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Stability Evaluation and Degradation Kinetics of Ascorbic Acid in Baobab Fruit Pulp Formulated with the Seed Oil

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMD designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors MT, JS and DA edited the manuscript. Author AY managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Baobab (*Adansonia digitata*) fruit pulp and the seed oil contain appreciable amount of vitamins and nutrients which help fight off diseases and afford commendable source of nourishment. It is essential to screen for the stability of the high vitamin C contents and validate the mechanism of its kinetic degradation in the fruit pulp with and without the oil extracted from seeds, during heat treatment. Experiments were planned according to standard methods and practices.
Methodology: Ascorbic acid degradation in both raw baobab fruit pulp and the pulp formulated with baobab seed oil were investigated at varying temperatures (25–80ºC) and at different time intervals. Kinetic data analysis was then conducted by utilizing the absorbance data collected and the validated calibration curve of standard method using DCPIP to determine the ascorbic acid contents.
Results: The results showed that reaction kinetics through heat treatments of the fruit pulp were well characterized by zero-order reactions. The activation energy (Ea) for the ascorbic acid degradation in the raw fruit pulp and the pulp treated with the seed oil were 0.000274 and 0.001903 Kcal/mol respectively. The shelf life of ascorbic acid in the formulated fruit pulp at 25ºC was approximately seven times that of the raw fruit pulp.

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Conclusion: The results indicate that the baobab seed oil exhibits both antioxidant enrichment and preservative properties.

Keywords: Baobab; seed oil; antioxidant enrichment; kinetics; activation energy; shelf life.

1. INTRODUCTION

Baobab (*Adansonia digitata*) fruit pulp is usually expended in Africa by children, expectant mothers and senior citizens due to the high content of vitamins and nutrients which help fight off diseases and afford admirable source of nourishment. In traditional medicine baobab fruit pulp is used in the treatment of fevers, diarrhea and malaria. Due to its high vitamin C content, baobab fruit pulp has a well-documented antioxidant capacity [1].

Vitamin C is a vital nutrient and is needed for the development of biological tissues. Humans, as well as other species, cannot synthesize this nutrient due to the absence of the enzyme L-gulonolactone oxidase. Vitamin C is the L-enantiomer of L-ascorbate, an ion of ascorbic acid, being the reduced form of vitamin C [2]. Vitamin C is very unstable in aqueous solutions and tests have shown ascorbic acid to be a weak, monobasic acid and a strong reducing agent, therefore, it oxidizes to form a product known as dehydroascorbic acid, a substance easily absorbed across cellular membranes. This way, due to our inability to synthesize ascorbic acid, we absorb it through active transport and passive diffusion. Although this step is reversible it does not take long for this substance to change into 2, 3-diketo-L-gulonic acid which is an irreversible process and therefore very necessary to avoid. Even though this substance is named “acid” it is a lactone; therefore, its acidity and ease of oxidation are due to the presence of an enediol group [3].

Vitamin C is mainly used for tissue growth and repair and also necessary for the creation of collagen which is a protein molecule responsible for the formation of the skin, tendons, ligaments and blood vessels [4]. Vitamin C is also very important for bone maintenance and it is believed to be responsible for wound healing and osteogenesis, and therefore suggested to be an effective antiviral agent and its antioxidant properties are well known as it interrupts other molecules’ oxidation. Oxidation damages cells when free radicals are created in the beginning of reactions and when food decomposes or when it is exposed to radiation or even cigarette smoke [5,6]. If free radicals accumulate in the body, the ageing process may be stronger, and can lead to fatal diseases, such as cancer or heart attacks. Antioxidants eliminate these free radicals making them unable to take part or trigger other unwanted immunological reactions. Hence, vitamin C is essential with respect to the ageing of the body. However, there are secondary effects when an excess amount of vitamin C is taken in, these are, amongst others, gastric irritation, taste deterioration and renal problems due to the action of the vitamin’s metabolic product, particularly, oxalic acid. This can lead to inhibition of natural processes, thus, vitamin C is an essential nutrient necessary for the maintenance of biological tissues, yet its intake needs to be controlled. Further, vitamin C is soluble in water, making its consumption easier and the excess amount consumed can be eliminated by the body, nonetheless controlling vitamin C consumption is very much essential. Several food groups contain vitamin C, amongst them, citrus fruits, such as the orange, lemon, lime or grape fruit but there are foods with comparable substantial vitamin C content and baobab fruit pulp is no exception. Fruits and fruit products in many varieties are a significant world produce and fragment of economic essence of many countries [7]. At the mention of orange or orange juice, what comes to mind is the vitamin C or ascorbic acid

because it happens to be the most important and readily available vitamin in citrus [8]. In this light, ascorbic acid could be used as a chemical marker for shelf life of orange and other fruits from plants, for example, baobab fruit pulp and the seed oil with equally high vitamin C content, since it would be easier to measure ascorbic acid concentration than measuring sensory acceptability directly [9].

