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Reverse-phase HPLC analysis of Vitamin C in two different varieties of pineapple found in Manipur, India

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Abstract

Vitamin C or ascorbic acid is an essential nutrient and important water-soluble antioxidant that plays a vital role in combating reactive oxygen species and maintaining human health. *Ananas comosus* (L.) Merr. is a tropical fruit which is famous for its nutritional, antioxidant content and therapeutic properties. Manipur in India is well known for the cultivation of two different cultivars of pineapple namely Queen and Kew which differ in fruit size, sweetness, texture and fruit quality. Depending on the cultivar of pineapple, growing conditions, maturity level, location and environmental factors, and post-harvest condition, vitamin C content in pineapple may vary. The study focuses on the determination and evaluation of vitamin C content in Queen and Kew varieties of pineapple grown in Manipur. The quantification of Vitamin C was done by reverse-phase HPLC method. The analyses show that the two cultivars have variations in vitamin C concentration. Vitamin C (ascorbic acid) acts as a potential antioxidant and protects human cells from reactive oxygen species. Moreover, evaluating vitamin C content in different cultivars of pineapple can give insightful information about the fruit quality, nutritional improvement and selection of high quality cultivars. Quantification of vitamin C content in Queen and Kew varieties of pineapple by Reverse-phase HPLC method showed that ripe pineapple contains higher vitamin C levels (ranging from 141.7 to 143.2 PPM) than unripe pineapple (131.3 to 135.5 PPM). Among the two different varieties of pineapple, the core part of ripe Kew variety showed the highest vitamin C content.

Keywords: *Ananas comosus*, vitamin C, reverse-phase HPLC, ripe, unripe, Queen, Kew pineapple

Introduction

Pineapple **(*Ananas comosus* (L.)Merr.)** is an important horticultural crop cultivated in Manipur which contributes to the state's agricultural economy, nutritional security, rural livelihood and cultural identity. The climatic conditions, suitable soil and adequate rainfall of Manipur provide favourable environmental conditions for pineapple cultivation. Queen and Kew varieties of pineapple are cultivated in Manipur. Pineapple contains bioactive compounds, vitamin C, organic acids, minerals, dietary fibers and antioxidant compounds [1]. Vitamin C is important

for its natural defence mechanism and immune support and it can function as antioxidant [2]. Overall, the estimation of vitamin C content in pineapple signifies the natural sources of antioxidants and essential nutrients. Large quantities of peel, core, crown, leaves and stalk generated during cultivation and consumption that can be utilized for composting, fiber extraction, animal feed, extraction of bioactive compounds, and production of value-added products like cellulose [3]. Utilization of such wastes can reduce environmental problems and in return, it can create additional economic opportunities for farmers and small-scale industries [4]. Pineapple contributes to the agricultural sector and promotes cultural identity of Manipur. Promoting improved practices of pineapple cultivation, post-harvest management, processing facilities and scientific research can further increase the pineapple's contribution to the economy of Manipur [5]. It is an important link between agriculture, rural livelihood, nutrition and sustainability of resource utilization [6].

Method

Reverse-phase HPLC method for determination of Vitamin C from Queen and Kew varieties of pineapple

A Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method developed by Csaba Horvath(1970) for determining of Vitamin C from Queen and Kew varieties of pineapple quantifies the vitamin in extracted pineapple sample by passing through a non-polar column [7]. It is followed by isocratic elution on a C_{18} column with UV detection at 254nm. It then measures the concentration against a calibration curve [8].

a. Reagents and solvents

HPLC-grade water, methanol, and acetonitrile were used as solvents. 1-hexanesulfonic acid sodium salt which is an ion-pairing agent and orthophosphoric acid were used as reagents. 3% metaphosphoric acid which is a weak acidic solution was used to prevent oxidation of vitamin C. For mobile phase, 0.1% aqueous orthophosphoric acid and methanol were used and P^H was adjusted between 2.2 - 2.8 to suppress ionization.

