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# Cell Wall Changes of Papaya Fruit Treated with 1-Methylcyclopropene: Postharvest Analysis through SEM and FT-IR Spectroscopy

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Authors SS and PJ, both planned and executed the experiment. Author SS wrote the first draft of manuscript which was corrected and approved by author PJ. Both the authors read and approved the final manuscript.*

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## ABSTRACT

1-Methylcyclopropene (1-MCP), is a widely researched and commercially used molecule to delay fruit ripening and improve the shelf life, both climacteric and non-climacteric fruits. Increasing the shelf life and keeping quality, especially of important climacteric fruits like papaya using 1-MCP, which undergoes a rapid softening during the ripening process required a detailed investigation of the structural changes of fruit cell wall. These changes include modifications in the cell wall polysaccharide structure due to increased activity of hydrolyzing enzymes, pectin, hemicellulose and cellulose degradation that results in fruit softening. Pectin degradation in papaya determines the extent of softening during ripening. Hence, the present investigation describes the role of 1-MCP in retaining the firmness, postharvest. The ultrastructural changes of papaya peel and pulp were studied using Scanning Electron Microscope (SEM). The pectin degradation in untreated and 1-MCP treated fruits was analyzed through a rapid and reliable technique, FTIR spectroscopy (Fourier transform-infrared (FT-IR)). The study revealed that postharvest application of 1-MCP at 900 ppb to papaya fruits, stored under cold storage (14°C) was effective in retaining higher fruit firmness by suppressing the rapid cell wall modifications and activity of pectin degradation, which are triggered by ethylene.

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*Keywords: 1-Methylcyclopropene (1-MCP); softening; ethylene; postharvest; Scanning Electron Microscope (SEM); Fourier Transform-infrared (FT-IR).*

## 1. INTRODUCTION

Papaya (*Carica papaya* L.) is an important tropical, but a highly perishable climacteric fruit. Climacteric fruits undergo rapid changes during ripening process that include dramatic rise in respiration rate, faster peel color changes, rapid loss of firmness leading to softening and quality loss. Postharvest softening basically occurs due to the enhanced activity of cell wall degrading enzymes, triggered by ethylene (major hormone that regulates ripening associated changes), especially in climacteric fruits. A characteristic feature of the softening process, is degradation of the ordered arrangement of cell wall and middle lamella polysaccharides, due to an increase in the activity of the cell wall degrading/hydrolyzing enzymes [1,2]. Hence, in other terms cell wall modification during ripening is accompanied by pectin solublization and hemicellulose depolymerization [3,4]. Pectin hydrolyzation is attributed to significantly affect fruit firmness, among the alterations in cell wall [5]. Retardation of faster ripening, associated rapid morpho-physiological and biochemical changes have been achieved by application of 1-Methylcyclopropene (1-MCP) in several climacteric and non-climacteric fruits. 1-MCP is a non-toxic molecule (approved by USEPA) that inhibit the ethylene action and improve shelf life of several fruits and vegetables. 1-MCP delays fruit ripening and softening by suppressing the activities of cell wall hydrolytic enzymes [6]. Hence, the present investigation is aimed at (1) studying the ultra-structural changes in the cell wall of papaya fruits as affected by 1-MCP, revealed through Scanning Electron Microscope technique (SEM) (2) pectin solubilization and degree of methyl esterification (DME) of pectin using Fourier transform-infrared (FT-IR) spectroscopy. FT-IR is a reliable, rapid and highly sensitive method to analyze the DME of pectin [7,8].

## 2. MATERIALS AND METHODS

### 2.1 Application of 1-Methylcyclopropene

Papaya (TNAU var. CO.8) fruit harvested at color break stage were selected for uniformity, devoid of injury or diseases manually and sorted into two groups for application of 1-MCP treatments. 1-MCP application procedure is as previously described [9]. Fruits were then loaded into plastic crates and cooled down to attain a pulp temperature of 15-20°C (measured using stick thermometer). One batch was treated with 1-MCP at 900 ppb, while the other batch was left untreated. The fruits were pre-treated with 100 ppm ethylene to trigger the ethylene receptor in order to avoid rubbery texture as reported in previous studies [10]. 1-MCP (obtained from Agrofresh Inc., Mumbai) at 900 ppb concentration was applied to papaya fruit by vaporization in a closed container for a duration of 14 hours @ 14°C, RH 90-95 per cent. After 1-MCP vaporization, half of the fruit from each treatment box were loaded into crates and kept at ambient temperature of 27±2°C and rest of the fruit were kept at cold storage (14°C). Thus, the treated and untreated fruits were allowed to undergo normal ripening at the respective storage conditions. The samples were then subjected to SEM and FTIR analysis. To determine the effects 1-MCP on papaya fruit morphology stored under ambient and cold storage, the peel samples were collected from all set of treatments on day seven (since ambient stored untreated fruit had achieved the last stage of consumer acceptance).

### 2.2 Ultrastructural Peel Changes Using SEM

Ultrastructural changes revealed through SEM in papaya fruit peel can be complemented to cell wall changes as observed using FT-IR spectroscopy. The papaya fruit peel sample was collected from the middle portion of the fruit. At the time of analysis, sample was placed to a carbon tape and affixed to a carbon stub which was then vacuum desiccated for 2 min to remove excess moisture from the stub. The peel sample was then viewed under a Scanning Electron Microscope (SEM; Quanta 250, FEI, Hillsboro, OR, USA) using an ETD detector. The SEM was operated at 5-15KV, with a working distance of 50 µm. The SEM facility at Department of Nanoscience and Technology, TNAU, Coimbatore, India was utilized for this study.

