

Table III. Average Grain Yield, Grain Protein Percent, and Grain Protein Production of NB 65317 and Triumph 64

	NB 65317	Triumph 64	LSD _{0.05}
Yield, kg/ha			
67 kg N/ha	1851	2661	
134 kg N/ha	3171	2751	
Average	2511	2706	311
Grain protein percent			
67 kg N/ha	19.5	12.8	
134 kg N/ha	19.0	15.6	
Average	19.3	14.2	1.8
Grain protein, kg/ha			
67 kg N/ha	361	341	
134 kg N/ha	602	429	
Average	481	385	58

for differences in nitrogen reduction. In contrast, the grain protein was greater in the high protein line, which is consistent with the higher protease after heading in the high protein line.

LITERATURE CITED

- Beevers, L., *Phytochemistry* **7**, 1837 (1968).
 Beevers, L., Hageman, R. H., *Annu. Rev. Plant Physiol.* **19**, 495 (1969).
 Croy, L. I., Hageman, R. H., *Crop Sci.* **10**, 280 (1970).
 Duffield, R. D., Master's Thesis, Oklahoma State University, Stillwater, Okla., 1971.
 Hageman, R. H., Flesher, D., Gitter, A., *Crop Sci.* **1**, 201 (1961).
 Haunold, A., Johnson, V. A., Schmidt, J. W., *Agron. J.* **54**, 121 (1962).
 Johnson, V. A., Schmidt, J. W., Mattern, P. J., *Econ. Bot.* **22**, 16 (1968).
 Lowry, O. H., Roseborough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 257 (1951).
 Middleton, G. K., Bode, E. C., Bales, B. B., *Agron. J.* **46**, 500 (1954).
 Mounfield, J. D., *Biochem. J.* **30**, 1778 (1936).
 Rao, S. C., Croy, L. I., *Crop Sci.* **11**, 790 (1971).
 Schlehuter, A. M., Tucker, B. B., *Cereal Sci. Today* **4**, 240 (1959).
 Seth, J., Hebert, T. T., Middleton, G. K., *Agron. J.* **52**, 207 (1960).
 Welch, L. F., Johnson, R. F., Pendleton, J. W., Miller, L. B., *Agron. J.* **58**, 271 (1966).
 Woolley, J. T., Hicks, G. P., Hageman, R. H., *J. Agr. Food Chem.* **8**, 481 (1960).

Received for review January 28, 1972. Accepted June 22, 1972.
 Journal Article 2402 of the Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma 74074.

Reactions of Heated Milk and Amino Sugars with *p*-Dimethylaminobenzaldehyde

Surinder Kumar and Poul M. T. Hansen*

Milk heated at sterilizing temperatures developed chromogens producing a red color upon reaction with *p*-dimethylaminobenzaldehyde (*p*-DMAB) in a reaction mixture of formic acid and chloroform. Results for heated milk indicated that the *p*-DMAB reactivity was proportional to the heating time for temperatures in the range 100–120°. Precursors for the reaction were identified as predominantly *N*-acetylhexosamines on the basis of the specificity of the reaction and by the absorption spectra of the

chromophores. Products formed by nonenzymatic browning were also reactive, but their contribution to the total *p*-DMAB reactivity was small. The principal chromogens formed in heated milk may be the mono- and dianhydro derivatives of *N*-acetylhexosamines. A secondary reaction with *p*-DMAB occurred when milk was kept in contact with the reaction mixture for several hours, but this reaction was not heat induced and did not appear to involve amino sugars.

Heat processing of milk and other fluid food products leads to complex changes and interactions among the constituents. During a study of heat effects on milk at elevated temperatures (Hansen, 1967), the observation was made that the product, after heat sterilization, developed a capacity for reacting with *p*-dimethylaminobenzaldehyde (*p*-DMAB). The reaction resulted in a pink coloration similar to the color produced by certain amino sugars following heating under alkaline conditions (Morgan and Elson, 1934). Although *p*-DMAB is commonly used for detecting amino sugars, the reagent is nonspecific since it produces color with a number of components, including pyrroles, phenols, many indole derivatives, and sialic acids. However, the solvent for *p*-DMAB and the conditions of reaction may be selected to permit the use of *p*-DMAB as a diagnostic tool (Kent and Whitehouse, 1955).

The purpose of this paper is to present a method for measuring the *p*-DMAB reactivity of heated milk and to identify the components responsible for this heat-induced change.