b. Sample Preparation

The core part of ripe and unripe Queen and Kew varieties of pineapple were taken out, weighed and extracted in methanol extraction solution using a sonicator. The supernatants were passed through 0.45 µm membrane syringe filter and loaded to the HPLC vials.

c. Conditions

Column should be C_{18} or C_8 reverse-phase column and size of particle must be 5 µm and temperature of the column should range from 20ºC - 30ºC. Injection Volume can hold from 10

µL to 20 µL and flow rate should be from 0.7 to 1.0mL/min . The detection of UV/Vis was measured at 243nm to 255nm.[9]

d. Calibration of Standard Solution

A stock solution of L-ascorbic acid was prepared as standard solution in the mobile phase. The standard solution and pineapple sample were injected and elution of vitamin C was observed at a retention time after 4 minutes.[10]

- The Calibration equation : y=mx+c

  where, x = concentration of compound in the injected extract

  y = peak area
  m = slope
  c = intercept

e. Calculation

The concentration of the peak areas obtained from pineapple samples were compared with the standard curve of pure L-ascorbic acid. The concentration in the pineapple sample was calculated by taking the dilution factor, extraction volume and weight of pineapple sample.[11]
Compound concentration = C×V×DF / W
Where:
C = concentration obtained from calibration curve
V = final volume of extract
DF = dilution factor
W = sample extract

Results and Discussion

Reverse-phase HPLC method for determination of Vitamin C from the core of ripe and unripe Queen and Kew varieties of pineapple

The Reverse phase HPLC analysis gave a chromatogram which comprised of a distinct peak corresponding to ascorbic acid. Using the authentic and standard ascorbic acid, the retention time was established. The peak area of the vitamin C increased with the increasing concentration of the ascorbic acid standard. The vitamin C content of ripe and unripe Queen and Kew varieties were calculated from the calibration curve.

Sl. no	Sample Code (Pineapple)	Site (Manipur)	Status	Amount (ppm)	Amount in 1 mg of extract
1	Queen Core	Andro	Ripe	141.747	28. 2 µg
2	Queen Core	Andro	Unripe	135.452	27.0 µg
3	Kew Core	Waithou Chiru	Ripe	143.156	28.6 µg
4	Kew Core	Waithou Chiru	Unripe	131.341	26.2 µg

Table1: Vitamin C Content in the core part of ripe and unripe Queen and Kew varieties of pineapple

Calibration Details	VITAMIN C					
Calibration Type	Lin, WithOffset			*Offset (C0)*		-14.7458
Evaluation Type	Area			*Slope (C1)*		0.1180
Number of Calibration Points	5			*Curve (C2)*		0.0000
Number of disabled Calibration Points	0			*R-Square*		0.9952

