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Antioxidative Effect of Tocopherol Isomers Against the Oxidation of Fish Lipid under Various Water Activity Conditions

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Abstract

α-, γ- and δ-Tocopherols were added to a mixture of sardine oil and casein with and without hemin, and the antioxidative effect of tocopherol isomers was compared at 20°C during storage at various water activities (≈0, 0.32, 0.75 and 0.86). γ-Tocopherol showed the longest induction period, and the lowest kinetic constants of lipid oxidation throughout the experiment. And it was considered to be the most effective tocopherol in preventing oxidation of fish lipid.

Introduction

Recently, synthetic antioxidants for food stuffs such as BHT (butylated hydroxytoluene) have been restricted due to their carcinogenic effect. On the other hand, naturally occuring antioxidants have been widely accepted. Tocopherol is representative of one of the widely spread naturally occuring antioxidants and at the same time it is a very useful synthetic antioxidant. Studies of tocopherols (Vitamin E) have been shifted on α-tocopherol (α-Toc) since it is the main tocopherol isomer in the human body. γ-Tocopherol (γ-Toc) has been found in many natural sources, but few studies have been made comparing the biological and antioxidative effect of tocopherol isomers.[1–3] In our preliminary study, we have found that γ-Toc is the most effective antioxidant among the three isomers i.e. α-, γ- and δ-, in a dehydrated model food system using three parts of sardine oil to seven parts of egg albumin (w/w), under various water activity (Aw) conditions. The purpose of this study is to verify the results obtained in the preliminary study by using casein instead of egg albumin, before beginning the study of stability of tocopherol isomers under various conditions.

Materials and Methods

Sardine oil was supplied by the Central Research Institute of Nippon Kagaku Shiryō Co., Ltd., Hakodate. Casein (acc. to Hammarsten) was purchased from Wako Pure Chemicals. α-, γ- and δ-Toc were supplied from Eisai Co., Ltd., Tokyo. All other chemicals used were reagent grade.

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Model Mixtures

An ether solution of sardine oil was added to casein, and a final mixture of three parts of sardine oil to seven parts of the casein (w/w) was prepared following evaporation of the ether. The pro-oxidant systems consisted of 10 ppm of hemin based on the amount of the oil used. The antioxidants used were 200 ppm of α-, γ- or δ-Toc added to the pro-oxidant free and to the pro-oxidant systems. Five grams of each of the mixtures were transfered into separate 100 ml Erlenmyer flasks. The samples were humidified in vacuum desiccators over a salt solution and adjusted to desired water ativities (Aw$\approx$0, 0.32, 0.75 and 0.86) at 20°C. This was done by evacuating the desiccators and allowing the samples to equilibrate for one week. After equilibration, the Erlenmyer flasks containing the mixtures were sealed with a rubber stopper and placed in a chamber at 20°C to measure the oxygen absorption.

Oxygen Absorption

Oxygen and nitrogen contents in the head space of the Erlenmyer flasks were measured by using a Hitachi 164 gas chromatograph equipped with a thermal conductivity detector, on a 3 mm$\times$2 m stainless steel column packed with Molecular Sieve 5A, at 70°C, and flow rate 50 ml/min. The oxygen absorbed in the mixture was calculated from the formula:

$$\text{Absorbed } O_2\ (\%) = 100\ (1 - A_t/A_o)$$

where A_t and A_o are the ratios of O_2 peak areas at zero time and time t, respectively, to the sums of the N_2 and O_2 peak areas in gas chromatograms.

