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Effect of thermal treatment on ascorbic acid content of pomegranate juice

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Pomegranate (*Punica granatum)* is one of the important fruit crops in India and the juice from pomegranate is one of the nature's good antioxidants source. The loss in antioxidant property in response to vitamin C and total phenolic content over the temperature range of 70-90°C was studied. The degradation kinetics of ascorbic acid in pomegranate juice during thermal treatment of 70-90°C was also studied. The loss of ascorbic acid followed the first order kinetic model. The temperature dependence of this degradation reaction can be described well by Arrhenius equation. The activation energy for this reaction was 81.67 kJ/mol but the retention of ascorbic acid and total phenolic content of the juice treated at 70°C for 90 min was more than 69 and 90%, respectively as that of the fresh pomegranate juice.

Keywords: Activation energy, Arrhenius equation, ascorbic acid, kinetics, pomegranate, thermal treatment

Introduction

Fruits and vegetables are a major source of dietary antioxidants that impart health benefits beyond nutrition[1]. The presence of antioxidants in fruits and vegetables has been attributed to protect cells against the damaging effects of reactive oxygen species[2] resulting strong protection against major disease risk including cancer, atherosclerosis, inflammation, neurodegenerative and cardiovascular diseases[3]. Collectively vitamin C, vitamin E and β-carotene are referred to as the antioxidant vitamins[4,5]. However, numerous studies have consecutively shown that consumption of food and beverages rich in phenolic content can reduce the risk of heart disease by acting as antioxidants against low density lipoprotein (LDL)[6]. Therefore, mostly the current focus is on the antioxidant action of phenolics as well as antioxidative nature of fruits and vegetables.

Pomegranate is one of the important crops in India[7]. The juice from pomegranates is one of the nature's good source of antioxidants[8] which contains phytochemicals, such as, flavonoids[9] and anthocyanidin, resulting in inhibition of cancer, cardiovascular diseases[10], and reduction in stomach disorder and systolic blood pressure[11]. Ascorbic acid is another important components of pomegranate juice. However, thermal treatments are believed to cause destruction of natural antioxidants in food[12]. Therefore, the purpose of this study was to investigate the thermal degradation of ascorbic acid as well as to determine the kinetic parameters for ascorbic acid degradation in pomegranate juice over a temperature range of 70-90°C.

Processing of fruit juice means production of juice and essence concentrates as valuable coproducts. Prolonged exposure to high temperature results in degradation reaction and ultimately in poor quality final products. One of the commonly occurring degradations in fruit juices is ascorbic acid loss. A general reaction rate expression for degradation kinetics can be written as follows[13,14,15].

$$d[C]/dt = k[C]^m \quad \ldots (1)$$

Where 'C' is the quantitative value of the degraded product under consideration. 'K' is the reaction rate constant and m is the reaction order. The rate of degradation will be of first order if 'm' = 1 and the equation of first order reaction is obtained upon integration of equation (1) and can be written as the following expression:

$$\ln ([C]_t/[C]_0) = -kt \quad \ldots (2)$$

$[C]_0$ is the value of the product at time 0 and $[C]_t$ is the value after reaction time t.

The relationship of reaction rate to temperature was quantified by the Arrhenius relationship *i.e.*,

$$k = A_0 \exp^{(Ea/RT)} \quad \ldots (3)$$

Where 'E_a' is the activation energy of the reaction, 'R' is the universal gas constant, 'T' is the absolute temperature and 'A_0' is a pre exponential constant.

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Materials and Methods

Extraction of Pomegranate Juice

Fresh pomegranates procured from local market were washed under running water. After removal of skin, seeds are separated and juice extracted using juice extractor (Bajaj Mixer Grinder, GM-550). The juice was then filtered through cheese cloth.

