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Determination Of Vitamin C Stability In Different Packaging Materials At Refrigerated Conditions By Hplc Analysis

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ORIGINAL STUDY

Determination of Vitamin C Stability in Different Packaging Materials at Refrigerated Condition by HPLC Analysis

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Abstract

The stability of Vitamin C depends on storage temperature, time, and packaging material. Vitamin C is known to prevent the oxidation of low-density lipoprotein primarily by scavenging the free radicals and other reactive oxygen species in the aqueous milieu, ascorbic acid combats cancer by promoting collagen synthesis and thus prevents tumors from invading other tissues.

Aim: **The present study aims at investigating the Vitamin C content of two citrus fruits under refrigerated conditions after a specific time interval, in different packaging materials.**

Material: **In the present study, two citrus fruits − Amla & Lemon were used to study the stability of Vitamin C in different packaging materials viz., perforated zip lock cover, brown paper cover & plastic container under refrigerated conditions (5−6 °C) at 0^{th} day, 15th day, 30th day and 45th day. Vitamin C was estimated by the HPLC method and compared with the standard Ascorbic Acid.**

Results: **The stability of Vitamin C in both Amla and Lemon stored in a perforated Zip lock cover was better than the other 2 packaging materials viz., Brown paper cover and Plastic container during the period of 0^{th} to 45th day. The vitamin C content of Amla at 0^{th} day - 200 mg and 45th - day 163 mg, and Lemon at 0^{th} day - 43 mg and 45th day - 26 mg packed in perforated zip lock cover.**

Conclusion: **From the present study it can be concluded that a perforated Zip lock cover is the most suitable packaging material for the retention of vitamin C under refrigerated condition.**

Keywords: Vitamin C, Packaging material, Amla, Lemon, HPLC

1. Introduction

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement. Humans, unlike most animals, are unable to synthesize vitamin C endogenously, so it is an essential dietary component [1]. Vitamin C is required for the biosynthesis of collagen and certain neurotransmitters; and it is also involved in protein metabolism [1,2]. Collagen is an essential component of connective tissue, which plays a key role in wound healing. Vitamin C is also an important physiological antioxidant [3] and has been shown to regenerate other antioxidants within the body, including alpha-tocopherol [4]. It is likely to be higher in early fruits than in late fruit [1]. The stability of ascorbic acid decreases with an increase

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Fig. 1. Amla in perforated zip lock cover.

Fig. 2. Lemon in perforated zip lock cover.

Fig. 3. Amla in brown paper cover.

Fig. 4. Lemon in brown paper cover.

in temperature and pH [5]. This destruction by oxidation is a serious problem in that a considerable quantity of the vitamin C contents of food is lost during processing, storage, and preparation [5,6]. Vitamin C decreases gradually during storage, especially at a temperature above 0 °C [7]. In various ways fruits and vegetables containing vitamin C decrease their vitamin C retention, for instance, bruising, peeling, heating, and cutting into pieces, and exposure to air decreases vitamin C retention [8,9]. Peeling apples may result in the loss of 8–25% of their vitamin C. It is evident that the vitamin C retention of fruits varies with the treatment but in general, fruits are valuable when they are used raw and have a minimum of bruising, cutting, peeling, heating, and exposure to air [10–12]. Lemon is one of the most popular world food crops and contains active phytochemicals that can protect health. In addition to this, it provides enough supply of vitamin C, folic acid, potassium, and pectin. The contribution of citrus species to the deterrence of life-threatening diseases has been assessed [13–16]. Amla is highly nutritious and is one of the richest sources of ascorbic acid, amino acids, and minerals [17]. The present study aimed at the stability studies of vitamin C in citrus fruits stored in different packaging materials at refrigerated temperatures (5–6 °C).

2. Materials and methods

2.1. Chemicals and column used

Acetonitrile (ACN), Glacial acetic acid (CH3COOH), Methanol, and Trifluoroacetic acid (TFA) of HPLC Grade. The column used was Superspher RP-18 (250 × 4.6 mm).

2.2. Materials to store food samples

Re-usable, re-sealable perforated zip lock covers were procured from S.C. Johnson & Son. Brown

Fig. 5. Amla and Lemon in a plastic container.

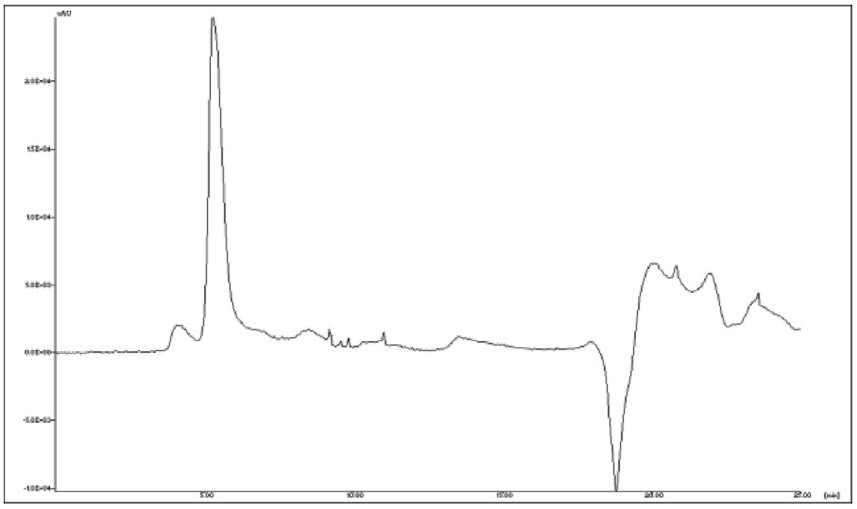

Fig. 6. Chromatogram for Standard Ascorbic acid solution (0.1 mg/ml).

paper covers were procured from Paper Kraft industries. Plastic containers were procured from Rainbow industries.

