EFFECT OF SUPPLEMENTARY IRON ON VITAMIN A STABILITY IN FOOD

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SUMMARY: Iron was fortified with vitamin A alcohol and suspended on microcrystalline cellulose (MCC). The mixture was stored in a desiccator at very low controlled relative humidity (a_w 0.11) with 30, 40 and 50°C. The loss of vitamin A alcohol was determined by normal phase HLPC. It was observed that storage time and temperature had great effect on vitamin A alcohol degradation. The rate of loss was found to be first order kinetics in iron fortified model dehydrated system. Approximately 50% of the vitamin A alcohol was destroyed in 55, 44 and 29 hours in iron fortified system stored at 30, 40 and 50°C respectively. Arrhenius activation energy (Ea) was found to be lower ($24.8x10^3$ $Jmol^{-1}$) in iron fortified food system as compared to non-fortified food system($28.3x10^3$ $Jmol^{-1}$).

Key Words: Vitamin A, iron, dehydrated food systems.

INTRODUCTION

There are a number of published reports concerning the loss of vitamin A acetate in solutions and foods (1-8). Vitamin A loss during thermal processing is not only dependent upon time and temperature relationship but also upon pH, media composition, the presence of metals, moisture, oxygen and oxidation-reduction potential (9). Manan *et. al.* (10) reported that the assay method employed also affected the kinetic behavior of vitamin A alcohol degradation. Finkel'shtein *et. al.* (11) found that the solid films of beta-carotene are readily oxidized by molecular oxygen and reported the oxidation of beta-carotene in solid films is a chain process.

Higuchi and Reinstein (12) studied the order of reaction of vitamin A with water in dehydrated systems and found the reaction to be second order with respect to ethanol concentration, slowly shifted to first order as the ethanol concentration was decreased. Other workers reported similar complex kinetics (13-15). For the control experimental work, microcrystalline cellulose (MCC) was chosen as an inert solid support. Many investigators (16,17) have used MCC as a solid support for a carotene model system. Very few reports have been published on the stability of vitamin A alcohol and its esters in food systems. Therefore, it was necessary to study the vitamin A alcohol stability in a model food system at very low water activity (a_w 0.11) to investigate its kinetic behavior. At the same time, the supplementary effect of iron was also studied.

MATERIALS AND METHODS

Microcrystalline cellulose (MCC Avicel PH 101) was obtained from Honeywell and Stein Company (UK). Pure standards vitamin A alcohol were purchased from Fluka AG (Cuchs, Switzerland). HPLC grade methanol, hexane and isopropanol were purchased from Rathburn Chemical Company (UK). Iron sulphate was of Anala A Grade obtained from BDH Chemical Company (UK).

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Preparation and storage of model system (MS)

MCC was washed with ethylene diamine tetra acetic acid (EDTA) solution to remove metals present in the model system (18). The MCC was dried after washing with distilled water and passed through a plastic mesh sieve (125 um aperture).

A solution of vitamin A alcohol in methanol; ethanol; acetone (MEA) (6;3;1 v/v) was prepared and transferred to the rotary evaporatory flask over pre-washed MCC. The ratio of vitamin A alcohol/MCC was chosen based on the procedure of Manan (18). Nitrogen was bubbled through the flask and the mixture was homogenized using a glass rod. The solvent was then evaporated under vacuum at 40°C using rotary evaporator. To the dried mixture, iron was added to the system as ferrous sulphate with the ratio as described in the Present Day Practice in Infant Feeding (19). The mixtures were homogenized in a food processor with a plastic blade and bowl in a nitrogen atmosphere.

The mineral fortified model system was then stored in a controlled relative humidity atmosphere in desicator under vacuum at very low a_w 0.11 at three different temperatures (30, 40 and 50°C) according to the method of Greenspan (20). After equilibration to the desired humidification and temperature, the vacuum of the system was broken using air of the same relative humidity. Samples were taken at 0 time and a series of samples at different intervals for the degradation study of vitamin A alcohol. All operations were done under subdued light and in the presence of nitrogen gas, wherever necessary.

Extraction and analysis of sorbed vitamin A alcohol

The vitamin A alcohol contents in the MS was extracted according to the method as described by Manan (18), with the addition of MEA reagent containing an antioxidant (BHT, 20mg/100g). The final volume of the residue in the flask was made in HPLC grade methanol. The extract from the MS was analyzed by normal phase HPLC (HPLC column Lichrosorb Si-60, E. Merck, Durmstedt, FR, Germany; column length 250 mm and internal diameter 4 mm) with the conditions as described by Manan (18).

