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Kinetics of Ascorbic Acid Degradation in Un-Pasteurized Iranian Lemon Juice During Regular Storage Conditions

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Abstract: The aim of this research was to determine shelf life stability of un-pasteurized lemon juice filled in clear or dark green glass bottles. Presence of light, time and temperature affect the ascorbic acid retention in citrus juices. Bottles were stored at room temperature (27±3°C) and in the refrigerator (3±1°C). Total soluble solids, total titrable acidity and pH value were measured every three weeks and analysis was carried out on ascorbic acid content by means of titration method in the presence of 2,6-dichlorophenol indophenol. The study was carried out for 12 weeks after which slight changes in color, taste and apparent texture in some samples were observed and ascorbic acid content reduced by 50%. Soluble solids content, pH value and total acidity were 5.5° Brix, 2.73 and 5 g/100 mL, respectively which appeared not to be significantly influenced by storage time or conditions. Ascorbic acid content initially at 38.50 mg/100 mL was sharply reduced to about 22 mg/100 mL within the first three weeks of storage. The final ascorbic acid content of all samples was about 15 mg/100 mL. The deteriorative reaction of ascorbic acid in the juice at all conditions followed a first-order kinetic model with activation energy of 137 cal mol^{-1}.

Key words: Ascorbic acid, un-pasteurized lemon juice, stability, reaction kinetics, storage condition

INTRODUCTION

The nutritional quality of lemon juice is related largely to its content of vitamin C and its antioxidant capacity. The juice is used as food additive, ingredient in salad dressing and as a popular drink because of its rich flavor and aroma. 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 (Williams *et al*., 1995). The main source of vitamin C for many consumers in Iran is usually pasteurized or un-pasteurized lemon juice or fruit. Vitamin C (ascorbic acid) is consisted of an enediol structure which is conjugated with carbonyl group in a lactone ring (Zerdin *et al*., 2003). Temperature, pH, oxygen concentration, enzymes, vitamin initial concentration as well as ratio of ascorbic to dehydroascorbic acid are the factors which influence the nature of degradation mechanism of vitamin C (Kimball, 1991). The decomposition of ascorbic acid and non-enzymatic browning, are the main deteriorative reactions that occur during processing, packaging and storage of citrus juice (Lee and Nagy, 1988). Ascorbic acid degradation proceeds through both aerobic and anaerobic pathways. During storage, the juice may experience number of deteriorative reactions including vitamin C loss, microbial spoilage, cloud loss, development of off-flavor, changes in color, texture or appearance, resulting in quality degradation of the product (Handwerk and Coleman, 1988). The vitamin C loss during storage in pasteurized citrus juice concentrate (orange, lemon, grapefruit and tangerine) and their reaction kinetics have been investigated by Burdurlu *et al*. (2006). Zerdin *et al*. (2003) examined rate of oxidation of ascorbic acid in the orange juice samples stored at 25 and 4°C. Kavousi (1997) studied the stability of ascorbic acid in commercially pasteurized limejuice. Kabasakalis *et al*. (2000) investigated ascorbic acid content of commercial fruit juices and its rate of loss upon storage. Vitamin C content of citrus fruit and their products was analyzed by Nagy (1980). Robertson and Samaniego (1986) examined the effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage.

In many places in Iranian Southern provinces, consumers store large volume of un-pasteurized juice at room or low temperature for consumption when the fresh fruit is not available. The aim of this study was to examine

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the shelf life stability of un-pasteurized lemon juice which was stored under conditions similar to those used by consumers at home. Variation in ascorbic acid content, pH, total titrable acidity (based on citric acid) and Total Soluble Solids (TSS) were recorded for 12 weeks after which slight changes in color, texture and taste were appeared in some of the samples stored at room temperature. Kinetic of ascorbic acid loss in the un-pasteurized lemon juice during storage was also studied.

MATERIALS AND METHODS

Sample preparation: Fresh lemons of almost equal size and weight were obtained from a garden in city of Minab, a town in proximity of Persian Gulf region, during the harvest season in April 2005. The fruit was transferred to the Pilot Plant of Food Science and Technology Department of Shiraz University where all the experimental runs were carried out. The lemons were then washed, peeled and hand-squeezed for juice. The Juice clarification was carried out with an ordinary kitchen filter. The clarified juice initially had Total Soluble Solids (TSS) of 5.5° Brix and pH value of 2.73. The juice was stored in 300 mL PET bottles (clear or dark green) for 12 weeks in storage conditions shown in Table 1. The sample bottles were under filled, resulting in about 5-10 mL of headspace. Variation in ascorbic acid content, pH, total titrable acidity (on the basis of citric acid) and TSS were observed and data analysis were carried out on three replicates.

