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NEW ANALYTICAL METHODS FOR THE DETERMINATION OF ASCORBIC ACID CONTENT IN AQUEOUS EXTRACTS OF FLESH, PEEL AND SEEDS OF PUMPKIN (*CUCURBITA MAXIMA*)

Mulu Hagos, Mesfin Redi-Abshiro*, Bhagwan Singh Chandravanshi and Estifanos Ele Yaya

Department of Chemistry, College of Natural and Computation Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

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ABSTRACT. The main objective of this research was to develop a simple, rapid, cost-effective, and environmentally friendly methods for determining ascorbic acid in flesh, peel, and seeds of pumpkin. Ascorbic acid was extracted from the different parts of the pumpkin in water. The content of ascorbic acid in three parts of the pumpkin was determined using ATR-FTIR and UV-VIS methods. The ATR-FTIR method was validated to determine ascorbic acid at 1046 cm^{-1}, and the results indicated a linear range of 5 to 50 g/L; R^2, 0.999; LOD, 1.7 g/L; LOQ, 5.2 g/L; RSD, 0.09% to 0.65%, and %recovery of 95.86%. Ascorbic acid was also determined at 265 nm by the UV-VIS method that revealed a linear range of 1 to 12 mg/L; R^2, 0.999; LOD, 0.25 mg/L; LOQ, 0.75 mg/L; RSD, 0.12% to 0.43%, and %recovery of 96.96%. Accordingly any of the two newly developed methods can easily be applied for quantitative determination of ascorbic acid in pumpkin flesh, peel, and seeds. Both methods yielded higher ascorbic acid content in pumpkin seeds than in pumpkin flesh and peel. Both ATR-FTIR and UV-VIS spectroscopic methods are green, low cost and rapid method. However, UV-VIS method showed higher sensitivity than the ATR-FTIR method.

KEY WORDS: Pumpkin, *Cucurbita maxima*, Ascorbic acid, ATR-FTIR spectroscopy, UV-VIS spectrophotometry

INTRODUCTION

Plants are natural sources of many components, responsible to cure various kinds of diseases. In the modern lifestyle, use of synthetic drugs has gained importance but their adverse effects are always present in the human beings. The role of plants, especially fruits and vegetables are highly recognized towards good health and reduced risk of diseases [1]. Citrus fruits, strawberries, green peppers, red peppers, tomatoes, broccoli, turnip, and pumpkin are rich sources of ascorbic acid [2].

Pumpkin, belonging to the genus Cucurbita and family Cucurbitaceae refers to any one of the species: *Cucurbita moschata*, *Cucurbita mixta*, *Cucurbita maxima*, and *Cucurbita pepo*. Pumpkin (*Cucurbita maxima*) is mostly used to refer to cultivars with round fruits which are used in the mature state for baking or feeding livestock in Ethiopia. Pumpkin is among the economically most important vegetable crops worldwide and is grown in both temperate and tropical regions. Peel, flesh and seeds can be used for food. Pumpkin different parts are rich source of biologically active compounds like vitamins, beta-carotens, phenolics, flavonoids, flavones, tocopherols and tocotrienols [1, 3, 4].

Vitamins are organic components that the human body need to maintain healthy life. Because these compounds cannot be manufactured in the human body, they must be obtained through diet [5]. Vitamins are split into two groups: fat-soluble vitamins and water-soluble vitamins. Vitamins A, D, E, and K are fat-soluble chemical compounds, while vitamins C and the entire vitamin B complex are water-soluble compounds. L-ascorbic acid is the most biologically active form of vitamin C. Ascorbic acid, also known as hexuronic acid, is an organic compound having the formula $C_6H_8O_6$ [6]. It is a valuable food component because of its antioxidant and therapeutic

*Corresponding author. E-mail: mesfin.redi@aau.edu.et

properties. As a potent antioxidant, it has the capacity to eliminate several different free radicals. It also has important biological and metabolic functions, particularly with respect to its role in the biosynthesis of connective tissue. Ascorbic acid aids the metabolism of tyrosine, folic acid, and tryptophan. Ascorbic acid lowers LDL (bad) cholesterol and triglyceride levels in the blood, which are both risk factors for heart disease [6-8].

Scurvy, anemia, and numerous infections and mental illnesses are all caused by a lack of ascorbic acid in the body [6]. However, according to Pisoschi *et al.* [9], too much ascorbic acid can cause stomach discomfort, and some of its metabolites, such as oxalic acid, can cause renal issues. Furthermore, if used in excess, it can block natural food processes and contribute to the loss of taste and scent in food and beverages.