### 2.3 Measuring the Degree of Methyl Esterification (DME) of Pectin Using FOURIER Transform-Infrared (FT-IR) Spectroscopy

FT-IR spectroscopy was chosen to analyze the pectin properties in papaya fruit. FT-IR works on

the principle that, absorption intensity of different functional groups changes at different wave numbers at near infra region, particularly the ionized carboxylate group ($COO^-$) and ester carbonyl group (C=O) [7,11], without hydrolyzing the ester linkage [12]. Isolation of papaya peel and pulp pectin was done adopting the methods of [13]. The dried powder obtained from the samples after blanching (95°C for 5 min) was used for the isolation of alcohol insoluble residue (AIR) cell-wall components as described in the protocol. Water soluble pectin (WSP) was extracted from AIR and used for the analysis following the procedure described by [8]. Approximately, 10 mg of the WSP was dissolved in MilliQ water and pH was adjusted to 6 with 0.1N NaOH (since pH of 6 gives total ionization of carboxylic groups). The samples were then freeze dried for further analysis. The FT-IR (Fourier Transformation- Infrared Analysis) was performed using Jasco FT/IR 6800 model to measure the degree of methyl esterification of pectin in 1-MCP treated and untreated papaya fruit stored under different storage conditions. The standard curve was obtained using pure esterified pectin from Sigma. For degree of methyl esterification (DME) determination, a sample was placed on sample holder devoid of any air. After loading the sample, it was analyzed in Attenuated Total Reflectance (ATR/FT-IR) and the spectral data were recorded at 64 scans $second^{-1}$ with resolution of 4 $cm^{-1}$ at the spectral range of 400-4000 cm. Spectra was changed to absorbance mode for baseline correction and peak splitting was done to correct for any protein interference. The intensity of peaks around 1740-1745 $cm^-$ indicates ester (C=O) stretching, and peaks around 1630-1600 $cm^{-1}$ ($COO^-$ stretching) were used as reference to predict the pectin loss or degree of methyl esterification. The DME was calculated based on [8], using the equation DME=124.7*R+2.2013, where R = A1740/ (A1740+A1600-1630), %A= 2-log (%T).

## 3. RESULTS AND DISCUSSION

### 3.1 Ultra-structural Peel Changes in 1-MCP Treated and Control (Untreated) Papaya Fruit Using SEM

SEM images (at 600X and 1200X) revealed notable difference between the 1-MCP treated and control papaya fruit peel (adaxial and abaxial surface view) modifications. In Figs. 1 & 2, at day seven of analysis, remarkable variations were observed among the fruit stored at ambient (AS) and cold conditions (CS). 1-MCP (900 ppb) treated fruit at AS appeared intact with homogenous cell matrix with well-defined/identifiable layers and less degraded than the control fruit. The fruit samples treated with 1-MCP, stored under CS clearly depicted clearly all the cell wall matrix with less intercellular spaces between the pectin matrixes. This clearly shows (from the magnified images) that 1-MCP treated fruit at CS maintained greater peel integrity, while the control fruit stored at cold storage showed higher depolymerization with many intercellular spaces. The control fruit peel (abaxial view) at AS displayed shrunken, incomplete and degraded structures (due to highly esterified pectin). These disintegrated, loosened cell wall structures were also visible under untreated fruit kept under CS, but it could be clearly identified to more intact than the ambient stored fruit. Hence, the extent of cell wall modification seemed to be more intact and coherent due to 1-MCP treatment which suppressed the ethylene action, than the storage conditions. The untreated fruit stored at ambient condition, depicted cracked surface with visible fractures, separations of the wall materials due to rapid ripening associated changes contributing to softening, and loss in firmness. The results in the study were similar to the findings of [14], where depolymerization of pectin polymers in papaya fruit, was due to ethylene associated ripening and softening changes, and 1-MCP application significantly improved the fruit firmness retention. Solubilization and depolymerization of cell wall polysaccharides (pectins; hemicelluloses) causes further dissolution of the cell wall materials, resulting in fruit firmness [15,16,17].

SEM images of adaxial view of the peel (Fig. 3) depicted that under AS (which were at post-climacteric phase), the untreated fruit peel had thinner layers, with deeper cracks, rough surface, loosened matrix, striated and shrunken compared to the 1-MCP treated fruits. This breakdown of middle lamella and loss of structural unity of cell walls could be presumed to be due to enhanced activity of pectinases enzymes like PME and PGase. Similarly, the untreated fruit under CS had disintegrated and coarse surface with cracks. 1-MCP treated fruit under CS had a smooth homogenous surface with no cracks. At day seven, treated fruit at AS had a coarse skin structure and lesser wall degradation than untreated fruit indicating the role of 1-MCP in decreasing the activity of

cell wall hydrolyzing enzymes. The cross-sectional view (Fig. 4) of the peel showed that the 1-MCP treated fruit at CS was intact with lesser tissue degradation, indication a decrease in the degree of polymerization and reduction in the solubilization of the hemi cellulosic and pectic cell wall polysaccharides. These structural differences would explain the higher firmness shown by the fruit treated with 1-MCP (recorded in the present investigation). [18] had also described the changes in papaya ultrastructural changes due to ripening and physiological disorders. Also, EDAX analysis (Fig. 5a, b) shows that 1-MCP treated fruit retained higher levels of potassium (K), calcium (Ca) and magnesium (Mg) when compared to control fruit. Studies on the effect of 1-MCP on K, Ca and Mg contents have not yet been reported but effect of 1-MCP in maintaining higher nutrient levels than the untreated fruits have been reported in few studies including mango [19] and tomato [20,21]. Hence, the present SEM examination showed that papaya fruit that received treatment with 1-MCP remained firmer, had displayed higher peel integrity than the untreated fruit.

### 3.2 Analysis of Pectin FT-IR Spectra and Degree of Methyl Esterification (DME) in 1-MCP Treated and Untreated Papaya Fruits

