Int.J.Curr.Microbiol.App.Sci (2018) *7*(5): 887-898

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 05 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.705.108

FTIR Spectroscopic Characterization of Almond Varieties (*Prunus dulcis*) from Himachal Pradesh (India)

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Keywords

Almonds, FTIR, Katha, Thin Shell, Dhebar, Telangi selection, Functional groups

Article Info

Accepted:
10 April 2018
Available Online:
10 May 2018

A B S T R A C T

Almond is known for its nutritive value and is popularly consumed in India after overnight soaking. It is rich in lipid profile, polyphenols content followed by vitamin-E and also found rich in minerals like magnesium, potassium, calcium and phosphorus which play important biological roles in the human body. There are different types of almond varieties present in India which include commercial types and wild types. The present study aims at Fourier Transform Infrared Spectroscopy (FTIR) characterisation of the four popular varieties of almond from Kinnaur, Himachal Pradesh (India) viz. Katha, Thin Shell, Dhebar and Telangi Selection. FTIR spectroscopic studies will enable us to assess the nutritionally important functional groups present in almonds which in turn will support the variety specific compositional chemistry and nutritional characterization of selected almond varieties. FTIR of four different varieties of almonds viz. Katha, Thin shell, Dhebar and Telangi Selection showed characteristic peak values referring the presence of diverse class of functional groups like esters, amines, carboxylic acids, aromatics, phenols and amides.

Introduction

Almond belongs to Rosaceae family which also includes prunes, apple, raspberries and pears. Almond (*Prunus dulcis*) is one of the nutritive tree nuts with a rich lipid profile, polyphenols content followed by vitamin E and also found rich in minerals like magnesium, potassium, calcium and phosphorus which plays an important biological roles in the human body.

In humans it lowers down the total LDL cholesterol if half of the daily diet intake is replaced by nuts (Abbey *et al.,* 1994). Almond and the other tree nuts are called as nutrient dense food due to the presence of lipids and proteins in them (King *et al.,* 2008) and therefore of vital importance for the human health and makes it a potential ingredient in nutrition based food industry. Besides this they also provide a healthy nutritional profile by providing dietary fibre, Vitamin-E, phytosterols, micronutrients which leads to diverse benefits in terms of blood lipid profile, cardio-protective benefits (Griel and Kris-Etherton, 2006; Esfahlan *et al.,* 2010; Richardson *et al.,* 2009; Jenkins *et al.,* 2008; Coates and Howe, 2007, Esfandan, *et al.,* 2010).

Diverse ancient medical and scientific records of Greeks, Persians, Chinese and Indian ayurveda describes about properties of sweet almonds and their role in nourishing the health and having the brain strengthening properties (Albala, 2009). Almonds are mainly grown in Jammu and Kashmir followed by Himachal Pradesh and some parts of the Uttarakhand (APEDA database). Globally, USA is the leading producer in the world for almond production and its production is mainly confined to the California (Bolling *et al.*, 2010). The environmental conditions like variability in the climate impacts the composition, nutritional value followed by the health attributes in almonds (Jahanban *et al.*, 2010; Piscopo *et al.*, 2010). Almonds are rich in monounsaturated fatty acids and possess 21% protein followed by 4% carbohydrates (Mexis and Kontominas, 2010). A latest FTIR study on the medicinal varieties of almonds (chinese origin) exhibited strong presence of alkene functional group, weak aldehyde groups followed by medium intensity functional groups like alkanes indicative of its pharmacological and medicinal significance (Cheng *et al.*, 2017). Various studies have also indicated that the sweet flavours and bitter flavours are inheritable traits in almond (Dicenta and Garcia, 1993 and Wirthensohn *et al.*, 2008). The bitter almonds possess (3-9%) of amygdalin which is a diglucoside by nature and on enzymatic hydrolysis, it leads to the development of hydrocyanic acid and benzaldehyde. Chaouali *et al.*, (2013) also reported about presence of toxic levels of cyanide in almonds. Thus bitter almonds are used for extracting flavors. In current pretext, very limited information is available about the varietal and nutritional characterization of indigenous (Indian origin) almond varieties.

Since, the scientific information regarding these underexplored varieties (Katha, Dhebar, Telangi Selection, Thin Shell) of Himachal Pradesh is limited, the aim of current study is an attempt to identify the of functional groups of these selected almond varieties. The information obtained as a result of this study could be beneficial to food and nutraceuticals industries for the new product development, extraction of flavours, medicinal compounds, etc.

Materials and Methods

The four local almond varieties namely Katha, Thin shell, Dhebar, and Telangi Selection were procured from Regional Horticultural Research and Training Station (Dr Y.S. Parmar University of Horticulture and Forestry, Solan), Sharbo, Distt. Kinnaur Himachal Pradesh. The samples were prepared immediately before analysis by grinding them in an electric grinder followed by sieving through 1mm sieve to achieve homogeneity. The homogenous samples were subjected to FTIR analysis using FTIR (Cary 630 FTIR Spectrometer, Agilent Technologies). A minute quantity of freshly ground sample was used on the ATR crystal area (1x1mm) ensuring the complete coverage of crystal area for error free analysis. The clamp over the sample was tightened for better functioning of the instrument. Transmittance mode was used for recording the spectra. Crystal was made clear by using acetone followed by its drying by a soft tissue paper before placing the subsequent samples. The interpretation of the FTIR results has been done on the basis of reference information as compiled by Coates (2000) and García *et al.*, (2013).