Thermal Treatment

Thermal degradation kinetics of ascorbic acid was studied by isothermal heating at 70, 80 and 90°C respectively. 10 mL samples were taken in sealed glass tubes and heated by placing them in a hot water bath. At regular time interval of 15 min, the tubes were taken out and rapidly cooled by plunging them into ice water and analyzed for ascorbic acid content. To study the degradation of ascorbic acid in pure solution, the ascorbic acid concentration was taken as the same as that of initial concentration of pomegranate juice (0.198 mg/mL) and followed the same method of heat treatment and analysis of ascorbic acid content.

Analytical Parameters

TSS was measured by hand refractometer (Erma Inc., Tokyo, Japan) and expressed in terms of °B, *p*H was determined by PH meter (Electronic Measurement India Pvt. Ltd., Calcutta, India). Total acidity of the sample was measured by titration of juice against 0.1 *N* sodium hydroxide using phenolphthalein as indicator and expressed as % citric acid[16]. Total phenolic content of pomegranate juice was determined by folin-ciocalteu method[17] at a wavelength of 765 nm using gallic acid standard and expressed as mg of gallic acid/100 mL of juice. Reducing sugar was estimated by DNS (3,5-dinitro salicylic acid) method[18] and expressed as % glucose. Ascorbic acid was determined by titrimetric method[19] and the value expressed as mg of ascorbic acid/100 mL juice.

Statistical Analysis

Data were analyzed using ANOVA in EXCEL. Significance level at $p \leq 0.05$ was applied to the results to test the significant difference.

Results and Discussion

Thermal degradation kinetics of ascorbic acid both in pomegranate juice and in pure ascorbic acid solution were studied by isothermal heating at 70, 80 and 90°C, respectively. Physico-chemical characteristics of the pomegranate juice were evaluated in normal juice and those heated at 70°C (for 60 min) and the results are presented in Table 1. The heat treatment has been found to decrease all the parameters of physico-chemical characteristics (TSS, *p*H, total acidity, ascorbic acid and total phenolic content) of the juice, except the reducing sugar, which was found to be increased. Thus, results clearly show that heat degrades the ascorbic acid and phenolic contents of the juice. The effect of heat treatment (70, 80 & 90°C) on the concentration of ascorbic acid in pomegranate juice and in pure ascorbic acid solutions for different time periods have been studied and the results showed that degradation of ascorbic acid increased with the increase of temperature but the degradation had been slower in pomegranate juice as compared with pure ascorbic acid solutions (Table 2). This was probably due to the presence of various phytochemicals in pomegranate juice and their

Table 1—The properties of fresh and heat treated (70° C, 60 min) pomegranate juice

Sample	TSS (°B)	*p*H	Total acidity (citric acid; mg/mL)	Ascorbic acid (mg/mL)	Reducing sugar (mg/mL)	Total phenolic content (gallic acid; mg /mL)
Fresh	14.5±0.6*	1.52±0.3	6.65±0.26	0.198±0.15	0.6±0.3	2.52±0.42
Treated	13.6±0.5	1.2±0.25	2.64±0.32	138±0.14	0.75±0.3	2.275±0.34

*Data are mean of 3 replicate observations
*Significance of variance is adjusted at 5% level

Table 2—Effect of heat treatment on ascorbic acid concentration (mg/mL) in pomegranate juice and in standard ascorbic acid solution

Time (min)	Temperature (°C) 70		80		90	
	Pomegranate juice	Ascorbic acid standard	Pomegranate juice	Ascorbic acid standard	Pomegranate juice	Ascorbic acid standard
15	0.173±0.50*	0.168±0.32	0.158±0.35	0.15±0.34	0.116±0.36	0.114±0.45
30	0.166±0.61	0.16±0.45	0.15±0.38	0.144±0.45	0.108±0.25	0.107±0.36
45	0.158±0.36	0.155±0.28	0.142±0.43	0.137±0.26	0.103±0.43	0.102±0.23
60	0.152±0.40	0.148±0.62	0.135±0.24	0.129±0.25	0.0907±0.65	0.095±0.45
75	0.145±0.42	0.144±0.34	0.129±0.26	0.123±0.32	0.0901±0.23	0.088±0.43
90	0.138±0.53	0.138±0.23	0.122±0.33	0.117±0.18	0.0869±0.35	0.083±0.27

