Oxygen Uptake of Vitamin A in a Model Dehydrated Food System

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Summary: The amount of oxygen consumed per mole of vitamin A alcohol lost was studied on microcrystalline cellulose (MCC) similar to the conditions of dehydrated foods (a_w 0.11, 0.23 and 0.32 at 30°C). In these experiments the average uptake of oxygen was 2.68 moles per mole of vitamin A alcohol or its degradative products. These findings are compatible with the reaction mechanism proposed by Evteeva and Gargarina [1] in which oxidation take place by chain reaction involving loss in unsaturated bonds. The mechanism may have more in common with the oxidation of solid thin layers than solutions, in which reduced molecular mobility causes intra-molecular chain reaction than intra-molecular propagation.

Introduction

Vitamin A, a fat soluble vitamin, plays an important role in human nutrition. Its deficiency in the developing world is devastating particularly among children. Of an estimated 700,000 pre-school children who develop severe corneal xerophthalamia each year, as many as 400,000 die from the deficiency and with other associated factors such as PCM, diarrhaea, respiratory tract infection and measles [2]. Susan [3] reported that there is a serious underlying and undetermined degree of vitamin A deficiency in Pakistan and 60% child death are strongly associated with vitamin A deficiency along with other factors. He also reported that in Indonesia child mortality has direct link with vitamin A deficiency.

To overcome these problems, fortification of vitamin A to basic food commodities such as tea, sugar, bread and salt has been proposed [4,5]. The stability of supplemented vitamin A are affected by oxygen, heat, light, water activity (a_w) and the presence of acids or mineral elements [6,7]. The above factors are the major cause of vitamin A degradation and isomerisation to cis isomers which have lower biological activity. However, the amount of oxygen taken up during vitamin A loss in dehydrated foods need critical experimental appreciation. Uptake of oxygen by vitamin A in solutions has been reported as 2.3 and 3.0 moles oxygen per mole of vitamin A lost [8,9]. Similarly the uptake of oxygen by beta-carotene has been studied both in solutions and on solid supports (i.e., cellulose) and the molar ratio of oxygen consumed per mole of beta-carotene lost has been reported as 7.0 and 8.0, respectively [10,11]. This project was therefore, undertaken in order to assess the amount of oxygen consumed by vitamin A alcohol on a range of solid model system related to the storage of dehydrated foods.

Results and Discussion

The Tables 1, 2 and 3 representing the experimental conditions used (a_w, storage temperature and storage time) and results showed the absorption of oxygen, vitamin A alcohol loss and formation of peroxides. The results in Table 1 indicated that 2.45 moles of oxygen were consumed per mole of vitamin A alcohol oxidised. This average molar ratio found by Pekkarinen and Montonen [8] and Finkel'shtein *et al.* [9] in studies of retinyl acetate or retinol oxidation in solutions. The mean value found was 2.3 moles per mole retinol in the oxidation of retinol in liquid paraffin at 50, 60, 70 and 80°C. They also reported the ratio to be greater than 1 and found by varying the concentration of retinyl acetate.

The results in Table 2 and 3 showed the average moles of oxygen consumed by per moles of

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Table 1: Vitamin A alcohol content and uptake of oxygen in the oxidation of vitamin A alcohol on MCC*. (a_w = 0.11, Temperature = 30°C)

Storage Time (min)	Vitamin A alcohol in MS after storage[a]	Moles of O_2 absorbed per mole of vitamin A alcohol lost	% initial O_2 present as peroxide
20	5.11	2.31	42.8
60	5.03	2.29	38.0
120	4.44	2.41	36.1
200	3.91	2.30	39.5
260	3.43	2.61	26.8
340	2.89	2.52	32.1
400	2.60	2.73	36.5

* = Initial vitamin A alcohol content in MS was 5.869 mole x 10^{-4} g^{-1} MCC.
a = mole x 10^{-4} g^{-1}

Table 2: Vitamin A alcohol content and uptake of oxygen in the oxidation of vitamin A alcohol on MCC*. (a_w = 0.23, Temperature = 30°C)

Storage Time (min)	Vitamin A alcohol in MS after storage[a]	Moles of O_2 absorbed per mole of vitamin A alcohol lost	% initial O_2 present as peroxide
30	5.00	2.67	36.1
60	4.72	3.00	42.7
120.15	4.06	2.68	32.3
150.09	3.68	3.12	35.2
210	3.22	3.09	37.9
330	2.48	2.75	42.4
480	1.65	2.56	31.9

* = Initial vitamin A alcohol content in MS was 5.501 mole x 10^{-4} g^{-1} MCC.
a = mole x 10^{-4} g^{-1}