EXPERIMENTAL SECTION

Materials. *N*-Acetylglucosamine, *N*-acetylgalactosamine, and *N*-acetylmannosamine were obtained from Calbiochem and formic acid and chloroform (A. R.) were obtained from Baker Chemical Company. The buffer (pH 6.8) was the lactose-free, synthetic milk salt system of Jenness and Koops (1962). This buffer was selected due to its similarity with the salt system of milk. Sterile milk dialysate was prepared by the method of Koka and Mikolajcik (1967) with frequent transfers over several days of the dialysis bags into fresh milk in order to approach equilibrium of dialyzable components with the intact milk system. This system was considered to be approximately equal in composition to the native, protein-free milk system described by Murphy and Whitney (1956). Milk ultrafiltrate was prepared using the LKB suction-ultrafiltration support, Model 6301 A, equipped with Visking No 32 dialysis tubing.

***p*-DMAB REAGENT.** *p*-Dimethylaminobenzaldehyde was obtained from Matheson Coleman and Bell, and a 4% (w/v) solution was prepared in chloroform and stored in amber bottles.

Preparation of Samples. Reconstituted skim milk (9.1%)

Department of Food Science and Nutrition, The Ohio State University, Columbus, Ohio 43210.

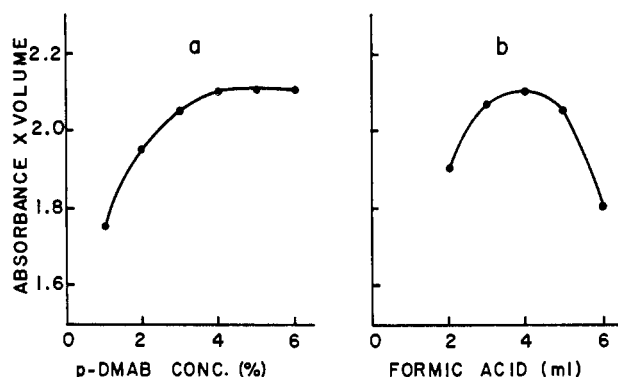


Figure 1. Effect of (a) concentration of *p*-dimethylaminobenzaldehyde and (b) volume of formic acid on the *p*-DMAB reactivity of a milk sample heated at 115° for 15 min

was prepared from low-heat, spray-dried skim milk powder and used throughout the study in order to maintain samples of uniform composition. For model studies, individual *N*-acetylhexosamines were dissolved in the simulated milk salt system to concentrations of 70 $\mu\text{g}/\text{ml}$. The prepared samples were distributed in ampoules with a 5-ml capacity. Precautions were taken to avoid contact of the solution with the neck of the ampoule. The open tips of the ampoules were heat-sealed by flame. The samples were then heated at selected temperatures for specified time intervals. During heating, the ampoules were kept completely immersed in the constant temperature baths. The temperature of heating was controlled within $\pm 0.5^\circ$. Cooling was effected by transferring the ampoules immediately to ice water after completion of heating. The time required for temperature equilibrium of the ampoules was 10 sec as determined with Thermo-melt pellets of various melting points (Markal Company, Chicago, Ill.).

The *p*-DMAB Reactivity Test. Two milliliters of the *p*-DMAB solution and 4 ml of formic acid were accurately measured into a screw-cap test tube. One milliliter of the heated sample was added slowly over the reaction mixture. The tubes were capped and the contents were completely mixed and incubated at $37 \pm 1^\circ$ for 20 min. At the end of the incubation period the tubes were cooled in ice water ($2-4^\circ$) for 10 min and centrifuged at $1000 \times g$ for 5 min. The clear supernatant was withdrawn and warmed to room temperature, and the absorbance was measured against a blank prepared from unheated milk within 15 min at 545 nm, using 10-mm square cuvettes.

Absorption Spectra. The clear supernatants from the *p*-DMAB test were scanned in a Coleman 124, double-beam spectrophotometer equipped with a Coleman Model 56 recorder using 10-mm square cuvettes.

Reducing Capacity. The development of reducing substances in heated milk and amino sugars was measured by a modified ferricyanide method (Crowe *et al.*, 1948) using a sample size of 2 ml.

RESULTS

The basis for the *p*-DMAB reactivity test was the observation that strongly heated milk contained substances which gave positive reaction for amino sugars by the Elson and Morgan (1933) and Morgan and Elson (1934) procedures without prior treatment with alkali or condensing reagents. The possible involvement of native or heat-induced amino sugars was suspected but spectrophotometric examination was precluded because the reaction mixtures obtained in these

Table I. The Effect of Varying the Incubation Time on the *p*-DMAB Reactivity of Heated Milk (Heated at 115° for 15 Min)

Incubation time at 37°, min	Cooling time, min	Time for centrifugation, min	Holding time, min	Total time, min	Absorbance at 545 nm, min
0	10	5	15	30	0.287
10	10	5	15	40	0.447
20	10	5	15	50	0.451
30	10	5	15	60	0.444
40	10	5	15	70	0.436
50	10	5	15	80	0.424

tests were generally turbid when milk samples were used. Although high-speed centrifugation did clarify the aqueous phase, this approach was abandoned because the sediment was observed to contain some of the color. Various modifications, including the methods of Aminoff *et al.* (1952) and Reissig *et al.* (1955), were also found to be unsuitable for milk.

