Browning of Galacturonic Acid in a Model System Simulating Fruit Beverages and White Wine

Anantharaman Jayaraman* and Jerome P. Van Buren

Browning of D-galacturonic acid could be a potential problem in fruit beverages and wines as seen from the kinetic studies in water and dimethyl sulfoxide systems. Galacturonic acid browned much faster than glucose, even in the absence of amino groups. Alcohol enhanced both the browning at 98° C and the bleaching of brown pigments in sunlight. Light exposure prior to browning retarded browning. Acid pH increased the induction period, but caused a slight increase in the browning reaction rate. Browning was slowest in DMSO system and fastest in a 3:1 mixture of DMSO and water. Natural organic acids generally retarded browning.

Nonenzymatic discoloration in foods has received much attention since Maillard (1912). Excellent reviews on various hypotheses of discoloration have appeared (Hodge, 1953; Reynolds, 1963, 1965); however, detailed studies on the mechanism and kinetics have been carried out only in the case of Maillard reactions involving carbonyl and amino groups (Song and Chichester, 1966). Nonenzymatic reactions other than Maillard also contribute significantly to browning. Sugars, polysaccharides, polyhydroxycarboxylic acids, reductones, α-dicarbonyl compounds, and quinones brown considerably on heating, in the absence of amino groups (Hodge, 1953). Uronic acids are reported to brown much faster than monosaccharides with and without added amino groups (Seaver and Kertesz, 1946). The faster rate of browning of D-galacturonic acid suggests that it may be important in the browning of fruit beverages and white wines which have been clarified with pectolytic enzymes. Darkening in enzyme-treated white wines (Cruess, 1948) led to a study of factors contributing to the discoloration (Cruess, 1951; Devillers, 1961; Berg and Akiyoshi, 1956; Caputi and Peterson, 1965; Quintanilla, 1967). White wines contained 6.0–14.1 mg per 100 ml of galacturonic acid (Cruess, 1951) and 0.56–3.55 g per l. of pectins (Reed, 1955). Natural derivatives of galacturonic acid were found in New York State wines (Rice, 1968). The above evidences suggest the possible role of galacturonic acid in contributing to the discoloration in foods in addition to Maillard browning. A simple model system is used to study the browning due to galacturonic acid in the absence of amino groups, as influenced by the various constituents of fruit beverages and white wine. The reported inhibitory effects of water on Maillard browning (Schroeder *et al.*, 1955) led us to study our model in aqueous and nonaqueous systems.

EXPERIMENTAL

Preparation of Samples. One-percent solutions of thin-layer chromatographically pure D-galacturonic acid (Nutritional Biochemical Corp.) were prepared in deionized water, dimethyl sulfoxide (DMSO), citrate buffer of pH 2, citrate–phosphate buffers of pH 3, 4, 5, 6, and 7 and borate buffers of pH 8 and 9. Portions (10 ml) of the test solutions were transferred to glass-stoppered tubes for experiments carried out in air and to Thunberg tubes for experiments conducted in vacuum. Vacuum pump was used to obtain a vacuum of 27 in. Browning was carried out at 98° C.

Color Measurement. Absorbance was measured at 400 nm in a Spectronic 20 (Bausch & Lomb).

Browning of Light Exposed Samples. Freshly prepared solutions of D-galacturonic acid were kept in sunlight for 30 hr until there was no further drop in absorbance. The solutions thus exposed to light were incubated at 98° C and the absorbance was measured at known intervals of time.

Bleaching of Browned Samples. Solutions browned at 98° C were kept in sunlight for 8 hr each day for 20 days and the changes in absorbance were measured at the end of each day.

Determination of Induction Period and Calculation of the Reaction Rate Constant (k). Absorbance and time were plotted on semilog graph paper on the X and Y axes, respectively. The change in absorbance at short exposure times was not significant. So, the rate constant during this induction period could not be calculated. However, after the induction period, there was linear correlation between absorbance and time following first-order kinetics. This linear portion of the graph was extrapolated back to intercept the X axis at 0.1 absorbance and the corresponding time was considered as induction period. k, the reaction rate constant, was calculated as

$$k = \frac{\log C_2 - \log C_1}{t_2 - t_1} \times 2.303$$

where C_1 and C_2 were the absorbances at times t_1 and t_2 after the induction period.

RESULTS AND DISCUSSION

Galacturonic acid browned more readily than most common carbohydrates. It was found that 0.55 M glucose showed an absorbance of 0.06 after 25 hr at 98° C, while 0.052 M galacturonic acid under the same conditions browned to show an

New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456.

