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Congress Secretary

Investigation of the effect of ultrasound on ascorbic acid of fresh orange juice

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Abstract

In this study the effect of high power ultrasound in degradation of ascorbic acid (Vitamin C) in fresh orange juice was investigated. Fresh orange juice was sonicated at various temperatures and acoustic amplitudes for different times at the constant frequency of 30 kHz. Then ascorbic acid was measured. The results showed that ultrasound at the high temperature, had a significant effect on vitamin C. Degradation of vitamin C was found to follow first order kinetics. It was also demonstrated that the efficiency of vitamin C increased with decreasing temperature. This paper will also present how the thermodynamic parameters of $\Delta S^{\#}$ and $\Delta H^{\#}$ changed with acoustic amplitude.

Keywords: Ascorbic acid, Ultrasound.

Introduction

The objectives of the present work are to investigate the effect of power ultrasound on the ascorbic acid in a natural and fresh condition of orange juice and also to study the effects of cavitation and temperature on ascorbic acid. Orange juice is the most well-known and rich source of vitamin C, which this component is an important nutrient known for its potential antioxidant, anticancerous and other health promoting properties. The researchers reported that vitamin C helps in the prevention of cancer [1].

The fresh orange juice normally contains 400-600 mg ascorbic acid per 100 ml orange juice [2]. Pasteurization has a great effect on the reduction of the ascorbic acid in the processed juice and cannot provide the product requirement [2]. Therefore, the synthesis of vitamin C needs to be added to the final product. In this study, the ascorbic acid has been evaluated after the TS treatment immediately.

Experimental

Chemical Reagents

Standard iodine solution (0.01N) was supplied Merck,Germany.

Ultrasonic Source

A laboratory ultrasonic processor (Dr. Hielscher GMbH, Model UP 50H, Germany) with an operating frequency of 30 kHz and maximum nominal power output of 50 W was used for sonication. A tapered titanium sonotrode of 3mm tip diameter was used an ultrasound applicator in all experiments.

Sample Preparation

Oranges of unknown source and variety purchased from local market. They were washed and cut into four pieces and pressed using a household orange squeezer. The extracted juice was centrifuged using a laboratory centrifuge (Hettich, Model EBA20,Germany) at 6000×g for 5 minutes. The supernatant was separated and clarified further by a vacuum pump (Büchi, Model B-169,Switzerland) through Whatman filter paper No 1.

Sonication of Orange Juice

Orange juice (5 ml) was sonicated for 10, 15, 20 and 25 minutes at various acoustic amplitudes (60%, 80% and 100%) and temperatures (50 ºC, 70 ºC and 80 ºC). Samples were cooled immediately after sonication and ascorbic acid was measured.

Heat Treatment of Orange Juice

5 ml fresh orange juice was heated in a round bottomed glass test tube in a water bath (Memmert, Germany) set at various temperatures ranging from 50ºC to 80ºC with 10 ºC increment for 10, 15 , 20 and 25 minutes. Samples were cooled immediately after heat treatment and ascorbic acid was measured.

Determination of Ascorbic acid

The iodimetric method was used to measure ascorbic acid. The orange juice sample (5ml) was added into a 25ml Erlenmeyer flask. Then starch solution 0.5% (6 drops) was added. The solution was titrated by iodine 0.01N until the endpoint reached, i.e. the first sign of blue color that remains after at least 20s of swirling and final volume was recorded. Since each ml of used iodine equals 0.88mg of ascorbic acid, the amount of existing ascorbic acid in samples was calculated.

Kinetic data analysis

Nutrient destruction is described using the reaction rate and the dependence of reaction rate on temperature. Parameters used are the reaction rate constant (k) and the Arrhenius activation energy (E_a). The kinetic data were analyzed as described by Van Boekel (1996) [3]. Vitamin loss is gernally considered to follow first order kinetics.

