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Effect of Metal Salts and Antioxidants on the Oxidation of Fish Lipids during Storage under the Conditions of High Moistures

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Abstract

Cu++, Fe+++ and hemin were added to a mixture of mackerel-sardine oil and egg albumin, and the pro-oxidative properties were determined at 20°C during the storage at water activities (a_w) 0.75, 0.86 and 0.95, respectively. At a_w=0.95, hemin system was the most pro-oxidative as was Cu++ systems at a_w=0.75 and 0.86.

Antioxidative effect of ethylene diamine tetraacetic acid (EDTA) was slightly observed only in the mixture of Cu++ at a_w=0.95. α-Tocopherol was effective against the oxidation caused by hemin and Cu++. But butylated hydroxyanisole (BHA) was more effective compared with α-tocopherol especially at a_w=0.75 and 0.86 against the oxidation caused by Cu++.

Introduction

In the previous paper[1], the effects of metal salts and antioxidants on the oxidation of fish lipids during storage under the conditions of low and intermediate moistures were reported. In the present study, the oxidation of fish lipids under the conditions of high moistures was studied. It has been reported by Labuza et al.[2] and Tjhio et al.[3] that a water soluble antioxidant such as ethylene diamine tetraacetic acid (EDTA) is effective under the existence of free water, and in contrast to this, under the condition of very little amount of free water, a water insoluble antioxidant such as butylated hydroxytoluene (BHT) becomes effective.

In this study, α-tocopherol, butylated hydroxyanisole (BHA) and EDTA were examined to compare the effectiveness on the oxidations induced by Cu++, Fe+++ and hemin, in addition to the studies on pro-oxidative properties of these metals under the conditions of high moistures, since most foods are classified as high moisture food.

Materials and Methods

Mackerel-sardine oil was supplied by the Central Research Institute of Nippon Kagaku Shiryō Co. Ltd., Hakodate, Japan. Egg albumin (Difco Labolatories) was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. All other chemicals used were reagent grade.

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Model Mixtures

Model mixtures were prepared in the same manner as described in the previous paper[1], and adjusted to desired water activities (a_w=0.75, 0.86 and 0.95) at 20°C.

Oxygen Absorption

Oxygen absorption of the prepared mixture was measured in the same manner as described in the previous paper.[1]

Results and Discussion

The effect of high water activities (a_w=0.75, 0.86 and 0.95) on the catalytic rate of oxidation induced by metal ions in the model mixtures, are shown in Fig. 1 and Table 1. At a_w=0.95, hemin system showed an extremely short induction period of lipid oxidation. And there was a tendency for the higher water activity to produce a correspondingly shorter induction period of lipid oxidation. Cu^{++}

