



Research article

Simultaneous Extraction and Pasteurization (SEPA) of a drink from “blood root” (*Justicia secunda*) leaves part 1: kinetic studiesNoveta Neba^a, Martin Ngwa^b, Claris Anuanwen^a, Divine Nde Bup^{a,*}^a Department of Food and Bioresource Technology, College of Technology, University of Bamenda, P.O. Box 39, Bamenda, Cameroon^b Department of Agricultural and Environmental Engineering, College of Technology, University of Bamenda, P.O. Box 39, Bamenda, Cameroon

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ABSTRACT

Juice produced from the leaves of *Justicia secunda* is claimed to have potentials for treating anemia but is highly perishable while the production conditions are not yet mastered. Meanwhile in juice production from fruits or vegetables, key processing steps include extraction and pasteurization which are usually carried out separately with an attendant consequence on the production cost. This work was aimed at combining these unit operations in order serve time and energy. The effect of Conventional Extraction followed by Pasteurization (CEP) in comparison with Simultaneous Extraction and Pasteurization (SEPA) on the Vitamin C content of the juice was investigated. Kinetic models indicated that, the variation of Vitamin C occurred in two phases; a first phase corresponding to the accumulation of Vitamin C in the extraction medium and a second phase corresponding to its degradation. Average Vitamin C in the juice from the SEPA process was significantly greater than that from the CEP process. Major gains from the SEPA process include gain in energy, reduction in processing times and higher retention of Vitamin C in the juice.

1. Introduction

The role played by non-alcoholic beverages on most diets worldwide today has been strongly emphasized. This is because the beverages have been and continue to be viewed as drinks that can conveniently be taken after meals and for refreshments during the hot seasons in both rural and urban settings. Majority of the non-alcoholic beverages are made up mostly of water, sugar, flavoring agents and to an extent preservatives (Osuntogun and Aboaba, 2004). Because of the positive contributions of food and drinks to human health, consumers' demands for these have increased considerably. This increase in demand for such foods and drinks is attributed to the rising cost of health care, life expectancy as well as the desire for the old people in particular and the society at large to improve on their health (Mollet and Rowland, 2002). Apart from test satisfaction, drinks are also consumed to provide the needed nutrients and to prevent or avoid diseases related to nutrition (Babajide et al., 2013).

Justicia secunda M. Vahl (Acanthaceae) is a plant used in traditional medicine in Cameroon in the treatment of anemia (Fongod et al., 2013). Some studies have also shown that the plant possesses anti-anemic (Moswa et al., 2008; N'Guessan et al., 2010) and antimicrobial (Rojas

et al., 2006) properties. Compared to many plants whose iron contents are known, *Justicia secunda* appears to be a very important source of iron. These high iron contents (240 mg/g \pm 19) justify its use as anti-anemic agent in the Congolese popular medicine (Moswa et al., 2008).

Decoctions of the leaves of *justicia secunda* are used traditionally for the treatment of anemia in humans (Fongod et al., 2013). Usually these decoctions are prepared only when need arises and in quantities that can be consumed before deterioration. It is worth noting that empirical observations in the field have shown that it is difficult to store this beverage beyond 3 days. Since the leaves are equally very perishable, the availability of the leaves and/or its decoctions out of season is very limited. To prolong the shelf-life of this drink, there is need to pasteurize and to stabilize it by the addition of an anti-bacteria agent which can delay the development of microorganisms during storage. The major steps involved in the production of a drink from plant leaves include harvesting and cleaning, particle size reduction, extraction, pasteurization and packaging. The conventional method of carrying out extraction before pasteurization may have a negative impact on the quality of the drink because of double heating carried out during the separate extraction and pasteurization stages. As a consequence time and energy demands

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will be bigger. Several studies have been reported in the literature on the preparation of non-alcoholic beverages from fruits and vegetables in which conventional extraction is followed by pasteurization (Sanchez-Moreno et al., 2006; Ullah et al., 2015; Zhang et al., 2016) but none has focused on the simultaneous extraction and pasteurization of the juice.

