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Reaction Mechanisms and Kinetics of Antioxidant Using Arrhenius Equation in Soybean Oil Oxidation

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Abstract

The reaction mechanisms and kinetics of tertiary butylhydroquinone(TBHQ) as an antioxidant in soybean oil oxidation were studied. The oxidation reaction of soybean oil at 55, 60 and 65°C was a first order. The activation energies of soybean oil containing 0, 25, 50, and 75ppm TBHQ were 12.15, 6.05, 6.15 and 6.17kcal/mole, respectively. The addition of TBHQ to soybean oil decreased the temperature dependency on oxidation reaction. The frequency factor of soybean oil containing 0, 25, 50, and 75ppm TBHQ were 1.88×10^7, 4.10×10^2, 4.32×10^2, and 3.97×10^2, respectively. The decrease of frequency factor in the presence of TBHQ may be due to the combination of (a) the radical quenching by TBHQ and (b) the loss of rotational freedom in the transition state. The addition of TBHQ influenced the frequency factor rather than the activation energy. The effects of antioxidants on the temperature dependency of lipid oxidation could be effectively evaluated by measuring their effects on the activation energy of lipid oxidation.

Key words: mechanisms and kinetics of antioxidant, TBHQ, soybean oil, temperature dependency

INTRODUCTION

Oxidation of foods could influence flavor quality, nutritional quality, consumer acceptance and toxicity of food products(1,2). Lipid oxidation is mainly responsible for the development of rancidity by the production of low molecular weight compounds that imparts undesirable flavor. Oxidation of fat and oil is a chain reaction which is initiated and propagated by the formation of free radicals. A free radical is any species that contain one or more unpaired electrons. The free radicals of oils react with diradical molecular oxygens to form peroxy radicals. The peroxy radical forms hydroperoxide which is decomposed to produce low molecular weight volatile off-flavor compounds. The abstraction and addition of free radicals yield a second radical that can participate in more radical reactions. The process of radical reactions of repeat creation of new and reactive radicals is named a free radical chain reaction and several different radical species may participate. The termination of this reaction is accomplished by removal of free radicals before they can catalyze the autoxidative process which leads to the rancidity of fats and oils. The antioxidants action may be described as follows: $R\cdot$ or $ROO\cdot + AH \rightarrow RH$ or $ROOH + A\cdot$ where $R\cdot$ is the free radical and AH is the antioxidant. The resultant antioxidant free radical($A\cdot$) is a stable resonance compound which does not initiate the free radical formation of other molecules and does not propagate chain reaction. Antioxidants are often described as free radical inhibitors since they act by interfering with free radical mechanism of autoxidation. Antioxidants are frequently added to foods to minimize the oxidation of fats and oils during storage and normal marketing distribution.

Player(3) reported that more than 15 million pounds of antioxidants were used by U.S. food manufactures in 1990. Due to the importance of antioxidants in extending shelf life of many foods, the qualitative and quantitative effects of synthetic and natural antioxidants on the oxidative stabilities of numerous foods have been extensively studied. However, the specific antioxidant reaction mechanisms and kinetics in the lipid oxidation have been rarely reported.

The purpose of this research is to study the reaction mechanisms and kinetics of TBHQ as an antioxidant using Arrhenius equation in the oxidation of soybean oil.

MATERIALS AND METHODS

Materials

Soybean oil was obtained from Karlsham Inc.(Columbus, Ohio, USA). TBHQ was obtained from Eastman Chemical Products, Inc., Kingsport, TN, USA.

Preparation for soybean oils containing 0, 25, 50 and 75ppm TBHQ

Soybean oils containing 0, 25, 50, and 75ppm TBHQ were prepared. All the oil samples were stirred slowly with a glass rod for 5 minutes at room temperature. Fifteen ml of each sample was transferred to 35ml serum bottles. The bottles were sealed air-tight with Teflon-coated rubber septa and aluminum caps and placed in the air-forced ovens at 55, 60 and 65°C. The oxidation of oil was determined by measuring the oxygen disappearance in the headspace of sample bottle daily for 6 days. The analysis was carried out in triplicate.

