That formaldehyde vapor followed by heat treatment caused intense fluorescence of this otherwise uv-absorbing substance suggests that it is a catecholamine. The other two substances that could be oxidized were leucoanthocyanidins, substances closely related to catechins. These were found to precipitate readily as gums and thus may influence the formation of dark gums that often are associated with oxidizable substances.

LITERATURE CITED

Bate-Smith, E.C., *Food* **23**, 124 (1954).
Bjorklund, A., Falck, B., Hakanson, R., *J. Chromatogr.* **40**, 186 (1969).
Imakawa, S., *Hokkaido Daigaku Nogakubu Hobun Kiyo* 6(62), 181 (1967).
Satoh, I., and Tanabe, K., *Tottori Daigaku Nogakubu Kenkyu Hokoku* **23**, 47 (1971).
Tono, T., *Tottori Daigaku Nogakubu Kenkyu Hokoku* **22**, 13 (1970).

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Participation of Amadori Rearrangement Products and Carbonyl Compounds in Oxygen-Dependent Browning of Soy Sauce

Hironaga Hashiba

The oxygen-dependent browning of Amadori compounds was studied since it was one of the most important factors contributing to the browning of soy sauce during storage. The browning of all Amadori compounds except fructose–arginine was accelerated notably by oxygen and Fe^{2+} (40 ppm), while every mixture of a parent amino compound and sugar exhibited no browning under experimental conditions. Amadori compounds composed of aromatic or heterocyclic amino acids, such as fructose–tyrosine, fructose–phenylalanine, fructose–histidine, and fructose–tryptophan, were especially reactive in oxygen-dependent browning, and this type of browning was synergistically accelerated by the presence of Fe^{2+} and Mn^{2+} (30 ppm). On the other hand, the presence of Mn^{2+} showed an inhibitory effect on the browning of fructose–serine, fructose–glutamic acid, and fructose–leucine. The oxygen-dependent browning of fructose–glycine, fructose–lysine, fructose–β-alanine, and xylulose–glycine was not affected by Mn^{2+}. Oxygen was thought to accelerate the breakdown of Amadori compounds to liberate amino acids and glucosone. When α-hydroxycarbonyls were stored together with glycine, significant browning also occurred. However, in this case, oxygen did not contribute to browning.

Oxygen accelerates the darkening of many foodstuffs; therefore, oxygen-dependent browning has been studied by many workers using materials such as ascorbic acid (Clegg, 1964), polyphenols (Burton et al., 1963), and furfural (Dunlop et al., 1946). Soy sauce also darkens rapidly in contact with atmospheric oxygen (Figure 1). Furthermore, oxygen-dependent browning (oxidative browning; Hashiba, 1975) of soy sauce is developed remarkably in the presence of Fe^{2+} and Mn^{2+} (Hashiba et al., 1970). The oxidative browning of soy sauce is considered to have a different mechanism from those of ascorbic acid, polyphenols, and furfural because the amount of these compounds in soy sauce is very small (Omata et al., 1955). Omata et al. (1955) have reported an important participation of carbonyl compounds in the browning of soy sauce, and Kato et al. (1961) have isolated 3-deoxyglucosone (3-DG) from soy sauce as an important precursor of the browning reaction. However, the effect of oxygen on browning is obscure in these reports. Previously, the author (Hashiba, 1974) has suggested that Amadori compounds play an important role in the browning of soy sauce in the presence of oxygen. In addition, the author (Hashiba, 1975) has reported the oxidative browning of Amadori compounds, fructose–glycine (F–Gly) and fructose–diglycine (F–diGly). Because soy sauce contains many kinds of amino acids and sugars (Hashiba, 1974), many kinds of Amadori compounds must exist in soy sauce, and participate in the oxidative browning. From this point of view, the present paper deals with the examination of the browning of some Amadori compounds and compares the browning with that of carbonyl compounds which were known as highly reactive compounds in browning.