The FT-IR method was used to analyze the methylation level of soluble pectin fractions isolated from the 1-MCP treated and untreated fruit peel as well as pulp, at day seven, when a significant change (climacteric peak) in the ambient storage condition was observed. The spectra of pectin standard showed a distinct peak associated with methylated esterified pectin (Fig. 6) at wavenumber 1730-1745 $cm^{-1}$ (due to stretching of ester carboxyl group (-$COOCH_3$) (Fig. 6). With the hydrolysis of pectin due to ripening, we could observe two peaks; a peak at wavenumber ($cm^{-1}$) 1630-1645 = corresponding to asymmetric–COOH stretch and a peak at wavenumber ($cm^{-1}$) 1420-1425 = corresponding to symmetric –COOH stretch among the untreated fruit (Figs. 9 & 10), however the absence of specific methylated peak confirms the activity of cell wall degrading enzymes. In the untreated papaya peel and pulp spectra, the carbohydrate peaks are visible, which have been reported to have a spectrum between 1200-950 $cm^{-1}$ wavenumber, that is highly specific for each polysaccharide. In 1-MCP treated papaya fruit pulp and peel had displayed peaks at wavenumber 1736 and 1742 $cm^{-1}$ respectively, similar to that of the standard indicating suppressed or lesser pectin hydrolysis (Figs. 7 and 8) DME (degree of methyl-esterification) values were calculated from absorbance spectra of the samples, plotted on a graph. The spectral analysis showed that DME percentage was lower in the pulp and peel of control fruit stored at ambient (AS) and cold storage (CS) conditions, signifying rapid softening process. Higher the DME values, lesser is the rate of cell wall hydrolysis (indicates reduced softening). DME was calculated using the equation DME=124.7*R+2.2013, where R= A1740/ (A1740+A1600-1630), %A= 2-log (%T). The degree of methyl esterification (DME) of pectin changes during ripening process, more predominantly in climacteric fruits. Compositionally, pectin is poly α-1-4-galacturonic acids, with varying degree of carboxylic acid residues present as methylated esters [22,23]. Loss of fruit firmness is therefore attributed to an increase in pectin solubility and depolymerization, changes in PG activity during ripening process [5,2]. [24] described that upregulation of endo-PGase was directly proportional to the firmness loss in fruit, signifying the crucial role of 1-MCP in downregulation of these endo-PGase and firmness retention in papaya fruit. Application of 1-MCP at 900 ppb significantly delayed the ripening process by suppressing softening, which was evident from the above conducted experiments.

Estimating the DME is thus an important indicator which reveals the significant softening and associated changes during fruit ripening, especially in a climacteric fruit like papaya. The lesser DME (%) in 1-MCP treated papaya fruits observed in this study was similar to the findings reported by [25]. the results from this corroborate with the GCMS profiles conducted during the study [9] where higher proportion of ester compounds were identified in control compared to 1-MCP treated. Therefore, this investigation furthers confirms that modifications in the carbohydrate matrix of cell wall, due to the activities of various hydrolytic enzymes are responsible for the tissue softening during fruit ripening [26,27,28,29,30,31].

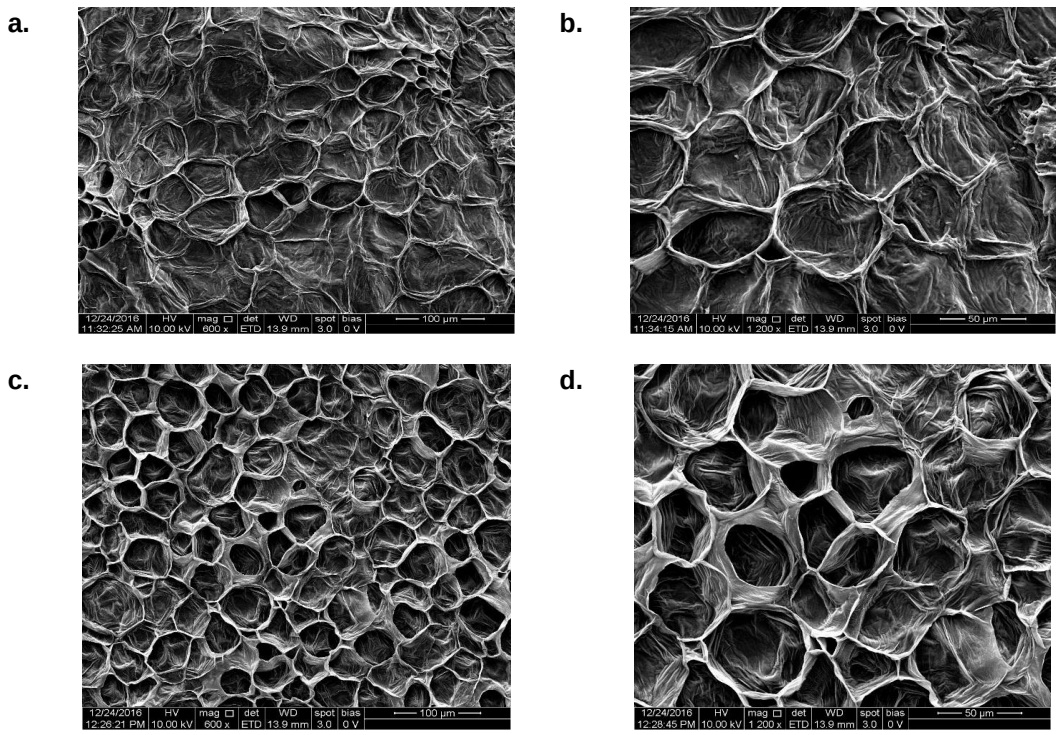



**Fig. 1. SEM images of control (a- 600X; b- 1200X) and 1-MCP treated (c- 600X; d-1200X) papaya fruit peel (abaxial view) under Cold Storage (CS)**

