RESEARCH PAPER | International Journal of Agricultural Engineering | Volume 11 | Issue 2 | October, 2018 | 282-288
ISSN–0974–2662 ■ Visit us : *www.researchjournal.co.in* ■ **DOI: 10.15740/HAS/IJAE/11.2/282-288**

Effect of thermal treatment on phenolic and antioxidant content of fresh bael juice

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Received : 24.04.2018; **Revised :** 03.08.2018; **Accepted :** 20.08.2018

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■ **ABSTRACT :** Bael (*Aegla marmelos*) is one of the important fruit in India and bael juice is most important source of antioxidants. The loss of phenolic compound and antioxidant content over the temperature range of 55-85^0 C was studied. Degradation kinetics was best fitted by first order reaction kinetic model for both phenolic compound and antioxidants. Arhenius and Erying – polany models had been used to determine the temperature dependent degradation. Following the Arhenius model, the activation energy for the phenolic compound and antioxidants were 18.52 and 45.08 KJ mol^{-1}, respectively. The retention of phenolic compound and antioxidants of bael juice treated at 55^0 C for 90 min was more than 61 and 68 per cent, respectively as that of fresh bael juice.

■ **KEY WORDS :** Phenolic compound, Antioxidant, Degradation kinetic, Arhenius, Erying-Polany, Activation energy

■ **HOW TO CITE THIS PAPER :** Banerjee, Ipsita and Ghosh, Uma (2018). Effect of thermal treatment on phenolic and antioxidant content of fresh bael juice. *Internat. J. Agric. Engg.*, **11**(2) : 282-288, **DOI: 10.15740/HAS/IJAE/11.2/282-288.**

The bael (*Aegla marmelos*) is a wellknown fruit found widely throughout India (Rahman and Pravin, 2014). Every part of the tree, root, bark, leaf, fruit, seed, latex are useful for their medicinal properties (Patel *et al*., 2012). The bael pulp consist of vital bioactive compounds such as carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids and terpenoids (Maity *et al*., 2009; Suvimol and Pranee, 2008). Bael is one of the nature's rich source of antioxidants and is beneficial for health beyond nutrition. It also has excellent aroma. This fruit has a lot of potential to be processed to value added products (Singh *et al*., 2014) such as squash, nectars, toffy, jam, powder, preserves, juice and refreshing beverages (Singh *et al*., 2013). Due to increaseing demand of health drinks based on indigenous fruits bael fruit can be processed for making juice and RTS bevarages. Singh *et al.* (2013) reported that commercial and crude pectinase enzyme were used for optimization of bael juice extraction (Singh *et al*., 2013 and Singh and Nath, 2004). Bael juice was used for preparation of RTS drink, nectar, squash (Verma and Gehlot, 2006) and blended beverages (Nidhi *et al*., 2008; Kenghe and Zambre, 2009).

However, thermal treatments cause degradation and may be the reason of destruction of natural antioxidants in food (Vikram *et al*., 2005; Mazur *et al*., 2014 and Remini *et al*., 2015). Two important variables to be controlled are temperature of treatment and its duration (Loannou *et al*., 2012). Therefore, degradation kinetic modelling is important to control changes of physico-chemical parameter during processing (Remini *et al*., 2015). In addition to that kinetic model can be utilized for economic evaluation of food quality. The effect of several experimental variables on nutritional values

(Lanny and Lie, 2014) can also be predicted by kinetic model. Hence, the purpose of this experiment is to determine the kinetic parameters of both total phenolic and antioxidant content during thermal treatment at different temperature by using Arrhenius and Eyring-polany model. This approach will give an idea about suitable time and temperature of processing for developing a bael juice having better nutritional quality.

METHODOLOGY

Preparation of bael juice:

Fresh baels procured from local market were washed under running water and shells were broken to collect pulp. Then water (1:10 w/v) was added to the pulp followed by blending in a juicer and centrifuged at 4000 rpm for 5 mins. The clear centrifugate collected and used as fresh bael juice.