Numerous studies have been done on the determination of vitamin C content in different fruit juices and model systems. Some gave only qualitative or semi-qualitative information because only initial and final vitamin C concentration values were reported [10] thereby making intermediate predictions difficult to make. Other researchers determined the order of the reaction based on inadequate data points, however, a number of conditions comprising temperature, pH and oxygen affect degradation of ascorbic acid during processing and storage. The vivid way to study the degradation of a compound is to determine the kinetics of its degradation reaction. Vitamin C draws attention of the research community and consumers as a nutrient with an extensive biological activity, significant for human health. The objective of this research was to determine the degradation kinetics of ascorbic acid in both raw baobab fruit pulp and the pulp formulated with oil extracted from the seeds in selected baobab fruits from Navrongo, in the Upper East Region of Ghana.

2. MATERIALS AND METHODS

2.1 Materials

Commercial pure L-ascorbic acid, 100 g, 5.0 go f 2, 6-dichlorophenolindophenol (DCPIP), and 12.5 cm size of Fluted Filter Paper were purchased from Benburto Chemical Enterprises Ltd, Accra, Ghana. Baobab fruit was harvested from Navrongo in the Upper East Region of Ghana. Fruits were cracked and the seed kernels were manually removed from the seed shell using a knife. Sonicator, UV-Vis Spectrophotometer, centrifuge, and Sohxlet extractor were obtained from the laboratory of the Department of Applied Chemistry & Biochemistry, Faculty of Applied Sciences, University for Development Studies.

2.2 Extraction of Seed Oil

The seed kernels were pulverized into fine powder using mortar and pestle and the powdered seed kernel, 40 g, was used for the extraction process applying a Sohxlet extractor. Organic solvents hexane and petroleum ether, 200 ml each was measured into separate 250 ml round bottom flask and the Sohxlet with a thimble containing the seed powder and a condenser were assembled. The solvent mixtures of both the hexane and the petroleum ether fractions were refluxed for 4 hours each. The mixture of each fraction was concentrated using rotary evaporator to obtain light yellowish oil, yield of 11.48 g and 5.24 g for oil extract from petroleum ether and hexane respectively.

2.3 Kinetics Procedures of the Baobab Fruit Pulp at Different Temperatures

Applying simple procedure, the fruit was ruptured into two halves and the pulp scrapped out with a plastic spoon. The semi-powdered pulp sample, 10 g was weighed, pulverized and dispersed in 250 ml of deionized water using a porcelain pestle and mortar. The mixture was then sonicated, centrifuged at 5000 rpm for 10 minutes and the supernatant filtered through rapid fluted filter paper and kept at 10ºC for the next experiment. The process was repeated

at time period of two and three hours respectively. The same process continued at variable temperatures (40 - 80ºC).

Baobab seed oil, 2 ml was added to 10 g/100 ml of raw baobab fruit pulp extract in a flask. The content was sonicated continuously at room temperature for one hour, centrifuged and the supernatant was decanted. The mixture was further filtered through sterile cotton wool into a scintillation vial and the filtrate was kept in a freezer for the next experiment. The process was repeated at an hour interval up to three hours and the filtrates were collected. The formulation process continued with samples heated at various temperatures ranging from 25, 40, 60 and 80ºC respectively. Kinetics data analysis were then conducted by utilizing the absorbance data collected and the validated calibration curve of standard method using DCPIP to determine the ascorbic acid (AA) contents.

2.4 Determination of Degradation Constant

The observed pseudo first-order degradation rate constants, k_{obs}, were calculated from the slopes of semi-logarithmic plots of the drug fraction remaining versus time in accordance with Equation 1.

$$\begin{aligned} &\ln[C] - ln\,[C]_0 = -k_{obs}t \\ &ln[C] = ln[C]_0 - k_{obs}t \\ &C = Co\,e^{-k_{obs}t} \\ &\log C = log\,Co - \frac{k_{obs}t}{2.3030} \end{aligned} \quad (1)$$

A plot of ln[C] vs. time *t* gives a straight line with a slope of $-k_{obs}$. Where C_0 was the initial concentration and C was the remaining concentration of AA at time, t. Summary of ln[C] data at variable temperature is given in Tables 1 and 2. Similarly, summary of rate constant for degradation of AA at variable temperature is given in Tables 3 and 4.