Table 3: Vitamin A alcohol content and uptake of oxygen in the oxidation of vitamin A alcohol on MCC*. (a_w = 0.32, Temperature = 30°C)

Storage Time (min)	Vitamin A alcohol in MS after storage[a]	Moles of O_2 absorbed per mole of vitamin A alcohol lost	% initial O_2 present as peroxide
10	5.74	3.00	34.11
20	5.09	2.98	36.20
60	4.75	2.75	42.00
120	4.06	2.65	46.12
180	3.34	2.50	38.14
240	2.75	2.86	39.12
300	2.30	2.77	41.00
320	2.03	2.99	42.12

* = Initial vitamin A alcohol content in MS was 5.501 mole x 10^{-4} g^{-1} MCC. a = mole x 10^{-4} g^{-1}

vitamin A alcohol were 2.79 and 2.81, respectively. The relatively large and variable number of moles of oxygen consumed under the conditions described may be associated for if vitamin A alcohol was reacting by way of a mechanism analogous to that described by Evteeva and Gargarina [1], where oxygen is consumed in the loss of unsaturated bonds and further peroxides were formed by primary oxidation products. They proposed that the oxidation took place by way of a sequence of chain reactions as shown by the following reaction scheme:

$$A_n \longrightarrow P_{n-} \longrightarrow P_{n-2} \longrightarrow P_{(0)}$$

Where A_n is the original polyene, vitamin A alcohol and P_{n-1} etc. are the reaction products. The oxidative product of vitamin A alcohol was first described by Karrer and Jucker [14]. The principal product of this reaction was 5,6-epoxy vitamin A alcohol with UV max 272-275 nm. In 1947, Karrer and Jucker [15] revised their original work and reported 7,8-epoxy vitamin A alcohol with an absorption max 264 nm. Scott [16] revised in 1964, the work of Karrer and Jucker and reported that 11,12-epoxy vitamin A alcohol was the major product resulting from the autoxidation of vitamin A alcohol.

The average value for the uptake of oxygen is 2.68 moles per mole of vitamin A, a value considerable greater than those reported by Ogata et al [17]. Texeiro Neto *et al.* [10] studied the uptake of oxygen by beta-carotene suspended on MCC and found 6 to 7 moles of oxygen per mole of beta-carotene decolourised. Thus, it can be proposed that due to reduced molecular mobility in the system under test, the uptake of oxygen by vitamin A alcohol showed characteristics in common with mechanism undergone by solid films rather than by solutions.

In solid films molecular mobility affects the rate of oxidation [18] as show by the high activation energy (E_a) for the oxidation of thin films of retinyl acetate between 10 and 25°C. Reduced mobility in the solid state might also be expected to increase the relative importance of intramolecular reactions and hence the uptake of oxygen.

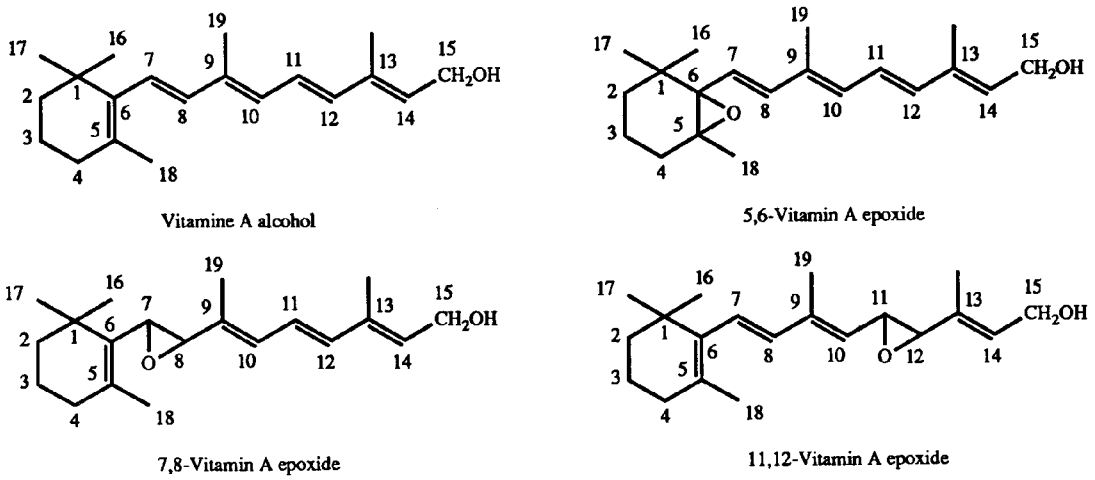

Fig. 1: The structure of vitamin A alcohol and vitamin A epoxides.

