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MINERAL COMPOSITION OF VEGETABLES

Mineral Elements in Fresh Vegetables from Different Geographic Areas

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The concentration of ten elements in nine different fresh vegetables collected in the Washington, D. C., wholesale market is reported. Marked divergencies between different producing areas were found for the sodium content of lettuce and onions, and for manganese in carrots and celery, among others. Copper and manganese in sweet corn showed statistically significant differences between means for individual lots grown on the same farm and illustrate the problems inherent in reporting averages for highly variable elements for a given producing area.

W I'III THE INTRODUCTION of new varieties and the use of different cultural practices in crop production during the last decade, there is interest among nutritionists and others regarding the nutrient content of present-day foods *(7. 70.* 77). This paper reports on the mineral composition of nine fresh vegetables grown in widely different sections of the Ynited States during 3956 and 1957.

Materials and Methods

Among the 27 or more commercial fresh vegetables available to consumers in this country, nine were chosen for study: asparagus, snap beans, cabbage, carrots. pascal celery, yellow sweet corn. iceberg letruce. dry yello\v onions, and tomatoes. The selection was based on data from the Department of Agriculture's 1955 food consumption survey **(79)** which show these to be the vegetables. other than poiatoes. consumed in the largest quantities by households in the United States. Market grades of all vegetables studied were designated as **I_-.** S. *So.* 1 or better.

Three or more lots of each vegetable were collected equally from major producing areas represented in the Washington. D. C., wholesale market during 1956 and 1957. By contacting as many as six wholesale merchants sufficiently in advance. it was possible to locate those who could furnish the needed vegetables. The producing areas for each vegetable studied in 1957 were chosen to account for 90% or more of the volume reaching the Washington wholesale market, except for sweet corn and tomatoes (76).

Most of the vegetables chosen for this study had a seasonal delivery pattern in the local market *(76).* Because of this. not more than five different vegetables made up each set collected in any one year. Difficulties in collection arose even with this small number, particularly for vegetables coming from a large number of producing areas.

Each lot collected came from a large population with respect to volume shipped into the Washington market. The smallest carrot population encountered in the wholesale market during 1957, among five carrot shipping areas, was 100,000 pounds shipped from Arizona, while the largest carrot population was 675,000 pounds shipped from Texas (76, 78).

In cases where the same shipper's label appeared during successive weeks of collecting the replicate lots. the wholesaler would make available other railway car or truck shipments from which it was possible to collect another lot. The shipper's name. railway car or truck number, name of wholesaler, date of collection. shipper's label on the carton, and other pertinent information were recorded on the receipt of each lot of vegetables. During 1957, much of this information was transferred to a postcard questionnaire and mailed to the various shippers and growers requesting more information on the origin of the samples.

Upon arrival of the vegetables in the laboratory. the entire sample was weighed to obtain the "as purchased" weight. Lot size for each vegetable corresponded to units normally found in the wholesale market, such as crates, cartons: or bushels. The entire lot or portion thereof was trimmed to the "edible portion," rinsed in demineralized water, and blotted with clean filter

paper. Rinsing was not done on sweet corn. The discarded portion was weighed, and the percentage of edible yield was calculated. In handling the lots of lettuce, cabbage, tomato, and onion each head, fruit, or bulb was quartered and opposite quarters were allocated to four sublots. With celery, one stalk was severed at a time, starting on the outside of the bunch and allocating it to one of the four sublots, until the entire cluster had been dismembered. With snap beans the entire lot was thoroughly mixed on a large tray and one fourth of the entire lot was used.

All samples were blended in a stainless steel blender. One portion of the blend was removed for determination of the percentage of total solids, and another portion, calculated to give about 100 grams of dry weight, was spread on a porcelain or stainless steel tray and dried under a battery of heat lamps. The dried sample was then ground in a Wiley mill fitted with a 20-mesh stainless steel screen and thoroughly mixed. For each vegetable lot, 2 grams of the dried sample were weighed into tared, borosilicate glass beakers for charring in a muffle furnace at 300' C. Another 2 gram portion was weighed into a tared Vycor crucible, quickly ashed at 475' to 500' C. in a muffle furnace fitted with a quartz liner, and used for potassium determination in the flame photometer. ***4** third portion was removed for determination of the percentage of residual moisture. The charred sample was cooled, thoroughly ground in an agate mortar, and used for spectrographic determinations. None of the steps from the time the vegetables were received in the laboratory contributed any measurable contamination.

