GRAIN STORAGE

Physical and Chemical Consequences of Advanced Spontaneous Heating in Stored Soybeans

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Spontaneous heating of soybeans in commercial storage has been observed to progress through characteristic biological, chemical, and physical phases. Under unfavorable moisture conditions frequently encountered in commercial grain, heating initiated by mold growth which reaches a maximum of 55° C. is carried forward to higher temperatures by nonbiological oxidation with progressive browning of the seeds. In the final stages of heating which occurs in an oxygen-deficient atmosphere due to physical factors, the grains resemble compressed pellets of oil-soaked coke. Accompanying chemical changes involved alterations in ash, total, reducing, and nonreducing sugars, crude protein, peptizable nitrogen, nonprotein nitrogen, crude fat, peroxide value and iodine value of the oil, free fatty acids, water-soluble acidity, fluorescence and absorbance of aqueous extracts, and urease activity. When such grain or its physical environment is undisturbed, heating to the point of ignition does not occur. Soybeans heated artificially as in a furnace show chemical properties markedly different from those of grain heated by natural spontaneous processes. These observations amplify information gained in previous laboratory studies of spontaneous heating of soybeans and clarify the interactions in commercially stored grain among the environmental, physical, biological, and chemical factors which cause advanced spontaneous heating. It is concluded that the exothermic nature of protein-sugar interactions (Maillard reaction), probably catalyzed by protein–trace metal complexes, is a major cause of spontaneous chemical heating in agricultural materials.

THE INCREASE IN PRODUCTION AND PROCESSING OF SOYBEANS in the United States in recent years has directed attention to problems associated with their commercial storage. Spontaneous heating is being encountered more frequently with this grain than with other seed crops. As losses due to this cause may reach considerable magnitude, scientific information gained from observations of this phenomenon should be made generally available.

In the course of investigations of a number of such commercial losses during several years, the authors have accumulated analytical and other data which appear to clarify this problem considerably. The circumstances involved in two typical cases, and the implications of the data obtained in terms of an integrated theory explaining the events which may lead to the severe deterioration observed, are dealt with in this paper.

Causes of Spontaneous Heating

The so-called "spontaneous" heating of agricultural materials, which occurs readily in stored soybeans, has been widely investigated and speculated upon, most frequently with reference to hay (*6–8, 11–14, 18, 24*). Laboratory studies with grains have been reported more recently (*21, 25, 26, 30, 31, 35, 36*). It is generally agreed that two distinct phases of heating exist, the first initiated by the growth and respiration of microorganisms normally present on all agricultural products. At humidity values between 75 and 95% (or moisture contents in equilibrium with such values) molds, principally of the *Aspergillus* and *Penicillium* species, predominate as causative agents. Under conditions where water activity is not limiting (95 to 100% humidity), bacteria may be the principal thermogenic and respiratory factors. Since commercial grain rarely attains moisture values as high as those in equilibrium with humidities of 95%, bacterial growth is infrequent and mold is thus the major thermogenic agency in the initial heating. The physical bulk of the commercially stored material also plays a vital part in heating, as insulation must be present to permit the accumulation of the heat due to microbiological respiration, within local zones of the grain mass. Under moisture conditions where fungi predominate, the maximum temperature produced by these microorganisms is approximately 55° C., whereas at higher humidities such as may be encountered in hay but not in stored grain, thermophilic bacteria may carry on until their inactivation temperature in the range of 70° to 80° C. is reached. This initial biological phase of spontaneous heating has been clarified by considerable experimental evidence including information on specific microorganisms involved, the nature and extent of the physical and chemical deterioration, as well as the characteristics of the respiratory process (*25–31, 35, 37*).

Less clearly understood is the second or purely chemical phase of spontaneous heating which goes forward after the thermal death of the microflora responsible for the initiation of heating. Although there have been studies of changes occurring in hay when this material is maintained artificially at temperatures beyond the thermal death range of microorganisms (*14*), only recently has self-sustained chemical heating following the biological phase been demonstrated in soybeans (*30*).

These studies indicate that the first heating stage, due primarily to mold respiration, ends when the thermal death range of this biological agency is attained (50° to 55° C.). In this phase the

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species *Aspergillus glaucus* and *Aspergillus flavus* predominate. Normally, seed visability is destroyed at temperature between 45° and 50° C.

