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DEGRADATION KINETICS OF ANTIOXIDANTS IN DEHYDRATED MATRIX OF INDIAN GOOSEBERRY AND GUAVA POWDER

MuditaVerma, RadhaKushwaha, GK Rai, and Devinder Kaur*

Centre of Food Technology, University of Allahabad, Prayagraj , Uttar Pradesh, India.

*Email ID: devinderkaur02@gmail.com

Correspondence address: Devinder Kaur*

Centre of Food Technology, University of Allahabad

Email Id: devi_sonu@yahoo.com

ABSTRACT

The stability of antioxidants in any material is essential for delivering health benefits even after their additional thermal processing for their preparation itself, for example, their incorporation in biscuits, cookies, bread, breakfast cereal mixes or many other functional foods for the end product's nutritional enhancement and value addition. In this study degradation of antioxidants were studied in Indian gooseberry and guava powder prepared by the cabinet and freeze-dried techniques at temperature 65 to 850C for 8 hours in the hot air oven. Polyphenolics, flavonoids, ascorbic acid content decreases in both the fruits as the temperature increased. Degradation of polyphenolics, flavonoids and ascorbic acid during processing, followed first-order degradation kinetics. Rate constants increased with increase in the temperature range. Activation energies calculated for cabinet dried Indian gooseberry powders were 31.04, 41.87 and 44.55 kJ/mol and 36.13, 45.23,48.65 kJ/mol while in case of guava, activation energies found to be 34.15, 74.62 and 42.78 for cabinet dried and 38.42,79.11, 52.47 for freeze-dried powder for total phenolics, flavonoids and ascorbic acid. The degradation followed first-order reaction model with the higher value of $R^2 > 0.927$. The Arrhenius law was used to relate the coefficient k (drying rate constant) with the air drying temperature.

Keywords: Antioxidants; degradation kinetics; activation energies; Indian gooseberry; guava

Introduction

The focus of nutrient enrichment has shifted from the provision of nutrient deficiency to the pursuit of optimal health and dietary intake. Modern consumers are now more interested in healthy foods and looking for foods that have added beneficial compounds such as antioxidants, phenolics and phytosterols. Addition of functional ingredients to food products also attracts the attention of health-conscious consumers (Shaviklo *et al.*, 2011). Indian gooseberry and guava have many bioactive compounds viz. include polyphenolics, flavonoids and ascorbic acid which show antioxidative properties. However, issues including seasonal availability, market accessibility, cost, high perishability and time restraints may limit consumption of these fruits on a daily basis. Therefore, frozen, canned or dried products may replace fresh products because of convenience and storage life. These products undergo a variety of processing methods to preserve freshness and physical integrity that could alter or diminish bioactive components in the food products (Negi, and Roy, 2001; Sato *et al.*, 2006; Turkmen *et al.*, 2006). There are many drying techniques available like sun/solar drying, oven drying, drum drying, microwave drying, spray drying and low temperature drying. These drying processes can show an adverse effect on the nutrients within the food product.

The kinetic parameters namely, rate constant and activation energy provides useful information on the nutrients change, which occur during thermal processing. Numerous researchers have studied the kinetics of pigment and color degradation of fruits and vegetables during thermal processing. However, limited information is available on the antioxidants degradation kinetics of Indian gooseberry and guava during thermal processing.

The application of fruit powder in food is a promising area for the food industry. The addition or replacement of wheat flour by fruit powders can be done which provides a high content of fiber and bioactive compounds in products as bakery other food products (Bhol *et al.*, 2016; Singh *et al.*, 2016; Lima *et al.*, 2015). It can be a method to supply of these compounds which is so crucial to the health of people (Elleuch *et al.*, 2011). Losses of these nutrients from the product should be verified and controlled while developing new food products. To find out the losses of nutrients during processing study about kinetics, reaction order, reaction rate and activation energy could be a best way. Keeping this in mind kinetic study was conducted to find out the changes in bioactive components and antioxidants activity in Indian goose berry and guava powder.

Material and Methods

Indian gooseberry (*chakaiya*) and guava (*Allahabad safeda*) were obtained from local market of Allahabad city and stored at $15 \pm 2^\circ\text{C}$ with relative humidity of 90-95%. Fruit were selected for uniformity of size and color, and blemished and diseased fruit were discarded. Procured fruits were identified from Botanical survey of India, Allahabad.