In this work we propose a new method in which extraction and pasteurization are carried out as a single unit operation in an effort to limit quality degradation, time and energy consumption during processing. Meanwhile it has been clearly demonstrated that pasteurization of different juices has a significant effect on the degradation of vitamin C (Mericali et al., 2012; Zhang et al., 2016). The evolution of Vitamin C content was therefore used as a marker in all the processes. Reaction kinetics of the Conventional Extraction followed by Pasteurization (CEP) as well as those of the Simultaneous Extraction and Pasteurization (SEPA) process are reported in this research.

2. Material and methods

2.1. Sampling and sample preparation

Before harvesting the plant was identified and authenticated by the Department of Botany and Plant Physiology, Faculty of Science, University of Buea-Cameroon as *Justicia secunda*. It has a voucher specimen catalogued number of MOUAU/VPP/2015/14. The fresh young leaves of *Justicia secunda* used in this work were harvested from Bambili (5° 59'N and 10° 15'E) Cameroon. Only the youngest 5 leaves on the twig of the plant were harvested, packaged in clean and dry polyethylene containers and transported to the laboratory within 1 h of harvest. On arrival in the laboratory, samples collected were thoroughly mixed to ensure uniformity and picked to separate flowers, stalk and other dirt from the leaves. The thoroughly mixed leaves were then washed repeatedly under running tap water and rinsed with distilled water and then subjected to the extraction process on the same day. Powdered ginger was bought from the Agyati Food Processors Cooperative Society Ltd, in Agyati-Bafut (6° 09' N and 10° 06'E) Cameroon and used without any further treatment.

2.2. Kinetic studies

This was carried out in two different treatments (Figure 1). In the first treatment, juice extraction was carried out separately and the resulting juice was then pasteurized. This is referred to in the text as Conventional Extraction followed by Pasteurization (CEP). The second treatment consisted of simultaneously extracting and pasteurizing the juice in a single reactor referred to later as Simultaneous Extraction and Pasteurization (SEPA) process of the juice.

For the CEP process, extraction was first carried out as follows: 50mL of distilled water was measured with a measuring cylinder and poured into a 100 mL beaker and then placed in a water bath (B. Bran Scientific and Instrument Company, England) already set to the desired temperature. Temperature of the water was monitored with the help of a thermocouple until the desired value of 50 °C. 5g of fresh leaves of *Justicia secunda* was weighed using an electronic mass balance of 0.01 precision (XY200S, Textile Electronic balance) and transferred to the 100 mL beaker in the water bath. Extraction was then carried out at different time intervals (10, 20, 30, 60, 90, 150 and 210 min). After the reaction time, samples were withdrawn from the beaker and the mixture filtered through a clean Whatman filter paper No. 1. The vitamin C content of the various samples was then analyzed as described below. Similar experiments were carried out at 60 and 70 °C. Experiments were carried out in triplicates at each temperature.

After the separate extraction kinetics, a bulk juice was prepared following the reaction temperature and time which yielded maximum vitamin C content. 50 mL each of the juice was measured using a measuring cylinder into a polyethylene container and 1 g of powdered ginger added into it as preservative. The samples were sealed and pasteurized in a water bath (B. Bran Scientific and Instrument Company, England) for predetermined temperatures and time. Pasteurization was carried out at 50, 70 and 90 °C for 10, 20, 30, 60, 90, 150 and 210 min. During the pasteurization process samples were withdrawn at each time interval, filtered and the quantity of vitamin C in the juice analyzed.

The SEPA experiments were carried out in a similar manner as described for the CEP process but for the fact that there was no separate pasteurization step (Figure 1) using the same time intervals as for the CE. Kinetics of SEPA process was carried out at pasteurization temperatures of 50, 70 and 90 °C. 1 g of ginger powder was also added to the same reactor as natural stabilizer.