$$-dC/dt=-kt \qquad (1)$$

where C is the concentration of nutrient, t is time and k is the reaction constant (time $^{-1}$). If C_0 is the concentration of the vitamin C at time zero, integration of the Eq (1) yields:

$$C = C_0 \exp(-kt) \tag{2}$$

Taking natural logarithm, we have:

$$\ln C = \ln C_0 (-kt) \tag{3}$$

For the first order reaction a plot of lnC against time (t) will be a straight line, and the rate constant is represented by the slope. Temperature dependence of a reaction is described by the Arrhenius equation [4]:

$$k = k_0 \exp(-\frac{E_a}{RT}) \tag{4}$$

where, k_0= frequency factor or the Arrehenus constant ($time^{-1}$), R is the universal gas constant (8.3145 J $mol^{-1}K^{-1}$) and T is the absolute temperature (K). The decimal reduction time (D-values), the time required to reduce the vitamin C concentration by 90% was related to reaction rate constants by [5]:

$$D = \frac{2.303}{k} \tag{5}$$

The D-values were then described as direct exponential function of temperature [5]:

$$\ln\left(\frac{D_1}{D_2}\right) = \frac{T_2 - T_1}{Z} \tag{6}$$

where D_1 and D_2 are decimal reduction time at temperatures T_1 and T_2 , respectively, with Z representing the temperature required for 1 log cycle reduction D value.The Eyring equation in chemical kinetics relates the reaction rate constant to temperature, from which the activation parameters can be estimated. The linear form of Eyring equation is [6]:

$$\ln\frac{k}{T} = \frac{-\Delta H^{\#}}{R}.\frac{1}{T} + \ln\frac{K_b}{h} + \frac{\Delta S^{\#}}{R} \tag{7}$$

where $\Delta H^{\#}$ is enthalpy of activation, kb is Boltzmann constant, h is Planck's constant and ΔS# is entropy of activation. The plot of ln (k/T) versus 1/T gives a straight line with a slope $\frac{-\Delta H^{\#}}{R}$, from which the enthalpy of activation can be derived, and an intercept $\ln\frac{K_b}{h} + \frac{\Delta S^{\#}}{R}$, from which the entropy of activation can be calculated.

Statistical Analysis

The effect of experimental variables on the degradation of vitamin C was studied using a completely randomized factorial design. The impact of sonication time, temperature and acoustic amplitude on the degradation of vitamin C was investigated in 4, 3and 3 levels with 3 replications. Duncan's multiple mean comparison test ($P<0.05$) was employed to calculate the significant difference among the degradation of vitamin C due to each factor. Microsoft Excel 2003 and SAS 8.2 (SAS Institute In., 2001) were used for data analysis.

Results and Discussion

The thermal influance on the amount of vitamin C

Fruit juice pasteruzation is a widely applied method to preserve fruit juices. As the consumption of such products continues to increase their contribution in providing important amounts of vitamin C, provitamin A and antioxidant activity in the diet will increase too[7].

In this study, the ascorbic acid has been evaluted after the thermal and thermosonication treatment immediately. Figure 1 illustrates semilogarithmic plot of the variation of vitamin C versus time depending on different temperatures. As Figure 1 shows 50 and 70 ºC have no significant effect on vitamin C and the degradation is rapid at higher temperature (80 ºC). Duncan's multiple range also confirmed the results of this study. The change of vitamin C was linear which proved that vitamin C destruction followed first order kinetics.

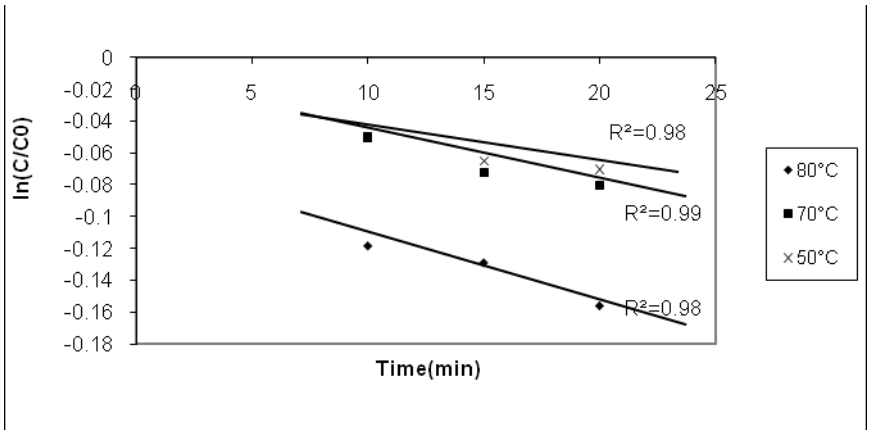


Figure1. Degradation of ascorbic acid in orange juice versus time at various temperatures.

The reaction rate constants (k) and thermal resistance value (D) for vitamin C degradation during method of heating and at different temperatures are indicated in Table 1. The k value further confirmed the influence of temperature. The comparison with the amount of D-values in thermal method, the Z-value was calculated to be 69.83 ºC which revealed the stability of vitamin C.

