

2. Methyl alcohol is not the only compound which will yield formaldehyde by oxidation in the manner prescribed. Even ethyl alcohol may furnish a small quantity of this aldehyde. Certainly methyl esters may yield the same, and a number of the higher alcohols show a like behavior. Hence quantitative results require confirmation as has been already stated. Still, in general, it may be said that the indications of the test as applied to alcoholic beverages point to impurities likely to be not less harmful than wood spirit.

LABORATORY OF NELSON, BAKER & Co.,
MAY 1922.

PEPSIN—METHODS USED FOR ITS QUANTITATIVE ESTIMATION— ITS PERMANENCE AND EFFECTIVENESS IN SOME OF ITS PREPARATIONS.*

BY ELMER J. TRAUT[†] AND H. W. VAHLTEICH.

It is the intention of the authors to discuss the subject matter indicated by the above title as a continuation of a previous paper¹ from this laboratory.

In accordance with our earlier results with different methods of pepsin assay, we have made the U. S. P. method the basis for the work done on the various preparations which were assayed. It was found practical, however, to use modifications of the method whereby considerable time was saved and yet as satisfactory results attained as if official directions had been strictly adhered to. The first modification was made in the method of adding the 0.3% HCl solution. The U. S. P. IX directs that it be added in small increasing portions, finally using a portion to rinse the stirring rod. In our assays we added the entire amount of 35 cc at one time, and then, instead of disintegrating with a glass rod, simply shook each bottle a sufficient number of times until upon inspection the albumen was found to be as thoroughly disintegrated as if the U. S. P. IX directions had been followed.

Also it was found most desirable to run a number of controls containing the known with each batch of tubes. The strength of the known pepsin used in these control tubes was determined by running a considerable number (150 or more) of determinations on it, following the U. S. P. IX method. This, then, was our control and solutions of it were prepared each time that we ran an unknown, the fourth, eighth, sixteenth, etc., bottles in the bath containing the known pepsin. At the end of the digestion period the digestion mixtures were poured into the sedimentation tubes and the amount of the residue in cc noted upon standing one-half hour. However, instead of considering a 1-cc residue as the basis for computing the strength of the unknown pepsin, we used the average of the residues in the tubes from the known pepsin. In our estimation this is a permissible modification in an investigation of this nature. The principle is essentially the same as for the comparative method outlined in our previous work and is intended to eliminate variations due to the variations in the composition and texture of the coagulated albumen, with which every manipulator of this method is familiar.

* Scientific Section A. Ph. A., Cleveland meeting, 1922.

[†] Work done as holder of Stearns Fellowship for 1921-22, in partial fulfilment of the requirements for the degree of Master of Science.

¹ JOUR. A. PH. A., 10, 595, 1921.

Avoidance of undue exposure to air and thorough mechanical mixing when large batches of albumen (200 to 300 grams) are being worked with, are also essential precautions.

The comparative method can also be applied to the use of commercial egg albumen as a substrate to supplant hens' egg albumen. There are commonly found on the market two kinds of egg albumen, the one a powder, labeled "soluble" and usually put up in original containers of various sizes; the other, in the form of chips, obtainable in any quantity in bulk. Of these two, the latter is the more desirable for the assay of pepsin, because the former, though labeled "soluble," is difficult to get into solution. The chips (said to be dried duck egg albumen imported from China) are more easily soluble and give a more homogeneous solution. A procedure found satisfactory is as follows:

Into a large porcelain evaporating dish are placed 100 Gm. (1 part) of chip albumen, and 300 cc (3 parts) distilled water poured upon it. It is stirred occasionally or allowed to stand over night and kept covered (in a cool place in warm weather). Solution being complete, it is poured into any type of flat vessel such as a good-sized crystallizing dish, preferably into a single dish large enough to hold it all, in a layer one and one-half to two centimeters deep (not over 2 cm. deep). The rate of coagulation and the uniformity of the coagulated mass is dependent upon the thickness of the walls of the vessel and also upon the depth of the layer of liquid. Large thin-walled crystallizing dishes are admirably suited for the purpose and a layer not over 2 cm. deep gives good results. The dish containing the solution is placed in a water-bath and its bottom supported by three or four small metal supports resting upon the bath. Thus heat is transmitted to the solution over the comparatively large area exposed to the bottom of the dish in the water. The dish is covered by a suitable glass plate and a temperature of 80° to 85° maintained until the mass has coagulated to the consistency of ordinary hard-boiled egg white, 30 to 45 minutes usually being required. It is well to occasionally remove condensed moisture from the covering plate.