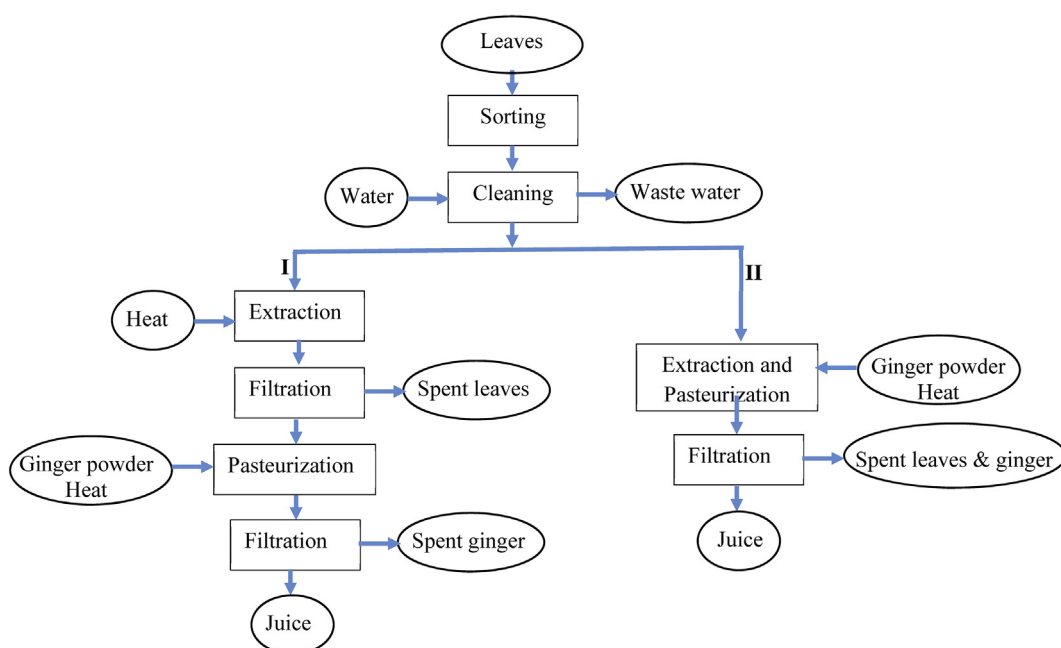


Figure 1. Block diagram for juice processing (Route I: CEP and Route II: SEPA).

2.3. Determination of vitamin C (ascorbic acid) content

Vitamin C content was estimated by the method described by [Mussa and El Sharaa \(2014\)](#). Briefly, 5mL aliquots of the juice were withdrawn and prepared as indicated by [Mussa and El Sharaa \(2014\)](#). The samples were then incubated at 37 °C for 3 h in a thermostatic water bath (B. Bran Scientific and Instrument Company, England). After incubation, the sample solutions were cooled in an ice bath for half an hour, treated with 5 mL of 85% H₂SO₄ and the absorbance of the sample was read at 521 nm using a UV –Visible spectrophotometer (UV 752(D), PEC Medical, USA). The concentration of the ascorbic acid in the juice sample was then calculated from the standard calibration curve.

2.4. Modelling of kinetic data

Kinetic analysis was done as described by [Peng et al. \(2014\)](#). Degradation of ascorbic acid was followed using the process reaction rate at different temperatures.

Generally, the kinetic data can be represented by the equation:

$$\frac{dC}{dt} = kC^n \quad (1)$$

where C is the concentration of vitamin C at time t, K and n are the rate constant and reaction order respectively. Eq. (1) can be integrated to give Eqs. (2) and (3) for n = 1 and n ≠ 1 respectively.

$$kt = -\ln \frac{C}{C_0} \quad (2)$$

$$kt = \left(\frac{1}{n-1} \right) \left(\frac{1}{C^{n-1}} - \frac{1}{C_0^{n-1}} \right) \quad (3)$$

To determine the rate constants (k), corresponding plots of t against $\frac{1}{C^{n-1}}$ or lnC (n = 1) and linear regressions were carried out on Microsoft excel. The coefficient of determination (R²) were compared in order to select the best-fitted reaction order for all the studied temperatures. The influence of temperature on ascorbic acid degradation was determined using the Arrhenius equation (Equation 4).

$$k = Ae^{\frac{E_a}{RT}} \quad (4)$$

The activation energy E_a (kJ/mol) was obtained from the slope of the plot of lnk against 1/T of the linearized form of Eq. (4). A, T and R are the pre-exponential constant, absolute temperature (K) of the medium, and the universal gas constant (8.314 kJ/mol.K) respectively.