The second heating stage, which is apparently already under way by the time the maximum temperature achieved by the molds is reached, and which is accompanied by a progressively increasing rate of gas exchange, accelerates markedly at temperatures higher than those at which the seeds and molds are killed. A plateau in the heating curve between 50° and 55° C. and a reduction in gas exchange at this stage indicate the transition from the biological to the chemical form of heating. At the termination of the experiments, when the temperatures were as high as 101° C., the soybeans were coffee-brown to almost black in color, with no evidence of the mold mycelia which were numerous at early stages of heating. Certain chemical properties were also shown to be characteristic of the various stages.

Spontaneous Heating of Soybeans In Commercial Storage

Only two of several cases involving spontaneous heating in bulk storage (those in which extensive physical and chemical data were obtained) are reported here. However, virtually all cases of this kind are strikingly typical. One of the purposes of these studies was to establish whether spontaneous heating, as previously described, or combustion involving flame, might have caused the deterioration observed.

Case A. In the late summer of 1948 symptoms of spontaneous heating were noted in a concrete bin containing 100,000 bushels of soybeans (56 pounds per bushel) at a soybean processing plant in Illinois. The first warning was the appearance of vapors rising from the top of the bin accompanied by characteristic odors of heating grain. Several days later, soybean oil began to exude through the walls of the bin to such an extent that pools of oil formed around the base on the outside. This indicated that an advanced stage of heating was in progress, although no evidence of flame or glow in the bin was apparent to the elevator personnel. No temperature measurements of the heating grain were taken. When attempts were instituted to remove the grain, only a fraction of it would flow from the unloading spouts at the bottom. As a last resort a large hole was opened in an outside wall of the bin. Large solidified masses of grain were then observed in various stages of heating and decomposition, ranging from apparently undamaged seeds to material which resembled oil-soaked coke (see Figure 1). During salvaging operations an attempt was made to segregate the grain into various stages of deterioration; nevertheless, much intermixing inevitably occurred. However, numerous samples of each truckload of grain removed from the bin were obtained and were shipped to the warehouse of the Underwriters Salvage Co. in Chicago. Here they were inspected by the authors, who selected 128 samples on the basis of widest range of apparent deterioration.

Case B. In the last week of 1950 an employee working above the storage bins in an elevator connected with a soybean processing plant in Kentucky noted an evolution of vapor or smoke from one of the bins. Subsequent investigations, particularly after normal grain was withdrawn from the bottom of the tank, revealed the presence of a congealed steaming column of dark brown to black grain standing in the center of the bin. This mass of tightly packed grain had to be broken up mechanically to permit removal through the unloading spouts.

A few days later other bins in the same elevator began to exude vapors and similar conditions in the grain were found upon unloading, with the uncovering of columns of extremely hot, compressed grain in the center of the bin as the sound grain at the periphery was withdrawn. These hot masses or columns of grain appeared to extend almost the full depth of the bin (90 feet). Fairly accurate temperature recording-were made on the hottest grain by thrusts ing thermocouple leads into the center of the heating mass, and correcting the temperatures recorded for losses due to conduction in the thermocouple probe and the leads. Maximum temperatures in the range of 145° to 170° C. were obtained in this manner.

Examination of records of the commercial characteristics of the grain as received by this elevator revealed that many samples had contained more than 15% moisture when placed in storage approximately 6 to 8 weeks before these events. This value is above that considered safe for the storage of this crop. At the time the bins were emptied, numerous samples, representing all conditions encountered in the bin, were taken and sealed immediately in quart Mason jars. Within a few hours these were placed in freezing storage in a food locker plant, where they were kept until the examination and analyses reported herein were made.

Materials and Methods

In order to re-establish for sampling purposes a cross section of the heating zones in the bins, the 128 samples obtained in Case A were carefully segregated by hand into nine groups on the basis of visual judgment of progressive deterioration, taking into account color, extent of mold growth, intensity of browning, organoleptic properties, etc.

Sample A-1. Normal soybeans.

Sample A-2. Slight browning. All

Table I. Progressive Changes in Composition of Spontaneously Heating Soybeans

(Case A)