Zero-order kinetics was investigated and the degradation rate constants were calculated from the slopes of concentration plots of the AA fraction remaining versus time in accordance with Equation 2.

$$Ct - Co = -kt \quad (2)$$

A plot of [C] vs. time *t* gives a straight line with a slope of –k, *w*here C_0 was the initial concentration and Ct was the remaining concentration of AA at time, t. Summary of concentration remaining and rate constant for degradation of AA at variable temperature is given in Tables 5 and 6

Arrhenius noted that the *k*(*T*) data for many reactions fit the equation below:

$$\begin{aligned} &k(T) = Ae^{-\frac{E_a}{RT}} \\ &\ln k = \ln A - \frac{E_a}{RT} \end{aligned} \quad (3)$$

where A and k are constants characteristic of the reaction and R is the gas constant.

Ea is the activation energy and A is the pre-exponential factor or the Arrhenius factor. The units of A are the same as those of k. Ea is usually expressed in kcal/mol or kJ/mol. In most cases, A is considered to be temperature-independent (Starink, 1996). This will also be applicable in this current manuscript. If the Arrhenius equation is obeyed, a plot of logk versus 1/T gives a straight line with slope $-\frac{E_a}{2.303R}$ and intercept log A. This allows Ea and A to be evaluated. Summary of Ea and the shelf-life for AA in both the raw fruit pulp and the formulated pulp for the zero order kinetics were found to be 0.000274 and 0.001903 kcal/mol respectively.

Table 1. Drug concentration (mg), at variable temperatures, T = 25ºC, 40ºC, 60ºC and 80ºC for raw baobab fruit pulp extract from first order kinetics

Time/hour	25ºC		40ºC		60ºC		80ºC	
	Conc. (mg)	Ln C	Conc. (mg)	Ln C	Conc. (mg)	Ln C	Conc. (mg)	Ln C
0	29.99	3.40	29.99	3.40	29.99	3.40	29.99	3.40
1	26.54	3.28	18.40	2.91	13.43	2.60	10.67	2.37
2	25.76	3.25	16.98	2.83	13.03	2.57	8.51	2.14
3	22.13	3.10	15.41	2.74	11.02	2.40	8.20	2.10

Table 2. Drug concentrations (mg), at variable temperatures, T = 25ºC, 40°C, 60°C and 80ºC for baobab fruit pulp formulated with the seed oil from first order kinetics

Time/hour	25ºC		40ºC		60ºC		80ºC	
	Conc. (mg)	Ln C	Conc. (mg)	Ln C	Conc. (mg)	Ln C	Conc. (mg)	Ln C
0	38.35	3.65	38.35	3.65	38.35	3.65	38.35	3.65
1	37.67	3.63	34.20	3.53	29.44	3.38	28.87	3.36
2	37.24	3.62	33.67	3.52	27.87	3.33	19.33	2.96
3	36.73	3.60	32.17	3.47	25.57	3.24	16.67	2.81

Table 3. Temperature (K), inverse temperature, 1/T (K^{-1}), Rate Constant, k ($hour^{-1}$) and Ln k for raw baobab fruit pulp extract from first order kinetics

Tempt (K)	1/T (K^{-1})	k ($hour^{-1}$)	Ln k
298	3.356×10^{-3}	-0.090	2.408
313	3.195×10^{-3}	-0.085	2.465
333	3.003×10^{-3}	-0.100	2.303
353	2.833×10^{-3}	-0.135	2.002

Table 4. Temperature (K), inverse temperature 1/T (K^{-1}), rate constant, k ($hour^{-1}$) and Ln k for baobab fruit pulp formulated with the seed oil from first order kinetics

Tempt (K)	1/T (K^{-1})	k ($hour^{-1}$)	Ln k
298	3.356×10^{-3}	-0.015	4.200
313	3.195×10^{-3}	-0.030	3.507
333	3.003×10^{-3}	-0.070	2.659
353	2.833×10^{-3}	-0.275	1.291

Table 5. Data for drug amount (mg), at temperatures 25ºC, 40ºC, 60ºC and 80ºC for the raw baobab fruit pulp and the pulp formulated with the seed oil from zero order kinetics

