Radiation Response of Vitamin A in Aqueous Dispersions

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The radiation destruction of vitamin A acetate was monitored in isooctane, coconut oil, and aqueous dispersions. The G(−vit. A), i.e. the number of vitamin A molecules destroyed per 100 eV of energy absorbed in lipid solvents and aqueous preparations, increased with the concentrations of vitamin A used. In the freely dissolved state, as in isooctane or coconut oil, the extent of destruction of vitamin A was more or less identical. However, a marked reduction in the radiation destruction of vitamin A was observed in aqueous dispersions at all concentrations except at 1×10^{-4} M. Incorporation of sugars, starch, and egg albumin in aqueous preparations offered considerable protection to vitamin A from radiation damage which could be discerned even at the lowest concentration (1×10^{-4} M). The protective influence of aqueous dispersion as noted for vitamin A was also observed for β-carotene, vitamin A alcohol, and ubiquinone-30. The significance of the above findings in radiation processing of foods has been discussed.

The radiation chemistry of food constituents has been extensively studied in order to understand the reaction mechanisms associated with the changes in color (Franceschini et al., 1959; Sawant et al., 1970; Madhavan et al., 1973; Kung et al., 1953), texture (Gore and Kumta, 1970), and flavor (Merritt, 1972; King et al., 1972) of radiation processed food products. Vacuum packaging and irradiation at cryogenic temperature are adopted to minimize the radiation-induced physicochemical changes in such products. Much of the information on the radiation sensitivity of the food constituents was obtained using either aqueous (Augenstine, 1962; Drake and Giffee, 1957) or lipid solvent systems (Kumta et al., 1970; Knapp and Tappel, 1961), depending upon the solubility of the test compounds. To explain the relative stability of β-carotene in mango pulp and orange juice as compared to its stability when dissolved in isooctane (Sawant et al., 1970), we investigated radiation degradation of β-carotene when dispersed in aqueous systems containing complexes of β-carotene with sucrose and albumin (Ramakrishnan et al., 1970; Brij Bhushan et al., 1971). It was implicated from these studies that enhanced stability of β-carotene in naturally occurring systems is probably due to the occurrence of β-carotene as complexes with sugars (Friend and Mayer, 1960) and protein (Faludi-Daniel et al., 1965; Nishimura and Takamatsu, 1957; Subbarayan and Cama, 1966). The dose modifying influence of aqueous dispersion was further confirmed with vitamin A, which showed only 13% destruction at 1.0 Mrad as compared to 90% destruction when dissolved in isooctane (Brij Bhushan et al., 1971).

Knapp and Tappel (1961) compared relative radiation sensitivities of fat-soluble vitamins dissolved in lipid solvents. These authors employed the ratio of D_{10} to initial concentration (D_{10}/C_0) referred to as "specific D_{10}" to normalize the differences in radiation destruction related to concentration of the compound. As there are no other detailed reports on the radiation damage to vitamin A in aqueous dispersions, the relative stability of vitamin A at varying concentrations has been examined. This paper provides further evidence substantiating the protective influence of aqueous dispersions on the stability of vitamin A acetate, vitamin A alcohol, ubiquinone-30, and β-carotene.

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EXPERIMENTAL SECTION

Materials. Vitamin A acetate was a gift from Roche Products Ltd. Vitamin A alcohol was prepared by saponification of vitamin A acetate. Both the preparations were purified on alumina column prior to use. In the succeeding pages vitamin A refers to vitamin A acetate unless otherwise specified. β-Carotene (all-trans) and Tween-80 were procured from Koch-Light Laboratories Ltd. (London). Antimony trichloride used for colorimetric estimation of vitamin A was the product of May and Baker Ltd. Pure coconut oil, packed in tin, was purchased from the local market. All the solvents and chemicals used in the present studies were of analar grade and solvents were distilled before use. Bausch and Lomb Spectronic-20 and Hitachi–Perkin-Elmer spectrophotometers were used for colorimetric and spectrophotometric studies, respectively. For preparation of aqueous dispersions, all-glass double-distilled water was used.