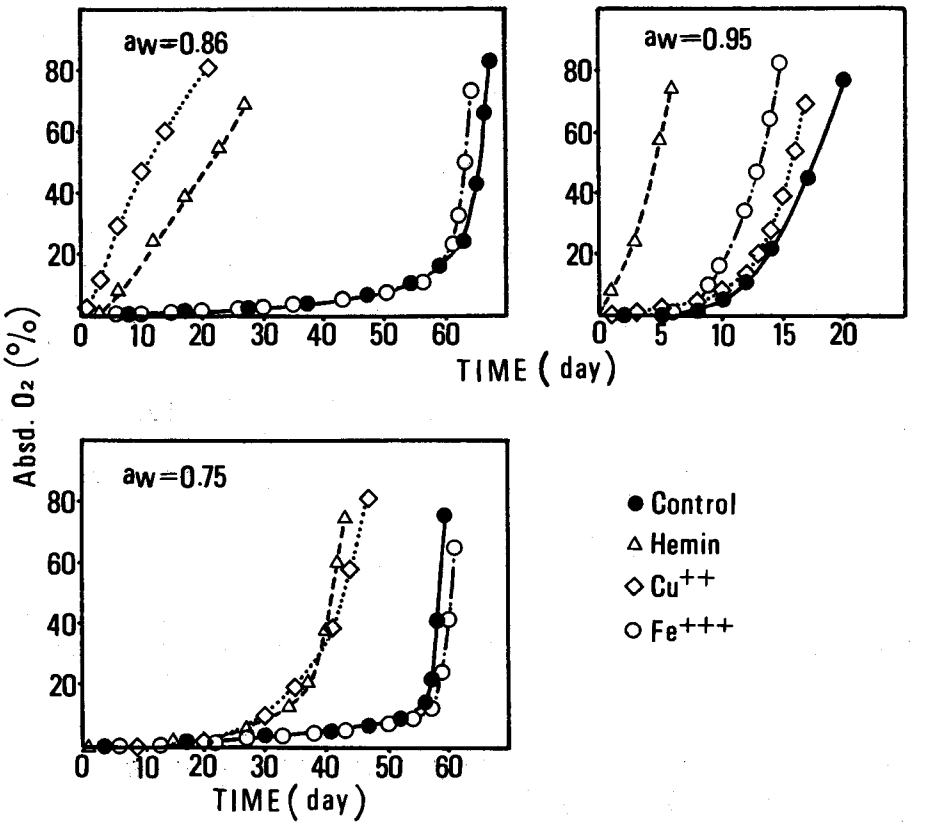


Fig. 1. Oxygen absorption of model system as a function of a_w at 20°C.

Table 1. *Effect of humidification on time required to reach 2% oxidation in mackerel-sardine oil - egg albumin model system at 20°C.*

	a_w		
	0.75	0.86	0.95
	Time (day)	Time (day)	Time (day)
Control	50	50	11
Fe^{+++}	51	51	10
Cu^{++}	29	2	10
Hemin	31	7	1

systems were characteristic since it showed a shorter induction period than control at a_w=0.75 and 0.86, whereas at a_w=0.95, the induction period was almost the same with the control.

Maloney et al.[4] and Labuza et al.[2] studied the kinetics of the effect of water on lipid oxidation using model mixtures, and demonstrated the kinetic plots. Figure 2 shows the plot of the square root of oxygen absorbed in moles of oxygen, per mole of lipid against the storage time. The plots indicated a straight line up to 1~2% of the oxidation level at a_w=0.75 and also at a_w=0.86 except in the case of Cu^{++}