Sample preparation

Sample preparation of cabinet and freeze dried guava and Indian gooseberry powder was done by removing seeds from fruit, dried through cabinet and freeze driers at different temperature and time combinations (65-85 °C for 0-8 h). The dried guavas were grind in amixer grinder and filtering through muslin cloth, and the whole process were repeated thricetimes.

Total phenolic content

The total phenolic content (TPC) of Indian Gooseberry and Guava powder was determined by using the Folin-Ciocalteu method (Slinkard, and Singleton, 1977). An aliquot (0.05 ml) of sample or standard was placed in test tube and the volume was adjusted to 6 ml with deionised water. Then 0.3 ml of FolinCiocalteu was added to all tubes. After 8 minutes 0.9ml of 20% sodium carbonate was added to the mixture and then incubated for 30 minutes at 40°C . Absorbance of the resultant blue color was measured at 765 nm in UV-spectrophotometer. Polyphenols were expressed as milligrams of gallic acid equivalents per gram of cabinet and freeze dried sample (mg GAE/g).

Total Flavonoid Content

The total flavonoid content (TFC) of samples was investigated by using the aluminum chloride colorimetry method described by Chang *et al.* (2002). Ethanolic extracts (2 ml) were mixed with 150 μl of sodium nitrite (5%). After 5 minutes, 150 μl of aluminum chloride (10%) was added. Then, after 10 minutes, 1 ml of 1M sodium hydroxide and 1.2 ml of distilled water were added in the mixture. The mixture was vortexed and incubated for 10minutes then the absorbance was read at 510 nm by spectrophotometer. A calibration curve was prepared using a standard solution of quercetin (0.05-0.5 mg/ml). TFC was expressed as milligrams of quercetin equivalent per gram of powder sample (mg QE/g).

Ascorbic acid

Ascorbic acid was estimated by 2, 6 dichlorophenol indophenol titration method (AOAC, 2005). Sample (10 g) was prepared in 3% (w/v) metaphosphoric acid and the volume was made up to 100 ml with metaphosphoric acid. Filtered aliquot (5 ml) of sample was titrated against standard 2, 6 dichlorophenol indophenol dye solution until the pink color developed completely. Results were expressed as milligrams per gram of powder sample (mg/g).

Kinetics model of antioxidants degradation

The kinetics of degradation of antioxidants has been reported to follow first order reaction adequately (Ahmed, and Shivhare, 2001; Gunawan, and Barringer, 2000). The

first order kinetic model based on antioxidants (Polyphenolics, ascorbic acid, flavonoids) concentration is

$$\ln(C/C_0) = -k_1 \cdot t \quad \dots(1)$$

Where,

C = amount of Polyphenolics/ascorbic acid/flavonoids content at time t (mg/g).

C₀ = initial amount of Polyphenolics/ascorbic acid/flavonoids (mg/g).

k₁ = reaction rate constant (1/h)

t = heating time (h)

Fractional conversion is a suitable variable and generally used in place of concentration

(Ahmad, *et al.*, 2002 a, b) and Gunawan, and Barringer, (2000) reported that it increases the accuracy of the calculation. The first-order reaction concerning the fractional conversion has been represented as

$$\ln(1-f_1) = -k_2 \cdot t \quad \dots(2)$$

Where, $f_1 = (C_0 - C) / (C_0 - C_\alpha)$

k₂ = Reaction rate constant (1/h)

C_α = Polyphenolics/ascorbic acid/flavonoids content at infinite time (mg/g)

Effect of temperature on antioxidants degradation

The Arrhenius model was applied to describe the temperature dependence of antioxidants reaction rate constant

$$k = k_0 \exp(-E_a/RT) \quad \dots(3)$$

Where, k₀ = frequency factor (1/h)

E_a = activation energy (kJ/mol)

R = universal gas constant (8.314 J·K⁻¹·mol⁻¹)

T = absolute temperature (K)

Statistical analysis

Regression analysis and Pearson's correlation coefficient was computed with the statistical package statistic a version 12 (Stat Soft Inc USA). Each experiment was replicated thrice and the average was used for the statistical analysis.