Solubilization of Vitamin A in Coconut Oil and Tween-80. A known amount of vitamin A was dissolved in 2–3 ml of petroleum ether (40–60 °C) and stirred with an amount of coconut oil required to give the needed concentration. Petroleum ether was completely evaporated off from the mixture with a stream of nitrogen gas and the sample was set aside for irradiation. The concentration of vitamin A with respect to coconut oil varied from 1×10^{-4} to 125×10^{-4} M. In Tween-80, vitamin A was directly solubilized to a concentration of 10×10^{-4} M.

Preparation of Aqueous Dispersions. The following types of aqueous dispersions were prepared.

(a) *With Coconut Oil.* Vitamin A was dissolved in coconut oil to get the concentration ranging from 10×10^{-4} to 1250×10^{-4} M. The mixture was homogenized with 10 vol of distilled water containing 0.5% (w/v) Tween-80, in oil emulsion homogenizer (Arthur Thomas Co.). Thus, a 10% coconut oil dispersion containing vitamin A concentrations ranging from 1×10^{-4} to 125×10^{-4} M was prepared. Preparations of β-carotene, vitamin A alcohol, and ubiquinone-30 in coconut oil and water were made as described above, except that only a single concentration (10×10^{-4} M) was employed.

(b) *With Carbohydrates and Proteins.* Vitamin A in petroleum ether was separately added to egg albumin, sucrose, glucose, lactose, or starch. After adequate mixing, in a mortar and pestle, petroleum ether was completely evaporated off with a stream of nitrogen gas and the residue was dispersed in distilled water to obtain a turbid preparation. Thus, aqueous dispersions were prepared with the concentration of vitamin A at 10×10^{-4} M and

of dispersing agents at the 10% level with the exception of egg albumin and sucrose where the concentrations were 0.1 and 15%, respectively. For the preparation of dispersion with starch, warming at 50 °C for 5 min was necessary.

(c) *With Tween-80.* A clear aqueous system of vitamin A (10×10^{-4} M) was prepared by using Tween-80 (10%) as dispersing agent.

Irradiation. To examine the radiation destruction pattern of vitamin A, 5-ml aliquots of vitamin A in isooctane, coconut oil, Tween-80, or aqueous dispersions were separately exposed to γ-radiation doses ranging from 0.1 to 3.0 Mrad under the atmosphere of N_2. Irradiation was carried out in a Gamma cell-220 (AECL) with a ^{60}Co source having a dose rate of 0.56 Mrad/h.

Extraction of Fat-Soluble Compounds from Aqueous Dispersions. A poor recovery of less than 10% of vitamin A was obtained by employing petroleum ether alone as extracting solvent. Recovery of vitamin A was, however, more than 95% when petroleum ether containing 30% methanol was used on aqueous dispersions. From aqueous Tween-80 dispersion, vitamin A was quantitatively extracted by using a diethyl ether, alcohol, and petroleum ether mixture (2:1:1). Moisture from the ether extract was removed by treatment with anhydrous sodium sulfate and the aliquot was used for quantitative determination of vitamin A.

Determination of Vitamin A, β-Carotene, and Ubiquinone-30. Vitamin A from coconut oil or aqueous extract was determined by employing the colorimetric method (Carr and Price, 1926). Destruction of vitamin A was checked by the UV spectrophotometric method employing the Morton and Stubbs (1948) correction for irrelevent absorption. Isooctane solvent was used for recording spectra of vitamin A in the UV region. Spectra of vitamin A in aqueous preparation were recorded using blanks containing a solution of dispersing agents only. β-Carotene was determined spectrophotometrically by recording absorbancy at 450 nm. The spectrophotometric method of Crane and Dilley (1963) was used for the determination of ubiquinone-30.

Calculation of *G* and DMF. The *G*(–vit. A), i.e. number of vitamin A molecules destroyed per 100 eV, was estimated from the initial slope of the best straight line through the yield–dose plots (Figure 1). Dose modifying factors (DMF) were calculated from the ratio of *G*(–vit. A) in isooctane or coconut oil to that in aqueous dispersion and were termed as DMF_I and DMF_O, respectively.