Peroxides determination indicates that in the conditions under which this study was carried out reaction products other than epoxides, 13-cis vitamin A alcohol was also observed. The percent of initial oxygen present as peroxides at the end of storage agreed with the findings of Koslov *et al.* [19], working with retinol acetate in n-decane to 40% loss of vitamin A alcohol, who found that the formation of peroxide compounds was inversely proportional to the concentration of vitamin A acetate and approximately equivalent, as a molar basis to the loss of retinol acetate.

Differences in oxidation mechanism between solid and liquid phases and between amorphous and crystalline states have been observed by Koslov *et al.* [19]. They reported that in solutions the amount of peroxide is approximately equimolar to the loss of polyene in the early stage of oxidation. In solid films of retinyl acetate on average only 1.16 moles of oxygen is present as peroxide per mole of polyene lost in the oxidised film. Crystalline films oxidise 15 to 20 times more slowly than amorphous films, and the oxidation proceeds without the formation of peroxides [20]. Oxidation in the amorphous state gives a shift of infrared (IR) frequency attributable to C = C bonds.

Experimental

Model system

A model system approach was used in order to control variables. 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.

MCC was washed with 0.05M ethylene diamine tetra-acetic acid (EDTA) to remove metal ions and rinsed with distilled water. The detail of the procedure has been described in previous publications of Manan [6] and Manan *et al.* [7]. The dried MCC was mixed with an ethanolic solution of vitamin A alcohol and the solvent has been removed by evaporation under reduced pressure. The residue was transferred to a food processor and homogenised for 2-5 minutes. The powder was then transferred to an airtight darkened glass jar to minimze loss of vitamin A.

Water activity equilibration

Water activity (a_w) equilibration was carried out by the procedure as described by Manan *et al.* [7]. Five grams of samples were weighed into glass dishes and transferred to desiccators containing saturated solutions of either lithium chloride (a_w = 0.11) or potassium acetate (a_w = 0.23) or magnesium chloride (a_w = 0.32). The desiccators were evacuated and flushed with N_2 gas after which they were placed in a water bath at 30°C for 48 hours in the dark.

Storage test

An accurately weighed (1-2 g) humidified samples were taken into clean dry conical flasks

(100 ml), ground neck, mean volume 0.127 dm^{-1}) and stoppered with suba-seal caps. The flasks were placed in a shaking water bath at 30°C and the atmosphere evacuated and replaced by air bubbled through saturated solution of the respective salts. The initial concentration of oxygen in the headspace was measured and the cellulose extracted for vitamin A assay. Control tests contained MCC which had been treated identically except for the omission of vitamin A. During storage the system was protected from light. Flasks were removed at suitable intervals, the oxygen content of the headspace measured and vitamin A and peroxide measurements made on sub-samples of the model system.

Vitamin A assay

The residual vitamin A content held on MCC was extracted with a mixture of methanol: ethanol: acetone (6:3:1 v/v) as described by Manan *et al.* [12]. The filtrate was evaporated to dryness under vacuum and the residue dissolved in ethanol (100 ml). The concentration of vitamin A alcohol was determined using high performance liquid chromatography (HPLC) by the procedure as described by Manan [6].

Oxygen uptake measurement

Uptake of oxygen was measured with a low resolution mass spectrometer marketed as a residual gas analyser (Model 5 x 200, VG Gas Analysis Ltd), pumped by an air cooled oil vapour diffusion pump (Model EO_2, Edwards High Vacuum) backed-up by a two stage rotary pump (Model E_2M_2, Edwards High Vacuum). The instrument was calibrated with oxygen-nitrogen mixtures containing from 1 to 20% oxygen and air. The oxygen consumed during storage was calculated from the difference between the oxygen mole fraction at the start of storage and the oxygen mole fraction found in the headspace at the time of sampling. From the difference in mole fraction, oxygen partial pressure difference corresponding to the changes in mole of oxygen were calculated using the equation below:

$$nO_2 = PO_2 \times V/RT$$

Where nO_2 is the moles of oxygen consumed, V is the volume of storage flasks (m^3), T is the temperature ^0K, R is the ideal gas constant 8.3 J $^0K^{-1}$ $mole^{-1}$ and PO_2 is the oxygen partial pressure change Nm^{-2}.

From measurement of the moles of oxygen consumed and the moles of retinol lost during storage, the molar ratio was calculated.

Determination of peroxide value

The peroxide value of the samples was determined by the method as described in IUPAC standard methods [13] on duplicate sub-samples of the model system before and after storage on both samples and controls.

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