The FDA recommendations for vitamin C intake are 75–90 mg daily for adult females and males, respectively, with increase of 10 mg for pregnancy, 45 mg for lactation, and 35 mg for smokers. Children require around 15–45 mg daily and adolescents 65–75 mg, while infants (12 months or less) require 40–50 mg daily. While youth do not have differences in dosage based upon gender until adolescence is reached. Vitamin C is outright vital for cognitive development of infants during pregnancy, and there are higher dietary requirements of vitamin C during pregnancy and lactation which have to be met wholly through dietary intake of vitamin C, and any deficiency may result in cognitive impairment to the child [10]. Ascorbic acid is also useful as a nutritious food additive, a reducing agent, a stabilizer, and a color stabilizer [6, 9]. As a result, figuring out what this molecule is crucial for pharmaceutical and biological purposes.

Different analytical methods have been reported for the determination of ascorbic acid in fruits and vegetables. These include HPLC-UV [8, 11, 12], titration [13-16] and voltammetry [17, 18]. However, HPLC-UV and voltammetric methods are expensive, complicated, laborious, destructive, and time-consuming methods that require skilled personnel and large amounts of solvents and reagents. While titration methods are not selective and less sensitive.

Mid-FTIR-ATR method has been proposed and proven to be an excellent alternative to traditional methods due to the simple, rapid and non-destructive technique which is playing a great role for the rapid determination of various components present in different samples [19, 20].

Mallah *et al.* [20] employed ATR-FTIR spectroscopy to determine the protein and tannin content of sorghum grains on a qualitative and quantitative level. FTIR-ATR spectroscopy was also used by Anjos *et al.* [21] to determine the flavonoid/phenolic acid profile in bee pollen samples. Sherazi *et al.* [22] developed the ATR-FTIR method for rapid screening of total polyphenols in lemon juice samples using infrared spectral data in combination with multivariate calibration, eliminating the use of toxic solvents and providing the benefit of green chemistry. Clark [23] used ATR-FTIR method for determining soluble solids and titratable acidity in citrus juices. Valderrama and Rojas [24] used an ATR-FTIR spectroscopic technique to quantify three active chemicals (carvacrol, thymol, and *p*-cymene) in thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*). According to Mohamed *et al.* [25], sugars, pectin, and organic acid (citric acid) levels were all detected in natural and processed fruit products at the same time. Domínguez-Martínez *et al.* [26] have reported the determination of capsaicin, ascorbic acid, total phenolic compounds, and antioxidant activity of pepper (*Capsicum annuum* L.) using the Mid-FTIR method.

UV-Visible spectrophotometry was utilized to determine ascorbic acid in a variety of matrices using a variety of reagents and organic solvents. The determination of ascorbic acid utilizing bromine water in the presence of acetic acid coupling with 2,4-dinitrophenylhydrazine was reported in the literature [27-31]. Abera *et al.* [32] published their findings based on the effect of ascorbic acid on the absorbance of hexavalent chromium solution in the presence of catalytic amounts of Mn(II). Pancham *et al.* [33] developed and evaluated a UV-VIS method for determining ascorbic acid in bulk powder using methanol:water (50:50 v/v) as a solvent.

However, there is no report in the literature on the use of Mid-FTIR-ATR spectroscopy and UV-spectrophotometry for determining ascorbic acid in the aqueous extracts of pumpkin flesh,

peel, or seed samples. Thus the purpose of this work was to develop rapid, cost-effective, and environmentally friendly analytical methods for determination of ascorbic acid in the aqueous extract of pumpkin flesh, peel, and seed. The direct assessment of ascorbic acid in aqueous extract of pumpkin flesh, peel, and seed was done in this study using Mid-FTIR-ATR spectroscopic and UV-spectrophotometric methods. The results obtained by the newly developed methods were compared with that of the redox titration method reported by Satpathy *et al.* [34].

EXPERIMENTAL

Instruments and apparatus

The standards and samples were weighed using an electronic balance (ARA520, OHAUS CORP., China). Dry pumpkin samples were ground with a mortar and pestle. Magnetic stirrer with hot plate (04803-02, USA) was used to stir the mixtures magnetically. The samples were centrifuged using a centrifuge (800D, China). FTIR spectrometer (Perkin Elmer, spectrum 65 Spectrophotometer, USA) with a sample holder of zinc selenide crystal in the attenuated total reflectance (ATR) mode were used for determination of ascorbic acid. In addition, a double-beam spectrophotometer (Lambda 950-UV-Vis-NIR, Perkin Elmer, UK) interfaced with a computer using 2 nm resolution in a 1 cm path length quartz cell were used for determination of ascorbic acid. The titration of ascorbic acid was done with a burette (Super Tec 25 mL, Switzerland).

Chemicals

Standard ascorbic acid (ACS reagent, reagent ISO, Ph. Eur., 99.7-100.5%), potassium iodide (98.5%, EU), iodine (99.9%, England) and starch (90%, India) were used in the study. Distilled water was used as solvent in the preparation of standard and sample solutions.

