COPPER MEDIATED AUTOXIDATION OF VITAMIN A IN FOOD

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SUMMARY: Vitamin A (retinol) was fortified to microcrystalline cellulose (MCC) and exposed to oxidative decomposition in the presence of copper ion under different storage temperatures at water activity (a_w) 0.11. It was observed that copper ion and temperature had great effect on the stability of vitamin A. About 50% of the fortified vitamin A was lost in 41, 34 and 24 hours when stored at 30, 40 and 50°C, respectively at a_w 0.11. The rate of reaction associated with copper ion was of first order character. The 'k' value was the highest at 30°C followed by 40°C and the minimum rate at 50°C. Model system (MS) fortified with copper ion had a lower activation energy (Ea) value ($20.1x10^3$ $Jmol^{-1}$) as compared to unfortified MS, which had an Ea value of $28.4x10^3$ $Jmol^{-1}$.

Key Words: Copper, vitamin A, trace elements.

INTRODUCTION

Copper is a trace element and is an integral part of many enzymes, involved in (i) development and maintenance of cells and tissues, (ii) cardiovascular and skeletal integrity, (iii) central nervous system structure and functions, (iv) erythropoietic function and (v) hair keratinization and pigmentation. Its deficiency may produce failure of iron absorption, bone demineralization, failure of erythropoiesis and finally death (1). Copper deficiency is very rare in humans consuming a variety of foods. The RDA of daily intake for adult is 2-3 mg and 0.08 mg/kg per day for infants and children. Although our diet is mainly based on cereals, pulses and green leafy vegetables, which provides sufficient quantity of copper for good nutrition but its role on other nutrients particularly on the destruction of vitamin A in the diet is of vital importance.

Certain metal-catalyzed destruction of vitamin A in food has been reviewed previously to a limited extent (2,3). Pekkarinen (4) reported that the catalytic efficiency of the univalent cations was lower than divalent cations at concentrations of less than 10 nM. He found that oxidation of vitamin A acetate in 1% aqueous and pure acetic acid is decreased by copper acetate. This antioxidant effect of cupper acetate was less for vitamin A acetate than for eleostearic acid. Similarly, Ogata *et. al.* (2) demonstrated the dependence of vitamin A alcohol autoxidation in paraffin on the concentration of cobalt stearate. As no work has been reported on copper-catalyzed degradation of vitamin A in foods, it was considered necessary to study the effect of copper ion on the destruction of vitamin A alcohol in a low moisture food systems.

MATERIALS AND METHODS

A model system (MS) approach was used to control composition variable because vitamin A losses during thermal processing are not only dependent upon the temperature

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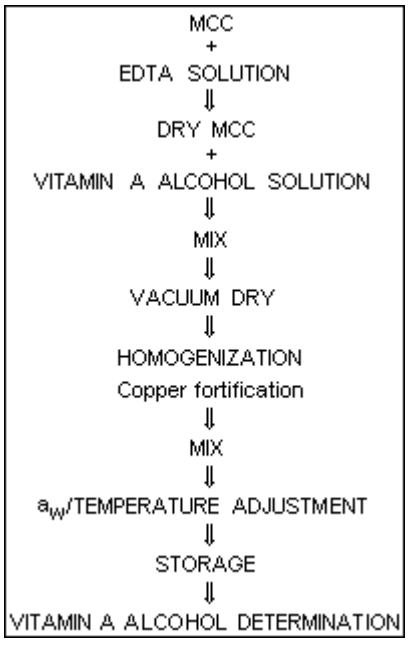
relationships but may also depend upon the presence of metals, oxygen, moisture, pH and media composition (5). Microcrystalline cellulose (MCC) was used as a solid support to simulate similar conditions to dried foods within impermeable packages and allowed conditions free from possible interaction from other food components.

Preparation and storage of model system (MS)

All possibly necessary precautions were taken to prevent adventitious destruction or isomerization of vitamin A, by treatment designed to remove metal contaminants by controlling the temperature and by working in subdued light under nitrogen during the preparation of MS and in the subsequent analytical procedures. It was ensured that all the starting materials received the same treatment and that randomization was accomplished in sample preparation, selection and analysis. The fortification procedure, the level of copper fortification to the MS and the storage conditions has been described by Manan (6) and is outlined in Figure 1.