RESULTS

Radiation Response of Vitamin A in Lipid Solvents and Aqueous Dispersions. The rates of destruction of vitamin A were studied in three systems, i.e. isooctane, coconut oil, and coconut oil–water dispersions, over a concentration range of vitamin A varying from 1×10^{-4} to 125×10^{-4} M. The results incorporated in Figure 1 reveal that at all the concentrations, except at 1×10^{-4} M, the number of vitamin A molecules destroyed was the least in coconut oil–water dispersion when compared to that in coconut oil or isooctane alone. Also, the destruction patterns of vitamin A in coconut oil or isooctane were almost identical; the values in coconut oil served as a control for the coconut oil–water dispersion while those in isooctane served as a comparison with literature values.

It is also apparent that at the lowest concentration (1×10^{-4} M), the radiation damage to vitamin A in aqueous dispersion was more than that observed in lipid solvents.

As the above data showed concentration-dependent radiation response, *G*(–vit. A) values were calculated for each of the concentrations. The results presented in Figure 2a suggest that in all three media, the *G*(–vit. A) increased with the concentration of vitamin A. In coconut oil, *G*(–vit. A) values were similar to those obtained in isooctane. In a coconut oil–water dispersion, however, *G*(–vit. A) values

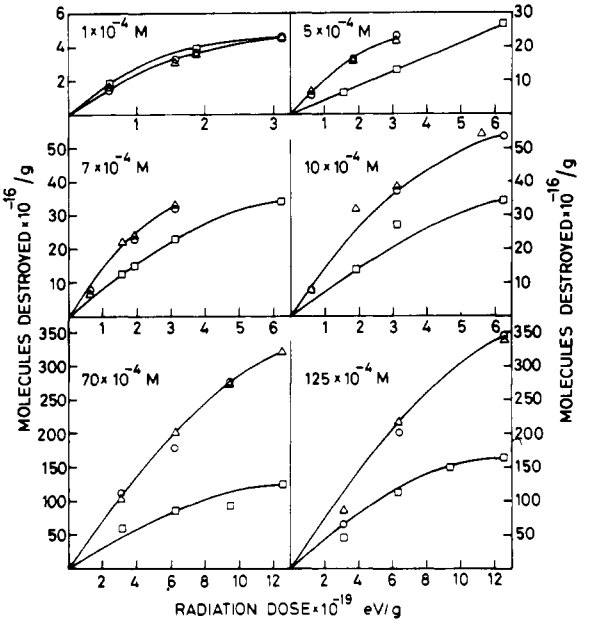

Figure 1. Differential radiation response of vitamin A in lipid solvents and oil–water dispersion. Coconut oil (△), isooctane (○), or coconut oil–water dispersions (□) containing vitamin A at various concentrations were exposed to different doses of γ irradiation. The amount of vitamin A destroyed was determined as stated in the text and plotted against the radiation dose to obtain the dose–response curves.

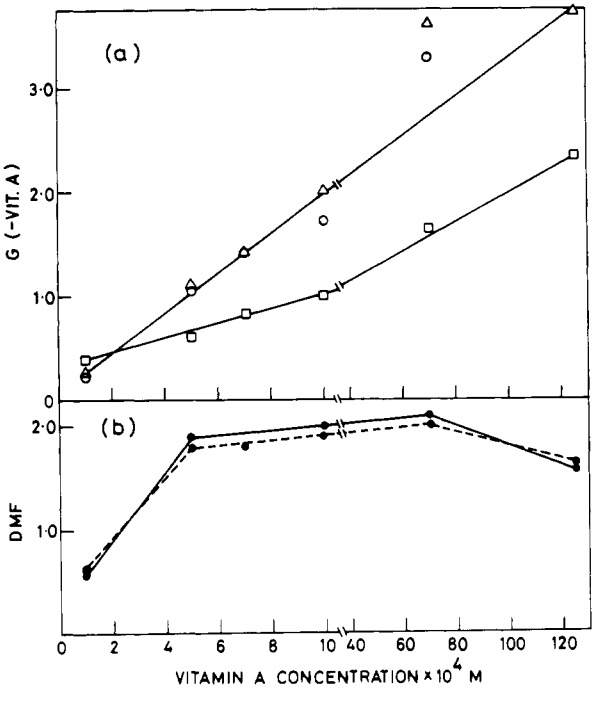

Figure 2. Effect of initial concentration on radiation response of vitamin A. (a) *G*(–vit. A) values plotted here against the concentration of vitamin A for coconut oil (△), isooctane (○), or coconut oil–water dispersion (□) were computed from Figure 1 according to the method outlined in the Experimental Section. (b) Shows dose-modifying factor, DMF_O (–•) and DMF_I (---•) against the initial concentration of vitamin A.

