COMMUNICATIONS

Vitamin C Concentration in Whole Orange Puree

Whole orange [*Citrus sinensis* (L.) Osbeck] purees prepared by a standarized procedure were found to contain 55–75 mg of total vitamin C (ascorbic and dehydroascorbic acids) per 100 mL of puree. No significant differences were found to exist between purees prepared from Marrs, Hamlin, Pineapple, and Valencia varieties. Purees made from smaller fruit had a higher vitamin C concentration than those made from larger fruit. Those purees prepared from fruit during the early portion of its harvest season had considerably more vitamin C than those purees prepared late in the season. After six months of storage at 20 °C Valencia puree retained only 66% as much vitamin C as the same puree stored at 4 °C.

Citrus fruits and their processed products are the most reliable and economical sources of natural vitamin C among those foods widely consumed around the world (Kefford, 1973). This importance is due not only to the amount of vitamin C in fresh fruit but also to its stability in most processed citrus products.

Considerable research has been conducted on the variability of vitamin C in citrus fruit and their main processed products as influenced by variety, climate, cultural practice, and parameters of processing and storage (Nagy, 1980). Relatively little work has been done on such new products as whole orange puree.

A citrus whole fruit puree process was developed by Cruse and Lime (1970) which utilized 85–90% by weight of citrus fruits to produce a product for use by the baking and dairy industries or as a beverage base for direct consumer consumption. The puree contains pulp and peel components in amounts up to one-third by weight of the total product. What effect this proportion of pulp and peel has on total vitamin C concentration and stability is largely unknown. Kefford and Chandler (1977) found conflicting evidence from a literature review on vitamin C concentration of comminuted citrus products, most of which contained lesser amounts of peel than did whole orange puree. While citrus peel is known to contain about a 3 times greater concentration of vitamin C than does the juice, it also contains ascorbic acid oxidase systems and natural antioxidants which could effect the stability of vitamin C in the product (Nagy, 1980). How these factors influence vitamin C content of whole orange puree is unknown.

It is the purpose of the research reported here to determine the vitamin C concentration of whole orange puree prepared from several orange varieties and to assess the effect of seasonal influences on long-term storage.

MATERIALS AND METHODS

Whole orange puree was prepared by a standarized procedure as described by Cruse and Lime (1970) and "Example 1" in the patented process of Lime and Cruse (1973). Forty to fifty kilograms of fruit was randomly selected from four or more trees of known scion variety on sour orange rootstock in groves maintained according to the practice of good commercial management. These trees were selected as being typical of normal development and were free of any off-bloom fruit. Acid and °Brix of the juice from subsamples of the fruit were analyzed to assure that the fruit had reached legal maturity and were characteristic of fruit of that variety which were being picked for commercial sales at that time. For cleaning, the fruit were soaked in water, subjected to a water spray and brush rollers, and then drained. Because the fruit were being processed by use of pilot plant equipment, the entire picking was run through the pureeing process as a single batch and then divided into individual canned samples of 0.5 kg each. The canned samples were immediately cooled under running water to room temperature before being randomly placed in temperature-controlled storage of either 4 or 20 °C (40 or 68 °F). Purees were prepared from Marrs, Hamlin, Pineapple, or Valencia varieties at various times during their respective season of maturity. Samples prepared during the eight harvest seasons of 1969–1970 through 1976–1977 were used to obtain estimates of long-term storage effects. Since the preparation of most of these purees predated the recognition of the need to make vitamin C determination of samples following long-term storage, no "zero-time" determinations were made of freshly prepared purees prior to the 1976–1977 season. "Zero-time" vitamin C determinations were made on Valencia purees prepared in Feb and May 1977.

Total vitamin C was determined after oxidation to dehydroascorbic acid with Norit (Deutsch and Weeks, 1965). The microfluorometric procedure using *o*-phenylenediamine was employed for quantitating the dehydroascorbic acid (Association of Official Analytical Chemists, 1975). Two or more replicate samples of each puree were analyzed with triplicate fluorometric readings made on the separate extracts of each sample. The fluorometric readings for a sample were averaged, and the vitamin C concentration of that sample was calculated. The average vitamin C concentration of the replicate samples was calculated along with their standard deviation.

RESULTS AND DISCUSSION

The microfluorometric method of vitamin C determination provided an improvement over previous methods of determining total vitamin C (ascorbic plus dehydroascorbic acid) in whole orange purees. The standard deviation of vitamin C concentration among all puree samples averaged about 2.5 mg/100 mL. This standard deviation represented about a 5% relative standard deviation for the majority of the samples in the range of 30–70 mg of vitamin

Table I. Influence of Fruit Size and Storage Temperature on Vitamin C Content of Whole Orange Puree

variety, prepn date, months of storage	vitamin C, mg/100 mL					
	4 °C storage			20 °C storage		
	sml frt[a]	lrg frt[b]	diff	sml frt	lrg frt	diff
Marrs						
Dec 1970, 80	50	34	16	17	17	0
Dec 1973, 42	38	19	19	28	15	13
Dec 1974, 31	54	42	12	27	20	7
Pineapple						
Dec 1973, 42	58			24	17	7
Valencia						
Feb 1975, 32	46	28	18	29	15	14
Feb 1977, 6	54	42	12	35	28	7

[a] Small fruit are field-run fruit with diameters of less than 7.0 cm. [b] Large fruit are field-run fruit with diameters of greater than 9.0 cm.

