VITAMIN C: CONTENT VARIABILITY IN SAMPLES: IMPLICATIONS FOR DIETARY STUDIES

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For the past several years, our laboratory has been involved in the analysis of the total vitamin C content of foods which are the major source of this nutrient in the American diet. The foods were prepared as they normally would be prepared in the American household and the amounts of ascorbic acid and dehydroascorbic acid determined by high performance liquid chromatography with fluorescent detection.[1]

One of the interesting results of this study was the wide variability of the vitamin C content found in food samples which are supposedly identical in nature. The foods were purchased at different local markets on different days and different seasons and analyzed on the day purchased. This variability is shown for some foods in Table 1. Note that the variations in the mean can be of the order of a factor of two or more in some foods. Some biological variability had been expected, but not to the extent that was found.

In Table 2, the range of values found in this study is tabulated along with the ranges calculated from the Agricultural Handbooks 8-9 and 8-11.[2] These latter ranges were calculated on the assumption that any outliers greater than ± 2 (s.d.) have been rejected and that the observed values fill the range x ± 2 (s.d.) where x is the average value and (s.d.) is the standard deviation. The standard deviation, in turn, has been calculated from the values of the standard error of the mean and the number of observations reported in Handbook #8. As a matter of fact, the range will vary with the number of samples studied. It will change from 3.7 (s.d.) for twenty samples up to 6.5 (s.d.) for 1,000 samples.[3] The four (s.d.) used in the present tabulation represents a suitable compromise. It would seem, on the basis of this comparison, that the ranges observed in this study are in line with the ranges inferred from Handbook #8 data. Casual users of Handbook #8 data may not realize the range of variation for the vitamin C content in a given type of food.

As this report is being written, this laboratory is involved in a human diet study on vitamin C being conducted jointly by the National Cancer Institute and the Beltsville Human Nutrition Research Center. The study includes two depletion phases and two repletion phases with the sources of vitamin C being raw and cooked broccoli, oranges, orange juice, and vitamin pills. The broccoli and oranges are shipped fresh from a distributor in California and are analyzed on a crate by crate basis. The orange juice is prepared daily from one lot of frozen concentrate and analyzed periodically throughout the study. The vitamin pills, all from the same lot, were analyzed at the beginning of the study. A minimum of stalk was included in the broccoli samples and the oranges were peeled so that no rind or pith remained. The cooked broccoli samples were boiled for 12 minutes in plastic bags.

Despite all efforts to standardize sample preparation, large variations in food composition of vitamin C were still found and it is of interest to see what implications this has for future human dietary studies involving vitamin C. We will concentrate only on the contribution to the variance from the food variability and disregard other variances due to biological variability of subjects, bioavailability, etc.

Analytical data on the foods is only available for the first half of the diet study (21 days of repletion) and this is shown in Table 3. Here, the data for broccoli and oranges are shown as an average value ± (s.d.) among crates and within crates. The vitamin C content of orange juice and vitamin pills, each from one lot, has not varied appreciably over the course of the study. Also shown are the range of values obtained for the different food products. Note the difference in the (s.d.)'s (or the variances) for the case of broccoli between crates and within crates. In the case of oranges, the (s.d.)'s are the same whether it is between crates or within crates.

In Table 4, the overall results obtained in this diet study are compared with those reported in Handbook #8 and the corresponding German Handbook.[4] Our results are definitely higher than those given in Handbook #8. This is not surprising in the light of our sample preparation and cooking methods. For broccoli, for example, a minimum of stalk was used and all cooking was done in plastic bags. All food samples were obtained fresh from the distributor. It is likely that the Handbook values are more representative of the content of these food products as purchased and cooked by most American consumers. There is a definite bias in our data as a result of the more stringent sample collection, sample handling and preparation, and cooking methods. The effect of this bias can be seen in Table 5. Here are shown the net intake of vitamin C by the subjects using different sources. The vitamin pill had a label value of 100 mg/tablet. If this were taken at its face value and the weights of the other sources adjusted using handbook #8 data to give 100 mg intake, it would result in actual average intakes shown in the first column. Clearly, there is at least a thirty percent variation in intake. If the overall average values observed in the present study were used to determine intake, then the values will be those shown in the second column. If the daily analyses were used to determine intake, the results shown in the third column are obtained. Note the difference in the (s.d.)'s (or variances) for broccoli between the second and third columns. Briefly, in moving from column 1 to 2, one corrects for bias while in moving from column 2 to column 3 one reduces the variance attributable to the vitamin C content of the foods.