Kinetics study and data analysis

The kinetic parameters of the data obtained from the storage studies were calculated using the integrated kinetic equations as described by Laidler (21) and the best equation was selected, which gives the largest correlation coefficient (r). The temperature dependence for vitamin A alcohol degradation was analyzed according to the Arrhenius equation:

$$k = A_{exp} (E_a / RT)$$

where k is the first order rate constant, A is the Arrhenius pre-

Table 1: Concentration of vitamin A alcohol in model food system at a_w 0.11, fortified with $FeSO_4 . 7H_2O$ and stored at 30°C.

Storage time (hr)	Vitamin A alcohol in model food system after storage*	Vitamin A alcohol (%)
0	6.56	100
10	6.07	92.60
30	4.82	73.49
50	4.24	64.65
70	3.53	53.79
90	3.06	46.59
100	2.73	41.85
120	2.38	36.33

Mean of three determinations. Initial vitamin A alchol in model food system was 6.56 mol x 10^{-4} g^{-1} MCC.
*= mol g^{-1} x 10^{-4}.

Table 2: Concentration of vitamin A alcohol in model food system at a_w 0.11, fortified with $FeSO_4 . 7H_2O$ and stored at 40°C.

Storage time (hr)	Vitamin A alcohol in model food system after storage*	Vitamin A alcohol (%)
0	7.56	100
10	6.55	89.69
20	5.61	74.26
30	5.01	66.31
40	4.14	54.81
50	3.38	44.71
60	2.82	37.29
70	2.69	35.59
80	2.16	28.54

Mean of three determinations. Initial vitamin A alchol in model food system was 7.56 mol x 10^{-4} g^{-1} MCC.
*= mol g^{-1} x 10^{-4}.

Table 3: Concentration of vitamin A alcohol in model food system at a_w 0.11, fortified with $FeSO_4 . 7H_2O$ and stored at 50°C.

Storage time (hr)	Vitamin A alcohol in model food system after storage*	Vitamin A alcohol (%)
0	7.05	100
5	5.95	84.43
10	5.60	79.50
15	5.00	70.95
20	4.65	65.96
30	3.23	45.85
40	2.95	41.92
45	2.40	33.99
50	1.99	28.34

Mean of three determinations. Initial vitamin A alchol in model food system was 7.05 mol x 10^{-4} g^{-1} MCC.
*= mol g^{-1} x 10^{-4}.

Table 4: Rate constant (k) for vitamin A alcohol degradation at a_w 0.11, fortified with iron and stored at three different temperatures.

a_w/Temperature	k $hr^{-1} \times 10^{-2}$	SD $\times 10^{-1}$	r	95 % Confidence level of k $\times 10^{-2}$
0.11/30/Control	0.20	0.09	0.99	0.19-0.20
0.11/30	1.25	0.82	0.99	1.2-1.33
0.11/40/Control	0.31	0.09	0.99	0.30-0.31
0.11/40	1.58	1.30	0.99	1.53-1.63
0.11/50/Control	0.41	0.07	0.98	0.40-0.41
0.11/50	2.40	0.82	0.99	2.37-2.44

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.

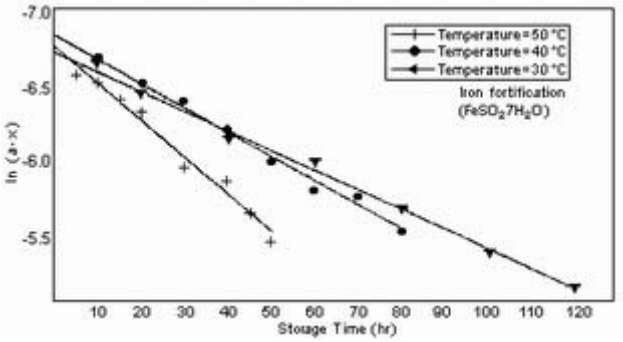
exponential factor, Ea is the activation energy ($Jmol^{-1}$). R is the gas constant (8.314 $Jmol^{-1}$) and T is the absolute temperature (°K). The standard deviation and the 95% confidence level of the sample data were calculated by using the equation as described by the Ractliffe (22).