Results and Discussion

Degradation Kinetics of Antioxidants in Indian gooseberry Powder

Polyphenolics Degradation

In the present study, the polyphenolic content of freshly prepared (0hr) cabinet and freeze-dried Indian gooseberry powder was 223.23 and 334.13 GAE, mg/g dry matter. After thermal treatment in cabinet dryer at 65°C to 85°C for 8 hrs polyphenolic content decreased from 105.09 mg/g to 58.10 mg/g (Table 1). While when the sample was prepared by freeze drying it shows less degradation during processing compared to cabinet dried samples i.e. from 334.13 GAE, mg/g to 100.1 GAE, mg/g after 8 hrs at 65°C to 85°C. The logarithms of total phenolic content vs. processing time was

plotted for different temperatures and shown in Fig.1A and 1B.

Linear relationship between total phenolic content vs. processing time demonstrated that degradation of phenolics followed a first order, kinetic model. Polyphenolic content decreased as the temperature increased from 650C to 850C. A decline in polyphenolic content was probably due to the damage from heat and oxidation. The degradation rate constants were found to vary from 0.08138 to 0.1511 h⁻¹ for cabinet dried and 0.0921 to 0.165 for freeze-dried samples (Table 2). The rate constants for polyphenolics increased with increasing temperature from 650C to 850C. The R² and standard error values for cabinet and freeze-dried samples were shown in Table 2 which validated the first order model for explaining degradation changes.

In a comparison between Indian gooseberry powders prepared by two different drying processes, higher degradation rates and shorter half-life values of polyphenolics observed in freeze-dried samples. Furthermore, because of the porous structure of freeze-dried powder, oxygen can easily be allowed to reside in the powder granules. As a result, oxidation of polyphenolics may occur; this could be a reason for the faster decrease of polyphenolics in freeze-dried samples. The activation energy obtained for polyphenolics in cabinet dried powder was 31.04 and in freeze-dried 36.13 kJ/mol. Kyi *et al.* (2005) studied the degradation kinetics of polyphenols during drying of Malaysian cocoa beans and found that polyphenols in cocoa beans decline rapidly during drying under air conditions. They found that process followed pseudo-first-order reaction kinetics with a rate constant in the range of 0.055 to 0.200 and activation energies between 27.8-30.31 kJ/mol which is comparable with the present study.

Flavonoids Degradation

Flavonoid content of freshly prepared (0hr) Indian gooseberry cabinet and the freeze-dried powder was 67.48 and 75.57 QE mg/g, respectively (Table 1). After thermal treatment in cabinet dryer at 650C to 850C for 8 hrs flavanoid content decreased from 67.48QE mg/g to 7.27QE mg/g. While when the sample was prepared under low temperature it shows less degradation during processing compared to cabinet dried samples i.e. from 75.57 QE, mg/g to 10.23 QE, mg/g after 8 hrs. The degradation data were analyzed using linear regression (Eq.3) to conclude the order and rate constant of the degradation reaction (Fig. 1C and 1D).

Higher correlation coefficients (R²= 0.99) confirmed that the degradation of flavonoids in Indian gooseberry powder follows the first-order reaction for all the selected temperatures. As processing time and temperature increased, the flavonoid content decreased. The fastest rate of degradation observed at 850C of which is evident from the half-life values also (Table 2). The freeze-dried powder has higher activation energy of flavonoids degradation i.e. 45.23kJ/mol as compared to cabinet dried (E_a=41.87). This shows that the flavanoids are very sensitive to higher temperature, reinforcing the importance of optimizing processing conditions for fruit powders. This was observed that trend of flavonoid content degradation in both the

powders had a similar behavior to polyphenolic content, which tends to higher degradation at higher temperatures. Aoyama, and Yamamoto, (2007) reported similar results for green Welsh onion that flavanoid content decreased significantly following after boiling for 15 min or more. Dewanto *et al.* (2002) had found contradictory result that total flavonoids content increases with time and temperature at 880C for 30 min from 9.38µg/g to 10.38µg/g in tomatoes. They also suggested the heat-processed tomatoes might maintain their total phenolics, flavonoids, and total antioxidant activity although the loss of vitamin C.