Figure 2 is Arrehnious plot that indicating thermal degradation of vitamin C. With applying thermal method, the value of activation energy due to vitamin C degradation was obtained 21.07 k J mol^{-1} by semilogarithmic plot of k variations against absolute reversal temperature.

Table1. Rate constants and thermal resistance parameters for vitamin C degradation

Temperature (°C)	Inactivation rate constant (k) (min^{-1})	D-value (min)
50	0.00399	577.2
70	0.00441	522.22
80	0.0086	267.79

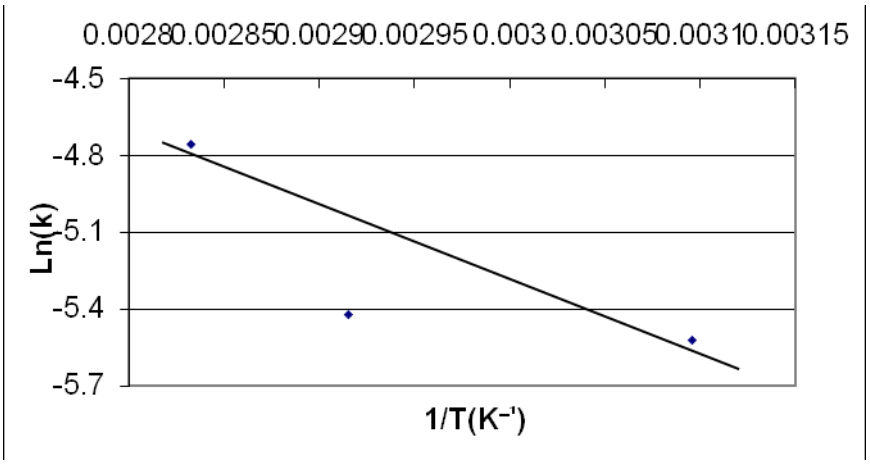


Figure2. Arrehnious plot for thermal destruction of vitamin C

Eyring plot relating to thermal destruction of vitamin C was plotted as shown in Figure 3.

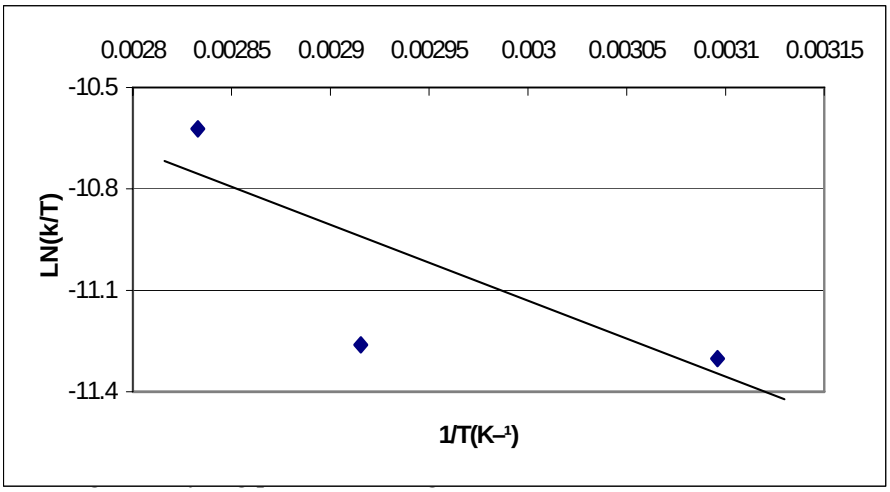


Figure3. Eyring plot relating to thermal destruction of vitamin C

Entropy, Enthalpy and Gibbs free energy to destruct vitamin C were calculated and given in table2.