3. Results and discussion

3.1. Kinetic studies

3.1.1. Conventional extraction followed by pasteurization (CEP)

3.1.1.1. Extraction. Figure 2 presents the variation of vitamin C as a function of extraction temperature. It is observed that in a general manner, irrespective of the extraction temperature, vitamin C content increased rapidly within the earlier period of extraction and then decreased slightly afterwards. At 50 °C the vitamin C increased from 0 – 2.63 mg/100 mL then decreased to 1.75 mg/100 mL. The corresponding variations at 60 °C and 70 °C were an increase from 0 – 2.38 mg/100 mL then decrease to 1.75 mg/100 mL and an increase from 0 – 4.90 mg/100 mL then decrease to 2.70 mg/100 mL respectively. The maximum vitamin C content was obtained when extraction was carried out at 70 °C for 20min which was significantly higher (p < 0.05) than that obtained at 50 °C and 60 °C throughout the extraction period. From Figure 2, it can be observed that, there was no significant difference (p < 0.05) between the vitamin C contents when extraction was carried out at 50 °C compared to that at 60 °C within the entire extraction period. Vitamin C is water-soluble as such is easily leached into the water. With increasing temperature, the solubility of vitamin C in water also increases thereby enhancing its leaching rate into the solution and consequently its concentration in the juice. This explains the higher contents of vitamin C obtained at 70 °C compared to those obtained at 50 °C and 60 °C. These results agree with a previous study carried out by [Diengdoh et al. \(2015\)](#) on the effect of cooking time on the ascorbic acid content of some selected green leafy vegetables (mustard leaves, cabbage, radish leaves, spinach, and beet leaves). They concluded that cooking temperature affected the percentage of vitamin C significantly as it is easily leached into the boiling medium. As the boiling time increases above 30 min the vitamin C content of the juice was degraded to constant values ([Igwemmar et al., 2013](#)). From Figure 2, extraction time for maximum extraction at 70 °C is about 20 min.

3.1.1.2. Pasteurization. Figure 3 presents the variation (degradation) of vitamin C with extraction time at 50°, 70° and 90°. It is observed that at all pasteurization temperatures vitamin C content decreased steadily and attained stable values after about 140 min of pasteurization. Some researchers have demonstrated that, during pasteurization, vitamin C concentrations gradually decreased with time at a rate depending on the pasteurization temperature ([Khalil et al., 2015](#); [Mercali et al., 2012](#); [Cinquanta et al., 2010](#)). Vitamin C content of carrot (*Daucus carota*), pumpkin (*Cucurbita maxima*), green peas (*Pisum sativum*), pepper

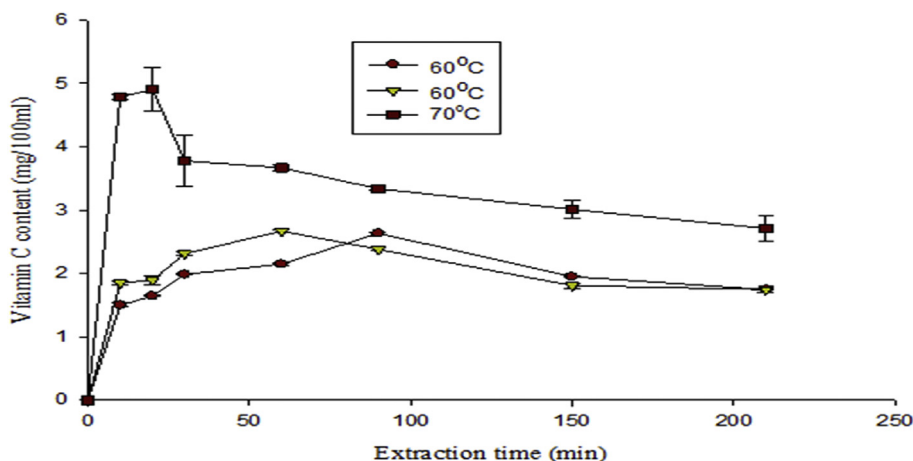


Figure 2. Influence of temperature on Vitamin C content during conventional extraction of juice.

