

Shelf life of unpasteurized sour orange juice in Iran

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Abstract — Introduction. In Iran, sour orange is available between mid-October and March. Frequently, consumers store a large volume of unpasteurized juice at room or low temperatures for consumption when the fresh fruit is not available. The aim of our research was to determine the shelf life of unpasteurized sour orange juice which was stored under conditions similar to those adopted by consumers at home. **Materials and methods.** Sour orange juice was prepared by hand-squeezing fresh fruit; it was filtered, and then poured into clear or dark green glass bottles. Bottles were stored at room temperature [(28 ± 2) °C], in the refrigerator [(4 ± 1) °C] and in the freezer [(-12 ± 1) °C] for 12 weeks. Additional samples were prepared by supplementing juice with 2% (w/w) citric acid and they were stored in the refrigerator. Total soluble solids and pH values were measured every 2 weeks and analysis was carried out on ascorbic acid content by means of the titration method in the presence of 2,6-dichlorophenol indophenol. The study was performed for 12 weeks. **Results and discussion.** Total soluble solids content and pH value of the initial juice were 11.5 °Brix and 3.18, respectively. pH appeared not to be significantly influenced by storage time or conditions; however, total soluble solids content in a few samples was reduced to about 9.5 °Brix. The initial ascorbic acid content was 130 mg·100 mL⁻¹; after 2 weeks, it was reduced by nearly 50% for all unfrozen samples. The final concentration of ascorbic acid in the juice was approximately 20 mg·100 mL⁻¹, regardless of storage conditions. The deteriorative reaction of ascorbic acid in the juice at each temperature experienced followed a first-order kinetic model with activation energy of 2.67 kJ·mol⁻¹.

Iran Islamic Republic / *Citrus aurantium* / fruit juices / packaging / storage / keeping quality / ascorbic acid

Durée de conservation du jus d'orange amère non pasteurisé en Iran.

Résumé — introduction. En Iran, l'orange amère est disponible de mi-octobre à mars. Les consommateurs stockent fréquemment de gros volumes de jus non pasteurisé, à basse température ou à température ambiante, en prévision des périodes où le fruit frais ne sera pas disponible. L'objectif de nos recherches a été de déterminer la durée de conservation du jus non pasteurisé de ces oranges amères, stocké dans des conditions semblables à celles utilisées, chez eux, par les consommateurs. **Matériel et méthodes.** Du jus d'orange amère a été préparé en pressant à la main le fruit frais ; il a été ensuite filtré, puis versé dans des bouteilles en verre clair ou en verre vert-foncé. Les bouteilles ont été stockées à la température ambiante [(28 ± 2) °C], au réfrigérateur [(4 ± 1) °C], et au congélateur [(-12 ± 1) °C] pendant 12 semaines. D'autres échantillons ont été préparés en ajoutant au jus 2 % (poids / poids) d'acide citrique ; ils ont été stockés au réfrigérateur. La matière sèche soluble et les valeurs du pH ont été mesurées toutes les 2 semaines et la teneur en acide ascorbique a été analysée par titration en présence d'indophénol 2,6 dichlorophénol. L'étude a été réalisée pendant 12 semaines. **Résultats et discussion.** La teneur en matière sèche soluble et la valeur du pH du jus initial ont été de 11,5 °Brix et 3,18, respectivement. Le pH a semblé ne pas être significativement influencé par le temps ou les conditions de stockage, toutefois la teneur en matière sèche soluble dans quelques échantillons a été réduite à environ 9,5 °Brix. La teneur en acide ascorbique initiale a été de 130 mg·100 mL⁻¹ ; après 2 semaines, elle a été réduite de presque 50 % pour tous les échantillons non congelés. La concentration finale en acide ascorbique des jus a été approximativement de 20 mg·100 mL⁻¹ indépendamment des conditions de stockage. La réaction de détérioration de l'acide ascorbique dans le jus, pour chaque température testée, a suivi un modèle cinétique de premier ordre avec une énergie d'activation de 2.67 kJ·mol⁻¹.