Ascorbic Acid Degradation

After processing at 65 to 850C for 8 hours cabinet and freeze-dried samples exhibited a first order kinetic model for loss of ascorbic acid at all temperatures (Fig. 1E and 1F). The initial content of ascorbic acid in freshly prepared (0 hr) cabinet dried Indian gooseberry powder was 24.23 mg/g, and 45.56 mg/g for freeze dried (Table 1). The correlation coefficient (R²) in the range of 0.939 to 0.997 confirms that the first order model fits on the data of powder prepared by both the drying techniques (Table 2). It observed that the degradation rate constants were higher as the temperature range increased. The half-life values were maximum at 650C and minimum at 850C for both the samples. Processing temperature and time both affects significantly on the degradation kinetics of vitamin C. The activation energy of ascorbic acid is 44.55 and 48.65 kJ/mol in the cabinet and freeze-dried respectively, which shows the higher temperature sensitivity of ascorbic acid. Some studies have been reported on the degradation of ascorbic acid in thermally processed fruits and vegetables like green asparagus, watercress (Cruz *et al.*, 2005), amla (Nisha *et al.*, 2004), drumstick (Bineesh *et al.*, 2005) and strawberry products (Castro *et al.*, 2004). Considerable losses of ascorbic acid have also been reported during the production of dried tomato halves and tomato pulp at high temperatures (Toor, and Savage, 2006). Authors (Nisha *et al.*, 2004; Castro *et al.*, 2004; Vikram *et al.*, 2005), have found in various foodstuffs vitamin C degradation appears that follows first-order kinetics.

Degradation Kinetics of Antioxidants in Guava Powder

Polyphenolics Degradation

Polyphenolic content of freshly prepared (0 hr) guava powder was 119.10 and 268.07GAEmg/g for cabinet and freeze-dried samples respectively (Table 1), and it degraded with increasing temperature. By fitting the data to the first-order reaction kinetics, 'k' were obtained by a graph was plotted concentration versus time (Fig. 2A and 2B). It was found that rate constants for degradation of polyphenolics increased with increasing temperature range 65 to 850C from 0.1386 to 0.2733 k/h in cabinet dried. Activation energy value of 34.15kJ/mol indicates less heat sensitivity of polyphenols in cabinet dried samples as compared to freeze dried. Table 3 show that half-life values decreased as the processing temperature increased. At all temperatures coefficient of determination, R² was satisfactorily high that shows it follows first order reaction. Many colored carotenoid vegetables, like green chilli puree (Ahmed, 2000), mango puree (Ahmed, 2002a), and red chilli puree (Ahmed,

2002b), seem to be less affected with thermal processes, presenting lower E_a values (range from 11.4 to 36.8 kJ/mol). Maillard and Berset (1995) suggested three hypotheses to explain the decrease of bound phenolic acids: the release of bound phenolic compounds; partial degradation of lignin which could lead to the release of phenolic acid derivatives; and/or the beginning of thermal degradation of the phenolic compounds. In this case, the fact that a maximum reduction of the polyphenol content of both cabinet and freeze-dried samples observed at 850C, suggests that thermal degradation is the primary mechanism. In case of freeze-dried guava powder again higher degradation rates of polyphenolics as compared to cabinet dried samples is attributed to the effect of the porous structure of the freeze-dried samples which causes higher losses of polyphenolics at a faster pace.

Flavonoids Degradation

Freshly prepared (0hr) cabinet and freeze-dried guava powder had 95.34 and 127.97 QE, mg/g respectively. After thermal treatment in cabinet dryer at 650C to 850C for 8 hrs flavanoid content decreased from 95.34 QE mg/g to 5.47QE mg/g (Table 1). While when the sample was prepared under low temperature it shows less degradation during processing compared to cabinet dried samples i.e. from 127.97QE, mg/g to 23.16QE, mg/g after 8 hrs.

Degradation of flavonoids in thermal processing of guava powder followed the first order reaction kinetics. Variation of degradation rate constants with temperature obeyed the Arrhenius relationship. The kinetics parameters of flavonoids degradation given in Table 3. Degradation rate constants for the freeze-dried samples were higher than cabinet dried. The calculated values of the activation energy for flavonoids are found to be higher in freeze dried (79.11 kJ/mol) samples as compared to cabinet dried (74.62 kJ/mol). Correlation coefficients (R^2) were found to be in the range of 0.927 to 0.995. Standard error values ranged from 0.099 to 0.287 for cabinet and 0.098 to 0.183 for freeze-dried powder, as shown in Table 3.