Results and Discussion

The antioxidative effect of tocopherols under various Aw are shown in Fig. 1 and 2 and Table 1. Except at Aw$\approx$0, γ-Toc showed the most antioxidative effect since it had the longest induction period when water exists. This was also supported from the results of kinetic constants which had been calculated from the following formula:[4–7]

$$[O_2]^{1/2} = [\text{ROOH}] = \frac{K_M}{2} \cdot t$$

$$\ln \frac{[O_2]}{1-[O_2]} = K_B \cdot t$$

where K_M is the monomolecular rate constant and K_B is the bimolecular rate constant of the oxidative reactions. Table 2 shows the K_M and K_B of the model system. With only one exception in K_B at Aw=0.32, γ-Toc showed the lowest K_M and K_B when water exists. α-Toc was relatively effective in prolonging the induction peroid of oxidation especially when there is no water (i.e. Aw$\approx$0). δ-Toc seems to be effective in inhibiting monomolecular reaction when there is no water. But it could not inhibit the bimolecular reaction when there is water.

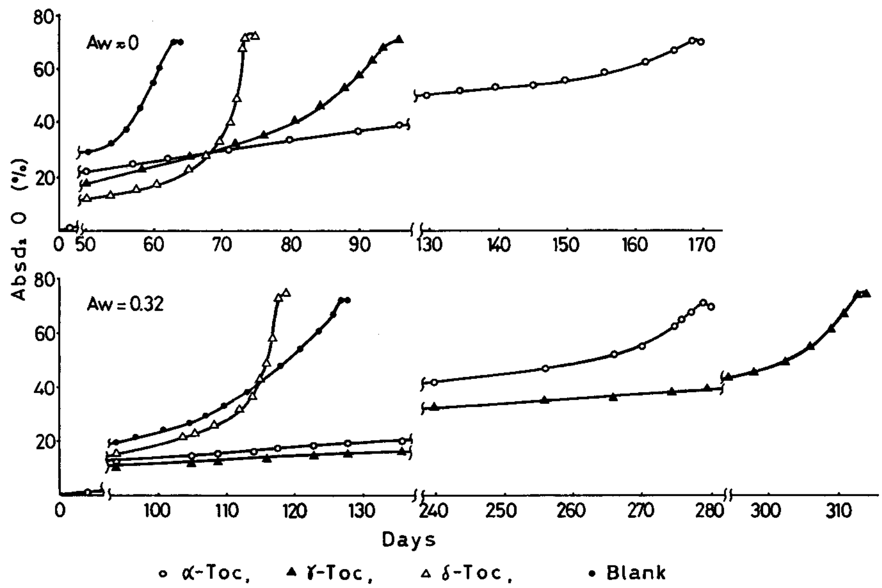

Fig. 1 Oxygen absorption in sardine oil-casein model system without hemin at 20°C, under Aw≈0 and 0.32.

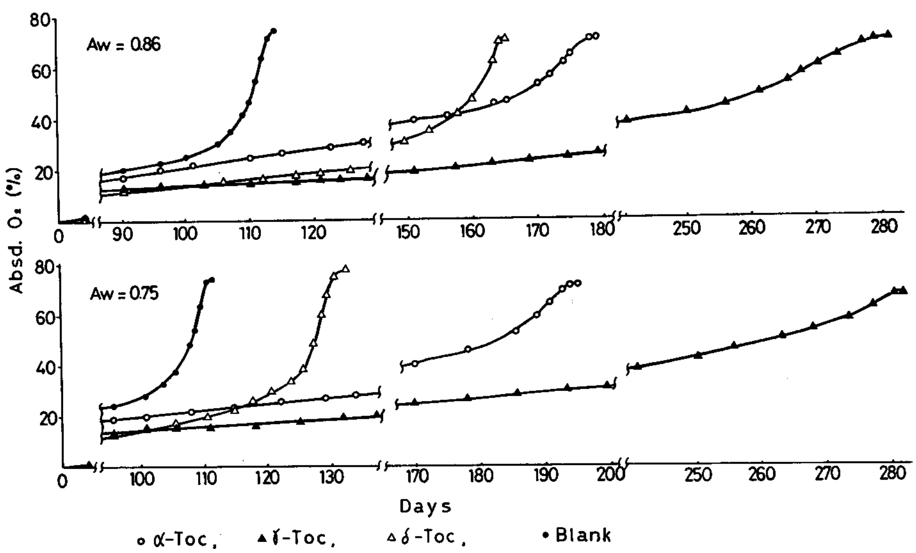

Fig. 2. Oxygen absorption in sardine oil-casein model system without hemin at 20°C, under Aw=0.86 and 0.75.

