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The Effects of Frozen Storage Conditions on Lycopene Stability in Watermelon Tissue

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The purpose of this investigation was to evaluate the rate of deterioration of lycopene in watermelon tissue during frozen storage, because little is known about the stability of watermelon tissue lycopene under cold storage conditions. Heart tissue from each of nine individual watermelons was stored at −20 or −80 °C as either small chunks or puree and periodically sampled over a year's time. Initial freeze−thaw experiments indicated that a small percentage of lycopene, ∼4−6%, degraded during an initial freeze−thaw. Analyses of the samples showed a loss of ∼30−40% lycopene over a year's storage at −20 °C and a loss of ∼5−10% over the same period at −80 °C. Lycopene was slightly more stable in pureed compared with diced watermelon tissue at −20 °C, but not at −80 °C. The kinetic data were best fitted by application of two simultaneous, first-order decay processes. HPLC analysis of the samples after a year's storage suggested that β-carotene was more stable during storage at −20 °C than was lycopene.

KEYWORDS: Carotenoids; lycopene; lycopene stability; watermelon; watermelon frozen storage

INTRODUCTION

Lycopene, a fat soluble carotenoid, is a precursor of β-carotene (*1*) and has at least twice the antioxidant capacity of β-carotene (*2*). A number of epidemiological studies have suggested that positive health benefits can be derived from the consumption of diets high in lycopene (*3*), although a consensus for either its beneficial or detrimental role in the modulation of carcinogenesis remains to be established (reviewed in ref *4*).

Like the more widely recognized lycopene source, tomato, watermelon is a rich source for this carotenoid. Because of its value as a phytochemical, many breeders want to maximize lycopene content in their watermelon breeding lines, and growers want to utilize production methods to increase lycopene content. The need for specialized, expensive equipment, toxic organic chemicals, and trained technicians for the current lycopene detection assays makes quantification of lycopene content impractical for some breeders, producers, and researchers. Thus, in many cases, samples have to be collected, stored, and shipped to a laboratory equipped to perform these analyses.

Tomato lycopene has received virtually all of the investigative attention with respect to its stability under various processing conditions (*5*, *6*, *7*) and storage conditions (*8*). Conversely, there is only limited information published on the stability of lycopene in cut watermelon tissue stored at refrigerator temperature (*9*). It may be that the stability of lycopene will be different in the two systems, because of differences in the attending tissues and compounds with which the lycopene is associated.

Two questions that the watermelon industry must address are as follows: (1) has the lycopene content of watermelons been underestimated as a result of degradation during storage, and (2) what are the optimal storage conditions that will minimize lycopene deterioration during storage? The purpose of this investigation was to evaluate the effects of tissue preparation, temperature, and duration of frozen storage on total lycopene (all-trans plus cis-isomers) in cut watermelon tissue, with the goal of finding practical long-term storage conditions optimal for lycopene preservation between tissue sampling and analysis.

MATERIALS AND METHODS

Sample Preparation. Watermelons used for this study were from the 2001 crop grown at Lane, OK, under standard cultural practices that followed Oklahoma State Extension Service guidelines (*10*). Melons were cut and tissue was removed the day after harvest. All steps from the time watermelons were cut lengthwise were performed in subdued lighting at room temperature, unless stated otherwise. Heart tissues from nine watermelons (*Citrullus lanatus* cv. Sangria) were individually processed. The excised tissue was cut into approximately 3 cm^3 chunks or smaller. The collective chunks from an individual melon were mixed by hand to attempt to obtain as homogeneous a distribution as possible. One-half of the chunks were divided into ∼10-g samples in zip-closure plastic bags for the chunk storage studies. The other half of the chunks from each melon were placed in a blender and homogenized to a puree. The puree from each individual melon was proportioned out in ∼10-mL aliquots in 15-mL screw-top plastic conical centrifuge tubes for the puree storage studies. We elected not to exclude oxygen from the frozen samples for two reasons. First, nothing is known about the susceptibility to degradation of lycopene in watermelon tissue at low temperature under atmospheric O_2, and lycopene susceptibility during processing or storage appears to vary with the attending tissue matrixes (*5*, *8*). Second, sample processing in preparation for storage and its influence on lycopene degradation needed to be addressed. Nine of the replicate purees and nine of the replicate chunk samples for each

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individual watermelon were frozen at −20 °C. Similarly, nine puree replicates and nine chunk replicates for each watermelon were frozen at −80 °C. The remaining two replicates of fresh tissue from each watermelon were assayed for lycopene, and their average served as the lycopene content of fresh tissue from each fruit and against which the lycopene contents of the frozen samples were compared. The lycopene contents of fresh heart tissue from the nine individual watermelons ranged from 42.9 mg/kg to 78.2 mg/kg. The average lycopene content of the nine watermelons was 53.6 ± 10.8 mg/kg (mean ± s.d.), and the median lycopene content was 53.2 mg/kg. Samples were stored for various lengths of time at −20 °C or −80 °C. For the long-term storage study, each sample was thawed only once before analysis. Thawed samples were pureed using a Brinkmann Polytron Homogenizer (Brinkmann Instruments, Inc., Westbury, New York) with a 20-mm O. D. blade to produce a uniform slurry with particles smaller than 3 mm^3. The samples were not allowed to heat or froth during homogenization by chilling them on ice and using short durations of homogenization.

