SOURCES OF VARIABILITY IN THE CAROTENOID LEVEL AND VITAMIN A ACTIVITY OF FOODS

Gary R. Beecher

USDA/ARS
Nutrient Composition Laboratory
Beltsville, Maryland

Introduction

Most foods are products of biological systems and as a result the levels of nutrients and other constituents are expected to vary. Estimates of the magnitude of the variability of a nutrient within a food are tabulated in the various sections of the United States Department of Agriculture (U.S.D.A.) Handbook No. 8. This statistic is the standard error. A more meaningful statistic is the range which together with the mean, establishes minimum, and maximum values. The expected range of values can be calculated from the standard error and number of samples using simple equations and appropriate factors (Steele and Torrie, 1960). These values are tabulated in Table I for the vitamin A activity for selected foods. As can be seen, the estimated ranges vary widely about the mean for each food but are about the same order of magnitude as those reported by Johnson et al. (1985). In part, this may be due to the small number of samples that were analyzed (Table I), but more likely it is due to the "natural" variability of vitamin A activity in each food.

There are many potential sources of variation relative to the nutrient content of foods. For foods of plant origin these include cultivar, length of growth period, soil type and fertility, climate, and storage conditions of the harvested food. Similarly, for foods derived from animals, animal species, maturity, and feed are a few of the factors that influence the nutrient content of the resulting food. Finally, food processing and preparation as well as analytical procedures used to measure nutrient levels also contribute to the variability of the nutrient content of all foods.

The vitamin A activity of foods is unique in that nearly a dozen related molecules contribute to its activity (Beecher and Khachik, 1984). The most active compound is retinol which is also the most prominent form of vitamin A in animal products. In plant foods, vitamin A activity is vested in a family of compounds called carotenoids. In general, the most abundant compound with vitamin A activity in these foods is β-carotene, which has about one-sixth the activity of retinol. Beta-carotene is also prevalent in selected animal products, e.g., liver, eggs and butter. Other vitamin A active carotenoids common to plant foods include α-carotene, γ-carotene and β-cryptoxanthin. Each of these compounds has less vitamin A activity than β-carotene. The level of each of the carotenoids or retinol in food has the potential to be altered by factors discussed above. Thus the variability of vitamin A activity is the summation of the variability of each of the components that contribute to the vitamin activity.

During the past decade, epidemiologic studies have associated the ingestion of a number of plant foods i.e., cruciferous vegetables, with reduced incidence of cancer (Micozzi, 1989). Although the cancer modifying effects of these foods was originally attributed to vitamin A activity, these foods are all relatively low in β-carotene but high in non-vitamin A active carotenoids, e.g., lutein, and other nutrients, e.g., vitamin C. These observations suggest that selected foods must be analyzed for all carotenoids and the impact of environmental and other factors on the variability of carotenoid content evaluated.

In this paper, examples of the effect of selected factors on vitamin A activity are discussed. The variability of the vitamin A content of individual foods is discussed in the perspective of mixed diets and recommended dietary allowances (RDA) in the last section of the paper.

Influence of Environment, Fertility, Genetics, and Maturity on Variability

Climate. Simon et al. (1982) have identified climate as one of the primary contributors of variation in the carotene content of carrots. An example of this effect is shown by data from a Swedish study (Hårdh et al., 1977) where carrots grown in southern Sweden (Alnarp, 55° 05' north latitude) had nearly twice the level of α- and β-carotene as the same cultivar grown in northern Sweden (Ojebyn, 65° 03' north latitude). Khachik and Beecher (1988) also reported climatic/geographic effects on the carotenoid level of squash. Foods grown in North Carolina (35° 37' north latitude) had more than twice as much lutein and about 1.5 times the level of β-carotene as the same cultivar grown in Michigan (43° 27' north latitude). These observations support the postulation of Hårdh et al. (1977) that the primary environmental factors responsible for alteration of carotene levels are temperature and total radiation.

Soil type and fertility. Hårdh et al. (1977) reported that carrots grown on peat and mineral soil had similar levels of α and β-carotene. Similarly, the application of fertilizer containing nitrogen, phosphorous and potassium, increased the total carotene levels of carrots only ten percent (Evers, 1989). The yield of carotenes per hectare showed about the same effect of fertilization which suggests that the size of carrots was not influenced by fertility. Organically grown carrots also had similar levels of carotenes and similar yields as carrots grown on unfertilized soils (Evers, 1989).