*Significance of variance is adjusted at 5% level

synergistic effect, which helped in the slower degradation of vitamin C. The changes in total phenolic content of pomegranate juice with the temperature at different time intervals were studied and results are represented in Fig. 1. It is clear from the data that total phenolic content in pomegranate juice was decreased as the temperature and time of heat treatment increases. As the total phenolic content is a measure of the antioxidant activity, it can be conferred that antioxidant activity of the juice decreases with the increase in temperature. The present results are in conformity with the previous studies where total phenolic content of some vegetables was found decreased with the increase in temperature and time of thermal processing[20].

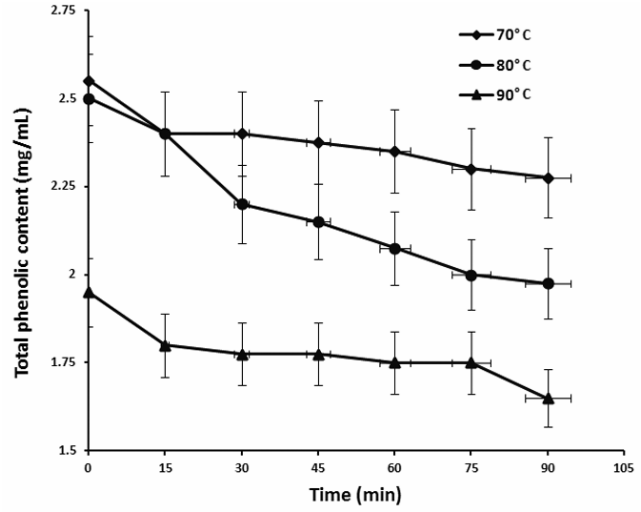

Fig. 1—Change in total phenolic content of pomegranate juice with temperature (5% error bar has been adjusted for the graphs)

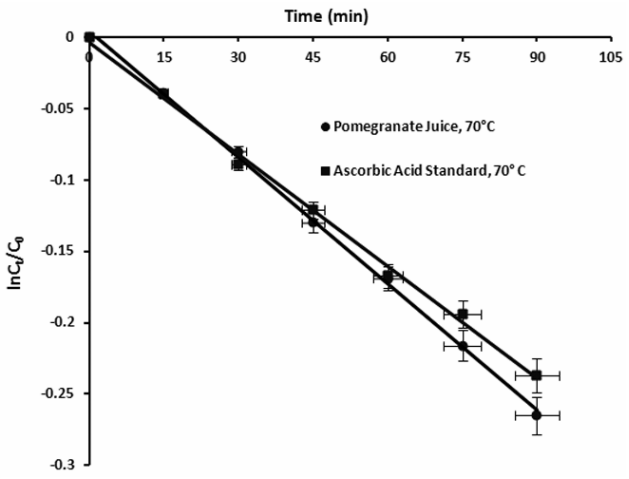

Fig. 2—Kinetics of ascorbic acid content of pomegranate juice and standard ascorbic acid solution at 70°C (5% error bar has been adjusted for the graphs)

The plots of $\ln([C]_t/[C]_0)$ versus t are shown in Figs 2-4 and the first order rate constants k were calculated from the slope of the straight line. In all the cases, a correlation coefficient >0.9 confirms that the degradation of ascorbic acid in the juice and pure solution follows a first order reaction at all the temperatures. Earlier studies also reported that the ascorbic acid degradation follows first order kinetics in aqueous solutions[21,22]. $T_{1/2}$, the time required for ascorbic acid to degrade 50% of its original value was calculated from the rate constant as 0.693/K. Table 3 represents the rate constants and half-lives of ascorbic acid in juice and in pure ascorbic solutions. The rate constant for ascorbic acid degradation in juice