Low-Volume Hexane Extraction Quantitative Analysis of Lycopene. The low-volume hexane extraction method was performed as described in Fish et al. (*11*). Once pureed, samples were stirred on a magnetic stirrer during sampling for analysis. Approximately 0.6-g (determined to the nearest 0.01 g) duplicate samples were weighed from each puree into two 40-mL amber screw-top vials (Fisher, #03-391-8F) that contained 5 mL of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 mL of 95% USP grade ethanol, and 10.0 mL of hexane. Samples were extracted on an orbital shaker at 180 rpm for 15 min on ice. After shaking, 3 mL of deionized water was added to each vial, and the samples were shaken for an additional 5 min on ice. The vials were then left at room temperature for 5 min, to allow for phase separation. The absorbance at 503 nm of the upper hexane layer was measured in a 1-cm path length quartz cuvette after zeroing the instrument with pure hexane. The lycopene content of each sample was then estimated using the absorbance at 503 nm and the sample weight (*11*, *12*).

Fitting of Experimental Data With Kinetic Equations. Our data for lycopene deterioration during frozen storage could not be fitted with simple, single-step rate equations. We were able, however, to fit the data by resolving them into two first order decays of the general form

$$\mathrm{Ct/Co} = P_1 \exp(-k_1 t) + P_2 \exp(-k_2 t)$$

In this equation, Ct is the concentration of lycopene in tissue after storage for time, t, in days. Co is the concentration of lycopene in fresh tissue minus 4% for freeze−thaw destruction. The rate constants, k_1 and k_2, are the respective first-order rate constants for each of the two first-order processes. P_1 and P_2 are constants for each process and are related to lycopene measurement and the proportions of lycopene degrading by each process. The value of the smaller rate constant, k_2, (slower rate) was determined from the slope of the linear portion of a plot of ln(Ct/Co) versus t at large values of t. The intercept upon extrapolation to $t = 0$ yielded values for P_1 and P_2 (because $P_1 + P_2 = 1$) (*13*). The contribution of $P_2 \exp(-k_2 t)$ (slower process) to Ct/Co was then subtracted from the left-hand side of its corresponding equation, and the ln of the residual was plotted against time. These plots were linear, within the uncertainty of the data, and their slopes yielded the value of k_1, the larger rate constant (faster rate).

HPLC Analysis of Carotenoids. The distribution and relative levels of trans-lycopene, its collective geometric isomers, and other carotenoids in several selected samples were determined by HPLC and diode array detection by the method of Tonucci et al. (*14*). This method does not resolve the individual cis-isomers, but collectively separates them from all-trans-lycopene.

Statistical Analysis. Statistical analyses that included analysis of variance, mean, and standard deviation determinations were performed with the aid of Statistica software, version 6 (StatSoft, Tulsa, OK)

RESULTS

Effect of Freeze−Thaw on Lycopene Levels. The potential effect of a freeze−thaw process on watermelon tissue was evaluated by thawing a chunk sample and a puree sample from each watermelon after the samples had been frozen for 2 days and assaying for lycopene content. The unused portion of each sample was then refrozen and assayed again about 24 h later to evaluate the effect of a second freeze−thaw cycle. The lycopene content of flesh from each melon expressed as a percent of the lycopene content of the fresh tissue was averaged over the nine melon samples (**Table 1**). Inspection of the standard deviations of the treatments reveals that the puree form produces a more uniform set of samples than does the chunk form (s.d. for puree ∼1.2; s.d. for chunks ∼4.3). Analysis of variance indicated that there was a significant difference ($p < 0.05$) between the frozen-thawed samples and the fresh tissue. However, there was no significant difference ($p > 0.05$) in the means of once frozen-thawed and twice frozen-thawed or between puree and chunk samples. We were unable to determine the cause of this ∼4−6% decrease in lycopene as a result of freeze−thaw. We have observed this behavior previously (*15*) but did not report the amount of degradation.

Table 1. The Effect of Freeze–Thaw Cycles on Lycopene Content of Watermelon Tissue Samples

number of freeze–thaw cycles	lycopene content (% of fresh tissue)[a] puree −20 °C	puree −80 °C	chunks −20 °C	chunks −80 °C
one	96.7 ± 1.2	97.4 ± 1.2	93.6 ± 3.3	92.9 ± 4.3
two	96.0 ± 1.0	96.1 ± 1.4	92.1 ± 4.8	92.3 ± 4.6

[a] Average ± standard deviation for samples from nine watermelons.