Preparation of ascorbic acid standard solutions for ATR-FTIR

To make a 50 g/L standard stock solution, 2.5 g of ascorbic acid was dissolved in 50 mL volumetric flask in distilled water. From this stock solution, serial dilutions were made to obtain 5, 10, 20, 30, 40, and 50 g/L of ascorbic acid solution. About 1 mL of the standard solution was placed in the zinc selenide crystal to fully cover its surface for the spectral measurements. For the identification purpose the standard solutions of ascorbic acid were scanned in the reasonably free spectral range (900 to 2000 cm^{-1}) chosen for this study. The working standard solutions were scanned in triplicate in the range of (950 to 1100 cm^{-1}) and the band corresponds to the C-O-C stretching at 1046 cm^{-1} was used for plotting the calibration curve.

Preparation of ascorbic acid standard solutions for UV-VIS spectrophotometry

To obtain 1000 mg/L of stock solution, a 100 mg of ascorbic acid was weighed and placed into a 100 mL volumetric flask, and the volume was made up to the mark using distilled water. From this stock solution, serial dilutions were made to obtain 1, 3, 6, 9, and 12 mg/L solutions of ascorbic acid. The working standard solutions were scanned in the spectral range (200–400 nm) selected for this study. The absorption spectral data were collected from their typical absorption peak maximum obtained at 265 nm for plotting the calibration curve according to Pancham *et al.* [33].

Pumpkin sample collection and preparation

Riped pumpkins (*Cucurbita maxima*) were collected from a local market in Addis Ababa, Ethiopia, in February 2021. The procedure reported by Hagos *et al.* [4] was used to carry out the

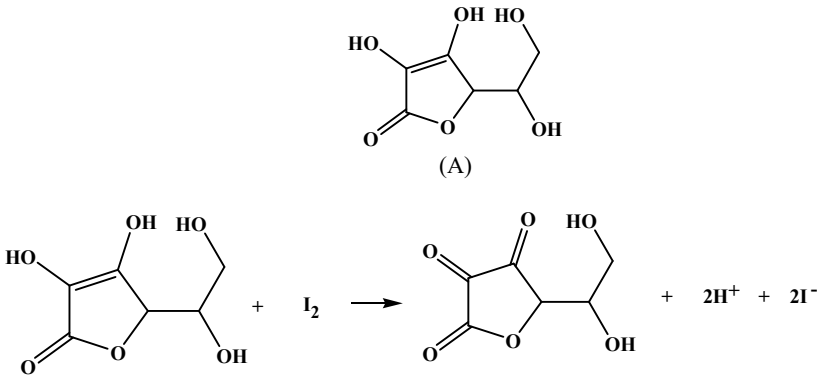
sample preparation process. To remove any foreign matter, pumpkins were cleaned in distilled water. After that, it was sliced into two parts and the seeds were carefully separated. The peeling procedure was done sequentially with a knife because the pumpkin peel and flesh are two distinct layers. The three parts (peel, flesh, and seed) of pumpkin were thoroughly cleaned to remove any fibrous waste. Comminuting and passing small sized pumpkin flesh, peel, and seed parts through a screen with 6 mm holes assisted the drying process. All the samples were dried at room temperature for seven days until they reach a constant weight. The dried sample were ground and screened through 250 μm sieve to get a uniform texture. Finally, the powdered samples were used to extract ascorbic acid.

Extractions of ascorbic acid from pumpkin peel, flesh and seed samples

Accurately weighed 5 g of powdered pumpkin peel, flesh and seed samples were dissolved in distilled water. The solution was stirred continuously using magnetic stirrer over a hot plate for 30 min at room temperature to facilitate the solubility of ascorbic acid. The mixture was filtered through Whatman filter paper number 41 (110 mm). The filtrate was transferred into clean dried centrifuge tubes and centrifuged at 4,000 rpm for 10 min at room temperature. The supernatant was collected into vials while the residue was discarded. Finally, the extract was directly used for qualitative and quantitative analysis using ATR-FTIR and UV-VIS spectroscopic methods.

Procedure for redox titration using iodine solution

Redox titration using iodine solution was used to determine ascorbic acid concentration in the extract of flesh, peel, and seed of pumpkin samples according to the method described by AL Majidi and Y-AL Qubury [29] with minor modification. Iodine solution was added in the course of titration; ascorbic acid was oxidized to dehydroascorbic acid, while the iodine was reduced to iodide ions (Figure 1). Accordingly, the added iodine is instantly reduced to iodide as long as there is any ascorbic acid present in the solution. When all the ascorbic acid has been oxidized, the excess iodine is free to react with the starch indicator, forming a blue-black starch-iodine complex indicating the endpoint of the redox titration.



(B) $C_6H_8O_6 + I_3^- + H_2O \rightarrow C_6H_6O_6 + 3I^- + 2H^+$

Figure 1. (A) Sructure of ascorbic acid and (B) oxidation of ascorbic acid with iodine.