Time (hour)	25°C		40°C		60°C		80°C	
	Conc. (mg)		Conc. (mg)		Conc. (mg)		Conc. (mg)	
	Raw	Formulated	Raw	Formulated	Raw	Formulated	Raw	Formulated
0	29.99	38.35	29.99	38.35	29.99	38.35	29.99	38.35
1	26.54	37.67	18.40	34.20	13.43	29.44	10.67	28.87
2	25.76	37.24	16.98	33.67	13.03	27.87	8.51	19.33
3	22.13	36.73	15.41	32.17	11.02	25.57	8.20	16.67

Table 6. Data for inverse of temperature, 1/T and rate constant, K for Arrhenius plots of raw baobab fruit pulp and the fruit pulp formulated with the seed oil from first and zero order kinetics

Temperature (K)	$1/Kx10^{-3}$ (K^{-1})	K (h^{-1}) Zero Order		K (h^{-1}) First Order		lnK Zero Order		lnK First Order	
		Raw	Formulated	Raw	Formulated	Raw	Formulated	Raw	Formulated
298	3.356	2.436	0.529	0.090	0.015	0.890	- 0.637	- 2.408	- 4.200
313	3.195	4.516	1.907	0.085	0.030	1.508	0.646	- 2.465	- 3.507
333	3.003	5.731	3.991	0.100	0.070	1.746	1.384	- 2.303	- 2.659
353	2.833	6.753	7.458	0.135	0.275	1.910	2.009	- 2.002	- 1.291

3. RESULTS

The study was to evaluate the kinetics of degradation of ascorbic acid in both raw baobab fruit pulp and the pulp treated with oil from the seeds at variable temperatures. From the data collected at different temperature, reduction in ascorbic acid concentration was monitored with 2, 6-dichlorophenolindophenol (DCPIP). The result of the kinetic experiments showed reduction in vitamin C concentration in both samples, although the rate was far slower at all temperatures for the formulated fruit pulp than the raw fruit pulp extract.

Figs. 1 and 2 showed the degradation profile of ascorbic acid in baobab fruit pulp at varying temperatures for the raw pulp extract and the pulp formulated with the seed oil respectively. As projected, higher temperature promoted higher ascorbic acid degradation in the raw extract. The curves for the formulated pulp at temperatures, 25, 40 and 60°Cgave straight line indicating seemingly zero-order kinetics but deviated at a temperature of 80ºC (Fig. 2).

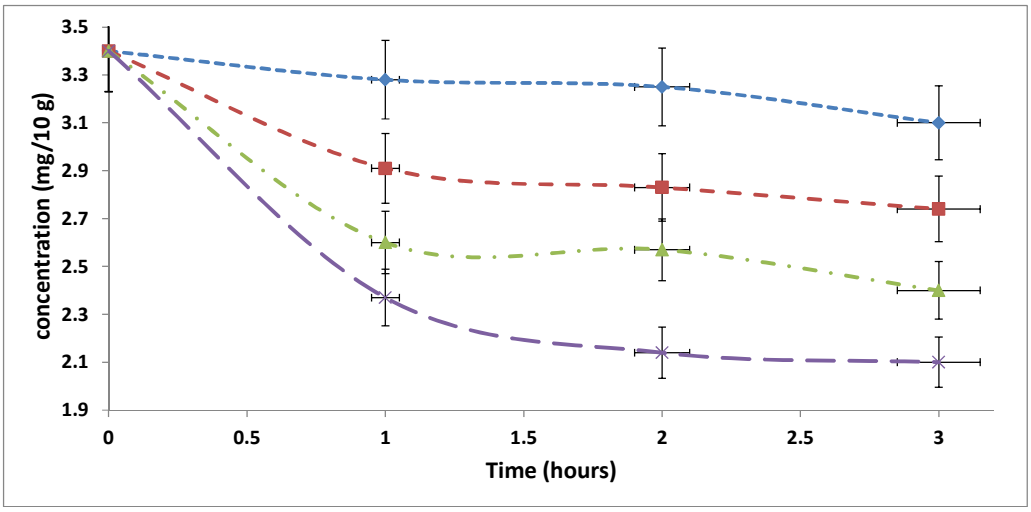

Fig. 1. Plots of zero order degradation kinetics of ascorbic acid in raw baobab fruit pulp extract at variable temperatures (♦) T1 = 25ºC, (■) T2 = 40ºC, (▲) T3 = 60ºC, (×) T4 = 80ºC

Although ascorbic acid content of baobab fruit pulp was reported to range from 300 mg/100 g [11], different values from baobab fruit procured from Blue Nile State had the highest ascorbic acid content (370.66 mg/100g) followed by sample from Kordofan (357.33 mg/100g), while that from Darfur showed the lowest vitamin C level (347.33 mg/100 g) [12].