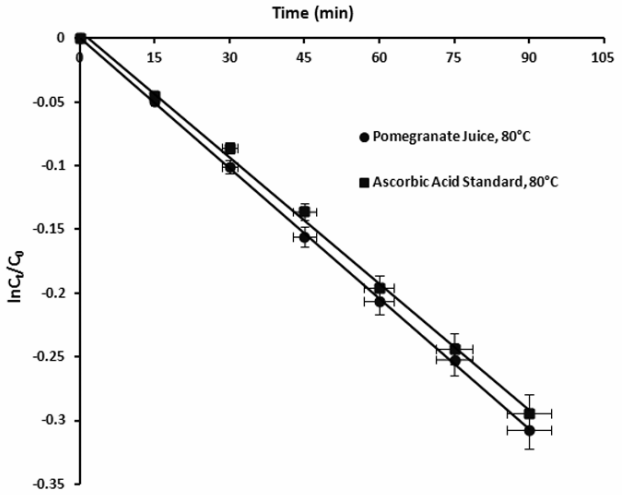

Fig 3—Kinetics of ascorbic acid content of pomegranate juice and standard ascorbic acid solution at 80°C (5% error bar has been adjusted for the graphs)

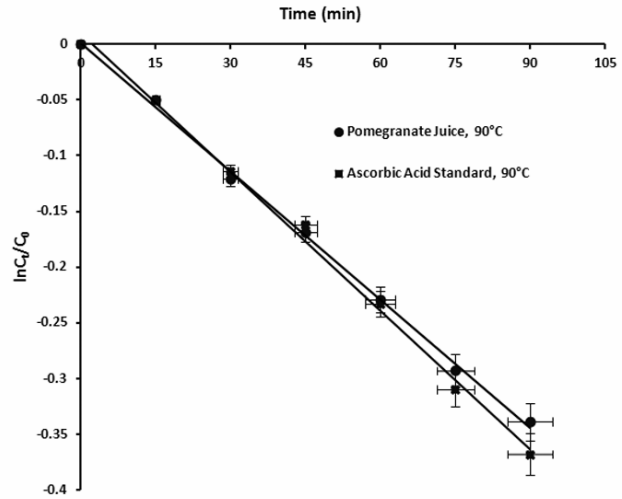

Fig. 4—Kinetics of ascorbic acid content of pomegranate juice and standard ascorbic acid solution at 90°C (5% error bar has been adjusted for the graphs)

Table 3—Kinetic and Arrhenius parameters for ascorbic acid degradation in pomegranate juice and ascorbic acid solution

	Kinetic parameter					
	Pomegranate juice			Ascorbic acid standard		
Temperature (°C)	k (min^{-1})	R^2	$t_{1/2}$ (min)	k (min^{-1})	R^2	$t_{1/2}$ (min)
70	2.880×10^{-3}	0.998	0.240×10^{3}	2.630×10^{-311}	0.996	0.263×10^{3}
80	3.466×10^{-311}	0.999	0.199×10^{3}	3.266×10^{-3}	0.997	0.212×10^{3}
90	3.816×10^{-3}	0.998	0.181×10^{31}	4.088×10^{-31}	0.995	0.142×10^{3}

Arrhenius parameters					
Pomegranate juice			Ascorbic acid standard		
A_0 (min^{-1})	E_a (kJ/mol)	R^2	A_0 (min^{-1})	E_a (kJ/mol)	R^2
0.512	81.67	0.972	0.138	62.49	0.999