Preliminary experimentation revealed that the turbidity was caused by milk fat and coagulated milk proteins, and that a combination of formic acid and chloroform could be used as a solvent mixture. These reagents, when mixed with milk, formed a binary mixture from which a clear supernatant, free of turbidity, could be obtained by centrifugation at low temperatures. The small amount of insoluble material settling at the interphase did not appear to bind the red color.

Optimum Reaction Conditions. The effects of variable factors in the *p*-DMAB reactivity test were studied to obtain a set of conditions that would yield maximum color intensity. Figure 1 shows the *p*-DMAB reactivity of a heated milk sample as influenced by variations in the reagents. For studying the effect of *p*-DMAB concentration (Figure 1a), the volumes of chloroform and formic acid were kept constant at 2 and 4 ml, respectively. A plateau in the curve for the color intensity was attained at a concentration of 4% (w/v) *p*-DMAB in chloroform. Similarly, from Figure 1b, it is evident that within the test conditions 4 ml of formic acid in the reaction mixture produced maximum color.

The effect of incubation conditions of the reaction mixture on the *p*-DMAB reactivity is shown in Table I. From the absorbance values, it appears that a variation in incubation time of ± 10 min could be tolerated without appreciable errors in the results. For the *p*-DMAB reactivity test, an incubation period of 20 min and a total span of 40–60 min for the entire test was found to be satisfactory, since the variation in this range was small. Beyond an incubation time of 30 min, slow fading of the color was observed. However, upon prolonged incubation (several hours), the color intensified and developed even in unheated samples. This secondary reaction should not be confused with the heat-induced *p*-DMAB reactivity.

Compounds Responsible for the *p*-DMAB Reactivity. The developed test was applied to milk and to milk fractions which had been exposed to heat treatment either before separation into fractions or immediately thereafter. The results in Table II show that the intact milk system possessed a greater reactivity than any of the subfractions. The data suggested that the responsible factors may be associated to an appreciable degree with the casein micelles which are removed by ultracentrifugation and to a lesser degree with the fat globules and the whey proteins removed by cream separation and ultrafiltration, respectively. Evidently, heating did not alter the distribution of the chromogens or their precursors in the

Table II. Distribution of *p*-DMAB Reactivity^a between Various Fractions of Milk

Fraction	Unheated	Heated 100°/60 min	
		After fractionation	Before fractionation
		Absorbance at 545 nm	
Whole milk	0.090	...	0.355
Skim milk	0.085	0.335	0.340
Ultracentrifuge serum ^b	0.020	0.185	0.170
Ultrafiltrate	<0.015	0.140	0.100
Milk dialysate ^c	<0.015	0.150	...
Dialyzed milk ^d	<0.095	0.135	...

^a Measurements made relative to reagent blank using water in place of sample. ^b Prepared by centrifugation at 40,000 rpm for 30 min in a refrigerated preparative ultracentrifuge. ^c A native, protein-free milk system prepared by dialysis of water against fresh milk (Murphy and Whitney, 1956). ^d Exhaustively dialyzed against a synthetic milk salt solution (Jenness and Koops, 1962).

Table III. *p*-DMAB Reactivity^a of Milk Proteins in Phosphate Buffer at pH 6.8

Component	Before heating	Heated at 100°/60 min
	Absorbance at 545 nm	
α_s -Casein (1%)	0.070	0.075
β -Casein (1%)	<0.015	<0.020
κ -Casein (1%)	<0.015	<0.020
β -Lactoglobulin (0.5%)	<0.015	<0.020

^a Measurement made relative to reagent blank using buffer in place of sample.

fractions studied. The *p*-DMAB reactivity of protein-free milk ultrafiltrate and milk dialysate may be taken as evidence that the substances involved are in part soluble and dialyzable and that proteins are not required for the formation of chromogens. Some residual *p*-DMAB reactivity (about 16%) remained in milk after prolonged dialysis, suggesting once again that some of the precursors may be firmly associated with the proteins.

Relatively high absorbance values were obtained for unheated milk and fractions containing casein but not for other samples. The data in Table III show that α_s -casein alone contains a factor which is capable of producing color with *p*-DMAB without prior heating. The nature of this substance remains obscure because we have found that none of the common protein constituents, including amino acids and amino sugars, possess significant *p*-DMAB reactivity under these conditions.