2.3. Sample collection and preparation

Amla and Lemon samples were procured from the local market of Mysuru on the same day. Amla and Lemon were first washed in water, wiped, and packed in a perforated zip lock cover, brown paper cover, and plastic container. The packed materials were tightly closed and kept in a refrigerator. Two samples were packed in each material in duplicates (Figs. 1–5).

2.4. Mobile phase

0.1% TFA in water – 1 ml of TFA was added to the 1-liter volumetric flask, the solution was made up to 1000 ml with distilled water, filtered using 0.44 µm filtrate and sonicated for 20 min.

0.1% TFA in ACN – 1 ml of TFA was added to the 1-liter volumetric flask, the solution was made up to 1000 ml with ACN, filtered using 0.44 µm filtrate and sonicated for 20 min.

2.5. Vitamin analysis by HPLC method for sample

a) Extraction solution:

50 ml ACN + 10 ml glacial acetic acid

↓

Volume made up to 1000 ml with HPLC water.

b) Sample extraction:

10g sample + 50 ml extraction solution

↓

Kept in shaking water bath for 45 min at 50°C

↓

The sample was centrifuged at 5000 rpm for 20 min

↓

Filtered using 0.22µm filter

↓

20 µl sample is injected
(Each sample will take 25min)

2.6. Vitamin C analysis during the storage period

Vitamin C was analyzed in Amla and Lemon stored in three different packaging materials viz., perforated Zip lock cover, Brown paper cover & Plastic container at refrigerated conditions (5–6 °C) on 0th day, 15th day, 30th day, and 45th day (see Fig. 5).

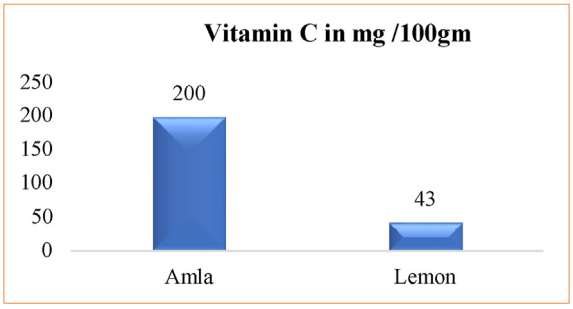

Fig. 7. Vitamin C content of fresh samples.