Table2: Calibration results of Vitamin C

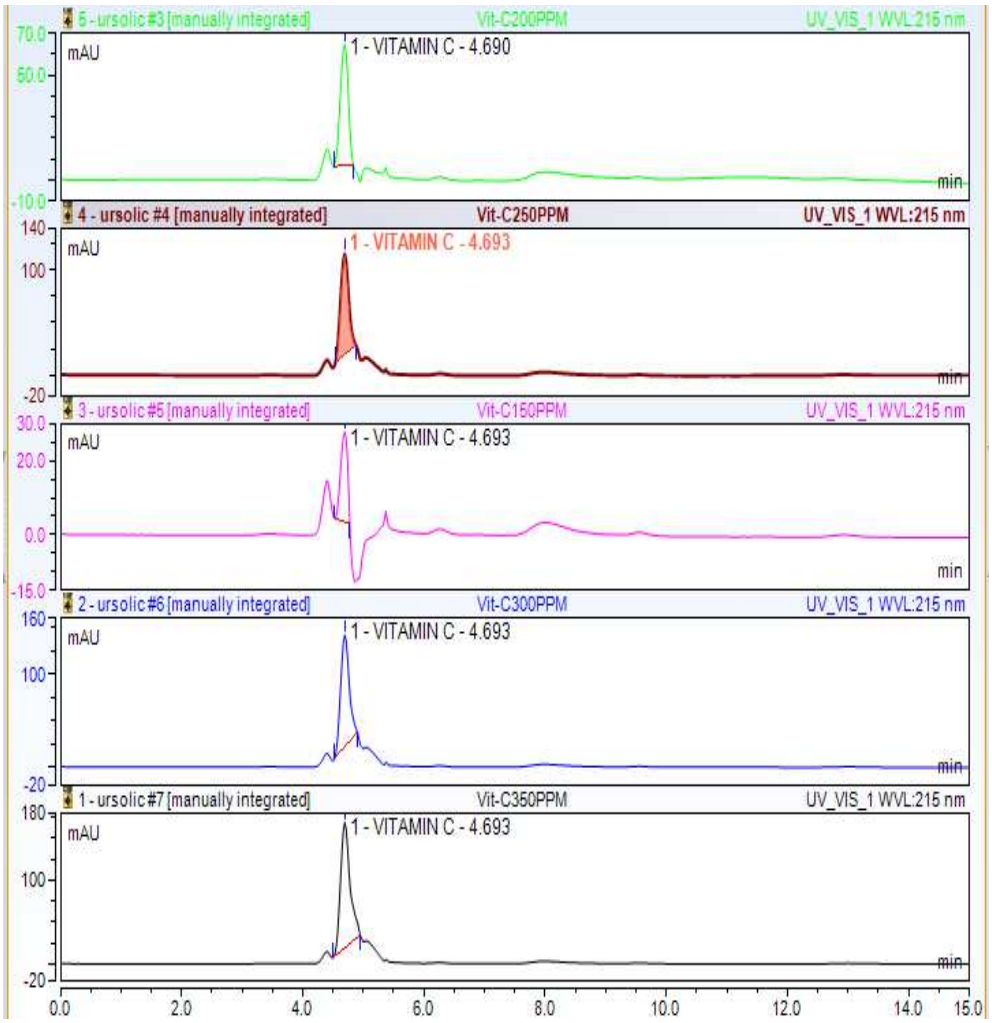


Fig1: Chromatogram of the standard vitamin C

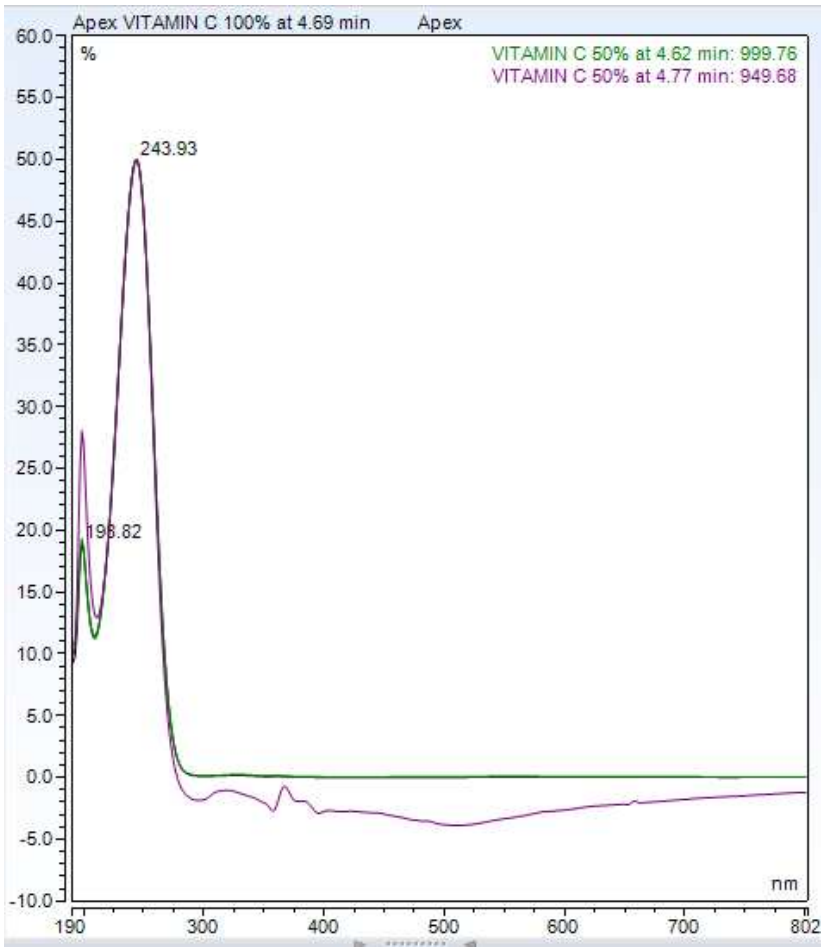