Thermal treatment:

Thermal degradation kinetics of total phenolic content and antioxidant activity were studied by isothermal heating at 55^0, 65^0, 75^0 and 85^0, respectively. 10 ml samples had been taken in each sealed glass tubes and then heated by placing them in thermostatic water bath (Scientific instrument and chemical company, India). At every 15 min interval the tubes had been taken out and immediately cooled by dipping them into ice water and analyzed for total phenolic content (TPC) and total antioxidant activity.

Analytical parameters:

Total soluble solid (TSS) was measured by Hand Refractometer (Erma Inc., Tokyo, Japan) and expressed in terms of ^{0}B.

Total phenolic content was determined by folin-ciocalteu method (Singleton and Rossi, 1965) at a wavelength of 765 nm using gallic acid standard and expressed as mg of gallic acid/g of fruit.

Determination of Ascorbic acid done by titrimetric method (Rangana, 1986) and the value expressed as mg of ascorbic acid /g fruit.

Antioxidant activity was measured by FRAP method (Benzie and Strain, 1996) at a wave length of 593nm.

Thermal degradation of nutrients:

Nutrients degradation in foods during their thermal processing has been described in terms of zero, first or second order kinetics (Corradini and Pleg, 2006). A general reaction rate expression for degradation kinetics can be written as follows (Paul and Ghosh, 2012).

$$\frac{d[c]}{dt} = k_x [c]^n \quad \ldots (1)$$

where, c= quantitative value of the degraded product under consideration. k_x = the reaction rate constant and 'm' is the reaction order, 't' is the reaction time (min).

The reaction order was determined by linear regression through graphical analysis, where exponent 'n' in eq. (1) was set to 0, 1, and 2 to compare the co-efficients of determination (R^2). The integrated forms of zero, first and second order models are given in eq.:

Zero order: $X_t = X_0 - k_x t$ … (2)

First order: $\ln\left(\frac{[X]_t}{[X]_0}\right) = -k_x t$ … (3)

Second order: $1 / X_t - 1 / X_0 = k_x t$ … (4)

Using the experimental data, the co-efficients of determination (R^2) were observed to be minimum for n = 1, predicting a first order reaction.

The relationship of reaction rate to temperature was evaluated by the Arrhenius equation (Paul and Ghosh, 2012):

$$K_x = A_0 \exp^{-(Ea/RT)} \quad \ldots (5)$$

where 'k_x' is the rate constant (min^{-1}), 'E_a' is the activation energy (kJ mol^{-1}) of the reaction, 'R' is the universal gas constant (8.314 J mol^{-1} k^{-1}), 'T' is the absolute temperature and 'A_0' is a pre exponential constant.

In Eyring-polany model enthalpy of activation (ΔH^*) and entropy of activation (ΔS^*) are the model's parameters (Eq. 6)

Entropy (ΔS^*_{xi}) and enthalpy (ΔH^*_{xi}) were obtained from the Eyring-polany model:

$$\frac{\ln K_x}{T} = \frac{\Delta H^* xi}{R} \cdot \frac{1}{T} + \frac{\ln K_B}{h} + \frac{\Delta S^* xi}{R} \quad \ldots (6)$$

where K_B = the Boltzmann constant (1.381 x 10^{-23} J K^{-1}), T = absolute temperature, h=the planck constant (6.626 x 10^{-34} J s).

Statistical analysis:

Data were analysed using student t-test (origin 6.1). Significance of differences was defined at $P \leq 0.05$.

