

solution was, in turn, poured into the spectrometer sample holder and D_{\max} determined several times over a period of 24 hours. During this period both solutions formed a stiff gel, but the value of D_{\max_0} remained essentially constant. The only effect noted was a decrease in relaxation time, T_1 , as the viscosity increased, an effect discussed by Bloembergen, Purcell, and Pound (7).

Because D_{\max_0} is unaffected by gelation of the sugar-gelation solutions, it is concluded that the experimental method used by Shaw and Elsken for determining the moisture content of hygroscopic materials may give a value for D_{\max_0} which includes the contributions from the hydrogen nuclei of both water and soluble solids in liquid samples and samples as rigid as a stiff gel.

Conclusions

The observed value of D_{\max} obtained under the conditions described by Shaw and Elsken (2) is influenced by

soluble solids which contain hydrogen nuclei. In all cases investigated, the hydrogen nuclei of the soluble solids contributed to D_{\max} in direct proportion to their concentration. In order to calculate the moisture content of a sample that contains soluble solids it is necessary, therefore, either to make a calibration curve or to use Equation 2. The latter method requires that the soluble solids content be known, while the former method requires that the soluble solids composition be the same for all standard and unknown samples. The uncorrected data for the two skim milk samples shown in Figure 1 illustrate what may occur when the solids composition varies. In specific cases it may be necessary to have separate calibration curves for each variety of a product as well as for different maturity grades.

It is also evident from the data presented in Figure 1 that for these particular samples the nuclear magnetic resonance results change by only 4.7% when the moisture content changes by 14.5%;

consequently the percentage change in D_{\max} , the value measured in the nuclear magnetic resonance spectrometer, is only about one third as much as the percentage change in water content.

Acknowledgment

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CROP STORAGE

Feedstuffs Values and Chemical Changes in Spontaneously Heated Alfalfa Pellets

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Alfalfa pellets from fresh alfalfa hay have become a major feed ingredient. Recently serious losses have occurred, due to spontaneous heating in grain elevators. A comprehensive analytical study, showing progressive deterioration from sound through brown to black pellets, indicated that spontaneous heating is initiated and sustained by sugar-protein interactions of the Maillard type, accompanied by rapid utilization of sugars, slower destruction of fat, and increases in ash, total nitrogen, and crude fiber. In preliminary and intermediate phases, sharp reduction of carotene and regular increases in fluorescence occur. Fat acidity increases rapidly only with onset of advanced browning. Riboflavin values are influenced by fluorescence due to browning products. Retention of nitrogen in heated pellets is attributed to melanoidins resistant to thermal destruction and nutritive utilization. Decomposition is advanced in terms of color, chemical deterioration, and nutritive losses before flaming combustion.

SPONTANEOUS HEATING IN STORED HAY resulting in the production of brown to black residues appears to be a common occurrence (4). Impairment of the feeding value of alfalfa hay for livestock due to spontaneous heating has been noted (3). Recently, spontaneous heating to the point of ignition in commercial alfalfa pellets was brought to the attention of the authors.

Alfalfa pellets are prepared by extrusion pelleting of chopped, artificially dried alfalfa. This product has become

a major feed ingredient and is handled and stored in considerable quantities by methods common to bulk grain.

It appeared that the physical and chemical factors which play a part in the spontaneous heating of other bulk granular agricultural materials might also be involved in the spontaneous heating of this product. It was also of interest to compare the chemical changes occurring in pellets at progressive stages of heating with those in soybeans, concerning which considerable information is available (7).

Materials and Methods

Samples of alfalfa pellets at various stages of deterioration were obtained from a commercial elevator bin in which spontaneous heating of pellets occurred in November 1954. This bin had been filled with 1100 tons of bulk pellets about 3 months prior to this event. One of the authors (MM) inspected the material shortly after the advanced heating had commenced and while salvage operations were in progress. The pel-