Fig2: Peak of Vitamin C at Lamda max 243

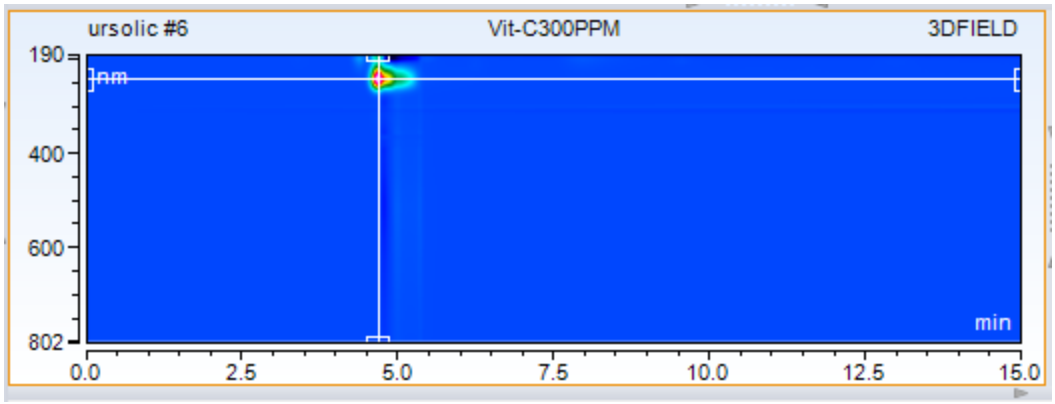


Fig3: 3D field of Vitamin C Detection at 210 nm.