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EXPERIMENTAL SECTION

Synthesis and Isolation Procedure of Amadori Compounds. One-half mole of glucose, 0.1 mol of an amino acid, 10 g of sodium metabisulfite, and 10 ml of water were placed into a 500-ml conical flask and heated in boiling water for 1–5 hr with frequent shaking. When the mixture became yellowish brown, heating was stopped and the mixture was dissolved into about 1 l. of water and sent through a 2.5 × 45 cm column of Amberlite CG-120 (H^+). After the resin was washed with 1 l. of water, the absorbed substances were eluted with 0.1 *N* ammonia using a fraction collector. The amount of the Amadori compound was measured by a test which consisted of reduction of ferricyanide at pH 6.6 at 50°C for 20 min (Adachi's method; Adachi, 1958). The fractions reducing ferricyanide were pooled (about 400 ml) and then half was applied to preparative ion exchange chromatography (Hashiba, 1975). The fractions positive to ninhydrin, phenol–H_2SO_4 tests, and reducing ferricyanide at pH 6.6 were collected as Amadori compounds. Two such preparations obtained

Table I. Preparation and Some Characteristic Properties of Amadori Compounds

Amadori compd[a]	Reaction time[b]	Position of emergence[c]	Neut. equiv Calcd	Neut. equiv Found	Phenol-H_2SO_4 test[d]	Reduction of ferricyanide at 100°C[e]	Reduction of ferricyanide at 50°C[f]	Elementary anal. of Amadori compds, % Carbon Calcd	Carbon Found	Hydrogen Calcd	Hydrogen Found	Nitrogen Calcd	Nitrogen Found
F-Ser	1	Cys acid-Asp	267.1	267.3	6	115	113	40.5	40.3	6.42	6.43	5.24	5.23
F-Glu	1	Cys acid	309.3	309.6	7	112	111	42.7	42.6	6.20	6.14	4.53	4.40
F-Gly	1	Cys acid-Asp	237.2	237.2	12	110	100	40.5	40.5	6.37	6.37	5.91	5.90
F-Leu	2	Asp-Thr	293.2	293.3	13	83	107	49.2	49.1	7.91	7.92	4.78	4.77
F-Tyr	5	Pro-Gly	343.3	344.5	12	96	112	52.5	52.2	6.17	6.30	4.08	4.29
F-Phe	1	Pro-Gly	327.3	327.4	12	85	114	55.0	55.0	6.47	6.50	4.28	4.27
F-Trp	1	Leu-Tyr	366.3	369.5	17	110	109	55.7	55.5	6.05	6.32	7.65	7.91
F-Lys	0.5	Ala-Cys	308.3	309.3	17	98	97	46.8	46.9	7.85	7.92	9.09	9.21
F-His	0.5	Phe	317.3	317.6	13	123	125	45.4	45.4	6.04	6.15	13.2	13.3
F-Arg	1	Lys-NH_3	366.2	366.5	23	75	90	42.9	42.7	7.20	7.43	16.7	16.9
F-β-Ala	0.5	Val-Met	251.2	251.2	7	95	96	43.0	43.0	6.77	6.78	5.58	5.60
F-di-Gly	0.5	Ala-Cys	294.3	294.4	13	101	102	40.8	40.6	6.12	6.25	9.52	9.30
X-Gly	0.5	Cys acid-Asp	207.2	207.2	12	108	100	40.6	40.5	6.32	6.33	6.76	6.75
Glucose					100	100	0						

[a] Abbreviations used are: F-Ser, fructose-serine; F-Glu, fructose-glutamic acid; F-Gly, fructose-glycine; F-Leu, fructose-leucine; F-Tyr, fructose-tyrosine; F-Phe, fructose-phenylalanine; F-Trp, fructose-tryptophan; F-Lys, fructose-lysine; F-His, fructose-histidine; F-Arg, fructose-arginine; F-β-Ala, fructose-β-alanine; F-diGly, fructose-diglycine; X-Gly, xylulose-glycine. [b] Hours in boiling water. [c] Position of emergence in Hitachi KLA-5 amino acid analyzer. Abbreviations used are: Cys acid, cysteic acid; Asp, aspartic acid; Thr, threonine; Pro, proline; Gly, glycine; Ala, alanine; Cys, cysteine; Val, valine; Met, methionine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Lys, lysine. [d] Percent of glucose. [e] Percent of glucose by Somogyi-Nelson's method. [f] Percent of F-Gly by Adachi's method.