The absorption spectra of the colored supernatant from milk and reference compounds were examined (Figure 2). The spectrum for heated milk exhibited characteristic absorption peaks at 545 and 584 nm and a shoulder around 510 nm. The absorbance below 450 nm was excessive and no comparisons were made in this region. Solutions of *N*-acetylglucosamine, *N*-acetylgalactosamine, and *N*-acetylmannosamine were observed to react with *p*-DMAB after heating at elevated temperatures and producing absorption spectra nearly identical to that by milk. *N*-Acetylneuraminic acid, hexosamines, and tryptophan failed to react with the *p*-DMAB under the conditions of the test. This is noteworthy because these components are capable of reacting with *p*-DMAB under altered reaction conditions (Elson and Morgan, 1933; Mauzerall and Granick, 1956; Werner and Odin, 1952).

Since some of the products resulting from nonenzymatic browning are known to react with *p*-DMAB (Aminoff *et al.*, 1952; Gottschalk and Partridge, 1950), an attempt was made to evaluate the extent of contribution by these products in the

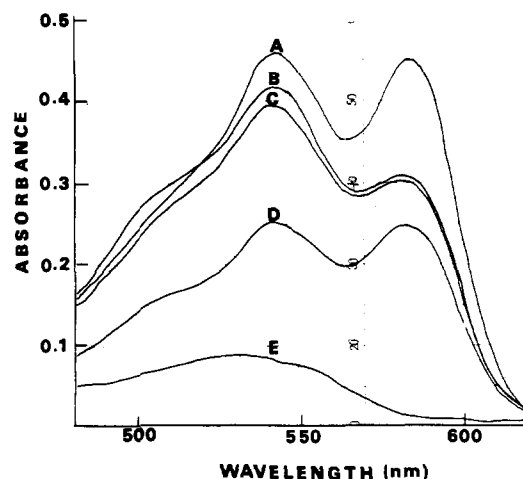


Figure 2. *p*-DMAB absorption spectra of milk and reference compounds. (A) *N*-Acetylglucosamine in a milk salt system (16 mg/100 ml); (B) Skim milk; (C) Whole milk; (D) *N*-Acetylgalactosamine in a milk salt system (16 mg/100 ml); and (E) Casein (3%) and lactose (5%). All samples were heated at 120°/15 min and the color was developed according to the *p*-DMAB reactivity test. The spectrum for *N*-acetylmannosamine overlapped the one for *N*-acetylglucosamine

overall *p*-DMAB reactivity of heated milk. A model solution containing 5% lactose and 3% sodium caseinate in phosphate buffer at pH 6.8 was heated at 120° for 15 min and reacted with *p*-DMAB under the conditions of the test. A positive *p*-DMAB reaction was observed but the resulting absorption spectrum was distinctly different, exhibiting a single broad peak with maximum at 520 nm. This type of spectrum was also obtained when lactose was heated in the presence of lysine or alanine. Comparison of the absorbance values in Figure 2 revealed that the contribution by the caseinate-lactose interaction was approximately 1/3 of the total *p*-DMAB reactivity of heated milk at 545 nm, but was insignificant at 584 nm. Thus, milk contains, or may develop upon heating, several components that are potentially capable of reacting with *p*-DMAB. However, specific conditions determine which particular components take part in the reaction. Under the conditions used in this study, it appears that *N*-acetylhexosamines in milk or closely related compounds, such as *N*-acetylglucosamine or possibly phosphorylated derivatives (Hoff, 1963) of *N*-acetylhexosamines, are the major contributors to the *p*-DMAB reactivity of heated milk. The *p*-DMAB reactive substances arising as a result of nonenzymatic browning contribute substantially at 545 nm and are presumably the cause of the nonidentical 584/545 peak ratios for milk and for *N*-acetylhexosamines which are apparent in Figure 2.

Protein-bound *N*-acetylhexosamines, such as those in κ -casein (Jollès, 1966), apparently do not participate in the reaction because heating of κ -casein (Table III) produced no measurable effect. Similarly, polysaccharide-bound amino sugars in hyaluronic acid and in chondroitin sulfate did not possess any *p*-DMAB reactivity upon heating.

The results in Table IV show that heating under identical conditions of milk and of a solution of *N*-acetylglucosamine of a selected concentration leads to nearly identical *p*-DMAB reactivity, which supports our contention that it is the *N*-acetylhexosamines in milk which are the target for the heat-induced change. The amount of 15 mg of *N*-acetylglucosamine per 100 ml of milk is in substantial agreement with the values reported by Hoff (1963). However, the severe heating conditions necessary to induce the *p*-DMAB reactivity also

Table IV. *p*-DMAB Reactivity and Ferricyanide Reducing Values for Heated Milk and *N*-Acetylglucosamine^a at pH 6.8

Heat treatment at 120°, min	<i>p</i> -DMAB reactivity		Ferricyanide ^b reducing value	
	Milk	NAGL	Milk	NAGL
	Absorbance at 545 nm		Absorbance at 660 nm	
0	0.000	0.000	0.000	No measurable absorbance
10	0.370	0.335	0.250	
15	0.485	0.500	0.400	
20	0.620	0.650	0.640	
25	0.745	0.775	0.790	
30	0.885	0.840	0.870	

^a Concentration 15 mg/100 ml in a milk salt system. ^b The values were corrected for the reducing value of the unheated sample.