RESULTS AND DISCUSSION

It was observed in Fig. 1 that the total phenolic content decreased with increasing time and temperature

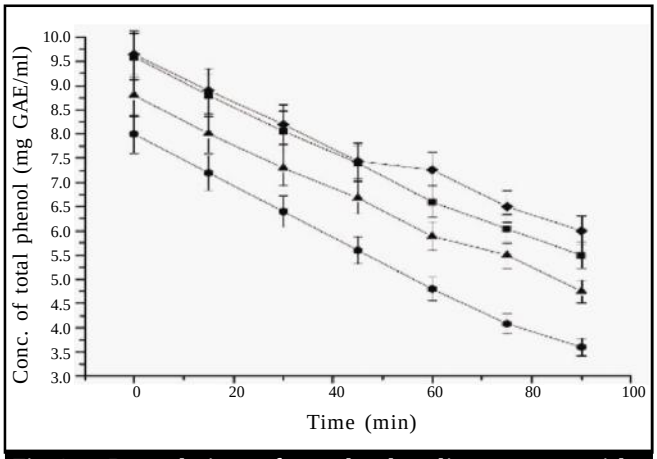

Fig. 1 : Degradation of total phenolic content with increasing time and temperature

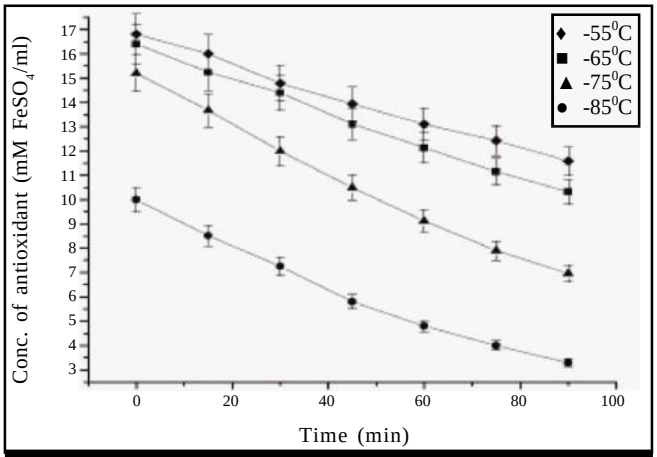

Fig. 2 : Total antioxidant activity with increasing time and temperature

Table 1 : Main characteristic of fresh bael

Analysis	Fresh bael
Juice yield (%)	20±0.26
TSS (^{0}B)	32±0.12
Moisture (%)	66±0.15
Total phenol (mg GAE/ml)	9.7±0.4
Ascorbic acid (mg/ml)	0.22±0.01
FRAP (mM $FeSO_4$/ml)	17±0.2
Carotenoid (µg/ml)	14.65±0.33

of thermal treatment. Results were similar with the previous findings where total phenolic content of some vegetables decreased with the increasing time and temperature of thermal processing (Lin and Chou, 2009). Thermal breakdown of polyphenols can happen during heat treatment that can affect the cell structure (Youssef and Mokhtar, 2014). Phenolic compounds degraded due to the effect of polyphenol oxidase (PPO) enzymatic activity (Sonawane and Arya, 2015). Polyphenols were utilized as substrates for the PPO protein (Janovitz-Klapp *et al*., 1990). The decrease in the content of total phenolic compounds during thermal treatment were also explained with previous studies that polyphenolics were heat labile and extended heat treatment could cause irreversible chemical modification to phenolic compounds (Mrad *et al*., 2012) .

As shown in Fig. 2, the antioxidant capacity of bael juice decreased with increasing thermal temperature and time. Normally, high temperature could enhance degradation of bioactive compounds and decrease the antioxidant capacity of sample (Zhou *et al*., 2016). Garau *et al*. (2007) also observed that the antioxidant capacity in orange peel and pulp both decreased by air drying. A similar trend was observed by Zhang and Hamauza (2004) that the antioxidant content in broccoli decreased with duration of thermal processing. Oxidation and isomerisation are the most important causes of antioxidants degradation during thermal processing (Shi and Le Maguer, 2000).