Table I. Feedstuffs Analysis of Heat-Damaged Alfalfa Pellets

Sample	Moisture, %	Ash ^a , %	Crude Protein ^a , %	Crude Fiber ^a , %	Ether Extract ^a , %	N.F.E. ^{ab} , %	Carbo- hydrates ^{ac} , %
N-1	4.4	11.4	17.6	25.7	3.5	41.8	67.5
N-2	4.5	11.4	17.7	27.2	3.4	40.3	67.5
N-3	4.3	11.4	17.3	25.8	3.3	42.2	68.0
A	5.8	11.7	17.8	25.9	3.4	41.2	67.1
B	5.6	12.0	18.2	27.4	2.8	39.6	67.0
C	5.3	12.6	19.1	33.3	2.2	32.8	66.1
D	4.4	12.8	21.0	33.7	2.5	30.0	63.7
E	4.5	22.3	24.2	49.6	0.8	3.1	50.0
F	3.0 ^d	32.6	20.4	48.8	0.2 ^d	...	48.8

^a Dry weight basis.^b Nitrogen-free extract (by difference).^c Nitrogen-free extract plus crude fiber.^d Single determination, due to limited sample.

lets being removed exhibited various degrees of heating damage, although apparently sound pellets predominated. In general, the physical characteristics and color were strikingly similar to those observed in the spontaneous heating of soybeans, with the major exception that there was no visual or organoleptic evidence of fungal growth.

As the material in the bin was being removed, it became apparent that, as has been reported for soybeans, the large heating mass consisted of a dark, congealed, and very hot central column, surrounded by decreasing gradations of deterioration and temperature toward the outer edges, where the pellets were cool and normal. The congealed mass of pellets would not flow from the unloading spouts in the bottom of the bin, and an opening was made into the concrete wall to facilitate removal. The time sequence (about 3 months) between loading of the bin and the onset of advanced stages of heating occurred during a cooling trend in the weather in the fall of the year. The significance of this factor has been discussed (7).

Toward the end of the salvage operation, other observers noted that flames occasionally burst out when large lumps of very dark heating pellets, exuding smoke and vapor, were broken up and their interiors exposed to air. There had been no evidence of flame under other circumstances.

Samples from this salvage operation were sealed in metal cans and shipped to Kansas State College, where they were held in frozen storage until the sampling and analyses reported here were completed. Most of the samples were mixtures of pellets representing various stages of heating, particularly material that was loaded and shipped out in railroad cars. However, a few samples of undamaged pellets were obtained from areas of the bin not affected by the heating as well as from other bins in the same elevator in which no heating had occurred.

To develop information concerning compositional changes as heating progressed, it was deemed necessary to

segregate the salvaged material into distinct visual stages of heating damage. By this means it would be possible to re-establish the sequence of chemical changes that occur in passing from sound normal material (as in the periphery of the heating zone) to the hottest very dark material (as in the center of the congealed column of pellets). The following series of samples was therefore prepared for analysis:

I. Normal Series N-1, N-2, and N-3.

These samples were taken from bulk lots which were apparently entirely normal and not affected by heating. At least one member of this normal group was known to be taken from a bin that was not involved in this loss.

II. Graded Series Representing Progressive Heating Deterioration

- A. Apparently normal pellets.
- B. Slight browning.
- C. Brown.
- D. Very dark brown, but original form of pellet retained.
- E. Black, with pitted surfaces.
- F. Pellets black, also shrunken. Many apparently covered with light deposit of ash.

The pellets in this series were hand-picked out of mixtures of various stages of heating to represent the sequence of deterioration through which pellets had progressed, from apparently normal pellets, A, through intermediate brown stages B, C, and D, to black pellets in sample E and material in F which appeared to be affected by fire.

Complete feedstuffs analysis including moisture, ash, crude protein, crude fiber, ether extract, and nitrogen-free extract (by difference) were conducted by methods of the Association of Official Agricultural Chemists (1). Procedures from the same source were employed for total, reducing, and nonreducing sugars, fat acidity, and riboflavin.

Carotene was determined by the method of Silker, Schrenk, and King (8), amino acid nitrogen by the technique of Beauchene and others (2), and nonprotein nitrogen by the method of Loomis and Shull (5). Fluorescence measurements of aqueous extracts were made as

described for soybeans by Milner and Thompson (7).