This study shows that flavonoids of freeze-dried guava powder are more susceptible to heating losses than cabinet dried (Fig. 2C and 2D). According to Schieber *et al.* (2001), the loss of macromolecules like flavonoids during heat treatment might be due to the harsh drying conditions, in particular, the temperature and duration used.

Ascorbic Acid Degradation

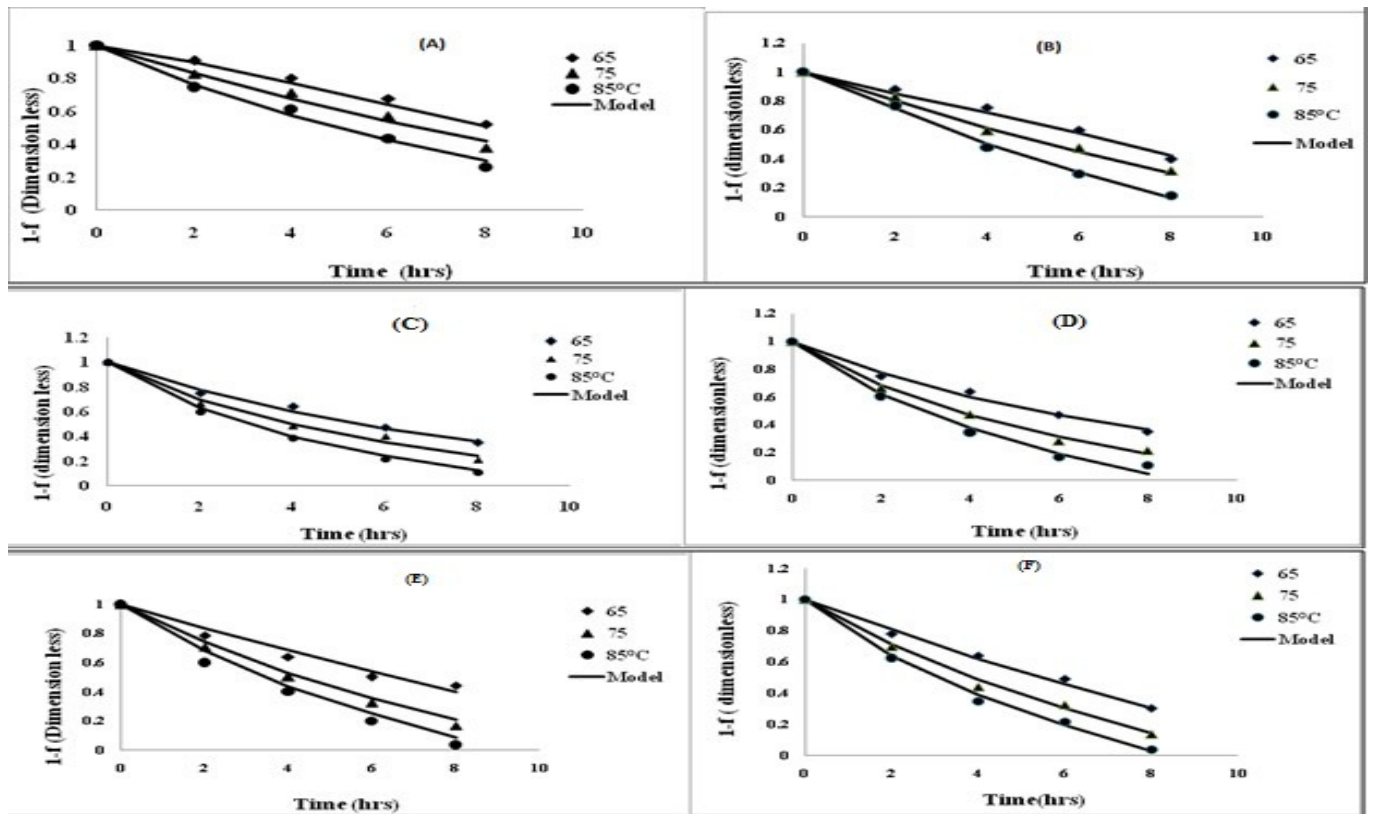
In this current study, the ascorbic acid content of freshly prepared (0hr) cabinet and freeze-dried guava powder was 6.10 and 12.29 mg/g, respectively (Table 1). Thermal processing resulted in degradation of ascorbic acid in both the samples. The changes in the ascorbic acid content after thermal processing summarized in Table 3. It is well established that high temperatures are conducive to the breakdown of vitamins. Good fitting results ($R^2 > 0.95$) are obtained for the first-order model for ascorbic acid degradation in guava powder. At 650C, ascorbic acid content