The order of the thermal degradation of total phenol content was estimated by examining the co-efficient of determination (R^2) from plots of total phenol versus treatment time over the temperature range of 55^0-85^0 C (Table 2). On the basis of the mean R^2 (0.99) it can be said that the thermal degradation of total phenol follow first order kinetics. Earlier studies also reported that total phenolic content degradation follows first order kinetic model (Jaiswal *et al*., 2012 and Jaiswal and Abu-Ghannam, 2013).

The order of reaction for antioxidant activity was determined by comparing R^2 obtained from plots of antioxidant versus treatment time over the temperature range of 55^0-85^0 C (Table 2). Antioxidant degradation showed a high degree of fit for first order kinetic model being most suitable with highest R^2 value, ranging from 0.995-0.998. Similar finding were seen by Jaiswal and Abu-Ghannam (2013). On the basis of R^2 values, the degradation of total phenol and antioxidant fits better with first order model with R^2 (0.99). From Table 2 and Fig. 3-8, it can be concluded that degradation of total phenol and antioxidant content could commonly be fitted by first order reaction model.

The kinetics parameters K_x, $t_{1/2}$ and R^2 of first order

Table 2 : Reaction order determination of total phenol and antioxidant degradation based on R^2 from zero, first and second order modal

	Temp	Zero order		First order		Second order	
		T.P.	FRAP	T.P.	FRAP	T.P.	FRAP
R^2	55^0C	0.986	0.986	0.991	0.998	0.964	0.977
	65^0C	0.979	0.989	0.997	0.997	0.988	0.975
	75^0C	0.987	0.982	0.997	0.995	0.966	0.957
	85^0C	0.986	0.987	0.998	0.997	0.956	0.962

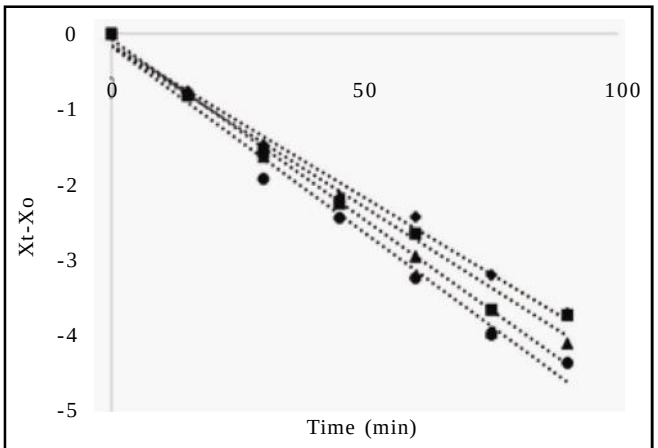

Fig. 3 : Zero-order plot for the degradation of phenolic compound from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

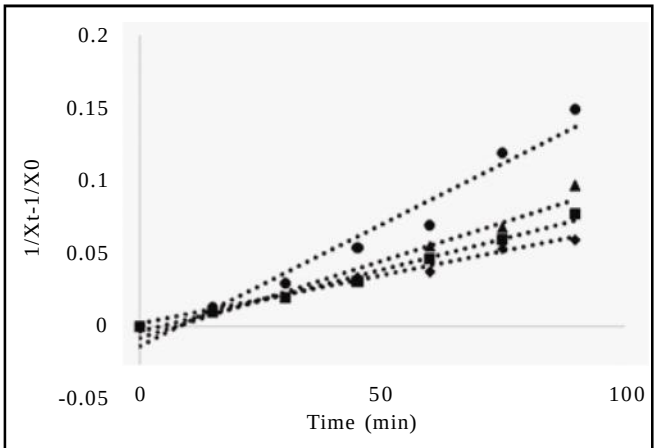

Fig. 5 : Second-order plot for the degradation of phenolic compound from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