Analysis of soybean oil oxidation by gas chromatography

The oxidation stability of soybean oil was determined by measuring the headspace oxygen reacted with soybean oil in the air-tight serum bottle. Thermal conductivity gas chromatography was used to determine the headspace oxygen content of sample bottle. Forty ml of headspace was injected into a gas chromatography(Hewlett Packard 5890) equipped with a thermal conductivity detector(4). The temperatures of oven, injection port and detector were 40, 120 and 180°C, respectively. The flow rate of helium was 24ml/min. A stainless steel column(1.83×0.32cm) packed with 80/100 mesh Molecular Sieve 13X(Alltech, Deefield, IL, USA) was used. The gas chromatographic oxygen peak area of headspace gas was determined using an electronic integrator(H.P. 5880). The oxygen content in the headspace was expressed as %, assuming that the oxygen content of air was 21.0%.

Statistical analyses

The headspace oxygen contents are mean values of triplicate samples. Tukey's Test(5) was used to ascertain the differences of headspace oxygen contents of soybean oil. Fisher's Least Significance Difference Test (6) was used to ascertain the differences of rate constants, activation energy, frequency factor, and Q_{10} of the oxidation of soybean oils containing 0, 25, 50 and 75ppm TBHQ stored at 55, 60 and 65°C under darkness.

RESULTS AND DISCUSSION

The coefficient of variation for the determination of headspace oxygen content of soybean oil for 6 replicate samples was 0.82% which was considered as excellent.

The effects of 0, 25, 50 and 75ppm TBHQ, and 55, 60 and 65°C storage temperatures on the headspace oxygen contents of soybean oil bottles are shown in Table 1. The headspace oxygen contents of the bottles containing 25, 50 or 75ppm TBHQ were significantly higher than the control containing no TBHQ($p<0.05$). The un-

Table 1. Turkey's test for the effects of 0, 25, 50, and 75ppm TBHQ on the headspace oxygen of soybean oil bottles during storage at 55, 60 and 65°C under darkness[1)]

TBHQ(ppm)	Headspace oxygen content(%)						
	1day	2day	3day	4day	5day	6day	Mean[2)]
Storage at 55°C							
0ppm	18.8	16.2	13.9	12.3	10.3	8.9	13.4[a]
25ppm	20.4	19.6	19.2	17.7	17.6	16.8	18.6[b]
50ppm	20.6	19.7	9.4	18.6	17.9	17.0	18.9[b]
75ppm	20.7	20.0	19.5	18.7	18.2	17.5	19.1[b]
Storage at 60°C							
0ppm	18.6	14.5	12.1	9.9	8.7	6.0	11.6[a]
25ppm	20.2	19.3	18.5	17.7	16.9	16.2	18.1[b]
50ppm	20.3	19.5	18.7	18.0	17.3	16.6	18.4[b]
75ppm	20.5	19.7	19.1	18.0	17.8	16.9	18.7[b]
Storage at 65°C							
0ppm	17.7	11.9	10.1	7.7	6.0	4.6	9.6[a]
25ppm	20.0	19.1	18.1	17.2	16.3	15.7	17.7[b]
50ppm	20.1	18.8	18.0	17.1	16.8	15.9	17.8[b]
75ppm	20.2	19.5	18.6	17.8	17.5	16.3	18.3[b]

[1)]The initial headspace content was 20.9%

[2)]Means in a column with different superscripts are significantly different at $p<0.05$

reacted and remained headspace oxygen increased as the concentration of TBHQ increased from 25 to 50 and 75ppm, but were not significant($p>0.05$). The headspace oxygen of samples containing 0, 25, 50 or 75ppm TBHQ decreased as the temperature increased from 55 to 60 and 65°C. The effects of 55, 60, and 65°C on the headspace oxygen contents of sample bottles containing no TBHQ were significant($p<0.05$) but, were not significant among the samples containing 25, 50, or 75ppm TBHQ($p>0.05$). The temperature dependence of lipid oxidation decreased among the soybean oils in the presence of TBHQ.