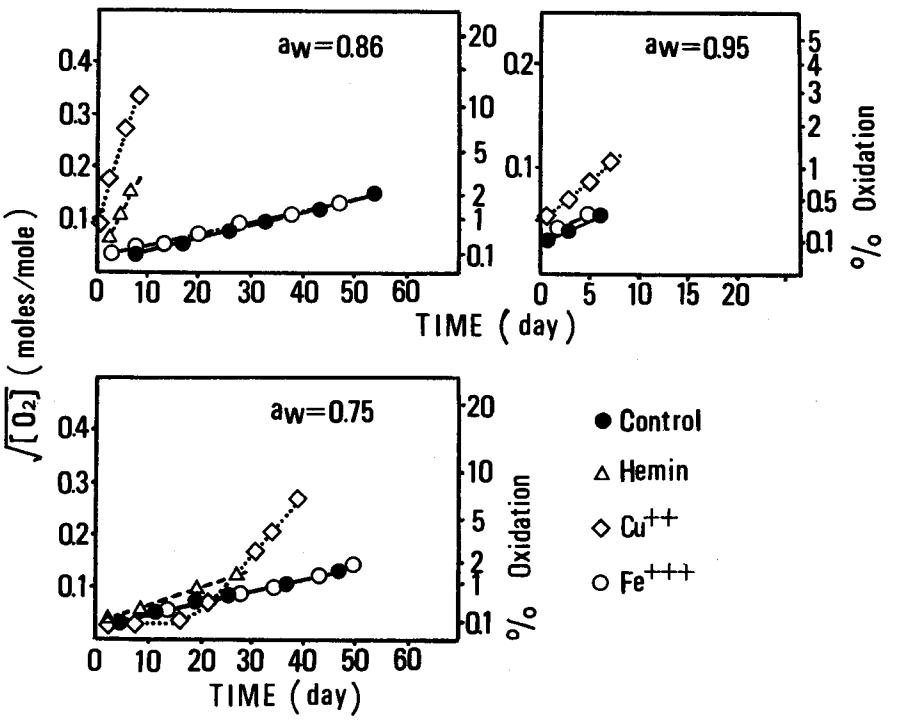
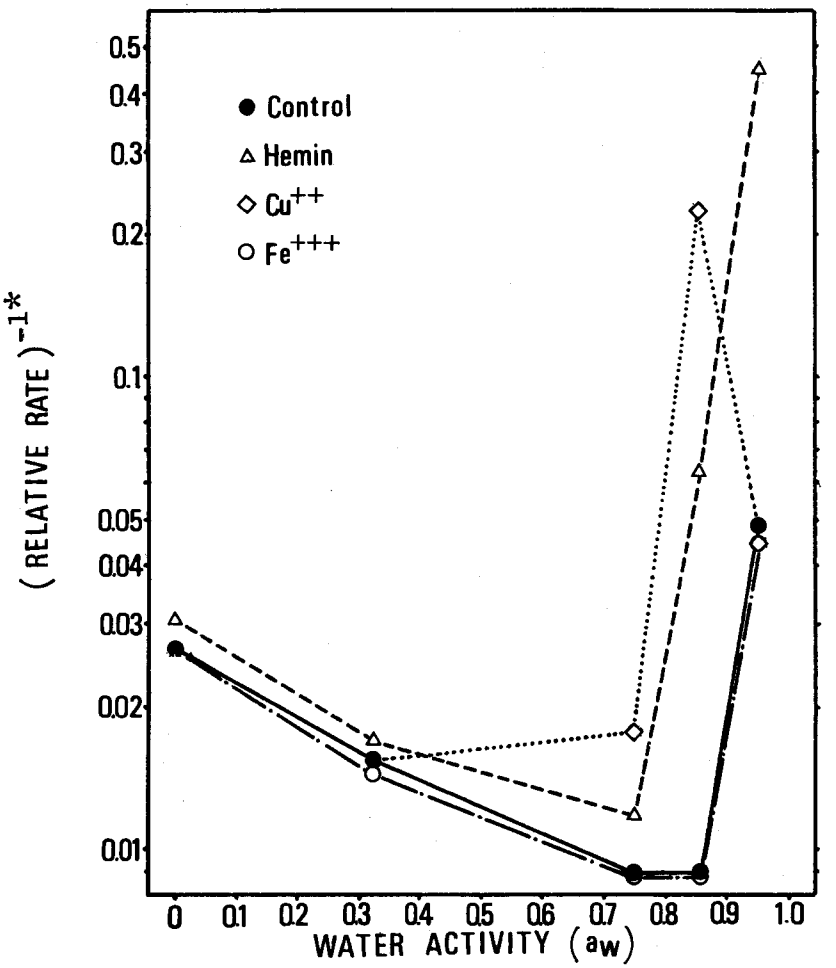


Fig. 2. Oxidative reaction kinetic plot for the monomolecular rate period in model system as a function of a_w at 20°C.

added mixture. And it is assumed that the oxidation in this region proceeds as monomolecular decomposition reaction of hydroperoxides. On the other hand, Cu^{++} added mixtures indicated a straight line up to 7% of the oxidation level, and for this reason it was characteristic. At a_w=0.95, the oxidation rate of hemin system was so high that it was impossible to see a monomolecular reaction stage. It is considered that hemin becomes most active as a catalyst in accordance with the increase of free water amount, and this fact is attributed to the largest mobilization in free water among the metal salts examined.

Figure 3 illustrates the relationship between reciprocal value of induction period (relative rate) and a_w including low and intermediate moistures. As shown



* Reciprocal value of the ratio against the induction period of control at a_w=0.75.

Fig. 3. Relation between reciprocal value of induction period and a_w.

by the dotted line, two critical water activity points were observed in the case of added Cu^{++}; whereas in the cases of the control and the mixtures with hemin, there was only one each. This characteristic of Cu^{++} was hard to explain by the difference in the solubility[1]. At $a_w \fallingdotseq 0$, oxidation proceeded to a considerable degree followed by a general decrease in oxidation up to the point around $a_w=0.75$. It was assumed that at these low and intermediate moisture levels, the mobilities of metals might be very low. When water activity is at the high level, the mobility of the metal might be extremely high, and as a result, pro-oxidative effect of metal might exceed the antioxidant effect of water to give totally pro-oxidative effect.