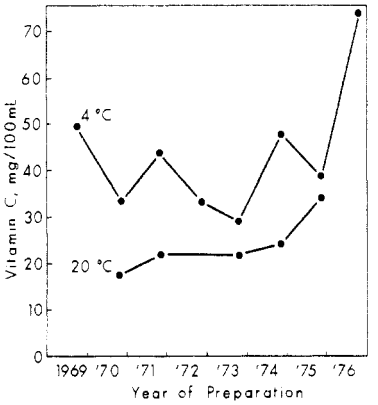

Figure 1. Vitamin C concentration of Marrs whole orange puree prepared in December of each indicated year and stored at 4 and 20 °C. Samples were analyzed in mid-1977.

C/100 mL of puree. For those puree samples which fell below this range the relative standard deviation ranged up to 10%.

Because of the limitations imposed by the lack of a large number of sample replicates, sample variability, and decrease in vitamin C with increasing fruit age after maturity, it was not possible to show any significant differences in purees prepared at the same time from the Marrs, Hamlin, Pineapple, or Valencia varieties of oranges. When purees were prepared from field-run fruit shortly after reaching maturity, they generally fell in the range of 55–75 mg of vitamin C/100 mL. Samples prepared later in the season or from larger fruit trend downward from these values.

For the purpose of this study all fruit samples employed had a minimum °Brix of at least 10.0% and a minimum ratio of °Brix to total (titratable) acid of at least 10.0.

Table I illustrates the influence of fruit size on puree vitamin C concentration. Eleven pairs of purees prepared from either small or large fruit from among three varieties, five preparation dates and storage times, and two storage temperatures were used to compare the vitamin C content between the two members of the pair. Purees prepared from field-run fruit of 7-cm diameter or less contained 12–19 mg/100 mL more vitamin C than did purees prepared from field-run fruit of 9-cm diameter or greater, this after prolonged storage at 4 °C. These same samples, when stored for corresponding times at 20 °C, lost sufficiently more vitamin C so that absolute differences in vitamin C concentration between purees from the two fruit sizes became narrower. This indicates that although purees from the smaller fruit have the higher vitamin C concentration they also tend to experience the greatest rate of

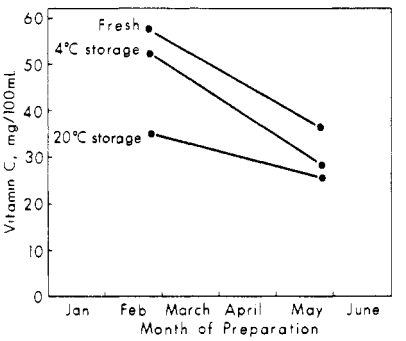

Figure 2. Vitamin C concentration of Valencia whole orange puree prepared during Feb and May 1977. Analyses were made when freshly prepared and after 6 months storage at 4 and 20 °C storage.

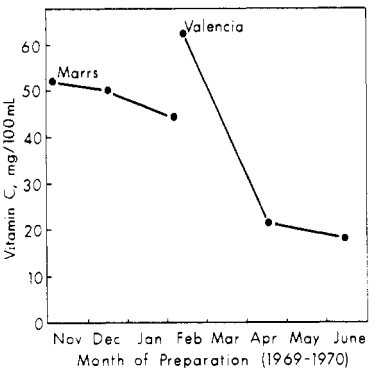

Figure 3. Vitamin C concentration of Marrs and Valencia whole orange puree after 7 years of storage at 4 °C.

loss during prolonged storage at ambient temperatures. This difference was not significant during the shorter storage periods. Valencia purees stored at 20 °C for 6 months retained about 66% of the vitamin C concentration retained in the same purees stored at 4 °C, regardless of whether the purees were prepared from small or large fruit.

Among the available puree samples having undergone long-term storage only those from the Marrs variety processed in December of previous years provided sufficient samples to assess the relative effects of long-term storage at 4 and 20 °C (Figure 1). With extended storage both the absolute vitamin C concentration and the relative difference between 4 and 20 °C storage tended to remain nearly constant with values of around 40 and 20 mg/100 mL, respectively. The variation in vitamin C concentration between years does not correlate with maturity as measured by °Brix and °Brix/acid ratio of the juice from the source fruit. The values for these years are as follows: for 1969, 12.9 and 23.0; for 1970, 12.7, and 21.5; for 1971, 11.6 and 24.2; for 1972, 12.9 and 23.4; for 1973, 10.1 and 19.1; for 1974, 11.1 and 20.2; for 1975, 12.0 and 24.0; for 1976, 12.2 and 18.5.