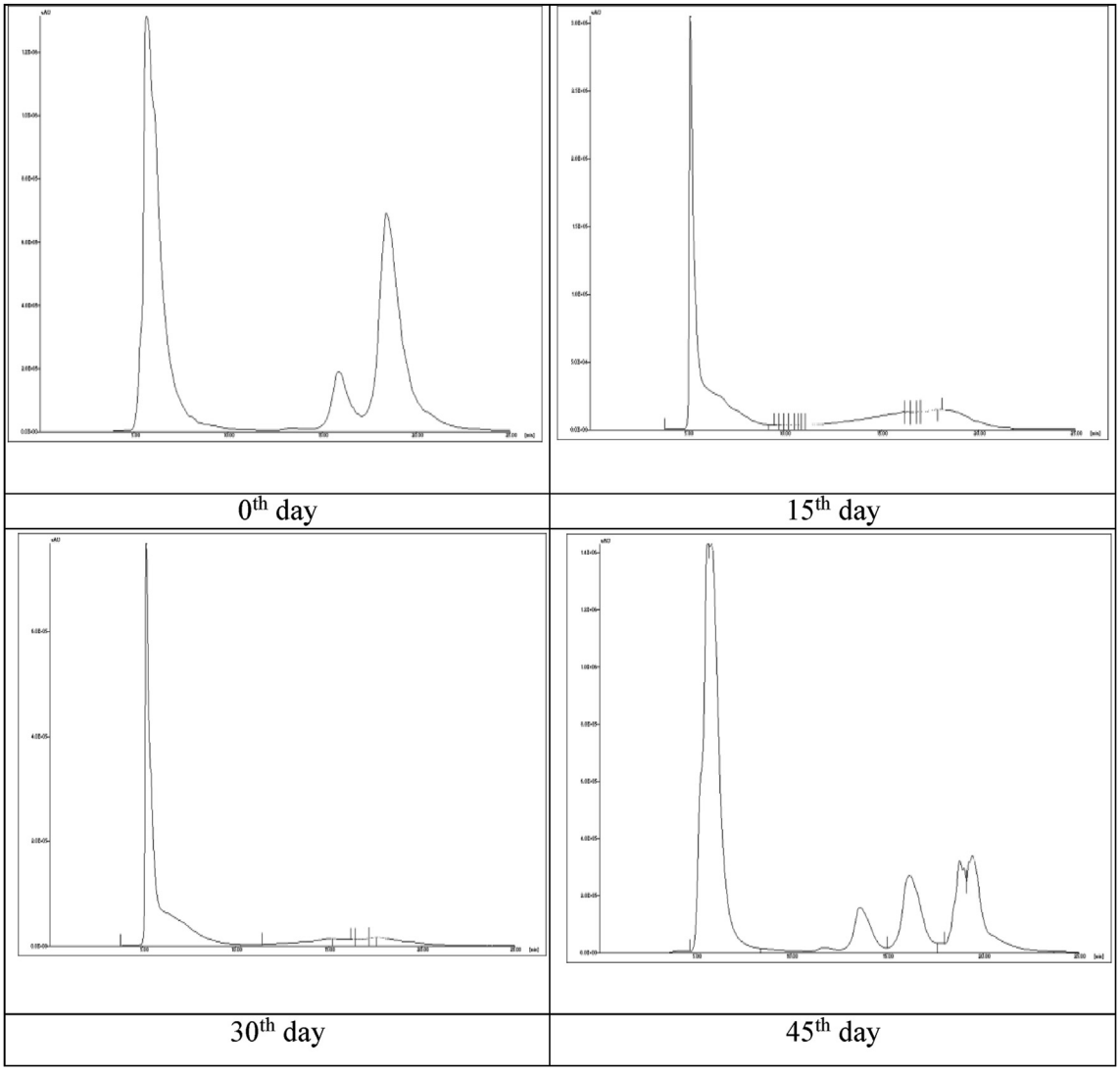

Fig. 8. Chromatograms of vitamin C in Amla packed in perforated zip lock cover.

3. Results

The samples viz., Amla and Lemon were initially analyzed for vitamin C content. Stability studies of the samples were studied by storing at refrigerated temperature (5–6 °C) and analyzed periodically for vitamin C retention. A chromatogram for standard ascorbic acid solution (0.1 mg/ml) is given in Fig. 6. The vitamin C content of fresh Amla and Lemon was found to be 200 mg and 43 mg per 100g respectively and is given in Fig. 7.

3.1. Vitamin C retention in amla stored in different packaging materials

There was a gradual reduction in the Vitamin C observed from 0th to 45th day at an interval of 15 days in amla and stored in different packaging materials. Vitamin C content of amla in the perforated zip lock cover was reduced from 200 mg to 163 mg, in the brown paper cover it was reduced from 200 mg to 156 mg and in the plastic container, it was reduced from 200 mg to 144 mg/100g of sample. On the 15th day retention of Vitamin C in the perforated zip lock cover was more i.e., 196 mg compared to other packaging materials viz., brown paper cover (192 mg) and plastic container (193 mg). On the 30th day, Vitamin C retention in brown paper cover was more i.e., 179 mg compare to other packaging materials viz., perforated zip lock cover (172 mg) and plastic container (172 mg). On the 45th day retention of Vitamin C in the perforated zip lock cover was more i.e., 163 mg compared to other packaging materials viz., brown paper cover (156 mg) and plastic container (144 mg). However,

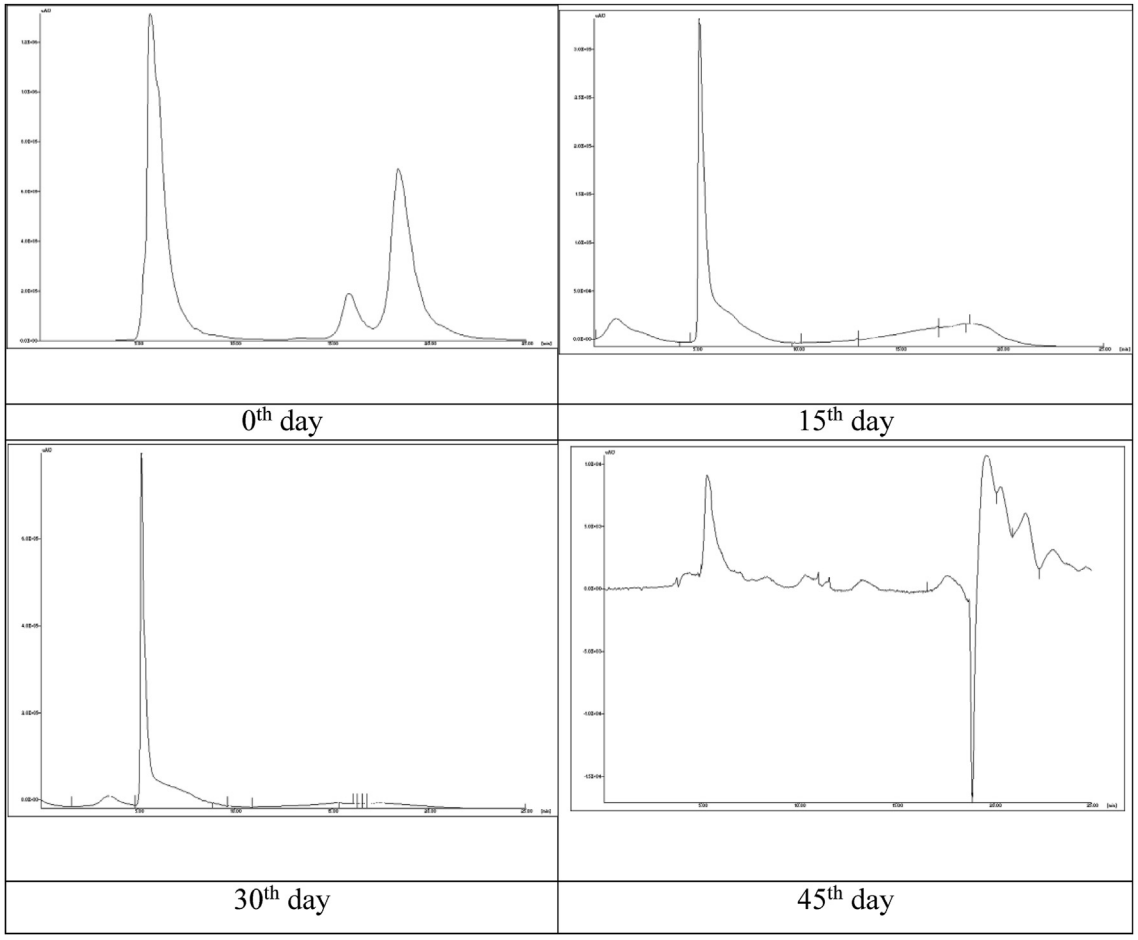

Fig. 9. Chromatograms of vitamin C in Amla packed in brown paper cover.

after the 21st day, Amla was soft and spoiled with white and brown patches. Chromatograms of Amla in different packaging material viz., zip lock cover, brown paper cover, and plastic container at different time interval was represented in Figs. 8–10. The retention percentage of vitamin C in Amla in different packaging materials is represented in Fig. 11.