Iran République islamique / *Citrus aurantium* / jus de fruits / conditionnement / stockage / aptitude à la conservation / acide ascorbique

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1. Introduction

A species of multiple use, the sour orange (*Citrus aurantium* L.), native to southeastern Asia, is also known as bitter or Seville orange. It is a round, oblate or oblong-oval, rough-surfaced fruit, with a fairly thick, aromatic, bitter peel becoming bright reddish-orange on maturity, and with minute, sunken oil glands [1]. Twenty-three varieties have been described and illustrated in Europe [2]. In Iran, this fruit is called *narenj*. In Spain, Italy and India, it is identified as *naranja ácida*, *melangolo* and *khatta*, respectively. The nutritional quality of sour orange juice is largely related to its vitamin C content and its antioxidant capacity [3]. The juice is used as a food additive, ingredient in salad dressing and as a popular drink because of its rich flavor and aroma [4].

Vitamin C is an essential nutrient for the human being and has a high antioxidant power, providing protection against the presence of free radicals and, consequently, participating in the prevention of many degenerative diseases. Because of the instability of vitamin C and its nutritional importance, its content guarantees the presence of other nutrients and is considered an indicator of the nutritional quality of foods [4]. The main source of vitamin C for consumers is usually citrus fruits.

Vitamin C consists of an enediol structure, which is conjugated with a carbonyl group in a lactone ring [5]. Temperature, salt and sugar concentration, pH, oxygen, enzymes, and vitamin initial concentration, as well as the ratio of ascorbic to dehydroascorbic acid, are factors that could influence the nature of degradation mechanisms [6]. The decomposition of ascorbic acid and non-enzymatic browning are the main deteriorative reactions that occur during processing, packaging and storage of citrus juice [7]. This degradation proceeds through both aerobic and anaerobic pathways [8]. Processing of juice is mainly responsible for oxidation of ascorbic acid, while anaerobic degradation occurs during storage of juice which has undergone some sort of thermal preservation [3]. During storage, the juice may experience a number of deteriorative reactions including vitamin C loss, microbial

spoilage, cloud loss, development of off-flavor, and changes in color, texture or appearance, resulting in quality degradation of the product [9]. Vitamin C loss during storage in various citrus juice concentrates (orange, lemon, grapefruit and tangerine) and their reaction kinetics have been investigated by Burdurlu *et al.* [8]. Other authors determined the effects of fluorescent light on flavor and ascorbic acid content in refrigerated orange juice [10].

Several approaches are applied to extend shelf life of fresh-cut fruits, vegetables or fruit juices. One such approach is to use chemical inhibitors, without toxic effects, to control browning [11]. Citric acid, with its ability to chelate metal ions such as copper and iron, has been used as an effective inhibitor in many circumstances [11]. In citrus juice, binding of these metal ions limits available ions that are necessary for polyphenol oxidase activation [12]. No literature regarding shelf life stability and kinetics of vitamin C in sour orange juice was found. Most of the references cited here discuss other citrus fruits.

In Iran, the fresh fruit is available between mid-October and March. Frequently, consumers store a large volume of unpasteurized juice at room or low temperatures (usually in the refrigerator, occasionally in the freezer) for consumption when the fresh fruit is not available. The aim of our research was to determine the shelf life stability of unpasteurized sour orange juice which was stored under conditions similar to those adopted by consumers at home.

2. Materials and methods

2.1. Sample preparation

Fresh fruits were collected in a garden in Shiraz, Iran; then, they were washed, peeled and hand-squeezed for juice. Juice clarification was carried out with an ordinary kitchen filter. The clarified juice initially had total soluble solids (TSS) of 11.5 °Brix and a pH value of 3.18.

The juice was stored in glass bottles (clear or dark green) with plastic caps, for 12 weeks, in five different conditions:

- clear glass bottles at room temperature of (28 ± 2) °C,
- dark green glass bottles at room temperature of (28 ± 2) °C,
- dark green glass bottles in a refrigerated storeroom at (4 ± 1) °C,
- supplementation with 2% (w/w) citric acid in dark green glass bottles in a refrigerated storeroom at (4 ± 1) °C,
- dark green glass bottles in a freezer at (-12 ± 1) °C.

