Browning of Spray-Processed Lactose

By CHARLES A. BROWNLEY, JR., and LEON LACHMAN

The relationship that exists between the discoloration of spray-processed lactose and the presence of 5-(hydroxymethyl)-2-furaldehyde (HMF) was investigated. The HMF was determined by reaction with 2-thiobarbituric acid using procedures developed by Keeney and Bassette. The results obtained indicate that spray-processed lactose contains significant quantities of HMF, while conventionally processed lactose contains essentially no HMF.

FOLLOWING THE appearance of spray-processed lactose on the market in 1956, a serious problem soon developed for the users of this common pharmaceutical excipient. This sugar, unlike the lactose prepared by the conventional recrystallization methods, was occasionally received from the supplier brown instead of white, or else turned brown in storage or in systems where such color change had not been previously experienced.

The literature on browning reactions has, in general, emphasized the complexity of the subject and the lack of specific knowledge of the chemical reactions and intermediates involved (1, 2). It is recognized today that browning of food products can be broadly classified into three types.

The most common type—carbonyl-amine reactions—includes the reactions of aldehydes, ketones, and reducing sugars with amines, amino acids, peptides, and proteins. The term "Maillard reaction" (3) has been attached to this type of browning in honor of the man who made the first definitive study of the phenomenon. This type of browning requires a relatively low order of energy for its initiation and exhibits autocatalytic qualities once it has begun.

Another type, called caramelization, occurs when polyhydroxycarbonyl compounds (sugars, polyhydroxycarboxylic acids) are heated to relatively high temperatures in the absence of amino compounds. Dutra, Tarassuk, and Kleiber (4) found that this type of discoloration characteristically requires more energy to get started than carbonyl-amino reactions, other conditions being equal. Both acids and bases are known to catalyze caramelization reactions, but little more than this is known. Patton (5) made the interesting commentary that pure caramelization is rarely encountered in the food field.

A third type of browning frequently encountered by the food processor is the group of oxidative reactions which, for example, convert ascorbic acid and polyphenols into dicarbonyl and polycarbonyl compounds. Oxidative browning is exemplified by the so-called enzymatic browning in fruits and vegetables, but is not necessarily limited to enzyme-initiated systems.

The literature of the dairy chemists and food technologists is abundant with experiments concerning discoloration and degradation of manufactured dairy products, especially dried milk products (4–15). A considerable amount of evidence has implicated Maillard-type browning as the main contributing factor in these products.

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It becomes apparent, after considering the literature on the types of browning, that in studying the browning experienced with lactose U.S.P. prepared by spray drying, investigation of the Maillard-type reaction provides a good starting point.

It is the purpose of this paper to show that a relationship exists between the concentration of 5-(hydroxymethyl)-2-furaldehyde (HMF), a reaction product of the Maillard-type browning, and the degree of browning of spray-processed lactose. In addition, an attempt is made to relate the presence of HMF to the relative stabilities of spray-dried and conventionally processed lactose.

EXPERIMENTAL

Materials

Lactose U.S.P., prepared by spray drying and conventional drying after recrystallization; 5-(hydroxymethyl)-2-furaldehyde, (Aldrich Chemical Co. Inc.), purified by recrystallization from ether and petroleum ether, m.p. 31.5°; 0.3N oxalic acid ($H_2C_2O_4 \cdot 2H_2O$) (Fisher Scientific Co.); 40% trichloroacetic acid (Fisher Scientific Co.); and 0.05M 2-thiobarbituric acid (Bios Laboratory, Inc.) were utilized.

2-Thiobarbituric (TBA) solution is prepared by warming to 60° and cooling to room temperature before use. The reagent must be prepared fresh. It remains relatively unchanged for a 6-hour period. After this period of time, the absorbance values decrease.

Equipment

Water baths at 40 and 100°, Beckman model DK-1 recording spectrophotometer, Beckman zeromatic pH meter, and a Beckman model DU spectrophotometer with fitted reflectance attachment were employed.

Test Methods

TBA Reaction.—The analytical procedure used for determining the concentration of HMF in lactose includes the selective digestion procedure developed by Keeney and Bassette (15) to convert the early intermediates of the Maillard-type browning reaction into HMF. The HMF is then determined by spectrophotometric measurement of its 2-thio barbituric acid (TBA) reaction product.

The TBA combines with furfural compounds by simple condensation reaction to yield yellow pigments. The reaction provides a sensitive method of measuring the extremely small quantities of the decomposed products of milk—namely, the carbonyl compounds.