Results

Data for feedstuffs analysis corrected to a dry weight basis, as well as the moisture content of the various samples, are presented in Table I. While exposure of samples to atmospheric conditions during salvage and prior to sealing in containers would be a variable factor influencing the moisture content, it is of interest to note a tendency toward decreasing moisture content as visual deterioration increased. This drop in moisture content with increasing intensity of heating would appear to be related to the strong evolution of vapor from this bin, which was the first indication of deterioration noted by the supervisory personnel at the elevator.

The ash content of the classified series shows small regular increases with browning through stages A to D and an abrupt, two- to threefold increase in black samples E and F. The ash factor in the pellets probably was subject to the least change by spontaneous heating and combustion, so that this increase provides an inverse measure of the relative disappearance of other more sensitive constituents.

The crude protein content increases from samples A to E and then decreases in F. The relative increase in crude protein in samples B, C, and D is greater than the rate of increase of ash in these samples. Thus samples B, C, and D probably represent the progressive transformation of nitrogen into more stable forms—e.g., melanoidin pigments characteristic of heating agricultural materials—as the more labile constituents of the pellets are utilized or destroyed in the heating process. It is clear that nitrogen, in whatever organic combination it may exist as heating progresses, is unusually stable. In the late stages of spontaneous heating, represented by samples E and F, where the color is black rather than brown, it would appear that nitrogen is actually being lost, probably by combustion.

Crude fiber, which represents materials digestible with difficulty even by ruminants, exhibited an apparent increase with progressive heating similar to that shown by crude protein. In fact, the rate of increase up to the dark brown stage (sample D) was considerably greater than for either the ash or the protein content. It is believed that this increase may be accounted for on the basis of one or more of the following possibilities:

1. Greater stability of cellulose, hemicellulose, pentosans, etc., to chemical changes accompanying heating.
2. Chemical inertness of melanoidins formed during heating, which would therefore contribute to the crude fiber analysis.

3. Free carbon which appears in advanced stages of heating, which would also contribute to the crude fiber values.

Changes of the type indicated by 2 and 3 would appear to be the most likely explanations for the apparent increase in crude fiber values until the end of the dark brown heating stage.

Ether extract values decreased regularly from sample A to D, and drastically in E and F. This decrease could be accounted for by:

Combustion or volatilization of the fats or of fatty acids, following hydrolysis.

Oxidative polymerization of unsaturated fats to produce ether-insoluble materials.

Studies on soybeans subjected to spontaneous heating indicate that similar decreases in fat content can occur (6).

Nitrogen-free extract is calculated by difference from 100%, taking into account all the other complete feedstuff analyses performed. It presumably represents the more readily available constituents (sugars, dextrins, starch, etc.). The carbohydrate values are the sum of the nitrogen-free extract values and the values for crude fiber.

Table II. Sugar Content of Heat-Damaged Alfalfa Pellets

Sample	Reducing Sugars ^a , %	Total Sugars ^a , %	Nonreducing Sugars ^{ab} , %
N-1	1.5	3.1	1.6
N-2	1.1	2.4	1.3
N-3	1.4	3.1	1.7
A	1.3	2.9	1.6
B	0.6	1.4	0.8
C	0.3	0.4	0.1
D	0.2	0.3	0.1
E	0.1 ^c	0.3 ^c	0.2 ^c
F

^a Expressed as invert sugar.

^b Uncorrected for water of hydrolysis.

^c Values extrapolated from Hammond table (7).

The results for nitrogen-free extract demonstrate clearly the rapid disappearance of available carbohydrates with heating. Even more direct evidence of utilization of sugars in the heating process appears in the data of Table II.

Surprisingly little change occurred in the carbohydrate fraction proceeding from samples A through D. Even sample E, which was completely black, apparently contained 50% or more carbohydrates. These values are misleading, however, as the destruction of sensitive carbohydrates with heating appears to be overshadowed by the apparent increase in crude fiber which is included with the carbohydrate value, but which in turn seems to represent at least through the dark brown (D) stage the conversion of some of the sugars to nitrogen-containing complexes that are relatively unavailable for the inter-

mediate stages of heating, and would disappear rapidly only with the onset of flaming combustion (stage F).