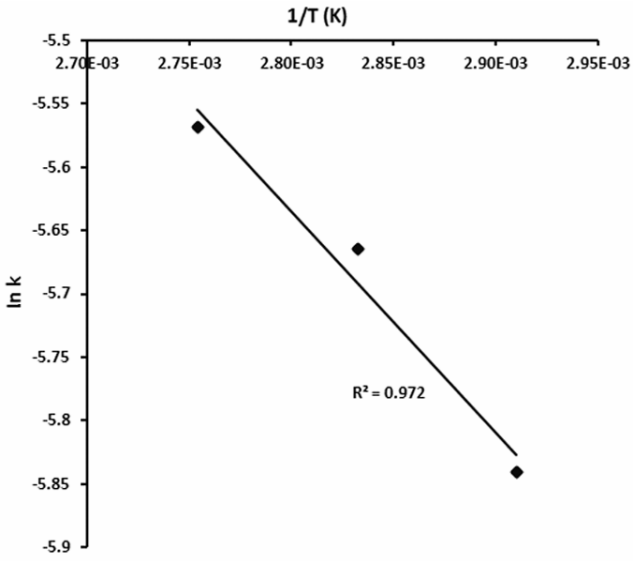

Fig. 5—Arrhenius plot of the ascorbic acid loss rate of pomegranate juice (5% error bar has been adjusted for the graph)

increased from 2.880×10^{-3} min^{-1} (for 70°C) to 3.816×10^{-3} min^{-1} (for 90^0C) and the half-life decreased from 0.240×10^{3} min to 0.181×10^{3} min as the temperature increased from 70-90°C. A similar trend was observed with pure vitamin C solutions and the rate constants obtained in the present study are in the range of 2.630×10^{-3} to 4.088×10^{-3} min^{-1}.

Arrhenius plot for degradation of ascorbic in pomegranate juice and in pure ascorbic acid solution are shown in Figs 5 and 6, respectively. Activation energies E_a (kJ/mol) were calculated as a product of gas constant R and the slope of the graph obtained by plotting lnk versus 1/T. The activation energies of juice and pure ascorbic acid solution are calculated to be 81.67 and 62.49 kJ/mol, respectively. Although thermal processing gives microbial safety, the addition of heat causes the loss of flavour, vitamins, and other heat labile nutrients. In the present study, 30.30% loss in vitamin C was observed for processing at 70°C for 90 min.

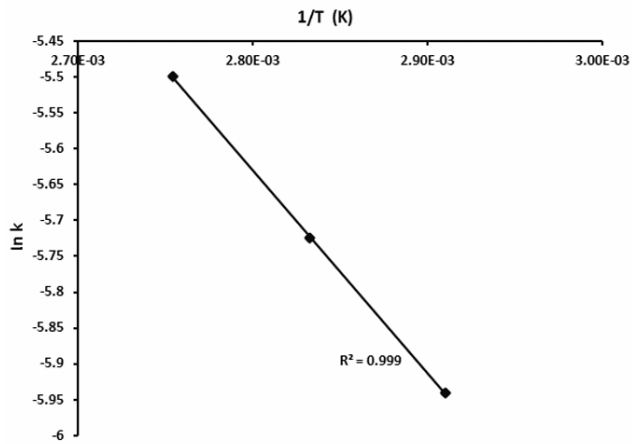

Fig. 6—Arrhenius plot of the ascorbic acid loss rate of standard ascorbic acid solution (5% error bar has been adjusted for the graph)

Conclusion

The study revealed that the loss of ascorbic acid content increases with time and temperature of processing. The ascorbic acid degradation in pomegranate juice can be described by an overall first order reaction and about 69% retention of vitamin C could be obtained after processing at 70°C for 90 min. The total phenolic content of pomegranate juice also decreases with the increase in time and temperature of thermal processing and about 90% retention of total phenolic content obtained after processing at 70°C for 90 min. Such information would be helpful for establishing appropriate processing and storage protocols to minimize the degradation of vitamin C and total phenolic content, the natural antioxidants, in pomegranate juice as well as other fruit juices contained ascorbic acid and phenolic antioxidant compounds.

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