Nonenzymatic Browning in Model Systems Containing Sucrose

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Freeze-dried systems containing sucrose and organic acids underwent rapid nonenzymatic browning, even at low relative humidities. Addition of protein reduced the rate of browning, especially at low humidities. The browning was due to reducing sugars produced by acid-catalyzed hydrolysis of sucrose, which occurred even at water contents below 1% and below Brunauer-Emmett-Teller monolayer coverage.

During a study of deteriorative reactions in freeze-dried model systems at 55° C., experiments were performed on the browning of model systems containing carbohydrates, lipids, and proteins. Many foods of similar composition brown at high temperatures and humidities. The reaction is usually attributed to interaction between reducing sugars and free amino groups of the protein (Lea, 1958). Our results indicate, however, that hydrolysis of sucrose may occur at relatively low water contents, and that the reducing sugars formed participate in browning.

EXPERIMENTAL

Sample Preparation. The model systems shown in Table I were prepared by mixing the components in an Omni-Mixer, with subsequent freeze-drying of 4- to 5-gram samples. After drying, the samples were humidified to the desired relative humidities over saturated salt solutions and then stored at 55° C. The procedures for preparation of the model systems were similar to those reported by Maloney *et al.* (1966).

Browning Pigments [modified procedure of Choi *et al.* (1949)]. Two- to 5-gram samples of dry material were dispersed in 20 ml. of water, and 2.5 ml. of a 10% fresh trypsin suspension were added. After 1 hour of incubation of 45° C., 2 ml. of 50% trichloroacetic acid and 0.1 gram of Filter Aid were added. After mixing and filtration, the absorbance at 400 mμ was measured on the clear solution, with the enzyme blank set at 100% transmittance. The results are reported as (absorbance per gram of sample) × 100.

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Reducing Sugars. Two- to 5-gram samples of dry material were extracted with 25 ml. of water for 1/2 hour and then filtered. A modified Somogyi reducing sugar test (oxidation of copper to Cu_2O) was performed on 5 ml. of the filtrate (Bates, 1942).

Moisture Content. Moisture content was determined by a procedure in which samples were extracted with anhydrous methanol, and the water content was determined by gas chromatography. Details of the procedure have been published (Karel and Labuza, 1967).

RESULTS AND DISCUSSION

Two model systems were studied in run 1. Their composition is shown in Table I, and the extent of browning at three different relative humidities (RH) is shown as a function of time in Figure 1. As expected, substantial browning occurred in the system containing glucose. However, the system containing sucrose as the only added carbohydrate also browned considerably, even at very low water contents.

The possibility that the browning was due to carbonyl compounds produced during autoxidation of the lipid was considered, but sensitive thin-layer chromatographic tests for peroxide content (Privett and Blank, 1962) showed that oxidation level of the methyl oleate was negligible. Therefore, the possibility that sucrose was being hydrolyzed was considered, and a further study was undertaken in run 2.

In this run, three model systems were studied (Table I). Lipid and glucose were eliminated and citric acid instead of malic acid was used because of its higher solubility. In addition, one system was prepared without any protein or citric acid (model C) and one without protein but with citric acid (model D).

Figure 2 shows the extent of browning for the three

Table I. Composition of Model Systems

	Weight of Component, Grams				
	Run 1		Run 2		
	Model A	Model B	Model C	Model D	Model E
Component					
Carbohydrates					
Glucose	...	66.8	...	...	...
Sucrose	133.6	66.8	150	150	150
Malic acid	4.0	4.0	...	...	...
Citric acid	...	...	...	5.0	5.0
Avicel[a]	9.6	9.6	10	10	10
Egg albumin[b]	2.8	2.8	...	...	5
Methyl oleate[b]	3.8	3.8	...	...	...
Water	46.0	46.0	40	40	40
Moisture content, grams water/100 grams solids					
0.1% RH	<0.31	5.42	0.11	0.22	0.39
31% RH	0.50	7.39	0.33	0.47	3.85
50% RH	0.98	10.24	...	...	...
75% RH	...	...	0.95	3.72	5.67

[a] American Viscose Corp., Md.
[b] Mann Research Co., New York, N. Y.