Table I. Effect of Initial Concentration on Radiation Response of Vitamin A Irradiated in Liquid or Frozen State of Coconut Oil

Concn of vit. A			Molecules destroyed × 10^{-16}/g at radiation dose (Mrad)							G(−vit.	
a	*b*	Medium	0.1	0.25	0.3	0.5	1.0	1.5	2.0	A)	DMF
1	6.02	Liquid	1.38	3.19	3.49	4.64				0.22	1.10
		Frozen	1.08		3.31	4.55				0.19	
7	42.14	Liquid	6.74	20.23	23.18	33.08				1.36	1.86
		Frozen	3.37		12.64	18.96				0.73	
70	421.4	Liquid				101.14	198.06	278.12	328.69	3.70	1.61
		Frozen				65.74	84.28	149.60	183.48	2.30	

[a] $\times 10^4$ M. [b] Molecules/g × 10^{-16}. Vitamin A diluted in coconut oil at three different concentrations was exposed to varying doses of γ radiation in the liquid state (34–38 °C) and solid state (0 °C). The destruction of vitamin A was estimated at each radiation dose. G(−vit. A) and DMF were calculated as described in the Experimental Section.

Table II. Dose-Modifying Influence of Aqueous Media on Radiation Sensitivity of Fat-Soluble Compounds

Test compds, 10 × 10^{-4} M (60.2 × 10^{16} molecules/g)	Medium	Molecules destroyed × 10^{-16}/g 0.1 Mrad	0.3 Mrad	0.5 Mrad	DMF_O[a]
Vitamin A acetate	Coconut oil	13.24	32.51	39.13	2.2
	Coconut oil-water dispersion (10:90)	6.02	13.85	25.28	
Vitamin A alcohol	Coconut oil	19.08	40.64	51.17	1.3
	Coconut oil-water dispersion (10:90)	16.25	37.81		
β-Carotene	Coconut oil	50.15	58.09		2.4
	Coconut oil-water dispersion (10:90)	24.08	57.79		
Ubiquinone-30	Coconut oil	15.05	35.52	46.72	1.2
	Coconut oil-water dispersion	13.24	30.1	40.45	

[a] DMF_O is expressed as the ratio of G(−vit. A) values obtained in coconut oil alone to that from the coconut oil-water dispersion.

were significantly lower than those obtained in lipid solvents (concentration 5 × 10^{-4}–125 × 10^{-4} M). The dose-modifying influence of the aqueous system becomes much more clear when these results were computed as ratios of G(−vit. A) in coconut oil (DMF_O) and in isooctane (DMF_I) compared to that in aqueous dispersion. Figure 2b shows the relationship of DMF to the concentration of vitamin A. It can be seen that DMF_O increased nearly by threefold as the concentration of vitamin A was raised from 1 × 10^{-4} to 5 × 10^{-4} M and remained almost constant up to 70 × 10^{-4} M. Above this concentration, a slight drop in the DMF was observed.

In order to examine the extent of protection afforded by the frozen state of media, vitamin A in coconut oil at 1, 7, and 70 × 10^{-4} M concentrations was irradiated at 0 °C. Results incorporated in Table I show that the protection offered by the frozen state of media was almost negligible at 1 × 10^{-4} M concentration while the same was quite significant at higher concentrations.

Protective Influence of Coconut Oil–Water Dispersion on Other Fat-Soluble Compounds. Vitamin A acetate, vitamin A alcohol, β-carotene, and ubiquinone-30, dispersed in a coconut oil–water system along with their respective controls in coconut oil, were irradiated at different doses as stated in Table II. Each of these compounds showed consistently lesser radiation destruction in oil–water dispersion compared to that observed in coconut oil. The DMF_O value for vitamin A alcohol was found to be less than that observed with vitamin A acetate.