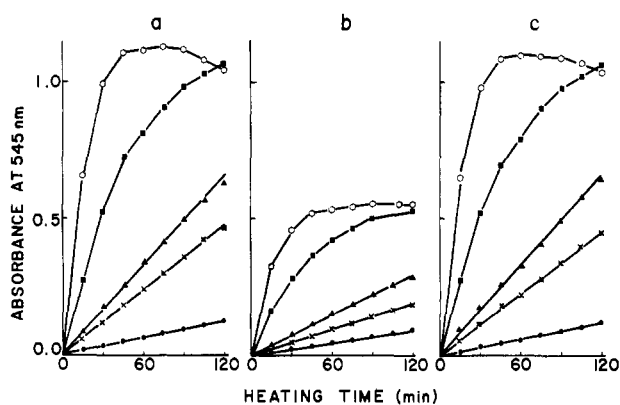


Figure 3. Effect of heat treatment on the *p*-DMAB reactivity of heated solutions of (a) *N*-acetylglucosamine, (b) *N*-acetylgalactosamine, and (c) *N*-acetylmannosamine. The temperatures of heating were 100° (●—●), 110° (×—×), 120° (▲—▲), 130° (■—■), and 140° (○—○)

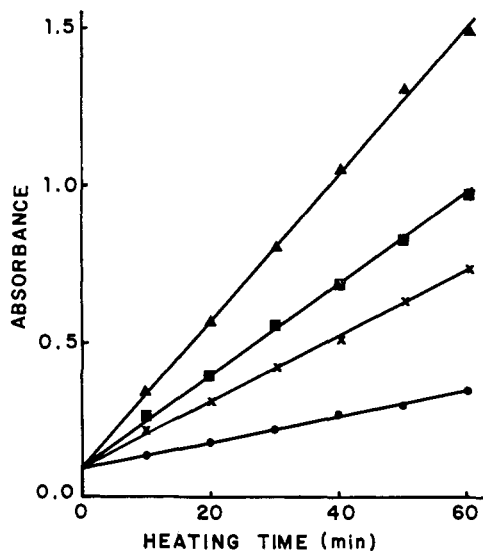


Figure 4. Effect of heat treatment on the *p*-DMAB reactivity of skim milk. ●—●, 100°; ×—×, 110°; ■—■, 115°; and ▲—▲, 120°

cause the accumulation of reducing substances (Chapman and McFarlane, 1945) and this reaction apparently closely parallels the *p*-DMAB reactivity of milk, although not of *N*-acetylhexosamines. Crowe *et al.* (1948) have reported that the reducing capacity of milk arises principally from thiol groups in proteins, from ascorbic acid, and from browning

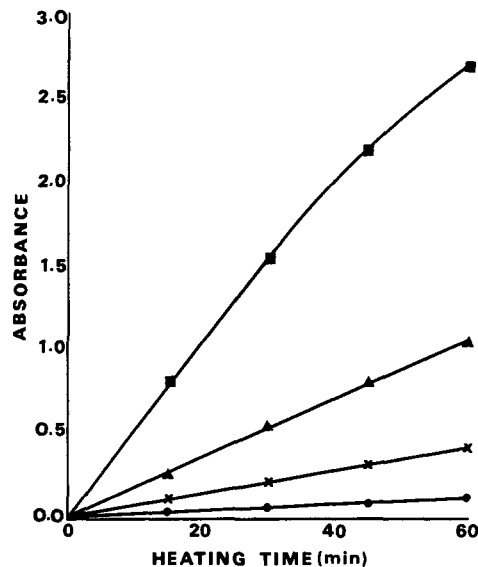


Figure 5. Effect of heat treatment of the *p*-DMAB reactivity of milk dialysate. ●—●, 100°; ×—×, 110°; ▲—▲, 120°; ■—■, 130°