Table I. Browning of 1% Galacturonic Acid at 98° C

Details	Induction period, hr	$k \times 10^{-2}$/hr
Water system		
Water solution, in air	6.9	11.50
Water solution, in vacuum	6.3	12.30
5% Alcohol, in water, in air	15.0	11.20
10% Alcohol, in water, in air	16.0	13.79
15% Alcohol, in water, in air	12.5	16.45
15% Alcohol, in water, in vacuum	14.5	10.54
DMSO system		
DMSO solution, in air	8.0	9.65
DMSO solution, in vacuum	3.2	10.70
5% Alcohol, in DMSO, in air	6.3	4.95
10% Alcohol, in DMSO, in air	4.2	4.76
15% Alcohol, in DMSO, in air	4.2	4.95
15% Alcohol, in DMSO, in vacuum	9.4	5.47
Phosphate buffer system (pH 2.75)		
Phosphate buffer solution, in air	11.0	10.35
Phosphate buffer solution, in vacuum	6.0	38.70
5% Alcohol, in phosphate buffer, in air	5.5	10.30
10% Alcohol, in phosphate buffer, in air	8.0	10.80
15% Alcohol, in phosphate buffer, in vacuum	8.6	19.60

Table II. Browning of 1% Galacturonic Acid Solutions Exposed to Sunlight and Incubated at 98° C

Details	Induction period, hr	$k \times 10^{-2}$/hr
5% Alcohol, in water, in air	15.0	7.44
10% Alcohol, in water, in air	13.0	12.54
15% Alcohol, in water, in air	12.5	10.32
5% Alcohol, in DMSO, in air	1.5	0.66
10% Alcohol, in DMSO, in air	4.0	1.59
15% Alcohol, in DMSO, in air	5.0	1.95
5% Alcohol, in phosphate buffer, in air	12.5	10.74
10% Alcohol, in phosphate buffer, in air	14.0	11.02
15% Alcohol, in phosphate buffer, in air	15.0	11.62

Table III. Bleaching in Sunlight of 1% Solutions of Galacturonic Acid Browned at 98° C

Details	$k \times 10^{-2}$/day[a]
Water solution, in air	5.09
Water solution, in vacuum	2.59
5% Alcohol, in water, in air	16.53
10% Alcohol, in water, in air	25.53
15% Alcohol, in water, in air	8.53
DMSO solution, in air	5.42
DMSO solution, in vacuum	5.19
5% Alcohol, in DMSO, in air	24.26
10% Alcohol, in DMSO, in air	18.66
15% Alcohol, in DMSO, in air	13.05

[a] Bleaching per day is equivalent to bleaching in sunlight for 8 hr.

Table IV. Effects of Fruit Beverages and Wine Constituents on the Browning at 98° C

Constituents	15% alcohol		No alcohol	
	Induction period, hr	$k \times 10^{-2}$/hr	Induction period, hr	$k \times 10^{-2}$/hr
Water	12.6	16.60	6.5	11.5
5% Tartaric acid	7.6	9.24	7.2	10.4
0.25% Citric acid	7.0	12.44	8.2	7.86
0.25% Malic acid	8.4	10.73	9.0	8.67
0.20% Succinic acid	6.0	9.80	10.0	9.31
0.4% w/v lactic acid	7.4	14.57	9.0	8.67
0.4% w/v glycerol	3.0	14.82	9.2	8.67
10% Glucose	9.4	18.77	7.4	9.79

absorbance of 1.3 at 400 nm. Seaver and Kertesz (1946) reported that 0.1 *M* solution of glucose did not brown at 98° C after 4 hr while 0.1 *M* galacturonic acid browned to give a color equivalent to 3 mg% of $K_2Cr_2O_7$ at 370 nm.

In initial experiments, it was found that galacturonic acid browned faster at 98° C than at 55° C and the reaction followed first-order kinetics after the induction period. During the induction period, changes in the absorbance were not high enough to be accurately measured at 400 nm. Hence the rate constants for the initial reaction during the induction period could not be calculated. However, the induction period, as deduced by extrapolating the linear portion of the graph correlating absorbance with time, served as an indirect measure of the rate of the initial reaction. The whole process of browning of galacturonic acid might be postulated as follows.

$$\text{galacturonic acid} \xrightarrow[\text{period}]{\text{induction}} \text{precursors} \xrightarrow{k} \text{brown pigment}$$

Both induction period and k were affected by conditions as seen in Tables I–IV.

In the DMSO system containing alcohol, browning began sooner (reduced induction period) and continued at a slower rate than in the aqueous system (Table I). It might be postulated that DMSO favored esterification of galacturonic acid in the presence of alcohol, under elevated temperature. Esterified galacturonic acid has been found to brown less than free galacturonic acid even in the presence of an amino group (Seaver and Kertesz, 1946). In the DMSO alcohol system, a compound that yielded galacturonic acid on alkaline hydrolysis was isolated.

The browning of galacturonic acid seemed to be nonoxidative as the absence of air did not have any significant effect on either induction period or k in the aqueous system. Similar findings were reported in the cases of starch (Puddington, 1948), amylopectin (Bryce and Greenwood, 1963), and ascorbic acid (Kurata and Sakurai, 1967). However, in the phosphate buffered system, exclusion of air resulted in a threefold increase in k and reduction in induction period to one-half. Phosphates were known to accelerate browning in the Maillard reaction, regardless of any buffering effect (Burton and McWeeny, 1963).

