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Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: a storage study

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Abstract

A storage study of frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice was conducted to determine vitamin C loss on a monthly basis over a period of 24 months. Vitamin C content declined from an initial value of 40.6 mg/100 ml to 32.8 mg/100 ml in the final analysis for a loss of 19.2% over the storage period. A regression analysis showed a decrease of about 0.34 mg/100 ml of the vitamin C per month at this condition. The estimated shelf-life of this juice to meet the label claim of vitamin C of 130% DV is about 22 months at −23°C. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Frozen orange juice; Daily value; Vitamin C; Shelf life

1. Introduction

Orange juice has long been known to be an excellent source of vitamin C and is a product desired by many consumers who are interested in maintaining a healthy diet. For many years, orange juice has been produced in numerous forms such as frozen concentrate, orange juice from concentrate and pasteurized juice. Although these products must conform to strict guidelines which prevent unnatural change in the juice, concern for diet and nutrition has led consumers to seek a more natural product. Hand squeezed juice is often inconvenient in the fast pace of today's society which has many consumers seeking convenient forms of juice for daily consumption. The manufacture of fresh squeezed, unpasteurized orange juice has become more common and fills this consumer preference. Increased consumer awareness has also contributed to the increasing demand for orange juice which is as natural as possible and without extensive processing. According to market research carried out in Europe, use of fresh squeezed, unpasteurized orange juice has increased by 50% a year (Decio, 1993). Production of unpasteurized orange juice is a small-scale operation but packers market fresh squeezed, unpasteurized orange juice in retail size containers along with their traditional citrus juice products. This product is currently marketed throughout the United States and to some other countries.

Refrigerated, fresh squeezed orange juice is reported to have a relatively short shelf-life up to 14 days (Fellers, 1988) based on subjective flavor evaluation. The absence of pasteurization and lack of preservatives allow for the opportunity for the growth of bacteria and yeasts, spoilage, certain enzyme activity causing off-flavors, and oxidation (Attaway, Carter, & Fellers, 1989). Fellers, who conducted a shelf-life study of fresh squeezed orange juice, reported that staleness was the primary off-flavor limiting shelf-life at refrigerated storage temperature. Storage temperature was the prime limiting factor for shelf-life of this product; however, various low temperatures can be considered to make freshly squeezed citrus juices economically feasible to pack and market (Kopelman & Rauchwerger, 1985; Fellers, 1988). However, even with refrigerated temperature producing a shelf-life of about 2 weeks under the best of production conditions, refrigeration could not compare to the advantages of marketing the product in a frozen state where a longer shelf-life was achieved.

Maintenance of high sanitation standards and low temperature (−1 to 0°C) throughout the production, storage, distribution and retailing have been suggested to extend the shelf-life for a longer period (Gray, 1989). Thus, frozen storage of fresh squeezed orange juice has a longer shelf-life than refrigerated fresh squeezed orange juice, providing the distributor with a more

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flexible time schedule for distribution. But once thawed, the thawed frozen orange juice has a refrigerated life of 7–10 days (Rose, 1993).

Reports previously presented for fresh squeezed orange juice have been mostly concerned with specific procedures for production and handling (Attaway et al., 1989; Beasley, 1997; Carter, 1988; Gray, 1989), microbial populations and flavor estimation (Fellers, 1988; Kopelman & Rauchwerger, 1985), and supply and distribution (Whiteaker, 1988). Little information, however, exists for vitamin C loss in unpasteurized orange juice during frozen storage. Fellers reported vitamin C retention in freshly squeezed, unpasteurized citrus juices at four different storage temperatures (from −1.7 to 7.8°C) with limited time periods but was not indicative of vitamin C loss due to the interferences by microbial activity/production in the dye titrimetric estimation of vitamin C content. Since vitamin C is one of the principal nutritional components of citrus juices, and is known to degrade under less desirable storage conditions, quantitative analysis of vitamin C content was considered as one of the simple approaches to predict the shelf-life of citrus products. The objective of our study was to determine vitamin C in frozen, fresh squeezed, unpasteurized orange juice during frozen storage to determine the shelf-life.

2. Materials and methods

2.1. Samples

Commercial production line samples of frozen, fresh squeezed, unpasteurized orange juice were provided by a local Florida processor in one liter high density polyethylene bottles (HDPE). The samples were immediately placed in a walk-in storage room set at −23°C for storage test. Two samples were withdrawn for vitamin C analysis each month for a period of 24 months. Initially, the samples were found to have vitamin C content of 40.6 mg/100 ml, a pH of 3.7, and 11.4 °Brix.