The headspace oxygen contents of soybean oils containing 0ppm TBHQ during storage at 55, 60 and 65°C are shown in Fig. 1. The correlation coefficient of the linear regression equation between Ln(headspace oxygen content) and storage time were greater than 0.98. The high value of correlation coefficient suggests that soybean oil oxidation was a first order reaction(7). The figures(not shown) between Ln(headspace oxygen content) and storage time of soybean oils containing 25, 50 or 75ppm TBHQ show the similar patterns as shown in Fig. 1 and the correlation coefficients of the regression lines were greater than 0.98.

The rate constants of first order oxidation reaction of soybean oils containing 0, 25, 50 and 75ppm at 55,

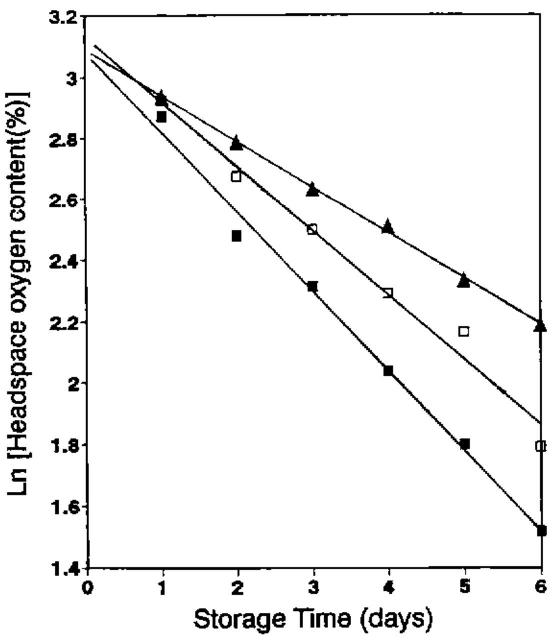

Fig. 1. Plot of Ln(headspace oxygen content) versus storage time for soybean oil containing 0ppm TBHQ at 55, 60 and 65°C.
▲: 55°C, □: 60°C, ■: 65°C

Table 2. Fisher's least significance difference test for the oxidation rate constants per day of soybean oils containing 0, 25, 50, and 75ppm at 55, 60 and 65°C

Temperature(°C)	TBHQ concentration(ppm)			
	0	25	50	75
55	0.150[a]	0.038[d]	0.035[d]	0.031[d]
60	0.211[b]	0.044[d]	0.039[d]	0.036[d]
65	0.260[c]	0.050[d]	0.046[d]	0.041[d]

Means in a vertical column with different superscripts are significantly different at $p<0.05$
Means in a row column with different superscripts are significantly different at $p<0.05$

60 and 65°C are listed in Table 2. The rate constants of soybean oil oxidation increased as the storage temperature increased and decreased as the TBHQ concentration increased. The oxidation rate constants of soybean oils containing 25, 50 or 75ppm TBHQ were significantly lower than the control containing no TBHQ ($p<0.05$). The oxidation rate constants of soybean oil containing 25, 50 or 75ppm TBHQ were not significant ($p>0.05$). The effects of 55, 60, and 65°C on the oxidation rate constants of soybean oils containing 25, 50 or 75ppm TBHQ were not significant($p>0.05$), but were significant in the oils containing no TBHQ(Table 2). The effects of reaction temperature on the oxidation rate constants were analyzed according to the Arrhenius equation: $k=k_0\exp(-E_a/RT)$ where k is the rate constant and k_0 is the frequency factor, E_a is the activation energy, R is the gas constant, and T is the absolute temperature. Arrhenius equation has been widely used in kinetic studies for foods(8,9). Levenspiel(10) reported that the reaction temperature effects on the frequency factor is rather minor and can be ignored in Arrhenius equation. Since $k=k_0\exp(-E_a/RT)$ and k_0 is not generally influenced by reaction temperature, the increased oxidation rate constant as a function of increased temperature in Table 2 might be mainly due to the increased $\exp(-E_a/RT)$.