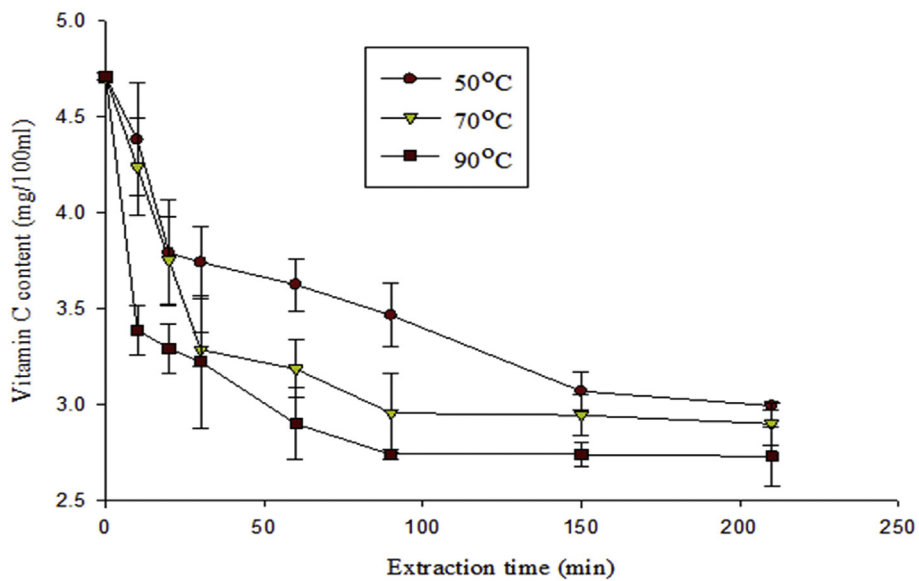


Figure 3. Influence of pasteurization temperature on the Vitamin C content of the extracted juice.

(*Capsium annuum*) and spinach (*Spinacia oleracea*) has also been reported to decrease considerably during heat processing (Khalil et al., 2015; Igwemmar et al., 2013). Vitamin C degradation was significantly affected ($p < 0.05$) by temperature with the degree of degradation increasing with temperature. From Figure 3, it can be estimated that time and temperature required to limit vitamin C degradation will be 30 min and 50 °C respectively. This indicates that the CEP process requires a total of 50 min (20 for extraction and 30 min for pasteurization).

3.1.2. Simultaneous extraction and pasteurization (SEPA)

The variation of Vitamin C content of the juice with time at different temperatures obtained for the SEPA process is shown in Figure 4. Similar to the CEP process, it was observed that for the SEPA process, in a general manner, irrespective of the extraction temperature vitamin C content increased rapidly within the earlier period of extraction and then decreased slightly afterwards. At 50 °C the vitamin C increased from 0 –

3.57 mg/100 mL then decreased to 2.46 mg/100 mL. The corresponding variations at 70 °C and 90 °C were: an increase from 0 – 4.69 mg/100 mL then decrease to 3.01 mg/100 mL and an increase from 0 – 4.72 mg/100 mL then decrease to 3.74 mg/100 mL respectively. In the first 50min of the SEPA process, vitamin C content at 70 and 90 °C were significantly higher than those obtained at 50 °C. The maximum vitamin C content was obtained when SEPA was carried out at 90 °C for 10min. Temperature equally had a significant effect ($p < 0.05$) on the Vitamin C content for the SEPA process.

3.1.3. Comparison between CEP and SEPA

Figures 5, 6, and 7 compares the vitamin C profiles obtained during the conventional pasteurization and SEPA processes at 50, 70 and 90 °C respectively. At 50 °C the Vitamin C content of the juice obtained from the CEP process was significantly higher ($P < 0.05$) than that of SEPA in the first 30 min. At 70 and 90 °C the vitamin C content of the juice