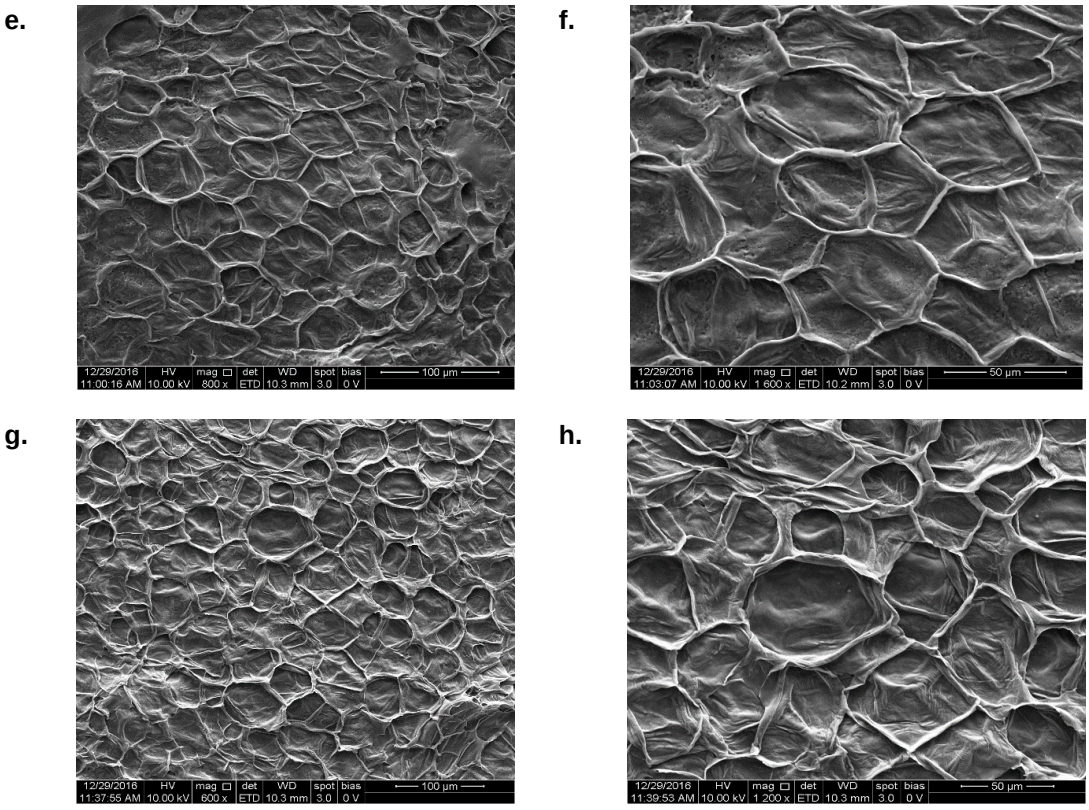



**Fig. 2. SEM images of control (e- 600X; f- 1200X) and 1-MCP treated (g- 600X; h- 1200X) papaya fruit peel (abaxial view) under Ambient Storage (AS)**

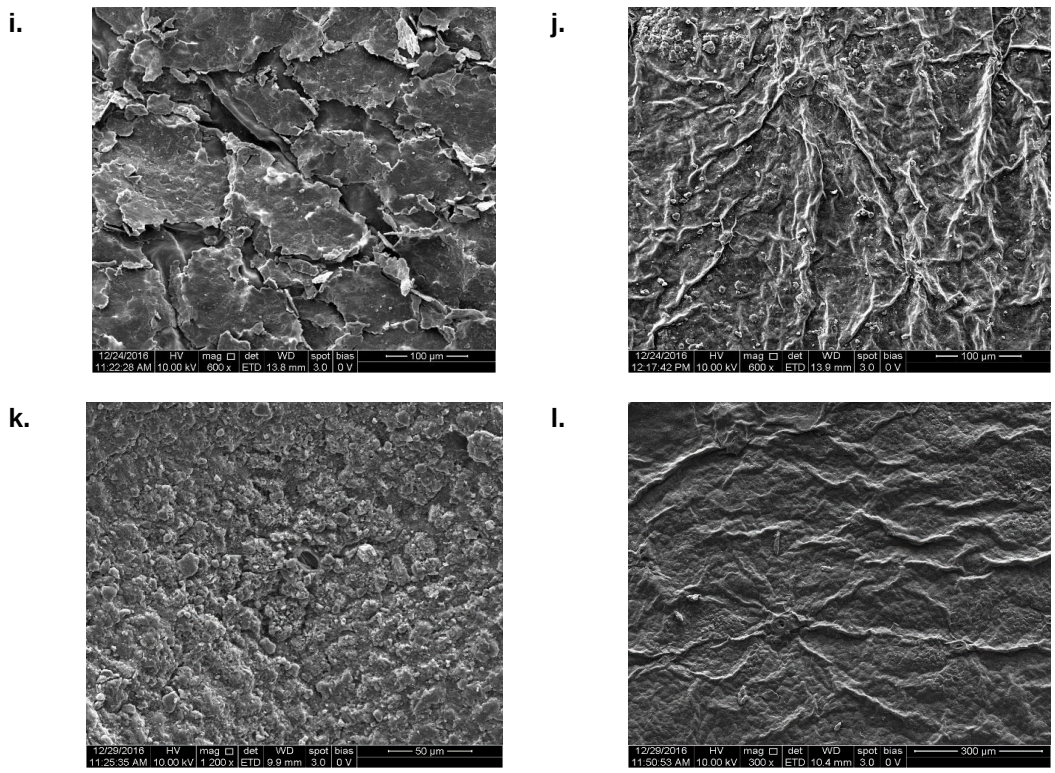



**Fig. 3. SEM images of control (i, k at 600X) and 1-MCP treated (j, l at 600X) fruit peel (adaxial view) under AS (i, j) and CS (k, l)**