The bias of the data and its effect on a given biological marker is illustrated in Fig. 1. Here are plotted the urinary rates of excretion of vitamin C as a function of vitamin C intake for human studies during an earlier repletion study by Baker et al. who reported the excretion rate in terms of millicuries of C^{14} measured.[5] These have been plotted as a function of (vitamin C intake)^2/(n-1) where n is 21, the number of day in the repletion stage in the present study. The latter function was chosen not only because of its variance-like characteristic but also to spread out the experimental points so that differences would be apparent. The projected urinary excretion rates are calculated on the basis of Handbook #8 data. The projected experiment would indicate a much larger urinary excretion rate for those subjects being fed broccoli and oranges than those being fed orange juice or vitamin pills. Since Baker et al. related this urinary excretion rate with the build-up of the body pool of vitamin C, one might conclude that broccoli and oranges were more bioavailable than either orange juice and the vitamin pill. Also shown in Figure 1 is the actual average range of intake that the subjects would be receiving on a daily basis. Since this scale is of a variance type, this suggests a large value of variance attributable to intake which could perhaps mask other effects that are of interest. If our average values were used, the average intake would be the same and the bias eliminated but the range of values would still be relatively large.

After elimination of bias, it would be further desirable to reduce the variance due to sample content to as low a value as possible. In other words, what would it take to reduce the variance down from that of the overall average to that obtained with the daily analyses? The variance calculated with standard statistics could be reduced by either expanding the number of subjects or by extending the length of the repletion study. This is shown in Table 6 for broccoli. The present study involved 11 and 12 subjects for raw and cooked broccoli respectively and the length of the repletion study was 21 days. To increase the precision up to the level one would have with daily analyses values, the number of subjects would either have to be increased to 41 or 27 for raw and cooked broccoli, respectively, or the length of the study increased to 84 or 51 days for raw and cooked broccoli, respectively. Both options represent a considerable financial, personnel, and facilities commitment. These estimates were based on 95% confidence intervals using the student t test.

On the basis of the present data, then, daily analysis of data will eliminate bias and substantially reduce the size and scope of the diet study in order to reduce the variance due to sample content. One should mention that the coefficient of variability in the analytical method is 1% and the coefficient of variability due to the extraction procedure is 3%. Neither affect the results given in Table 6.

For the dietitian not involved in human diet studies but involved in day-to-day menu planning, the above concerns do not apply. Beaton[6] has addressed this problem and has indicated that in a diversified diet, vitamin C comes from many sources and the variation in intake is counterbalanced by the variation in intake from other sources. In addition, the Recommended Dietary Allowances are normally set at 2 (s.d.) above the mean which should compensate for those foods whose nutrient content is at the lower end of the observed range.

In summary, our preliminary results indicate that daily analysis of the diet is essential if one wishes to eliminate bias in the data and reduce the variance to the lowest possible level. The alternative to this approach (assuming bias has been corrected for) is to expand the length and breadth of the study which in the long run is likely to be more expensive than the cost of nutrient analysis on a daily basis.

References

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Table 1. Variability of Vitamin C Content in Some Foods[1]

	(mg/100 g)		
SAMPLE	AA	DHAA	TOTAL
BANANA	9.5 ± 0.4	2.5 ± 0.6	12 ± 1
	4.5 ± 1.0	7.7 ± 2.6	12 ± 3
	15.3 ± 2.5	3.3 ± 0.6	19 ± 3
BROCCOLI, FRESH	89.0 ± 2.0	7.7 ± 0.6	97 ± 2
	119.5 ± 5.4	9.1 ± 0.7	129 ± 5
	123.2 ± 3.1	7.6 ± 1.0	131 ± 3
	121.2 ± 4.4	10.7 ± 1.2	131 ± 5
	139.1 ± 8.0	9.1 ± 0.7	148 ± 8
	145.9 ± 2.1	13.4 ± 0.6	159 ± 2
	148.2 ± 3.0	15.2 ± 0.5	163 ± 3
BROCCOLI, BOILED	37.0 ± 1.0	2.6 ± 0.6	40 ± 1
	61.3 ± 1.7	7.1 ± 0.8	67 ± 2
	74.1 ± 4.9	9.0 ± 0.5	83 ± 5
	78.8 ± 2.9	7.5 ± 0.4	86 ± 3
	83.2 ± 4.1	6.8 ± 0.4	90 ± 4
GRAPEFRUIT	13.7 ± 1.2	7.3 ± 0.6	21 ± 1
	21.3 ± 0.6	2.3 ± 0.6	24 ± 1
	23.0 ± 0.0	5.6 ± 0.6	29 ± 1
	27	4	31
ORANGES, CA NAVAL	56.3 ± 4.2	12.0 ± 0.0	68 ± 4
	66.0 ± 1.0	6.7 ± 2.5	73 ± 3
	75.0 ± 4.5	8.2 ± 1.6	83 ± 5
ORANGES, CA NAVEL VERY FRESH	52.1 ± 1.6	0	52 ± 2
	59.7 ± 2.1	3.2 ± 0.3	63 ± 3
	78.1 ± 1.4	0	78 ± 1
ORANGES, FLORIDA	44.0 ± 3.0	9.0 ± 1.7	53 ± 3
	54.7 ± 2.5	8.3 ± 1.2	63 ± 3
ORANGE JUICE, FROZEN RECONSTITUTED	38.7 ± 1.5	2.3 ± 0.6	41 ± 2
	43.0 ± 1.5	3.3 ± 0.6	46 ± 1
	43.3 ± 0.4	3.2 ± 0.6	47 ± 1
	45.3 ± 2.5	3.3 ± 0.6	49 ± 3
SPINACH, RAW	22.5 ± 3.5	2.7 ± 1.2	25 ± 4
	43.3 ± 0.4	3.2 ± 0.6	47 ± 1
	49.3 ± 10.0	1.3 ± 1.1	51 ± 10
	67.7 ± 3.8	2.3 ± 0.6	70 ± 4