The glass bottles were underfilled, resulting in about 5–10 mL of headspace. Variation in ascorbic acid content, pH and TSS were observed and data analysis was carried out on three replicates.

After 12 weeks, changes in color, texture and taste appeared in some of the samples stored at room temperature and a few patches of mold growth were observed.

2.2. Analytical measurements

Variations in ascorbic acid content, pH and total soluble solids were recorded for the duration of the experiment. In addition, the capability of citric acid to influence inhibition of the ascorbic acid degradation reaction in the refrigerated samples was also examined. Kinetics of ascorbic acid loss in sour orange juice during storage were also studied.

The total soluble solids were determined as °Brix by a temperature control refractometer (Carlzeiss Jena, model 711849, Germany), and pH was measured with a pH meter (Metrohm, Model 623, France). Ascorbic acid content, expressed in $\text{mg}\cdot 100\text{ mL}^{-1}$, was determined by the titration method using 2,6-dichlorophenol indophenol according to AOAC [13]. The accuracy and precision of the titration method for ascorbic acid determination was tested by running analysis of standard solutions prepared from ascorbic acid, pro-analysis reagent. It is noteworthy that this titration method only determines ascorbic acid and not dehydroascorbic acid [14].

Data analysis was carried out by analysis of variance in randomized complete blocks.

3. Results and discussion

3.1. Ascorbic acid content

The value of ascorbic acid measured at the start of storage in freshly prepared sour orange juice was about $130\text{ mg}\cdot 100\text{ mL}^{-1}$.

Evolution in ascorbic acid during the 12 weeks of observation showed that ascorbic acid of all samples decreased during storage (*table I*). The degradation, particularly for all unfrozen samples, was sharp for the first 2 weeks of storage. Such an initial rapid and immense loss of ascorbic acid is attributed to oxidation by the air dissolved in the juice (no liquid flashing was carried out prior to bottling) and by the layer of air trapped in the headspace of the bottle. The incorporation of air in the juice during extraction, clarification and bottling was already recognized by previous authors [15]. The high loss in ascorbic acid content, mainly in the first 2 weeks of storage, may well be due to activity of cytochrome oxidase, ascorbic acid oxidase and peroxidase [15]. The ascorbic acid loss in the subsequent 10 weeks of storage was steady but at a much slower pace. After 12 weeks, the ascorbic acid contents of all samples were reduced to values less than $20\text{ mg}\cdot 100\text{ mL}^{-1}$. This is well below the minimum values recommended for processed orange juice ($40\text{ mg}\cdot 100\text{ mL}^{-1}$) [4]. Choi *et al.* [16], studying the retention of ascorbic acid with storage in blood orange juice, observed a linear reduction in concentration with time. They noticed that more than 50% of ascorbic acid was lost within 3 weeks of refrigerated storage, and that ascorbic acid was completely degraded in the control juice after 5 weeks of storage.

3.2. Soluble solids content and pH

The initial total soluble solids value for bottles which were without citric acid was 11.5 °Brix, which was reduced to about 10 °Brix over 12 weeks (*figure 1*). The reduction in TSS was tied with formation of gel lumps in samples. The formation of gel-like lumps, especially for unfrozen samples, may be attributed to activity of natural

Table I.

Ascorbic acid content (mg ascorbic acid·100 mL⁻¹ of sample) of unpasteurized sour orange juice stored in various conditions (mean of three replicates).

Storage time (weeks)	In clear glass bottles at (28 ± 2) °C	In dark green glass bottles			
		at (28 ± 2) °C	at (4 ± 1) °C	at (-12 ± 1) °C	at (4 ± 1) °C with 2% citric acid
0	130.00 Aa ± 9.8	130.00 Aa ± 9.8	130.00 Aa ± 9.8	130.00 Aa ± 9.8	130.00 Aa ± 9.8
2	65.65 Ba ± 6.5	76.96 Bb ± 3.5	69.68 Ba ± 2.0	114.19 Bc ± 8.5	56.09 Bd ± 4.1
4	50.17 Ca ± 3.5	56.21 Ca ± 4.5	55.17 Ca ± 3.6	82.61 Cb ± 5.2	45.17 Cc ± 2.1
6	35.17 Da ± 3.9	37.21 Da ± 4.2	32.00 Da ± 4.6	30.52 Da ± 2.0	23.55 Db ± 3.2
8	23.65 Ea ± 3.4	25.80 Ea ± 3.2	21.51 Ea ± 2.1	22.94 Ea ± 2.5	17.96 Ea ± 2.5
12	19.65 Ea ± 2.5	19.88 Ea ± 3.1	19.18 Ea ± 3.0	18.82 Ea ± 2.9	16.44 Ea ± 3.7