Methods of analysis: The pH was measured with a pH meter (Metrohm, Model 623, France). TSS was determined as °Brix by a temperature controlled refractometer (CARLZEISS JENA, Model. 711849, Germany). The total titrable acidity (based on citric acid) was examined according to the standard method stated in AOAC (1975). Ascorbic acid content expressed in mg/100 mL was determined by titration method using 2,6-dichlorophenol indophenol according to AOAC (1975). 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 (DHAA) (Kabasakalis *et al*., 2000).

Table 1: Storage conditions for samples of un-pasteurized lemon juice

Sample	Storage conditions
LA	Samples in 300 mL clear PET bottles at room temperature (27±3°C)
DA	Samples in 300 mL dark green PET bottles at room temperature (27±3°C)
DC	Samples in 300 mL dark green PET bottles in refrigerated store room (3±1°C)

Data analysis was carried out by analysis of variance in randomized complete blocks and the COSTAT statistical programme. Excel® was used for preparing the plots.

RESULTS AND DISCUSSION

Effect of storage duration on ascorbic acid loss: The value of ascorbic acid present at the start of storage in freshly prepared lemon juice was 38.50±0.2 mg/100. An important parameter in degradation of ascorbic acid is storage duration (Lee and Nagy, 1988). Table 2 shows evolution in ascorbic acid content of the juice during 12 weeks storage. The ascorbic acid of all samples decreased during storage. The large decrease in ascorbic acid concentration was evident from day 0 to day 14 at both storage temperatures. In general, the results show that there was a rapid degradation of vitamin C in the early stage of storage, followed by a gradual loss. Same results reported by other investigators (Kennedy *et al*., 1992; Soares and Hotchkiss, 1999) and can be attributed largely to the oxygen that is dissolved in the juice (no liquid flashing was carried out prior to bottling) and in the headspace in the early stages of storage. The incorporation of air in the juice during extraction, clarification and bottling is recognized by previous worker (Rassis and Sam Saguy, 1995). The high loss in ascorbic acid content, mainly in the first two weeks of storage, may also be attributed to activity of cytochrome oxidase, ascorbic acid oxidase and peroxidase, too (Nagy, 1980). The ascorbic acid loss in the subsequent 10 weeks of storage was steady but at much slower pace. After 12 weeks the ascorbic acid content of all samples were reduced to mere 14 mg/100 mL. Choi *et al*. (2002) studied the retention of ascorbic acid with storage in blood oranges juice, observed a linear reduction in concentration with time. More than 50% was lost within three weeks of refrigerated storage and it was completely degraded after five weeks storage in the control juice.

Effect of light on ascorbic acid retention: The analysis of data indicated that throughout the 12 weeks storage, lemon juice stored in dark bottles had marginally better retention of ascorbic acid in comparison to samples in clear bottles (Table 2). Variation were however observed between samples LA and DA over 12 weeks ($p<0.05$). The slightly higher degradation of ascorbic acid in LA samples in comparison to DA samples was attributed to better light penetration through clear bottle. This was perhaps due to a synergistic effect caused by combination of air trapped in the headspace and dissolved in the juice and that of light penetration through the bottle.

Table 2: Ascorbic acid content of un-pasteurized lemon juice stored in various conditions

	mg ascorbic acid/100 mL sample at storage time (weeks)				
Samples	0	3	6	9	12
LA	38.50±1.20^{*a**A***}	21.20±1.50^{bB}	17.60±1.02^{cB}	16.00±0.90^{dB}	12.90±0.85^{eA}
DA	38.50±1.20^{aA}	23.80±1.10^{bA}	22.40±2.00^{bA}	22.17±1.20^{bA}	13.50±1.11^{cA}
DC	38.50±1.20^{aA}	22.00±2.00^{bB}	20.30±1.80^{cA}	16.90±1.80^{dB}	14.00±1.30^{eA}

*: Data are mean of three replicates, **: Different small superscript letter(s) in a row means the values differ significantly $p<0.05$, ***: Different capital superscript letter(s) in a column means the values differ significantly $p<0.05$