The analysis is performed as follows. Dissolve 5 Gm. of lactose in sufficient purified water to make 100 ml. of solution. Pipet 10 ml. of the solution into a 50-ml. test tube, and add 5 ml. of 0.3 N oxalic acid.

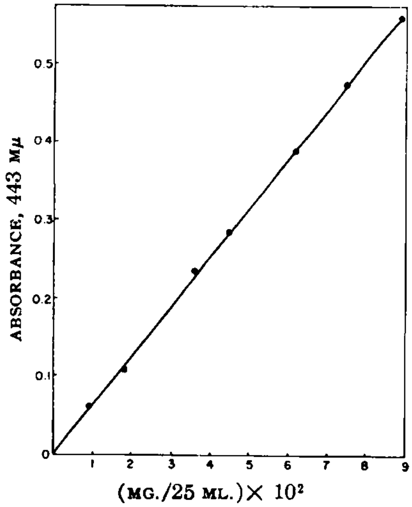

Fig. 1.—Colorimetric assay for 5 -(hydroxymethyl)-2-furaldehyde showing Beer's law relationship.

TABLE I.—CONCENTRATION OF HMF IN LACTOSE U.S.P. PREPARED BY SPRAY DRYING

Sample Age, Mo.	Mg. Free HMF/Kg. of Lactose	Mg. Total HMF/Kg. of Lactose	Relative Degree of Browing[a]	Reflectance, %
2	11.71	13.73	0	93.0
18	14.98	19.98	+	...
20	19.31	20.91	+	75.6
22	17.95	20.60	+	...
24	19.98	25.60	+++	69.8
26	21.23	26.84	+++	...
27	18.73	23.43	+	75.0
27	26.54	36.84	+++	...
29	25.77	33.74	++	70.4
29	32.78	41.52	+++	...
30	23.43	31.24	+++	72.7
30	29.66	39.34	+++	...

[a] From 0 to +++ shows increase in browning.

Cover the tube with an inverted 20-ml. beaker, and place it into a boiling water bath. Immerse it so that the top of the tube and the cover can be kept cool by a gentle flow of air. Remove the tube after 1 hour; cool with cold water to room temperature. (This heating step is omitted in the determination of the free HMF.)

Add 5 ml. of 40% trichloroacetic acid to the digested solution. Mix the solution and filter through Whatman No. 42 paper. Collect the filtrate into a 25-ml. volumetric flask; add 2.5 ml. of 0.05 *M* TBA to the flask. Wash the filter paper with sufficient purified water to bring the volume to 25 ml. Heat the flask and its contents to 40° in a water bath, and keep at this temperature for 30 to 40 minutes. Remove the flask and cool to room temperature. Measure the absorbance of the solution at 443 mμ against a blank prepared in the same manner as the sample, substituting purified water for the lactose solution. Figure 1 shows that the absorbance of TBA/HMF reactant conforms with Beer's law.

The HMF in the sample is converted to milligrams per kilogram of lactose by mg. HMF/Kg. lactose = (absorbance/6.36) × 2000.

Test solutions for spectrophotometric measurement are stable for approximately 1 hour.

The concentrations of HMF found in the sample of lactose are expressed as free HMF or as total HMF. Free HMF value is the quantity found by direct analysis. The total HMF value is a measure of the free HMF plus HMF developed as a result of the selective digestion procedure used.

Reflectance.—Color change in the powder samples was measured by reflectance at 480 mμ. The reference standard was a block of reagent grade magnesium carbonate, and the results are given as per cent reflectance.

pH.—The pH of the test solutions were checked periodically. In the test procedures, the oxalic acid is employed to provide an optimum pH of 3.2 to produce the maximum amount of HMF from its precursors. The trichloroacetic acid provides a pH of 0.8 to 1.0, which is the acidity necessary to carry out the TBA/HMF reaction.

RESULTS AND DISCUSSION

The relationship that exists between the presence of HMF and the browning of spray-processed lactose was investigated. The data presented in Tables I and II show the HMF concentration and extent of browning for various samples of spray-processed and conventionally dried lactose, respectively. These samples are representative of materials obtained from quality control reference stock and have been stored under ambient conditions for varying time intervals.

It is evident from the data in Table I that as the concentration of free HMF increases, the spray-processed lactose darkens in color. The data in Table II for conventionally processed lactose show the presence of no free HMF or color change for storage up to 36 months.