The coagulated mass is passed through a No. 40 sieve in the usual (U. S. P. IX) manner. If two or more batches are coagulated separately they should be mixed well after sieving to assure uniformity. In running pepsin determinations only 6 $\frac{2}{3}$ Gm. of this sieved material are necessary instead of the 10 Gm. of egg albumen usually used for each bottle run. A solution of a known strength of pepsin is also made up and 5 or more control tubes containing this solution are run side by side with the unknowns. It is not necessary to run the digestion with this albumen longer than 2 hours, and it seems from our experience that 1 $\frac{1}{2}$ hours may also be sufficient to give reliable results. This may be due to the fact that this substrate is more uniform, as a rule, than that obtainable from storage eggs, and that after being passed through a No. 40 sieve it is much more readily suspended in *N*/12 HCl than the latter.

As a substrate to replace storage egg albumen in the comparative method its advantages may be summed up as follows:

1. Even when storage eggs are low in price the cost of albumen alone per bottle run is one-half or less of the cost of usual hens' egg albumen.
2. It entirely eliminates using a stirring rod to disintegrate the albumen since

it rapidly suspends itself uniformly by shaking the bottle once or twice (absence of membranous material).

3. It gives more consistent results in less time for digestion.

Its principal disadvantage is that it requires additional (though simple) apparatus, and labor.

The possibility of pepsin assay by the milk-curdling process has been brought to our attention by one of the manufacturers.¹ Several investigations have been carried out in an effort to determine the possible identity of pepsin and rennin.^{2,3,4} For the purposes of our work, however, it was of interest especially to determine whether the proteolytic activity of pepsin paralleled its milk-curdling power, and under what conditions.

Our procedure included the following:

Solutions:

1. 0.1 Gm. of known pepsin (determined by U. S. P. assay) in 150 cc of distilled water.
2. 0.1 Gm. of unknown pepsin in 150 cc of distilled water.
3. Fresh skimmed milk.
4. Lactic acid (88%) and hydrochloric acid (36%).

Apparatus:

1. Thermostat regulated to 35° C., preferably with black interior and suitable illumination to facilitate the determination of the end-point.
2. Two wide-mouthed 250-cc bottles.
3. Two stop watches.
4. Two 150 cc beakers.
5. Measuring flask.

METHOD.

The milk used in this laboratory was pasteurized and about one day old, although investigations have shown that milk not pasteurized is more susceptible to coagulation by pepsin.⁵ The milk is brought to 35° C. and made up to the proper acidity by the addition of two minims (0.10 cc) of lactic acid and one minim (0.05 cc) of hydrochloric acid⁶ to each 700 cc of milk, these amounts having been found adequate. The acidity of the original milk was taken by means of a Leeds and Northrup student potentiometer and the p_H value found to be 6.4. The original milk is therefore only slightly acid—almost imperceptibly so. Similar results are shown by Wendt.⁶

Into one of the two wide-mouthed bottles of 250 cc capacity are placed 5 cc of the known solution of pepsin (Solution No. 1) and into the other are placed 5 cc of the unknown solution (Solution No. 2). The two bottles are then placed in the constant temperature bath. Two 100-cc portions of the adjusted milk are poured into suitable containers. One of these is added to the 5 cc of known pepsin solution and the time of addition recorded with a stop watch, the two are thoroughly mixed, and then the other 100 cc are added to the 5 cc of unknown pepsin solution

¹ Mr. H. M. Adams of Frederick Stearns & Co., Detroit, Mich.

² Hammarsten, *Zeit. physiol. Chem.*, 56 18-185.

³ Effront-Prescott, "Biochemical Catalysts," 181-185, 204.

⁴ *Zeit. physiol. Chem.*, 74, 242, 1911.

⁵ *Fermentforschung*, 3, 81-192, 1920.