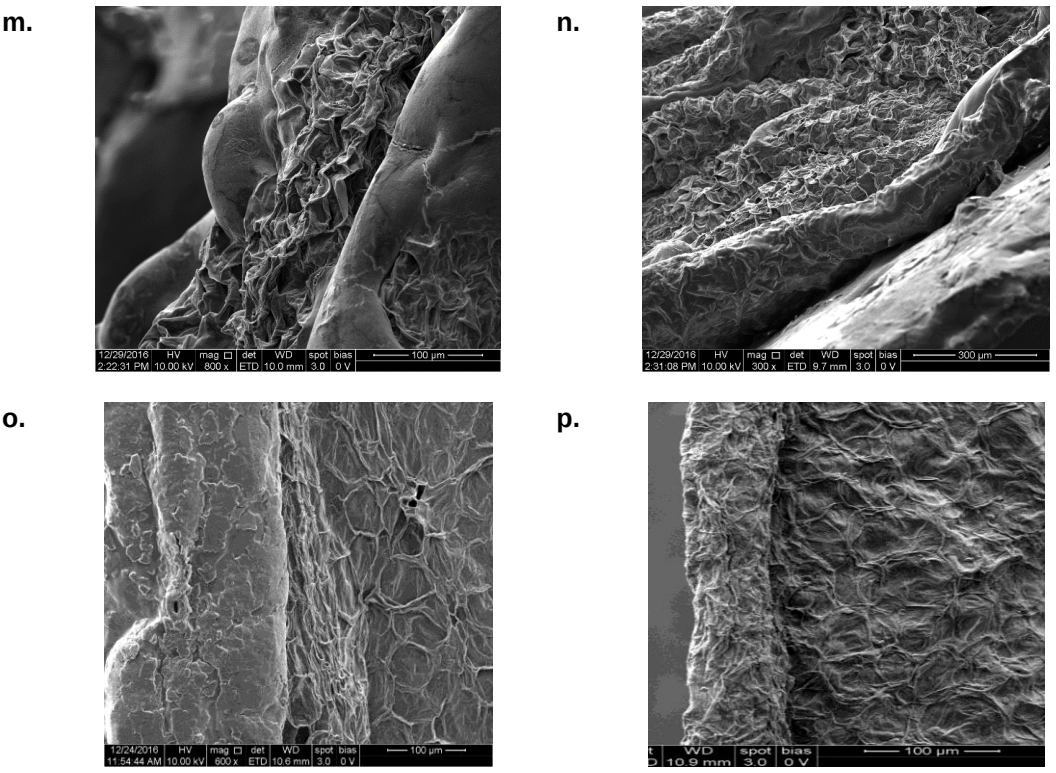



**Fig. 4. SEM images of control (m,o at 600X) and 1-MCP treated (n, p at 600X) fruit peel (cross sectional view) under AS (m,n) and CS (o, p)**

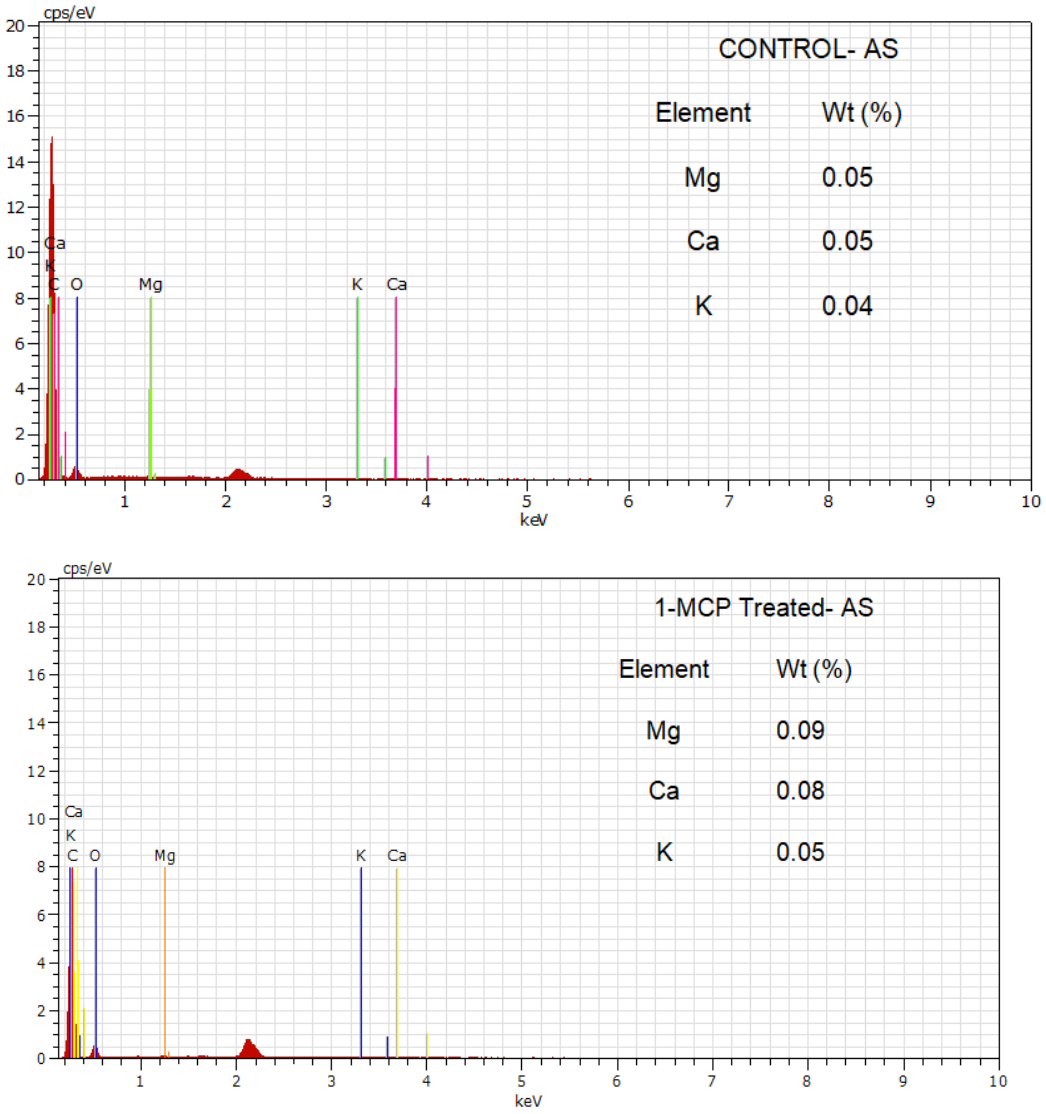
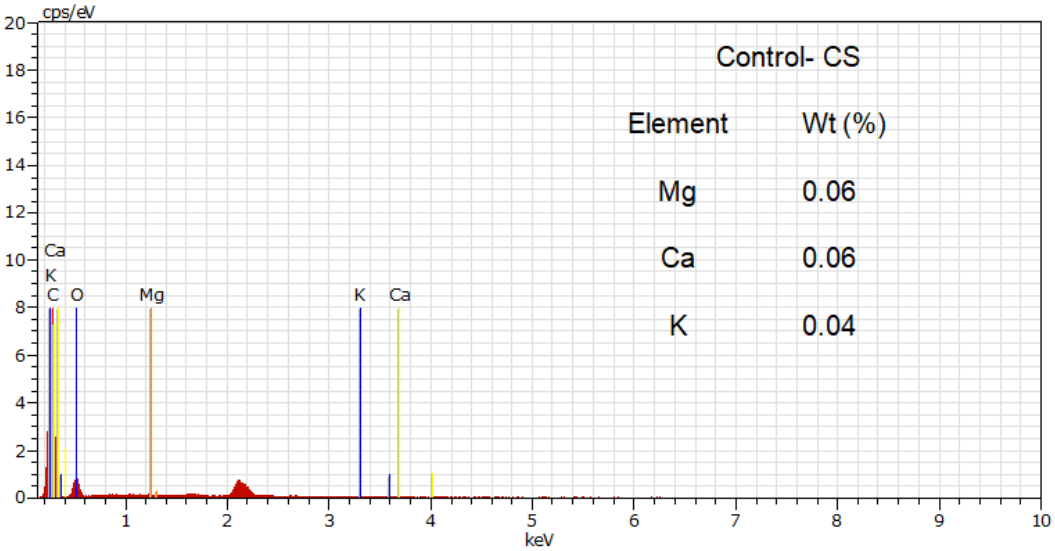