2.2. Reagents

L-Ascorbic acid (vitamin C), metaphosphoric acid, and potassium phosphate monobasic were obtained from Fisher Scientific. A stock 1000 ppm solution of ascorbic acid was prepared and a dilution with 2.5% metaphosphoric acid to 10 ppm was made for standardization.

2.3. Vitamin C determination

Vitamin C determination was done by an HPLC method (Lee & Coates, 1987) with a slight modification. A juice was thawed and 5 ml was pipetted into a 50 ml, centrifuge tube containing 5 ml of 2.5% metaphosphoric acid. The sample was centrifuged for 5 min at 6500 rpm and 5°C. A 0.5 ml aliquot of the supernant was then pipetted into a 10 ml volumetric flask and filled to volume with 2.5% metaphosphoric acid. The flask was shaken to mix thoroughly and filtered using a 0.45 μm nylon filter (Fisher Scientific) and placed in an autosampler vial. The chromatographic system consisted of a Waters model 6000A solvent delivery system, LDC/Milton Roy SpectroMonitor D UV detector, a Waters model 717+ autosampler with chiller, and Waters Millennium integration software. A Zorbax ODS (250×4.6 mm) column fitted with a Zorbax ODS guard column was utilized with a mobile phase of 2% KH_2PO_4 (pH 2.4) at a flow rate of 0.5 ml/min. A 10 μl injection was made for standards and samples. Results were calculated as ppm and reported as mg/100 ml. Each sample bottle was prepared and analyzed in duplicate. Statistical analysis was performed using SigmaStat 2.0 PC software from SPSS (Chicago, IL) with significance at $p < 0.05$.

3. Results and discussion

Fig. 1 presents a semi-log plot of vitamin C contents of frozen, fresh squeezed, unpasteurized orange juice as determined on a monthly basis over a period of 24 months. This study was extended in time because frozen orange juices are usually stored for long periods of time in the market. Regression analysis has been applied and vitamin C loss follows a linear equation. Correlation between vitamin C loss with increasing storage time was significant ($p < 0.05$). Fig. 1 also shows the 95% confidence interval for the regression line of means, which is analogous to reporting the standard error of the mean. This additional confidence interval of 95% can increase confidence in the accuracy of the data. Initially, the juice

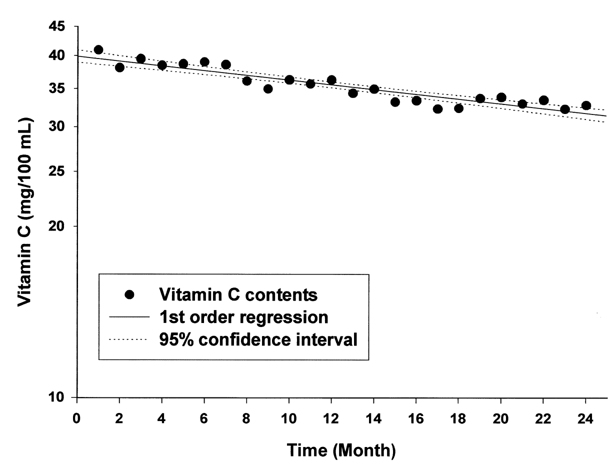

Fig. 1. Vitamin C contents (mg/100 ml) of fresh squeezed, unpasteurized, polyethylene-bottled orange juice as a function of storage time at −23°C.

was found to contain 40.6 mg/100 ml or 162% DV (daily value). Over the 24 month period, the rate of decline was about 0.8% per month providing a total retention of 80.8% with a final ascorbic acid content of 32.8 mg/100 ml. Characterization of kinetics of vitamin C loss at this temperature was not the intention for this study, but the vitamin C loss was known to follow first order reaction for orange juice stored below 50°C (Nagy & Smoot, 1977).

The loss of vitamin C in this frozen, fresh squeezed orange juice sample, however, was significant compared to the loss in canned, frozen concentrated orange juice (FCOJ) in which no measurable loss was detected for storage over a 60 month period at −20°C (Kew, 1957), and in a similar study conducted by Huggart, Harman, and Moore (1954). Oxidative enzyme reaction was considered responsible for loss of vitamin C in the fresh squeezed, unpasteurized orange juice sample during frozen storage. By comparing the data with frozen concentrate, we can surmise that the enzyme activity in frozen concentrate has been terminated by the heat required for the concentration process, whereas in frozen, fresh squeezed, unpasteurized orange juice, no heat processing has taken place and the enzyme activity, although slower at colder temperatures, is still occurring. Cytochrome oxidase, ascorbic acid oxidase, and peroxidase are known to be present in citrus fruits and are responsible for the oxidation of vitamin C (Nagy, 1980). These enzymes are readily destroyed during pasteurization of juice, but the lack of pasteurization in frozen, fresh squeezed orange juice offers the opportunity for enzyme activity to occur. Furthermore, the poor oxygen barrier property of polyethylene bottles (Bissett & Berry, 1975) is assumed to be another factor for vitamin C loss even though slow mass transfer was expected at this frozen state.

