宮 崎 大 学 農 学 部 研 究 報 告 55 (87－90) (2009)
Bulletin of the Faculty of Agriculture, University of Miyazaki 55 (87－90) (2009)

Original Articles

Lipid Oxidation of Bread Containing α-Tocopherol

Tadashi SAKAI and Naoko MIKI

Division of Biotechnology and Biochemistry, Department of Biochemistry and Applied Biosciences, Faulty of Agriculture, University of Miyazaki

(Accepted on November 28, 2008)

Summary : Plain breads containing 0, 0.1, 0.2 and 0.5 % of α-tocopherol were stored at 4°C for 4 days and changes in malonaldehyde contents were analyzed.

After baking, malonaldehyde contents in the breads containing 0.1 and 0.2 % α-tocopherol were significantly lower than that of control and 0.5 % α-tocopherol containing breads. After 4 days of storage, malonaldehyde contents in breads containing α-tocopherol were significantly lower than those in control. Present results indicate that α-tocopherol may act as anti-oxidant in the bread.

Key words : Bread, Lipid oxidation, Malonaldehyde., α-Tocopherol

Introduction

The main problem which occurs with reactions of lipid oxidation is the development of unpleasant odors and flavors which reduce the consumers' acceptance and also the shelf life of many foodstuffs (Ladikos & Lougovios, 1990 ; Pearson *et al.* 1983 ; Pearson *et al.* 1977). Like other foodstuffs, bakery products, especially those with high lipid content, are subjected to rancidity (Smith *et al.* 2004 ; Yordanov & Mladenova, 2004). Rancidity is characterized by oxidation of polyunsaturated fatty acids. During lipid oxidation, polyunsaturated fatty acids yield malonadehyde (MA). MA is directly or indirectly involved in multistage process of carcino-genesis and is mainly involved in DNA damage leading sometimes to mutations in tumour suppres-sor genes (Marnett, 1999).

Although both natural and synthetic antioxidants are widely used to prevent lipid oxidation in foods, there has been a general increase on the part of consumers to reject all synthetic additives in food, including antioxidants. In Japan, several natural antioxidants, such as α-tocopherol are added into bread to prevent lipid oxidation. There are many papers reported lipid oxidation in several foods (Shamberger et al. 1977 ; Saeed & Howell, 2002 ; Ladikos & Lougovios, 1990). However to our knowledge, only few papers deal with lipid oxidation in breads (Hsu *et al.* 2004 ; Yordanov & Mladenova, 2004 ; Frutos & Hernandez-Herrero, 2005 ; Sakai & Kawahara, 2005). Fritos and Hernandez-Herrero have reported that effects of rosemary extract on lipid oxidation in breads (Frutos & Hernandez-Herrero, 2005). In other paper, effects of natural antioxidants on lipid oxidation in breads were not examined. In addition, α-tocopherol does not prevent lipid oxidation in meat products when it was added before processing (Benedict *et al.* 1975 ; Aksu & Kaya, 2005). Therefore it is uncertain why it act as antioxidant in breads or not. Therefore, we examined MA contents in some breads and changes in its content in these breads stored at 4°C.

Materials and Methods

Materials and Storage Experiments

The bread was prepared using the following ingredients: 1 kg hard flour, 610 mL warm water, 50 g sugar, 50 g margarine, 30 g whole egg, 20 g dry skim milk, 20 g yeast and 20 g salt. α-Tocopherol (Wako Pure Chemicals, Tokyo, Japan), was added to each

責任著者：境 正
宮崎大学農学部草生物機能科学講座
〒889-2192 宮崎市学園木花台西1-1

Corresponding author : Tadashi Sakai
Division of Biotechnology and Biochemistry
Faculty of Agriculture, University of Miyazaki
1-1 Gakuen Kibana-dai Nishi Miyazaki-shi, 889-2192, Japan

300 g dough and final concentrations were adjusted to 0, 0.1, 0.2, and 0.5 %, respectively. Dough was fermented for 40 min in room temperature. Bread was baked in an oven at 230°C for 20 min. These breads were stored at 4°C and 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* (1999). One g of each bread 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 mixture was finally made up to 0.8 ml with distilled water. After 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 then cooled quickly with running tap water. To extract the DETBA-MA adduct, 4 ml of ethyl acetate was added and the mixture was shaken vigorously. An 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 × 4.6 m i.d. ; GL Sciences, Tokyo, Japan) ; mobile phase, acetonitrile-0.1 mol/l sodium chloride (75 : 25, v/v) ; flow rate, l.0 ml/min ; detection, excitation 515 nm and emission 555 nm.

Statistical Analysis

All data were expressed as the mean±SE. Significant differences among means were determine by the Duncan (1955) multiple range tests.

Results and Discussion

Table 1 shows the changes of MA contents in the breads containing a-tocopherol. As shown in the Table, the contents in the bread containing 0.1 and 0.2 % α-tocopherol were significantly lower than those of control just after the baking. The contents in the bread containing 0.5 % α-tocopherol were lower than those of control although the difference was not significant. These results suggest that α-tocopherol acts as an antioxidant in breads during baking process. MA contents in the breads containing α-tocopherol were significantly lower than those of control after 4 days of storage. During storage α-tocopherol also acts as a antioxidant in breads.

We have recently reported that MA contents in some breads increased during the 4 days of storage at 4°C, indicating that lipid peroxidation occurred in the breads during the storage period (Sakai & Kawahara, 2005). MA is thought to be carcinogenic (Marnett, 1999 ; Ray & Husain, 2002). MA is also known to accelerate the formation of nitrosoamine when coexists with nitrous acid or secondary amine (Ohshima, 1994). Nitrosoamie is also thought to be carcinogenic (Ray & Husain, 2002). Because breads are recently one of the stanple food in Japan, it is necessary to prevent lipid oxidation in breads, from the view point of food hygiene. Therefore, it is noteworthy that addition of α-tocopherol to plain bread suppresses lipid oxidation in the bread.

Table 1. Changes in MA contents in bread of control, those containing 0.1, 0.2 and 0.5 % α-tocopherol stored at 4°C

Days	0	2	4
MA (μmol/g tissue)			
Control	0.17 ± 0.02^{a,x}	0.19 ± 0.02^{a,x}	0.26 ± 0.01^{b,x}
0.1 % α-tocopherol	0.12 ± 0.01^{a,yz}	0.19 ± 0.01^{b,x}	0.20 ± 0.01^{b,y}
0.2 % α-tocopherol	0.11 ± 0.01^{a,y}	0.09 ± 0.00^{a,y}	0.16 ± 0.02^{b,y}
0.5 % α-tocopherol	0.16 ± 0.01^{a,xz}	0.07 ± 0.00^{b,y}	0.18 ± 0.02^{a,y}

a-c Means (n=3) ±SE in the same row with no common superscript differ significantly (P<0.05).

x-z Means (n=3) ±SE in the same column with no common superscript differ significantly (P<0.05).

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α-トコフェロールを添加したパンの脂質酸化

境　正・三木直子

宮崎大学農学部応用生物科学科生物機能科学講座

要　約

発酵前の食パン生地にα-トコフェロールを各0，0.1，0.2および0.5％添加して焼成した後，マロンアルデヒド（MA）含量を経時的に測定した．

0.1および0.2％α-トコフェロール添加パンのMA含量は焼成直後，無添加パンのそれに比べ有意に低くかった．すべてのα-トコフェロール添加パンのMA含量は貯蔵4日目では無添加パンのそれに比べ有意に低く，α-トコフェロールはパン中で抗酸化機能を示した．

キーワード：パン，脂質酸化，マロンアルデヒド，アルファトコフェロール．