Table 2. Range of Vitamin C Values (mg/100g) in Some Fruits and Vegetables

SAMPLE	HANDBOOK 8+	OUR STUDY
APPLE JUICE	0 - 2	<1
BANANAS	7 - 12	12 - 19
BEANS, SNAP, RAW	11 - 22	12 - 18
BROCCOLI, RAW	77 - 109	97 - 163
CABBAGE, RAW	31 - 64	42 - 83
CUCUMBER	0 - 10	13 - 14
GRAPEFRUIT, RED	28 - 48	21 - 31
LETTUCE	1 - 7	5 - 6
ORANGES, CA NAVEL	40 - 74	52 - 78
FLORIDA	45	53 - 63
POTATOES, IDAHO	0 - 30	11 - 13
RED SKINNED		27
SPINACH, FRESH	6 - 50	25 - 70
FROZEN	5 - 44	25
TOMATOES	9 - 26	14 - 19

+ Range obtained from mean value ± twice the std. dev. found from standard error and number of samples tabulated in handbook.

Table 3. Summary of Data During Nutrition Study[1]

SAMPLE	VITAMIN C CONTENT (mg/100g)++ AVERAGE OVER CRATES	RANGE	WITHIN CRATE
BROCCOLI, RAW	121.2 ± 18.8 (15.5%)	88-163	(7.4%)
BROCCOLI, COOKED	80.2 ± 15.4 (19.0%)	55-121	(11.7%)
ORANGES (NAVEL)	75.9 ± 10.6 (14.0%)	65-86	(12.9%)
ORANGE JUICE FROZEN RECONSTITUTE	43.8 ± 2.3 (5.0%)	42-46	--
VITAMIN PILL (NOMINALLY 100)	107.5 ± 1.3 (1.2%)	106-108	--

\+ Coefficient of Variation given in parenthesis.
++ Mean ± std. dev.

Table 4. Comparisons Among Sets of Data[1]

SAMPLE	VITAMIN C CONTENT (mg/100g)++ THIS STUDY	HANDBOOK #8++	GERMAN H.B. [WASTE]
BROCCOLI, RAW	121.2 ± 18.8	93.2 ± 15.6	114 [39]
BROCCOLI, COOKED	80.2 ± 15.4	62.8	90 [0]
ORANGES (NAVEL)	75.9 ± 10.6	57.3 ± 8.4	50 [28]
ORANGE JUICE	48.3 ± 2.3	38.9 ± 5.3	56 [0]

\+ Mean ± std. dev.
++ Std. dev. calculated from standard error and number of samples tabulated in handbook.

Table 5. Average Vitamin C Intake Based on Different Data Sets

	INTAKE (mg)+		
SAMPLE	HANDBOOK #8 PILL LABEL	THIS DATA (AVERAGE)	THIS DATA (DAILY)
BROCCOLI, RAW	129.7 ± 20.1	107.5 ± 16.7	107.5 ± 8.4
BROCCOLI, COOKED	127.5 ± 24.5	107.5 ± 20.6	107.5 ± 12.9
ORANGES (NAVEL)	132.8 ± 18.6	107.5 ± 15.0	107.5 ± 13.9
ORANGE JUICE	112.6 ± 5.9	107.5 ± 5.6	107.5 ± 5.6
VITAMIN PILL	107.5 ± 1.3	107.5 ± 1.3	107.5 ± 1.3

\+ Mean ± std. dev.