The analysis for early browning intermediates

TABLE II.—CONCENTRATION OF HMF IN LACTOSE U.S.P. PREPARED BY CONVENTIONAL RECRYSTALLIZATION TECHNIQUES[a]

Sample Age, Mo.	Mg. Total HMF/Kg. of Lactose	Reflectance, %
2	6.87	94.7
11	13.42	92.4
12	16.39	93.0
13	15.36	94.3
17	16.39	90.5
20	11.23	93.1
28	8.12	91.9
30	11.71	92.3
34	12.49	91.9
36	13.49	92.9

[a] No free HMF was detected and the lactose remained white with all samples.

TABLE III.—CONCENTRATION OF HMF AND PER CENT REFLECTANCE FOR SPRAY-PROCESSED LACTOSE STORED AT 80° C.

Time, Hr.	Mg. Free HMF/Kg. of Lactose	Mg. Total HMF/Kg. of Lactose	Reflectance, %
0	3.12	18.73	94
2	9.37	24.98	91
4	12.49	28.10	78
6	18.73	32.78	73
8	24.98	39.03	71
16	46.83	...	62
20	56.20	...	59
24	62.44	73.34	59

capable of being converted to HMF produced a positive TBA reaction for spray-processed and conventionally processed lactose. This is evident by the total HMF content of up to 16.39 mg./Kg. lactose for conventionally prepared and up to 41.52 mg./Kg. of lactose for spray-processed lactose. A definitive pattern of increase in total HMF with storage time for spray-processed lactose appears to exist, but no such relationship exists for conventionally processed lactose.

The data in these tables attempt to relate total HMF concentration to color development measured by reflectance readings for both conventionally and spray-processed lactose stored at ambient conditions. It is evident from these data that total HMF does not appear to be the criterion for color development since samples of conventionally processed lactose have considerably higher total HMF values than spray-processed lactose without affecting the color of the lactose. It would appear from these results that it is the concentration of free HMF in the samples that is related to color change, since none of the samples of conventionally processed lactose contained any detectable free HMF, while the spray-processed lactose samples contained varying amounts of free HMF.

It appears from these data that the presence of free HMF is an indication that the complex Maillard-type reaction has begun, and browning will take place once the concentration of HMF reaches a critical value. The data obtained by other investigators appear to substantiate this reasoning. Keeney and Bassette (14) made the general observation that once HMF in dry milk increased to a certain level, flavors associated with color change develop. Craig (12) reported that a quantitative relationship existed between storage stability and initial HMF concentration for vacuum foam-dried whole milk. Newth (16) also reported such a relationship for glucose solutions.

To evaluate the effect of the presence of HMF precursors in conventionally and spray-dried processed lactose on color development, samples of both sugars were heated at 80° for 24 hours. At the end of 24 hours, the total HMF present in conventionally processed lactose was approximately equal to that determined initially. However, in spray-dried lactose an increase in free and total HMF took place as illustrated in Table III. In addition, these samples exhibited a significant degree of darkening, evidenced by the decrease in reflectance values. The data presented in this table illustrate that, as HMF is formed, a concurrent production of coloring matter results.

In an attempt to determine whether the process of spray drying influences the HMF content of lactose, a sample of conventionally processed lactose, containing no free HMF and 13.42 mg./Kg. of total HMF, was spray dried. Testing of this spray-dried material for 1 year at ambient conditions gave no darkening. Spray drying a slurry containing 50% lactose, 5% dextrose U.S.P., and 5% galactose also showed no increase in total HMF after drying and no darkening after storage for 1 year.

These preliminary experiments indicate that the physical operations of spray drying and the addition of the common hydrolysis products of lactose give no additional quantities of HMF. It would appear that the simple hydrolysis products of lactose are not the major precursors of HMF in spray-processed lactose.

SUMMARY

The results obtained in this investigation implicate the presence of free HMF in lactose with resultant browning. Samples of conventionally processed lactose containing no free HMF and heated for 24 hours at 80° and stored at ambient conditions for 36 months showed no darkening. On the other hand, samples of spray-processed lactose, treated similarly, containing free HMF, darkened significantly.

The TBA reaction provides a suitable quantitative method for determining free HMF and should serve as a suitable technique for the quality control of lactose relative to browning possibilities.

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Centrifugally Accelerated Thin-Layer Chromatography

By B. P. KORZUN and S. BRODY

Centrifugal force has been used to accelerate the development of thin-layer chromatograms and the results compared with data on the standard ascending development technique.

Centrifugal force has been employed to accelerate paper chromatographic separation of compounds in mixtures (1–4). This method was applied to thin-layer chromatography on circular glass plates or aluminum plates. Comparisons were made between standard thin-layer and accelerated thin-layer chromatography. The accelerated method was completed in about 10 minutes, compared to the 30 to 40 minutes required for the standard ascending method.

Circular glass or aluminum plates 26 cm. in diam-

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