Table 1. Effect of tocopherols on time required to finish the induction period in sardine oil-casein model system at 20°C.

	Aw			
	≈0 Time (day)	0.32 Time (day)	0.75 Time (day)	0.86 Time (day)
Blank	65	128	110	115
α-Toc	173	280	195	180
γ-Toc	96	301	280	283
δ-Toc	75	118	135	165

Table 2. Kinetic constants in sardine oil-casein model system without hemin at 20°C.

Aw	$K_M \times 10^3$ (moles/mole)$^{1/2}$ day^{-1}				$K_B \times 10$ (day^{-1})			
	≈0	0.32	0.75	0.86	≈0	0.32	0.75	0.86
Blank	4.99	1.98	2.03	2.10	0.42	0.23	0.48	0.58
α-Toc	3.80	1.39	1.94	2.02	0.06	0.11	0.15	0.19
γ-Toc	3.43	1.20	1.33	1.34	0.17	0.19	0.08	0.10
δ-Toc	3.08	1.71	1.74	1.68	1.01	0.86	0.62	0.53

K_M. Monomolecular rate constant.
K_B. Bimolecular rate constant.

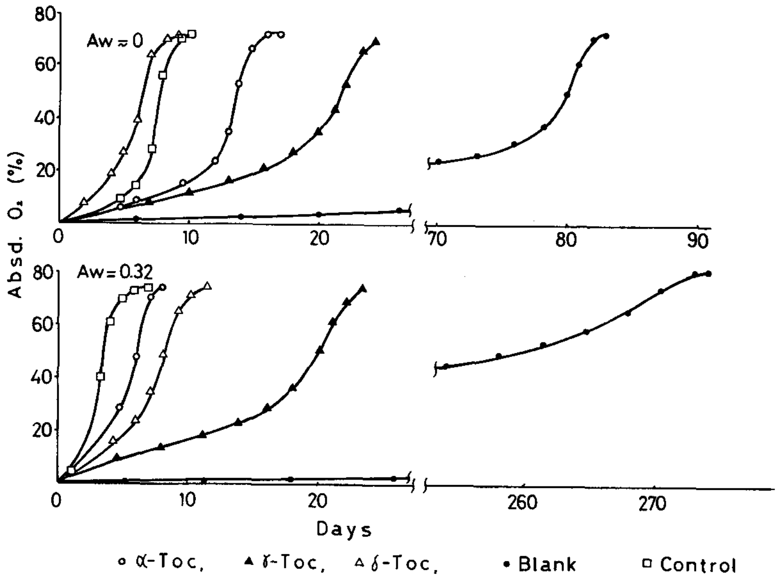

Fig. 3 Oxygen absorption in sardine oil-casein model system with hemin at 20°C, under Aw≈0 and 0.32.

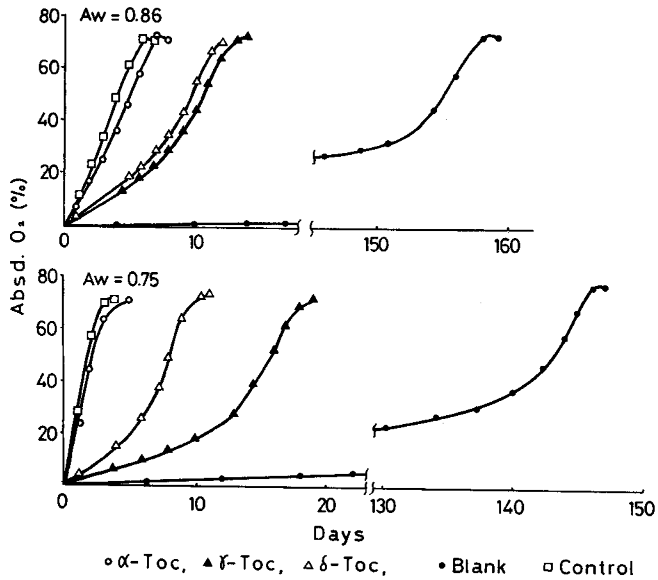

Fig. 4. Oxygen absorption in sardine oil-casein model system with hemin at 20°C, under Aw=0.86 and 0.75.