To prepare iodine solution (0.005 M) 2 g of potassium iodide was weighed and transferred in to 100 mL beaker and 1.3 g of iodine was added to it. A few mL of distilled water was added to dissolve the iodine, which was mixed well for a few minutes. The iodine solution was transferred to a 1 L volumetric flask and diluted with distilled water to the 1 L mark. A 0.5% starch indicator solution was prepared by weighed 0.25 g of soluble starch and mixed it with 50 mL of near boiling water in a 100 mL conical flask. The solution was stirred to dissolve and cooled before using.

Method validation

In this study, new ATR-FTIR and UV-VIS methods for quantifying ascorbic acid in pumpkin flesh, peel, and seed samples were developed and validated according to ICH guidelines for selectivity, linearity range, limit of detection (LOD) and limit of quantification (LOQ), precision, and accuracy.

By comparing the spectra of sample extracts to standard ascorbic acid, the selectivity of both ATR-FTIR and UV-VIS spectrometric methods were assessed. To verify the linearity of the newly proposed ATR-FTIR method, a series of standard ascorbic acid solutions in the concentration range of 5 to 50 g/L were prepared. Another series of standard ascorbic acid solutions in the concentration range of 1 to 12 mg/L were used to test the linearity of the newly designed UV-VIS method. Concentration vs. absorbance were plotted to establish calibration curves for the two methods. The correlation coefficient were computed using the regression equations to determine linearity.

The least amount of analyte that can be detected is referred to as the LOD. The LOQ is the least amount of analyte that can be quantified accurately and precisely. The standard deviation and slope were calculated using the linearity of calibration curve. LOD and LOQ were calculated from (3 × SD)/s and (10 × SD)/s, where SD and s were standard deviation of blank measurement (n = 6) and slope of calibration curve, respectively.

For both the newly developed ATR-FTIR and UV-VIS spectrometric methods, precision was assessed using repeatability, intra-day, and inter-day precision. Repeated measurements of a standard ascorbic acid solution (n = 6) were used to determine repeatability. Intra-day precision was determined by evaluating three duplicates of solutions containing varied concentrations of ascorbic acid and computing %RSD at various time intervals on the same day. Analysis of solutions containing varying concentrations of ascorbic acid in three replicates and calculation of the %RSD on three distinct days were used to estimate inter-day precision.

Using the standard addition method, the accuracy of the newly proposed FTIR-ATR and UV-VIS spectrometric methods were assessed. A known concentration of ascorbic acid standard solution was added to the pumpkin flesh, peel, and seed samples. The spiked solutions were analyzed three times in order to achieve an average recovery. The following formula was used to compute the percent recovery: $((C_S - C)/C_A) \times 100 = \%R$, where C_S is the concentration of the spiked sample, C means the concentration of the unspiked sample, and C_A the concentration of the spiked ascorbic acid.

RESULTS AND DISCUSSION

Determination of ascorbic acid content in pumpkin peel, flesh, and seed using Mid-FTIR-ATR method

Identification. A new ATR-FTIR method for quantifying ascorbic acid in pumpkin peel, flesh, and seed samples was developed using a liquid sampling methodology. The solvent for the entire ascorbic acid extraction method was distilled water. The ATR-FTIR spectrum of ascorbic acid was scanned from 900 to 2000 cm^{-1} (Figure 2). The absorption band at 1758 cm^{-1} corresponded to the stretching modes of the C=O functional groups. The absorption band found at 1688 cm^{-1} is

caused by C=C stretching modes. The absorption band at 1350 cm^{-1} is caused by C-O-H. There is a distinct band at 1146 cm^{-1} due to C-O-C stretching.

The peak at 1046 cm^{-1} corresponds to the C-O-C stretching vibrational mode. A slight shift in the absorption band in the ATR-FTIR spectra of sample extract and standard ascorbic acid is due to variation in the chemical environment of sample extracts. As can be seen in Figure 3, the peak in the absorption spectra of standard ascorbic acid and sample extract is almost matched at 1046 cm^{-1}, this absorption band was selected for quantitative determination of ascorbic acid. The newly developed ATR-FTIR method's selectivity is demonstrated by overlapping standard and sample spectra at 1046 cm^{-1}, which clearly shows the decrease in matrix interference to negligible levels.

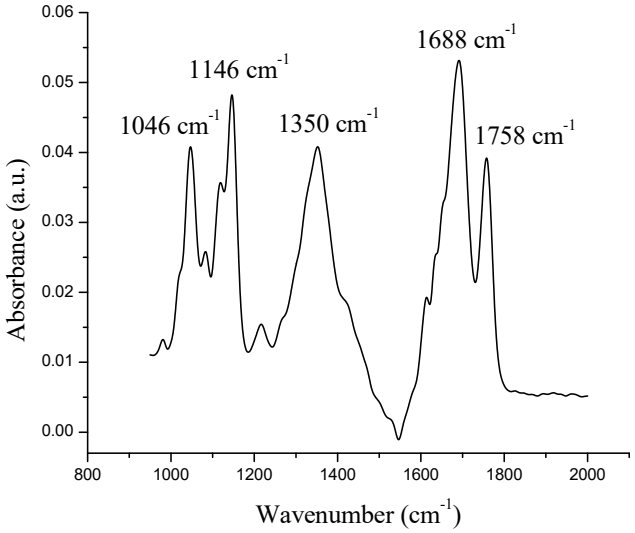


Figure 2. ATR-FTIR spectrum of standard ascorbic acid in distilled water (20 g/L).