3.2. Storage studies on vitamin C retention of lemon in different packaging materials

There was a gradual reduction in the Vitamin C observed from 0th to 45th day at an interval of 15 days in lemon and stored in different packaging materials. The Vitamin C content of lemon in the perforated zip lock cover was reduced from 43 mg to 26 mg, in the brown paper cover it was reduced from 43 mg to 25 mg and in the plastic container, it was reduced from 43 mg to 23 mg/100g of sample. On the 15th day retention of Vitamin C in perforated zip lock cover and brown paper cover was more i.e., 40 mg compare to a plastic container (38 mg). On the 30th day retention of Vitamin C in brown paper cover was more i.e., 35 mg compare to other packaging materials viz., perforated zip lock cover (32 mg) and plastic container (26 mg). On the 45th day retention of Vitamin C in perforated zip lock cover was more i.e., 26 mg compare to other packaging materials viz., brown paper cover (25 mg) and plastic container (23 mg). However, after the 21st day, the Lemon was soft and spoiled with white and brown patches. The outer layer of Lemon had turned brown and completely shrunken. The juice content drastically decreased on the 45th day. Chromatograms of Lemon in different packaging material viz., zip lock cover, brown paper cover, and plastic container at different time interval was represented in Figs. 12–14. The retention percentage of vitamin C in Lemon in different packaging materials

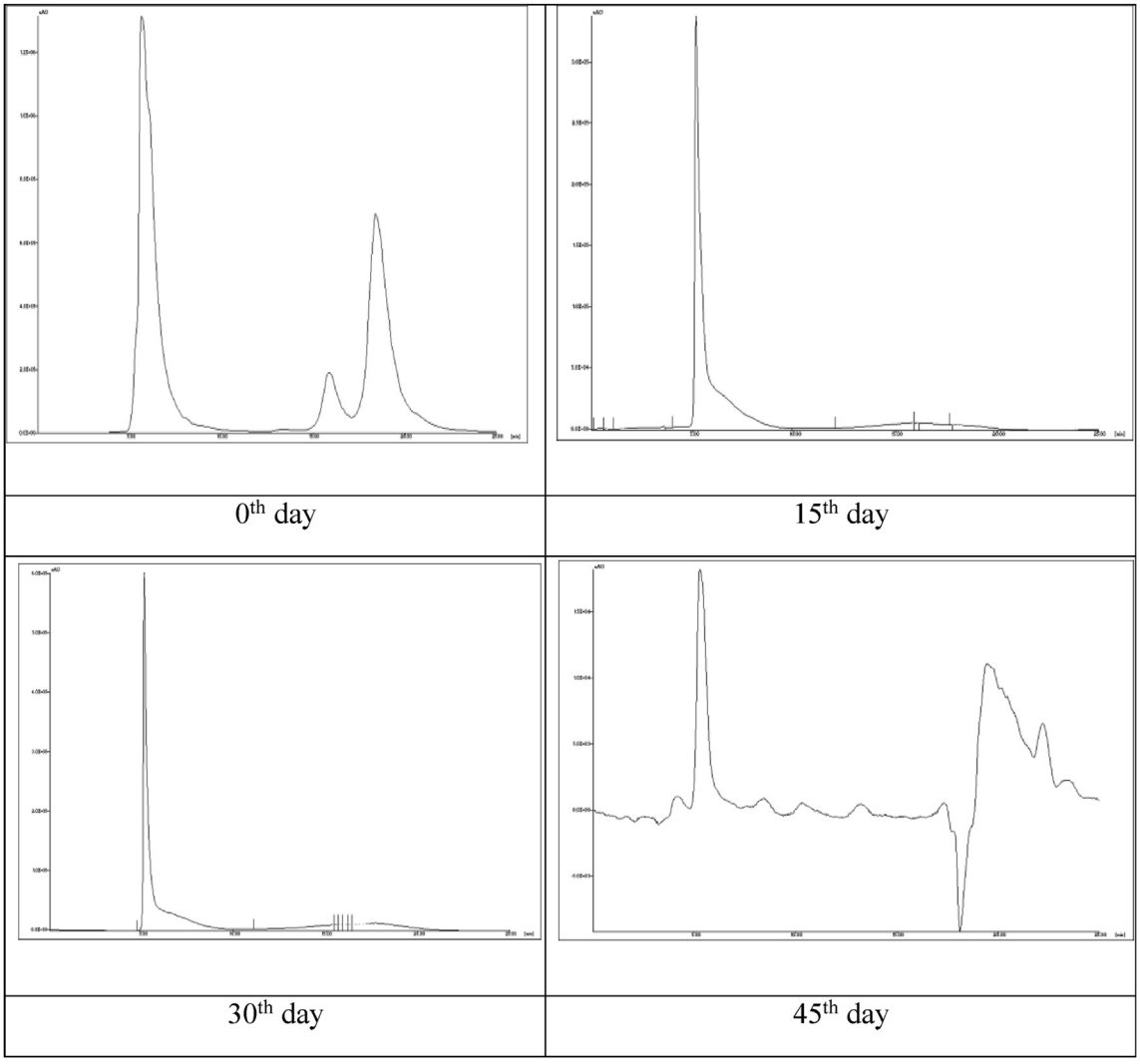

Fig. 10. Chromatograms of vitamin C in the Amla packed in the plastic container.

