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Stability of Vitamin C in Broccoli at Different Storage Conditions

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Abstract

In this study, the retention of vitamin C in fresh broccoli stored at different temperatures (i.e. chiller, room, cooking, and roasting or baking; 5-120^{o}C) was investigated. The thermal stability of vitamin C in broccoli was analysed at 5, 20, 45, 60, 70, 80, 110, and 120^{o}C. The vitamin C content was measured by the indophenol titration method. Vitamin C was affected negatively at all stored temperatures. The degradation of vitamin C was modelled by first-order reaction kinetics and the reaction rate constants were observed as 9.03×10^{-8} and 5.65×10^{-3} s^{-1} when stored at 5^{o}C and 120^{o}C, respectively. The activation energy was estimated as 74.2 kJ/mol within the temperature range used in this study. The lowest decay of vitamin C was observed during the chilling condition. The data on retention of vitamin C in broccoli could be used to determine their stability, when stored as raw, and when heated at different temperatures.

Keywords: Ascorbic acid; First-order reaction; Cooking; Chilling; Activation energy

1 Introduction

Vitamins serve as an essential component in metabolism and could be used to protect the human body against diseases, such as cancer, cataracts, and cardiovascular diseases. It is shown from epidemiological studies that a high intake of vegetables and fruits is correlated with a low risk of diseases due to their antioxidants, health functional components and vitamins, such as ascorbic acid (vitamin C), carotenoids, and tocopherols (Bergquist, Gertsson, & Olsson, 2006; Wootton-Beard & Ryan, 2011). Broccoli has gained considerable attention due to its health-promoting ability, which has been attributed to bioactive phytochemicals such as nitrogen-sulfur compounds (glucosinolates and isothiocyanates), phenolic compounds (chlorogenic and sinapic acid derivates and flavonoids) and vitamins (Dominguez-Perles, Carmen Martinez-Ballesta, Carvajal, Garcia-Viguera, & Moreno, 2010; Suresh, Al-Habsi, Guizani, & Rahman, 2017).

Among the vitamins, vitamin C is one of the most important for maintaining human health (Hamad, 2009; Kumar, Ajay Kumar, Raghu, & Manjappa, 2013). Fruits and vegetables contain a high amount of ascorbic acid. Vitamin C in the natural forms of L-ascorbic acid (L-AA) and L-dehydroascorbic acid (L-DA) is found in foods (Ismail, 2013). It is a highly unstable molecule and could decrease during domestic and industrial processing through enzymatic and non-enzymatic reactions (Munyaka, Makule,

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Oey, Van Loey, & Hendrickx, 2010). Therefore, processing treatments like peeling, bruising and cutting fruits or vegetables into pieces, and air exposure of the disrupted cells cause oxidation of L-AA in the presence of ascorbic acid oxidase (AAO) and this decreases the retention of ascorbic acid (Munyaka et al., 2010). The degradation can be triggered by many factors such as moisture, temperature, light, pH, metal ions, and oxygen (Munyaka et al., 2010; Spinola, Mendes, Camara, & Castilho, 2012). Enzymatic degradation involves oxidation of L-AA to dehydroascorbic acid (L-DA) and loss of its antiscorbutic activity (i.e. ability to prevent scurvy). The antiscorbutic activity can be lost rapidly and irreversibly by hydrolysis of L-DA to 2,3-diketogulonoic acid (2,3-DKG) (Jain & Mulay, 2014). The oxidation is catalyzed by the enzymes, AAO, and ascorbic acid peroxidase (AAP) (Venkatesh & Park, 2014).

The stability of vitamin C depends on many factors, such as the types of fruits and vegetables, growing conditions, level of maturity, storage and processing conditions (Masamba & Mndalira, 2013). For instance, about 8-25% of ascorbic acid is lost when apples are peeled. The best retention of vitamin C is possible with fresh or minimal processing compared with thermal and drying processing methods (Oyetade, 2012; Spinola, Mendes, Camara, & Castilho, 2013). The ripening of fruits causes gradual loss of vitamin; however, this can be slowed down by refrigeration (Oyetade, 2012). Phillips, Council-Troche, McGinty, Rasor, and Teresa Tarrago-Trani (2016) studied the stability of vitamin C in fruit and vegetable homogenates stored at different temperatures (10, -20 and -55^{o}C) and observed that the degradation depended on the storage temperatures, as well as the types of fruits and vegetables. Maximum losses were observed as 23% and 94% after 1 and 7 days of storage, respectively. The vitamin C stability in three types of juices (pineapple, guava and baobab) was influenced by storage temperature, storage time and types of preservatives used. At room temperature, vitamin C in pineapple juice (34.7 mg/100 ml) decreased to 89.9% after 2 months of storage.