⁶ Leach, "Food Inspection and Analysis," 4th Ed., 142, 1033.

and the time of addition again noted. The end-point of curdling is obtained by gently tipping the container in the bath at 35° at short intervals and watching the side which has been wet by the milk. When curdling begins an easily perceptible stringy precipitate will cling to the wall of the bottle and the time of its first appearance is taken as the end-point. Two stop watches, one for each solution, are the most practical, for the end-point is sharp and can be caught to the second or less. The strength of the pepsin may be given in the same terms as the present U. S. P. method, since a standard known pepsin is used with each assay. For instance, supposing we had:

Standard 1-3000—5 cc of dilution curdled in 4 min.

Sample ? 5 cc of dilution curdled in 3 min.

$4/3$ of 3000 = 1-4000 pepsin.

The unknown is then checked in the same way as the U. S. P. assay method, *i. e.*, the results as above show 1-4000, then $15000/4000 = 3.75$ cc instead of 5 cc should be used and this quantity should curdle the milk in the same time as 5 cc of the standard 1/3000 pepsin does.

TABLE.

	Date.	Time of curdling.	Strength milk by method.	Strength by U. S. P. method.
Elix. Lact. Pepsin No. 1 Standard	4/19/22	243 seconds	1-2404	1-2400
		208 "		
	6/15/22	466 "	1-2414	1-2400
Elix. Lact. Pepsin No. 2 Standard	4/19/22	375 "		
		257 "	1-2179	1-2300
	6/15/22	200 "		
Elix. Lact. Pepsin No. 3 Standard	4/19/22	430 "	1-2225	1-2300
		319 "		
	6/15/22	240 "	1-2368	1-2300
Elix. Lact. Pepsin No. 4 Standard	4/19/22	203 "		
		427 "	1-2114	1-2300
	6/15/22	301 "		
Elix. Pepsin & Iron No. 1 Standard	4/19/22	230 "	1-2532	1-2500
		208 "		
	6/15/22	402 "	1-2462	1-2500
Elix. Pepsin & Iron No. 2 Standard	4/19/22	330 "		
		738 "	1-1800	1-1800
	6/15/22	476 "		
Elix. Pepsin & Iron No. 3 Standard	4/19/22	646 "	1-1762	1-1800
		380 "		
	6/15/22	708 "	1-1641	1-1700
Elix. Pepsin & Iron No. 4 Standard	4/19/22	415 "		
		744 "	1-1645	1-1700
	6/15/22	375 "		
Elix. Pepsin & Iron No. 1 Standard	4/19/22	630 "	1-1781	1-1700
		401 "		
	6/15/22	639 "	1-1685	1-1700
Elix. Pepsin & Iron No. 2 Standard	4/19/22	359 "		
		640 "	1-1754	1-1800
	6/15/22	401 "		
Elix. Pepsin & Iron No. 3 Standard	4/19/22	624 "	1-1762	1-1800
	6/15/22	367 "		

DISCUSSION.

The method has been tried on various preparations made up in this laboratory and the results obtained seem to merit consideration. It cannot as yet be accepted as a standard but it is a very good approximation of what the U. S. P. assay results will be, some determinations showing identical results. The preceding data were obtained in the laboratory with Elixir Digestive Compound, N. F. III and Elixir Pepsin and Iron, N. F. IV. The basis for numbering the preparations was as follows:

No. I contained Aromatic Elixir made up according to U. S. P. IX.

No. II contained Aromatic Elixir with the alcohol replaced by glycerin and the sugar by saccharin.

No. III contained Aromatic Elixir with sugar replaced by saccharin.

No. IV contained Aromatic Elixir with alcohol replaced by glycerin.

The figures in the table show that the results of the method are well within the range of error of the U. S. P. method and show a relationship between the proteolytic strength of the pepsin and the time of curdling. It will be noted that the time of curdling for the standard varies, but in these experiments the milk was made up at various times and each batch had a slightly different time of curdling. Why it should vary for different batches when the same quantity of milk is used each time with the same addition of hydrochloric and lactic acids has not been determined. The fact that a control is run each time eliminates the possibility of error. The method has been found practical only in preparations which contain pepsin in concentrations of one gram per 100 cc of 1-3000 pepsin or higher, pepsin of lower strength at times has not coagulated even after 30 minutes. Thus the above results on Elixir Pepsin and Iron N. F. IV are good, considering that the pepsin in this preparation is below the above-mentioned concentration. Many other results obtained, but not listed here, were far more variable. This would eliminate this method as an accurate one for weaker pepsin preparations but still would answer the purpose of the manufacturers whose pepsin ranges from 1-3000 and up. The addition of the acids in such small amounts had no marked effect on the acidity of the preparation as determined by the potentiometer apparatus, but the pepsin did curdle the milk which it had not previously done on the original milk.¹ Again, instead of adding one minim of hydrochloric acid, three minims and more of lactic acid were added. Then no curdling resulted. The acidity of milk changes upon standing and the time of coagulation is shortened, so that it is best to make up only one liter or two at a time and use it at once. The time for coagulation should be between 5 and 6 minutes for the standard, and the concentration of lactic and hydrochloric acid in 700 cc of milk as used here approximates that time. In fact a more rapid curdling makes the method less accurate.