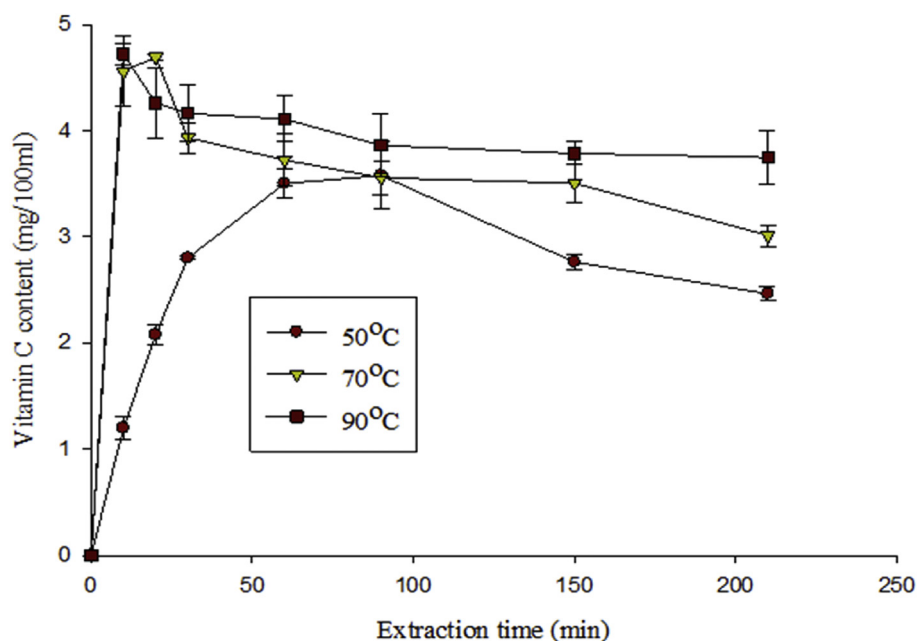


Figure 4. Influence of temperature on Vitamin C content during SEPA process of juice.

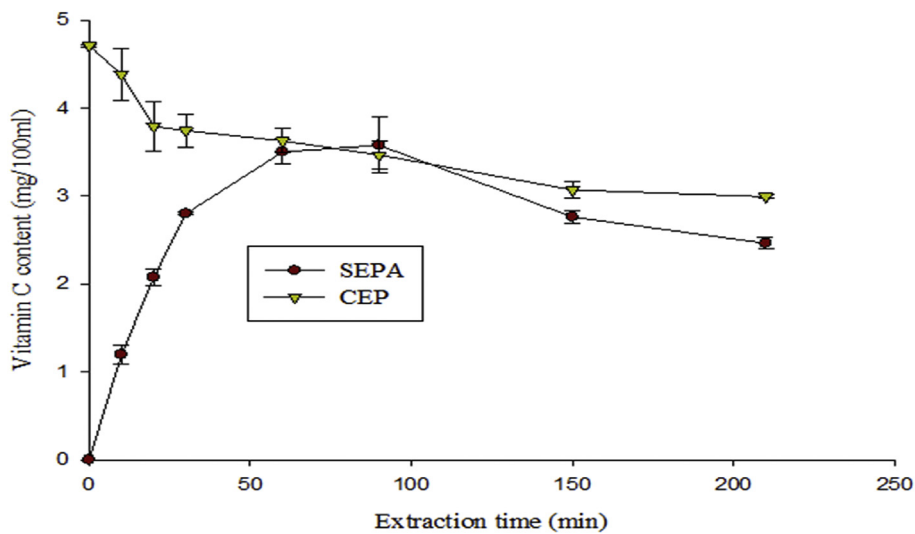


Figure 5. Comparison of Vitamin C content for conventional pasteurization and SEPA at 50 °C.

obtained from the SEPA process was significantly higher ($P < 0.05$) than that obtained from the CEP process by values up to about 4 % at 70 °C (Figure 5) and 38 % at 90 °C (Figure 6). The higher retention of vitamin C in the SEPA process is attributable to the reduction of double heating (extraction and then pasteurization) carried out on the drink during the CEP to a single round of heating in the SEPA process.

3.1.4. Modeling of the CEP and SEPA kinetics

The zero, first and second-order reaction equations were tested to model the kinetics of conventional extraction, conventional pasteurization as well as that of the SEPA processes. In all the three cases, it was not possible to model the entire kinetic data with the tested equations. The data was therefore divided into two phases. The first phase corresponded to vitamin C accumulation and while the second corresponded to the degradation of Vitamin C for both the conventional extraction and SEPA processes. For the conventional pasteurization process, these two phases corresponded to a first and second vitamin C degradation rates respectively. k and linear determination coefficient (R^2) values obtained for the various processes are presented in Table 1. Under a certain temperature,

the order of reaction was estimated by comparing (R^2) values. The higher the R^2 is, the better the reaction order is suited. It turned out that the degradation of vitamin C in the first degradation phase of the conventional pasteurization process and the accumulation of vitamin C in the SEPA process was successfully described by a zero order kinetic model. Accumulation of vitamin C in the separate extraction process was successfully described by a first order kinetic model. The degradation of vitamin C in the conventional extraction, SEPA and the second phase of the conventional pasteurization processes followed a second order kinetic model. Van Bree et al. (2012) and Lanny and Lie. (2014) showed that the degradation reaction of vitamin C some fruit juices during storage followed zero order kinetic models. Numerous studies have shown that vitamin C degradation of beverages at pasteurization and storage temperatures followed a first-order kinetic model (Jirasatida and Noipanta, 2015; Paul and Gosh, 2012 and Zhang et al., 2016).