Nath, Bagchi, Misra, and Deka (2011) studied the weight loss, ascorbic acid, chlorophyll, β-carotene, and total antioxidant activity decay in fresh broccoli stored at 15^{o}C and 4^{o}C. The ascorbic acid (i.e. 130 mg/100 g FW) decay of the stored samples at 15^{o}C and 4^{o}C (open atmosphere) were 92.9% and 29.2%, respectively, after 6 days. Favell (1998) studied the stability of vitamin C in peas (three varieties), green beans, broccoli, and spinach at ambient, chill, and frozen conditions. They observed that the decrease of ascorbic acid in peas after post-harvest varied from 26 to 31 mg/100 g, respectively, depending on the variety. The samples stored at 4^{o}C (chilled) showed little change in the first 3 days, but reduced steadily at 2-3% per day. On the other hand, the loss was much faster for the peas stored at ambient temperature (20^{o}C) and the loss was about 10% per day over the first 7 days. The ascorbic acid content of freshly harvested broccoli was reported within the 77.0-93.0 mg/100 g sample. A steady loss was observed for broccoli stored at ambient storage with only 44% ascorbic acid retained after 7 days of storage and 28% after 14 days. However, at chilled temperature, the retention was much better, with no loss after 7 days of storage and 80% retention even after 21 days. Most of the studies determined percent losses during a predetermined storage time. There are relatively few reports available in the literature on the degradation kinetics of vitamin C in fruits and vegetables (Ariahu, Abashi, & Chinma, 2011). Yet the reaction order, rate constant, and activation energy are essential for predicting food quality loss during storage, as well as thermal processing (Nisha, Singhal, & Pandit, 2005).

There are negligible studies in the literature on the loss of vitamin C in broccoli during storage and processing over a wide temperature range. The objective of this study was to determine the stability of vitamin C at different temperatures (i.e. 5-120^{o}C). The experiments were performed in isothermal conditions and the selected temperatures could be used to simulate the conditions of chilling and room temperature storage, cooking, and roasting or baking conditions. The reaction rate was modelled by first-order reaction kinetics.

2 Materials and Methods

2.1 Sample Collection and Preparation

Fresh broccoli (*Brassica oleracea*, variety: Calabrese) grown in Oman were purchased from a local supermarket. The vegetables were washed in tap water to remove the dirt adhering to them and was spread on tissue paper to absorb the excess surface water. Only florets were cut from the bunch and eight batches of 200 g samples were placed into different aluminium cells and stored at temperatures of 5, 20, 45, 60, 70, 80, 110, and 120^{o}C. Previous reports showed that depending on the type of vegetables and fruits and effects of temperature (low and high), more or less heating time would be needed to measure the vitamin C content (Hal, Bosschaart, Twisk, Verkerk, & Dekker, 2012; El-Ishaq & Obirinakem, 2015; Polinati, Kremer Faller, & Fialho, 2010). Considering this aspect, different time frames were used for different storage temperatures. The heating time and temperature for broccoli samples are shown in Table 1. Samples were taken from each cell at different time intervals and their vitamin C content was measured.

2.2 Measurement of Ascorbic Acid in Broccoli

Vitamin C was measured according to the AOAC (1990) using a titration method with 2,6-dichloro-indophenol reagent. Samples of 10.0 g broccoli were weighed at different time intervals from each storage temperature and blended with 100 ml metaphosphoric acid (3%). The solution was then vacuum filtered and transferred to a 100 ml volumetric flask. Ten ml of the diluted sample solution was then titrated against the standardized dye. The dye was standardized by a known concentration of ascorbic acid solution (0.1 mg AA/ml). The end-point was indicated by the appearance of a light pink colour. The result was expressed as mg/100 g fresh broccoli sample and replicated three times.