RESULTS AND DISCUSSION

The results obtained for the concentration of vitamin A alcohol in dehydrated food system (a_w 0.11) fortified with $FeSO_4$. $7H_2O$ and stored at 30, 40 and 50°C are shown in Tables 1, 2, 3 . It was observed that vitamin A concentration decreased with storage time and temperature studied. A high reduction in vitamin A contents was found at 50°C followed by 40°C and the least degradation at 30°C storage temperature.

The first order of the rate constant (k) in the iron fortified system equilibrated at a_w 0.11 and stored at three different temperatures are presented in Table 4, with standard deviation, correlation coefficient and 95% confidence limits. A great catalytic effect of iron on vitamin A alcohol at a_w 0.11 was found in all temperatures studied. The results obtained were in agreement with the work of Kirk (3) for vitamin A acetate stability in foods. He reported that iron had a significant effect on vitamin A acetate stability. Wilkinson *et. al.* (4) reported that mineral fortification increased the degradation of vitamin A in pureed beef liver. At a_w 0.11, the kinetic data from the study for iron catalysis is interpreted as

Figure 1: First order plot for iron fortified vitamin A alcohol at a_w 0.11 and different temperatures.



reflecting the reduced solubilities and motilities of the metal ions in the aqueous phase of the metal systems. A significant increase in the degradation rate of vitamin

Figure 2: Arrhenius plot for iron fortified vitamin A alcohol at a_w 0.11.

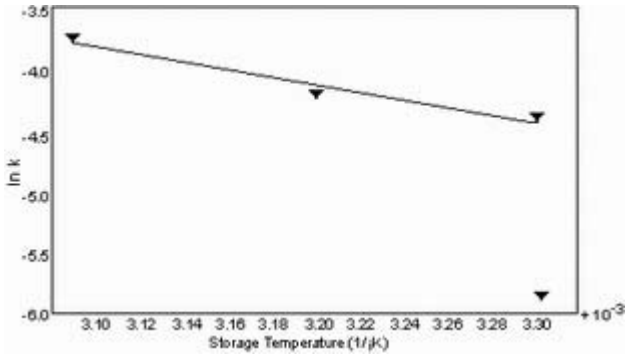


Table 5: Half life ($t_{1/2}$) and activation energy (E_a) for vitamin A alcohol at a_w 0.11, fortified with iron and stored at three different temperatures.

a_w	30°C	40°C	50°C
Half life ($t_{1/2}$) (hr)			
0.11/Control	345	226	169
0.11/Iron	55	44	29
Activation energy (Ea)			
0.11/Control	28.4×10^3 $Jmol^{-1} \pm 4.69 \times 10^2$		
	r= 0.99		
	95% Confidence level = $27.54\text{-}29.26 \times 10^3$ Jmol-1		
0.11/Iron	24.3×10^3 $Jmol^{-1} \pm 5.54 \times 10^2$		
	r= 0.98		
	95% Confidence level = $23.28\text{-}25.31 \times 10^3$ Jmol-1		

r= Correlation coefficient of Ea.

A alcohol is noted for supplemented iron to the system as compared to the system not fortified with iron.

The effect of iron supplementation on the half life ($t_{1/2}$) of vitamin A alcohol is presented in Table 5, which indicates that iron had great effect on vitamin A alcohol degradation in the all the systems studied. Approximately 50% of vitamin A alcohol was lost in 55, 44 and 29 hr in the systems fortified with iron at a_w 0.11 and stored at 30, 40 and 50°C, respectively. The results indicated that the amount of vitamin A alcohol decreased as the temperature increased.

The experimental data for the degradation of vitamin A alcohol in fortified model system was treated by first order kinetics (Figure 1). The results obtained agreed with the first order character reported by Manan *et. al*. (10) for simple model dehydrated food system. The first order model also agreed with the results as obtained by Kirk (3) for vitamin A acetate degradation in the fortified system was described by the Arrhenius equation (Figure 2). Comparison of the activation energy (E_a) values between fortified and non-fortified model systems (Table 5) showed that a lower E_a value was observed (relative to the control) for iron supplementation at all storage conditions studied. The standard deviation of the E_a values was less than 10% and the correlation coefficient was greater than 95%. A decrease in the E_a value may be due to increased salvation of the iron and increased effective charge on the transition state at the a_w 0.11 in the system.

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