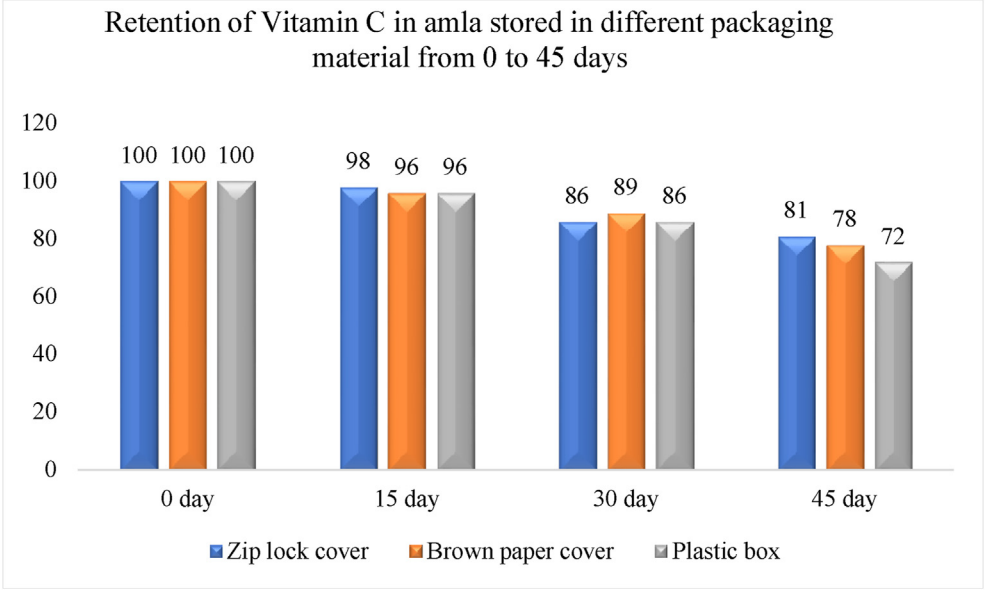

Fig. 11. Retention (%) of Vitamin C in Amla stored in different packaging materials from 0 to 45 days.

is represented in Fig. 15. Therefore refrigeration condition for storage of vitamin C sources can be suggested.

4. Discussion

Fresh is often best for optimal vitamin C content, as long as the fresh undergoes minimal storage at room temperature [7]. Studies have recommended that refrigeration conditions are more suitable for the stability of vitamin C under investigation [7,18]. Many components such as chemical reactions, temperature, packaging materials, and the storage period, contribute to the stability of vitamin C. The results of the present study showed that the samples packed in different packaging materials viz., perforated zip lock cover, brown paper cover, and plastic container at refrigerated temperature (5–6 °C) affected the stability of the vitamin C, however, each packaging material has shown varied retention of vitamin C. In the perforated zip lock cover, Vitamin C retention was more compared to the other two packaging materials at the end of the 45th day in both amla and lemon. Oyetade et al., 2012 [18], studied the effect of packaging materials and storage time on vitamin C in laboratory-grade vitamin C, grape juice, and vitamin C tablet (a pharmaceutical product) by the titrimetric method. Packaging