Table 3: TSS of un-pasteurized lemon juice stored in various conditions

	Total soluble solid content at storage time (weeks)				
Samples	0	3	6	9	12
LA	5.50±0.20^{*a**A***}	5.50±0.50^{aA}	5.00±0.45^{aA}	5.00±0.35^{aA}	5.50±0.41^{aA}
DA	5.50±0.20^{aA}	5.50±0.38^{aA}	5.00±0.48^{aA}	5.00±0.50^{aA}	4.50±0.40^{aB}
DC	5.50±0.20^{aA}	4.80±0.42^{aB}	5.00±0.37^{aA}	5.00±0.51^{aA}	5.00±0.45^{aA}

*: Data are mean of three replicates, **: Different small superscript letter(s) in a row means the values differ significantly ($p<0.05$), ***: Different capital superscript letter(s) in a column means the values differ significantly ($p<0.05$)

Table 4: pH of un-pasteurized lemon juice stored in various conditions

	pH at storage time (weeks)				
Samples	0	3	6	9	12
LA	2.73±0.15^{*a**A***}	2.69±0.10^{aA}	2.51±0.20^{aB}	2.42±0.15^{aA}	2.75±0.21^{aA}
DA	2.73±0.15^{aA}	2.69±0.18^{aA}	2.65±0.11^{aA}	2.37±0.20^{aA}	2.65±0.13^{aA}
DC	2.73±0.15^{aA}	2.67±0.12^{aA}	2.53±0.17^{aB}	2.56±0.18^{aA}	2.70±0.15^{aA}

*: Data are mean of three replicates, **: Different small superscript letter(s) in a row means the values differ significantly ($p<0.05$), ***: Different capital superscript letter(s) in a column means the values differ significantly ($p<0.05$)

Table 5: Acidity content of un-pasteurized lemon juice stored in various conditions

	g citric acid/100 mL sample at storage time (weeks)				
Samples	0	3	6	9	12
LA	5.30±0.18^{*a**A***}	5.15±0.15^{aA}	5.00±0.14^{aA}	5.20±0.18^{aA}	5.23±0.11^{aA}
DA	5.30±0.18^{aA}	5.20±0.18^{aA}	5.20±0.20^{aA}	5.08±0.17^{aA}	5.18±0.15^{aA}
DC	5.30±0.18^{aA}	5.00±0.22^{aA}	5.10±0.17^{aA}	5.14±0.11^{aA}	5.17±0.21^{aA}

*: Data are mean of three replicates, **: Different small superscript letter(s) in a row means the values differ significantly ($p<0.05$), ***: Different capital superscript letter(s) in a column means the values differ significantly ($p<0.05$)

Effect of storage temperature: The significance of temperature on rate of reduction of ascorbic acid in un-pasteurized samples was examined in temperature range of 3 to 28°C (Table 2). The results indicated that ascorbic acid content in samples kept refrigerated was marginally lower than those stored at room temperature. The difference over 12 weeks in this temperature range was not however significant ($p<0.05$). The marginally lower retention of ascorbic acid in refrigerated samples was probably caused by the higher oxygen solubility at lower temperatures, hence resulting in higher oxidation reaction within the containers. Furthermore, it is probable that the PET bottles were not an ideal oxygen barrier particularly in cold storage room where the convection fans were producing strong draft. The result in this study was in contrast with those of Zerdin *et al.* (2002) who found much better retention of ascorbic acid in pasteurized orange juice samples which were stored at 4°C as compared to those at 25°C. Findings of Kavousi (1997) on the stability of ascorbic acid in commercially pasteurized limejuice however showed lesser dependency of ascorbic acid retention on temperature in the range 5-25°C.

Soluble solids content and pH and total titrable acidity: The initial TSS value for samples was 5.5° Brix which did not vary significantly over 12 weeks ($p<0.05$) (Table 3). There was however formation of gel lumps in samples. The formation of gel-like lumps in samples may be attributed to activity of natural enzymes, such as PME. These enzymes catalyze the demethoxylation of the pectins, causing an increase in the free carboxyl groups which favors clarification of the juice (Rouse and Atkins, 1952). The pH values of these samples initially at 2.7 remained almost unchanged throughout the study (Table 4).

To a large extent, acidity protects against the development of pathogens. In lemon juice, citric acid is the most abundant, followed by malic, both being present mostly as free acids. The total acidity in the lemon juice samples initially at 5 g/100 mL remained almost unchanged (Table 5). This may be give an indication that no spoilage and fermentation takes place in the sample.