In each phase of the reaction, and for all models studied, k values increased generally with an increase in temperature which confirmed the variation of vitamin C with temperature as earlier discussed. It is also observed from Table 1 that k values for the vitamin C

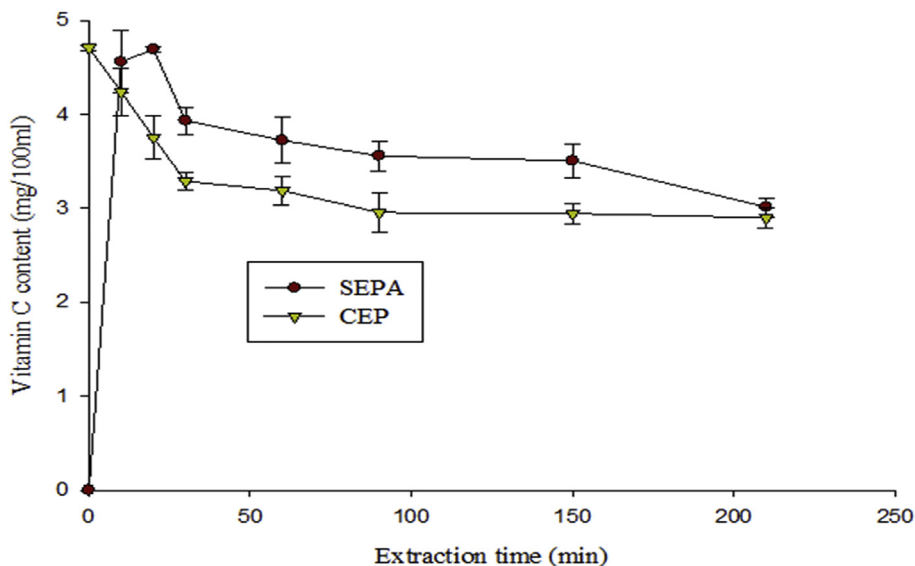


Figure 6. Comparison of Vitamin C content for CEP and SEPA at 70 °C.

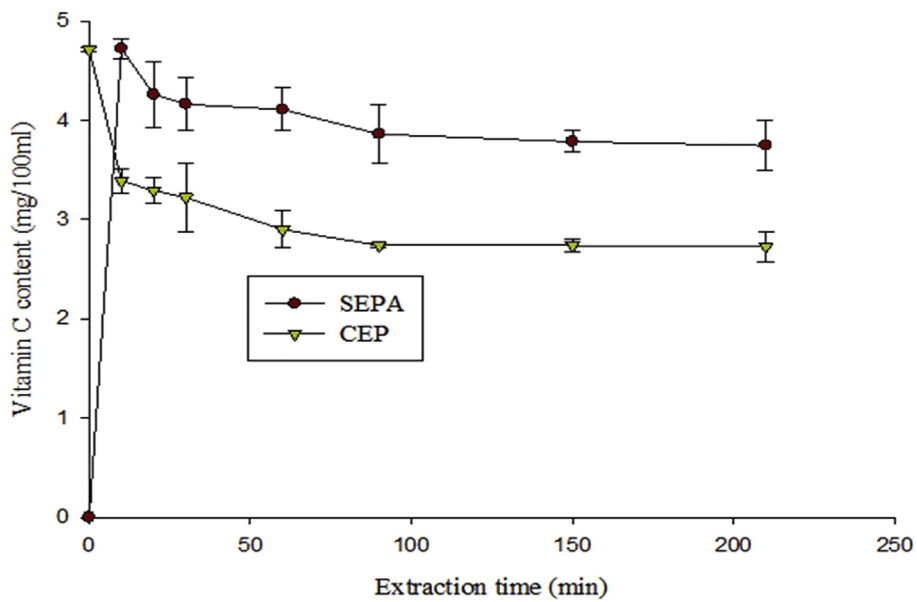


Figure 7. Comparison of Vitamin C content for CEP and SEPA at 90 °C.