Analyses[a]	*Sample No.* 1	2	3	4	5	6	7	8	9
Ash, %	5.2	5.3	5.3	5.8	5.5	5.6	5.6	6.7	6.7
Total sugars, %	9.0	8.8	7.5	2.9	4.4	2.9	2.1	0.5	....
Reducing sugars, %	0.47	0.52	0.77	1.4	2.1	1.3	....	....	....
Nonreducing sugars[b], %	8.2	7.8	6.3	1.4	2.1	1.3	....	....	....
Crude protein, %	41.9	42.5	40.7	43.8	45.6	43.1	43.1	43.8	45.6
Peptizable nitrogen, %	4.1	3.3	0.9	0.4	0.7	0.9	1.1	3.5	3.6
Nonprotein nitrogen, %	0.21	0.26	0.26	0.30	0.47	0.69	0.95	2.50	3.10
Crude fat, %	18.7	19.3	20.3	18.1	21.0	22.2	21.3	24.4	29.9
Peroxide value	4.8	3.5	5.0	19.6	12.4	15.2	29.4	55.5	31.6
Iodine number	130.5	131.9	130.5	128.0	129.5	128.0	128.2	108.0	114.0
Free fatty acids[c], %	0.9	2.0	4.1	9.4	8.9	19.5	31.3	14.9	....
Water-soluble acidity[d]	0.9	0.9	1.4	1.2	2.0	2.3	2.2	1.8	1.1
Absorbance[e],	0.10	0.11	0.13	0.20	0.32	0.52	0.83	1.40	1.54

[a] Moisture-free basis.

[b] As sucrose.

[c] As oleic.

[d] Ml. 0.1N NaOH per gram.

410 mμ.

Table II. Progressive Changes in Composition of Spontaneously Heating Soybeans

(Case B)

Analyses[a]	Sample No. 1	2	3	4	5	6
Ash, %	4.5	4.6	4.5	4.6	4.5	4.7
Total sugars, %	8.4	8.2	6.3	4.5	1.5	0.4
Reducing sugars, %	0.52	0.85	....	1.1	....	....
Nonreducing sugars[b], %	8.2	7.9	....	3.6	....	....
Crude protein, %	43.0	45.5	45.5	45.0	46.0	49.0
Peptizable nitrogen, %	2.8	2.1	0.6	0.9	0.9	....
Nonprotein nitrogen, %	0.15	0.14	0.15	....	0.90	1.50
Crude fat, %	16.8	18.3	16.6	14.2	14.8	13.6
Peroxide value	1.8	1.9	4.1	3.4	6.6	2.4
Iodine number	135	123	128	128	127	113
Free fatty acids[c]	4.1	4.9	6.1	9.4	28.0	16.1
Water-soluble acidity[d]	0.67	0.95	1.4	1.8	2.5	3.0
Absorbance[e]	0.05	0.06	0.14	0.27	0.44	0.68

[a] Moisture-free basis.
[b] As sucrose.
[c] As oleic.
[d] Ml. 0.1N NaOH per gram.
[e] 410 mμ.

beans molded or spotty. Slight musty odor.

Sample A-3. General browning evident. Browning throughout entire bean, not limited to surface. Appearance dull and lifeless, reminiscent of typical food browning. Slight musty odor.

Sample A-4. Similar to A-3 but extremely moldy. Beans matted together by mold mycelia. Shape of individual beans irregular, indicating beans have been deformed by pressure.

Sample A-5. Browning well advanced. First indication of heated odor. Some beans oily. Irregular or faceted in shape. Numerous depressions and cracking.

Sample A-6. Darker than A-5. Heated odor a little stronger and more acrid. Irregularities in shape more pronounced. Some beans oily.

Sample A-7. Decidedly dark brown to black beans. Less oily than either group 5 or 6. Beans generally intact, but a number of fragments present and numerous loose seed coats.

Sample A-8. Darker than group 7. Generally black. All beans badly deformed.

Sample A-9. Similar to 8 but heavily oil-soaked. Strong acrid odor. Some beans essentially intact, but for the most part this sample consisted of heavily oil-soaked, black carbonaceous masses. The badly deformed individual seeds could be distinguished only with difficulty.

A similar classification of seeds from Case B is as follows:

Sample B-1. Normal soybeans.

Sample B-2. Slight browning evident. Seeds darker than B-1. Slight musty odor. Physical shape normal.

Sample B-3. General browning evident. This off-color extends through the entire seed and is not limited to the surface. Beans appear dull and lifeless, typical of normal food browning. Evidence of surface wrinkling and depressions. Slight musty odor; also heated odor.

Sample B-4. Browning well advanced. Heated odor prevails but is not similar to the toasted or burned odors obtained by subjecting normal soybeans to elevated temperatures. Odor more sour in caked masses. Deviation from normal physical condition evidenced by depressions, wrinkling, and cracking of individual beans. No free oil evident.

Sample B-5. Darker than B-4. Odor more acrid. Deformities more frequent.

Sample B-6. Extremely dark brown soybeans, marked heated odor, acrid and sour. Numerous bean fragments.