Fig. 2 shows that the plot of Ln(k) for soybean oil containing 0ppm TBHQ as a function of 1/T. The activation energy of soybean oil oxidation containing no TBHQ can be calculated from the slope of the Fig. 2. The Arrhenius plots(not shown) for soybean oils containing 25, 50 or 75ppm TBHQ showed the similar patterns as Fig. 2 with different slopes.

The activation energies, frequency factors, and Q_{10} values of oxidation of soybean oils containing 0, 25,

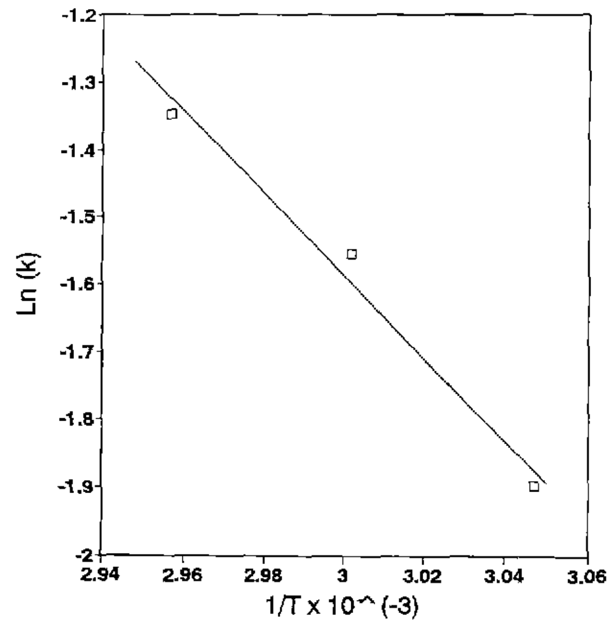

Fig. 2. Arrhenius plot of the oxidation rate constants in soybean oil containing 0ppm TBHQ.

Table 3. Fisher's least significance difference test for frequency factors, Q_{10} values and activation energies for the oxidation of soybean oils containing 0, 25, 50 and 75ppm TBHQ

TBHQ (ppm)	Activation energy (kcal/mole)	Frequency factor	Q_{10} value (55/65°C)
0	$12.15+1.61^{a}$	1.88×10^{7a}	1.73^{a}
25	$6.05+0.19^{b}$	4.10×10^{2b}	1.32^{b}
50	$6.15+0.41^{b}$	4.32×10^{2b}	1.32^{b}
75	$6.17+0.20^{b}$	3.97×10^{2b}	1.32^{b}

Means in a vertical column with different superscripts are significantly different at $p<0.05$