The data for sugar analysis in Table II indicate that these compounds are the most sensitive of all to utilization in the spontaneous heating process. Marked decrease in these components occurs before similar changes appear in other components. This rapid loss of sugars probably involves interaction with proteins and amino nitrogen to form the browning products characteristic of the deterioration. The heating condition is clearly dependent on the utilization of sugars entering into these browning combinations.

From Table III it is apparent that changes in amino acid nitrogen concentration are by no means so rapid as is the drastic loss of sugar, except at extremely advanced stages of heating as represented by sample E. The nonprotein nitrogen fraction includes not only the amino acid nitrogen but also other soluble forms such as amides and polypeptides. These data demonstrate even more clearly the marked resistance of the nitrogen fraction to destruction during heating, at least until the final extreme stages (combustion?) are reached.

Formation of fatty acids apparently precedes the utilization or destruction of the fats. This is evidenced in Table II by the very sharp increase in fat acidity at the C and D stages in the face of only a slow decrease in total fat (ether extract) until the dark brown D stage is reached (Table I). Apparently, extensive splitting of fat occurs only at advanced stages of heating, where chemical rather than biological or enzymatic agencies are involved.

The carotene analyses indicate not only that this component is extremely sensitive to destruction by spontaneous heating even in the preliminary phases, but also that even the normal samples were deficient in this important nutrient. Sound alfalfa pellets would need to contain a minimum of approximately three times the quantity which was present in

these normal samples, to be considered commercially acceptable. Thus, for example, alfalfa meal is normally used at about a 5% level in poultry rations to provide the necessary carotene. Even the normal samples of this group could not have supplied the minimum requirement of 2000 units of carotene per pound of ration.

Fluorescence is a sensitive index of the formation of products of the Maillard or protein-sugar interaction. In the classified series (A to F) this value increases sharply from samples A through D but decreases rapidly through samples E and F, indicating that a gross destruction of organic constituents is taking place in these very advanced stages of heating.

The increase in riboflavin with heating to the brown stage represented by the C sample appears entirely anomalous in view of the known sensitivity of this vitamin. The most reasonable conclusion is that this increase is only an apparent one, due possibly to the breakdown of riboflavin into fluorescent fragments, or to presence in the extracts of fluorescent browning reaction products. The manner in which the riboflavin concentrations parallel those for fluorescence favors the latter assumption. Thus, the question whether the apparent increase represents riboflavin or nonriboflavin fluorogens can be settled finally only by biological assay for this vitamin. Values for riboflavin determined by fluorometric measurements in materials damaged by spontaneous heating must be viewed with skepticism.

Discussion and Conclusions

The analyses reported indicate that the spontaneous heating of alfalfa pellets in initial and intermediate stages involves sugar-protein interactions accompanied by a rapid utilization of sugars and an initially slower destruction of fat with corresponding relative increases in the ash, protein nitrogen, and crude fiber fractions. There are also involved in

Table III. Amino Acid Nitrogen, Nonprotein Nitrogen, Carotene, Riboflavin, and Fluorescence Values of Heat-Damaged Alfalfa Pellets

Sample	Amino Acid Nitrogen, %	Nonprotein Nitrogen, %	Fat Acidity, Mg. KOH / G. Fat	Carotene, Mg./100 G.	Fluorescence ^a	Riboflavin, γ /G.
N-1	0.18	0.61	2.5	5.6	16.8	19.3 ^c
N-2	0.18	0.63	2.8	4.5	15.1	...
N-3	0.20	0.51	2.7	5.1	20.0	...
A	0.16	0.59	4.0	3.4	20.2	19.1
B	0.12	0.55	7.6	1.3	42.0	23.2
C	0.12	0.57	43.9	0.4	53.8	23.5
D	0.10	0.63	41.0	0.4	56.8	16.6
E	0.02	0.26	22.0	0.3	33.2	4.3
F	... ^b	... ^b	0	... ^b	5.0	... ^b

^a Fluorescence of extract obtained with 1-gram ground pellets to 25 ml. 0.2N HCl, diluted 1 to 500, using fluorescein standard at 60 (0.075 p.p.m. sodium fluorescein).