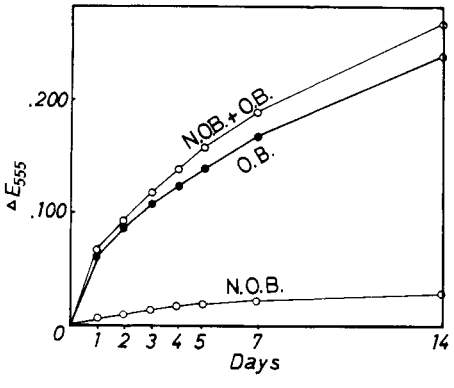


Figure 1. Oxidative and nonoxidative browning of soy sauce: N.O.B., nonoxidative browning; O.B., oxidative browning. The method of measurement of oxidative and nonoxidative browning is described in the Experimental Section.

from the preparative chromatograph were combined and charged on a 2.5 × 50 cm column of Amberlite CG-120 (H^+) to remove citrate. After the resin was washed with 2 l. of distilled water, the absorbed Amadori compound was eluted with 0.1 *N* ammonia, collecting 20-ml fractions. These fractions were tested on paper chromatography (solvent, butanol–acetic acid–water, 4:1:1) and fractions containing only Amadori compounds were collected and lyophilized. The product was a slightly hygroscopic white powder (yield was about 5 g). Attempts to crystallize Amadori compounds were unsuccessful, except for F–Gly, fructose–β-alanine (F–β-Ala), and xylulose–glycine (X–Gly). The high purity of the synthesized Amadori compound was indicated by its emergence in the amino acid analyzer as a single symmetrical peak uncontaminated by an amino acid. When examined by paper chromatography, the product appeared as a single spot at the identical position whether detected by ninhydrin or KIO_4–tetrabase reagent (Yoda, 1952). The following tests were carried out on all the compounds prepared: total sugar by Somogyi-Nelson's method (Nelson, 1944), reduction of ferricyanide by Adachi's method, effluent position in the amino acid analyzer, and liberation of a parent amino acid by acid hydrolysis (in 2 *N* H_2SO_4 at 100°C for 1 hr). Table I gives the calculated and found neutral equivalents and elementary analyses. All Amadori compounds we have prepared so far gave values for sugar close to that of an equivalent amount of the parent sugar. A more useful test, which distinguishes the Amadori compounds from both the *N*-glycosylamino acids and free sugar, is the ability of the former to reduce ferricyanide in Adachi's method. The *N*-glycosylamino acids and the free sugars give very low values by this test.

Analyses of Amino Compounds. Amino acids and Amadori compounds were analyzed with a Hitachi KLA-5

Table II. Effect of Oxygen, Fe^{2+}, and Mn^{2+} on the Browning of Amadori Compounds

	Browning (ΔE_{555})[a]				
	Oxidative				
Compds[b]	None	+ Fe^{2+} [c]	+ Mn^{2+}[c]	+ Mn^{2+},[c] Fe^{2+}[c]	Non-oxidative[d]
F-Ser	0.000	0.036	0.002	0.023	0.003
F-Glu	0.000	0.030	0.000	0.015	0.002
F-Gly	0.000	0.100	0.000	0.100	0.003
F-Leu	0.000	0.025	0.000	0.010	0.000
F-Tyr	0.003	0.385	0.020	0.620	0.005
F-Phe	0.003	0.150	0.004	0.260	0.004
F-Trp	0.025	0.230	0.020	0.350	0.010
F-His	0.006	0.210	0.007	0.320	0.008
F-Lys	0.005	0.045	0.003	0.046	0.010
F-Arg	−0.110	0.000	−0.100	0.050	0.115
F-β-Ala	0.006	0.095	0.005	0.096	0.003
F-diGly	0.000	0.102	0.000	0.102	0.015
X-Gly	0.006	0.120	0.005	0.120	0.020
Mixture of parent materials[e]	0.000	0.000	0.000	0.000	0.000