Table 3. Kinetic constants in sardine oil-casein model system with hemin at 20°C.

Aw	$K_M \times 10^3$ (moles/mole)$^{1/2}$ day^{-1}				$K_B \times 10$ (day^{-1})			
	≈0	0.32	0.75	0.86	≈0	0.32	0.75	0.86
Blank	3.58	1.16	1.18	1.46	0.41	0.29	0.68	0.45
Control	*	*	*	*	3.08	11.20	24.00	24.00
α-Toc	17.21	*	*	*	1.74	1.42	14.20	1.63
γ-Toc	12.01	9.36	16.25	25.10	0.49	0.77	0.79	1.00
δ-Toc	*	*	*	*	0.98	1.42	14.20	1.22

* Imposible to calculate the KM.
KM: Monomolecular rate constant.
KB: Bimolecular rate constant.

The antioxidative effect of tocopherols with hemin under various Aw are shown in Fig. 3 and 4 and Table 3. In this case, the pro-oxidative effect of hemin increased drastically in proportion to the increase in Aw, probably because of the increase in mobility of hemin as a catalyst of oxidation. Even under these conditions, γ-Toc acted as the most antioxidative tocopherol among the isomers. At all Aw examined, control systems, i.e. with hemin and without tocopherol, exhibited bimolecular reaction immediately after the measurement of oxygen absorption. The oxidation rate was so rapid in α- and δ-Toc systems with hemin at Aw more than 0.32, that it was impossible to determine the induction period and to calculate the K_M as shown in Table 3. γ-Toc was the only available tocopherol to give the induction period in hemin-containing systems. K_B was the lowest in γ-Toc at all

Aw followed by δ-Toc at Aw≂0 and 0.86. K_B of α- and δ-Toc at Aw=0.32 and 0.75 was almost the same, but that is to say nothing of γ-Toc which has the lowest K_B at those Aw. The fact that γ-Toc was the most antioxidative among tocopherols examined, coincided with the results of Reinton et al.,[2] but disagreed with the results obtained by Kajimoto et al.[1] and Ōtsuki.[3] This is presumably due to the differences in the analytical conditions. Namely, interactions between oil-water-protein- and tocopherol exist in the mixtures prepared in this study in contrast to the oil systems used in other studies. And another factor might be the differences in the oil used. In this study, sardine oil which is abundant in eicosapentaenoic acid and docosahexaenoic acid was used. Though further careful examination is required to give a conclusion, γ-Toc is considered to be the most effective antioxidant among the tocopherols examined for preventing oxidation of fish lipid which contains highly unsaturated fatty acid. Widicus et al.[8] studied the stability of α-Toc after storage under various Aw by checking the α-Toc content using high performance liquid chromatograph. He considered that the storage stability of α-Toc is dependent upon the lipid composition of the food product. From this aspect, it can be assumed that γ-Toc has a higher stability against the oxidation of highly unsaturated fatty acid and therefore it was effective when fish oil was used. Livingston et al.[9] reported that the rate of α-Toc loss increased as a function of the moisture content of alfalfa. Results shown in Table 1 support this phenomenon since at above Aw=0.32, the induction period of α-Toc mixtures decreased in proportion to the increase in moisture content. But further examination is required to see whether this decrease in the induction period is the result of the decrease in α-Toc remaining under high moisture. The relationship between the stability of tocopherol isomers in various moistures and their antioxidative effect should be examined in the next study.

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