In this study initial ascorbic acid concentration was 299.9 mg/100 g found in the raw pulp extract while 383.5 mg/100 g was determined in the pulp formulated with the seed oil. It was observed that there was no significant change in ascorbic acid content at 25º Cuntil the end of the storage time of three hours for the formulated pulp, that is, 376.7 g/100 g at one hour and 367.6 mg/100 g at three hours. The concentration in the formulated extract reduced gradually from 376.7 mg/100 gat 40°C, to 255.7 mg/100 g at 60ºC and 166.7 mg/100 g at 80ºC respectively during the three hour storage period. A similar trend with more rapid reduction was observed for the raw pulp extract. The ascorbic acid content reduced from 221.3 mg/100 g to 82 mg/100 g at temperature (40 – 80ºC) during the three hour storage period.

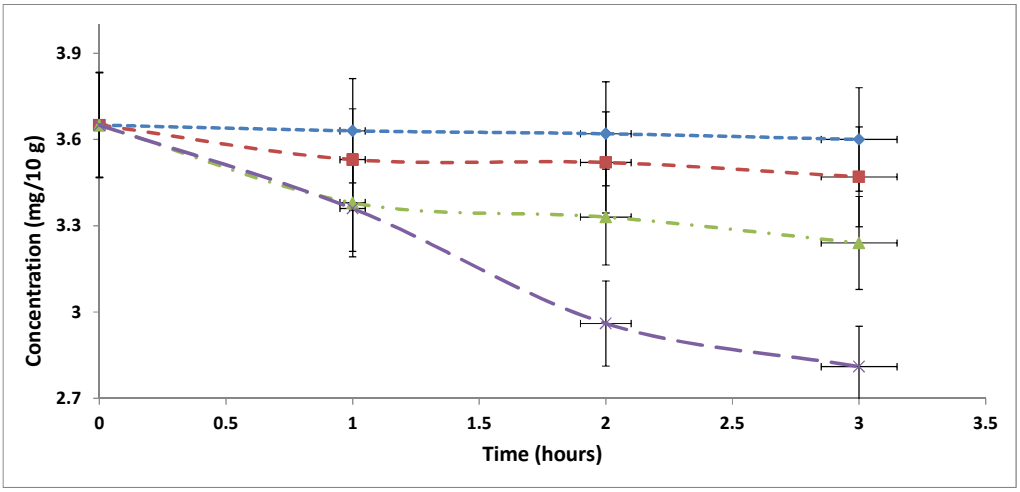

Fig. 2. Plots of zero order degradation kinetics of ascorbic acid in baobab fruit pulp formulated with oil from the seeds at variable temperatures (♦) T1 = 25ºC, (■) T2 = 40ºC, (▲) T3 = 60ºC, (×) T4 = 80ºC

The percentage reduction of the initial concentration in the raw pulp samples at the three storage conditions (1-3 hours) was from 11% to 14.10% and 26.2% at 25ºC respectively. At 80ºC, at storage time of 3 hours, the percent reduction was drastic, from 26.20% to 48% to 63.25% and 72.65 respectively (Fig. 3). The gradual degradation of the formulated sample is evident in the percent reduction distribution. At 25ºC, the percent reduction was 1.76 at the storage time of one hour. When the temperature was raised to 80ºC, and at three hour storage period the percent reduction was from 4.22% to 16.11% to 38.32 and 56.53% accordingly (Fig. 4). This supports the antioxidant capacity of the seed oil and its high ascorbic acid content. Research by [13], reported that low temperature storage is vital in order to safeguard L-ascorbic acid retention. Research by [14] and others have reported zero order kinetics in orange juice packaged in cans and Tetrabrik. Our research is in agreement with Davies and research group who reported that ascorbic acid degradation could be zero-order kinetics when the total destruction is less than 50%. First order kinetics of ascorbic acid degradation have been shown in some tropical leafy vegetables by [15] and thermal degradation of thiamine in periwinkle based formulated low acidity foods by [16].