a, b, c, ...: data followed by a different small letter in a row are significantly different ($P < 0.05$).

A, B, C, ...: data followed by a different capital letter in a column are significantly different ($P < 0.05$).

Figure 1.

Evolution of unpasteurized sour orange juice total soluble solids with time according to different storage conditions.

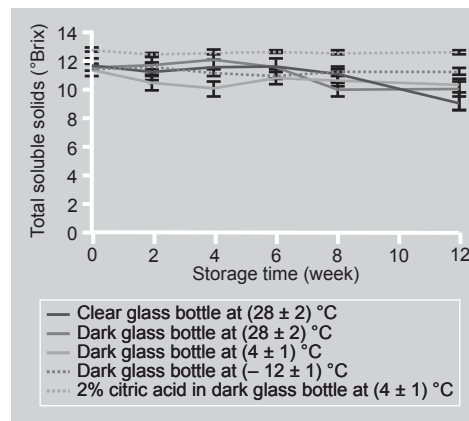
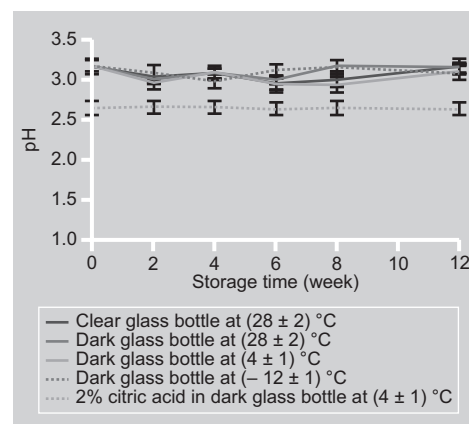


Figure 2.

Evolution of unpasteurized sour orange juice pH with time according to different storage conditions.



enzymes, such as PME (Pectin Methyl Esterase). These enzymes catalyze the demethoxylation of the pectins, causing an

increase in the free carboxyl groups, which favors clarification of the juice [17].

The pH values of these bottles which were without citric acid, initially at 3.18, did not vary significantly over 12 weeks ($p < 0.05$) (figure 1).

The TSS of refrigerated bottles containing citric acid was 12.7 °Brix, and their initial pH value was 2.6. TSS and pH of these samples remained almost constant throughout the study (figures 1, 2).

3.3. Effect of light on ascorbic acid retention

The analysis of data regarding ascorbic acid retention in sour orange juice indicated that, for the first 4 weeks, sour orange juice stored in dark bottles had marginally better retention in comparison with samples in clear bottles (table I). The retention was statistically significant for the first 2 weeks. However, no significant variation in the juice at room temperature, either in clear glass bottles or in dark green glass bottles, was observed over 12 weeks ($p < 0.05$). The higher degradation of ascorbic acid in clear glass bottle samples at (28 ± 2) °C in comparison with dark green glass bottle samples at the same temperature, at the beginning of our study, was attributed to better light penetration through clear glass, which probably resulted

in a synergistic effect, particularly when the concentration of ascorbic acid was the highest.