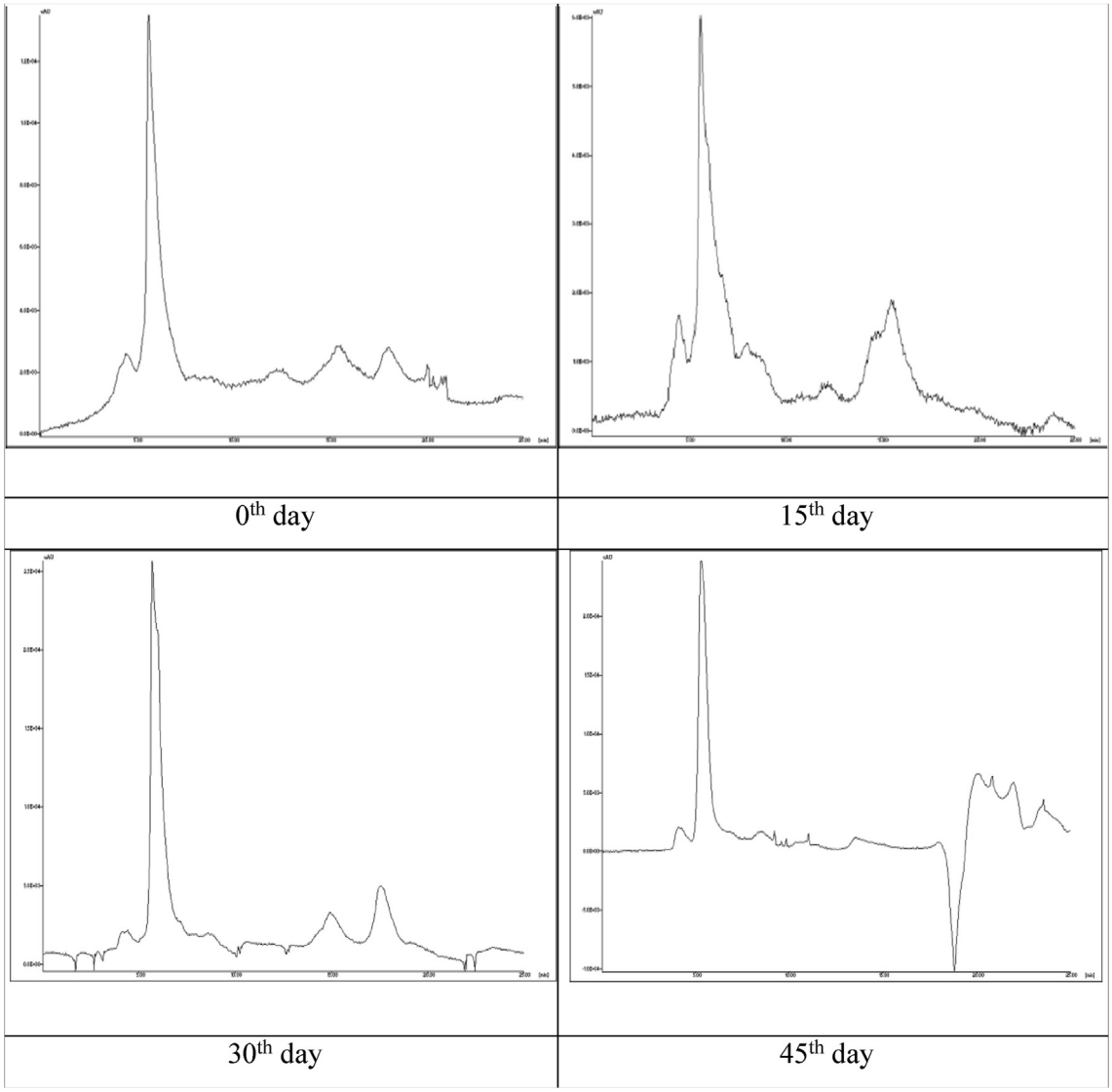

Fig. 12. Chromatograms of vitamin C in Lemon packed in perforated zip lock covers.

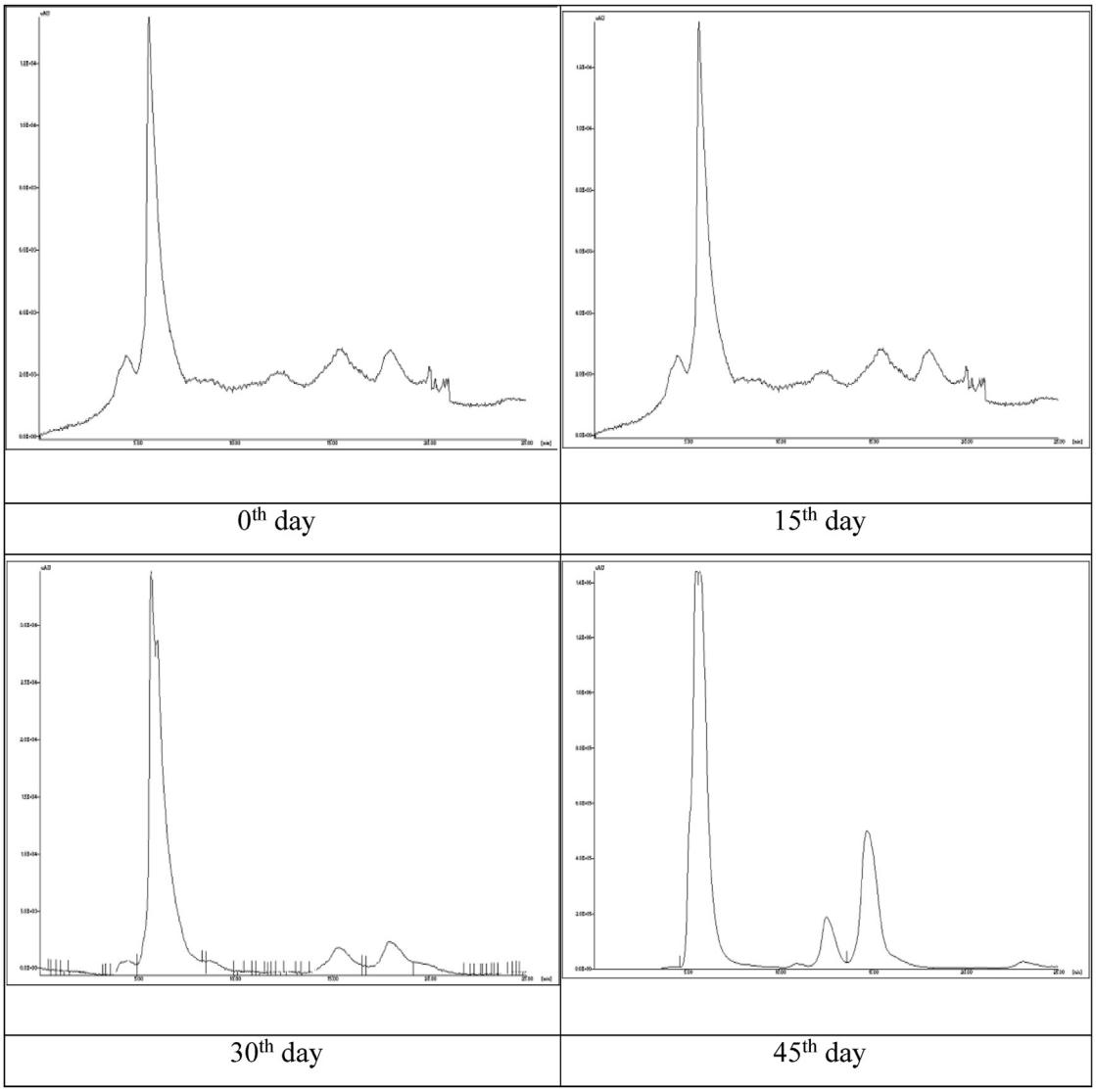

Fig. 13. Chromatograms of vitamin C in Lemon packed in brown paper cover.