Dose-Modifying Effect of Other Aqueous Dispersions. Aqueous dispersions of vitamin A (10 × 10^{-4} M), prepared with water-soluble compounds, were separately exposed to two levels of γ radiation, i.e. 1.0 and 3.0 Mrad. The results obtained (Table III) show that both doses of radiation caused damage to vitamin A, the effect being more pronounced at the higher dose. Nevertheless, the radiation destruction of vitamin A was dependent on the nature of dispersing compound employed. Thus, while lactose offered maximum protection to vitamin A, egg albumin seemed less effective. Even then, the retention of vitamin A at 1.0 Mrad was 6.5 times higher in aqueous egg albumin preparation compared to that in isooctane, suggesting the protective nature of aqueous dispersions.

Table III. Influence of Carbohydrates and Protein on Radiation Lability of Vitamin A Acetate in Aqueous Systems[a]

Dispersing medium	%	Vit. A destruction × 10^{-16} molecules/g 1.0 Mrad	3.0 Mrad
Lactose	10	4.2	13.9
Sucrose	15	7.8	17.5
Glucose	10	11.4	22.9
Starch	10	12.0	26.5
Egg albumin	0.1	16.9	37.4

[a] In isooctane, the destruction of vitamin A at 1.0 Mrad was 53.6 × 10^{16} molecules/g. Aqueous dispersions of vitamin A with the test compounds were prepared as described in the text. The initial concentration of vitamin A was 60.2 × 10^{16} molecules/g (10 × 10^{-4} M).

The dose-modifying effect of aqueous sucrose dispersion was also observed with vitamin A acetate, ubiquinone-30, vitamin A alcohol, and β-carotene (all at 1 × 10^{-4} M, i.e. 6.02 × 10^{16} molecules/g). The respective destructions of the above compounds at 1.0 Mrad (i.e., 6.24 × 10^{19} eV/g) were 1.63, 1.87, 5.36, and 0.72 × 10^{16} molecules/g. In isooctane medium, corresponding G values were found to be 0.25, 0.10, 1.25, and 3.6, respectively.

Radiation Response of Vitamin A in Aqueous Tween-80 System. Irradiation of an aqueous solution of vitamin A with Tween-80 (10%) resulted in a high G(−vit. A) value of 3.5 compared to 2, 1.7, and 1.6 in media containing Tween-80, coconut oil, and isooctane alone, respectively. Calculation of DMF by dividing the G(−vit.

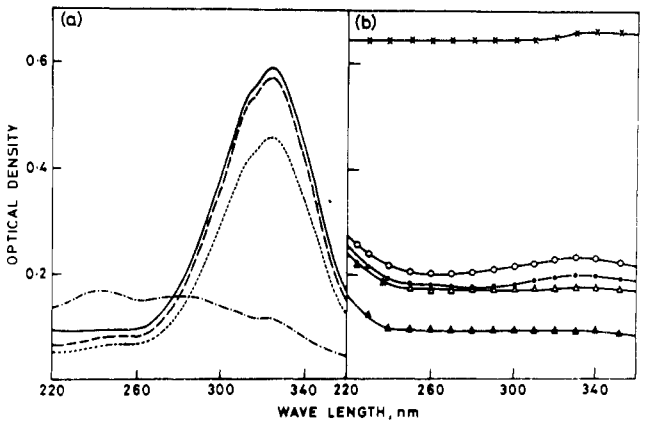

Figure 3. UV absorption spectra of vitamin A in lipid solvent and aqueous dispersion. Spectra of vitamin A in isooctane (a) and aqueous dispersions (b) were recorded as described in the text: (a) (—) unirradiated vitamin A; (-·-) irradiated (1.0 Mrad) in isooctane; (- - -) extracted from unirradiated aqueous dispersion with sucrose; (· · ·) extracted from irradiated (1.0 Mrad) aqueous dispersion; (b) (×) coconut oil and Tween-80; (○) glucose; (●) sucrose; (△) lactose; (▲) albumin.