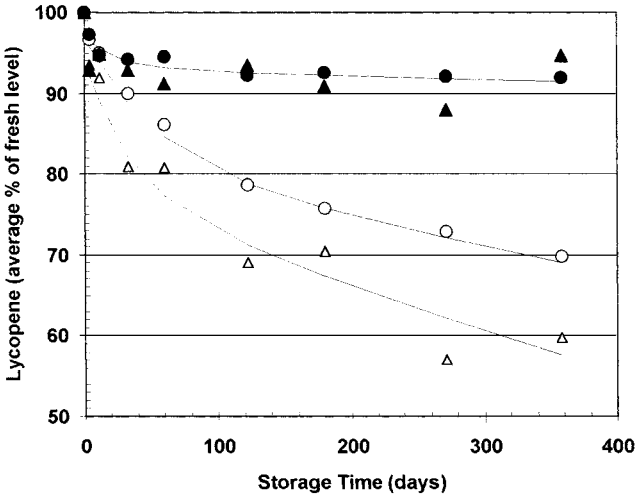

Figure 1. The rate of loss of lycopene during frozen storage of watermelon tissue. Lycopene levels for nine samples were normalized to percent of the fresh level, and the average of the nine samples for each time point was plotted versus the time of storage. Open circles, puree stored at –20 °C; solid circles, puree stored at –80 °C; open triangles, chunks stored at –20 °C; solid triangles, chunks stored at –80 °C. Best fit lines were generated for the corresponding set of data points by eqs 1, 2, or 3 in the text.

Lycopene Levels with Temperature and Time of Frozen Storage. Samples from each watermelon were removed from frozen storage at selected intervals over a year's time, and each was assayed for lycopene content. The results are summarized in **Figure 1**. Three observations are apparent from the results. First, lycopene in frozen watermelon tissue is more stable when stored at −80 °C than when stored at −20 °C. Second, again it is suggested by comparison of the scatter in the respective data that the puree form of tissue produces the more homogeneous

set of samples. Part of the source of variation in the chunk samples may have occurred by loss of variable amounts of water out of the chunks and into the plastic bags. Third, the form in which the tissue was stored has little effect on lycopene stability compared to the effect of temperature, because chunks and puree behave basically identically at −80 °C. However, at −20 °C, an apparently faster rate of lycopene deterioration was exhibited by chunks than by puree.

We attempted to fit the lycopene deterioration data with simple, single-step rate equations. The results could not be adequately fit with the linearized forms of zero-, first-, or second-order rate equations; in all cases, curvilinear plots were obtained. This was true whether the data were adjusted for the ∼4% lycopene loss by freeze−thaw or not. The results could, however, be resolved into two first-order decay processes. The equations are given below for three of the cases.

$$\text{Chunks, } -20\ ^\circ\text{C: } Ct/Co = 0.15\exp(-0.03t) + 0.85\exp(-0.0008t) \quad (1)$$

$$\text{Puree, } -20\ ^\circ\text{C: } Ct/Co = 0.15\exp(-0.018t) + 0.85\exp(-0.00045t) \quad (2)$$

$$\text{Puree, } -80\ ^\circ\text{C: } Ct/Co = 0.04\exp(-0.04t) + 0.96\exp(-0.000045t) \quad (3)$$

Each line in **Figure 1** was generated by its corresponding equation and offers a reasonable fit to the experimental data. No attempt was made to fit the data for chunks at −80 °C, because it was similar to that for the puree but was less precise. The fact that the experimental data may be satisfactorily described by a combination of two first order processes does not prove that this is actually what is occurring during storage and certainly does not imply a mechanism of degradation. The data do imply, however, that more than one deterioration process is likely operating on lycopene during its frozen storage. Equations 1−3, rather, provide a means to predict the amount of lycopene degradation expected for storage of frozen watermelon tissue at one of two temperatures over a given period of time.

Additionally, we evaluated if any portion of all-*trans*-lycopene was being converted to its collective forms of *cis*-isomers during storage. HPLC separation of the carotenoids from −20 °C and −80 °C puree samples from four watermelons was performed after one year of storage. No measurable increase in the collective quantities of *cis*-lycopene isomers was observed. One interesting observation, however, was that samples stored at −20 °C exhibited a ratio of β-carotene to lycopene about 10 percent greater than that of its counterpart stored at −80 °C. This held true for the four samples whose two storage temperatures were compared in this manner. The results suggest that deterioration of β-carotene is slower at −20 °C than is that of lycopene. This differential rate of degradation of lycopene and β-carotene should have no effect on the quanitative spectral assay for lycopene, because of the widely disparate levels of the two in watermelon, ca. 15 times more lycopene than β-carotene (*11*).