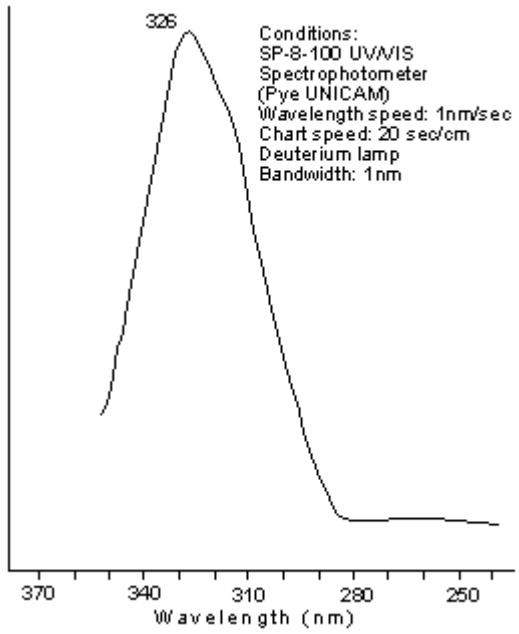
Figure 1: Schematic diagram of vitamin A alcohol assay in copper fortified MS stored at a_w 0.11.



Extraction and analysis of vitamin A

Triplicate samples (1-2 g) of the MS were taken randomly from storage cabinet into ground neck flasks and 20 ml of the methanol: ethanol: acetone (6:3:1 v/v) containing BHT (20 mg/100 ml) was added. The flasks were securely stoppered to avoid liquid loss and shaken for about 1 hour by means of a mechanical shaker (Gallenkanp). The detail of the extraction procedure has been described by Manan (6).

Figure 2: UV spectrophotometer of standard vitamin A alcohol.



UV-spectrometry of vitamin A

The uv-absorption spectrum of standard and extracted solutions was obtained by recording the uv-absorbance of the solution against wavelength on a double beam spectrophotometer (SP-800, Pye Unican Ltd) using methanol (BHT, 20 mg/100 ml) as a reference solvent at laboratory temperature.

Table 1: Rate equation and half-lives for simple reaction*.

Reaction Order	Rate Equation		Half-Life
	Differential form	Intergrated form	
0	$dx/dt = k_0$	$k_0 = x/t$	$a/2k_0$
1	$dx/dt = k_1 (a-x)$	$k_1 = 1/t \text{ in } (a/a-x)$	$\ln_2/k_1$
2	$dx/dt = k_2 (a-x)^2$	$k_2 = 1/t (a/a (a-x)$	$1/ak_2$

* 'a' is the concentration of reactant at time t=0
'x' is the concentration of reactant at time r=t
'k' is the rate constant.
Source = Laidler (8).

Scans were carried out between wavelength of 200-450 nm. A typical uv-absorption spectrum of standard vitamin A is shown in Figure 2. The absorption maximal of standard vitamin A and in MS was determined by uv-spectrometry using beer's lambert equation:

$$C = E_{corr} (el)^{-1}$$

where 'C' is the concentration of vitamin A in mol per liter, 'E_{corr}' is the absorbance obtained after correcting equation for eliminating irrelevant and interfering absorbance, 'e' is the molar extinction coefficient of maximum absorption and 'l' is the path length (cm). The corrected absorbance was calculated using the equation for eliminating irrelevant absorption and fixation given by Cama *et. al.* (7) by using equation:

$$E_{corr} = 3.28 (E_2 - E_1 - E_3)$$

where E_1, E_2 and E_3 are the recorded absorbances at 312, 324 and 325 nm, respectively.

Table 2: Concentration of vitamin A alcohol in model food system at a_w 0.11 and fortified with $CuSO_4$.

Storage time (hr)	Vitamin A alcohol in model Food system after storage*	Vitamin A alcohol (%)
Storage[1] temperature (30 °C)		
0	6.59	100
5	6.21	94.19
15	5.38	81.73
20	4.69	71.13
25	4.41	66.88
35	3.80	57.62
45	3.24	49.17
50	2.72	41.36
55	2.65	40.27
Storage[2] temperature (40 °C)		
0	6.55	100
5	5.86	89.41
10	5.20	79.39
20	4.41	67.27
30	3.45	52.68
35	3.36	51.34
40	2.99	45.76
45	2.74	41.78
Storage[3] temperature (50 °C)		
0	7.00	100
5	6.46	92.27
10	4.83	69.04
15	4.00	57.14
20	3.24	46.26
25	2.51	35.90
30	2.31	33.00
35	1.86	26.65

* mol g^{-2} x 10^{-4}.

1. Initial vitamin A alcohol in model food system was 6.59 mol x $10^{-4} g^{-1}$ MCC.
2. Initial vitamin A alcohol in model food system was 6.55 mol x $10^{-4} g^{-1}$ MCC.
3. Initial vitamin A alcohol in model food system was 7.00 mol x $10^{-4} g^{-1}$ MCC.