As a summary the following can be said:

1. Our results indicate that, under certain conditions of temperature and acidity, the proteolytic and the milk-curdling powers of pepsin are parallel and that the latter can be used to advantage in its assay.

2. The method is a good approximation and affords a rapid assay for the manufacturer who desires to know to a fair degree of accuracy the strength of his pepsin and does not care to spend considerable time with the U. S. P. assay.

¹ *J. Dairy Science*, 2482-6, 1919.

3. It is a rapid method, requires little time, and is less costly from a standpoint of apparatus and cost of milk as compared to the apparatus and fresh eggs required to determine the strength of pepsin and its preparation.

4. It is not applicable to preparations containing rennin, owing to the high curdling power of rennin.

5. It necessitates the preparation of a standard by the U. S. P. method, no method as yet being devised whereby that can be eliminated.

DETERIORATION OF PEPSIN IN ITS PREPARATIONS.

Experiments have shown that preparations of pepsin show more or less deterioration after being made up. The question as to why these preparations should deteriorate, some more rapidly than others, has caused many investigations and to this matter also the writers have been devoting their time. E. Buchner¹ pointed out that cane sugar was an inhibitant and we find a similar statement by Euler² in regard to alcohol above a certain concentration. On the assumption that these might be two of the chief causes, a number of preparations of pepsin were made up, namely, Elixir Pepsin, N. F. IV, Elixir Pepsin and Iron N. F. IV and Elixir Digestive Compound, N. F. III. Aromatic Elixir for the above was made up four different ways as mentioned in the discussion of the milk method above. First, strictly U. S. P.; second, substituting glycerin for the alcohol; third, saccharin for the sugar; and fourth, saccharin for the sugar and glycerin for the alcohol. The U. S. P. syrup calls for 850 grams of sugar and sufficient water to make 1000 cc of finished syrup. Saccharin, 1/350 of the total amount of sugar, was used and water added to make 1000 cc, and this was then the saccharin syrup used in the aromatic elixir incorporated in the preparations wherein saccharin was substituted. The preparations for each elixir were four in number: namely, one with Aromatic Elixir, U. S. P.; another with Aromatic Elixir containing saccharin and alcohol; another, saccharin and glycerin; and the fourth, syrup and glycerin. These preparations were then assayed at monthly intervals over a period of seven months.

Results show that the preparations containing saccharin for sugar in the Aromatic Elixir deteriorate even more rapidly than those made up with Aromatic Elixir U. S. P., which is in keeping with a statement previously made, that the addition of saccharin has no favorable effect in a hydrochloric acid and pepsin mixture unless the pepsin is present in excessive amounts.³ The substitution of only glycerin for alcohol and not saccharin for sugar has practically no effect on stability. Our preparation with this substitution kept just a trifle better than the official one.

We find in the literature a great deal about so-called "activators and paralyzers" of pepsin in its preparations. Effront⁴ and Euler⁵ make mention of alkalis, salts, and organic compounds as stabilizers, and it was with these suggestions that caffeine, boric acid and iodoform were used and their effect noted in the course of seven months. A quantity of Essence of Pepsin N. F. IV was prepared and divided into 250-cc portions. In one was placed caffeine in a concen-

¹ E. Buchner, *Ber.*, 30, 1, 1110, 1897.