Method validation for ATR-FTIR. The method was tested for linearity in a concentration range of 5 to 50 g/L. With a regression equation of y = 0.00129 + 0.02311x and a regression coefficient of R^2 = 0.999, the method demonstrated good linearity. The linear relationship between peak intensity and ascorbic acid concentration is shown in (Figure 4). Furthermore, the detection and quantification limits were found to be 1.7 and 5.2 g/L, respectively. The RSD for method repeatability was 0.09%. RSDs of 0.13% and 0.65% were found for intra-day and inter-day precision, respectively. The accuracy of the method were tested by adding a known amount of standard to the sample, and the results indicated good percent recovery (%R) of 90.9±0.4% to 99.7±0.6% (Table 1).

Table 1. Analytical parameters of the proposed ATR-FTIR method such as wavenumber, concentration range, regression equation, R^2, LOD, LOQ, RSDs, and recoveries.

Wavenumber (cm^{-1})	Concentration range (g/L)	Regression equation	R^2	LOD (g/L)	LOQ (g/L)	%RSD	%R
950-1100	5-50	y = 0.00129 + 0.02311x	0.999	1.7	5.2	0.09-0.65	90.9 ± 0.4-99.7 ± 0.6

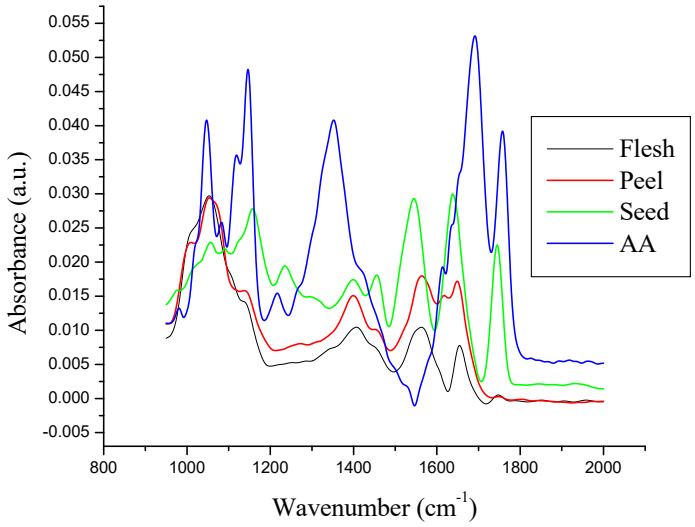


Figure 3. ATR-FTIR spectra of standard ascorbic acid (AA) and sample extracts in distilled water.

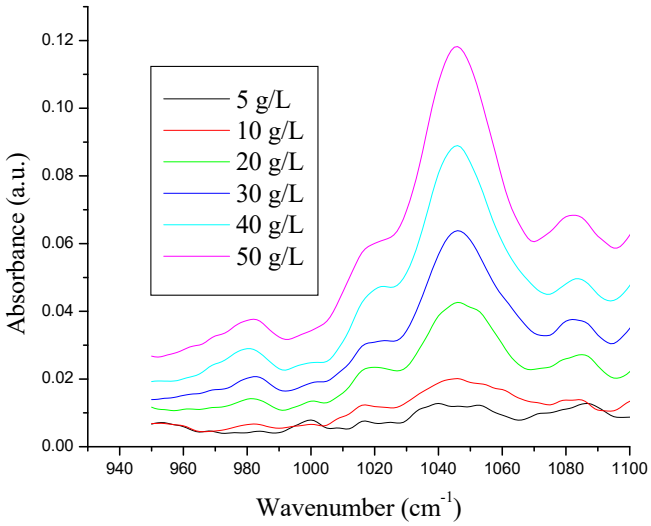


Figure 4. ATR-FTIR spectra of different concentrations of standard ascorbic acid in distilled water.

ATR-FTIR method for real sample analysis. The ATR-FTIR spectroscopy method was used to measure the amount of ascorbic acid in the pumpkin flesh, peel, and seed samples. Using a regression equation, the ascorbic acid content (mg/100 g) were estimated. Table 4 demonstrates that pumpkin seeds (32±0.7 mg/100 g) had the highest concentration of ascorbic acid, followed by pumpkin flesh (27±0.5 mg/100 g) and peel (24±0.3 mg/100 g). There is no published report on the determination of ascorbic acid in pumpkin using the ATR-FTIR method to our knowledge.