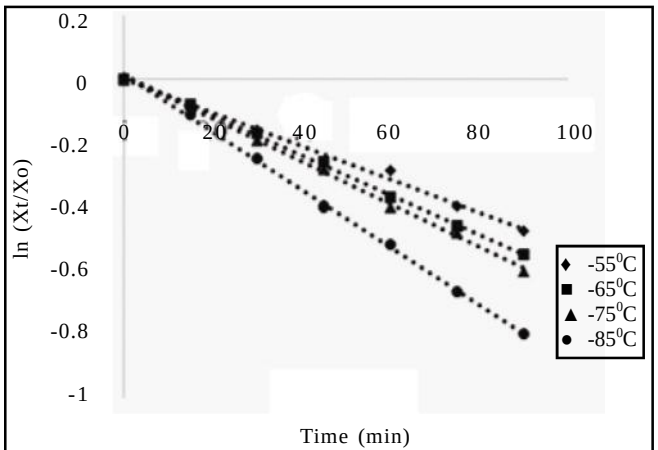

Fig. 4 : First-order plot for the degradation of phenolic compound from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

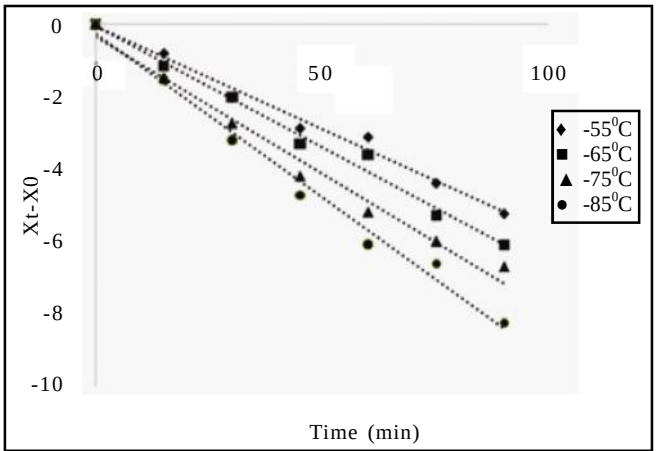

Fig. 6 : Zero-order plot for the degradation of antioxidant from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

model through a least square fitting procedure of total phenol and antioxidant degradation are given in Table 3. A good fit was obtained R^2 0.991 -0.998 from this first order model. The degradation rate constant (K_x) increased systematically with temperature (Table 3). The half-life ($t_{1/2}$) for phenolic compound decreased from 130.75- 78.75min and for antioxidant 173.25-57.27 min as the temperature increased from 55-85°C.The half – life ($t_{1/2}$) is the time required for phenolic compound and antioxidant to degrade to 50 per cent of its original value.

As shown in Table 3 that kinetic constant K_x of total phenol content and antioxidant compounds increase with increasing temperature which confirmed that with increasing temperature degradation become faster.

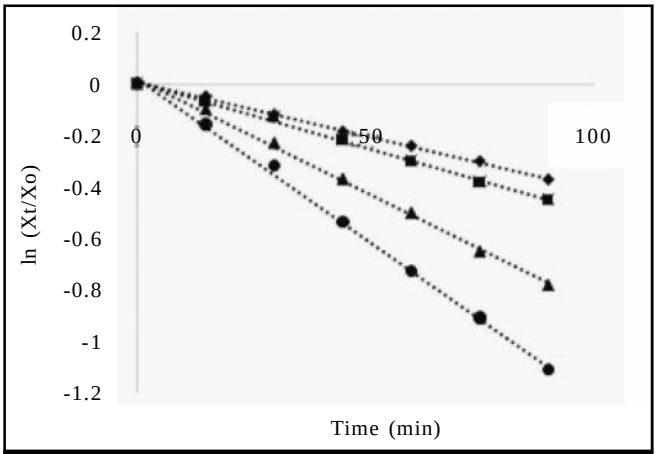

Fig. 7 : First-order plot for the degradation of antioxidant from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