Segregation of the samples in the manner indicated appeared to be justified on the basis of previous laboratory studies (*30*) which showed that gradation in color of the grain from the normal yellow of sound beans through tan and dark brown and finally to black had occurred as the spontaneous heating proceeded. In no case was ash visible. In later discussions the samples listed from A-1 to A-9 and B-1 to B-6 are referred to as "official" samples in order to distinguish them from damaged grain prepared in the laboratory for comparison.

An additional small series of three samples was obtained in connection with another case of this kind in Missouri in 1952. This series, pictured in Figure 1, consists (from left to right) of normal grain, soybeans in advanced stage of browning, and the final completely black stage. Here, no mixing of the various stages had occurred before sampling.

Analyses were conducted on the samples by the following methods: moisture content (*2*, paragraph 22.3); ash (*2*, paragraph 22.9); total nitrogen (*2*, paragraph 22.10); crude fat (ether extract), (*2*, paragraph 22.24); water-soluble acidity (*2*, paragraph 22.42); sucrose (*2*, paragraph 22.33); reducing sugars (*2*, paragraph 29.35); free fatty acids (*2*, 6th ed., paragraph 31.32 a); iodine number (*2*, paragraph 26.16); nonprotein nitrogen, method of Becker, Milner, and Nagel (*4*); peptizable nitrogen (sodium chloride extraction), method of Jones and Gersdorff (*19*); peroxide value, method of Horne *et al.* (*16*); urease activity, method of Jacobs (*17*). This qualitative method for urease activity was made roughly quantitative by recording the time required for litmus paper to turn blue and ranking samples accordingly.

Fluorescence determinations were made only on the small Missouri series (Figure 1) by a modified procedure used in the laboratory of the senior author in connection with studies of browning phenomena in stored wheat (*9*).

One gram of soybeans ground in a Wiley mill to pass the No. 30 screen is extracted for 1 hour in a 250-ml. Erlenmeyer flask with 50 ml. of 0.2N hydrochloric acid, swirling every 15 minutes. After filtering through Whatman No. 4 paper, 1 ml. of filtrate is diluted with 0.2N hydrochloric acid to 50 ml. in the case of the sound grain, and with as many as 1000 ml. in the case of the black seeds. Fluorescence is determined on an aliquot of diluted extract with the Coleman fluorometer using the B_1-S filter standardized with a 0.1 p.p.m. solution of sodium fluorescein, with the dial set at 60.

A special laboratory test was undertaken to demonstrate the difference in effect between spontaneous heating and the direct application of heat. Number one grade soybeans were placed in an electric furnace. The temperature was adjusted to rise at the rate of 100° C. per hour. Samples were withdrawn from the furnace when temperatures of 200°, 250°, and 300° C. were reached and examined for physical and chemical changes.

Results

An excellent degree of parallelism between the data for various analytical values obtained with the two series of official samples is evident in Tables I and II. Only the trends for crude fat differ.

The relatively slow disappearance of organic material in cases of true spontaneous heating in contrast to the rapid destruction which can be expected with flaming combustion is clearly illustrated in the data on ash content. In both series the ash content increased slowly until the final stages of heating approached, when a moderate increase occurred indicating a somewhat more rapid utilization of the organic components. However, no free ash could be found on even the most deteriorated samples, indicating that combustion temperatures were not attained.

Strong regular decreases in total and nonreducing sugars indicate that these compounds are utilized in the thermogenic process. In the initial phase of heating, reducing sugars increase sharply, indicating preliminary fragmentation of larger carbohydrate molecules, but this trend is reversed when the carbohydrate content is reduced to low levels.

The generally increasing trend for crude protein in the heating grain is probably a reflection of the greater relative stability of this nitrogen-containing compound in contrast to the labile and rapidly utilized carbohydrate materials. A minor reversal in this trend during intermediate stages of heating might be accounted for on the basis of the low respiratory quotient values noted in the laboratory studies (*30*) at temperatures beyond 55° C., indicating a marked uptake in oxygen and little disappearance of weight relative to the dry matter loss to be expected from the carbon dioxide production. Thus a period of relative increase in mass of other labile components of the grain would be reflected in some decrease in the relatively stable component,—i.e., the nitrogen-containing fraction. The data for peptizable nitrogen suggest that in the initial phases of heating, the protein loses solubility owing to heat denaturation, but that this process is reversed when the deterioration reaches a stage where the protein is sufficiently fragmented to result in a new increase in solubility. This interpretation appears to be borne out by the data for nonprotein nitrogen, which show a relatively slow initial rate of increase followed by a rapid acceleration in appearance of the soluble nitrogen. The marked survival of the organic nitrogen in increasingly soluble form may also reflect the utilization of the nonnitrogen portion of the amino acids for the thermogenic process, as the carbohydrates become exhausted.