2.3 Reaction Kinetics

The loss or degradation of vitamin C is commonly modelled by the first-order reaction as follows:

$$Ln\left(\frac{C}{C_0}\right) = -k_1 t \tag{1}$$

where, C is the concentration of vitamin C in a sample at time t (mg/g sample), C_0 is the initial concentration of vitamin C in the sample (mg/g sample), k_1 is the first-order rate constant (s^{-1}) and t is the storage time (s).
In most of the isothermal experiments, an initial lag period was observed and the above equation was modified with an intercept rather forcing the intercept to zero as Equation 1 (Rahman et al., 2015). The experimental data was fitted with a linear equation with an intercept as follows:

$$Ln\left(\frac{C}{C_0}\right) = -k_1 t + a \tag{2}$$

where, a is the intercept. The rate constant was determined from the slope of a linear plot Ln (C/C_o) versus t. Activation energy of first-order kinetics was estimated using the Arrhenius equation as:

$$Ln(k_1) = -\left(\frac{E_a}{R}\right)\left(\frac{1}{T}\right) + b \tag{3}$$

where, R is the universal gas constant (8.314 J/mol K), E_a is the activation energy (J/mol), T is the temperature (K), and b is the pre-exponent factor. The activation energy was estimated from the slope of the linear plot of $Ln\ k_1$ versus $1/T$.

2.4 Statistical Analysis

Each experiment was replicated three times and the regression analysis of Equations 2 and 3 were performed using Microsoft Excel (MS-Excel, 2016). The regression coefficient was considered as the goodness of the regression equation.

Table 1: Temperature and heating scheme for broccoli

	Broccoli							
Temperature (°C)	**5 (Days)**	**20 (h)**	**45 (h)**	**60 (min)**	**70 (min)**	**80 (min)**	**110 (min)**	**120 (min)**
Time	0	0	0	0	0	0	0	0
	1	5	5.25	10	5	4	3	2
	3	19.5	7.25	27	15	9	6	5
	21	25.5	8.75	40	25	14	9	8
	35	42.5	10.00	55	40	22	13	11
	49		11.25	70	50	32	19	16
	63		12.50	100	60	42	25	21
	77		14.50	115	70	52	31	26
			15.75	130	80	67	38	31
			17.00	145		82	45	36
			23.00			97		
			24.00					
			25.50					

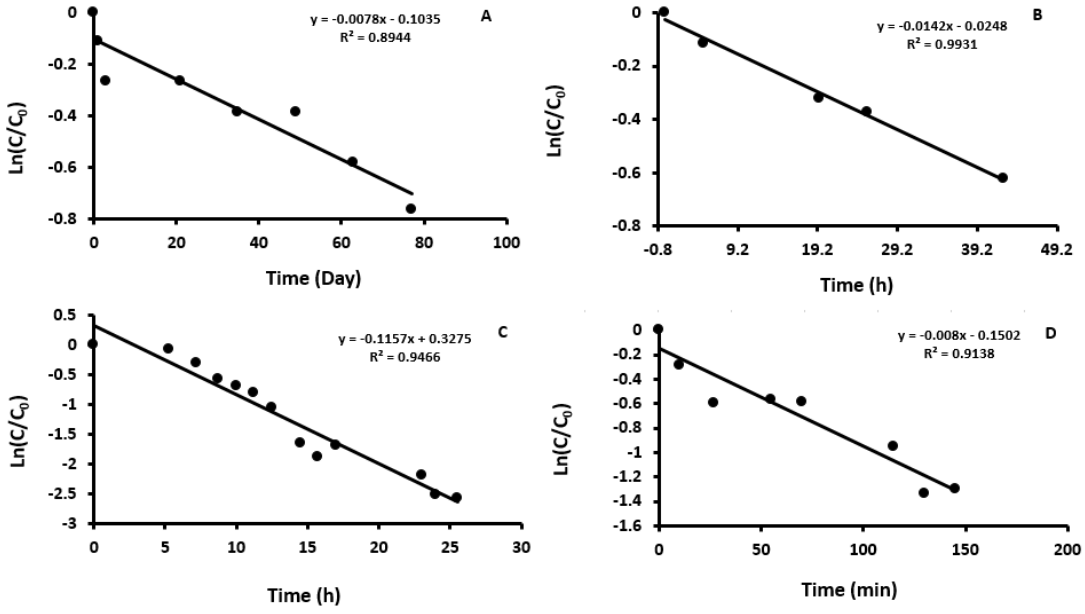

Figure 1: Plots of $Ln\,(C/C_o)$ versus time. A: 5^{o}C, B: 20^{o}C, C: 45^{o}C, D: 60^{o}C