However, using the FTIR method, Domínguez-Martínez *et al.* [26] found that the ascorbic acid concentration of serrano chilli ranged from 7 to 458 mg/100 g. Li *et al.* [36] identified 13.8, 15.1 and 15.1 mg/100 g of ascorbic acid in the seed, peel, and flesh of spaghetti squash (*Cucurbita pepo* L.), respectively, using an HPLC method.

Furthermore, Amin *et al.* [7] determined the concentration of ascorbic acid in pumpkin peel, flesh, and seed samples from native and hybrid types using the HPLC method. The concentration of ascorbic acid from the indigenous pumpkin was found to be 10, 12.5, and 15 mg/100 g in the pumpkin peel, meat, and seed samples, respectively. In the hybrid pumpkin cultivar, the peel contained (2.5 mg/100 g), the flesh included (39.5 mg/100 g), and the seed contained (10.7 mg/100 g). The concentration of ascorbic acid measured using the ATR-FTIR method in the present investigation was consistent with that measured using the HPLC method in the indigenous pumpkin reported by Amin *et al.* [7]. In both method, high ascorbic acid was detected in the seed parts of the pumpkin, followed by the flesh and peel parts.

Determination of ascorbic acid content in pumpkin peel, flesh, and seed using UV-VIS spectrophotometry method

Identification. The UV-VIS spectra of ascorbic acid in distilled water were scanned from 200 to 400 nm which showed maximal absorption at 265 nm (Figure 5). This was in line with the reported value by Pancham *et al.* [33]. The UV-VIS spectra of pumpkin flesh, peel, and seed extracts had the maximum UV-VIS absorption at the same wavelength as standard ascorbic acid (Figure 6). An overlapping standard and sample spectra, which clearly illustrates the reduction of matrix interference to minimal levels and demonstrates the newly developed UV-VIS method has good selectivity.

Method validation for UV-VIS. The linearity of the method was tested from 1 to 12 mg/L, and it exhibited good linearity with a regression of $y = 0.06609x - 0.00777$ and a regression coefficient $R^2 = 0.9998$ (Table 2). The linear relationship of absorbance with the concentration of ascorbic acid is shown in (Figure 5). Furthermore, 0.25 mg/L and 0.75 mg/L were calculated as the detection and quantification limits, respectively. The RSD% of the method repeatability was 0.12%. RSD 0.16% and 0.43%, respectively, for intra-day and inter-day precision. The accuracy of the method was tested by adding a known concentration of standard solution to the sample, and the results revealed good percent recovery (%R) of 91.3±0.5% to 98.3±0.6%.

Table 2. Analytical parameters of the proposed UV-VIS method such as wavelength, concentration range, regression equation, R^2, LOD, LOQ, RSDs, and recoveries.

Wavelength (nm)	Concentration range (mg/L)	Regression equation	R^2	LOD (mg/L)	LOQ (mg/L)	%RSD	%R
200-400	1-12	y = 0.06609x - 0.00777	0.999	0.25	0.75	0.12-0.43	91.3 ± 0.3-98.3 ± 0.6

UV-VIS method for real sample analysis. The ascorbic acid content in the distilled water extract of pumpkin flesh, peel, and seeds samples was determined using the established UV-VIS method. Using a regression equation, the ascorbic acid content in the sample (mg/100 g) was estimated. As shown in Table 4, the highest concentration of ascorbic acid was found in pumpkin seeds (28±0.4 mg/100 g), followed by flesh (23±0.2 mg/100 g) and peel (22±0.4 mg/100 g) samples. El Shara *et al.* [27] used UV-spectrophotometry with bromine water extraction in the presence of acetic acid coupling with 2,4-dinitrophenyl hydrazine and redox titration techniques to determine the ascorbic acid content in pumpkin flesh. UV-VIS method revealed 30.8 to 48.2 mg/100 g of ascorbic acid, while redox titration method found 18.2 to 38.2 mg/100 g of ascorbic acid. The amounts of ascorbic acid in mango, pawpaw, pear, banana, and plantain were evaluated using titrimetric and spectrophotometric methods with oxalic and orthophosphoric acid as extracting

solvents by Adebayo [30]. The titrimetric method yielded ascorbic acid in the range of 0.20 to 13.20 mg/100 g, while the spectrophotometric method yielded 7.76 to 87.1 mg/100 g.