Table III. Urease Activity of Spontaneously Heating Soybeans

Sample Designation	Case A	Case B
1	* * * * *	* * * * *
2	* * * *	* * * *
3	* * *	* * *
4	* *	*
5	* *	Trace
6	*	Trace
7	Trace	
8	None detected	
9	None detected	
Control, normal soybeans	* * * * *	

Although the trends in crude fat content do not agree in the two series, in the controlled laboratory studies (*30*), a progressive decrease in ether extract occurred without corresponding loss in weight of the seed material. This was taken to indicate that a progressive oxidation and polymerization of the unsaturated fatty acids rendered the fats increasingly less soluble in the fat solvent. The data for the B series thus fit the laboratory findings more closely than do those of the A series. However, the A series attained a more advanced stage of deterioration than did the B series, involving loss of oil from the blackened seeds. This increase in oil content may therefore reflect a reappearance of the oil in more soluble form when extreme stages of heating are attained.

Some corroboration of the laboratory findings appears in the data for the trends in peroxide value and iodine number. In both the A and B series there was a progressive increase in peroxide value of the oil, considerably more pronounced in the A than in the B series, followed at the end by some decrease. Iodine values suggest little initial oxidation of the fats, followed in the final stage by some disappearance of unsaturation. The data for both series indicate that oxidation of the points of unsaturation of fats is a component of the spontaneous heating process.

Hydrolysis of the fats, as reflected in increases in free fatty acids, is a major process which becomes reversed in the advanced stages of heating, corresponding to a similar decline in peroxide value and iodine number. The preliminary increase is doubtless caused by the biological activity responsible for the initial heating, whereas the marked subsequent drop in free fatty acids is due to thermal destruction or volatilization of the fatty acids at the more elevated temperatures.

Increase in water-soluble acidity, probably due to formation of organic acids other than fatty acids in the respiratory processes accompanying biological heating under relatively anaerobic conditions, parallels the increase in the free fatty acids. The decrease in water-soluble acidity in the late stages of heating suggests that, at more elevated temperatures, the organic acids produced by the microorganisms are either decarboxylated or volatilized.

The increasing absorbance (optical density) of aqueous extracts of the soybean samples is a direct reflection of the intense browning associated with spontaneous heating. Similar increases in spectrophotometric density in the ultraviolet region, of extracts of model browning systems containing purified proteins and sugars, have been reported (*32*).

It was frequently observed that the discoloration of the seeds at progressive stages of browning was of much greater intensity in the interior of the seed than at the surface, and that the seed coat might even appear almost normal under such conditions. This is further indication that the browning and heating phenomena occur within the seed itelf rather than in extraneous material in contact with it.

The data for urease activity summarized in Table III indicate that this enzyme survived in seeds showing advanced darkening and deterioration. This observation suggests that the extreme deterioration noted may occur at moderately elevated temperatures where even protein denaturation is incomplete.

A drastic increase in fluorescence associated with spontaneous heating is shown in the data of Table IV. An increase in this characteristic in other food products has been generally attributed to the formation of products of the browning reaction (*9*, *33*).

The physical changes observed during the artificial heating of soybeans in a laboratory furnace were:

200° C. General browning and breaking open of beans. Heavy moisture liberation. Toasted odor, not unpleasant.

250° C. Marked charring. Oil begins to distill from the beans. Burned odor.

300° C. Advanced charring and breakdown of the beans. Heavy oil distillation. Strong burned odor. Charring appeared near completion. Increase in volume of about 1.3 times.

These samples were subjected to the same chemical analyses as the official samples (Table V). The results indicate that the deterioration obtained by direct application of heat to soybeans, as might occur as the consequence of burning down of a storage structure, is radically different from that which occurs at considerably lower temperatures during the relatively slow process of natural spontaneous heating. Total sugars are rapidly and completely destroyed at temperatures below 250° C. Rapid increase in the ash content at temperatures beyond 250° C. indicates gross destruction of all organic constituents. The increase in fat content, which reflects preferential destruction of more labile chemical components, occurs at temperatures beyond 200° C., in contrast to the situation in the official samples (Case A) where some relative increase in fat content appeared from the beginning.