Charring was used to partially concentrate the samples, because the magnesium. manganese. copper, and aluminum levels were often low. **A** 10-mg. portion of the charred sample was weighed with a torsion-type microbalance, transferred to the crater of an electrode, and ashed in a furnace fitted with a quartz liner at a temperature of 475° to 500° C. over a 90-minute period. Ash residues in the electrodes were taken up in approximately $0.1N$ hydrochloric acid after waterproofing the electrodes with a paraffin-kerosine mixture (75).

The initial evaluation of variability of minerals in fresh vegetables was made during the summer of 1956. The experimental design involved five vegetables. three producing areas per vegetable, and three lots from within each producing area. Sine lots for each of five vegetables, snap beans, domestic round cabbage, pascal celery, iceberg lettuce, and dry yellow onion, were collected during the peak of the delivery cycle from the principal producing areas represented at that time in the wholesale market at Washington, D. C.

The experimental design used for each vegetable was a randomized block plan (12) consisting of four plates-blocks-for each of the nine lots.

Samples with four levels of standard solutions for all elements were included on each 4×10 inch spectrographic plate. Up to 16 spectra constituted one of the four replicates for each vegetable. To increase the accuracy of spectrographic determinations, the 16 samples were arced on four different days, making a total of 64 arcings for each vegetable (20) . The analytical system employed a total spectral energy technique, and a sufficient range of known standard concentrations to bracket unknown samples on each plate. It was thus possible to construct analytical curves relating intensities of the spectral lines to known concentrations of the respective elements in the standard solutions. From these analytical curves the concentrations of the elements in the unknown samples were read. **As** the spectra of the standard solutions were photographed on every plate, the emulsion response characteristics were easily established, making it possible to eliminate calculations necessary in plate calibrations (15). Statistical evaluation of the spectrographic data was then carried out by analysis of variance on logarithmically transformed data (72). The spectrographic assay system is known to be subject to a number of random variations (2, *3, 8).* In a study designed to measure the frequency distribution of the spectrographic error employing the direct current arc, the arcing error was found to be normally distributed (8) . Therefore, tests of significance based on the normality hypothesis are applicable to investigations employing the direct current arc.

The spectrographic analytical system used in these assays was similar to that described by Specht *et al.* (75), with the following modifications: **A** medium quartz spectrograph was used and the motor generator provided an excitation current of 225 volts and 20 amperes, with an average of 40 volts across the arc during the burn. Nine elements determined simultaneously on each sample were : boron, 2496.8 A,, phosphorus, 2535.6 or 2553.3 A., magnesium, 2781.4 A., manganese, 2801.1 **A,,** iron, 3020.6 **A,.** aluminum, 3082.2 A., calcium 2997.3 or 31 58.9 **A.,** copper, 3247.5 or 3273.9 **A..** and sodium, 3302.3 **A.** Both sodium and potassium were determined on a Beckman DU flame photometer equipped with a photomultiplier attachment. Sodium values determined by the two instruments agreed well. Prior to analysis of the experimental samples, check runs were made on a number of plant samples previously analysed by 10 other laboratories using both the spectrograph and the flame photometer. Results obtained on the medium spectrograph were in satisfactory agreement with the ones reported by the other laboratories (4).

Investigations in **1956.** Mean values and the upper and lower *95%* confidence limits for nine elements in the five vegetables studied in 1956 are shown in Table I. The variance of a producing area mean was regarded as a linear function of the variance components for lots, plates, and plate-lot interaction. In most cases Satterthwaite's method of determining the degrees of freedom associated with estimates of variance components was used *(9).*

Figure 1 shows the ratio between the average element content of six vegetables from different geographic areas. Among the nine elements studied, the sodium content of lettuce and onion, and the manganese content of celery exhibited the greatest divergence in values between producing areas. In each instance. the divergence occurred between an easrern and a western area.

Results of the analyses of variance revealed significant differences between lot means for certain elements in lettuce. celery. cabbage, and snap beans grown within the same general area. In most cases, there were significant differences between the mean values for producing areas included in the 1956 study. This implies that element averages for different areas should be reported separately. In the statistical analysis, variation was eliminated from differences between lot and area means.