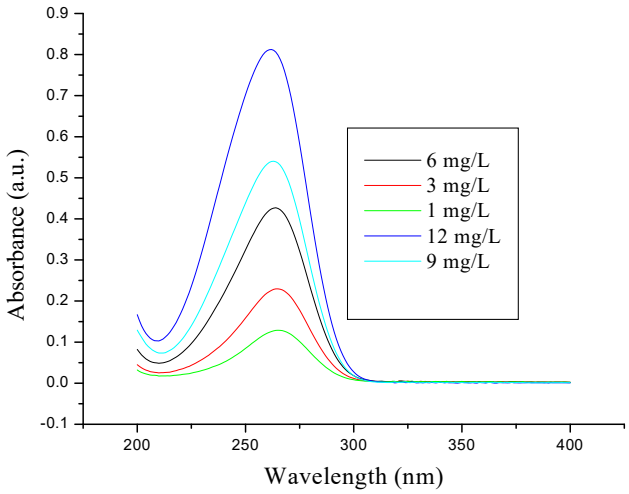


Figure 5. UV-VIS spectra of different concentrations of standard ascorbic acid in distilled water.

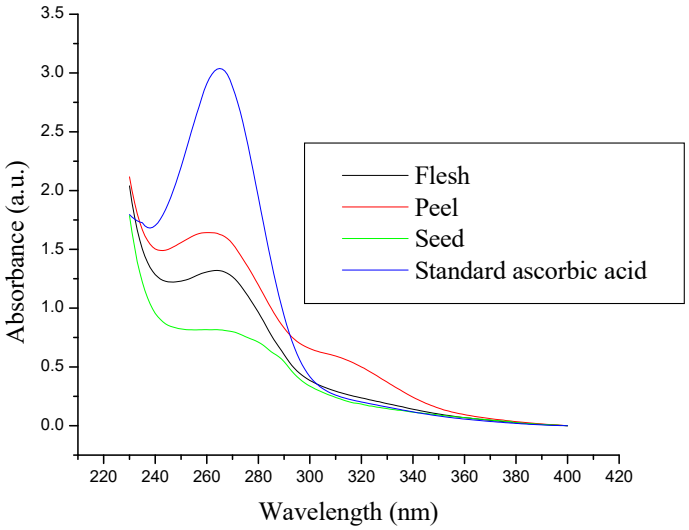


Figure 6. UV-VIS spectra of standard ascorbic acid and pumpkin extracts in distilled water.

In addition, Mehta *et al.* [31] found ascorbic acid levels ranging from 25.9 to 72.1 mg/100 g in fourteen distinct tomato varieties. In the present study, the concentration of ascorbic acid measured using the UV-VIS method was within the range of ascorbic acid in pumpkin flesh using UV-VIS methods and the 2,6-dichlorophenolindophenol (DCPIP) methodology, as described by Adebayo [30].

Comparison of analytical parameters obtained by two newly developed methods and previously published methods for determination of ascorbic acid from different samples

The analytical parameters of the two newly developed methods for determining ascorbic acid in different samples are compared to those of a previously published methods (Table 3). ATR-FTIR and UV-VIS methods for determining ascorbic acid in pumpkin flesh, peel, and seed parts were developed and applied in this research. The identical extraction process was used for all the procedures. The correlation coefficient (R^2), linear range, limit of detection (LOD), limit of quantification (LOQ), precision (%RSD), and percentage recovery (%R) are the analytical parameters of each method (Table 3). ATR-FTIR method has shown good accuracy and precision, and is regarded as a nondestructive, environmentally friendly, low-cost, and quick approach. Furthermore, the UV-VIS method demonstrated high sensitivity, accuracy, and precision, as well as being a green, low-cost, and quick method. In comparison, the new ATR-FTIR method has lower sensitivity than UV-VIS method. Domínguez-Martínez *et al.* [26] have reported the determination of ascorbic acid in pepper (*Capsicum annuum* L.) using the FTIR in conjunction with multivariate analysis. The sample was directly applied to the ZnSe crystal and pressed with a press accessory of the spectrophotometer to obtain the spectrum. While in the present method the spectrum was obtained in aqueous solutions of different parts of pumpkins. The correlation coefficient and precision for determining ascorbic acid using ATR-FTIR are in good agreement with that obtained by the newly developed ATR-FTIR method. Pancham *et al.* [33] developed and applied the UV-VIS method for determining ascorbic acid in bulk powder using methanol:water (50:50 v/v) as a solvent. While in the present method ascorbic acid was extracted in water from the different parts of the pumpkins. The analytical parameters obtained using the newly developed UV-VIS method are in agreement to those obtained by Pancham *et al.* [33]. The sensitivity of both UV-VIS techniques is good. The published UV-VIS method, on the other hand, did not reported the percentage recovery of the method.

Table 3. Analytical parameters of the proposed methods and previously published method for determination of ascorbic acid from different samples.

Samples	Methods	Linear range	R^2	LOD	LOQ	% R	RSD%	Reference
Pumpkin	FTIR	5–50 g/L	0.999	1.7 g/L	5.2 g/L	95.86	0.09–0.65	This study
Serrano chilli	FTIR	NR	0.998	NR	NR	NR	0.098	[26]
Pumpkin	UV-VIS	1-12 mg/L	0.999	0.25 mg/L	0.75 mg/L	96.96	0.12–0.43	This study
Bulk powder	UV-VIS	3-15 mg/L	0.997	0.96 mg/L	2.91 mg/L	NR	0.16–0.42	[33]

NR = Not reported.