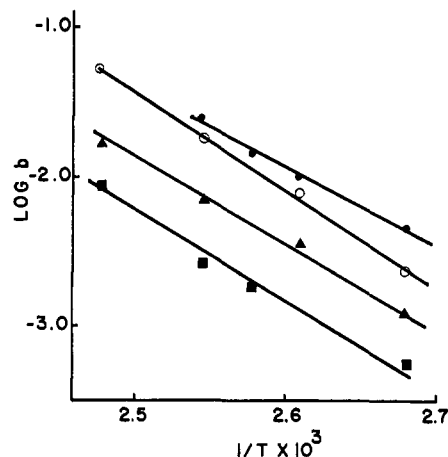


Figure 6. Temperature dependence of the rate of change of *p*-DMAB reactivity of: (●—●) whole milk, (○—○) milk dialysate, (▲—▲) *N*-acetylglucosamine and *N*-acetylmannosamine, and (■—■) *N*-acetylgalactosamine. (Regression analysis revealed that the slopes were not significantly different, $p > 0.05$)

reaction products. Evidently, the heat-induced changes in *N*-acetylhexosamines do not contribute to the formation of reductones.

Time-Temperature Effects. Model solutions of *N*-acetylhexosamines in the milk salt system were subjected to various heat treatment, and the *p*-DMAB reactivity of the heated samples was determined. In Figure 3 are shown the progressive heat conversions of these amino sugars. In all three cases, a linear relationship was observed between the *p*-DMAB reactivities and the time of heating, except for the heat treatments above 140° for 45 min. It should be noted that *p*-DMAB reactivity for *N*-acetylgalactosamine was considerably lower than for the other two isomers, although not as low as in the usual reaction mixture employed in the Morgan-Elson procedure (Roseman and Daffner, 1956).

Figure 4 shows the influence of heating of milk on the *p*-DMAB reactivity. For different temperatures of heating the reactivity increased linearly with time. Similar curves were obtained in Figure 5 in the case of protein-free milk dialysate, indicating once again that proteins are not primarily involved

Table V. Effect of pH on the *p*-DMAB Reaction of Milk Heated at 120°

pH (adjusted with 1 N NaOH or HCl)	$\frac{\Delta \text{ABS}}{\Delta t \text{ (min)}} \times 100$
6.3	3.30
6.5	3.55
6.7	3.63
6.9	3.94

in this heat-induced reaction. The rate of change for milk and for the three amino sugars, as determined from the linear portion of the curves (0–30 min), was found to have a logarithmic relationship with time (Figure 6). The nearly parallel lines suggest similar activation energies for the *p*-DMAB reactivity of the different systems.

RELATIONSHIP TO THE MORGAN-ELSON REACTION. The *p*-DMAB reactivity of *N*-acetylhexosamines in the classical Morgan-Elson procedure is dependent upon a pretreatment of the amino sugars in alkali. However, alkaline conditions are not strictly necessary for the formation of the chromogenic substances; but at the lower pH the rate of the reaction is considerably retarded. The reaction, which at pH 9 is brought to completion within minutes, requires hours of heating at physiological pH. Table V shows the effect of pH variations on the rate of the chromogen formation in milk as determined from appropriate regression equations.

The question arises as to whether or not the present *p*-DMAB reactivity test produces the same chromogens as those reported for the Morgan-Elson reaction (Horton, 1969). Comparison of the spectra in Figure 7 indicates the chromogens may be identical. The similarity in the overall profile is evident and the difference in the relative intensities of the two maxima at 545 and 584 nm appears to be a result of the selection of reaction mixture rather than conditions of pretreatment. On this basis, we suggest that the principal chromogens produced in heated milk are the same furan derivatives as formed in the Morgan-Elson reaction. These include mono- and dianhydro derivatives of *N*-acetylglucosamine, containing one and two double bonds, respectively. Just how much of the original amino sugar undergoes the heat-induced change under practical conditions of milk sterilization remains uncertain, because even under the severity of the Morgan-Elson reaction, the conversion may amount to only 15% (Horton, 1969).

DISCUSSION

This investigation has been devoted to a study of the color reaction of heated milk with the aromatic aldehyde *p*-DMAB in a reaction mixture of formic acid and chloroform. Conditions for reproducible color production have been established and a method for the quantitative determination of *p*-DMAB reactivity in terms of absorbance units has been developed.