Cultivar. The carotene content of fruits and vegetables is, in part, dependent on the genotype of the plant (Gabelman, 1974). Cultivars of carrots have been developed which have a total carotene content 4-5 times greater than most cultivars (Simon and Wolff, 1987). Heinonen (1990) also observed more than a two-fold range in the content of carotenes when nineteen varieties of carrots were compared. Cultivars of other fruits and vegetables also vary in their total carotene content. Different cultivars of mexican peppers varied by 50-fold (Mejia, et al., 1988), hybrid citrus by 20-fold (Stewart, 1977), Mangoes by four-fold (Rodriguez-Amaya, 1988) and cultivars of corn, squash and tomatoes varied by about two-fold within each vegetable (Lee et al., 1981; 1984, Lee and Robinson, 1980). In contrast, Watada et al. (1976) reported four cultivars of tomatoes were similar in their β-carotene content. These observations substantiate the potential variability introduced into vitamin A and carotenoid data when several cultivars of fruits and vegetables supply these foods for human consumption.

Species. Although there are only limited data, animal species and the associated variability due to nutritional status and other factors greatly influence the vitamin A content of liver. Schindler et al. (1987) reported the average vitamin A content of beef, calf and pig liver varied about two-fold. However, within each species values ranged from about five-fold to ten-fold. Liverwurst, a product of pig liver, also had an eight-fold range of values.

Maturity. Stage of ripeness or maturity may greatly influence the carotenoid and vitamin activity of fruits and vegetables. Lee (1986) reported that as carrots grew, vitamin A activity expressed per 100g fresh weight, about doubled during the last eighty days of growth. Because the size increased the yield of total vitamin A activity per unit area of land increased dramatically. Carotenoid levels were found to increase about 30-fold for squash and three-fold for lettuce, as these matured (Arima and Rodriguez-Amaya, 1988; Ramos and Rodriguez-Amaya, 1987). Whereas the level of abundant carotenoids tended to change in concert in the previous examples, as citrus ripens the levels of the most prominent carotenoids increased disproportionately (Stewart, 1977; Gross,1982). In the case of tangerines and hybrid cultivars, β-carotene increased to a greater extent than β-cryptoxanthin. However, for cultivars of oranges, the reverse occurred.

Influence of Food Storage, Fortification, Processing and Cooking on Variability

Food Storage. Carrots stored at 2°C for 155 days had only minor fluctuations (<10%) in the levels of α- and β-carotene (Lee, 1986). Similarly, the β-carotene and α-cryptoxanthin content of canned mango slices and

mango puree were constant for ten months when stored at room temperature (Godoy and Rodriguez-Amaya, 1987). However, during the subsequent fourteen months of storage, β-carotene levels declined more than 80% in both mango slices and puree. The concentration of α-cryptoxanthin also decreased in slices (>20%) but remained constant in puree during the same storage period. The same laboratory showed β-carotene in guava juice was stable ten months but lycopene gradually declined during storage to 75% of its initial level (Padula and Rodriguez-Amaya, 1987). Both retinol and β-carotene were stable in butter stored at 4°C for twelve months (Heinonen et al., 1988). These observations indicate that stability of carotenoids during storage is dependent upon the food matrix, the specific carotenoid and any processing that may have preceded storage.

Fortification. Several foods in this country and other countries are fortified with vitamin A. The level of vitamin A observed in margarines from several Finnish manufacturers was only 67-98% of the obligatory level (Heinonen et al., 1988). Tanner et al., (1988) investigated the levels of vitamin A in fortified fluid milks in the U.S. and reported that as the fat content of milk decreased (whole milk, 2% milk, skim milk), a greater proportion of samples had less than 50% of label claim for vitamin A. The incidence of whole milk with vitamin A at less than 50% of label claim was zero, whereas for skim milk 30-50% of the samples fell into this category. These results can be explained by the sequence of fortification (whole milk) and subsequent processing (removal of fat) with no addition of vitamin A to compensate for that removed with the fat (J. Tanner, personal communication).

Processing and Cooking. The effect of heat on carotenoids in food products is very complex. Firstly, selected classes of carotenoids (hydrocarbon and hydroxylated) are less susceptible to destruction by heat than other carotenoid classes (epoxides). Secondly, carotenoids undergo subtle stereoisomeric changes (all-trans to cis) in response to heating and as a result, vitamin A activity is reduced. Finally, changes in carotenoid levels and isomerism are directly correlated with the intensity and duration of heat treatment.

Frying (200°C) decreased the β-carotene content of several green vegetables by 8-81% (Speek at al., 1988). Canning at high temperatures (116-121°C for longer than one hour) reduced the total β-carotene content of sweet potatoes (Chandler and Schwartz, 1988) and decreased the level of several carotenes of winter squash (Lee et al., 1984). The β-carotene content of pumpkin processed under similar conditions was only slightly reduced (Khachik and Beecher, 1987). When the severity of canning conditions was reduced (100°C, 20-30 minutes), hydrocarbon and hydroxy carotenoids in mango slices and puree as well as in guava juice were not changed or only slightly decreased (Godoy and Rodriguez-Amaya, 1987; Padula and Rodriguez-Amaya, 1987). Speek et al., (1988) reported 47-63% loss of β-carotene as a result of sun drying several foods. This observation suggests sun drying is also a severe processing treatment perhaps due to a combination of heat and ultraviolet radiation.

