Note

Malonaldehyde Content in Some Breads and Its Change during Storage

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Malonaldehyde (MA) content in plain bread, French bread, and croissants was analyzed. MA was detected in all the analyzed samples. The MA and lipid contents in croissants were higher than those in other breads. Changes in MA content in plain bread, French bread, and croissants stored at 4°C were examined after 0, 2, and 4 days of storage. The MA content in all the bread samples increased during the storage period. The highest increase of MA content was observed in French bread.

Key words: malonaldehyde; lipid peroxidation; bread; fatty acid

Introduction

Bakery products, particularly those with a high lipid content, tend to become rancid after prolonged storage^{1,2)}, owing to the peroxidation of polyunsaturated fatty acids. During lipid peroxidation, polyunsaturated fatty acids yield malonaldehyde (MA), which is directly or indirectly involved in the multi-stage process of carcinogenesis, particularly DNA damage, sometimes leading to mutations in tumor-suppressor genes³⁾. In addition, when MA coexists with nitrous acid and secondary amines⁴⁾, it accelerates the formation of carcinogenic nitrosamine²⁾.

Numerous papers have reported lipid oxidation in various food items^{5,6)}; however, to our knowledge, only a few papers deal with lipid oxidation in breads^{7,8)}. From the viewpoint of food hygiene, it is important to analyze the MA content in breads. Therefore, MA content and its change were examined in several types of breads stored at 4°C.

Materials and Methods

Materials and storage experiments

Plain breads, French breads and croissants kept at room temperature for 2–4 hours after baking were obtained from two bakeries (A and B). Crusts were removed from plain and French breads before analysis. Each bread was minced in a food processor for 1 min to prepare samples. MA contents in five samples of each bread were analyzed. In addition breads just out of the oven were obtained from bakery A. In order to avoid the effects of temperature fluctuation, each bread was wrapped with plastic wrapping paper followed by aluminum foil and stored at 4°C. The MA contents of 3 samples from each bread were analyzed after 0, 2 and 4 days of storage.

MA analysis

The 1,3-diethyl-2-thiobarbituric acid (DETBA) assay is based on the method of Sakai *et al.*⁹⁾ One gram of each bread sample was homogenized with 9 mL of ice-cooled 10 mmol/L sodium phosphate buffer (pH 7.0) in a Polytron homogenizer at 0°C. An aliquot (less than 0.4 mL) of the homogenate was transferred to a screw-capped tube containing 0.2 mL of 8% SDS and 0.2 mL of 20 mmol/L butyl hydroxy toluene in ethanol, and the final volume of mixture was made up to 0.8 mL with distilled water. After the addition of 3.2 mL of 12.5 mmol/L DETBA in 0.125 mol/L sodium phosphate buffer (pH 3.0), the solution was mixed, heated in a water bath at 95°C for 15 min, and quickly cooled under running tap water. To extract the DETBA–MA adduct, 4 mL of ethyl acetate was added, and the mixture was shaken vigorously. The ethyl acetate extract (2.4 mL) containing the DETBA–MA adduct was transferred to another tube and evaporated in vacuo. The residue was dissolved in 150 µL of methanol, and 10 µL of the sample was subjected to HPLC under the following conditions: column, Inertsil ODS (5 µm particle size, 250 mm × 4.6 mm i.d.; GL Sciences, Tokyo, Japan); mobile phase, acetonitrile–0.1 mol/L sodium chloride (75:25, v/v); flow rate, 1.0 mL/min; detection, excitation at 515 nm and emission at 555 nm.

Total lipids and fatty acids analysis

Total lipids were extracted from the breads by the method of Folch *et al.*¹⁰⁾ and estimated by gravimetric analysis. The content of fatty acids was measured by gas chromatography using *n*-octadecane as internal standard according to Takenoyama *et al.*¹¹⁾

Statistical analysis

All the data were analyzed by ANOVA and significant differences among means were determined by the Duncan multiple range tests¹²⁾.

Table 1. MA Content ($\mu\text{mol/g}$) in Bread Samples

	Plain bread	French bread	Croissants
Bakery A	0.92 ± 0.22	0.65 ± 0.02	4.43 ± 0.39
Bakery B	0.86 ± 0.18	0.48 ± 0.04	4.36 ± 0.22

Mean ($n=5$) $\pm$ standard deviation

Results and Discussion

Table 1 shows the MA content in plain bread, French bread, and croissants.

The MA content in each bread sample obtained from bakery A was higher than that from bakery B; though the difference was not significant. The MA content in croissants obtained from both bakeries was significantly higher than that in other breads. Table 2 shows the lipid contents of these breads.

The lipid content of croissants was much higher than those of other breads. The composition of 18:2 fatty acid was low in croissants, but high in other breads. High 18:2 fatty acid content may be associated with high MA content, because fatty acids containing more than 2 double bonds generate MA during lipid peroxidation³.

Figure 1 shows the changes in MA content in plain bread, French bread, and croissants stored at 4°C. The MA content in these breads increased during 4 days of storage at 4°C, indicating that lipid peroxidation occurred during the storage period. Because the composition of 18:2 fatty acid is very high in French bread, the MA content of this bread may increase rapidly after 2 days of storage. After 4 days of storage, the MA content in croissants was $3.29 \pm 0.24 \mu\text{mol/g}$. The MA content in croissants shown in Table 1 were higher than that in the samples shown in Fig. 1, possibly because the former were kept at room temperature for 2–4 hours after baking. From the viewpoint of food hygiene, it is noteworthy that the MA content in croissants was extremely high and increased even when they were stored at 4°C.

Fig. 1. Changes in MA content in breads stored at 4°C. Error bars indicate the standard deviations of the mean of triplicate measurements. ■: Plain bread, ◆: French bread, ▲: Croissants. Script a–b indicate the difference between storage period in the breads. Script x–z indicate the difference between each bread. Different script indicate significantly different ($p < 0.05$).

As mentioned in the introduction, MA is a carcinogen^{2), 3)} and accelerates the formation of nitrosamine²⁾ in the presence of nitrous acid or secondary amines⁴⁾. From the viewpoint of food hygiene, it is important to analyze the MA contents in many types of breads and to find way to suppress MA formation in them, because bread is one of the staple food items in Japan. Studies of the effect of some antioxidants on MA content in the breads are in progress in our laboratory.

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Table 2. Total Lipid Contents (wt%) and Fatty Acids Composition (%) in Breads

	Plain bread ^a	Plain bread ^b	French bread ^a	French bread ^b	Croissant ^a	Croissant ^b
Total lipid	4.13	3.16	1.15	0.75	21.29	23.5
Fatty acid						
12:0	3.44	1.48	0.14	0.05	3.49	1.14
14:0	2.61	3.52	0.28	0.16	9	4.74
14:1 (n-5)	0.05	0.22	0	0	1.67	0.97
15:0	0.11	0.23	0.08	0	1.12	0.64
16:0	17.13	23.08	16.83	19.75	29.52	30.83
16:1 (n-7)	3.67	0.31	2.5	0.08	3.71	0.87
17:0	0.37	0.22	0.09	0.09	0.9	0.39
18:0	6.55	6.76	1.54	2.75	8.97	10.57
18:1 (n-9)	41.86	46.96	14.27	14.27	32.83	43.18
18:2 (n-6)	21.68	15.44	61.87	59.99	8.01	6.18
18:3 (n-3)	2.13	1.6	2.39	2.73	0.78	0.49
20:0	0.39	0.19	0	0.14	0	0

^a Obtained from bakery A^b Obtained from bakery B

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