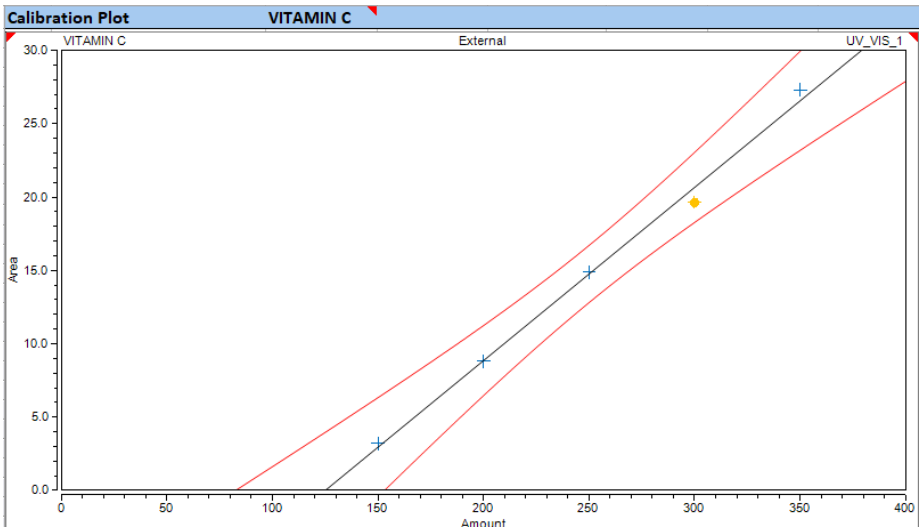


Fig4: Calibration details of Vitamin C

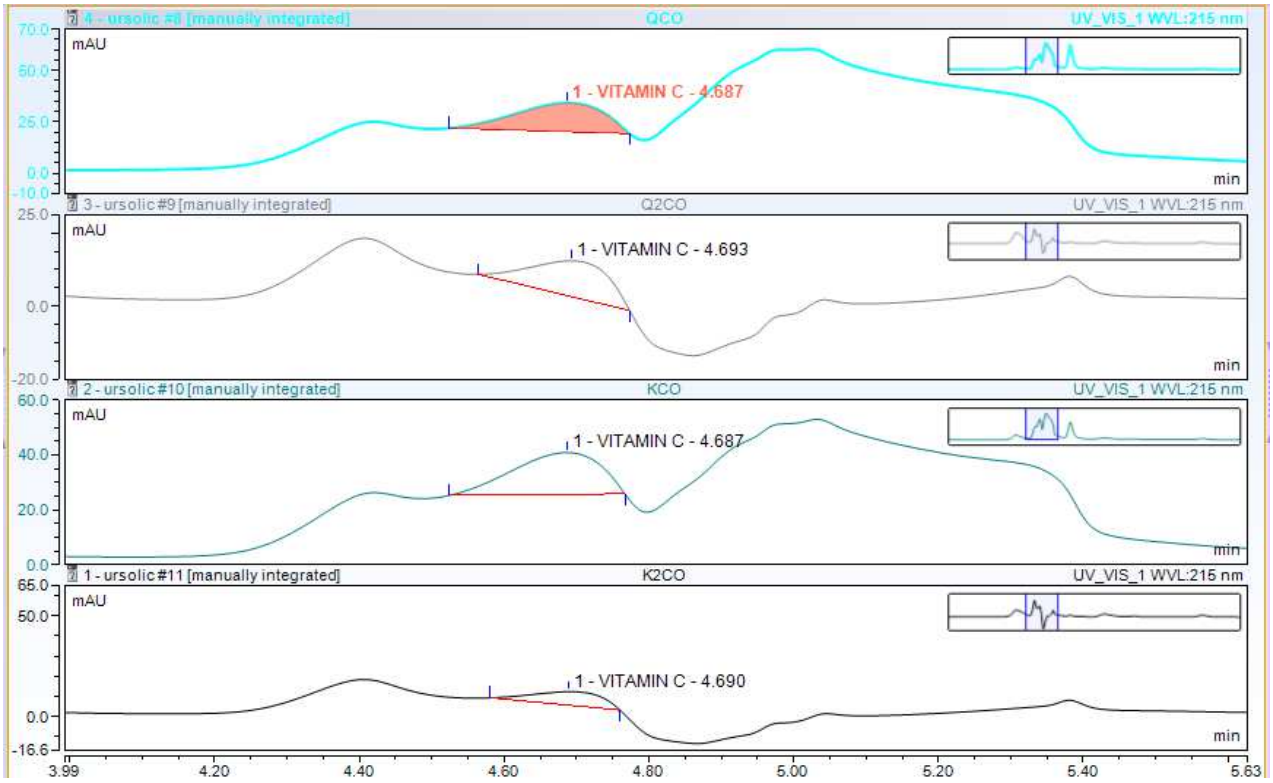


Fig5: Chromatogram showing peak of vitamin C against retention time.