Table II. Hydrolysis of Sucrose (Run 2)
(Per cent hydrolysis)

Time, Days	Model C			Model D			Model E		
	Dry	31% RH	75% RH	Dry	31% RH	75% RH	Dry	31% RH	75% RH
0	0.15	0.05	0.06	1.45	1.81	8.05	0.91	0.96	2.43
3	0.09	0.03	0.09	2.83	6.62	41.3	1.13	4.79	11.96
7	0.13	0.06	0.09	5.26	8.14	70.4	0.85	15.87	21.68
10	0.12	0.05	0.11	5.22	6.51	69.6	2.91	14.42	16.30
14	0.13	0.07	0.12	7.89	10.97	73.8	2.81	15.58	25.80
17							2.81	18.06	40.37
22							3.25	16.54	36.05

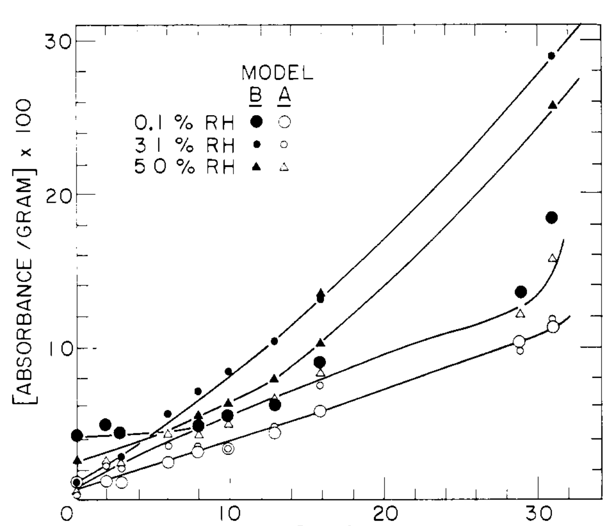

Figure 1. Nonenzymatic browning

Run 1, 55° C.

models, each held at three water activities. When acid is lacking, the model system is stable and does not brown (model C). In the presence of acid, but without protein (model D), significant browning occurs even at extremely low humidities (moisture content of 0.22 gram of H_2O per 100 grams of solids). The protein added to system E seemed to act as a buffer, possibly by reducing the concentration of hydrogen ions in the surface water.

These results support the hypothesis that hydrolysis of sucrose can occur in freeze-dried systems, producing reducing sugars which then participate in browning reaction. The hypothesis was confirmed further by direct determination of reducing groups (Table II). As browning becomes significant, products of this reaction also give a positive reducing sugar test. This fact may be responsible for the variability of results obtained at high moisture contents. In subsequent studies the authors have confirmed the occurrence of sucrose hydrolysis by demonstrating increases in glucose using the specific glucose oxidase test (Karel and Labuza, 1967).

Our study shows that in model systems containing sucrose and citric acid, at low moisture content hydrolysis

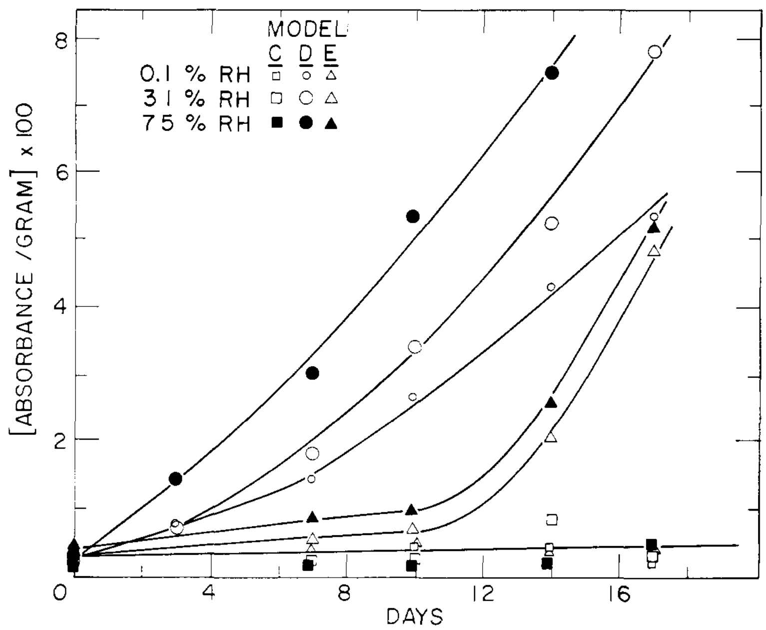

Figure 2. Nonenzymatic browning

Run 2, 55° C.

of sucrose can occur, leading to nonenzymatic browning. The mechanism of this reaction requires the participation of water as well as the dissolution of sucrose in the aqueous phase (Schoebel, 1968). The results presented here indicate that the reaction can occur even at very low water contents, and in fact occurs below the monolayer coverage as estimated from water sorption isotherms of these systems (Karel and Labuza, 1967).

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