^b Insufficient for analysis.

^c Composite of N₁, N₂, and N₃.

the preliminary and intermediate phases drastic destruction of carotene, regular increases in fluorescence, and sharp increases in fat acidity. The end of the intermediate stage referred to may be considered the change in color of the material from brown to black.

The data suggest that deterioration was extremely advanced in terms of color and chemical deterioration before flaming combustion appeared. The evidence for this is the regular change in most of the analytical values to a condition where the pellets are very dark brown in color. With the appearance of the final black stage of heating, however, these gradual and regular changes become very abrupt transitions and even reversals in trend in most of the analytical values, such as occurred here in passing from the D (dark brown) to the E and F samples. It seems probable that if flaming combustion were involved, it occurred at a stage of deterioration not earlier than that represented by sample E, which was entirely black. Sample F of this series exhibited what appeared to be ash on the surface of the pellets. It may also be surmised, as has been indicated for other spontaneously heating materials (7), that flaming combustion probably would not occur so long as the material in the dark brown stage was not exposed to oxygen, as happens in salvage operations. Undisturbed large masses of heating granular biological materials have been known to become "spent" and may eventually cool without attaining flame.

The present data parallel those previously published for spontaneously heating soybeans, with the significant exception that no visual evidence of storage molds could be found on any of the pellets, in spite of very careful examination of numerous samples at the moment of salvaging. The pattern of change in fat

acidity as well as that of other chemical values also argues against a major involvement of biological factors in the deterioration. It may be considered, therefore, that alfalfa pellets are even more susceptible to the spontaneous heating than are soybeans and that such heating may involve products of the Maillard reaction. This conclusion may not be surprising when consideration is given to the composition of alfalfa pellets and the fact that extensive mechanical rupture of the plant tissues and forceful mixing of cell constituents occur during the grinding, drying, and pelleting operations. Doubtless the elevated temperatures and pressures used in the pelleting operation also favor the initiation of browning phenomena.

The nutritive and commercial value of the pellets as represented by the various stages of deterioration from A to F and the influence on these values of various amounts of these materials mixed into otherwise sound pellets cannot be established by analyses of these kind alone. Only feeding trials with animals will indicate clearly the extent of nutritive impairment. Feeding value, as determined, say, with chickens, would not be necessarily pertinent to that for cattle. Nevertheless it is clear that the loss of carotene and sugars and the formation of browning products which are known to reduce the nutritive availability of the proteins, would seriously affect nutritional quality of these alfalfa pellets in proportion to the extent of heating. Furthermore, studies in the literature on the feeding value of brown, stack-burned, sun-cured alfalfa hay for dairy cows (3) show clearly that such material is decidedly inferior in nutritive value to green alfalfa hay and that this impairment is more or less proportional to the intensity of the brown or black color developed during heating. The similarity of the

analytical results obtained in the present study to those for the hay used in the cattle feeding experiment referred to suggests that impairment of the feeding value of the pellets would be of the same order. The melanoidin complexes of carbohydrate and nitrogen not only are apparently refractory to thermal decomposition but also probably represent nitrogen in a nutritionally unavailable form.

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NUTRITIVE VALUE OF RICE

Nutrients in Rice Bran and Rice Polish and Improvement of Protein Quality with Amino Acids

THE RICE GRAIN, as it is received at the rice mill from the farm thresher, is called rough or paddy rice and is contained in a hard siliceous hull which encloses the edible kernel. It is made up of the hull, the seed coat (pericarp), the starchy endosperm, and the embryo or germ (15). The hull is removed in the first step of the milling process and

leaves whole brown rice, which retains the bran coat and the germ. The light brown coat is attached firmly to the endosperm or starchy body of the kernel. This coat consists of seven layers of differentiated types of cells and at one end of the kernel lies the germ or embryo, the nutritive value of which has been reported (4). The seed coat layers and

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the embryo contain more than half of the mineral matter of the grain, a fourth of the protein, practically all of the vitamins, and about three fourths of the fat (12).

By means of suitable milling equipment rough rice is separated into milled rice, hulls, bran, and polish. The milled or white rice is usually marketed for