Several substances are present in unheated milk which are potentially capable of reacting with *p*-DMAB under appropriate reaction conditions. These include free (nonacetylated) hexosamines after treatment with condensing reagents such as acetyl acetone, *N*-acetylhexosamines after heating in alkaline solutions (Zuckerandl and Messiner-Klebermass, 1931), and sialic acids and tryptophan when these are exposed to strong mineral acids (Knowlton *et al.*, 1960; Werner and Odin, 1952). Upon heating of these compounds, only *N*-acetylhexosamines produced significant color reaction with *p*-DMAB under the conditions of the test. Strongly heated milk is known to develop substances by the browning reaction

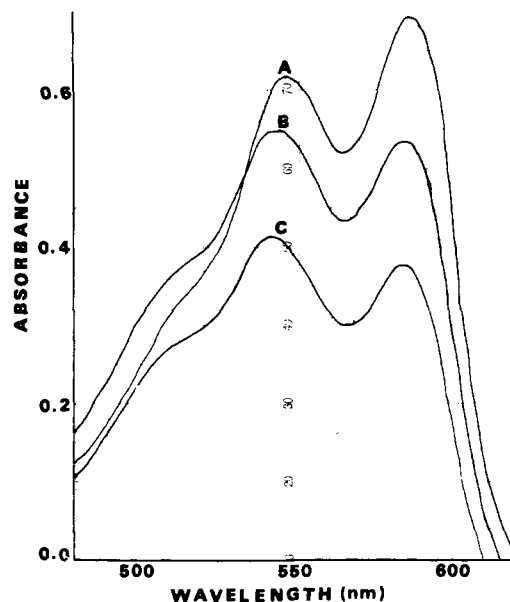


Figure 7. *p*-DMAB absorption spectra of *N*-acetylglucosamine developed under different conditions: (A) heating of sample and color development according to the method of Reissig *et al.* (1955); (B) sample heated (100°/10 min) with borate buffer (pH 8.9) and color developed with chloroform-formic acid reaction mixture; (C) sample heated (120°/30 min) at pH 6.8 and color developed with chloroform-formic acid

between lactose and free amino groups which would be expected to react with *p*-DMAB (Kent and Whitehouse, 1955); however, related spectra revealed that this contribution was comparatively small in moderately heated milk at normal pH. Therefore, the components responsible for a major part of the *p*-DMAB reactivity appear to be the *N*-acetylhexosamines present in milk in free and dialyzable form. Those amino sugars which are conjugated to proteins, for example in κ -casein, do not contribute to the formation of chromogens, nor do amino sugars contained in polysaccharides such as hyaluronic acid or chondroitin sulfate. Hoff (1963) reported that bovine milk contains the equivalent of about 23 mg/100 ml of nonprotein-bound *N*-acetylglucosamine, half of which may be phosphorylated in the C-1 position. In the present investigation, we found that the *p*-DMAB reactivity corresponded to the equivalent of approximately 15 mg/100 of *N*-acetylglucosamine. However, the exact content cannot be established by any of the present procedures because the *p*-DMAB reactivity of *N*-acetylgalactosamine is much lower than for its isomers. Furthermore, we have not established whether or not possible phosphorylated derivatives may serve as precursors. Their participation would require dephosphorylation, but there is evidence that other ester phosphates in milk are readily heat labile (Belec and Jenness, 1962; Sharma and Hansen, 1970).

A key point of this study has been the finding that *N*-acetylhexosamines may be altered to heterocyclic compounds by heat at physiological pH and the reaction, therefore, does not necessarily require alkaline conditions. This observation, in our opinion, may have nutritional significance. *N*-Acetylhexosamines have been shown to be growth-promoting factors for *Lactobacillus bifidus*, a microorganism of demonstrated importance in infant nutrition (Tomarelli *et al.*, 1949). The heat treatments such as those used in evaporated milk for sterilization may cause part of these amino sugars to be converted into substances, the nutritional role of which is not yet known.

According to a review by Horton (1969), alkali treatment of *N*-acetylhexosamines leads to the production of three furan compounds which subsequently can react with *p*-DMAB to yield the characteristic spectrum with two distinct maxima. Our observation that the spectra obtained from heated milk and from *N*-acetylhexosamines, heated in a simulated milk salt system, are similar to those obtained by alkali pretreatment suggests that the chromogens formed in milk are the same as those identified in the Morgan-Elson reaction, namely mono- and dianhydro derivatives of *N*-acetylhexosamines containing one and two double bonds, respectively. The relative amounts converted are not known, but may represent only a small portion of the original concentration present in milk (Horton, 1969).

The heat-induced *p*-DMAB reactivity of milk represents additional evidence for the effect of elevated temperatures on the milk system. However, the study has shown that the reaction is independent of the parallel reaction leading to the accumulation of reductones

Reference has been made to a secondary color formation by unheated or heated milk when kept in contact with the *p*-DMAB reaction mixture over a prolonged period (several hours). This reaction is not heat induced and does not appear to involve amino sugars and may have a bearing on the relatively high blank values obtained for unheated milk. This background color appeared to be a contribution mainly by an as yet unidentified compound found in α_s -casein but not in the other milk proteins.