Table2. Activation parameters for thermal destruction of vitamin C in temperature range 50-80 ºC

Temperature (ºC)	$\Delta G^{\#}(kJ.mol^{-1})$	$\Delta H^{\#}(kJ.mol^{-1})$	$\Delta S^{\#}(kJ.mol^{-1}.K^{-1})$
50	**95.83**	**18.27**	**-0.24**
70	**100.63**		
80	**103.03**		

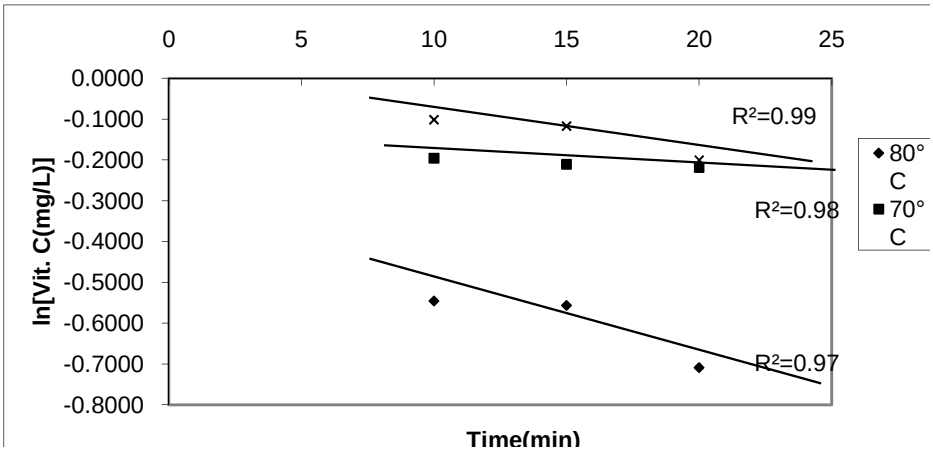
Thermosonication effect on the amount of vitamin C

Figure 4 showed the amount of vitamin C versus various acoustic amplitudes at different temperatures. As it can be seen the maximum amount of vitamin C in orange juice was obtained in the 50 ºC and amplitude 60% and the minimum was found in 80 ºC and amplitude 100%. According to Analysis-variance table, the optimum time and acoustic amplitude to preserve vitamin C were 10 minutes, 60% and 50 or 60 ºC respectively. Therefore, under the same condition, it was recommended that 50 ºC, 10 min, 60% were used to keep the maximum amount of vitamin C in orange juice. Figure 4, demonstrated the amount of vitamin C versus time at different temperatures regarding thermosonication.

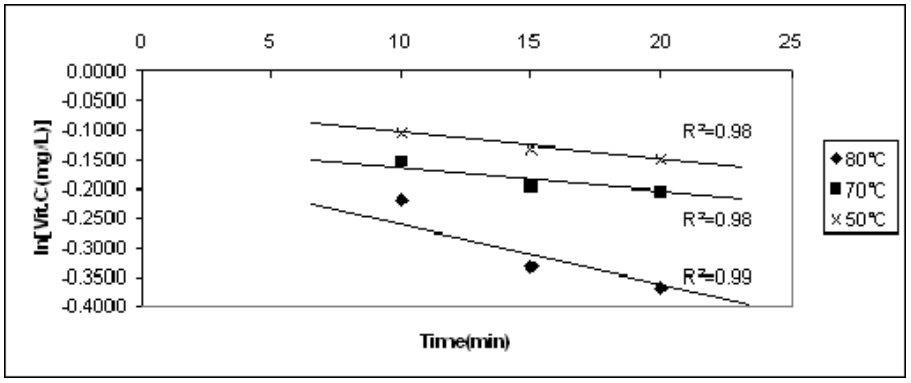
As can be observed in all experiments such as the reduction of thermal destruction of vitamin C versus time is linear. This fact indicates that kinetic reaction follows the first order. According to Figure 4 the minimum fall of vitamin C in acoustic amplitude is 60%, because cavitation collapse is mainly low. Reaction rate constant is the slope of straight lines of figure 4, given in table 3.

Table3. Reaction rate constants and D-values for thermosonical degradation of vitamin C at various temperatures