materials viz., brown bottle, transparent bottle, nylon, brown paper, and white paper were used and analyzed for 4 days intervals up to 20 days on vitamin C content of vitamin C tablet (a brand of pharmaceutical products) in mg/tablet. This study reported, a significant influence of packaging materials on the stability of vitamin C. Supporting the above, in the current study, similar results were found concerning the packaging material and vitamin C. They also studied the reduction in the vitamin C content of the samples viz., laboratory-grade vitamin C, grape juice, and vitamin C tablet with the length of exposure, also indicated that a significant reduction in ascorbic acid content was noticed within 60 min of exposure in tablet and grape juice, no such significant change was observed in the laboratory-grade vitamin C until 120 min, rapid loss of the vitamin C was noticed at 180 min of exposure. For tablet and grape juice, rapid losses were noticed at 240 min. Supporting the above, in the present study, similar results were found concerning the time of storage, as the storage time increases, Vitamin C retention was decreased in both the samples packed in different packaging materials.

5. Summary and conclusion

It is known that Vitamin C is heat sensitive-labile, lower temperature do enhance the stability of the

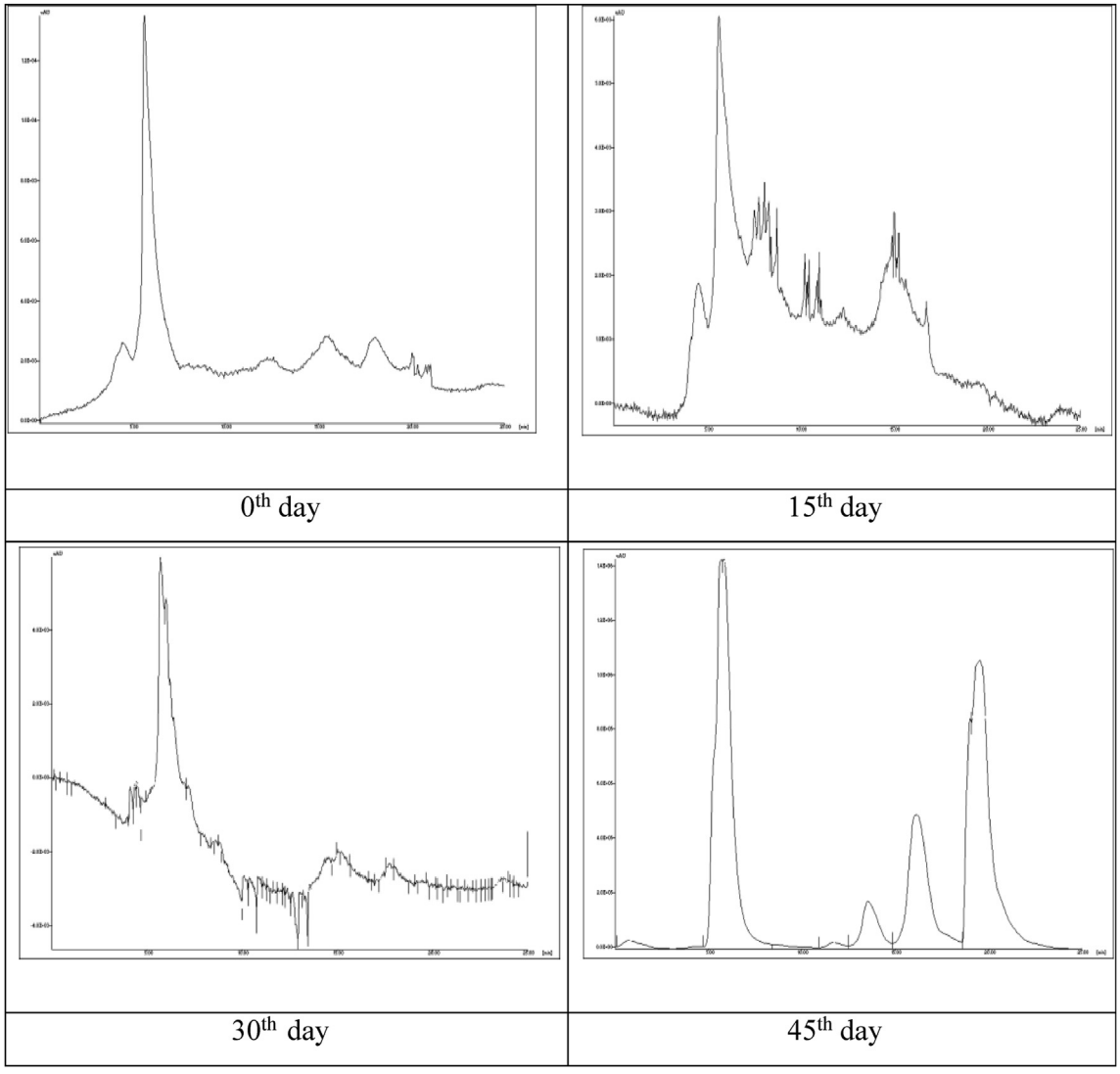

Fig. 14. Chromatograms of vitamin C in Lemon packed in the plastic container.