Table 1. Vitamin C accumulation and degradation rates, k, for the SEPA and CEP processes.

		Temperature/°C	Zero-order		First order		Second Order	
			k-value	R ²	k-value	R ²	k-value	R ²
Conventional extraction	accumulation phase	50	0.0291	0.683	0.0071	0.843	0.0028	0.822
		60	0.036	0.647	0.0171	0.928	0.0034	0.890
		70	0.2451	0.767	0.0118	0.677	0.0039	0.686
	degradation phase	50	0.0074	0.911	0.0034	0.933	0.0016	0.954
		60	0.0053	0.824	0.0026	0.835	0.0013	0.847
		70	0.0061	0.984	0.0019	0.986	0.0006	0.993
SEPA	accumulation phase	50	0.0556	0.872	0.0191	0.780	0.0093	0.670
		70	0.2345	0.771	0.0074	0.608	0.0017	0.692
		90	0.2129	0.669	-	-	-	-
	degradation phase	50	0.0045	0.925	0.0093	0.934	0.0011	0.970
		70	0.0045	0.925	0.0013	0.871	0.0004	0.860
		90	0.0024	0.832	0.0007	0.866	0.0002	0.872
PAS	First degradation period	50	0.0349	0.922	0.0083	0.922	0.002	0.921
		70	0.0475	0.999	0.012	0.997	0.003	0.728
		90	0.0457	0.692	0.0117	0.709	0.003	0.728
	Second degradation period	50	0.0044	0.926	0.0013	0.930	0.0004	0.935
		70	0.0015	0.634	0.0005	0.642	0.0002	0.649
		90	0.0009	0.507	0.0003	0.509	0.0001	0.510

accumulation phase were much higher than those in the degradation phases for both conventional extraction and SEPA processes. This indicates that vitamin C accumulation was predominant over degradation. For the conventional pasteurization process, k values were higher in the first phase of extraction indicating that most of the degradation occurred in the early minutes of pasteurization compared to the later periods.

3.1.5. Activation energies

The activation energies evaluated for the Vitamin C accumulation phases of the conventional extraction and the SEPA processes as well as the first degradation phase of pasteurization are presented on Table 2. Activation energies calculated using the zero order equation were found to be 14 times higher for the conventional extraction process compared to the SEPA process. That is, the accumulation of vitamin C has stronger

Table 2. Activation energies and Arrhenius constants for the extraction and pasteurization of the juice.

		Zero order			First order			Second order		
		E _a (KJ/mol)	A	R ²	E _a (KJ/mol)	A	R ²	E _a (KJ/mol)	A	R ²
Conventional extraction	accumulation phase	97.42	1.22E+14	0.81	23.99	65.36	0.35	15.30	0.84	1
SEPA	accumulation phase	6.73	0.450	0.72	-	-	1	-	-	0.99
Pasteurization	First degradation period	6.73	0.450	0.68	8.55	0.21	0.73	10.07	0.09	0.78

temperature dependency at conventional extraction temperatures compared to SEPA temperatures. Activation energy is the energy required for a reaction to begin. The higher activation energies for the conventional extraction process indicate that it is a more energy intensive process compared to the SEPA process.

4. Conclusion

Based on the methodology used in this study and the results obtained it can be concluded that the new method introduced (simultaneous extraction and pasteurisation (SEPA) process) for juice production from the leaves of *Justicia secunda* led to an 80 % reduction in processing time, higher retention of average vitamin C (26.17 %) content as well as the elimination of some unit operations without any loss on the quality of the juice produced. The process was successfully modelled with kinetic equations. Major innovation of the SEPA process remains the reduction of the number of unit operations compared to the CEP process. The SEPA process is promising because it results in substantial gains in energy, reduction in processing times and higher retention of Vitamin C in the juice compared to the CEP process.

Declarations

Author contribution statement

Noveta Neba: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Martin Ngwa: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Claris Anuanwen: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Divine Nde Bup: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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