**Fig. 5a. EDAX analysis of Mg, Ca and K (%) 1-MCP treated and control sample under AS**



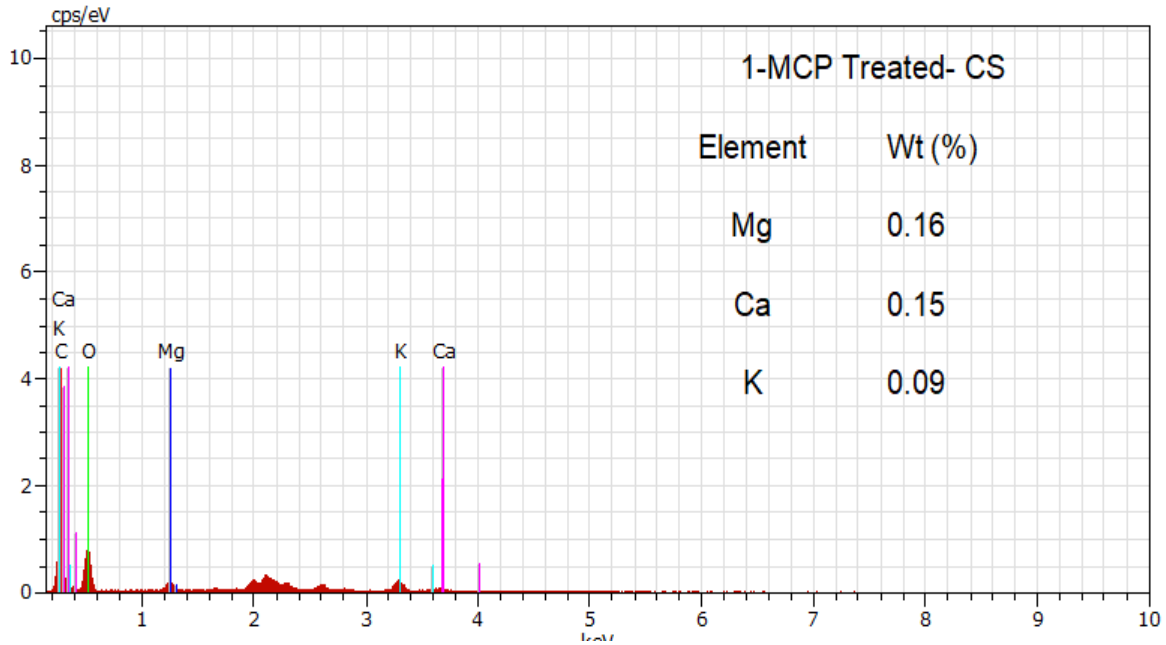



**Fig. 5b. EDAX analysis of Mg, Ca and K (%) 1-MCP treated and control sample under CS**

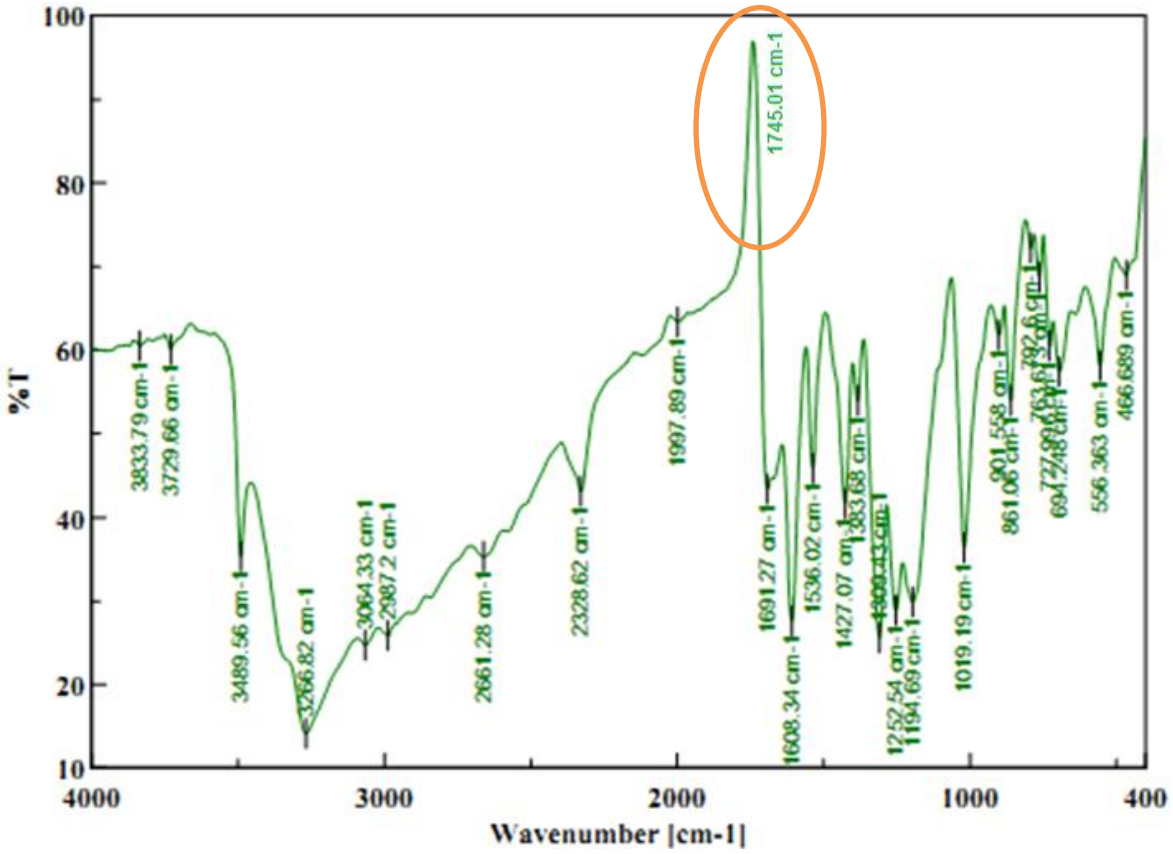



**Fig. 6. FT-IR spectrum of methylated pure pectin- standard (Sigma)**

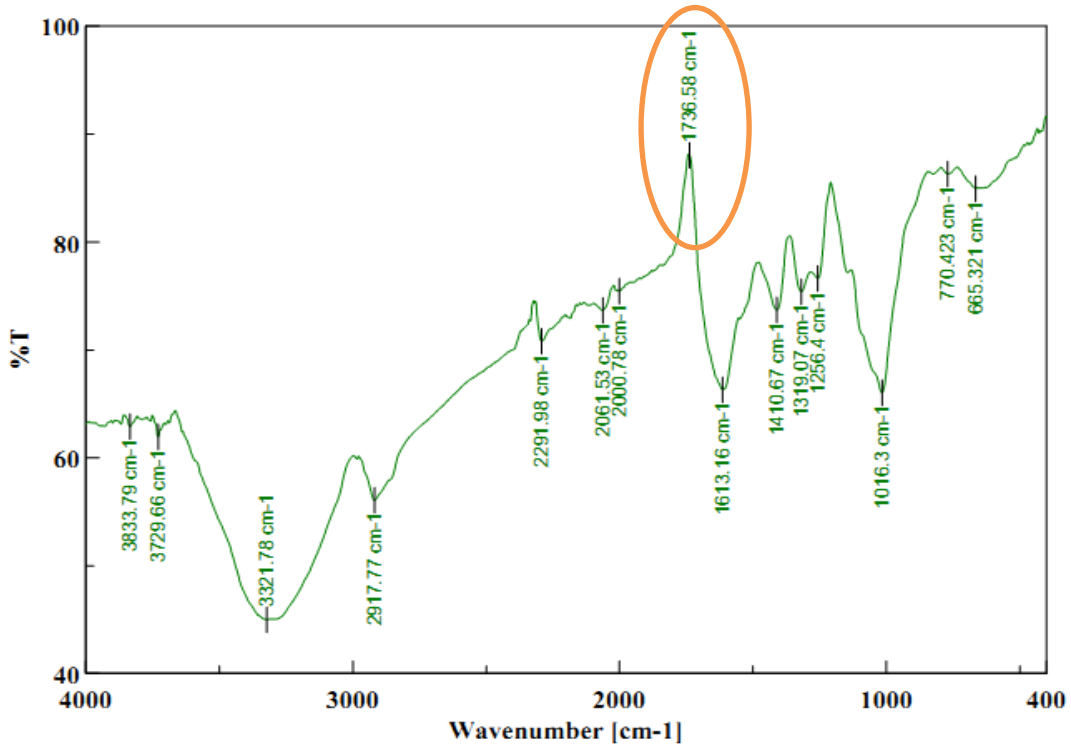



**Fig. 7. Pulp of 1-MCP treated fruit showing the characteristic peak similar to standard at around wavenumber 1736 cm$^{-1}$**