decreased from 6.10 mg/g to 2.11mg/g while at 850C it decreased from the initial value to 0.52 mg/g in cabinet dried samples for 8 hours. While for freeze-dried at 650C, the reduction in ascorbic acid content was found to be from 12.29 mg/g to 8.2 mg/g, while at 850C it decreased from initial value to 6.16 mg/g. Paul and Ghosh, (2011) also studied the degradation kinetics of ascorbic acid in pomegranate juice and found that its supported first-order kinetics in the temperature range of 70 to 900C. Degradation of ascorbic acid in fruits may associate with enzymes, such as cytochrome oxidase, ascorbic acid oxidase and polyphenol oxidase (Nagy, 1980). According to El-Gendy (2014), the main mechanism of the loss in vitamin C appears to be due to water solubility, mass transfer, heat sensitivity and enzymatic oxidation ascorbic acid to dehydro-ascorbic acid followed by further degradation to 2,3-diketogulonic acid and finally to furfural compounds. Different pathways, which give origin to different breakdown products, exist for the degradation of ascorbic acid. As many parameters will influence the kinetics of vitamin C decomposition, it is difficult to establish a precise precursor-product relationship. Various mechanism of deterioration might operate simultaneously. The rate constants for degradation of ascorbic acid increased with increasing temperature range 65 to 850C from 0.130- 0.304 k (h⁻¹) in cabinet dried and from 0.14-0.38 k (h⁻¹) for freeze-dried guava powder. Activation energy value of 42.78 kJ/mol indicates less heat sensitivity of ascorbic acid in cabinet dried samples as compared to freeze dried. Therefore, in both the powders ascorbic acid degradation followed first-order kinetics; this is evident from fig. 2E and 2F. All the antioxidants (polyphenolics, flavonoids and ascorbic acid) followed first-order degradation kinetics in the cabinet and freeze-dried Indian gooseberry and guava powders.

Conclusion

Processing of food industrially is often incriminated in lowering the nutritional value of products. Therefore, a demand is increasing to understand and prevent the degradation of nutrients during processing and storage. Regarding this first approach is related to understand the pathways which can lead to nutrient losses. In the case of phenolics it has been shown that Auto oxidation and/or breakdown could decrease the content of phenolics. Upon thermal treatment, antioxidant activity change. They followed first-order degradation processes. The Arrhenius model described the temperature dependence of the reaction rate constant of all the factors considered. Higher drying temperatures increased vitamin C degradation rates.

Cabinet drying showed more degradation among antioxidant as compared to freeze drying. All the antioxidants (polyphenolics, flavonoids and ascorbic acid) followed first-order degradation kinetics in the cabinet and freeze-dried Indian gooseberry and guava powders.



Figures

Fig. 1 : Degradation kinetics of Indian gooseberry powder at selected temperatures; Polyphenolics (A) Cabinet dried, (B) Freeze dried; Flavonoids (C) Cabinet dried, (D) Freezedried; and Ascorbic acid(E) Cabinet dried, (F) Freeze dried