3.4. Effect of storage temperature

The effect of temperature on the rate of ascorbic acid reduction in unpasteurized juice of sour oranges was examined in a comparatively broad temperature range of (–12 to 28) °C (*table D*). The ascorbic acid content of the juice decreased during storage, faster at 28 °C than at –12 °C. After 2 weeks of storage, ascorbic acid retention was 60%, 54% and 88% of the original value for juice at (28 ± 2) °C, (4 ± 1) °C and (–12 ± 1) °C, respectively. In the frozen samples, the retention of ascorbic acid was the highest for the first 4 weeks ($p < 0.05$) (*table D*). This was in good agreement with the observation of Nagi [15] who found better retention of ascorbic acid in frozen concentrated pasteurized orange juice between –22 °C and 0 °C. This trend, however, was not maintained until the end of our study. After the 4 weeks of storage, a sharp loss of ascorbic acid concentration was observed in the frozen samples, which was unexpected and cannot be easily explained. One may attribute this to the bigger headspace in these samples, which had to be provided to ensure the safety of the bottles against volume expansion and possible breakage. Differences in ascorbic acid concentration in juice stored in refrigerated conditions [(4 ± 1) °C] and that at room temperature [(28 ± 2) °C] over 12 weeks (except for the first 2 weeks) were not statistically significant ($p < 0.05$). This result was in contrast to those of Zerdin *et al.* [5] who examined the rate of oxidation of ascorbic acid in orange juice samples stored at 25 °C and 4 °C. Kavousi studied the stability of ascorbic acid in commercially pasteurized lime juice [18]. His results indicated a lesser dependency of ascorbic acid retention on temperature in the range 5–25 °C. The slightly lower retention of ascorbic acid for refrigerated samples (*table D*), particularly during the first two weeks, may be attributed to higher oxygen solubility at lower temperatures, hence resulting in a higher oxidation reaction within the containers.

3.5. Effect of citric acid addition

It is well known that citric acid stabilizes ascorbic acid by chelating prooxidant metals [11]. It is also recognized that the rate of ascorbic acid breakdown is inversely proportional to the square root of the hydrogen ion concentration in acid solutions [15]. According to Jiang *et al.* [11], the presence of citric acid above 0.1 M markedly inhibited the degradation activity. They further added that, at low acid concentration, stimulation of polyphenol oxidase might occur [11]. In our investigation, refrigerated juice supplemented with 2% (w/w) citric acid (0.3 M) not only enhanced the retention, but also increased the ascorbic acid degradation rate (*table D*). Since ascorbic acid decomposes more easily in acid solution, the lower pH of these juices (pH = 2.6 as compared with 3.18) may well be the reason for the increased loss. No other explanation could be given for the above phenomenon. TSS of these samples nonetheless remained almost unchanged throughout the 12 weeks and juice cloud loss was at a minimum.

Microbial examination of samples was not in the scope of our study; however, the low pH of samples (pH < 3.2) usually inhibits any pathogenic microorganism from growing. In some of the juices in clear glass bottles which were stored at room temperature, patches of mold growth were observed after 12 weeks.

3.6. Kinetic study of ascorbic acid loss in sour orange juice

The decrease in ascorbic acid concentration to levels unacceptable by legislation or industrial practice often defines citrus juice shelf life, rendering ascorbic acid an important indicator of citrus juice quality [19]. When ascorbic acid retention of unpasteurized juice was plotted versus storage time, polynomial curves were obtained, with determination coefficients of the curves ranging between 0.9425 and 0.9838. A representative graph for juice stored in a temperature range from –12 °C to 28 °C was plotted (*figure 3*). The plot of change in logarithms of ascorbic acid retentions yielded straight lines with correlation coefficients between 0.8921 and

Figure 3. Plots of ascorbic acid loss in unpasteurized sour orange juice stored under different conditions. C : ascorbic acid concentration at the determined storage time, C_0 : ascorbic acid concentration at the beginning of the storage.