Based upon this storage study, the vitamin C content of frozen, fresh squeezed, unpasteurized orange juice has been shown to provide about 131% of the daily value (DV) of vitamin C requirements, based on 8 fl. oz. serving (240 ml), even after 24 months of frozen storage at −23°C. Storage at this temperature for a 24 month period appears to be close to the storage limit to provide the amount of vitamin C claimed on the label (130% DV). It should be noted that it demonstrated that vitamin C content in juice was still within the label declaration which complies with the nutritional labeling law [Food and Drug Administration (FDA), 1993], but a vitamin C level below 130% DV (about 32.5 mg per 100 ml) can be defined as the level at which the product would no longer meet label claims.

Since there is no established, uniformly applicable definition of the shelf-life (Taoukis, Labouza, & Saguy, 1997), and no information was available about packer's target shelf-life for this product at this temperature, the declared vitamin C content (130% DV) was used to estimate a value for the end of shelf-life of this frozen, fresh squeezed orange juice for marketing purpose for this study. This regression line (vitamin C mg/100 ml = 39.88 − 0.34X, $r^2 = 0.85$, where X is a month) in Fig. 1 shows the estimated values for vitamin C retention. Slope of the regression line was interpreted as an estimation of the rate of the vitamin C loss at this storage condition. A slope of −0.34 shows a decrease of about 0.34 mg vitamin C per 100 ml of juice per month, and estimates that this juice probably has a shelf-life of 22 months at −23°C (Table 1). The estimated % DV of vitamin C and 95% confidence interval of % DV are presented in Table 1. It is estimated with 95% confidence that vitamin C in the juice is between 126 and 132% DV after 22 months at this storage condition (Table 1).

The juice is still judged organoleptically acceptable but quantitative analysis of vitamin C content in juice could be a more meaningful and objective measurable way of assessing juice quality and determining the shelf-life at this condition. The determination of end of shelf-life can be dependent on the definition's intention, for example, for regulatory or marketing purpose (Taoukis et al., 1997), but it is the packer's responsibility to declare on the label and provide the consumer the amount of nutrients claimed on their label.

Since early season orange fruit is known to have more vitamin C than oranges from later season, a noticeable difference in vitamin C in fresh squeezed juice from month to month can be expected. According to a 10-year study with production fine citrus juice samples

Table 1
Estimated % daily value (DV)[a] of vitamin C during storage at −23°C

Month	Estimated % DV	95% Confidence interval
1	158	154–161
2	157	153–160
3	155	152–158
4	154	151–156
5	153	150–155
6	151	148–153
7	150	147–152
8	149	146–150
9	147	145–149
10	146	144–147
11	145	142–146
12	143	141–144
13	142	140–143
14	141	138–142
15	139	137–140
16	138	135–139
17	136	134–138
15	135	132–137
19	134	131–136
20	132	129–134
21	131	128–133
22	130	126–132
23	128	124–131
24	127	123–130

[a] Values are based on 8 fl. oz. (240 ml) serving.

processed in Florida (Lee & Coates, 1997), the value of vitamin C contents found from single-strength, pasteurized, orange juice ranged from 18.6 mg/100 ml (74% DV) to 58.7 mg/100 ml (235% DV) in 319 samples. Since early season fruit is not likely to be blended with late season, target shelf-life could vary according to the initial vitamin C content, and variety of fruit used by the processors. Other possible factors such as temperature fluctuation during shipping and transportation of juice may vary the shelf-life.

In summary, the vitamin C loss in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice was greater than expected, a 19.2% loss after 24 months of storage at −23°C. The % retention of vitamin C after 24 months of storage at −23°C, however, was still sufficient to supply 100% DV for 8 fl. oz. of orange juice. The rate of vitamin C loss (about 0.34 mg/100 ml, of the vitamin C per month) for this study and % DV in Table 1 can be utilized to estimate vitamin C loss in fresh squeezed orange juice during frozen marketing, and may be able to help juice processors to ensure that their juice complies with the nutrition labeling law to provide the amount claimed on the label.

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