[a] Samples were stored at 37°C for 5 days, and browning was measured after diluting ninefold with water. [b] Concentration of compounds is 0.2 *M* in water. Abbreviations used are the same as Table I. [c] Concentrations of Fe^{2+} and Mn^{2+} are 40 and 30 ppm, respectively. [d] In this case Fe^{2+} and Mn^{2+} had no effect on browning. [e] All the combinations of a parent sugar (0.2 *M*) and an amino acid (0.2 *M*) for Amadori compounds in this table showed no browning under the conditions.

automatic amino acid analyzer using a Durrum single analyzer column (0.8 × 30 cm) with a Pico-Buffer system II. The peaks of amino acids were identified by (i) the elution volume in comparison with the results obtained for authentic amino acid and (ii) the increase in a peak resulting from the addition of an authentic amino acid to the samples.

Synthesis of 3-DG and Glucosone. These were prepared by Kato's (1962) and Bayne's (1963) methods, respectively. 2,4-Dinitrophenylhydrazones (2,4-DNPH) of the compounds gave a single spot on thin-layer chromatography on silica gel (solvent, benzene–ethyl acetate, 1:1).

Measurement of Oxidative Browning. The method is almost the same as previously reported (Hashiba, 1975). Two millimoles of compound was dissolved in 10 ml of water. One-milliliter aliquots were placed into two test tubes (1.5 × 16 cm) and (a) one was plugged with rubber (under aerobic conditions, headspace volume of 28 ml) and (b) another was sealed under vacuum (under anaerobic conditions, <0.3 mmHg). Both samples were held for 5 days at 37°C and the color increase (ΔE_{555}) was measured. The difference of ΔE_{555} between methods a and b was defined as oxidative browning and ΔE_{555} of b as nonoxidative browning in this experiment.

Determination of 3-DG and Glucosone. The method is the same as reported by Kato (1962), that is, 2,4-DNPH of these compounds were prepared and purified with acid–aluminum oxide chromatography. Subsequently, the purified DNPH of 3-DG or glucosone was dissolved in ethyl acetate and measured colorimetrically at 435 nm.

RESULTS AND DISCUSSION

Oxidative Browning of Amadori Compounds. Solutions of an Amadori compound (0.2 *M*) were kept at 37°C for 5 days under anaerobic or aerobic conditions. The color increase of samples (ΔE_{555}) during 5-day's storage at 37°C under both conditions was measured. The oxidative and nonoxidative brownings of the Amadori compounds are shown in Table II. The Amadori compounds browned only slightly without oxygen and in this case Fe^{2+} and Mn^{2+} did not affect the browning. On the other hand, the browning of all the Amadori compounds except fructose–arginine (F-Arg) was increased very significantly when oxygen and Fe^{2+} were present. F-Arg browned notably without oxygen and decolorization was observed in the presence of oxygen. Each mixture of a parent sugar (glucose or xylose) and an amino acid or a peptide did not at all undergo browning under both these aerobic and anaerobic conditions.

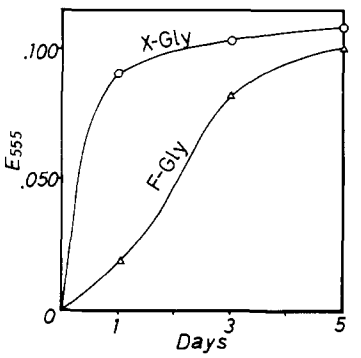


Figure 2. Comparison of the oxidative browning of F-Gly and X-Gly. The method of measurement of oxidative browning is described in the Experimental Section.