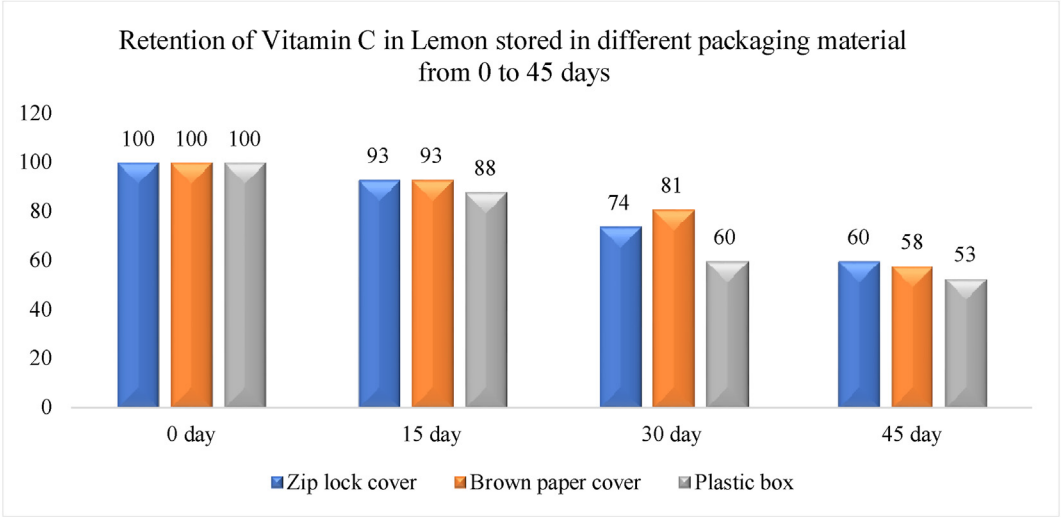

Fig. 15. Retention (%) of vitamin C in Lemon stored in different packaging materials from 0 to 45 days.

vitamin C. Keeping this in view, an attempt was made to check the impact of different packaging materials on vitamin C and it has been proved in the present study that, packaging materials is one of the major factor that can influence the stability of vitamin C in both Amla and Lemon. Simultaneously, the period influencing the retention of vitamin C at a lower temperature was also determined. At the domestic level, different packaging materials and storage conditions are used to store fruits and vegetables e.g., they are stored in plastic containers in refrigeration condition for more than two weeks which can affect the stability of many micronutrients especially vitamin C. Results of the present study indicated that, among the three packaging material, perforated zip lock cover used for storage was the most suitable material for vitamin C stability in refrigerated condition. In the present study storing in a perforated zip lock pouch showed better vitamin C stability compared to storing in the brown paper pouch and plastic container. Further studies can be conducted on more samples rich in vitamin C.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

[1] Li Y, Schellhorn HE. New developments and novel therapeutic perspectives for vitamin C. J Nutr 2007;137: 2171–84.
[2] Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am J Clin Nutr 1999;69:1086–107.
[3] Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci U S A 1989;86:6377–81.
[4] Jacob RA, Sotoudeh G. Vitamin C function and status in chronic disease. Nutr Clin Care 2002;5:66–74.
[5] Emese J, Nagymate PF. The stability of vitamin c in different beverages. Br Food J 2008;110:296–309.
[6] Davey MW, Van M, Montagn D, Inze M, Sanmartin A, Kanallis A, Smirnoff N. Plant l-ascorbic acid: chemistry function metabolism, bioavailability and effects of processing. J Sci Food Agric 2000;80:825–60.
[7] Ajibola VO, Babatunde OA, Suleiman S. The effect of storage method on the vitamin c content in some tropical fruit juices. Trends Appl Sci Res 2009;4:79–84.
[8] Allen MA, Burgess SG. The losses of ascorbic acid during the large scale cooking of green vegetables by different methods. Br J Nutr 2006;4:95–100.
[9] Maria G, Encarna IA, Kade AA. Quality changes and nutrient retention in fresh cut versus whole fruits during storage. J Agric Food Chem 2006;54:4284–96.
[10] Garard IO. Introductory food chem. seventh ed. Mc Graw Hill; 1987. p. 51.
[11] Franke AA, Custer LJ, Arakaki C, Murphy SP. Vitamin c and flavonoid levels of fruits and vegetables consumed in hawai. J Food Compos Anal 2004;17:1–35.
[12] Murcia MA, Opez BL, Ayerra M, Martinez T, Vera AM, Garcia-Carmona F. Evolution of ascorbic acid and peroxidase during industrial processing of broccoli. J Sci Food Agric 2000;80:1882–6.
[13] Proteggente AR, Saija A, De Pasquale A, Rice-Evans CA. The compositional characterisation and antioxidant activity of fresh juices from Sicilian sweet orange (*Citrus sinensis* L. Osbeck) varieties. Free Radic Res 2003;37:681–7.
[14] Gorinstein S, Cvikrova M, Machackova I, Haruenkit R, Park YS, Jung ST, Yamamoto K, Ayala ALM, Katrich E, Trakhtenberg S. Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. Food Chem 2004;84:503–10.
[15] Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN, Boskou D. Radical scavenging activity of various extracts and fractions of sweet orange flavedo (*Citrus sinensis*). Food Chem 2006;94:19–25.
[16] Guimaraes R, Barros L, Barreira JCM, Sousa MJ, Carvalho AM, Ferreira ICFR. Targeting excessive free radicals with peels and juices of citrus fruits: grapefruit, Lemon, lime and orange. Food Chem Toxicol 2009;48:99–106.
[17] Srivasuki KP. Nutritional and health care benefits of Amla. J Phar 2012;3:141–51.
[18] Oyetade OA, Oyeleke GO, Adegoke BM, Akintunde AO. Stability studies on ascorbic acid (vitamin c) from different sources. IOSR J Appl Chem 2012;2:20–4.