Figure 4 through 6 show the effects of antioxidants and chelating agent on oxygen absorptions of the model mixtures especially constituted Cu^{++} or hemin, as a function of a_w. The results were obtained by using a different batch of lipid from the experiments without those agents. Table 2 shows the relative rate of the time to reach 1% oxidation and 2.5% oxidation since most of the induction period of lipid oxidation finishes at a 1~2.5% oxidation level. As evident from

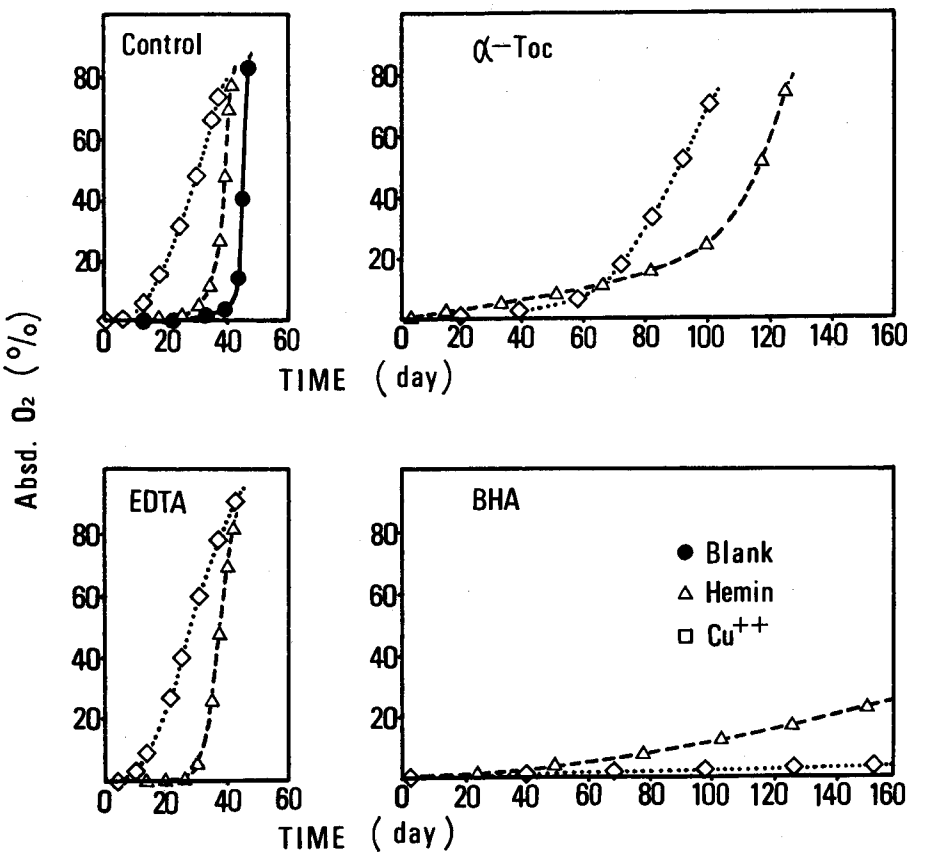


Fig. 4. Effect of antioxidant and chelating agent on oxygen absorption of model system at $a_w=0.75$ (20°C).

this table, BHA acted extremely synergetically with the antioxidative action of water against the catalytic action of Cu^{++}, especially at a_w=0.86. Time to reach 1% oxidation in Cu^{++} with BHA system was 5 times longer than that in Cu^{++} without BHA system at a_w=0.75 and 0.95, and about 25 times longer at a_w=0.86. And for 2.5% oxidation, 11 times, 3 times and 25.5 times longer at a_w=0.75, ≈0 and 0.86 respectively. In the cases of hemin system, BHA acted synergetically with water to lengthen the induction period 1.5～3 times at lower and higher water activities. But it was not so effective as it was in the Cu^{++} systems at a_w=0.75 and 0.86. α-Tocopherol also acted extremely synergetically with the antioxidative action of water against the catalytic action of Cu^{++} especially at a_w=0.86. Time to reach 1% oxidation in Cu^{++} with α-tocopherol systems were 3～5 times longer than Cu^{++} without α-tocopherol at a_w=0.75, 6 times longer at a_w=0.95 and 15 times longer at a_w=0.75 and 0.95, and 15.5 times longer at a_w=0.86. In the cases of hemin systems, α-tocopherol acted synergetically with water to lengthen the induction period 1.5～7 times up to reaching 1% oxidation level at lower and higher