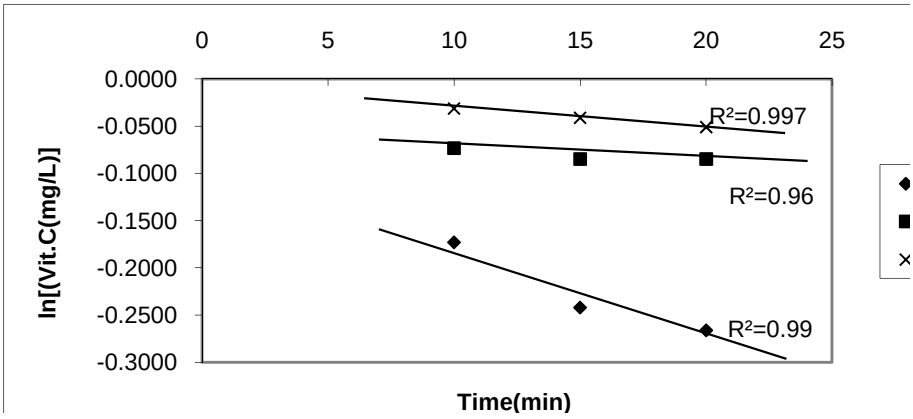
Amplitude(%)	**Temperature (ºC)**	**Inactivation rate constant (k)**	**D-value (min)**
100	**50**	**0.00936**	**246.05**
	70	**0.0131**	**176.1**
	80	**0.0386**	**59.66**
80	**50**	**0.00837**	**275.15**
	70	**0.0119**	**193.69**
	80	**0.0201**	**114.630**
60	**50**	**0.00459**	**501.7**
	70	**0.0051**	**451.57**
	80	**0.0147**	**156.35**



(a)



(b)



(c)

Figure4. The amount of ascorbic acid after ultrasound with amplitude a) 100% b) 80% c) 60%

Furthermore, this table shows reaction rate constants, D-values of vitamin C under thermosonication condition against various temperatures. Comparing the results obtained from the both methods namely thermosonication treatment (60%) and thermal method reveals that D-values are decreased from 577.19 min without ultrasound to 501.70 min with ultrasound at the same temperature (50 ºC).

Accordingly at low temperatures such as 50 ºC heating and thermosonication cause the fall of ascorbic acid. As Vercet et al (2001) confirmed that the reduction of ascorbic acid, under MTS and heating condition was only 10% [8]. They also stated that when pH is low vitamin C, against temperature is stable but it is sensitive to oxidation, therefore according to Sinclair theory in 1984 vitamin C decreased at low temperatures due to oxygen and enzyme activity dehydrogenization [2].
The variations of reaction rate constant, D-values relating to orange juice thermosonication under various acoustic amplitudes were given in table3. Activation energy of vitamin C destruction at different acoustic amplitudes was given in Table 4.

Table4. Activation parameters for thermosonical degradation of vitamin C within the temperature range 50-80°C

Amplitude (%)	**T(°C)**	$\Delta G^{\#}(kJ.mol^{-1})$	$\Delta H^{\#}(kJ.mol^{-1})$	$\Delta S^{\#}(kJ.mol^{-1}.K^{-1})$	$Ea(kJ.mol^{-1})$
100	**50**	**92.14**	**37.2**	**-0.17**	**39.99**
	70	**95.54**			
	80	**97.24**			
80	**50**	**92.48**	**23**	**-0.215**	**25.8**
	70	**96.78**			
	80	**98.93**			
60	**50**	**95.66**	**46.86**	**-0.151**	**49.66**
	70	98.48			
	80	100.19			

The changes of k versus absolute reversal semilogarithmic plot for the destruction vitamin C at different acoustic amplitudes (100%, 80%, 60%) were shown in figure 4. Higher activation energy implies that a smaller energy change is needed to degrade a specific compound more rapidly. The activation enthalpy and entropy for vitamin C degradation were found to vary. The change of entropy, enthalpy and Gibbs free energy under the mentioned condition were taken from the slope of above plots which they were shown in Table 4. In general the activation enthalpy is a of energy barrier that must be overcome by reacting molecules and is related to the strength of the bonds, which are broken and made in the formation of the transition state from the reactants. The activation entropy is related to the number of molecules with appropriate energy that can actually react. The value of activation entropy also includes steric and orientation requirements along with solvent effects [9].
The results revealed that after heating and thermosonication the amount of ascorbic acid decreased. At lower temperatures such reduction will be done at higher rate by ultrasound. That justifies what Mason and Lorimer did in 1988. They came to the condition that oxidation reaction was accelerated by ultrasound.

Conclusions

In the present work, the influence of different methods (TS treatment and heat treatment) on the vitamin C degradation was studied. The study indicated that the degradation of vitamin C for two methods followed first order kinetics. It has been found out that the ascorbic acid was

reduced after the TS treatment and heat treatment. The greater ultrasonic power induced greater reduction at low temperature. The oxidation reaction, which induced vitamin C to be lost during the process, was also accelerated by ultrasound. Further investigation needs to be carried out to optimize the nutrient (vitamin C) and thermosonication effect to arrive a suitable time, temperature and amplitude profile.

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