Table 6. Modification of Study Required to Achieve Equal Precision if Overall Instead of Daily Averages Used for Broccoli[1]

	SAMPLE	DIET STUDY	MODIFICATION
# SUBJECTS	RAW	11	41
	COOKED	12	28
LENGTH OF STUDY++	RAW	21	84
	COOKED	21	51

\+ At 95% confidence interval.
++ Repletion phase in first one-half of Human Diet Study.

Projected Urinary Excretion Rate - Handbook #8 Used to Determine Dietary Intake.

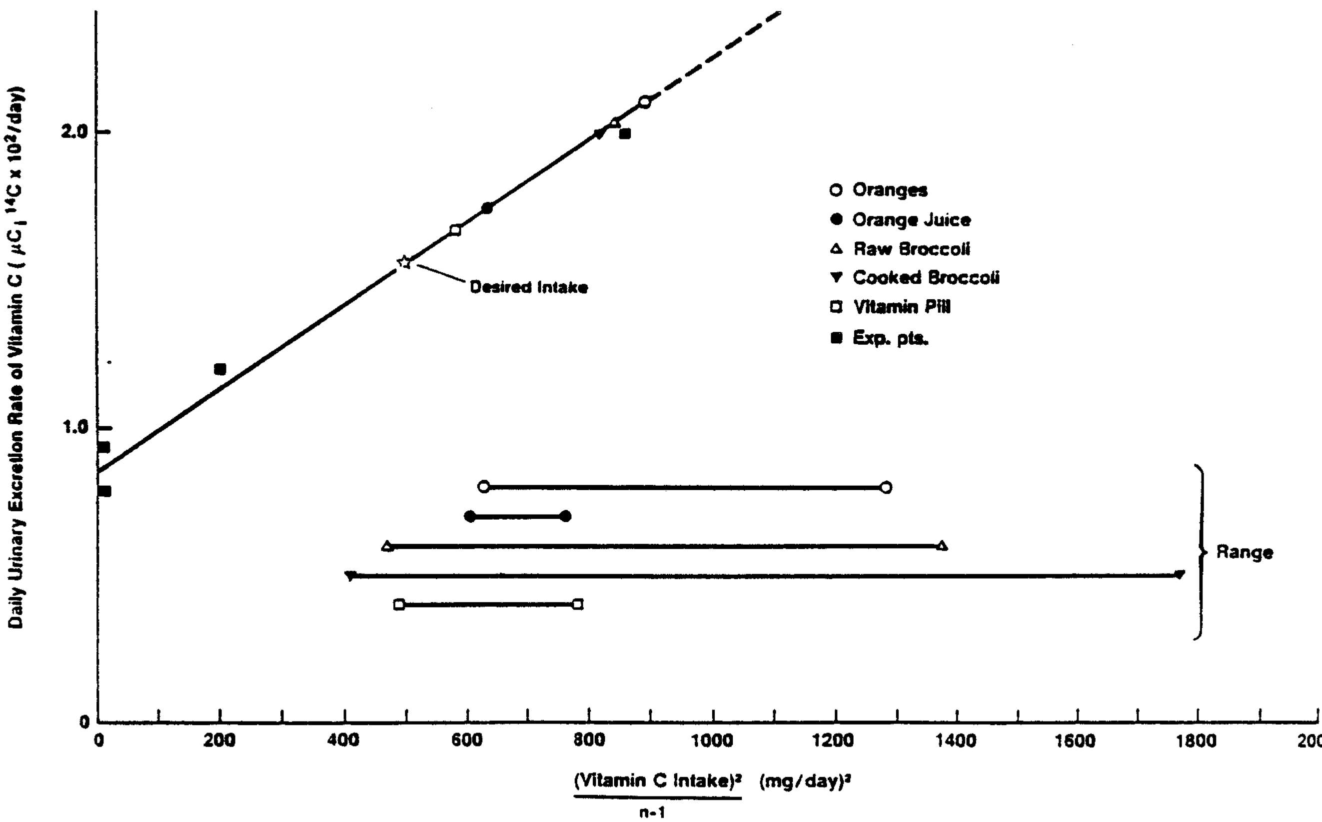

Fig. 1 Urinary excretion rates during repletion study by Baker et al. plotted vs (Vitamin C intake)^2/(n-1). Superimposed on this graph is the actual intake by subjects in the National Cancer Institute-Beltsville Human Nutrition Research Center study if Handbook #8 data had been used to calculate intake.