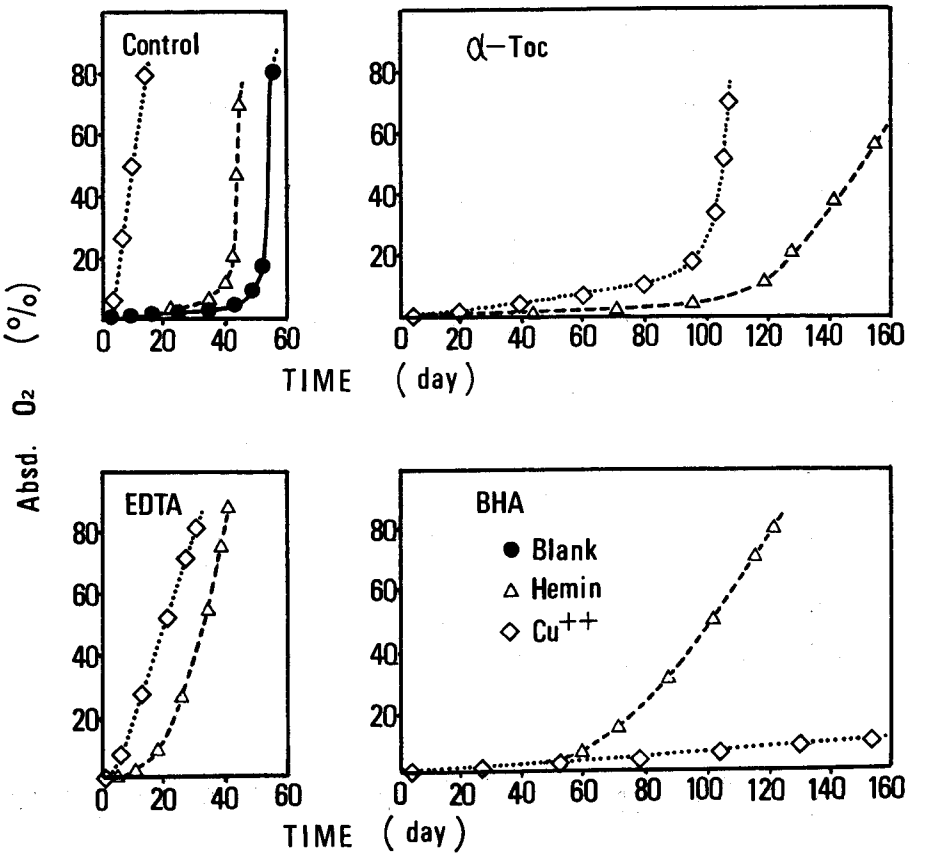


Fig. 5. Effect of antioxidant and chelating agent on oxygen absorption of model system at a_w=0.86 (20°C).

water activities. But it was not so effective as it was in the Cu^{++} system at a_w= 0.86. EDTA scarcely had any effect on the action of Cu^{++} and hemin except in the case of a_w=0.95 under the condition in this investigation as is evident from Table 2 and Figs. 4~6. Although EDTA is soluble in water and as such can be an effective antioxidant in high moisture conditions, it must be considered that at the lower end of high moisture condition (around a_w=0.75 to 0.86), EDTA effect could never surpass the catalytic effect of metals in the same high moisture conditions.