By Component		VITAMIN C				
No.	Injection Name	Ret.Time min UV_VIS_1 VITAMIN C	Area mAU*min UV_VIS_1 VITAMIN C	Height mAU UV_VIS_1 VITAMIN C	Amount PPM UV_VIS_1 VITAMIN C	Rel.Area % UV_VIS_1 VITAMIN C
3	Vit-C200PPM	4.690	8.806	58.184	199.532	100.00
4	Vit-C250PPM	4.693	14.883	96.071	251.015	100.00
5	Vit-C150PPM	4.693	3.199	24.378	152.031	100.00
6	Vit-C300PPM	4.693	19.632	120.487	291.252	100.00
7	Vit-C350PPM	4.693	27.295	150.453	356.171	100.00
8	QCO	4.687	1.985	14.124	141.747	100.00
9	Q2CO	4.693	1.242	9.854	135.452	100.00
10	KCO	4.687	2.152	15.310	143.156	100.00
11	K2CO	4.690	0.757	6.813	131.341	100.00

Table3: Quantification of Vitamin C and pineapple sample using RP-HPLC

	A	B	C	D	E	F	G	H	I	J	K	L	M
	Inj. No.	Injection Name Selected Peak:	Type	Ret.Time min UV_VIS_1 VITAMIN C	Amount PPM UV_VIS_1 VITAMIN C	Rel.Area % UV_VIS_1 VITAMIN C	Area mAU*min UV_VIS_1 VITAMIN C	Height mAU UV_VIS_1 VITAMIN C	Type UV_VIS_1 VITAMIN	Width (50%) min UV_VIS_1 VITAMIN C	Asym. EP UV_VIS_1 VITAMIN C	Resol. EP UV_VIS_1 VITAMIN C	Plates EP UV_VIS_1 VITAMIN C
	3	Vit-C200PPM	Calibration	4.690	199.5315	100.00	8.8060	58.18	BMB*	0.148	0.94	n.a.	5527
	4	Vit-C250PPM	Calibration	4.693	251.0146	100.00	14.8829	96.07	BMB*	0.148	1.06	n.a.	5567
	5	Vit-C150PPM	Calibration	4.693	152.0311	100.00	3.1993	24.38	BMB*	0.133	0.79	n.a.	6930
	6	*Vit-C300PPM*	*Calibration*	*4.693*	*291.2518*	*100.00*	*19.6323*	*120.49*	*BMB**	*0.150*	*1.16*	*n.a.*	*5389*
	7	Vit-C350PPM	Calibration	4.693	356.1709	100.00	27.2950	150.45	BMB*	0.157	1.30	n.a.	4923
	8	QCO	Unknown	4.687	141.7468	100.00	1.9854	14.12	BMB*	0.142	0.78	n.a.	6042
	9	Q2CO	Unknown	4.693	135.4515	100.00	1.2423	9.85	BMB*	0.126	0.83	n.a.	7721
	10	KCO	Unknown	4.687	143.1556	100.00	2.1517	15.31	BMB*	0.143	0.76	n.a.	5943
	11	K2CO	Unknown	4.690	131.3409	100.00	0.7571	6.81	BMB*	0.112	0.83	n.a.	9798
Maximum				4.693	356.1709	100.00	27.2950	150.45		0.157	1.30	0.00	9798
Average				**4.691**	**200.1883**	**100.00**	**8.8836**	**55.08**		**0.140**	**0.94**	**n.a.**	**6427**
Minimum				4.687	131.3409	100.00	0.7571	6.81		0.112	0.76	0.00	4923
Standard Deviation				0.003	81.4900	0.00	9.6187	54.36		0.014	0.19	n.a.	1523
Relative Standard Deviation				**0.06%**	**40.71%**	**0.00%**	**108.28%**	**98.70%**		**10.21%**	**20.49%**	**n.a.**	**23.70%**