Table IV. Fluorescence of Aqueous Extracts of Soybeans at Various Stages of Deterioration

(Missouri series shown in Figure 1)

Condition of Soybeans	Fluorescence
Sound	4.9
Brown	99
Black	1200

Crude protein content was only slightly affected at temperatures below 200° C., but increased markedly beyond this temperature (as sugars were destroyed). Gross destruction of protein appears beyond 250° C.

The trend for nonprotein nitrogen in the furnace-heated samples differed from that in the spontaneously heating grain. In the latter (Tables I and II) increasingly high levels of this factor appeared at relatively low temperatures in com-

Figure 1. Typical appearance of soybeans at progressive stages of advanced spontaneous heating

From left to right, dishes contain unheated sound beans, beans in a brown stage, and beans in the final black stage of heating. Note characteristic faceted condition of heated beans caused by elimination of the interseed air space following plastic deformation of the hot seeds.

parison with the moderate increase in the values for furnace-heated grain at high temperature (250° C.), which was followed by marked destruction. Peptizable nitrogen similarly was destroyed by temperatures beyond 250° C., a characteristic of furnace-heated grain which had no counterpart in the spontaneously heated grain.

The trends for water-soluble acidity, free fatty acids, and peroxide value are strikingly different from those in naturally heated samples, which suggests that these characteristics would serve to differentiate these two types of heated grain. The marked drop in iodine value and absorbance of the furnace-heated samples beyond 250° C. indicates that gross destruction of fats and sugar-protein browning products occurs at these temperatures, which was not a characteristic of spontaneously heated grain.

Obviously, the destruction of soybeans due to heating in a furnace from room temperature to 300° C. in 2.5 hours was greater than that which occurred during the entire prolonged process of spontaneous heating under natural conditions.

Discussion and Conclusions

The systematic changes in numerous chemical factors determined in this study indicate that the ranking of the samples of salvaged grain by physical, visual, and organoleptic characteristics did indeed represent progressive stages of deterioration.

The great sensitivity of soybeans to chemical deterioration is indicated by the drastic changes which can occur at relatively low temperatures. In previous laboratory studies (*28*) it was observed that at temperatures as low as 38° C. discoloration of the seeds can occur if exposure to this temperature is prolonged for several days. Soybeans can heat spontaneously even without preliminary intervention of mold growth, from temperatures as low as 54° C. (*30*), and by the time this temperature is attained through normal means involving preliminary activity of fungi, the beans are already brown. The commercial samples examined in this study retained some urease activity into advanced stages of physical deterioration and darkening. This indicates that when the exposure is prolonged, marked deterioration can occur at temperatures below that at which proteins are heat-denatured. In any event it is clear that the maximum deterioration observed in these commercial samples, where the grain resembled oil-soaked coke, occurred at temperatures well below that at which ignition can occur.

Browne (*7*) has suggested that ignition of spontaneously heating hay cannot occur until a temperature of 230° C. is reached. The physical conditions surrounding a heating bulk of soybeans are considerably different from those in hay, inasmuch as interseed air spaces have been eliminated at previous stages of the process by plastic deformation of the warm seeds by grain pressures in the bin. The diffusion or convection of air through the hot grain is, therefore, markedly reduced, and thus the heating process at advanced stages is slow and limited, because only small quantities of air can reach the heating zone. As long as such conditions are maintained, heating in a bin to the point of ignition of initially normal soybeans would probably be impossible. Indeed, it has been observed that grain which has reached this advanced stage, if undisturbed, will eventually cool off. One observer has reported that upon close examination of the hot grain masses being removed from a bin in a case of this kind, he noted that the hottest grain was dark brown to chocolate in color, rather than black. It would appear that the black oil-soaked material represents a final stage of the deterioration in which the labile chemical components necessary for heat production have been virtually exhausted. A marked resemblance is to be noted between these theories and observations and those which are generally accepted concerning the formation of deposits of peat and coal (*5*).

Ignition could occur, it would appear, only if sufficient air is brought into contact with the heating grain before the final black stage of decomposition. To accomplish this in an ordinary grain bin, while maintaining sufficient insulation to allow combustion to propagate, would be virtually impossible under practical conditions. However, it is conceivable that grain aerated as in a salvage operation and containing all stages of deterioration associated with spontaneous heating, when returned to a bin in sufficient bulk to provide insulation, could again heat up rapidly, even to the point of ignition. A belief prevalent in the trade is that foreign material, such as bean pods and stems normally present in commercial soybeans, is more sensitive to ignition than the beans themselves. An extreme sensitivity of defatted, heat-treated soybean meal to spontaneous ignition has been observed (*10*).

