EVIDENCE OF ENZYMATIC DESTRUCTION OF THE VITAMIN A VALUE OF ALFALFA DURING THE CURING PROCESS*

BY SIGFRED M. HAUGE

(From the Research Chemical Laboratory, Purdue University Agricultural Experiment Station, Lafayette)

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Numerous investigators (1–5) have shown that the drying of alfalfa by means of mechanical driers tends to preserve the vitamin A value, while the field curing process is more or less destructive to the vitamin A value. Hauge and Aitkenhead (3) made a study to determine the factors involved in the preservation or destruction of the vitamin during these processes, and presented evidence which indicated that enzymes are the important factor in the deterioration of the vitamin A factor during the curing process. They also showed that the sun’s rays were not directly responsible for the marked destruction during the field curing process.

It seemed desirable to make a further study of the relation of enzyme activity to the destruction of the vitamin A factor in alfalfa. Alfalfa was therefore treated by some methods which were favorable to enzyme activity and by other methods which inhibited enzyme action. The effect of these treatments on the vitamin A value of alfalfa was determined.

EXPERIMENTAL

Since earlier experiments (6) had shown that young alfalfa has a higher vitamin A value than alfalfa in the full bloom stage, the alfalfa selected for this experiment was cut from young plants (10 to 12 inches high) before they showed any bloom. The alfalfa was cut early in the morning in July, 1933, and removed imme-

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Enzyme Effect on Vitamin A Value

diately to the laboratory, where it was separated into eight portions and subjected to special treatments.

*Sample 1*—This portion of alfalfa was treated in an autoclave in the presence of live steam, at 17 pounds pressure, for 1 hour in order to inhibit all enzyme activity, and then dried on screens by direct exposure to the sun's rays.

*Sample 2*—This alfalfa was sterilized as in Sample 1, cooled, placed in closed jars to retain the moisture, and then incubated at 37° for 24 hours, which is a favorable temperature for enzyme action. It was then dried on screens by exposure to the sun.

*Sample 3*—This portion was treated in such a manner as to test the effect of the addition of a fresh supply of enzymes to alfalfa in which the natural plant enzymes had been inactivated. The alfalfa was first sterilized by treatment in the autoclave, in order to inactivate the enzymes present in the alfalfa, and then cooled. Since potato juice contains oxidative enzymes, 100 ml. of fresh potato juice were added to 500 gm. of alfalfa, thoroughly mixed,
enclosed in glass jars, and held at 37° for 24 hours. It was then spread on screens and dried in the sun.

*Sample 4*—This portion of alfalfa was placed in jars and immediately frozen at about −25° in order to rupture the cellular structure of the plant tissue and liberate the enzymes. It was then defrosted and held at 4° for 24 hours, after which it was sterilized, spread on screens, and dried in the sun.

*Sample 5*—This sample was given the same treatment as Sample 4, except it was held at 37° during the 24 hour digestion.

*Sample 6*—This sample was placed in jars without any preliminary treatment, then held at 4° for 24 hours, sterilized in the autoclave, and finally dried in the sun.

*Sample 7*—This alfalfa was treated as was Sample 6, except that it was held at 25° during the digestion period.

*Sample 8*—This portion was treated as was Sample 6, except that it was incubated at 37° for 24 hours instead of 4°.

After all the samples were dry, they were finely ground in a Wiley mill and stored in glass jars for the biological assays.

The vitamin A values of these samples were determined by biological assays and the use of the curative method. The technique was the same as has been previously described (6). The results of these tests are given in Chart 1, the values being expressed in Sherman and Munsell (7) vitamin A units.

**DISCUSSION**

In a previous report from this laboratory (3) it was shown that in any process whereby the enzymes were inactivated by heat, whether by mechanical driers, with either heated air or hot flue gas, or by sterilization by treatment in an autoclave, alfalfa was produced with high vitamin A value. This was confirmed in Sample 1, in which the enzymes were inactivated by sterilization in an autoclave. The vitamin A value of this sample is so high that it might indicate that there was practically no deterioration from that of the fresh plant. This appears probable in view of the recent work of Russell (5) who found that machine-dried alfalfa contained as much carotene, to which the vitamin A value of alfalfa is probably due, as freshly cut material from the same field.