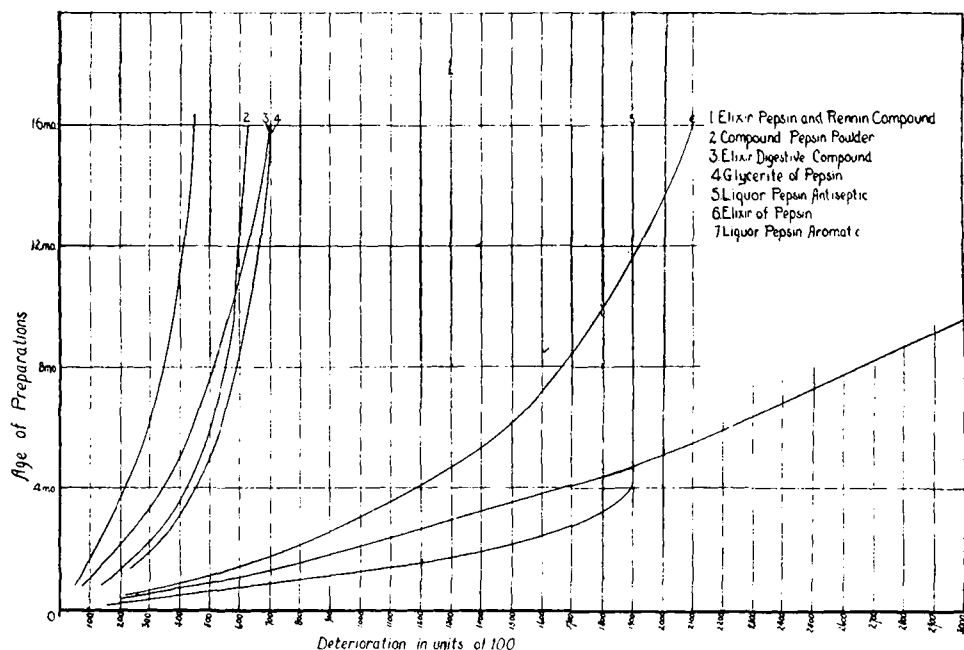
² Euler-Pope, "General Chemistry of Enzymes," 121.

³ *Arch. Med. Exp.*, 19, 497-504.

⁴ "Effront-Prescott, "Biochemical Catalysts," 181-185, 204.

⁵ "Euler-Pope, "General Chemistry of Enzymes," 121.

tration of 0.1 Gm. to 100 cc of Essence of Pepsin; boric acid 0.12 Gm. per 100 cc; iodoform, 0.05 Gm. per 100 cc of Essence. (Little attention was paid at this time as to whether the amounts were large enough for therapeutic effect, it being simply a trial for observing the keeping properties of any of the pepsin preparations containing the different-named ingredients.) These preparations and also that of Essence of Pepsin N. F. IV without preservatives were assayed over a period of seven months; it was found that although the Essence of Pepsin without preservatives dropped in peptic activity to 1-2700, the others remained 1-3000. Also a series of concentrations of caffeine in Essence of Pepsin were prepared as follows: 0.05 Gm. per 100 cc; 0.034 Gm. per 100 cc; 0.017 Gm. per 100 cc; 0.0085 Gm. per 100 cc and 0.00425 Gm. per 100 cc and assays run at monthly intervals over a period of four months. It was found that there was no dropping off of peptic activity in any of these, not even the one containing 0.00425 Gm. per 100 cc which is an amount so small that the therapeutic effect would be negligible. Likewise the boric acid in the one preparation is present in an amount which would have no therapeutic effect. Four doses a day of 1 fluidrachm of this Essence of Pepsin would mean the taking of 0.57 grain of boric acid per day. All the preparations remained clear and showed no evidence of precipitate. Their taste was not affected; if anything, the one containing the iodoform seemed more palatable.