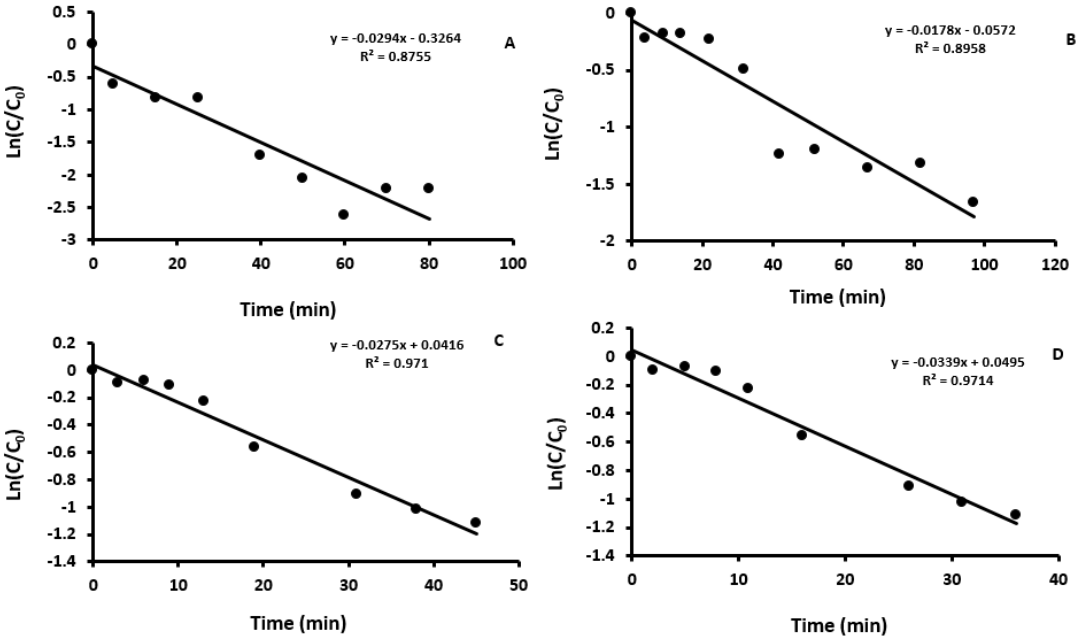

Figure 2: Plots of $Ln\,(C/C_o)$ versus time. A: 70^{o}C, B: 80^{o}C, C: 110^{o}C, D: 120^{o}C