50 and 75ppm TBHQ were listed in Table 3. The activation energy of soybean oil containing no TBHQ was 12.15±1.61kcal/mole, which is within the range of 10 ~15kcal/mole according to Labuza(11). The activation energies of soybean oils containing 25, 50, and 75ppm TBHQ were 6.05, 6.15 and 6.17, respectively. The activation energy of soybean oil containing no TBHQ was significantly higher than those containing 25, 50 or 75 ppm TBHQ at $p<0.05$. However, the activation energies of soybean oils containing 25, 50 or 75ppm TBHQ were not significant($p>0.05$). Levenspiel(10) reported that the chemical reactions with high activation energies are temperature sensitive; the reactions with low activation energies are temperature insensitive. That is, the reaction temperature change has significant effect on the chemical reaction rates in the temperature sensitive reactions. Since the addition of TBHQ decreased the activation energy of soybean oil oxidation, the addition of TBHQ to soybean oil decreased the temperature dependency on oxidation reaction. The frequency factor of soybean oil containing no TBHQ is 1.88×10^{7} and those of soybean oils containing 25, 50 or 75ppm TBHQ were 4.10×10^{2}, 4.32×10^{2} or 3.97×10^{2}, respectively(Table 3). The atmospheric molecular oxygen is a triplet state which contains two unpaired electrons. The electron arrangement of a triplet oxygen does not allow for a direct reaction with nonradical organic compounds such as unsaturated fats that exist in the singlet state. Bamford and Dewar(12) reported the frequency factor of reactions involving two organic radicals was approximately 1.0×10^{7}. The frequency factor of control sample was significantly higher than those of samples containing 25, 50 or 75ppm TBHQ($p<0.05$). The frequency factors of soybean oils containing 25, 50 or 75ppm TBHQ were not different at $p>0.05$(Table 3). Evans(13) reported that the frequency factor of Arrhenius equation would depend on the entropy of the activated complex formation. Evans and Szware(14) reported that the increased complexity of molecules and radicals leads to the loss of rotational freedom in the activated complex. The addition of TBHQ to soybean oil will decrease the rotational freedom in the activated complex and may decrease the entropy of the activated complex formation. Since the frequency factor of Arrhenius equation is exp ($\Delta S/R$), the decrease in ΔS of activated complex formation in the presence of TBHQ will decrease the frequency factor. The addition of 25ppm TBHQ decreased the frequency factor between soybean oil free radicals and molecular oxygen in the sample bottle from 1.88×10^{7} to 4.10×10^{2}(Table 3). The result agrees with the suggestions of Evans and Szware(14). The simultaneous decrease of frequency factor and activation energy in Table 3 agrees with Bolland et al.(15,16) who reported that the frequency factor of Arrhenius equation decreased with decreased activation energy in the chain propagation reaction. Bolland et al.(15,16) also reported that if there is a change in reaction rate due to the variation of olefinic structure in the absence of antioxidant, the change of activation energy plays more important role than the frequency factor change. Table 3 shows that TBHQ influenced the frequency factor more than the activation energy in the reaction rates of soybean oil. Uri(17) postulated that the greater changes in frequency factor than the activation energy in the lipid oxidation in the presence of antioxidant. The

loss of rotational freedom in the transition state due to the presence of antioxidants can be led to low frequency factors. Such low frequency factors would be experienced in collisions between two long-chain molecules, as is indeed the case in the propagation reaction of autoxidation of soybean oil. The addition of TBHQ to soybean oil must reduce the concentration of free radicals by donating the hydrogen. The reduction of free radicals by TBHQ is most likely responsible for the reduction of frequency factor of Table 3. The result in Table 3 was consistent with the theory of Uri(18). The decrease of frequency factor in the presence of TBHQ may be due to the combination of (a) the radical quenching by TBHQ, and (b) the loss of rotational freedom in the transition state. The Q_{10} values(55/65°C) were determined from the reaction rate constants in Table 2. The Q_{10} values of soybean oil containing 0, 25, 50 and 75ppm TBHQ were 1.73, 1.32, 1.32 and 1.32, respectively. The Q_{10} value of sample containing 0ppm TBHQ was significantly higher than those of samples containing 25, 50 or 75ppm TBHQ at $p<0.05$. Park(19) reported the Q_{10} value(55/65°C) of soybean oil oxidation without antioxidants was 1.77.

In summary, the oxidation reaction of soybean oil at 55, 60, and 65°C was a first order. The addition of TBHQ decreased the frequency factor, and also activation energy to minimize the reaction rate of lipid oxidation. The addition of TBHQ influenced the frequency factor more than the activation energy to minimize the lipid oxidation. The effects of antioxidants on the temperature dependency of lipid oxidation could be effectively evaluated by measuring their effects on the activation energy of lipid oxidation.

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