Previously, Hashiba et al. (1970) have reported that the oxidative browning of soy sauce is accelerated remarkably by the presence of Fe^{2+} and Mn^{2+} whose concentrations in soy sauce are about 40 and 30 ppm, respectively. The accelerating effect of Mn^{2+} on sugar–amine browning reaction has scarcely been reported. On the contrary, Bohart and Carson (1955) have found inhibition of browning with Mn^{2+} in a glucose–glycine model system. To elucidate the effect of Mn^{2+} on oxidative browning of soy sauce mentioned in the introductory statement, the oxidative browning of the Amadori compounds was investigated in the presence of 30 ppm of Mn^{2+}. As shown in Table II, Mn^{2+} had no effect on the oxidative browning of the Amadori compounds by itself, but together with Fe^{2+}, Mn^{2+} accelerated synergistically the oxidative browning of fructose–tyrosine (F-Tyr), fructose–phenylalanine (F-Phe), fructose–tryptophan (F-Trp), and fructose–histidine (F-His). In the case of F-Arg, Mn^{2+} also appreciably accelerated the oxidative browning synergistically with Fe^{2+}. On the other hand, the oxidative browning of F-Gly, fructose–lysine (F-Lys), F-β-Ala, F-diGly, and X-Gly was not affected and that of fructose–serine (F-Ser), fructose–glutamic acid (F-Glu), and fructose–leucine (F-Leu) was inhibited by the presence of Mn^{2+}. The effect of Mn^{2+} on the oxidative browning of sugar–amino acid systems is not considered to be uniform with the amino acids employed.

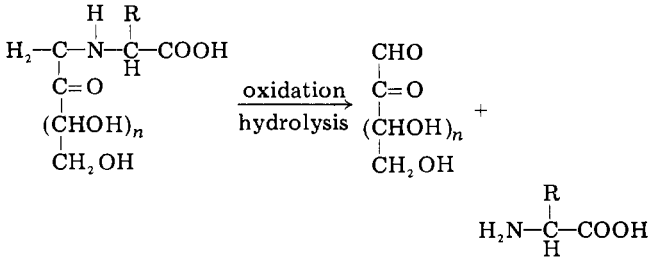
Contrary to many reports (Lewis and Lea, 1950; Spark, 1969) that xylose is more reactive than glucose in the browning reaction, the oxidative browning of X-Gly is close to that of F-Gly as shown in Table II. However, a more detailed investigation exhibited that the rate of the browning reaction of X-Gly is much higher than F-Gly in an early stage. Figure 2 showed that the amount of the browning occurring in 1 day is six times as much for X-Gly as for F-Gly. Amadori compounds from pentose are presumed to be more reactive in an initial velocity than those from hexose in the oxidative browning reaction.

Table III. Effect of Oxygen on the Degradation of F-Gly and F-Phe

	Conditions of storage	Remaining Amadori compds[b]	Liberated amino acid[b]	Glucosone[c]	3-DG[c]
F-Gly[a]	Anaerobic	120	4	0.060	0.360
	Aerobic	80	7	0.200	0.540
F-Phe[a]	Anaerobic	75	20	0.090	1.900
	Aerobic	36	31	0.410	2.600

[a] Water solutions of Amadori compounds (0.2 *M*) were stored at 37°C for 2 months. [b] Micromoles per milliliter. [c] E_{435}, 0.05 ml of samples was used.

Degradation of F-Gly and F-Phe by Oxygen. In order to relate the high oxidative browning of Amadori compounds and their susceptibility to oxygen, the changes of two Amadori compounds during storage under anaerobic or aerobic conditions were compared. F-Gly and F-Phe were chosen as representative Amadori compounds of the simplest and the most aromatic amino acids, respectively. Degradation products of the Amadori compounds are shown in Table III. F-Gly and F-Phe were decomposed by oxygen and a free amino acid (glycine or phenylalanine) and glucosone were found to be produced. The amount of 3-DG produced under aerobic conditions was larger than that under anaerobic conditions. However, even under the latter conditions the amount of 3-DG was appreciable. The results of Table III also support the idea that Amadori compounds were closely related to the browning reaction in the presence of oxygen. One of the interactions of oxygen to the degradation of Amadori compounds is considered as follows.