Several common household cooking methods appear to be sufficiently gentle so as not to drastically alter the level of carotenoids in foods. Microwave cooking (600 watts, 7 minutes; 1100 watts, 4 minutes) did not alter the level of β-carotene in sweet potatoes (Chandler and Schwartz, 1988) or the level of lutein and β-carotene in broccoli, green beans and spinach (Khachik et al., 1989). Khachik and co-workers (1986, 1987, 1988) reported a small reduction (5-15%) of β-carotene when acorn squash, Brussels sprouts, kale and sweet potatoes were cooked with microwave energy. For several of the same vegetables, lutein was decreased 18-25% as a result of microwave cooking. Boiling green beans for as long as an hour did not alter the levels of β-carotene or lutein (Khachik et al., 1989). However, boiling sweet potatoes for only ten minutes decreased levels of β-carotene about 26% (Almeida and Penteado, 1988) and boiling several green leafy Thai vegetables (two-eight minutes) reduced β-carotene 11-50% (Speek et al., 1988). Limited data from the baking of sweet potatoes (Chandler and Schwartz, 1988) and steaming of broccoli and spinach (Khachik et al., 1989) indicates that these cooking methods do not drastically change levels of β-carotene or lutein, if present, in the vegetable.

Several studies have shown that epoxides of carotenoids (generally not vitamin A active) are very sensitive to most common cooking and processing procedures (Godoy and Rodriguez-Amaya, 1987; Padula and Rodriguez-

Amaya, 1987; Almeida and Penteado, 1988; Khachik et al., 1989). Most susceptible are 5, 6 epoxides, e.g., violaxanthin, as they are converted to 5, 8 epoxides, e.g., luteoxanthin and auroxanthin. These compounds are subsequently destroyed by heat.

A very subtle change in carotenoids and subsequent reduction of vitamin A activity promoted by cooking and processing is isomerization of the all-trans double bond configuration to cis-stereoisomers that are not sterically hindered. Sweeney and Marsh (1971) showed canning, cooking and freezing decreased levels of all-trans β-carotene and concomitantly increased levels of several cis-isomers in vegetables. Several different processing procedures, including blanching, drying and baking, also had the same effect in sweet potatoes (Chandler and Schwartz, 1988).

Influence of Analyses on Variability. Traditional methods for the determination of vitamin A activity in plant foods rely on open column chromatography procedures of which the AOAC method is the most widely used (Williams, 1984). These procedures only separate carotenoids into major classes of compounds, i.e., hydrocarbon, mono-hydroxy, etc. Since α-carotene has about one-half the vitamin A activity as β-carotene, and since both of these compounds are eluted together, the estimation of vitamin A activity is inaccurate when foods containing both of these carotenoids, e.g., carrots, are analyzed with AOAC procedures. Unfortunately, most of the vitamin A data in U.S. Department of Agriculture handbooks and databases has been generated employing AOAC procedures (J. Weihrauch, personal communication).

The application of high performance liquid chromatography (HPLC) to the measurement of carotenoids in foods permits the resolution and accurate quantification of each carotenoid present. From these data, vitamin A activity for each food can be calculated. Bushway and Wilson (1982) compared vitamin A values of several foods measured by both AOAC and HPLC procedures. In general, vitamin A activities determined by HPLC ranged from 40% (green foods) to 90% (carrots) of the values determined by AOAC methods (Bushway and Wilson, 1982). These data raise a question as to the accuracy of vitamin A values for other plant foods.

Levels of retinol in animal foods can also be measured employing older colorimetric methods (Williams, 1984) or newer HPLC procedures. Again, most of the vitamin A values currently in tables and databases have been generated with colorimetric procedures. These methods are subject to interferences and false positive reactions from such compounds as carotenoids and other similar chromophobic compounds (Frolik and Olson, 1984). Measurement of retinol and retinol derivatives using HPLC methods (Ball, 1988) permits each vitamin A active component to be resolved from interferencing compounds and accurately quantified.

Variability of Vitamin A Activity in the Context of Mixed Diets and Recommended Dietary Allowances

The above discussion has focused on sources of variation and the presentation of data for individual foods as examples. Most foods consumed by human beings are consumed as combinations of foods in the form of meals or mixed diets. The question then is how does the combination of foods into mixed diets impact on the variability of vitamin A activity in the diet? Beaton (1985) has examined the variability of nutrients in mixed diets. This was accomplished by selecting a random value (restrained to the normal distribution defined by the mean and standard error) for each nutrient in each food and subsequently adding together the values for each nutrient to estimate the one day intake. This exercise was repeated 1,000 times using new random numbers of the populations of possible compositions and then the standard deviations and coefficients of variation for one day nutrient intakes were calculated. Results from this exercise showed that the coefficient of variation for vitamin A was about 7.4%. This is surprisingly low compared to coefficients of variation for individual foods which may be as high as 74% (calculated for beef liver in Table I).