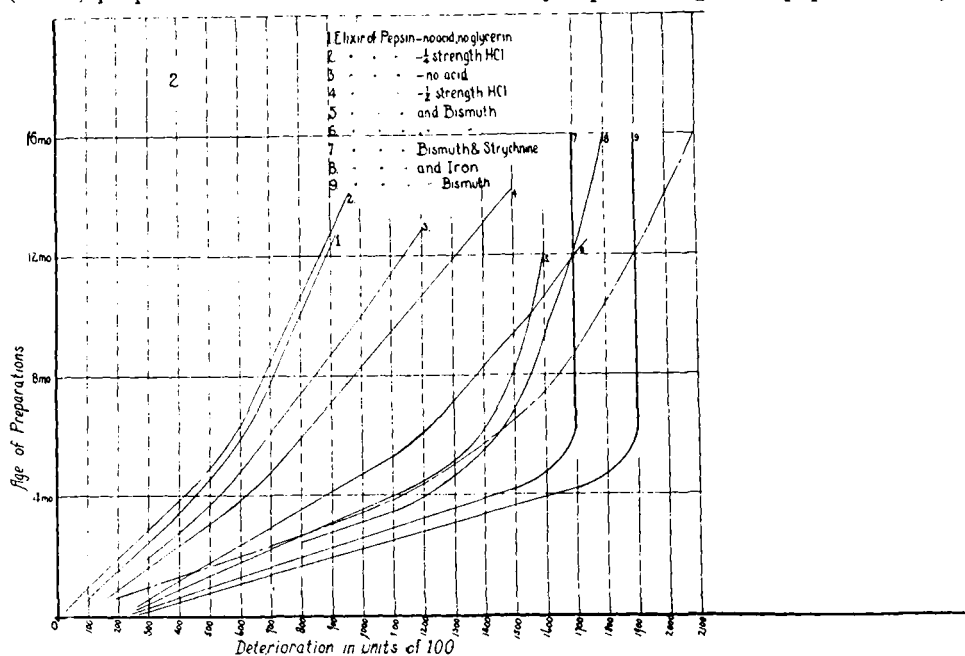
Graph 1.

Graphs 1 and 2 include most of the official (N. F.) pepsin preparations in use as medicaments, besides other special combinations, the same preparations on which a previous paper¹ presented data for a 6-month interval. They were assayed as described at that time. It is interesting to note that many of the preparations show very slow deterioration after 6 or 8 months. This seems to be especially

¹ JOUR. A. PH. A., 10, 595, 1921.

striking in those of the bismuth-pepsin combination which dropped off rapidly in peptic activity for the first six or eight months and have since remained constant. All four of the bismuth-pepsin elixirs (Graph 2), for which curves are presented, were made up according to the N. F. IV directions and hence are identical except for the addition of strychnine to the one of them. The purpose of recording data on all four of these elixirs was to get checks and to show that the results were duplicable within reasonable limits. It will be noticed that these four and the iron elixir run fairly parallel (Graph 2).

Liquor Pepsini Aromatici showed no noticeable peptic activity when assayed by the U. S. P. IX method less than a year after it was made. No other official (N. F.) preparation showed such an abnormally rapid falling off in peptic activity.



Graph 2.

Elixir Pepsin and Rennin Compound, N. F. IV, also commonly known as Essence of Pepsin, maintains its peptic activity unusually well. Several commercial preparations, described by their makers as of this type, were also assayed periodically and with one exception held their activity equally well. Pulvis Pepsini Compositus is an N. F. III preparation, and a powder of similar composition compressed into tablets is frequently prepared by manufacturing pharmacists. Pepsin in this dry combination seems to retain its activity quite well. Elixir Digestive Compound, also commonly known as Elixir Lactated Pepsin, though rapidly losing its diastasic value, retains its peptic activity comparatively well. Glycerite of Pepsin, used mostly as an ingredient in other preparations, is another preparation which stands up well. These four preparations are seemingly in a class by themselves with respect to the manner in which they retain their peptic activity.

Elixir of Pepsin was made up in several combinations, as designated in Graph 2. It is interesting to note that a preparation made up with only one-half as

much HCl as the official preparation retained its peptic activity much better than did the latter; similarly, in regard to the two elixirs made up with no acid at all, the one with glycerin, the other without it. The broken line curve of Graph 2 is a duplicate of Curve 6 of Graph 1 (Elixir of Pepsin), inserted to facilitate comparison.

Deterioration of the activity of pepsin in these combinations may be due to the action of the other ingredients. The action of various substances as inhibitors and paralyzers of enzymic activity has been the source of considerable interest and discussion in the past. In a previous paper¹ it was pointed out that Elixir of Pepsin, made up with only one-fourth as much HCl as the official preparation, retained its activity much better than the latter. The curve for this one-fourth strength HCl preparation is shown for a 14-month period as No. 2 on Graph 2. It is practically on a par with the similar preparation containing no added acid whatever, for retaining its peptic activity. A study of the relation of the p_H values of these preparations to their keeping qualities is interesting. Thus we get the following data:

Preparation.	E.	p_H .	C_H .
Elixir of Pepsin—no acid	0.548	4.485	2.91×10^{-5}
Elixir of Pepsin— $\left\{ \begin{array}{l} \text{no acid} \\ \text{no glycerin} \end{array} \right.$	0.537	4.35	4.47×10^{-5}
Elixir of Pepsin— $1/4$ HCl	0.481	3.35	4.47×10^{-4}
Elixir of Pepsin— $1/2$ HCl	0.435	2.58	2.63×10^{-3}
Elixir of Pepsin—N. F. IV (1 HCl)	0.387	1.760	1.74×10^{-2}
Elixir of Pepsin— $1 1/2$ HCl	0.353	1.185	6.57×10^{-2}
Elixir of Pepsin—2 HCl	0.340	0.964	1.085×10^{-1}
Elixir of Pepsin & Rennin Compound	0.500	3.67	2.14×10^{-4}
Elixir Digestive Compound (N. F. III)	0.475	3.25	5.62×10^{-4}
Glycerite of Pepsin	0.454	2.89	1.29×10^{-3}
Elixir of Pepsin & Iron, N. F. IV	0.380	1.728	1.708×10^{-2}

Also, the acidity of the U. S. P. IX digestion mixture, made up with 0.30% HCl, after $1/2$ hour at 52° and before the addition of any pepsin, is about 0.08% HCl by titration with $N/10$ KOH, using alizarine sulphonate as indicator, remaining practically constant throughout the digestion as indicated by the following data:

($N/10$ KOH Required for 10 Cc of the Digestion Mixture.)

Bottle No.	1 After 30 min. at 52° .	2 After $1/2$ hr. digm.	3 After 1 hr. digm.	4 After $1 1/2$ hrs. digm.	5 After 2 hrs. digm.	6 After $2 1/2$ hrs. digm.
1	2.20	2.50	2.45	2.40	2.30	2.30
4	2.00	2.30	2.30	2.25	2.25	2.25
15	2.00	2.20	2.20	2.15	2.20	2.20
16	2.10	2.30	2.25	2.25	2.20	2.20
37	2.10	2.30	2.30	2.25	2.25	2.20
48	2.30	2.50	2.40	2.30	2.30	2.25

Ten cc of 0.30% HCl used in the digestion mixture required for neutralization 8.40 cc of $N/10$ KOH. After $1/2$ hour at 52° in contact with the egg albumen, this dropped, as is shown in Column 1, to 2.10 cc, corresponding to about 0.08% titrable HCl. Then 5 cc of a solution of 0.1 Gm. pepsin in 150 cc of 0.30% HCl were added, and digestion begun. This addition accounts for the slight increase

¹ Jour. A. Ph. A., 10, 595, 1921.

in KOH required as shown in Column 2 and subsequent columns. The titrable acidity remains practically constant throughout the digestion.¹ Starting with both higher and lower concentrations of HCl than 0.30% gives higher residues in the sedimentation tubes, thus indicating that the 0.30% of HCl solution is optimum, although the albumen present brings the concentration down to about 0.08% HCl, which is optimum for the digestion at 52° with this substrate.

These data indicate that the digestion by pepsin in the U. S. P. IX assay method proceeds in a medium of about 0.08% titrable HCl, and that for preparations of the Elixir of Pepsin, N. F. IV type, including Elixir of Pepsin and Rennin Compound and Elixir Digestive Compound, an acidity of from 0.00% to 0.08% HCl is optimum for the retention of their peptic value. It also seems, when the hydrogen-ion concentration and superior keeping qualities of Glycerite of Pepsin are observed, that the medicinal pepsin preparations may be divided into three classes:

(1) Those of the type of Elixir of Pepsin N. F. IV, essentially preparations of pepsin in an aromatic elixir vehicle.

(2) Glycerite of Pepsin, which may be put in a class by itself because of its unusually high content of pepsin and glycerin, without any alcohol or sugar.

(3) All other pepsin elixirs and solutions consisting of pepsin in various combinations in which inhibitors or paralyzers are used for other added effects besides peptic value, *e. g.*, preparations containing aromatics, antiseptics, metals, etc.