Cyclic variations in climatic temperature appear to be important factors in promoting heating in commercial grain.

Table V. Progressive Changes in Composition of Soybeans Heated Artificially in a Laboratory Furnace

	Treatment			
Analyses[a]	Untreated	200° C.	250° C.	300° C.
Total sugars, %	8.5	7.5	[b]	[b]
Ash, %	4.9	5.6	5.7	8.1
Crude fat, %	19.7	20.2	21.1	24.2
Crude protein, %	40.7	41.9	46.3	46.9
Nonprotein nitrogen, %	0.13	0.18	1.1	0.32
Peptizable nitrogen, %	3.4	0.6	1.5	0.5
Water-soluble acidity[c]	0.59	0.60	0.91	[b]
Free fatty acids[d]	1.0	1.4	2.0	...
Peroxide value	0.7	1.3	1.8	1.2
Iodine number	130.0	129.5	128.5	70.0
Absorbance[e]	0.08	0.19	1.13	0.36

[a] Moisture-free basis.
[b] None could be determined.
[c] Ml. 0.1N NaOH per gram.
[d] As oleic.
[e] 410 mμ.

The role of seasonal changes in temperature in the initiation of spoilage in soybeans stored in farm bins has been analyzed by Holman (*15*). The effect of atmospheric temperature on grain in terms of rate of cooling of the grain bulk has been studied by Oxley (*34*) and Babbitt (*3*), while Kiesel *et al.* (*20*) and Anderson *et al.* (*1*) have clarified the mechanism whereby temperature differentials can produce moisture increases in localized areas of grain bulks. The migration of respiratory carbon dioxide and the propagation of temperature increases from a heating zone in stored soybeans have been observed by Milner and Geddes (*27*).

On the basis of information from the literature and the present observations, it is theorized that the following series of events may occur in grain stored in large quantities in vertical concrete silos, under conditions prevailing in the midwestern United States. Soybeans are generally harvested and placed in storage at a season of the year (October and November) when atmospheric temperatures are falling. Thus at almost any time in this period the average temperature of the grain bulk is higher than that of the surrounding atmosphere, except at points where it may come into close contact with the outside atmosphere: walls, girders, and the open top of the grain load. A gradient of increasing temperature is thus set up in grain bulk, not only from the top surface downward but also from the walls inward. Grains are good insulators and the temperature gradient existing between the warm grain in the central areas of the bulk and that at the periphery may persist for long periods of time, dissipating only slowly.

Within the interseed spaces of grain stored in bulk, convection currents of air are produced by these natural temperature differentials. A rising column of air appears in the intergranular spaces which in the case of soybeans amounts to about 45% of the grain bulk. There is evidence that this upward air movement causes cooler outside air to be drawn in at the bottom of the bin through and around the slide gate in the emptying spout at the bottom. The warm air moves as a continuous column at a rate depending on the temperature gradient existing between the warm grain and the cool outside air, as well as on the rate at which cooler air replaces the rising warm air at the bottom. This air movement can conceivably occur also in a cyclical fashion within the bin.

As warm air approaches cool grain at the top of the load, the temperature reduction causes a sharp increase in the relative humidity and thus two new factors appear. The cool grain near the top begins to absorb water from this moisture-laden air either as vapor or even as free water forming by condensation if the temperature drop is to the dew point. In any event, as soon as the relative humidity of the interseed air exceeds 75%, mold spores which are always present on the grain as well as within the seed coats will germinate and grow. This leads to a rapid matting together of the grain at or immediately below the surface, and vigorous heating due to mold respiration. Inserting a hand into such grain at the top of a bin discloses at once that such grain is hot (as high as 50° C.), even at a depth as little as 6 inches below the surface. This hot so-called "crust" was observed on several bins in Case B. In the case of many grains this may constitute the maximum deterioration due to moisture translocation, but another damaging development also may occur, particularly with soybeans.

