absolute hydrochloric acid by weight; most of the manufacturers turn out a product which runs higher, usually from 32% to 35%, sometimes as high as 38%. It is therefore absolutely necessary that each lot should be assayed before using for any purpose.

The U. S. P. requires an acid which assays exactly 0.3% hydrochloric acid. That this requirement should be strictly carried out, that no acid assaying lower or higher should be used, the following table will illustrate.

On using 5 cc. of the same pepsin solution and 35 cc. of diluted acid of different strengths the following results were obtained:

I. PEPSIN SCALES

(1)	Tested with 0.21%	HCl. left 5	Cc. undigested Egg Albumen
(2)	Tested with 0.30%	HCl. left 1	Cc. undigested Egg Albumen
(3)	Tested with 0.328%	HCl. left 1½	Cc. undigested Egg Albumen
(4)	Tested with 0.385%	HCl. left 2½	Cc. undigested Egg Albumen
(5)	Tested with 0.540%	HCl. left 19	Cc. undigested Egg Albumen

II. ESSENCE OF PEPSIN N. F.

(1)	Tested with 0.21%	HCl. left 6	Cc. undigested Egg Albumen
(2)	Tested with 0.30%	HCl. left 1	Cc. undigested Egg Albumen
(3)	Tested with 0.328%	HCl. left 1½	Cc. undigested Egg Albumen
(4)	Tested with 0.385%	HCl. left 3	Cc. undigested Egg Albumen
(5)	Tested with 0.540%	HCl. left 17	Cc. undigested Egg Albumen

Several other comparative tests were made with the same proportional results. This evidently shows that the egg albumen's digestive power of pepsin is lowered by using either a lower or higher percentage of hydrochloric acid than that of 0.3%.

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