A) value in Tween-80 by that in the aqueous Tween-80 system yielded a low value of 0.6 suggesting a lack of protection by the aqueous system of this type.

Spectral Characteristics of Vitamin A. Vitamin A dissolved in isooctane or coconut oil showed a characteristic peak at 325 nm. When exposed to a radiation dose of 1.0 Mrad in isooctane, there was considerable distortion in the spectra of vitamin A with a shift to the UV region (Figure 3a). Aqueous dispersions, however, owing to their turbidity, did not show the peak of vitamin A (Figure 3b); the retention of vitamin A could be demonstrated only when the compound was extracted after addition of methanol in petroleum ether (Figure 3a). Extracted vitamin A showed less radiation destruction in aqueous dispersion than in isooctane. A clear aqueous suspension of vitamin A with Tween-80 and water (10:90), however, gave a vitamin A peak similar to that observed in isooctane and coconut oil.

DISCUSSION

A comparison of the pattern of radiation damage to vitamin A in the freely dissolved system and in aqueous dispersion over varying concentrations of vitamin A unequivocally establishes the dose-modifying influence leading to enhanced stability of vitamin A in aqueous dispersions. The number of vitamin A molecules destroyed after exposure to varying doses of γ radiation in different media enables computation of G(–vit. A) values at each of the concentrations tested. It is evident from Figure 2a that two curves representing a relationship between G(–vit. A) and concentration exhibit different slopes in lipid solvents and in aqueous dispersion. Also, these curves intersect each other at a point corresponding to a concentration of 2×10^{-4} M. Thus, it becomes apparent that the oil–water dispersion system offers protection to vitamin A above this critical concentration but not at lower concentrations.

A molecule in solution undergoes radiation destruction possibly by two ways: (i) by direct absorption of radiation energy, direct effect, and (ii) by reaction with free radicals produced from the media, i.e. indirect effect (Bacq and Alexander, 1961). Irradiation in frozen media or with multiple selection of solute concentrations has been employed along with other techniques in order to ascertain the mode of radiation damage to a molecule (Bacq and Alexander, 1961; Dale, 1966). Apart from direct effect the radiation-induced reactions have been known to be mediated by H·, ·OH, HOO·, and e_{aq}^- species in aqueous systems (Draganic and Draganic, 1971), and by alkyl radicals and atomic hydrogen in irradiated hydrocarbons (Topchiev, 1964). Similarly, irradiation of tristearin has been shown to yield temperature-sensitive free radicals (Truby et al., 1962). Thus, it may be implied that direct effect as well as mobile free radicals may cause destruction of vitamin A in coconut oil. An increase in G(–vit. A) with concentration (Figure 2a) and reduction in vitamin A destruction by freezing the media during irradiation (Table I) support this view.

At low concentrations of vitamin A such as 1×10^{-4} M, however, direct effects of radiation may contribute maximally toward vitamin A loss for the following reasons. Theoretically, the extent to which the solute gets destroyed by irradiation in hydrocarbons follows a square-root relationship with the initial concentration (Warman et al., 1968). Thus, it follows that the ratio of the square of G(–vit. A) to the concentration, C [i.e., G(–vit. A)$^2/C$], is a constant. Computation of the ratios obtained from the data of Figure 2a for vitamin A destruction in isooctane gives fairly constant values of 0.22, 0.28, and 0.26×10^4 for 5, 7, and 10×10^{-4} M concentrations, respectively. However, in the case of very low (1×10^{-4} M) and high concentrations (70 and 125×10^{-4} M) of vitamin A, the ratio seemed to deviate greatly from the above values (the ratio being 0.06, 0.16, and 0.12×10^4 for 1, 70, and 125×10^{-4} M concentrations, respectively). The reason for the very low G(–vit. A)$^2/C$ ratio at 1×10^{-4} M could be that vitamin A concentration has been so low that primary radicals were lost by reacting with one another or with certain impurities in the medium instead of reacting with the solute. Studies of Dale (1940), Okada (1957), and Butler et al. (1960) with very dilute enzyme solutions also showed similar dilution effects. Results from present studies on frozen irradiated coconut oil also supported the above findings, in that, at low concentrations, there seemed to be hardly any protection to vitamin A from radiation destruction.