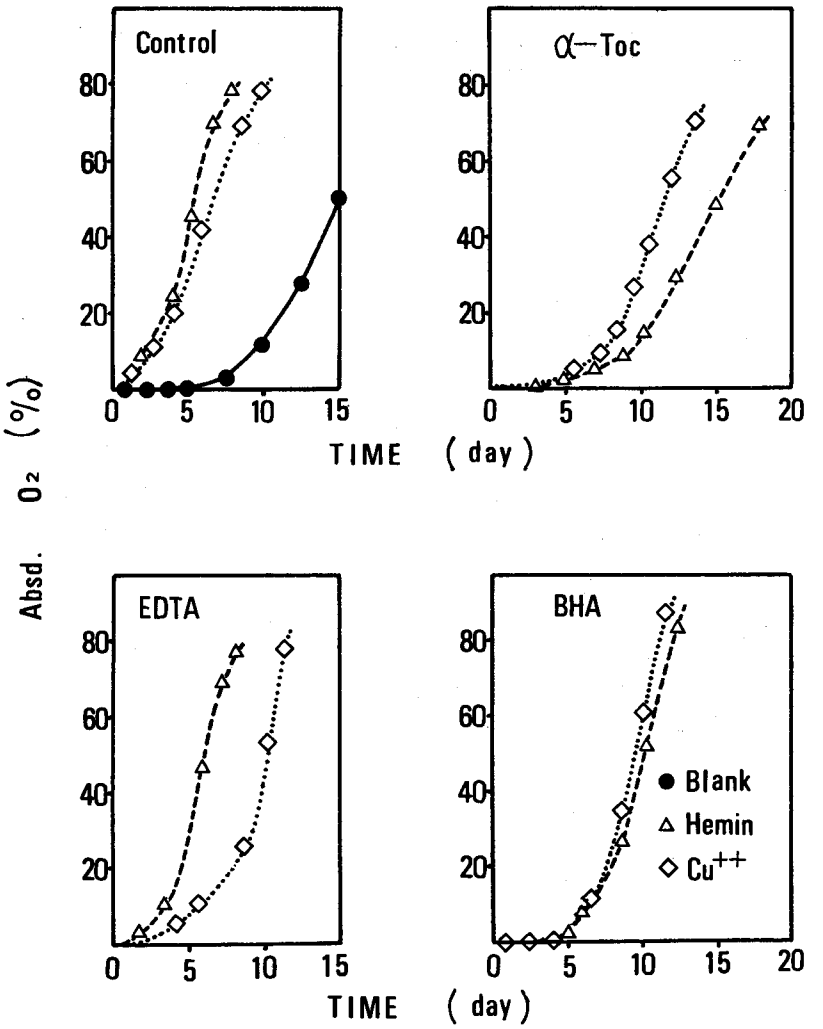


Fig. 6. Effect of antioxidant and chelating agent on oxygen absorption of model system at a_w=0.95 (20°C).

Table 2. *Effect of antioxidant and chelating agent on time required to reach 1% and 2.5% oxidation as a function of a_w in mackerel-sardine oil - egg albumin model system at 20°C*

Mixture \ a_w		Relative Rate to Reach 1% Oxidation					Relative Rate to Reach 2.5% Oxidation				
		≈0	0.32	0.75	0.86	0.95	≈0	0.32	0.75	0.86	0.95
Control	Cu^{++}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Hemin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
EDTA	Cu^{++}	1.0*	1.0*	0.8* 1.2	1.3	3.0	1.0*	1.0*	1.2* 1.4	1.5	2.5
	Hemin	0.9*	1.0*	0.6* 1.0	0.5	1.0	0.8*	1.0*	1.0* 1.0	0.5	1.5
α-Tocopherol	Cu^{++}	1.6*	1.0*	2.8* 3.2	15.0	6.0	2.4*	1.4*	3.1* 5.2	15.5	3.5
	Hemin	1.5*	1.8*	2.2* 1.0	3.6	7.0	2.1*	1.6*	2.4* 1.3	2.8	4.0
BHA	Cu^{++}	1.5*	1.0*	4.5* 5.0	24.7	5.0	3.2*	1.8*	11.0* 11.3	25.5	3.0
	Hemin	1.4*	1.0*	0.7* 1.2	1.6	5.0	2.9*	1.6*	4.5* 1.7	1.8	3.0

* Previous paper.

** Ratio of the time against the control of each a_w.

Oil used is a different batch from that in the experiments without antioxidants and chelating agent as shown in Figs. 1 and 2, and Table 1.

Acknowledgements

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References

1) Zama, K., Takama, K. and Mizushima, Y. (1979). Effect of metal salts and antioxidants on the oxidation of fish lipids during storage under the conditions of low and intermediate moistures. *J. Food Processing and Preservation* **3**, 249–257.

2) Labuza, T.P., Tsuyuki, H. and Karel, M. (1969). Kinetics of linoleate oxidation in model systems. *J. Am. Oil Chem. Soc.* **46**, 409–416.

3) Tjhio, K.H., Labuza, T.P. and Karel, M. (1969). Effects of humidification on activity of catalysts and antioxidants in model systems. *Ibid.* **46**, 597–600.

4) Maloney, J.F., Labuza, T.P., Wallace, D.H. and Karel, M. (1966). Autoxidation of methyl linoleate in freeze-dried model systems. 1. Effect of water on the autocatalyzed oxidation. *J. Food Sci.* **31**, 878–884.