Oxidative Browning of Carbonyl Compounds. Hodge (1953) has reported that compounds which have an α-hydroxycarbonyl group will undergo significant browning in the presence of amino compounds. Kato et al. (1961) have identified one carbonyl compound, 3-DG, in soy sauce as an important intermediate of the browning reaction. In addition, some carbonyl compounds such as furfural (Dunlop et al., 1946), ascorbic acid (Joslyn, 1957), and dehydroascorbic acid (Ranganna and Setty, 1968) have been studied as highly reactive compounds in the browning reaction. In order to investigate the contribution of these carbonyl compounds to the oxidative browning of soy sauce, the oxidative and nonoxidative browning of the carbonyl compounds is examined in Table IV. In good agreement with Hodge's, Kato's, and Ranganna's reports,

Table IV. Browning of Some Carbonyl Compounds

Carbonyls[b]	Browning (ΔE_{555})[a] Nonoxidative	Oxidative
Glyceraldehyde	0.330	0.000
Dihydroxyacetone	0.390	−0.045
Glucosone	0.130	−0.010
3-DG	0.120	0.005
Ascorbic acid	0.000	0.050
Dehydroascorbic acid	0.690	−0.150
Furfural	0.035[c]	0.110[c]

[a] Samples were diluted ninefold with water. [b] Water solutions of a carbonyl (0.2 *M*) and Gly (0.2 *M*) were stored at 37°C for 5 days. [c] Precipitate of dark materials was produced.

glyceraldehyde, dihydroxyacetone, 3-DG, glucosone, and dehydroascorbic acid are considerably reactive and exhibited a large amount of browning. However, the browning is not increased with oxygen; that is to say, the oxidative browning of these carbonyl compounds is almost negligible. Decolorization with oxygen was observed in some cases. Therefore, these carbonyl compounds are considered to take little part in the oxidative browning of soy sauce.

The acceleration of browning with oxygen was appreciable in furfural and ascorbic acid, but the contribution of these compounds to the oxidative browning of soy sauce is considered to be little because trace amounts only are present in soy sauce (Omata et al., 1955). On the whole, the experimental results in Tables II, III, and IV suggest that carbonyl compounds are less important than Amadori compounds in the oxidative browning of soy sauce.

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LITERATURE CITED

Adachi, S., *Nippon Nogei Kagaku Kaishi* **32**, 313 (1958).
Bayne, S., *Methods Carbohydr. Chem.* **2**, 421 (1963).
Bohart, G. S., Carson, J. F., *Nature (London)* **175**, 4454 (1955).
Burton, H. S., McWeeny, D. J., Pandhi, P. N., *Nature (London)* **199**, 659 (1963).
Clegg, K. M., *J. Sci. Food Agric.* **15**, 878 (1964).
Dunlop, A. P., Stout, P. R., Swadesh, S., *Ind. Eng. Chem.* **38**, 705 (1946).
Hashiba, H., *Agric. Biol. Chem.* **38**, 551 (1974).
Hashiba, H., *J. Agric. Food Chem.* **23**, 539 (1975).
Hashiba, H., Koshiyama, I., Sakaguchi, K., Iguchi, N., *Nippon Nogei Kagaku Kaishi* **44**, 312 (1970).
Hodge, J. E., *J. Agric. Food Chem.* **1**, 928 (1953).
Joslyn, M. A., *Food Res.* **22**, 1 (1957).
Kato, H., *Agric. Biol. Chem.* **26**, 187 (1962).
Kato, H., Yamada, Y., Isaka, K., Sakurai, Y., *Nippon Nogei Kagaku Kaishi* **35**, 412 (1961).
Lewis, V. M., Lea, C. H., *Biochim. Biophys. Acta* **4**, 532 (1950).
Nelson, N., *J. Biol. Chem.* **153**, 375 (1944).
Omata, S., Ueno, T., Nakagawa, Y., *Nippon Nogei Kagaku Kaishi* **29**, 259 (1955).
Ranganna, S., Setty, L., *J. Agric. Food Chem.* **16**, 529 (1968).
Spark, A. A., *J. Sci. Food Agric.* **20**, 308 (1969).
Yoda, A., *Nippon Kagaku Zasshi* **73**, 18 (1952).

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