If the major deterioration of vitamin A value of alfalfa during the curing process is due to other factors than enzymes, one would
Enzyme Effect on Vitamin A Value

naturally expect that if the moist alfalfa secured after inactivation of the enzymes was subjected to incubation at a warm temperature for 24 hours and then sun-dried, there would be marked deterioration of the vitamin A value. However, the biological assays of Sample 2 show that little or no deterioration took place. This would indicate that inactivation of enzymes by heat practically inhibited all deterioration of the vitamin.

Direct evidence that enzymes are destructive to the vitamin A value of alfalfa was obtained in Sample 3. This sample received the same treatment as Sample 2, except that active enzymes were introduced by the addition of fresh potato juice. These enzymes caused a destruction of more than 50 per cent of the vitamin A in this sample as in contrast to Sample 2 in which little or no deterioration took place. However, the destruction was not as great as that in Samples 5 and 8, although all samples were incubated at 37° for 24 hours. A possible explanation for these differences may be that the enzymes which were added by means of the potato juice were not of equal concentration to that of the naturally occurring enzymes in alfalfa or that they did not permeate the tissues sufficiently to be as effective in their destructive action as the naturally occurring enzymes in Samples 5 and 8.

Another method to gain an insight into this problem was to study the deterioration of vitamin A produced by heating to temperatures which more or less influence enzyme action. It is well known that enzyme activity is high at 37° and as the temperature is lowered the enzyme activity decreases. Therefore, if the activity of the naturally occurring enzymes of alfalfa were controlled by holding the material at definite temperatures, this might also affect the deterioration of vitamin A. In Samples 4 to 8 the natural plant enzymes were not inactivated until after the 24 hour digestion period. The results show that there is a direct correlation between the effect of temperature on the destruction of vitamin A and the effect of temperature on enzyme activity. In Samples 4 and 6, which were held at 4° for 24 hours before inactivation of the enzymes, the vitamin A value was somewhat lowered as compared with Sample 1, which is not surprising when one considers that there is some enzyme activity even at this low temperature. In Sample 7, in which the enzyme activity was increased by a temperature of 25°, a greater deterioration of
vitamin A occurred, while in Samples 5 and 8, which were held at temperatures most favorable to enzyme activity, the destruction was the greatest.

In Samples 4 and 5, the alfalfa was frozen at $-25^\circ$ for 2 hours, defrosted, and then digested at $4^\circ$ and $37^\circ$, respectively. The results show that the destruction of vitamin A in these samples was somewhat greater than in the corresponding Samples 6 and 8.

Every sample used in this experiment was subjected to sterilization in an autoclave at some time during the treatment period, which inactivated the enzymes, before being dried by exposure to the sun's rays. Since the drying conditions were identical with all samples, sunshine can be eliminated as a contributory factor toward the production of the differences found in these samples. Furthermore, since Sample 1 possessed greater potency than any sample hitherto tested in this laboratory, it appears evident that the sun's rays do not contribute directly to the destruction of vitamin A in alfalfa. However, in the field curing process, where natural enzymes are still active, the sunshine probably has an indirect effect by producing temperatures which accelerate enzyme activity.

**SUMMARY**

1. A study was made of the relation of enzyme activity to the destruction of the vitamin A factor in alfalfa.

2. By immediate inactivation of the enzymes in alfalfa, it is possible to produce dried alfalfa of very high vitamin A potency.

3. The digestion at $37^\circ$ of alfalfa in which the natural enzymes have been inactivated resulted in little or no deterioration, while digestion of such material to which active enzymes have been added resulted in marked destruction of this factor.

4. In samples containing the active plant enzymes, which were treated at temperatures which influence enzyme activity, there was found to be a direct correlation between the effect of temperature on the destruction of vitamin A and the effect of temperature on enzyme activity.

5. Evidence is presented which shows that enzymes are directly responsible for the destruction of the vitamin A value of alfalfa during the curing process and that sun’s rays have only an indirect effect by producing temperatures which accelerate enzyme activity.
Enzyme Effect on Vitamin A Value

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Sigfred M. Hauge


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