Apparently the cold air which is drawn up into the center of the warm grain from openings in the bottom of the bin results in the formation of a column of grain of lower average temperature than that of the remaining grain mass. Moisture vapor migration from the interseed air in the warmer surrounding bulk to this cold area then commences and within a relatively short time (depending on the temperature differences prevailing), the moisture content of the cooler initially dry grain can reach levels in excess of that critical for mold growth. With the propagation of fungal growth and with the excellent conditions of insulation prevailing, the temperature of the column of grain previously below that of the grain bulk rises rapidly and soon exceeds that of the bulk of the grain surrounding it. This phenomenon of moisture translocation due to temperature differentials is doubtless the cause of the inception of heating of grain whose average initial moisture value is too low to support active fungal growth. This state of affairs may be prevented in commercial storage by turning grain periodically as cold weather approaches, thus preventing the formation of temperature differentials. With a rising seasonal temperature trend, however, such cool grain need not be disturbed, since low temperature with its preservative effect will remain in the bulk for a considerable time. However, a definite hazard exists in turning cold grain in warm humid weather, since if the grain is at a temperature below the dew point of the atmosphere actual condensation of water on the grain will occur. Obviously, when a bulk of grain is placed in storage which either as a whole or in localized areas contains moisture values above that critical for mold growth, fungal growth with consequent heating can be initiated and spread from any point in the moist grain, and in any season. Good storage practice requires such damp grain to be dried to safe moisture levels prior to storage and to be turned thereafter in the manner and for the reason indicated for normal, dry grain.

In cereal grains (wheat and corn) the thermogenic effect of fungal respiration is virtually the only factor contributing to spontaneous heating (*26*) and thus in such grains temperature proceeds above the thermal death point of the fungi (55° C.) only with difficulty. [Isolated wheat germ, however, would probably undergo spontaneous chemical heating readily (*22*).] Soybeans, on the other hand, appear to be an exception among the grains in this regard, since the second or chemical stage of heating leading to high temperatures and advanced deterioration occurs readily. Formation of the hot, solidified core of brown to black grain in soybean bins appears to be a special characteristic of this seed, because the second purely chemical phase of heating is sustained easily beyond the highest temperatures achieved by the fungi. Thus biological respiration and heating are replaced rapidly by self-propagating chemical oxidation. It appears that such a heating core can appear as previously described within a few weeks after the bin is filled, particularly if a strong cooling trend in the weather exists.

With the initial rise in temperature due to fungal respiration and chemical oxidation, the individual beans become soft and plastic. The great pressures which exist within grain bulks cause these heat-softened grains in heating zones to deform and become compressed, with the virtual elimination of the intergranular air space originally constituting about 45% of the grain bulk. The chemical heating is therefore retarded to a rate regulated by the permeability of oxygen into the mass.

The active exothermic reaction which prevails in the postbiological heating phase appears to be due to nonenzymatic browning of the type originally postulated by Maillard (*23*), fluorometric and colorimetric evidence for which was obtained in this study. The soybean is an ideal medium for propagating this reaction, since it contains abundant quantities of proteins and sugars which are source materials for browning. Oxidation of reductones formed by this mechanism is probably a major factor in nonbiological heat production. Furthermore, in contrast to cereal grains, soybeans contain a relatively high concentration of those trace metals which probably catalyze the browning reaction. Analyses by one of the authors (J. B. T.) of more than 100 samples of soy flour has established a range of copper content of 15 to 24 p.p.m. (mean 19 p.p.m.). Analyses conducted on a few of the same samples indicates that the iron, manganese, and nickel contents also may be of a relatively high order of concentration (iron 80 to 100 p.p.m., manganese 29 to 35 p.p.m., nickel 8 to 11 p.p.m.).

It has been established that copper enters the nonenzymatic browning reaction and presumably other metals may also. In the case of copper a probable complexing reaction involving proteins has been suggested (*39*). Certain of the copper complexes so formed have been studied, and all possess marked browning characteristics such as color and fluorescence. Furthermore, it has been shown that the browned copper complexes once formed will catalyze a general browning (*38*).

Isolated wheat germ which is similarly high in sugar, protein, and minerals also browns readily owing to a typical Maillard reaction (*22*). It appears to the authors that this reaction is one of basic significance which has heretofore been overlooked as a cause of spontaneous heating in nearly all stored agricultural crops.

Data for the respiratory quotient of gas exchange involved in such deterioration which have been recorded in previous studies (*30*) show that marked differences exist between the carbon dioxide–oxygen ratio of biological activity and the gas exchange due to the chemical heating accompanying browning. The growth of fungi is associated with respiratory quotient values approaching unity, whereas the chemical oxidation is characterized by carbon dioxide–oxygen ratios of 0.5 or less and, in later stages of advanced heating, values well beyond unity may appear.

Investigation of the exothermic interactions among trace metals, proteins, sugars, and oxygen at elevated temperatures should provide important information concerning this browning phenomenon. The mode of production and nature of the pyrophors in spontaneously heating agricultural materials which Browne (*6, 7*) and Miehe (*24*) sought to explain by as yet unsupported hypotheses may well be found in the materials produced by these metal-catalyzed browning reactions.

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