Investigations in **1957.** Results from the work in 1956 pointed to the need for collecrion of more than three lots of vegeiables from known areas. In 1957, the plan was adopted of collecting six lots equally from producing areas delivering vegetables during the period April through October. The vegetables included asparagus, carrots, corn, tomatoes, and lettuce. Sample origins were checked and verified, Adherence *io* the plan of collecting six lots equally from each area was not always possible. however. Spring floods in Texas shortened what usually is a fairly long delivery season for carrots. Poor market conditions eliminated Virginia tomatoes earlier than expected, and North Carolina shipments of corn were limited because of bad weather and market conditions. These unforeseen difficulties. compounded with complications associated with the simultaneous collection of five different vegetables, illustrate some of the problems in carrying out a plan involving simultaneous collection of several vegetables.

Table I shows average fresh weight values and the upper and lower 95% confidence limits for the mean values for 10 elements in asparagus, carrots. corn, lettuce, and tomatoes collected in 1957. **A** twofold or greater spread occurred between certain area means for copper, sodium, and manganese in carrots;

Figure 1. Ratio between average element content of six vegetables from different geographic areas

manyanese and sodium in tomatoes; and sodium in asparagus (Figure 1) and Table I). The sodium content of California lettuce, collected in Washington, D. C., in 1957 was about tenfold higher than that from New York or New Jersey, duplicating the 1956 results. Year-to-year differences in the element content of lettuce were relatively small. Statistically significant differences were observed between lot means for sodium, manganese, iron, copper, magnesium, and calcium in practically all five vegetables.

Discussion and Conclusions

Coefficients of variation for most of the elements were less than 10% . There were 11 instances in which the coefficient of variation was equal to or greater than *207,.* In several of these cases-copper in lettuce from New York (1956), copper in carrots from New Jersey, copper in onions from Michigan, copper in sweet corn from central Florida, eastern Maryland, and northeastern North Carolina, and boron in sweet corn from New York-the estimated variance component for lots was considerably greater than that for plates. This suggests that better precision could have been obtained by increasing the number of lots. thereby permitting a decrease in the number of spectrographic plates for each lot.

The coefficient of variation for copper in onions from Michigan can be reduced from 31 to 21% by increasing the number of lots from three to eight and using four plates for each lot.

In the other four cases in which the coefficient of variation was equal to or greater than 20% -copper in onions from New York, boron in sweet corn from central Maryland and Delaware, and manganese in sweet corn from southeast North Carolina--the estimated

variance component for plates was larger than that for lots. This implies that the coefficients of variation can be reduced by increasing the number of spectrographic plates for each lot, thereby permitting a decrease in the number of lots. The coefficient of variation for boron in sweet corn from Delaware can be reduced from 23 to 19% if four lots and eight plates for each lot are uscd instead of five lots and four plates for each lot.

Content

Area or regional effects are evident for the sodium content of onions and lettuce from western and southwestern areas of the United States. This condition is probably associated with the higher sodium content in irrigation water and soils characteristic of these areas. However, because lettuce is low in sodium when compared to other foods in the diet, a tenfold increase in the averaqe sodium content of lettuce and onions from western areas would not constitute an alarming situation for those on low sodium diets (7).

Within limits, carrots and celery appear to be in a class apart with respect to their high sodium content. Both these vegetables belong to the same botanical family. The existence of physiological processes of selective exclusion of certain inorganic elements, by plant parts, is seen by the essential absence of sodium and calcium in sweet corn. These observations emphasize that, among the vegetables analyzed in this study, the existence of element binding specificity residing in different plant parts and between different species is probably more decisive than typical environment factors *per* **se** with respect to the relative inorganic composition of foods. This does not imply that environmental factors do not exert strong effects, for it has been shown that such effects are important *(73. 14).*

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Figure 2. Average manganese and phosphorus content of fresh carrots received in Washington, D. C., market from different geographic areas according to season, 1957

the different lots coming from the same specific area should not be ignored. The collections made in 1957 included from five to six lots of sweet corn delivered to the Washington, D. C., market at different times from each of two farms, one in central Delaware and one in eastern Pennsylvania. For lots from both farms, significant differences between the mean squares were found for boron, manganese, and copper.

The single and double asterisks denote significance at the 5 and 1% levels, respectively.

One aspect of the large differences among lots is that the significance of regional differences for certain elements becomes somewhat vague. The same conclusion was reached by others from a study of nutrients in field corn (6) and vegetables (11) .