In preparations of the first type it seems that those containing from 0.00% to about 0.10% added HCl, ranging in C_H^+ between 4.50×10^{-4} and 3.00×10^{-5} (approximately), retain their activity better than those of a higher HCl content. This is in agreement with the results obtained for the titrable acid content of the U. S. P. digestion mixture, and with the results of Jul. Schütz.²

In making Glycerite of Pepsin about 0.30% of its content of HCl is used. Still, the preparation made here 18 months ago showed a comparatively low acidity. A preparation of more recent manufacture, 4 months old, showed a similar emf. whereas, from the amount of acid added in its manufacture, the glycerite should have an emf. slightly below that of the N. F. IV Elixir of Pepsin. This can be explained by assuming that the HCl in the Glycerite is held in combination by the large amount of pepsin it contains (five times that of any other pepsin preparation) or that the large amount of glycerin present may reduce the hydrogen-ion concentration, either of which is a reasonable assumption.

In the third type of preparations, deterioration may be attributed to the ingredients added for secondary purposes, and which are known to be pepsin "paralyzers."

These data indicate that it is advisable to reduce the added HCl of Elixir of Pepsin by three-fourths or more, of the amount used at present, that the added HCl of Elixir Pepsin and Rennin Compound and of Elixir Digestive Compound be kept about the same or reduced slightly, and that the added HCl of the Glycerite of Pepsin might also be reduced slightly.

¹ H. T. Graber, *JOUR. A. PH. A.*, July 1921.

² Jul. Schütz, *Wiener klin. Wochenschrift*, 20, 1361, 1907.

We take pleasure in acknowledging at this point our indebtedness to Messrs. W. H. Blome and Mortimer Bye of the Frederick Stearns Company's laboratories for their helpful suggestions and interest in our work.

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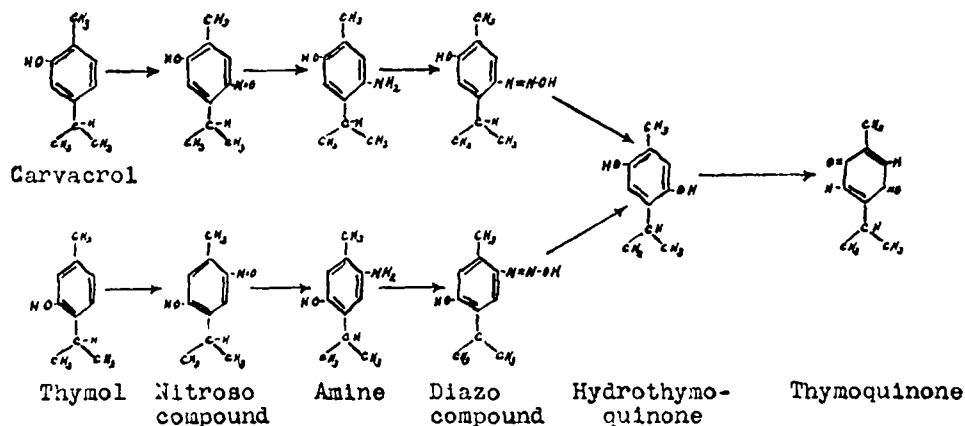
MISCELLANEOUS CHEMICAL PAPERS.*

PRODUCTION OF THYMOQUINONE ON A SEMI-COMMERCIAL SCALE IN THE LABORATORY.**

BY RALPH M. HIXON.***

A modification of the Liebermann-Illinski method¹ of preparing thymoquinone was developed in this laboratory,² whereby a yield of 90-93% of the theoretical amount of thymoquinone could be obtained from thymol. This reaction has now been tried on a larger scale (500-Gm. batches) with the object of ascertaining the feasibility of these proportions for the commercial production of thymoquinone from thymol or carvacrol.

The reaction can be expressed by the following series of intermediate products of which only the nitroso compound and the amine are actually isolated in the process:



The details of the method for the preparation of thymoquinone as originally developed in this laboratory were given as follows:²

"Dissolve 5 grams of thymol in 25 cc of 95 p. c. alcohol and add 25 cc of concentrated hydrochloric acid. Place this solution in a freezing mixture and add gradually, with constant stirring, crystals of sodium nitrite till 5 grams have been added. The solution at first becomes light green, then dark green, and, after a few minutes, solidifies to a bluish mass. Transfer this to a beaker containing about a liter of cold water and stir until the product becomes light yellow and fluffy in appearance. Wash this product with cold water and use it for the preparation of thymoquinone while still moist. Yield of nitrosothymol about 6 grams.

* From the Laboratory of Edward Kremers.

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