Alcohol increased the induction time in the water system and reduced it in DMSO and phosphate buffer system. In the DMSO system, the drastic reduction of the k values in the presence of alcohol may be due to ester formation as described earlier.

Solutions exposed to sunlight did not brown as fast as the unexposed solutions (Table II). Alcohol seemed to have increased the rate slightly. In DMSO systems both the induction period and k were reduced to a minimum. The photochemical changes taking place during the exposure to sunlight

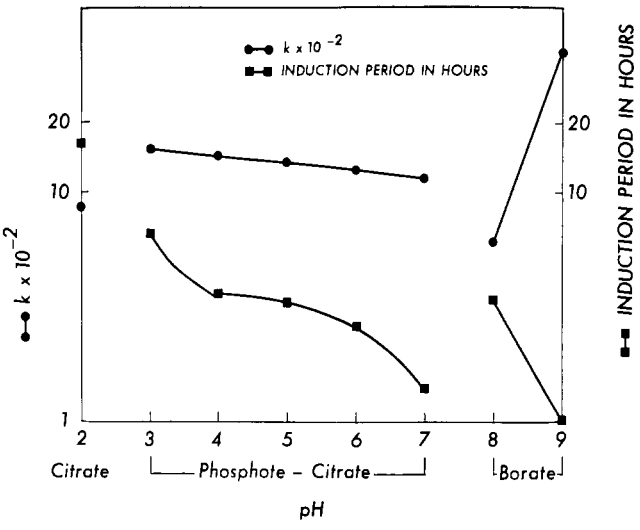

Figure 1. Effect of pH on the rate constant and induction period

and their subsequent effects on browning are not clear at the present time.

Table III shows that bleaching of solutions browned at 98° C in Table I was slower in DMSO and under vacuum. The presence of alcohol enhanced bleaching considerably in both water and phosphate systems. Further, the enhancing effects of alcohol, Table I, were reduced by holding in light.

Effects of pH on the browning are shown in Figure 1. The rate of reaction fell linearly with increase in pH from 3 to 7 in phosphate citrate buffers. However, the changes in the induction periods were not linear. At pH 7, induction period was minimum with k slightly less than that at pH 3.

Table IV lists the effects of various constituents of wine and fruit beverages on the browning. Generally the browning was increased in the presence of alcohol. In nonalcoholic systems browning was slightly reduced by the different constituents.

To further investigate the browning in aqueous and nonaqueous systems, different concentrations of DMSO and water, as compared with 100% DMSO and water, were tried and the results are shown in Figure 2. The rate of browning was enhanced in the mixture more than in either of the pure solvent systems. The fastest rate was in the system made up of 25% H_2O and 75% DMSO. DMSO is known to form a dihydrate at 66% concentration (Erlander and Tobin, 1968). But how this water and DMSO interaction influences the browning of galacturonic acid is not well understood.

The kinetic studies described above point to the possibility that browning due to galacturonic acid could be very significant, especially in acidic beverages stored for a long time and in the white wines stored in darkness.

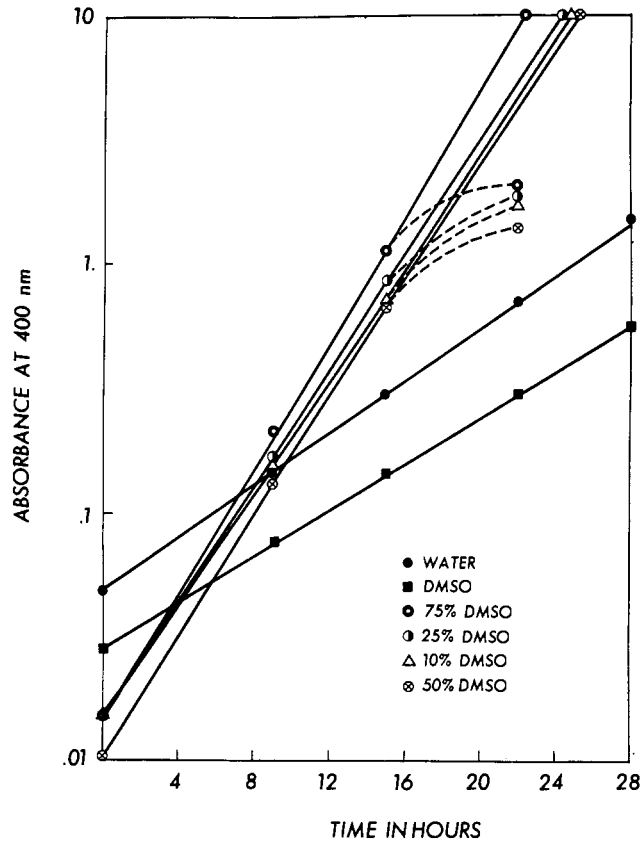

Figure 2. Browning of galacturonic acid at 98° C in DMSO/water systems

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