High performance liquid chromatography (HPLC)

Applied Chromatographic System (ACS) Model Series 300 pump, with a 20 μl loop injector model 7010 Rheodyne value (Jones Chromatography Ltd.) was equipped with a variable wavelength detector Varian Model UV-50. The HPLC column used was Lichrosorb Si-60 (E. Merck Durmstadt, F. R. Germany), column length 250 nm and internal diameter 4 mm supplied by BDH. The mobile phase was prepared by mixing 99.6 ml of hexane (HPC grade, Rathburn Chemical Ltd.) and 0.4 ml of propan-2-ol (special for chromatography, BDH Chemical Ltd.) The solution was degassed for 5 minutes with helium gas. A flow rate of 1.5 ml/min was maintained for all samples. Wavelength 326 nm was used and the column and detector temperature was 45°C. The detail of the HPLC procedure used is reported by Manan (6).

Kinetics study and data analysis

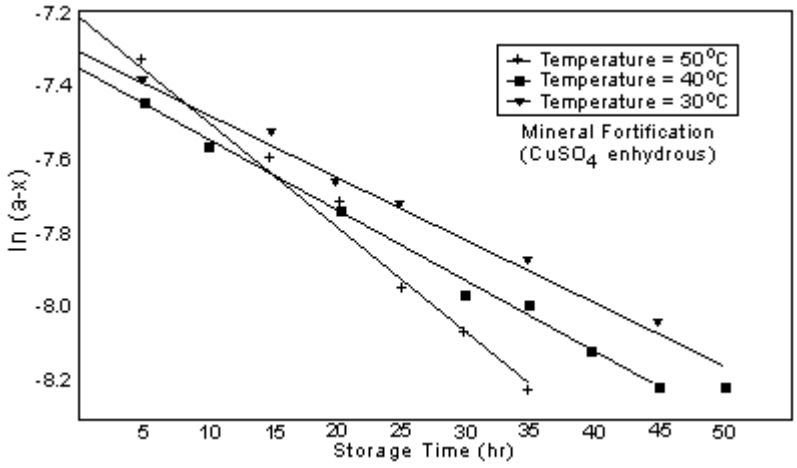
The data obtained from storage studies was fitted to the integrated kinetic equations (8) as shown in Table 1. The reaction order was determined by choosing the equation with which the data give the largest correlation coefficient and the

Table 3: Rate constant (k) for vitamin A alcohol degradation at a_w 0.11 fortified with copper and stored at three different temperatures.

a_w/Temperature °C	k hr^{-1} x 10^{-2}	SD x 10^{-2}	r	95% Confidence Level of kx 10^{-2}
0.11/30/Control	0.20	0.09	0.99	0.19-0.20
0.11/30	1.70	0.96	0.99	1.66-1.74
0.11/40/Control	0.31	0.09	0.99	0.30-0.31
0.11/40	1.92	1.41	0.99	1.86-1.97
0.11/50/Control	0.41	0.07	0.98	0.40-041
0.11/50	2.85	1.30	0.99	2.80-2.90

k = First order rate constant. SD = Standard deviation of k.
r = Correlation coefficient of regression line on sample data, as applies using integrated first order rate expression.

Figure 3: First order plots for fortified vitamin A MS stored at a_w 0.11 and different temperatures.



best graphical model. The temperature dependence for vitamin A degradation was analyzed according to the Arrhenius equation:

$$k = A_{exp} (-Ea/RT)$$

where 'k' is the first order rate constant, 'A' is the Arrhenius pre-exponential factor, 'Ea' is the activation energy (J mol^{-1}), 'R' is the gas constant (8.314 $Jmol^{-1}$) and 'T' is the absolute temperature (°K). The reaction rate constants and activation energies were calculated by linear regression analysis and by a computer programme. The 95% confidence level and standard deviation were calculated using the equation of Ractliffe (9).

RESULTS AND DISCUSSION

The geometric isomerization of vitamin A occurs spontaneously at 30, 40 and 50°C added on MCC in the presence of copper ion and stored at a_w 0.11. It is clear from the results (Table 2) that the reaction proceeds faster at 50°C followed by 40°C and the least rate at 30°C under the storage conditions studied. The vitamin A destruction occurs with storage time in fortified MS. Approximately 50% vitamin A was lost in 41, 36 and 24 hours at 30, 40 and 50°C respectively after storage at a_w 0.11. The rate of loss of vitamin A on storage is thus dependent on temperature. These data support the findings of Kirk (10), who reported that temperature increase of 10°C had a great effect on the stability of vitamin A acetate than an increase of 0.1 a_w in the range of 0.11-0.75.

The kinetic behavior is expressed satisfactorily by first order rate equation:

$$k_1 = 1/t \ln (a/a - x)$$

where 'k' is the first order rate constant, 'a' is the concentration of reactant at time, t=0 and 'x' is the concentration at time t=t.