Two direct practical applications of the developed *p*-DMAB reactivity tests are apparent. Since the change in the *p*-DMAB reactivity of milk is directly proportional to the heat treatment of milk, the test may serve as a heat index for high heat-treated milk products and in this respect would complement existing methods that demonstrate heat-induced alterations in milk; and since the solvent mixture used in the test produces a clear supernatant from milk, it may be used for determining *N*-acetylhexosamines in biological fluids contain-

ing relatively high concentrations of proteins and lipids. A method for the determination of *N*-acetylhexosamines based upon the formic acid-chloroform solvent mixture has already been reported (Kumar and Hansen, 1972).

LITERATURE CITED

- Aminoff, D., Morgan, W. T. J., Watkins, W. M., *Biochem. J.* **51**, 379 (1952).
 Belec, J., Jenness, R., *J. Dairy Sci.* **45**, 12 (1962).
 Chapman, R. A., MacFarlane, W. D., *Can. J. Res. Sect. B* **23**, 91 (1945).
 Crowe, L. K., Jenness, R., Coulter, S. T., *J. Dairy Sci.* **31**, 595 (1948).
 Elson, L. A., Morgan, W. T. J., *Biochem. J.* **27**, 1824 (1933).
 Gottschalk, A., Partridge, S. M., *Biochem. J.* **46**, vi (1950).
 Hansen, P. M. T., *J. Dairy Sci.* **50**, 952 (1967).
 Hoff, J. E., *J. Dairy Sci.* **46**, 573 (1963).
 Horton, D., in "The Amino Sugars 1 A," Jeanloz, R. W., Ed., Academic Press, New York, N. Y., 1969, pp 13-15.
 Jenness, R., Kooops, J., *Ned. Melk Zuivelijdschr.* **16**, 153 (1962).
 Jollès, P., in "Glycoproteins" Gottschalk, A., Ed., Elsevier, New York, N. Y., 1966, pp 335-350.
 Kent, P. W., Whitehouse, M. W., "Biochemistry of the Amino-sugars," Butterworths, London, 1955.
 Knowlton, M., Dohan, F. C., Sprince H., *Anal. Chem.* **32**, 666 (1960).
 Koka, M., Mikolajcik, E. M., *J. Dairy Sci.* **50**, 762 (1967).
 Kumar, S., Hansen, P. M. T., *Anal. Chem.* **44**, 398 (1972).
 Mauzerall, D., Granick, S., *J. Biol. Chem.* **219**, 435 (1956).
 Morgan, W. T. J., Elson, L. A., *Biochem. J.* **28**, 988 (1934).
 Murphy, G. K., Whitney, R. McL., *J. Dairy Sci.* **39**, 912 (1956).
 Reissig, J. L., Strominger, J. L., Leloir, L. F., *J. Biol. Chem.* **217**, 959 (1955).
 Roseman, S., Daffner, I., *Anal. Chem.* **28**, 1743 (1956).
 Sharma, K. K., Hansen, P. M. T., *J. Dairy Sci.* **53**, 640 (1970).
 Tomarelli, R. M., Norris, R. F., György, P., Hassinen, J. B., Bernhart, F. W., *J. Biol. Chem.* **181**, 879 (1949).
 Werner, I., Odin, L., *Acta Soc. Med. Upsal.* **57**, 230 (1952).
 Zuckerkandl, F., Messiner-Klerbermass, L., *Biochem. Z.* **236**, 19 (1931).

Received for review December 13, 1971. Accepted August 25, 1972. Article No. 9-71, Department of Food Science and Nutrition. This investigation was supported by Public Health Service Research Grants No. FD 00108 and FD 00462 from the Office of Research and Training Grants, FDA.

Effect of Southern Corn Leaf Blight on Composition and Selected Physical Characteristics of Corn

James F. Cavins, Ordean L. Brekke, Edward L. Griffin, Jr., and George E. Inglett*

The 1970 corn crop was significantly affected by southern corn leaf blight. We have analyzed heavily damaged, moderately damaged, and undamaged kernels from blight-damaged ears of corn. The kernels were generally smaller in size and lower in weight, and the grain was lower in test weight as

blight damage increased. Protein, ash, and fiber content increased with increased blight damage, while oil, starch, and pentosan content decreased. In general, amino acid data agreed favorably with those found for normal corn from years when no blight was present.

Helminthosporium maydis, Nisik et Miyake, Race T, the causative agent of southern corn leaf blight, markedly decreased the yield of corn grown during the 1970 crop year. This disease has been a problem in other years but

to a lesser extent. The effect of blight on composition and physical characteristics is important to the farmer, processor, and consumer. The farmer suffers a discount for blighted grain that is downgraded because of kernel damage and low test weight, while dry- and wet-millers encounter higher cleaning losses and lower yields of their principal products (Anderson *et al.*, 1972; Brekke *et al.*, 1972). Several investigators have shown that blighted corn presents no problem in feeding

*Northern Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois 61604.