At the low concentration of 1×10^{-4} M, aqueous dispersions apparently do not offer protection to vitamin A; while no definite explanation could be offered on the basis of present observations, it is relevent to point out that an exceptionally low ratio of G(–vit. A)$^2/C$ was obtained at a low concentration of vitamin A (1×10^{-4} M) in coconut oil. The ratio of G(–vit. A)$^2/C$ calculated from the data of Knapp and Tappel (1961) on irradiation of vitamin A (0.85×10^{-4} M) in isooctane was found to be 0.07, which is in close agreement with the values reported by us for a 1×10^{-4} M concentration of vitamin A.

It is difficult to explain the marked dose-modifying effect of the coconut oil–water dispersion or aqueous dispersions with protein and carbohydrates on β-carotene, vitamin A, or ubiquinone-30. In aqueous dispersions, possible reasons for observed protection could be (i) chemical adsorption of the lipid-soluble compounds with other molecules, (ii) formation of a complex with dispersing agents, (iii) the free-radical scavenging effect of dispersing agents, and (iv) the colloidal nature of aqueous lipid dispersions.

Evidence obtained from spectral studies and the need for the addition of polar solvents like methanol to accomplish maximal extraction of vitamin A in petroleum ether from the aqueous dispersions suggest that vitamin A may be present as complexes with the dispersing agents or may form oil-in-water types of colloidal dispersions in

such systems. In other types of aqueous preparation with 10% Tween-80, which does not offer protection to vitamin A, high concentrations of surface active agent may affect the molecular orientation and make vitamin A readily accessible to radiation damage.

These results reveal that, in addition to the effects of solvent and concentration of molecule, the mode of dispersion is important in determining the radiation response of vitamin A. Thus, our evidence points to one protective factor, occurrence of which in foods may be important in the consideration of radiation damage to compounds which are labile in the isolated state. Such and other protective mechanisms will tend to reduce the possibility of food toxicity arising from radiation degradation products.

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Temperature and Storage Effects on Percent Retention and Percent U.S. Recommended Dietary Allowance of Vitamin C in Canned Single-Strength Orange Juice

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Canned single-strength orange juice stored at 29.4, 37.8, and 46.1 °C for 12 weeks showed that the log % vitamin C retention was linearly related with time at 29 °C but not at 38 or 46 °C. Storage of 14 processed juices at 4.4 to 48.9 °C over 12 weeks showed that temperatures greater than ca. 27 °C markedly decreased the rate of vitamin C retention. Orthogonal polynomials were used to determine the equation for vitamin C degradation over 29.4 to 48.9 °C.

A major nutritional value of citrus fruit is their vitamin C content. Many nutritionists consider a daily intake of 50 to 150 mg per day is needed for good health maintenance. The National Academy of Sciences (Food and Nutrition Board, 1974) has recommended a daily intake of from 35 mg (infants) to 80 mg (lactating females) whereas the Food and Drug Administration (FDA, 1973) considers an intake of 60 mg per day (for adults and children 4 years or more in age) as meeting the 100% U.S. RDA requirement.

The retention of vitamin C potency in citrus products is important both to the consumer, concerned with maintaining good health, and to the citrus processor. Because of intensified interest in nutrient labeling, the citrus processor is concerned with the vitamin C content, which is expressed as percent U.S. RDA value, in orange juice products and its change during product storage.

Many studies (Moschette et al., 1947; Sheft et al., 1949; Freed et al., 1949; Blundstone et al., 1971; Bissett and Berry, 1975) have shown loss of vitamin C in single-strength orange juice (SSOJ) was related to storage temperatures. High-temperature loss of vitamin C may result from processing, unfavorable heat dissipation of the freshly processed juice (stack-burn), warehouse storage (heat pockets, poor air circulation, improper insulation), poor transit conditions (over-heated tractor trailers, railway cars, etc.), and poor handling at distribution centers and supermarkets (lack of rotation of flavor-sensitive foods).

A comparative study of the rates of vitamin C degradation at both low and high storage temperatures has

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