The reaction rate constant k, was determined for each temperature from the slope of the line obtained by least squares regression analysis. The reaction rate constant for the formulated sample is indicated in Table 6. The k values showed that, the degradation kinetics was zero order, substantiated by the change in k values at variable temperatures. Although, the curves for both first and zero order plots looked similar for the raw and the formulated samples, the zero order rate constants k varied drastically at different temperature, demonstrating zero order kinetics for the studies conducted. The curve was steeper at 80ºC than the lower temperatures for both raw and formulated samples, indicating higher degradation. This also confirms that temperature is one of the factors affecting ascorbic acid degradation. From the regression analysis between ascorbic acid degradation and length of time, R^2 was 0.991 and 0.952 at the lowest and highest temperatures respectively used for these studies. The coefficient in addition to the variation of the rate values for zero order kinetics suggested that the model was satisfactory in describing degradation of ascorbic acid in the formulated baobab fruit pulp. Zero order and

first order models have been used by various research groups to describe ascorbic acid degradation [17-19]. Applying the Arrhenius plots (Fig. 5), the activation energy of ascorbic acid in the raw pulp and the formulated sample were found to be 0.000274 and 0.001903 Kcal/mol respectively. The shelf live was also determined for both the raw and the formulated samples to be 1.2 and 7.0 hours respectively, suggesting the antioxidant enrichment capacity of the baobab seed oil. Figs. 6 and 7 show the first order kinetic profiles of ascorbic acid in the raw fruit pulp and the formulated sample respectively.

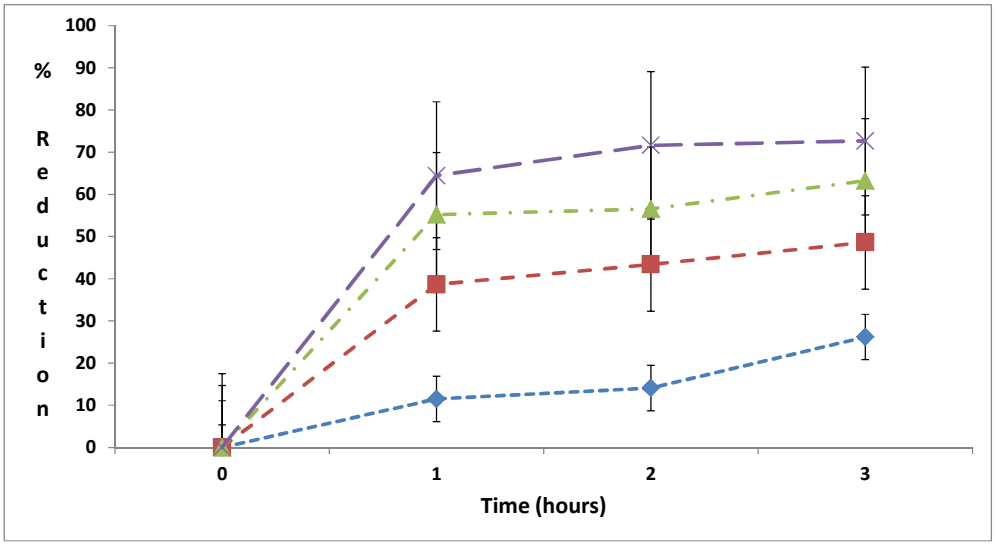

Fig. 3. Plots of percent reduction of ascorbic acid in raw baobab fruit pulp at variable temperatures (♦) T1 = 25ºC, (■) T2 = 40ºC, (▲) T3 = 60ºC, (×) T4 =80ºC