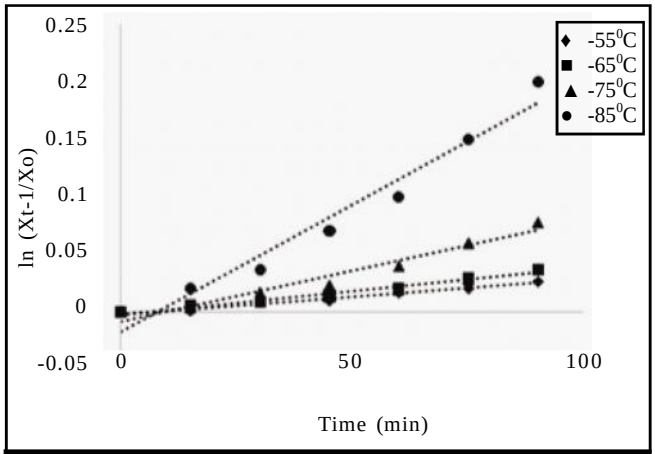

Fig. 8 : Second-order plot for the degradation of antioxidant from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

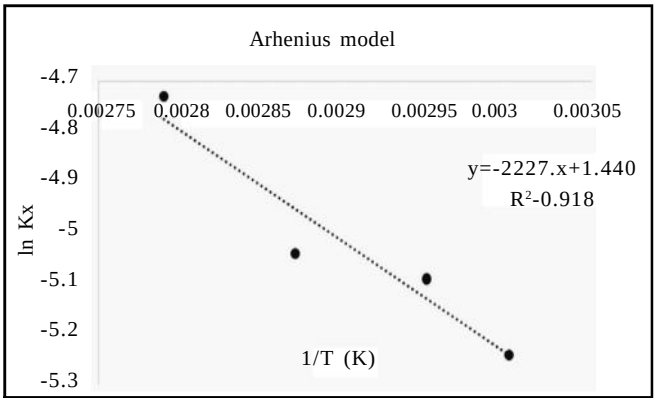

Fig. 9 : Plot of ln(k_x) versus (1/T) for degradation of flavonoid content from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

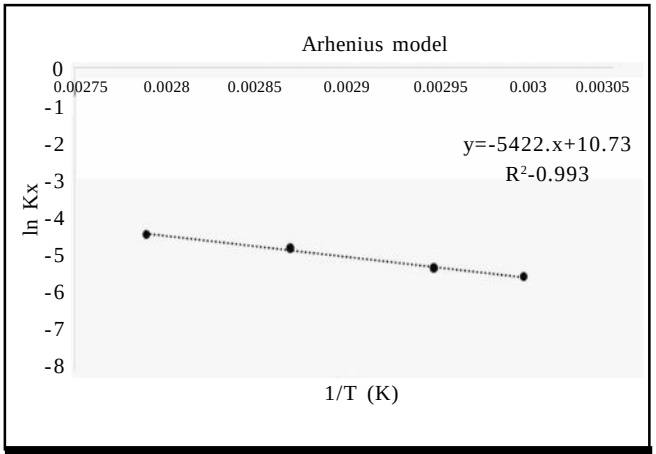

Fig. 10 : Plot of ln(k_x) versus (1/T) for degradation of antioxidant from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

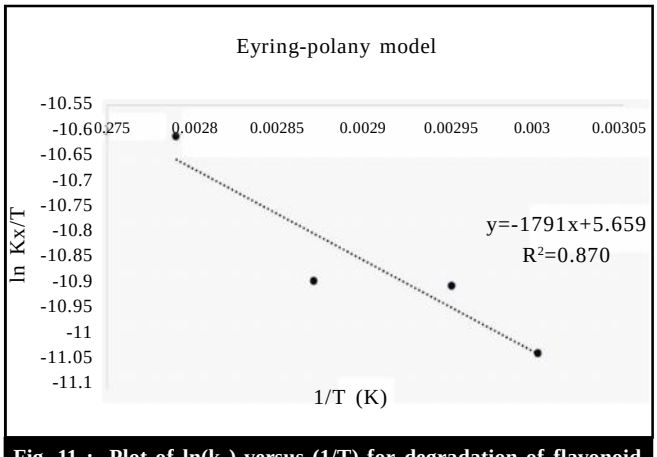

Fig. 11 : Plot of ln(k_x) versus (1/T) for degradation of flavonoid content from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