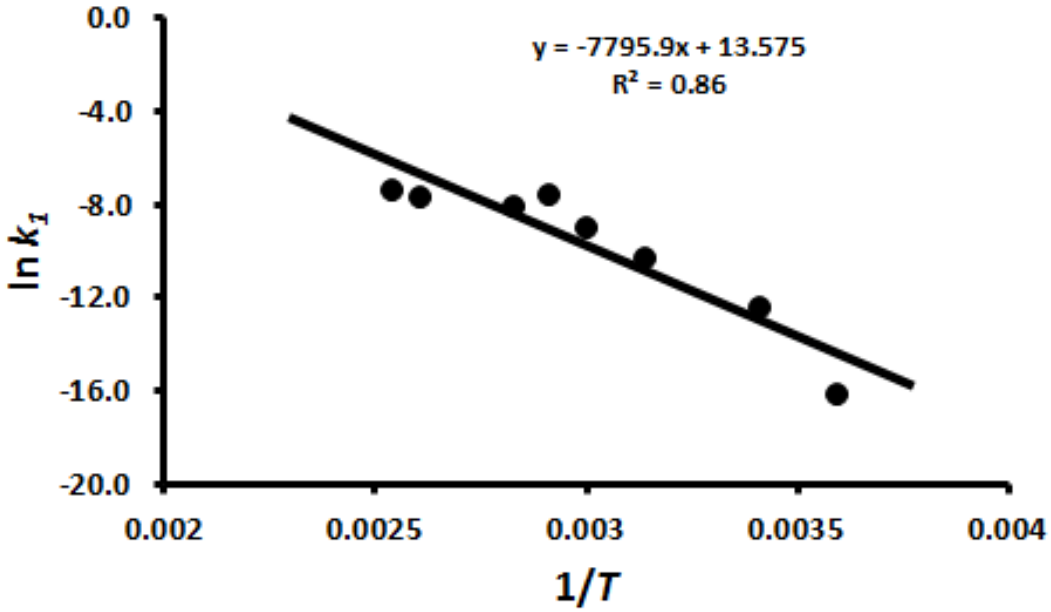

Figure 3: Arrhenius plot $Ln(k_1)$ versus $1/T$

3 Results and Discussions

The composition of fresh broccoli was determined and presented in our preliminary study (Suresh et al., 2017). Moisture, protein, fat, crude fibre, ash, and carbohydrate content of broccoli was 90.20, 2.08, 0.33, 0.75, 0.74 and 5.90 g/100 g sample, respectively; and pH was determined as 6.8. The vitamin C in the fresh broccoli varied from 53.6 to 64.3 mg/100 g sample. In order to study vitamin C stability with wide variations in temperature, different time frame experiments were used, as also identified earlier by Hal et al. (2012), El-Ishaq and Obirinakem (2015) and Polinati et al. (2010). The heating time frame at different temperatures is shown in Table 1. Different time frames could be used to compare vitamin C stability. For example, the vitamin C reduced to 40.4 mg/100 g sample (i.e. 62.8% loss) after 35 days of storage when stored in chilled conditions at 5^{o}C, whereas vitamin C reduced to 45.0 mg/100 g sample (i.e. 69.9% loss) after 7.2 h when stored at 45^{o}C. Similarly, vitamin C reduced to 38.4 and 36.0 mg/100 g sample (i.e. 59.7% and 56.6% loss, respectively) after 27 and 16 min when stored at 60 and 120^{o}C, respectively. Therefore, time to a specific reduction of vitamin C (for example above, 36-45 mg/100 g sample) could be used to compare data when stored at different temperatures. However, kinetics modelling with rate constants (s^{-1}) better standardize the processes when different time frames are used.

The vitamin C (ascorbic acid, AA) losses in broccoli, cauliflower, and cabbage were studied at different stages (delivery, cooked for 30 min and blast chilled, 0-3^{o}C) (Charlton, Patrick, Dowling, & Jensen, 2004). The AA content of broccoli, cauliflower, and cabbage from delivery to blast chilling varied from 2.9-24.0 mg/100 g sample. The dramatic AA losses occurred during cooking (33-81%), with broccoli showing the great loss (81%). During the 4-day chill storage, a steady decline of AA was observed with a loss/day of 4.2%. The loss of AA levels in broccoli during chilled storage was 3.6% per day. The loss/day for cauliflower, and cabbage was 7.6% and 2.3%, respectively.

Goncalves, Abreu, Brandao, and Silva (2011) studied the vitamin C loss in frozen broccoli (*Brassica oleracea* L. ssp. *Italica*) during storage. The vitamin C content significantly decreased after 121 days of isothermal storage by 80%, 60%, and 29%, respectively at -7, -15, and -25^{o}C. The major loss of vitamin C occurred during the first 55 days of storage. Shobham, Mudhavath, and Sukumaran (2017) studied the effect of microwave and pressure cooking on the stability of vitamin C in vegetables (carrot, potato, spinach, brinjal, cauliflower, green chilli, bitter gourd, and cabbage). The total content of vitamin C in raw vegetables ranged from 3.55 to 91.27 mg/100 g. Microwave and pressure cooking for 2 to 3 min resulted in considerable loss of vitamin C. The percentage loss of vitamin C content in cabbage subjected to microwave and pressure cooking was 9.9% and 49.5%, respectively, whereas it was 20.7% for cauliflower subjected to microwave and 46.2% loss during pressure cooking. A greater loss was observed for potato and spinach. Shams El-Din, Abdel-Kader, Makhlouf, and Mohamed (2013) studied the effect of cooking methods on natural antioxidants in Brassica vegetables. The result showed that boiling had a greater loss of vitamin C compared to microwave cooking. Boiling for 6 min caused a loss of 64.5% in broccoli, 70.7% loss in white cabbage, and 66.8% in cauliflower.