Figure 2 illustrates the effects of short-term storage (6 months) and seasonal decline in vitamin C concentration of Valencia whole orange puree prepared from small fruit. The early season puree contained half again as much vitamin C as the late season puree. Storage at 4 °C is seen to be considerably more advantageous to vitamin C retention during the early season than it is the late season. For the short term the time of puree preparation is as important as the storage temperature. Figure 3 shows that even after 7 years of storage at 4 °C the seasonal delcine of vitamin C concentration in Valencia and Marrs purees is quite apparent. The °Brix and °Brix/acid ratios for the juice of the source fruit for the Marrs samples are as follows: for Nov 1969, 11.7 and 18.0; for Dec 1969, 12.9

and 23.0; for Jan 1970, 13.4 and 22.7. For the Valencia samples the values are as follows: for Feb 1970, 12.8 and 10.8; for April 1970, 13.5 and 15.0; for June 1970, 10.9 and 10.9. Insufficient samples of Hamlin and Pineapple orange puree were available to establish a similar seasonal trend.

The present study confirms earlier studies (Kefford and Chandler, 1977) that comminuted orange bases contain about the same amount of vitamin C as orange juice and undergo the same degree of degradation at ambient storage temperatures. Since whole orange purees are diluted 10 to 1 for the preparation of citrus-flavored beverages (Lime and Cruse, 1972), such beverages are not nearly as useful a source of natural vitamin C as is orange juice.

Registry No. Vitamin C, 50-81-7.

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Received for review November 12, 1981. Revised manuscript received July 15, 1982. Accepted January 7, 1983. Names of companies or commerical products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endosement by the U.S. Department of Agriculture over others not mentioned.

Two Classes of Alkaloid Mycotoxins Produced by *Penicillium crustosum* Thom Isolated from Contaminated Beer

An apparent natural human intoxication resulted from consumption of beer contaminated with *Penicillium crustosum*. Under laboratory culture, the *P. crustosum* isolate produced two classes of toxic alkaloids consisting of roquefortine [10β-(1,1-dimethyl-2-propenyl)-3-(imidazol-4-ylmethylene)-5α,10β,11,11α-tetrahydro-2*H*-pyrazino[1′,2′:1,5]pyrrolo[2,3-*b*]indole-1,4(3*H*,6*H*)-dione], roquefortine A (isofumigaclavine A) (9α-acetoxy-6,8β-dimethylergoline), roquefortine B (isofumigaclavine B) (6,8β-dimethylergolin-9α-ol), and festuclavine (6,8β-dimethylergoline). Samples of the beer were not available for analysis.

This study was prompted by a clinical case that apparently resulted from a natural intoxication of a 44-year-old Caucasian male who consumed some commercial beer that was contaminated with a large mycelial mass of the fungus identified as *Penicillium crustosum* (Figure 1). Approximately 4 h after consuming the contaminated beer (approximately 30 cm^3 consumed), the individual became actuely ill with a throbbing frontal headache, feverish feeling, nausea, vomiting, diplopia, weakness, and bloody diarrhea. After 12 h, handwriting was illegible due to tremor. The symptoms prevented eating and other activities for approximately 30 h. After this time all symptoms disappeared and no apparent residual effects were noted. Five other family members and five visitors shared the evening meal but did not consume any beer and had no symptoms.

When the container was opened, there was no prior indication of contamination of the beer since the can of beer appeared to be properly filled and normally carbonated. Microbiological analysis of the pellicle-like mycelial mass showed a *Penicillium* sp. and a *Rhizopus* sp. to be present. Although accurate quantitation of the two isolates present was not possible, it appeared that the *Penicillium* sp. rather than the *Rhizopus* sp. mainly contributed to the mass of material. The *Penicillium* sp. was identified by Dr. Kenneth B. Raper as *Penicillium crustosum* Thom. However, the isolate did not form nearly as heavy crusts of conidia on agar plates typical of most isolates of this species.

This paper evaluated the toxin-producing potential of the isolate identified as *P. crustosum* when grown on two different media.

MATERIALS AND METHODS

The isolate of *P. crustosum* was cultured at 25–27 °C for 2 weeks in Fernbach flasks (2.8 L), each containing 100 g of shredded wheat and 200 mL of mycological broth supplemented with 2% yeast extract and 15% sucrose or 200 mL of a commercial brand of beer. The toxigenicity of the *P. crustosum* isolate and purification of the toxins were done by using the bioassay method of Kirksey and Cole (1974) with 1-day-old chickens dosed orally via crop intubation.

Mass production of the toxin was done in Fernbach flasks containing the shredded wheat medium under the cultural conditions described above.

The cultures were extracted with chloroform by homogenization with an Ultra-Turrax homogenizer (Tekmar Co.,