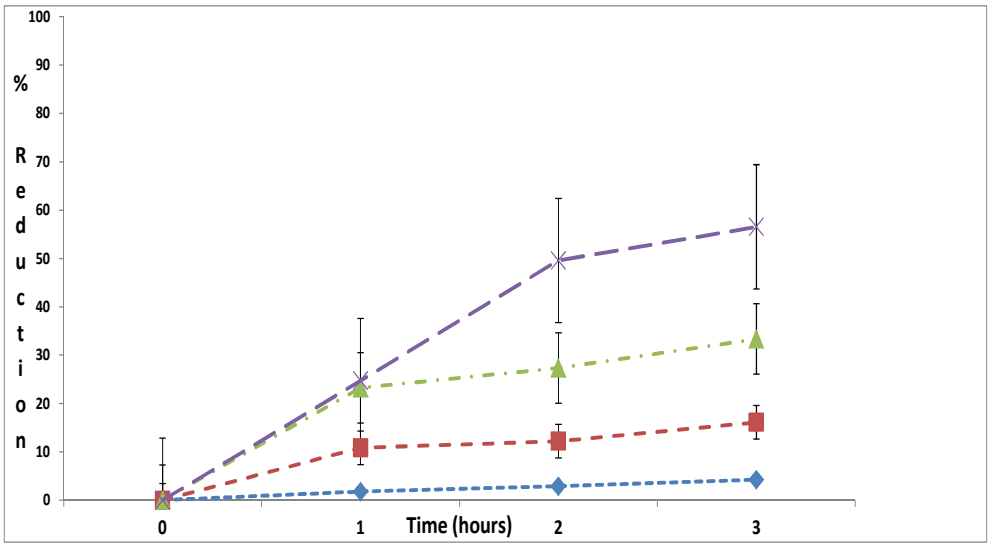

Fig. 4. Plots of percent reduction of ascorbic acid in baobab fruit pulp formulated with oil from the seeds at variable temperatures (♦) T1 = 25^{o}C, (■) T2 = 40ºC, (▲) T3 = 60ºC, (×) T4 =80ºC

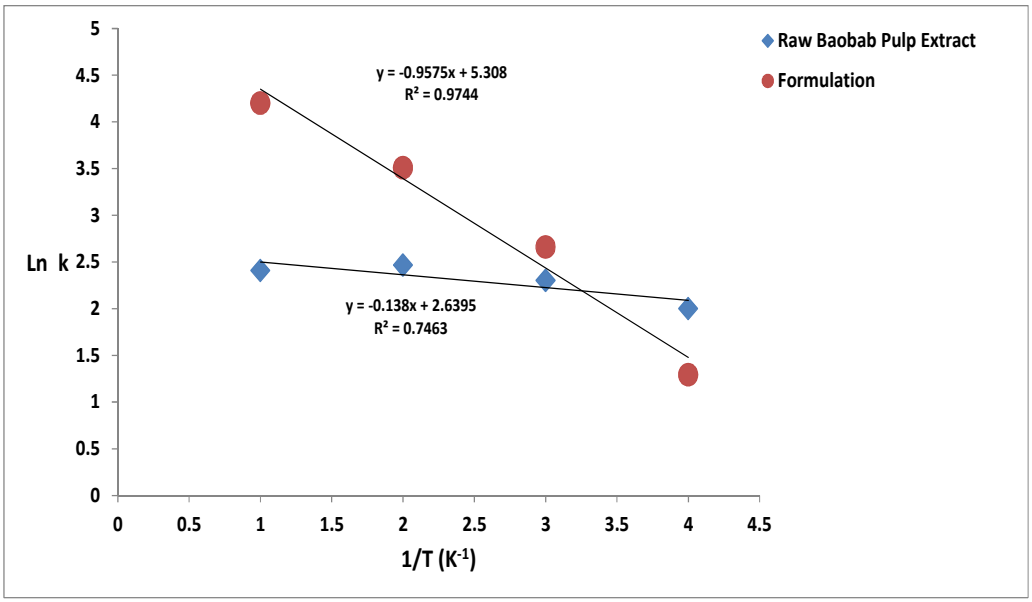

Fig. 5. Plots of Lnk against inverse of absolute temperature for first order degradation kinetics of ascorbic acid at variable temperatures, 25-80ºC (♦) raw fruit pulp extract (●) fruit pulp formulated with the seed oil

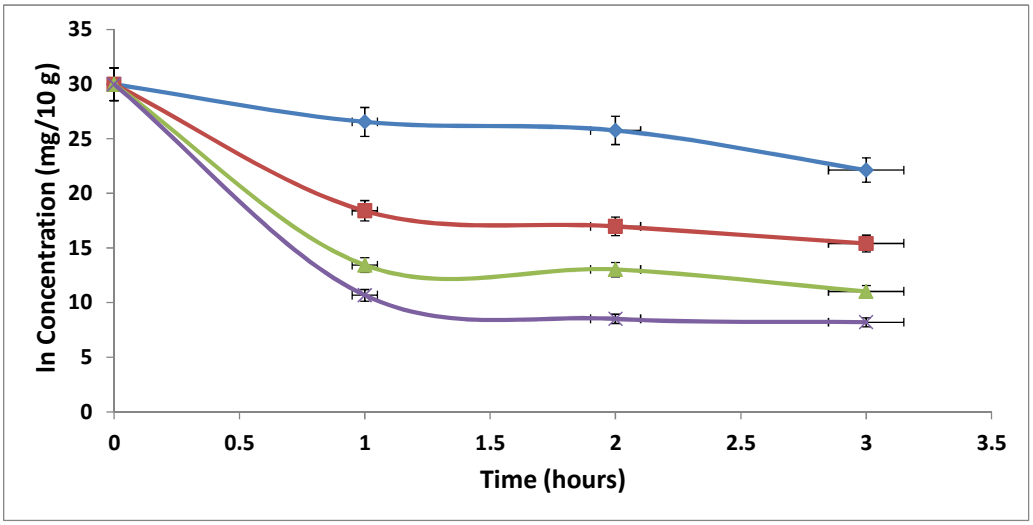

Fig. 6. Plots of first order degradation kinetics of ascorbic acid in raw baobab fruit pulp extract at variable temperatures (♦) T1 = 25ºC, (■) T2 = 40ºC, (▲) T3 = 60ºC, (×) T4 = 80ºC