What is the expected range of vitamin A values for mixed diets when the coefficient of variation is 7.4%? The

range of vitamin A values for diets was calculated following the procedures described above for individual foods. The average vitamin A value for all diets was set at the current RDA of 1,000 retinol equivalents (RE) per day for adult males (Food and Nutrition Board, 1989). The results are plotted as a normal distribution curve in Figure 1. Using this approach, the vitamin A values range from a little less than 800 to a little more than 1,200 RE per day. While this is a substantial range, the low value (~800) is considerably greater than the estimated requirement (600 RE/day) for adult males (Food and Nutrition Board, 1989). If it is assumed that the selection of foods is random and there is no systematic bias in food composition data, then there is an equal chance that daily vitamin A intake will fall between 1,000-1,200 RE as there is that it will be between 800-1,000. On average the RDA will have been met.

The curve in Figure 1 can also be used to predict the precision of 1) calculating dietary intakes from dietary records and 2) vitamin A levels of menus calculated from food composition data. This level of precision is probably adequate for assessing intakes and formulating menus for populations (perhaps even small populations) but it is inadequate for research situations. When human metabolic studies involving vitamin A are conducted the vitamin A and provitamin A levels in foods first must be measured and the resulting values used to formulate menus from those foods. Vanderslice and Higgs (1990) showed that large errors could result in the vitamin C content of menus when data from food tables were used in place of analytical data for the actual foods that were subsequently incorporated into meals. In the case of free choice meals, more accurate and precise estimates of vitamin A activity must be obtained by measuring appropriate components in duplicate meals.

Summary

Foods are derived from biological systems and, therefore, the vitamin A activity is expected to vary. The magnitude of this variation is tabulated in food tables and databases as the standard error. Ranges of values calculated from the standard error, number of samples, and the average are sometimes small but are often 5-to 10-fold. Several factors contribute to the variability of vitamin A activity. For plant foods, the most critical factors are cultivar, climate, length of growth period and storage conditions of the harvested food. For foods derived from animals, maturity, species, and feeding regimen are important contributors to variability. Processing and cooking also contribute to variability of vitamin A activity of foods. High temperature, long-term processing degrades most vitamin A active components in foods whereas low-temperature, short-term cooking preserves the stable, highly active vitamin A components, e.g., retinol, β-carotene. Fortification and method of analysis are two additional factors that contribute to variability of vitamin A activity as reported in food tables and databases.

While knowledge of nutrient variability within each food is important, the critical aspect relative to nutrition is the resulting variability after foods are combined into meals and daily diets. Using the theoretical coefficient of variation calculated by Beaton (7.4%) and setting the average vitamin A activity at 1,000 RE (RDA for adult males), vitamin A values ranged from about 800 to 1200 RE per day. This is the expected precision using current food composition data. This level of precision is probably adequate for assessing intakes and formulating menus for populations but inadequate for research situations. In the case of the formulation of menus for metabolic studies, each lot of food must be analyzed for vitamin A activity and the resulting data used to prepare menus. When metabolic studies involve free choice meals, more accurate estimates of vitamin A activity must be obtained by analyzing duplicate meals and diets.

Acknowledgements

The helpful suggestions of B. Gray, M. B. Goli, F. Khachik, J. Holden, and J. T. Vanderslice during the preparation of this paper are gratefully acknowledged. Thanks are also due C. D. Williams and S. C. Tinkcom for their assistance in the preparation of this manuscript.

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Table I.

Estimated Ranges of Vitamin A Levels for Selected Foods[@]

	RE/100 grams[b]			
Food Description	N[c]	Mean	SE[d]	Range[e]
Beef, liver, raw	28	10,503	1479	0 - 26,156
Broccoli, raw	5	154	4.5	135 - 173
Carrots, raw	162	2,813	15	2,307 - 3,319
Carrots, canned, solids and liquid	176	1,317	23	493 - 2,141
Sweet potatoes, cooked, baked in skin	8	2,182	136	1,470 - 2,894
Tomatoes, red, ripe, raw	259	113	1.8	30 - 196

[a] Data taken from various sections of the U.S. Department of Agriculture Handbook No. 8.

[b] Retinol equivalents per 100 grams food.

[c] Number of samples.

[d] Standard error of the mean.

[e] Estimated range calculated using the following formula: $f \bullet \sqrt{N} \bullet SE$, where f is the ratio of range to standard deviation (3.7 when N=20, 5.5 when N=200). Steele and Torrie, 1960.

Figure I.

Theoretical Variation of Vitamin A in Mixed Diets for Adult Males