DISCUSSION

Several factors need to be considered if reliable estimates of lycopene content of fresh watermelon tissue are to be derived from tissue that has been frozen and stored for a period of time before assay. First, a more homogeneous sample will be obtained if the tissue is homogenized before sampling and storing. This will result in fewer replicates needed to achieve a requisite precision level in the assay results. Second, the pureed form is at least as stable as the chunk form, and the lycopene appears to degrade more slowly at −20 °C in the puree than in the chunk form. Third, we observed a loss of ∼4−6% of the watermelon tissue lycopene as a result of a single freeze−thaw cycle. We hypothesize that this more labile form of lycopene may represent a small pool of tissue lycopene that is either environmentally or physiologically in an unstable state and is oxidized or otherwise degraded upon freezing−thawing. This small, but consistent, loss of lycopene has not been observed, to our knowledge, in the tomato system. It was also reported that both lycopene and β-carotene in liquid-frozen human serum remained stable through repeated freeze−thaw cycles (*16*). Together, these results suggest that this labile pool of lycopene does not exist in some systems or that the tissue matrix in watermelon does not afford the same level of protection as do the matrixes in tomato. For watermelon tissue, it appears that one should correct for this fractional loss of the fresh tissue lycopene when dealing with a frozen−thawed sample. Fourth, the temperature of storage exerts a substantial effect on the stability of lycopene in frozen watermelon tissue. Not surprisingly, the lower the temperature, the more stable the lycopene. Although −80 °C is the preferred storage temperature, realistically, such cold storage conditions will not be commonly available, and −20 °C will have to suffice. **Figure 1** or calculation with eq 2 shows that about 10 % (in addition to the ∼4% freeze−thaw loss) of the lycopene will degrade after 30 days storage of watermelon tissue puree at −20 °C, while less than this is lost after a year at −80 °C. Thus, storage time at −20 °C should be as short as possible, to minimize the uncertainty of the analysis. No shift was observed in the amount of all-trans-lycopene isomer to the collective cis-isomers during frozen storage. This is not totally unexpected in light of ab initio molecular modeling computational studies that indicate that all-trans-lycopene is more stable than its cis isomers, with the exception of 5-cis lycopene (*17*).

Because this storage study was conducted in the presence of an oxygen atmosphere (no effort was made to remove O_2 from the puree or from the atmosphere above the chunks or puree), it is highly likely that the degradation of lycopene occurred via oxidative processes. It is well documented that caratenoids can be oxidatively cleaved to form aldehydes, ketones, epoxides, and endoperoxides (*18*−*21*). Frozen storage of watermelon tissue in the absence of oxygen may slow the rate of lycopene degradation, but will likely not entirely eliminate the degradation. For example, tomato pulp samples stored in the dark at −20 °C still lost lycopene at one-half the rate in vacuuo as in the presence of air (*5*). Thus, ∼16% of the tomato pulp lycopene was lost during 60 days storage at −20 °C in the absence of O_2. In a model aqueous system, about 60% of the lycopene deteriorated after 7 h at 30 °C under a N_2 atmosphere (*18*).

Our data for frozen watermelon tissue are consistent with at least two lycopene degradation processes that occur simultaneously. The results may be described by two simultaneous first order decays (eqs 1−3), where one process is about forty times faster than the other. Obviously, this is a phenomenological kinetic description of what we observe and offers no mechanistic insight into what is actually happening. The rates and mechanisms of the degradation of lycopene in the presence of oxygen appear to be system dependent. Sharma and LeMaguer (*5*) observed that the degradation of lycopene in tomato pulp at −20, 5, and 25 °C could be described by a single, pseudo-first-order reaction at each of the three temperatures. Henry et al. (*18*) observed that the degradation of lycopene exhibited zero-order kinetics when adsorbed on a C_{18} solid phase in an aqueous system in the presence of O_2 or ozone at room temperature.

Agarwal et al. (*8*) observed no significant loss of lycopene in tomato juice stored at 4 °C for 12 months. Together, these results support the contention that the observed behavior of lycopene degradation will depend heavily on the medium in which it is stored and emphasizes that our results for watermelon tissue should not be extrapolated to lycopene stored in other watermelon systems (eg., juice).

Our observation that, in watermelon tissue, lycopene appears to degrade more rapidly than β-carotene during frozen storage is consistent with the observations of Lee and Coates (*22*) in a different food system. They observed >20% loss of lycopene and a 7% loss in β-carotene in pink grapefruit juice stored at −23 °C in plastic containers for 12 months. These observations with food systems are consistent with observations of in vitro model systems, in which the decreasing number of coplanar conjugated double bonds and the presence of hydroxy and keto groups in carotenoids decrease their reactivity in radical scavaging reactions (*23*, *24*).

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