Comparison of results obtained by the present developed methods with redox titration method

To further validate the newly developed methods the results of present study compare the results with accepted methods. The ascorbic acid concentration of pumpkin flesh, peel, and seed parts was determined using both the newly developed methods and also by the published by Satpathy *et al.* [34] method in the same sample. The titration methods have been reported by many researchers as preferred method of ascorbic acid determination because of its relatively low cost, rapidity and easy.

The results obtained using a titration method were compared to the results obtained using the present methods (Table 4). The results revealed that the amount of ascorbic acid determined by redox titration was lower than that determined by ATR-FTIR and UV-VIS methods. This is because titration only determines L-ascorbic acid, whereas ATR-FTIR and UV-VIS methods determine total L-ascorbic and L-dehydroascorbic. El Shara and Mussa [27] and Adebayo [30] have compared the determination of ascorbic acid using UV-VIS and titration methods and found that the UV-VIS method provided higher ascorbic acid than the titration method. In general, the ascorbic acid content obtained using the three methods in the present investigation is within the

range of data [7, 27, 30]. In comparison to the present study, Abera *et al.* [32] and Odeyemi *et al.* [35] found a higher ascorbic acid content. This is due to different factors such as: variety, geography, harvest time, ripening stage, etc. [36].

Table 4. Comparison of the concentrations of ascorbic acid obtained by the three methods of present study to the methods reported in the literature.

Methods	Ascorbic acid content (mg/100 g) in three parts of pumpkin (mean±SD (n =3)			Reference
	Flesh	Peel	Seed	
FTIR	27 ± 0.5	24 ± 0.3	32 ± 0.7	This study
UV-VIS	23 ± 0.2	22 ± 0.4	28 ± 0.4	This study
Titration (for comparison)	21 ± 0.9	19 ± 0.5	26 ± 0.8	This study
HPLC-UV	12 – 39	2 – 10	10 – 15	[7]
UV-VIS	30.8 - 48.2	NR	NR	[27]
Titration	18.2 - 38.2	NR	NR	[27]
UV-VIS	7.76 - 87.1	NR	NR	[30]
Titration	0.20 - 13.2	NR	NR	[30]
Titration	36	NR	NR	[32]
Titration	85	NR	NR	[35]

NR = Not reported.

The pumpkin seed parts had the highest levels of ascorbic acid, followed by the flesh and peel areas found by the three methods. Amin *et al.* [7] determined the concentration of ascorbic acid in the indigenous pumpkin using the HPLC method. The results reported are in good agreement with the results obtained in the present study with highest levels of ascorbic acid found in seed part of pumpkin followed by flesh and peel. In Ethiopia, most people discard the seeds and peels of pumpkins; nevertheless, this study discovered that the seeds and peels of pumpkins are rich in ascorbic acid. Ascorbic acid is the most biologically active form of vitamin C. It is a valuable food component due to its antioxidant and medicinal properties. Ascorbic acid aids the metabolism of tyrosine, folic acid, and tryptophan. It is also important for physiology and metabolism, especially when it comes to connective tissue production. Vitamin C lowers LDL (bad) cholesterol and triglyceride levels in the blood, which are both risk factors for heart disease. Because vitamin C is a water-soluble vitamin, it disintegrates when it comes into touch with water. Unlike fat-soluble vitamins, water-soluble vitamins do not accumulate in the body. Instead, vitamin C is supplied to tissues through body fluids, with any excess excreted in urine, as reported by Chambial *et al.* [6]. Because the human body neither store nor produce vitamin C, it is vital to consume vitamin C-rich foods on a daily basis, such as pumpkins.

CONCLUSION

In the present study, two methods were developed and applied for the determination of ascorbic acid in flesh, peel and seeds of pumpkin. The developed methods are simple, rapid, cost effective and do not involve organic solvent (water is used as solvent). ATR-FTIR spectroscopy method has shown excellent accuracy and precision, and is regarded as a non-destructive, environmentally friendly, low-cost, and quick approach. Furthermore, the UV-VIS method demonstrated high sensitivity, accuracy, and precision, as well as being a green, low-cost, and quick method. As a result, these methods can be used to determine the amount of ascorbic acid in pumpkin flesh, peel, and seeds quantitatively. The findings of this study indicated that diverse portions of the pumpkin (peel, flesh, and seed) are rich in ascorbic acid (vitamin C). This study could aid in determining whether the peel and seed from this pumpkin cultivar have the potential to be economically exploited for nutraceutical applications and included into food formulations for the benefit of

human health. Several nutrients are lost when the peel and seed of a pumpkin are discarded. According to this study, the unused parts (peel and seed) of pumpkin, in addition to the utilized component, may be valuable nutraceuticals.

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