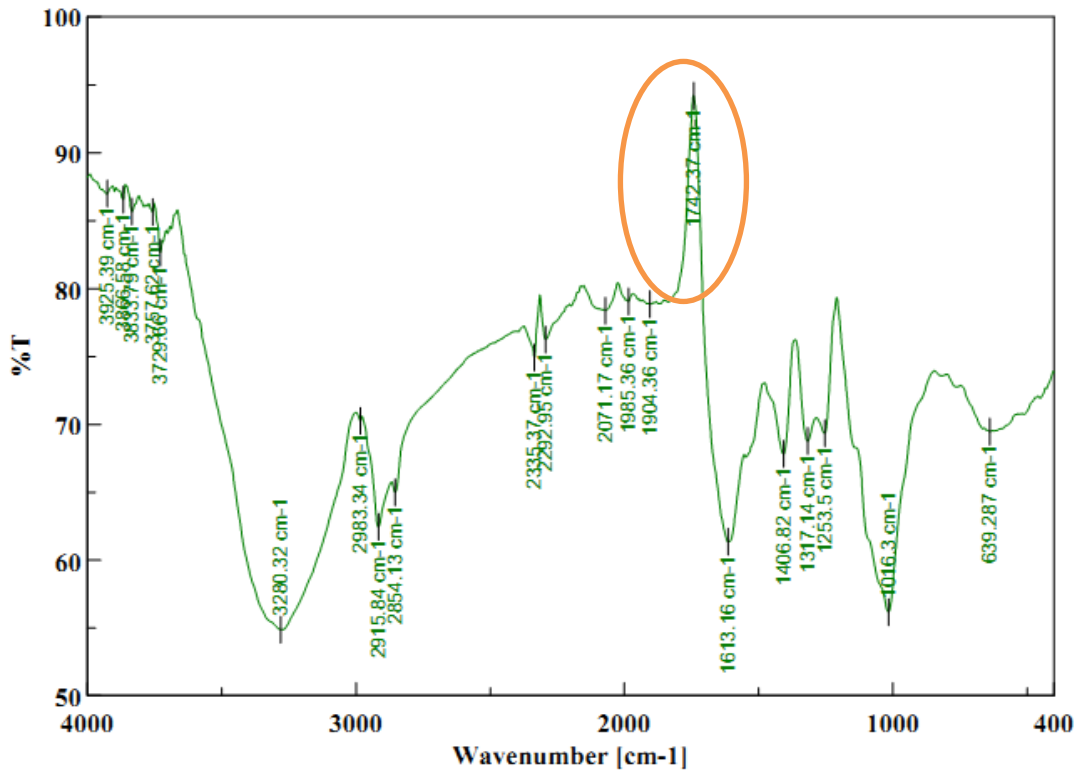



**Fig. 8. Peel of 1-MCP treated fruit showing characteristic peak at wavenumber 1742 cm$^{-1}$**

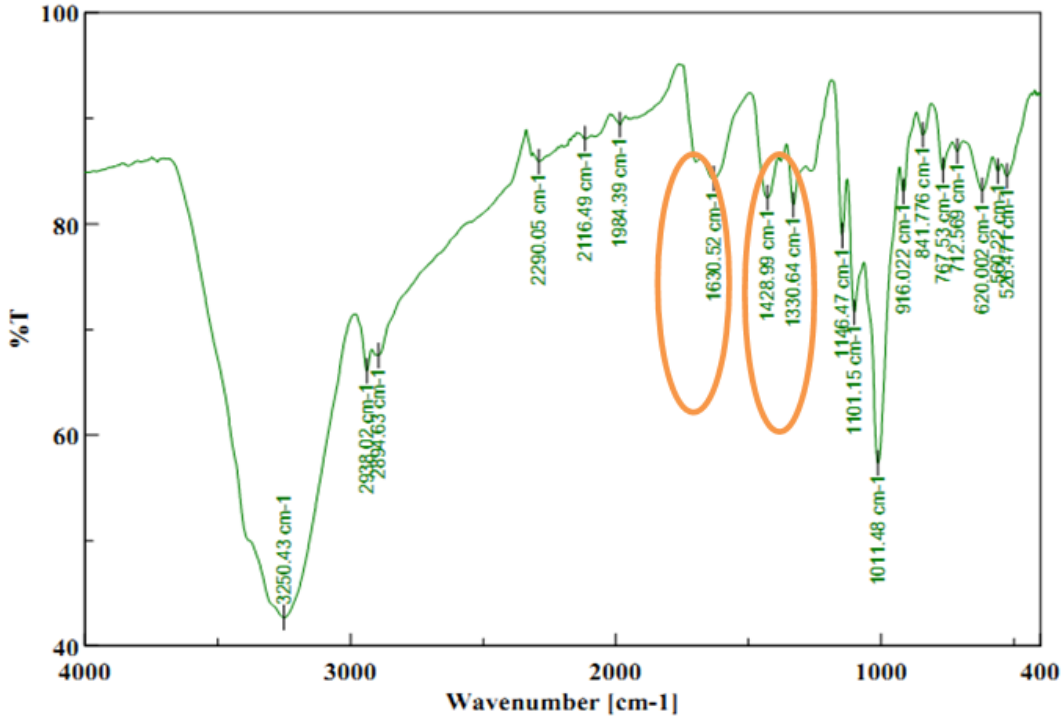



**Fig. 9. Pulp of untreated fruit depicting hydrolyzed pectin and more of carbohydrate peaks at around 1200-950 cm$^{-1}$**

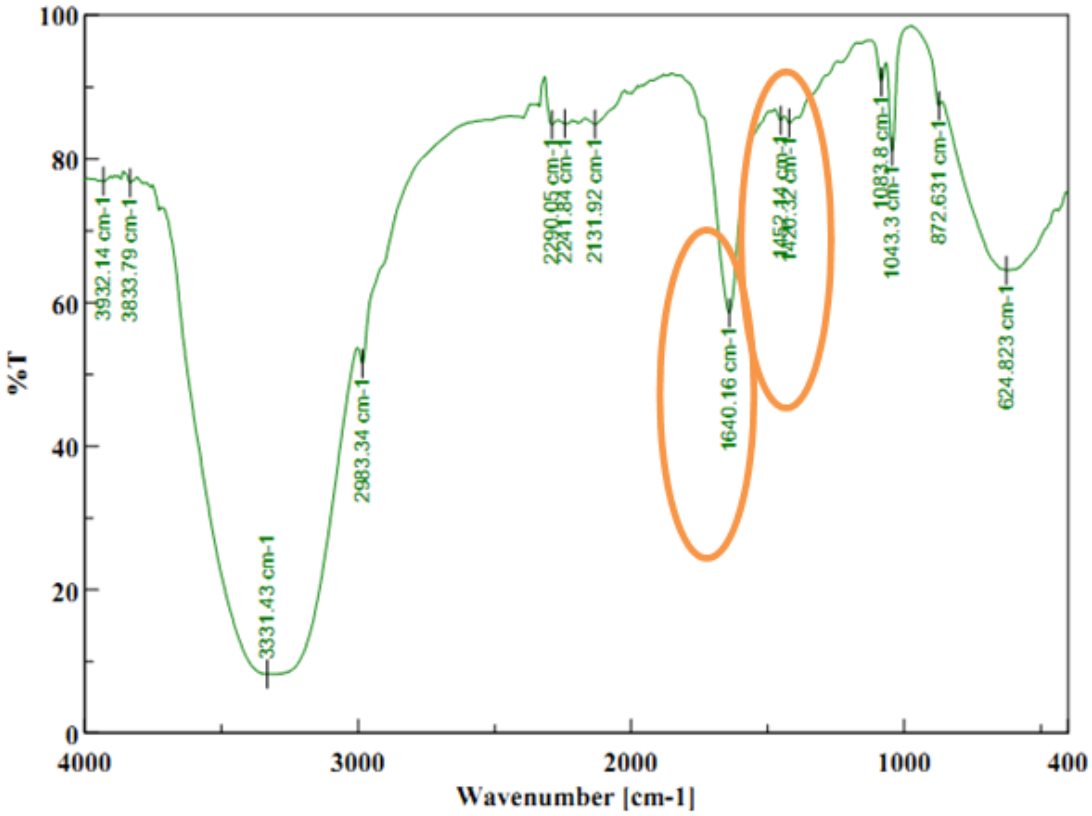



**Fig. 10. Peel of untreated fruit depicting hydrolyzed pectin spectra**

## 4. CONCLUSION

Application of 1-MCP had proved potential in improving the keeping quality of papaya fruit as revealed from the ultra-structural changes and FT-IR analysis. Studies to examine the papaya ripening associated characteristics using SEM and FT-IR spectroscopic were found to be very effective in studying the cell wall changes. The study further confirms that ethylene indued ripening and associated physiological, metabolic changes can be delayed using 1-Methylcyclopropene.

## DISCLAIMER

The 1-MCP product used for this research is and was approved by Environment and Protection Agency, since its use and is predominantly used product in our area of research and country to prevent postharvest losses. There is absolutely no conflict of interest between the authors and producers of the product because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the Agrofresh Inc., Mumbai company rather it was funded by personal efforts of the authors.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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