Table4: Calibration details of vitamin C and pineapple sample

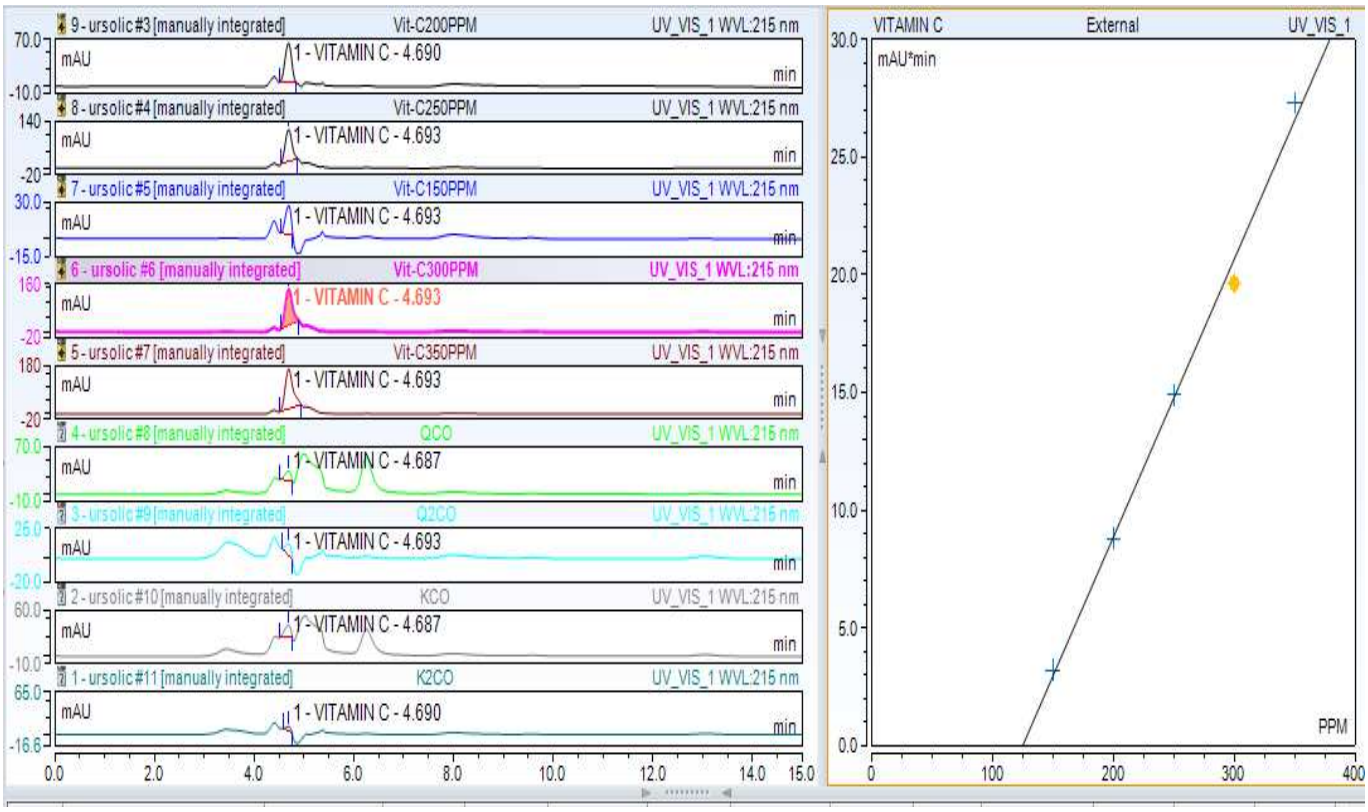


Fig6: RP-HPLC chromatogram of vitamin C and its calibration curve

The different concentrations of vitamin C at 150-350PPM shows the Vitamin C peak at 4.693 retention time. Kew pineapple has 143.156 PPM while Queen pineapple has 141.747 PPM of vitamin C. The reverse phase HPLC quantification indicates that the core of ripe pineapple contains higher vitamin C levels (ranging from 141.7 to 143.2 PPM) than the core of unripe stage of pineapple (131.3 to 135.5 PPM). The core part of ripe Kew variety from Waithou Chiru site shows higher vitamin C than the core part of ripe Queen variety. This suggests that for maximum nutritional benefit regarding vitamin C, the fruit should be harvested and consumed at the ripe stage.