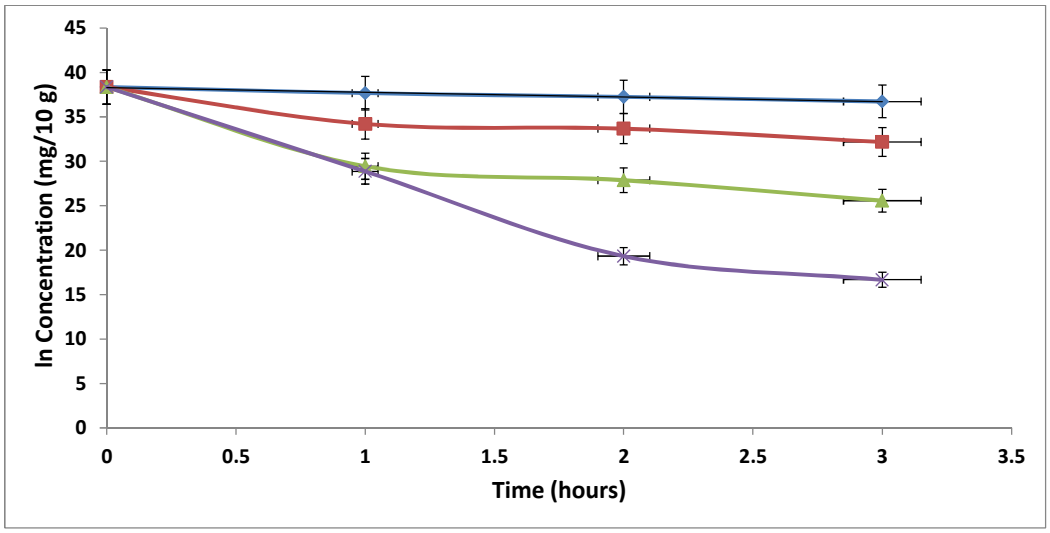

Fig. 7. Plots of first order degradation kinetics of ascorbic acid in baobab fruit pulp formulated with oil from the seeds at variable temperatures (♦) T1 = 25ºC, (■) T2 = 40ºC, (▲) T3 = 60ºC, (×) T4 =80ºC

4. DISCUSSION

In this study, data on ascorbic acid degradation in both raw baobab fruit pulp and the pulp treated with the seed oil are presented. The degradation was faster at high temperature but not very significant regarding the formulated sample compared with the raw fruit pulp. The knowledge acquired from this study is most specifically relevant to the food processing industry most especially food products with high vitamin C content but subject to varying degree of temperature during processing. Research by [20] showed that encapsulation is the key step of guarding against vitamin C degradation. For the application in solid food systems such as, cereals, bread, and biscuits spray-cooling, spray-chilling and fluidized bed appear the notable ways of encapsulation. In liquid food systems, liposomes represent the best form of encapsulation. The quest to finding more answers relating to healthy living is nonstop due reasonably to the increasing health alertness of the consumer. Ascorbic acid is a major provider to the increase patronage of most fruits and vegetables. The preservation of this nutrient would go a long way in curbing some of the debilitating disease as a result of inadequate supply of this all important nutrient.

5. CONCLUSION

The application of extracted oil from baobab seeds to the fruit pulp increased the total ascorbic acid content in the fruit pulp, enhanced antioxidant enrichment by protecting and stabilizing the ascorbic acid from degradation at higher temperatures. It seems reasonable to consider the baobab seed oil and the fruit pulp as an interesting food for diet supplement. Preservation of vitamin C in baobab fruit pulp by heat treatments, such as frying for producing fruit chips or high-temperature short-time treatments for producing concentrated or clarified juice, is a good alternative for enhancing the broad-spectrum of public's food quality intake.

COMPETING INTERESTS

It is hereby declared that the authors have no competing financial interests whatsoever in relation to the work described here. It is purely for academic and intellectual purposes.

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