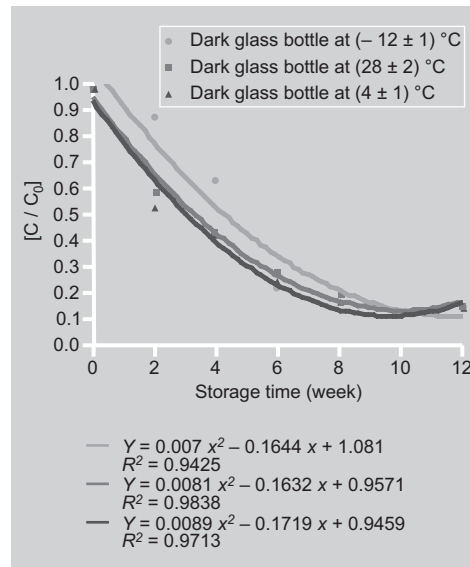
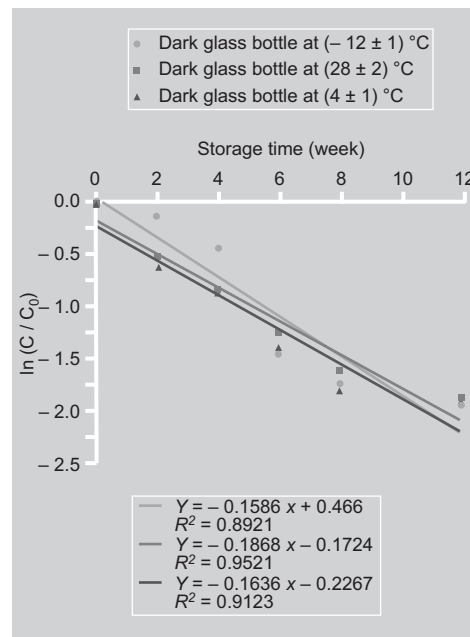


Figure 4. Linear ascorbic acid loss in juice plotted on a logarithmic scale. C : ascorbic acid concentration at the determined storage time, C_0 : ascorbic acid concentration at the beginning of the storage.



0.9521 (figure 4). Hence, the loss of ascorbic acid in unpasteurized juice, regardless of storage conditions, was described by a first-order reaction. The first-order kinetic model for ascorbic acid degradation determined in this study is in agreement with other studies for other citrus juices [8, 19, 20]. On the other hand, there have been studies that reported that ascorbic acid destruction follows a zero-order [21] or second-order reaction [22].

The loss rate of ascorbic acid in sour orange juice was calculated by the equation $[\ln(C) = \ln(C_0) - kt]$, the standard equation for a first-order reaction [23], where C is the concentration at time t (in $\text{mg}\cdot 100\text{ mL}^{-1}$); C_0 , the concentration at time zero (in $\text{mg}\cdot 100\text{ mL}^{-1}$); k , the ascorbic acid loss rate (per week); and t , the storage time (in weeks).

The values of k for the temperature range $(-12^\circ\text{C}$ to $+28^\circ\text{C})$ could be given in relation to the storage temperatures used (table II). The evaluated data, however, did not become statistically significant, as the final ascorbic acid contents of all juices, regardless of temperature storage conditions, were almost the same after 12 weeks.

The effect of storage temperature on the ascorbic acid degradation rate was described adequately by the Arrhenius equation [24]: $k_T = k_{\text{ref}} \exp[-E_a/R(1/T - 1/T_{\text{ref}})]$, where k_T is the ascorbic acid loss rate at a storage temperature T ; k_{ref} , the ascorbic acid loss rate at a reference temperature T_{ref} ; E_a , the activation energy ($\text{J}\cdot\text{mol}^{-1}$); R , the gas constant ($8.314\text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$); and T , the storage temperature on an absolute scale ($^\circ\text{K}$).

An Arrhenius plot of the natural log of the average rate constant (mg ascorbic acid loss $\cdot 100\text{ mL}^{-1}$ of sample) versus the reciprocal of storage temperature on an absolute scale (261 K to 301 K) was drawn (figure 5). One Arrhenius profile is evident in that figure which applied to the entire temperature range $(-12$ to $28^\circ\text{C})$. Activation energy (E_a) was determined to be $2.67\text{ kJ}\cdot\text{mol}^{-1}$.

4. Conclusion

The initial concentration of ascorbic acid in sour orange juice, of 11.5°Brix and a pH value of 3.18 , was $130\text{ mg}\cdot 100\text{ mL}^{-1}$. Ascorbic acid in unpasteurized sour orange juice stored under various conditions decreased with increasing temperature and time. For unfrozen juice, the highest rate of ascorbic acid loss was during the first 2 weeks of storage. The final ascorbic acid content in all juices studied was reduced to below $20\text{ mg}\cdot 100\text{ mL}^{-1}$ after 12 months of storage. The unpasteurized nature of the juice, head-space in juice bottles and air dissolved in the

Table II.