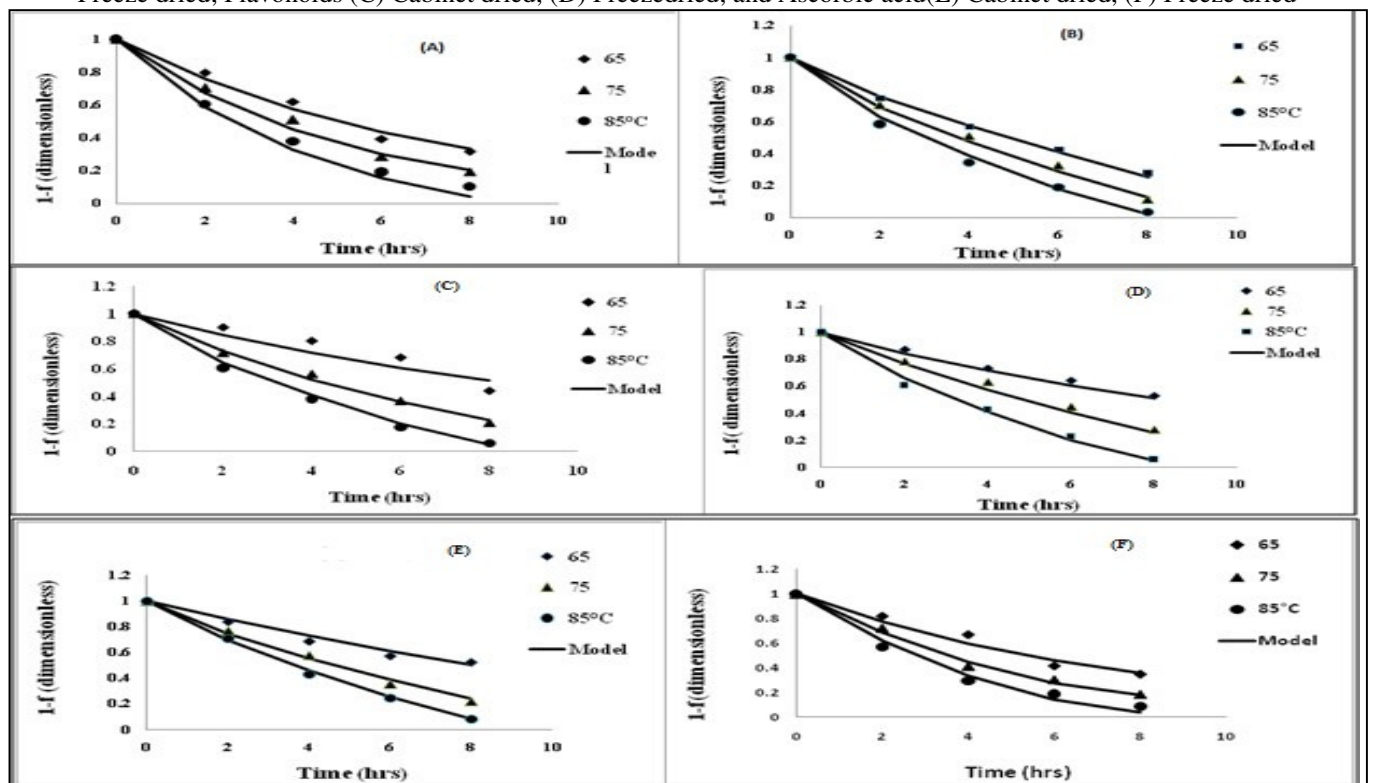


Fig. 2 : Degradation kinetics of Guava powder at selected temperatures; Polyphenolics (A)Cabinet dried, (B) Freeze dried; Flavonoids (C) Cabinet dried, (D) Freeze dried; andAscorbic acid(E) Cabinet dried, (F) Freeze dried

TABLES

Table 1 : Effect of heat on the antioxidants of Cabinet dried and freeze dried Indian gooseberry powder and guava powder at 65 to 85 °C