Conclusion:

Pineapple is an economically important fruit which is valued for its potential health benefits and nutritional content [12]. Among the nutrients, vitamin C which is chemically known as L-ascorbic acid acts as antioxidant that prevents biological molecules from oxidative damage [13]. The concentration of vitamin C is influenced by factors such as fruit maturity, storage temperature, exposure to heat, light, etc [14]. Vitamin C content in pineapple increases as the level of the ripeness increases. Several analytical methods have been employed for the determination of vitamin C such as spectrophotometry, titration method and chromatography techniques [15]. Out of these techniques, RR-HPLC is used as advanced separation technique that separates ascorbic acid from other constituents before quantification [16].

Reverse-phase HPLC provides a reliable result for identifying the chemical profile of pineapple. It is a powerful analytical method for characterization of pineapple since it gives information on identification, efficient separation and quantification of chemical constituents [17]. It can be used to compare two different cultivars of pineapple such as Queen and Kew variety and investigate vitamin C and many other phytochemicals present. For vitamin C content, RP-HPLC methods using C18 columns with aqueous phosphoric acid as mobile phase and UV detection at 245nm has been done to determine ascorbic acid content in Queen and Kew varieties of pineapple. The acid addition to the mobile phase can improve the peak shape and chromatographic behavior for many compounds like phenol. High or moderate vitamin C content in pineapple is influenced by maturity of the fruit, cultivar and parts of the fruit [18]. So, Queen and Kew variety of pineapple exhibit different chromatographic behavioral and peak slope even when they analyzed under the same condition. The two different varieties of pineapple at different stages of ripeness show substantial variations of vitamin C concentrations which depends on the maturity and early phase of the fruit sample, growing conditions, genotype, environmental factors and also in extraction conditions[19]. Reverse-phase HPLC provides a detailed chemical profile of pineapple and identified its nutritional value, quality assessment, antioxidant properties [20]. Pineapple has a valuable source of vitamin C, minerals and bioactive compounds which is accounted to its high quality content [21]. Consumption of pineapple can contribute to the improvement of human health, economic growth and a sustainable development[22].

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Author Contributions

Jenny R.: Literature Search, Writing Original Draft, Data Evaluation, Structure Design, Writing-Reviewing and Editing. Kh. Susheela: Writing-Reviewing and Editing, Conceptualization, Data Evaluation. All authors read and approved the final manuscript.

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Ethical declarations

The sample examined in this study comprised two varieties namely Queen and Kew varieties of pineapple (*Ananas comosus* (L.)Merr). The Queen pineapple was collected from Andro, Manipur and the Kew variety was collected from Waithou Chiru, Manipur, India. The local names assigned to the pineapple accessions were compiled in accordance with locally recognized and nationally applicable guidelines which were documented by the Department of Horticulture,

Government of Manipur, India. The collection sites of pineapple are situated on government-owned land area and accessible to local people and researchers. So, no specific permissions, licences, collection permits were required for this study. Moreover, the Queen and Kew varieties of pineapple are not listed among the endangered or legally protected plant species since they are grown vastly in Manipur. The conservation, handling and utilization of the plant species were conducted in accordance with national guidelines governing the use and collection of plant resources which includes Biological Diversity Act and relevant associated regulations, wherever applicable. The collected plant species were used exclusively for scientific researches and not subjected to commercial exploitation. To ensure compliance with applicable governmental requirements, appropriate care was taken during the collection, handling, maintenance and utilization of the plant species.

Consent to participate and publish

Not applicable

Competing Interests

The authors declare that there are no competing interests.

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