Ascorbic acid loss rate in unpasteurized sour orange juice stored in dark green glass bottles at three different temperatures (28 °C, 4 °C, -12 °C).

Ascorbic acid loss rate (k) ¹ (per week)	Ascorbic acid concentration ¹ (mg·100 mL ⁻¹)		Absolute temperature (T) (°K)	[10 ³ / T] value
	at time t (in weeks)	at time $t = 0$		
0.1757	18.82	130	261	3.83
0.1740	19.18	130	277	3.61
0.1707	19.88	130	301	3.32

¹ The values in the columns are not statistically different ($P < 0.05$).

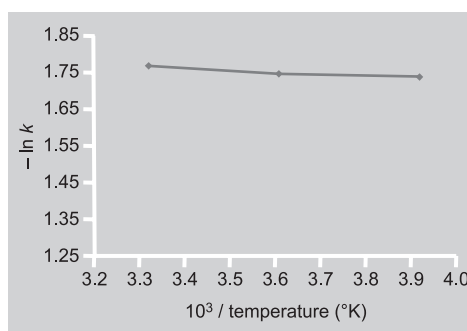
juice were thought to be the main reasons for the rapid and massive loss of ascorbic acid in samples, particularly in the first few weeks of storage. The loss of ascorbic acid in juice at all storage temperatures was described as a first-order reaction. Addition of citric acid to refrigerated samples did not improve the ascorbic acid retention but minimized the cloud loss in samples.

Acknowledgments

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**Figure 5.**

Arrhenius plot of ascorbic acid degradation in sour orange juice. The parameter k represents the ascorbic acid loss rate per week.

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Duración de conservación del zumo de naranja amargo no pasteurizado en Irán.

Resumen — Introducción. En Irán, la naranja amarga está disponible entre la mitad de octubre y el mes de marzo. Los consumidores almacenan frecuentemente grandes volúmenes de zumo no pasteurizado, a baja temperatura o a temperatura ambiente, en previsión de los periodos durante los cuales el fruto fresco no estará disponible. El objetivo de nuestros estudios fue determinar la duración de conservación del zumo no pasteurizado de estas naranjas amargas, almacenado en condiciones semejantes a las que se emplean localmente, por los consumidores.

Material y métodos. Se preparó zumo de naranja amarga exprimido con la mano; después se filtró, y se vertió en botellas de vidrio claro o en vidrio verde oscuro. Se almacenaron las botellas a temperatura ambiente [(28 ± 2) °C], en el refrigerador [(4 ± 1) °C], y en el congelador [(-12 ± 1) °C] durante 12 semanas. Se prepararon otras muestras añadiendo al zumo 2 % (peso / peso) de ácido cítrico, se almacenaron en el refrigerador. Se midieron la materia seca soluble y los valores del pH cada dos semanas; y el contenido en ácido ascórbico se analizó mediante valoración en presencia de indofenol 2,6 diclorofenol. Se realizó el estudio durante 12 semanas.

Resultados y discusión. El contenido en materia seca soluble así como el valor del pH de zumo inicial fueron de 11.5 °Brix y 3.18, respectivamente. Pareció que el pH no estaba influenciado significativamente por el tiempo ni por las condiciones de almacenamiento; sin embargo, el contenido en materia seca soluble en algunas muestras se redujo a aproximadamente 9 °Brix. El contenido en ácido ascórbico inicial fue de 130 mg·100 mL⁻¹; tras 2 semanas, se redujo de casi un 50% para todas aquellas muestras no congeladas. La concentración final de ácido ascórbico de los zumos fue aproximadamente de 20 mg·100 mL⁻¹ independientemente de las condiciones de almacenamiento. La reacción de deterioro del ácido ascórbico en el zumo, para cada temperatura testada, siguió un modelo cinético de primer orden con una energía de activación de 2.67 kJ·mol⁻¹.

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