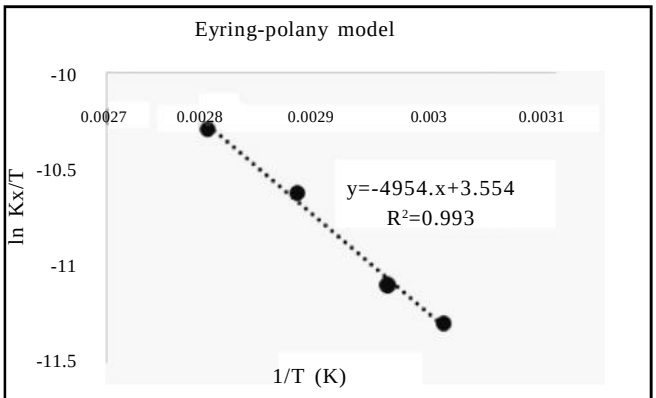

Fig. 12 : Plot of ln(k_x/T) versus (1/T) for degradation of antioxidant content from bael juice during heating over the temperature range of 55°-85°C for 0-90 min

Table 3 : Kinetics parameters for degradation of total phenol, antioxidant of bael juice due to thermal processing

	1st order model				Arrhenius model			Eyring-polany model		
	T(°C)	K_x(min^{-1})	$t_{1/2}$ (min)	R^2	E_a (KJ mol^{-1})	K_x (min^{-1})	R^2	ΔH(KJ mol^{-1})	ΔS (J mol^{-1} k^{-1})	R^2
TPC	55°	0.0053	130.75	0.991						
	65°	0.0061	113.63	0.997	18.52	2227.7	0.918	14.89	-244.60	0.87
	75°	0.0065	106.62	0.997						
	85°	0.0088	78.75	0.998						
Antioxidant	55°	0.004	173.25	0.998						
	65°	0.005	138.6	0.997	45.08	5422.7	0.99	41.19	-168.025	0.99
	75°	0.0085	81.53	0.995						
	85°	0.0121	57.27	0.997						

Which is similar with the finding of Henriquez *et al*. (2014). Kinetic parameters of total phenol and antioxidant degradation from Arrhenius model are shown in Table 3. The K_x value is 2227.7 for total phenol and 5422.7 for antioxidant. In case of total phenol R^2 (0.918) and for antioxidant R^2 (0.99). Activation energy (E_a) were 18.52 KJ mol^{-1} for total phenol and 45.08 KJ mol^{-1} for antioxidant. From Erying-Polany model (Table 3) we get R^2 (0.87) for total phenol and 0.99 for antioxidant. The activation enthalpy (ΔH) and entropy (ΔS) for total phenol was 14.89 KJ mol^{-1} and -244.60 JK^{-1}mol^{-1}, and for antioxidant activation enthalpy (ΔH) and entropy (ΔS) were 41.195 KJ mol^{-1} and -168.025 JK^{-1}mol^{-1}.

Conclusion:

This present study evaluated the effect of heat treatment on the kinetic behaviour of phenolic compounds degradation and antioxidant loss from bael juice which had been best defined through first-order kinetic model. Arrhenius and Eyring-Polany model well represented the temperature dependence of the degradation rate constant.

Acknowledgement:

The authors would like to acknowledge the UGC (University Grant Commission), New Delhi, for providing the financial assistance for the research work.

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