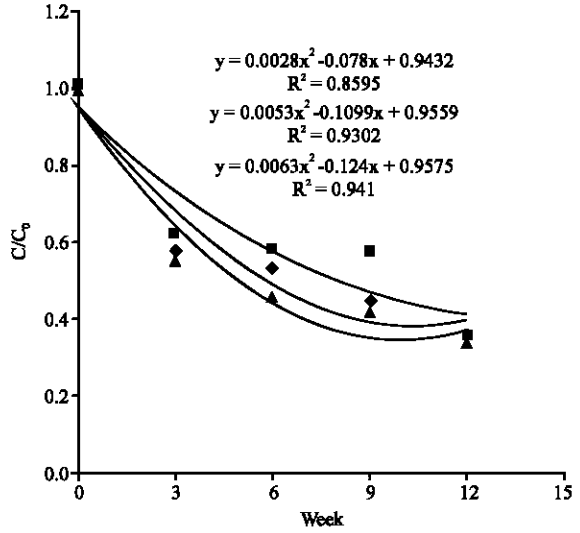

Fig. 1: Ascorbic acid loss in DA (■), DC (▲) and LA (◆) during 12 weeks storage

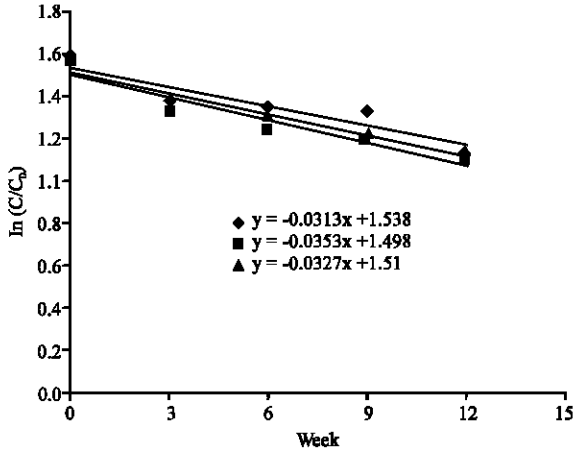

Fig. 2: Ascorbic acid loss in DA (■), DC (▲) and LA (◆) during 12 weeks storage

Kinetic study of ascorbic acid loss: When ascorbic acid retention of un-pasteurized lemon juice was plotted versus storage time, polynomial curves were obtained with determination coefficients of the curves ranged between 0.85 and 0.89 (Fig. 1). A representative graph for samples stored at temperature range 3 to 28°C is given in Fig. 1. The plot of change in logarithm of ascorbic acid retentions yielded straight lines with correlation coefficients between 0.86 and 0.91 (Fig. 2). Hence, the loss of ascorbic acid in un-pasteurized samples, 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 (Burdurlu *et al*., 2006; Kavousi, 1997; Polydera *et al*., 2003). On the other hand, there have been studies reported that ascorbic acid destruction follows a zero-order (Laing *et al*., 1987) or second-order reaction (Robertson and Samaniego, 1986).

The loss rate of ascorbic acid in samples was calculated by Eq. 1, the standard equation for a first-order reaction (Toledo, 1991):

$$\ln C = \ln C_0 - kt \quad (1)$$

Where:

C = Concentration at time t (mg/100 mL)
C_0 = Concentration at time zero (mg/100 mL)
k = Ascorbic acid loss rate (week^{-1})
t = Storage time (week)

The values of k for temperature range (3 to 28°C) is -0.032 and -0.031. The effect of storage temperature on ascorbic acid degradation rate was described adequately by Arrhenius equation (Labuza and Riboz, 1982):

$$k_T = k_{ref} \exp[- E_a/R(1/T - 1/T_{ref})] \quad (2)$$

Where:

k_T = Ascorbic acid loss rate at a storage temperature T
k_{ref} = Ascorbic acid loss rate at a reference temperature T_{ref}
E_a = Activation energy (J mol^{-1})
R = Gas constant (1.987 kcal mol^{-1} K^{-1})
T = Storage temperature in absolute scale (K).

Activation energy was calculated by using Arrhenius equation was 137.9 cal mol^{-1} for the temperature range of 3-28°C.

CONCLUSION

The initial concentration of ascorbic acid in un-pasteurized lemon juice of 5.5 °Brix and pH value of 2.7 was 38.5 mg/100 mL. The final ascorbic acid content in all samples was reduced to about 15 mg/100 mL. The un-pasteurized nature of samples, headspace in sample bottle and air dissolved in the juice were thought to be main reasons for the rapid and massive loss of ascorbic acid in samples particularly in the first two weeks of storage. The loss of ascorbic acid in samples at all storage temperatures was described as a first-order reaction.

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