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A Study of the Stability of Liquid Preparations Containing Pepsin*

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This work is an endeavor to study the stability of liquid preparations containing pepsin, and to investigate the possibility of stabilizing the activity of peptic systems. Heretofore, as mentioned by Worrell (1), stability studies of pepsin preparations have been inconclusive, due primarily to the lack of a satisfactory method of assay. Worrell (1) had this objective in mind, namely, to develop a procedure which could be employed successfully in the accurate assay of pepsin preparations for activity. In the experiments conducted in this research his method of assay for liquid preparations containing pepsin has been used entirely.

During the course of making an enzyme preparation, it is often noted that there is a rather acute loss in activity of the enzyme. Similar losses are noted when the enzyme solution is permitted to stand at room temperature for a period of time. Northrop (2, 3) indicates that a major source of inactivation in the case of pepsin and trypsin is due to denaturation of the enzyme protein.

In attacking the problem of stabilizing the activity of liquid preparations containing pepsin, it was deemed fundamental that a study first be made of the influence of individual factors or variables on the maintenance of peptic activity over an extended period of time. The factors which have received special consideration include temperature, p_H, antioxidants (maleic acid, hydroquinone, resorcinol), preservatives (alcohol, "Merthiolate"), protective agents (acacia) and low concentrations of amino acids (tyrosine).

EXPERIMENTAL

A number of preparations were made, each having one or more of the above variables which differentiated it from the standard. A standard preparation was designated as one having approximately five per cent of pepsin in distilled water, and which was stored at room temperature under nitrogen. Any substance added in order to study its effect on the stability of pepsin solutions was considered to be a variable. All of the samples were placed in ordinary clear glass bottles and stored in the dark.

Table I.—A Summary of Assay Results of Pepsin Solutions on Stability Tests. ("*n*" in the Number Designating the Sample Indicates Storage under Nitrogen; "*a*" Indicates Storage under Air)

Sample	Variable	Concentration of Pepsin (%) 1st Week	6th Week	14th Week	% Loss in Activity
3*n*	Storage at 5° C.	5.4	5.0	4.9	9.3
4*n*	1% maleic acid	5.2	3.4	2.5	51.9
5*n*	3% maleic acid	4.8	3.0	1.9	60.4
6*n*	5% maleic acid	4.8	2.5	1.3	72.9
7*n*	2% resorcinol	5.5	4.5	4.6	16.4
8*n*	2% hydroquinone	5.3	4.2	2.8	47.2
10*n*	10% U. S. P. Alcohol	5.7	4.8	4.9	14.0
16*a*	0.05% "Merthiolate," storage under air	4.6	4.4	4.2	8.7
19*n*	0.05% "Merthiolate"	4.5	4.0	4.1	8.9
21*n*	0.1% maleic acid, 10% U. S. P. Alcohol	5.3	5.2	4.7	11.3
22*n*	10% U. S. P. Alcohol	5.2	5.0	4.7	9.6
24*n*	0.05% "Merthiolate"	5.4	4.9	4.5	16.7
25*n*	0.05% "Merthiolate," 0.1% maleic acid	5.7	4.8	4.5	21.1
26*n*	0.05% "Merthiolate," 1% acacia	5.3	4.9	4.6	13.2
27*n*	0.05% "Merthiolate," satd. with tyrosine	5.3	4.9	4.6	13.2

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Assays were made weekly over a period of ten weeks on these preparations. An assay of the samples was also made a month following the ten-week period. A summary of the results of the assays is included in Table I.

Following these assays to determine the effect of storage on the activity of pepsin exposed to various factors, an experiment on the effect of shaking pepsin solutions was carried out.

A stock solution of pepsin approximately five per cent in strength was prepared. Portions of the stock solution were placed in several bottles to be shaken for various periods of time. For the purpose of finding out whether or not the effect of shaking was associated with air oxidation, identical samples were also stored under nitrogen. The stock solution was assayed at the beginning of the experiment, and twenty-four hours later each of the samples was assayed to determine the effect of shaking on the proteolytic activity.

Table II.—The Effect of Shaking on Pepsin Solutions

Pepsin lot 220799, Casein lot 33618

Sample	Period of Shaking	Gm. Pepsin per Cc. before Shaking	Gm. Pepsin per Cc. after Shaking	% Loss in Activity
Aa	1 hour	0.0488	0.0458	6.1
An	1 hour	0.0488	0.0462	5.3
Ba	2 hours	0.0488	0.0458	6.1
Bn	2 hours	0.0488	0.0462	5.3
Ca	6 hours	0.0488	0.0462	5.3
Cn	6 hours	0.0488	0.0462	5.3
Da	12 hours	0.0488	0.0441	9.8
Dn	12 hours	0.0488	0.0441	9.8
Ea	24 hours	0.0488	0.0387	20.7
En	24 hours	0.0488	0.0387	20.7
Fa	Not shaken	0.0488	0.0462	5.3

CONCLUSIONS

In general, it appears that the rate of loss in activity is greatest during the first six weeks of storage. The advantage of refrigeration is not sufficient to warrant its consideration commercially.

The addition of maleic acid presumably as an antioxidant leads to a marked loss in activity, the degree of inactivation paralleling increasing concentrations of the acid. Solutions containing sodium maleate were not included in the table of results because of putrefaction early in the assay period. However, the sample containing three per cent of sodium maleate was sufficiently alkaline to completely inactivate the pepsin by the end of the first assay.

Thus, it is obvious that ionization markedly influences the activity of pepsin. The presence of hydroxyl ions induces an immediate inhibition of pepsin. Hydrogen ions in excess also promote a gradual loss in activity. Apparently the optimum p_H for the storage of pepsin solutions is in the range of 4.5 to 5.0.

The antioxidant action of resorcinol and hydroquinone is of little value in preserving enzymatic activity. Both of the solutions containing these agents darken upon standing.

Alcohol and "Merthiolate" in the concentrations employed exhibit no discernible inhibition of peptic activity. The use of acacia as a sorbing or protective agent appears to be of no value. Saturating a pepsin solution with tyrosine apparently has no activating effect.

Vigorous shaking for a period of six hours or less produces no increased loss in activity of pepsin in a purely aqueous solution. However, it is noted that shaking for a period of twelve hours or longer results in a marked inactivation of the enzyme.

Storage under an atmosphere of nitrogen does not retard loss in peptic activity. From the experiment on the effect of shaking, samples stored under nitrogen (denoted by the letter "*n*") act the same as those stored under air. As a direct consequence, it appears that air oxidation has little to do with the inactivation of pepsin.

It is evident from these results that protein denaturation is primarily responsible for loss in peptic activity. Factors which promote protein denaturation also produce inactivation of pepsin. Apparently, the presence of water is conducive to protein denaturation or loss in activity.

A logical extension of this work is being made in an attempt to develop a pepsin preparation which will retain its initial activity over a long period of time.

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