Single values for the element content of various vegetables were obtained by computing averages weighted according to delivery volume. Weighted averages for the element content in all vegetables collected in 1957 are shown in Table 11. The assumption is made that the volume of vegetables shipped into a market approximates the level of human consumption of these vegetables within the market "reservoir." Information on delivery of carlot volumes of commercial fresh vegetables is published monthly by the Agricultural Marketing Service (16, 77).

,4 relatively large influx of Central California and Sew Jersey carrots with a low total manganese content and a decline in shipments of carrots from Texas and Southern California with a high manganese content apparently explains the marked depression in the seasonal curve for manganese (Figure 2).

The variation of certain elements in

Table 1. Mean Values and Upper and lower *95%* **Confidence limits for Means of Element Content in Nine Fresh Vegetables from Different Major Producing Areas** *No.*

Table I. Mean Values and Upper and lower *95%* **Confldence limits for Means of Element Content in Nine Fresh Vegetables from Different Major Producing Areas** *(Continued)*

^cIncludes seeds and skins.

Table II. Average Content of Elements in Edible Portion of Fresh Vegetables, Weighted According to Delivery Volume by Areas for All lots Collected in Washington, D. C., Market, April-October 1957

^a Including seeds and skins.

The same pattern was exhibited by the sodium content of lettuce (Table I), where a relatively large influx of New Jersey lettuce with one tenth the sodium content of California lettuce entered the market during the May-June 1957 period. The manganese curve for carrots varies considerably about the

weighted average of 0.29 mg. per 100 grams, fresh weight. On the other hand, the curve for phosphorus in carrots (Figure 2) varies but slightly about the Lveighted average of **34** mg. per 100 grams fresh weight. This is characteristic of the majority of the elements in vegetables collected in 1957.

Results of this study thus clearly indicate the existence of a series of average values for manganese conrent of carrots and sodium content of lertuce at different seasons. While the seasonal patterns for such highly variable elements as manganese in carrots and sodium in lettuce are exceptional in so far

as the present study is concerned, they illustrate the importance of knowing ahead of time something about the various market parameters, in order that collections of vegetable samples may be made at the proper seasons.

Information obtained from this study indicates the feasibility of estimating the inorganic element content of various vegetables in an urban market. Because many consumer groups obtain their food from rather well-defined urban markets, the extension of this approach to additional markets would seem desirable. This suggests that a market analysis could be made by sampling lots from different producing areas according to delivery volume at different seasons. The number of lots to be collected, and the number of spectrographic plates for each lot, would depend on the vegetable and the producing area sampled.

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SEPARATION OF MILK COMPONENTS

Chromatographic Isolation of Citric Acid and Lactose from Skim Milk

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A chromatographic procedure for separating citric acid and lactose from skim milk in pure form and sufficient amounts for carbon-14 measurements is described. Lactose in *7070* yield was separated from citric acid by ion exchange chromatography. The purity of the lactose was determined. Citric acid was recovered in 60% yield and was identified and its purity was determined,

I^N THE COURSE OF METABOLIC studies in the intact dairy cow using carbon-14 labeled metabolites, a method to obtain pure citric acid and lactose from skim milk for carbon-14 measurements was desired. Present methods for separating lactose (6) and citric acid (5) from milk are inadequate for obtaining pure compounds. Accordingly, an ion exchange chromatographic technique described by Busch *et al. (3)* has been modified to per-

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mit the isolation of citric acid and lactose from skim milk.

Apparatus and Reagents

The anion exchange resin employed was Dowex AG 2-X8 (Bio-Rad Laboratories. Richmond, Calif.) analytical grade, 50 to 100 mesh. with a capacity of 1.4 meq. per ml. of resin in water. Prior to use, the resin was converted to the formate form by eluting with $3N$ sodium formate until the eluate contained no chloride by the silver nitrate test. Excess formic acid was washed from the resin by distilled water

until the eluate was above pH 4. **A** 280 ml. resin bed was prepared by slurring with water and adding it to a chromatographic column 3×50 cm.

The cation exchange resin was Dowex AG 50 W-X8 (Bio-Rad Laboratories) analytical grade 20 to 50 mesh, with a capacity of 1.7 meq. per ml. of resin bed in water. The resin was converted to the hydrogen form by eluting with **3,V** hydrochloric acid until the effluent from the resin gave a negative calcium test with ammonium oxalate. Excess acid was washed from the resin with distilled water