The effect of copper on the reaction rate is given in Table 3. It appears from the 'k' values that there is a catalytic effect on vitamin A associated with copper supplementation. Thus 'k' values were the largest at 50°C followed by 40°C and the smallest rate at 30°C for a_w 0.11 in copper fortified MS. The kinetic data associated with the MS equilibrated at a_w 0.11 indicate that copper tested had a significant effect on the loss of vitamin A.

The first order kinetics observed in our study (Figure 3) agreed with the first order findings reported by Kirk (10) for vitamin A model dehydrated food system fortified with iron sulphate. Although the aw 0.11 is less than the BET monomolecular moisture content and therefore, provides limited mobility of the copper ion but the increase in the destruction rate by copper supplementation agreed with the work of Wilkinson *et. al.* (11) for vitamin A in pureed beef liver fortified with copper metal. They found that copper metal increased the destruction rate of vitamin A, and

Table 4: Half life ($t^{1/2}$) and activation energy (Ea) for vitamin A alcohol at a_w 0.11, fortified with copper and stored at three different temperatures.

a_w	30°C	40°C	50°C
Half life ($t^{1/2}$)	(hr)		
0.11/Control	345	226	169
0.11/Copper	41	36	24
Activation energy (Ea)			
0.11 / Control 28.4×10^3 Jmol^{-1} $\pm$ 4.69×10^2			
r= 0.99			
95% Confidence level = $27.54\text{-}29.26 \times 10^3$ Jmol-1			
0.11 / Copper 20.1×10^3 Jmol^{-1} $\pm$ 6.67×10^2			
r= 0.96			
95% Confidence level = $18.87\text{-}21.32 \times 10^3$ Jmol-1			

r = Correlation coefficient of Ea.

that the increasing concentration of copper being responsible for the increasing rate of vitamin A degradation. The results obtained in our study also agreed with the first order character reported by Ogate et. al. (5) for vitamin A in liquid paraffin MS catalyzed by cobalt ion.

In a similar system, Guevara (12) reported second order model for total vitamin A analyzed by reverse phase HPLC. This discrepancy in the order can be attributed to the formation of vitamin A isomers which are well separated by normal phase HPLC and are not separated by reverse phase HPLC assay, which might be degraded at different rates (13). The kinetics behavior in our study also contradict the results reported by Garrett (14) and Sattar *et. al.* (15). They observed zero order character for vitamin A in multivitamin preparations and foods.

It can be concluded from the above discussion that the great variations in 'kinetics results' may probably be due to differences in the degradative mechanism of vitamin A compound, and to the nature of vitamin A compound and environment for the reaction due to varied composition of the MS. Paquette and Kanaan (16) reported more than one degradative mechanism for vitamin A acetate in different solvent systems exposed to light, air, temperature and to the method of assay.

The half life ($t^{1/2}$) of vitamin A (Table 4) dropped approximately 1/8th of the unfortified values due to copper supplementation at a_w 0.11 and stored at 30, 40 and 50°C. The half life values were greater at 30°C as compared to 40 and 50°C storage temperatures. It is generally observed that 10°C increase in temperature reduced the half life of vitamin A by 30-50% at a_w 0.11.

The temperature dependence of vitamin A destruction in the MS was described by the Arrhenius equation. The activation energy (Ea) values calculated from a_w 0.11 below the monomolecular moisture region in the copper fortified MS was 20.1×10^3 Jmol^{-1} as compared to the unfortified MS which was 28.4×10^3 Jmol^{-1} (Table 4). The Ea values observed in our investigations were greater than the Ea values reported by Guevara (11) for vitamin A-MCC model system equilibrated at a_w 0.11 under storage conditions at 30 and 40°C. He found the Ea value of 6.67×10^3 Jmol^{-1} but this was based on two temperatures. The Ea values reported were also in disagreement with the values of 117.23×10^3 Jmol^{-1} reported by Finkel'shtein *et. al.* (17) for solid vitamin A acetate in atmospheric oxygen at 10, 20 and 25°C. While studying the vitamin A acetate in simple solvent systems, Paquette and Kanaan (16) reported the Ea value for the degradative reaction in absolute ethanol was 56 KJ mol^{-1}. The Ea value observed in the MS did not fall within the general range of the Ea values reported by Lund (18) for vitamins of 20-30 Kcal mol^{-1} .

Comparison of the activation energy (Ea) (Table 4) values between the copper fortified MS and the MS containing only vitamin A, showed a lower Ea value. The standard deviation (SD) of the Ea value was less than 10% and the correlation coefficient (r) was greater than 95%. The existence of a relationship of Ea values with copper supplementation in MS suggests that there is a change in the Ea values with copper fortification. A decrease in Ea value may probably be due to the effective charge on the transition state at a_w 0.11 in the MS studied.

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