Antioxidants	Tem p°C	Indian gooseberry powder				Guava powder			
		Cabinet dried		Freeze dried		Cabinet dried		Freeze dried	
		0 hr	8 hrs	0 hr	8 hrs	0 hr	8 hrs	0 hr	8 hrs
Polyphenolics (GAE, mg/g)	65	223.43±2.23	105.09±4.3	223.43±2.23	105.09±4.3	119.105±6.27	37.60±3.38	268.07±6.27	178.36±3.38
	75	223.43±2.23	85.07±2.15	223.43±2.23	85.07±2.15	119.105±6.27	22.88±3.89	268.07±6.27	156.2±3.89
	85	223.43±2.23	58.10±2.76	223.43±2.23	58.10±2.76	119.105±6.27	12.05±2.58	268.07±6.27	138.26±2.58
Flavonoids (QE, mg/g)	65	67.48±2.11	23.75±1.99	67.48±2.11	23.75±1.99	95.34±3.16	41.86±0.8	127.97±3.16	63.18±0.8
	75	67.48±2.11	10.50±0.71	67.48±2.11	10.50±0.71	95.34±3.16	10.35±0.46	127.97±3.16	30.56±0.46
	85	67.48±2.11	7.27±1.82	67.48±2.11	7.27±1.82	95.34±3.16	5.47±0.0	127.97±3.16	23.16±0.0
Ascorbic Acid (mg/g)	65	24.23±0.94	7.37±0.02	45.56±0.80	27.85±0.02	6.10±0.14	2.11±0.03	12.29±0.17	8.27±0.03
	75	24.23±0.94	3.33±0.04	45.56±0.80	24.74±0.04	6.10±0.14	1.10±0.01	12.29±0.17	6.99±0.01
	85	24.23±0.94	0.88±0.03	45.56±0.80	33.27±0.03	6.10±0.14	0.52±0.02	12.29±0.17	6.16±0.03

All data are mean ± SD of triplicate (n =3) analyses.

Table 2 : Kinetic parameters of first order model (Eq.1) for total phenolic, flavonoids and ascorbic acid content of cabinet dried and freeze dried Indian gooseberry powder

Antioxidants	Temp0 C	Cabinet dried				Ea (kJ/mol)	Freeze dried				Ea (kJ/mol)
		k (h-1)	t1/2 values (h)	R2	Standard error		k (h-1)	t1/2 values (h)	R2	Standard error	
Polyphenolics (GAE, mg/g)	65	0.08138	8.52	0.945	0.078	31.04	0.092	728	0.95	0.062	36.13
	75	0.10228	6.78	0.953	0.095		0.124	6.1	0.93	0.071	
	85	0.15110	4.59	0.978	0.096		0.165	3.52	0.94	0.084	
Flavonoids (QE, mg/g)	65	0.12652	5.48	0.99	0.045	41.87	0.153	4.86	0.96	0.052	45.23
	75	0.23465	2.95	0.99	0.043		0.281	2.31	0.99	0.045	
	85	0.28983	2.39	0.99	0.086		0.316	1.98	0.98	0.071	
Ascorbic Acid (mg/g)	65	0.16869	4.11	0.939	0.207	44.55	0.185	3.21	0.94	0.282	48.65
	75	0.27117	2.56	0.984	0.174		0.295	2.0	0.97	0.181	
	85	0.40893	1.69	0.997	0.094		0.431	1.38	0.98	0.087	

All data are mean ± SD of triplicate (n =3) analyses.

Table 3 : Kinetic parameters of first order model (Eq.1) for total phenolic, flavonoids and ascorbic acid of cabinet dried and freeze dried guava pulp

Antioxidants	Temp0C	Cabinet dried				Ea (kJ/mol)	Freeze dried				Ea (kJ/mol)
		k (h-1)	t1/2 values (h)	R2	Standard error		k (h-1)	t1/2 values (h)	R2	Standard Error	
Polyphenolics (GAE, mg/g)	65	0.1386	5.0	0.970	0.107	34.15	0.158	5.0	0.96	0.104	38.42
	75	0.1995	3.47	0.967	0.160		0.295	4.4	0.98	0.151	
	85	0.2733	2.54	0.99	0.109		0.333	3.0	0.99	0.109	
Flavonoids (QE, mg/g)	65	0.0831	8.34	0.931	0.099	74.62	0.093	7.25	0.95	0.098	79.11
	75	0.2237	3.10	0.927	0.287		0.223	2.10	0.97	0.183	
	85	0.36472	1.90	0.995	0.119		0.374	0.90	0.98	0.123	
	Ascorbic Acid (mg/g)	0.1300	5.33	0.970	0.097		0.140	4.33	0.97	0.084	
	65										
	75										
	0.2134	3.25	0.960	0.185	0.263						
85	0.3041	2.28	0.977	0.227	0.384	1.28	0.96	0.094			

All data are mean ± SD of triplicate (n =3) analyses.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethics approval

This article does not contain any studies with human participants or animals performed by authors.

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