Figures 1 and 2 show the plot of Ln (C/C_o) versus time according to first-order reaction kinetics (Equation 2). The high regression coefficient from 0.88 to 0.99 indicates that vitamin C decay can be predicted according to the first-order reaction. Similarly, first-order reaction was used for the degradation of vitamin C in cabbage and lettuce (Awagu, Ekanem, Kolo, & Adamu, 2017), cherry juice (Jirasatid & Noipant, 2015) and orange juice (Calligaris, Manzocco, & Lagazio, 2012). The first-order reaction rate constant was determined from the slope and these increased from 9.03×10^{-8} s^{-1} to 5.65×10^{-3} s^{-1}, when storage temperature varied from 5^{o}C to 120^{o}C. Vitamin losses in fresh capsicum at 5^{o}C and 20^{o}C were observed as 8.22×10^{-7} and 1.15×10^{-6} s^{-1} as compared to the results found in this study of 9.03×10^{-8} (at 5^{o}C) and 4.03×10^{-6} s^{-1} (at 20^{o}C) (Rahman et al., 2015). At refrigerated temperatures, the loss of vitamin C in broccoli was much higher than capsicum, whereas comparable losses were observed at 20^{o}C. In the case of orange juice

stored at 10^{o}C, the rate constant was observed as 4.63×10^{-7} s^{-1} (Calligaris et al., 2012). In the case hot water blanching of pumpkin at pH 6.5, the rate constant increased from 8.10×10^{-4} to 1.37×10^{-3} s^{-1} when blanching temperature increased from 60^{o}C to 90^{o}C (Ariahu et al., 2011). The reaction rate constant of cherry juice increased from 3.12×10-4 to 1.00×10-3 s-1 with increasing temperatures (75, 80, 85, 90 and 95 ^{o}C) (Jirasatid & Noipant, 2015). The rate constant observed in this study was similar to earlier reported values. The degradation of ascorbic acid in broccoli could be mainly due to the oxidation of ascorbic acid by oxidizing enzymes, e.g. ascorbic acid oxidase, peroxidase, catalase, and polyphenol oxidase (Mapson, 1970; Venkatesh & Park, 2014). Two types of vitamin C degradation could occur: aerobic and anaerobic degradation. In aerobic degradation, the AA is oxidised to L-dehydro-ascorbic acid (L-DA) followed by hydrolysis and further oxidation, whereas anaerobic degradation has not been clearly studied and reported (Wang, Law, Mujumdar, & Xiao, 2017). During processing, matrix disruption could occur thus facilitating the oxidation of L-AA to L-DA by the enzyme AAO. The L-DA can then be further hydrolysed to 2,3-diketogulonic acid, thus losing its antiscorbutic activity. Other possible chemical reactions associated with changes in flavour, colour, and odour could have occurred with time due to interactions among the components and this resulted in changes in pH (El-Ishaq & Obirinakem, 2015; Munyaka et al., 2010). In this study, low temperature likely caused minimal destruction of structure, thus enzymatic degradation reaction was observed at a slower rate as compared to high temperature. At high temperature, structural damage could enhance enzymatic degradation as well as increase interaction with other released components.
Figure 3 shows the Arrhenius plot of Ln (k_1) versus 1/T, and activation energy was estimated from the slope as 74.2 kJ/mol. Jirasatid and Noipant (2015) observed a similar activation energy of 69.4 kJ/mol for cherry within the temperature range 75-95^{o}C. The higher activation energy indicated that temperature could affect degradation at a faster rate. The activation energy was observed as 58.0, 39.0, and 29.0 kJ/mol, respectively, for guava, mango, and marula pulps within the temperature range of 80-150^{o}C (Hal et al., 2012). The high activation energy of vitamin C in broccoli implied that the deterioration was more sensitive to temperature as compared to the vitamin C degradation in guava, mango, and marula pulps. The activation energy of ascorbic acid loss during pumpkin blanching (60-90^{o}C) varied from 16.9 to 41.2 kJ/mol, while pH increased from 5.0 to 6.5, respectively (Ariahu et al., 2011). The pH of the fresh broccoli was measured as 6.8 (Suresh et al., 2017). Therefore, the activation energy of broccoli could be expected to be higher than the reported values for pumpkin. In the case of heat treatment (80-90^{o}C) of apple, the activation energy was observed as 88.9 kJ/mol. The activation energy varied from 10.7 to 99.2 depending on the type of fruits and vegetables (Courtois, Vedrenne, & George, 2009). Therefore, the activation energy of vitamin C loss depended on the types of fruits and vegetables, as well as their physicochemical properties and pH.

4 Conclusion

The loss of vitamin C increased with storage time and temperature (5-120^{o}C). Vitamin C degradation in broccoli followed a first-order reaction and the temperature dependence of rate constants was described using the Arrhenius model. The activation energy was estimated as 74.2 kJ/mol. Data on the retention of vitamin C could be used when broccoli is stored as raw and when it is cooked at different temperatures. The rate constants at different temperatures could be used in simulation and optimization of thermal processes.

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