Results and Discussion

FTIR analysis of four almond varieties native of the district Kinnaur (Himachal Pradesh) was done. The FTIR results obtained regarding spectra analysis of selected almond varieties have been presented in the Table 1 (A-D) and the peaks obtained for each variety

have also been presented in Figure 1-4, respectively. The Plates 1(A) and 1(B) depict the varieties under study.

The important constituents of almonds indicating presence of specific functional groups are proteins, carbohydrates, alcohol, esters and water. The spectra of each variety showed specific bands indicating the presence of specific functional groups. The results presented are among the first of its kind report on FTIR spectroscopic analysis of the selected varieties from Himachal Pradesh.

In Katha variety (Table 1A) and as shown in the Figure 1, the different bands falling between the different spectra are presented. In case of spectra region 3000-3200cm^{-1} shows the presence of band 3279cm^{-1} which indicates the presence of Normal "Polymeric" OH group (Alcohol and hydroxyl compounds group frequencies) is also supported by the previous studies done by Thygesen *et al.,* (2003) and Subramanian *et al.,* (2009).

Besides this, the latest study done on the Chinese medicinal almonds also showed the high intensity peaks for alkenes (Cheng *et al.,* 2017) and therefore confirms our results. Further, it is also to mention that the values above 3000cm^{-1} indicates the presence of unsaturated or aromatic groups (Medial *cis*- or *trans*-C-H stretch) whereas the values below 3000cm^{-1} indicate the prevalence of aliphatic groups (C-H Group). The presence of aliphatic groups have also been reported by Sanahuja *et al.,* (2009), Hern´andez and Zacconi (2009), Klaypradit *et al.,* (2010).

The range 2800-3000cm^{-1} spectra region showed the presence of two bands, one having the frequency 2923 cm^{-1} and 2855cm^{-1} which are assigned for the presence of Methylene C-H asymmetric/symmetric stretch (Saturated aliphatic alkane/alkyl group frequencies). The similar results were obtained by Hern´andez and Zacconi (2009); Maqsood and Benjakul (2010). Cheng *et al.,* (2017) also reported medium intensity peaks for alkane functional group in their study on medicinal almonds in China.

A sharp peak having frequency 1631cm^{-1} could be observed between the frequency range of 1600-1800cm^{-1} which indicated the presence of amides. The results are supported with the findings of Thygesen *et al.,* (2003), Subramanian *et al.,* (2009) and there is another peak within the same frequency range at 1742cm^{-1} which indicates the presence of Carbonyl C=O functional group frequencies of triglyceride esters and the result is supported with finding of Sanahuja *et al.,* (2009). In case of spectra region falling in range of 1400-1600cm^{-1} shows presence of peak frequencies 1536cm^{-1} which indicates the presence of Aromatic nitro compounds (Simple hetero-oxy compounds). The results are supported by the studies undertaken by Martinez *et al.,* (2003).

The frequency range 1200-1400cm^{-1} showed three peaks wherein peaks at 1396cm^{-1} and 1314cm^{-1} indicated the presence of Carboxylates (Carboxylic acid salts); Carbonyl compound group frequencies whereas peak at 1237cm^{-1} is related to the presence of Aromatic ethers (Oxy compounds group frequencies. The studies conducted by Zahm *et al.,* (2011) and Dimick *et al.,* (1983) also indicate the presence of such compounds in nuts and fruits.

Another frequency band of 1041cm^{-1} was found between the frequency ranges 1000-1200cm^{-1} which indicated the presence of Primary amine followed by presence of aliphatic phosphates (P-O-C stretch) grouped under simple hetero-oxy compounds as indicated by peak at 998cm^{-1} in the spectral frequency range of 800-1000cm^{-1}. Presence of such compounds has also been reported by Maga and Katz (2012) and Lott *et al.,* (2000) in their respective studies.

Table.1A FTIR spectroscopy results of Katha variety

Katha Almonds				
Sl No.	**Spectral frequency range**	**Peak values**	**Origin of Peak**	**Reference**
1.	3200-3400	3279	Normal "Polymeric" OH group (Alcohol and hydroxyl compounds group frequencies)	Thygesen *et al.*, (2003), Subramanian *et al.*, (2009)
2.	2800-3000	2923	Methylene C-H asymmetric/symmetric stretch (Saturated aliphatic alkane/alkyl group frequencies)	Hernandez and Zacconi ´ (2009), Maqsood and Benjakul (2010)
3.	2800-3000	2855	Methylene C-H asymmetric/symmetric stretch (Saturated aliphatic alkane/alkyl group frequencies)	Hernandez and Zacconi ´ (2009), Maqsood and Benjakul (2010)
4.	1600-1800	1631	Amide	Thygesen *et al.*, (2003), Subramanian *et al.*, (2009)
5.	1600-1800	1742	Carbonyl C=O functional group frequencies of triglyceride esters	Sanahuja *et al.*, (2009)
6.	1400-1600	1536	Aromatic nitro compounds (Simple hetero-oxy compounds)	Martinez *et al.*, (2003)
7.	1200-1400	1396	Carboxylates (Carboxylic acid salts); Carbonyl compound group frequencies	Zahm *et al.*, (2011)
8.	1200-1400	1314	Carboxylates (Carboxylic acid salts); Carbonyl compound group frequencies	Zahm *et al.*, (2011)
9.	1200-1400	1237	Aromatic ethers (Oxy compounds group frequencies)	Dimick *et al.*, (1983)
10.	1000-1200	1041	Primary amine	Maga and Katz. (2012)
11.	800-1000	998	Aliphatic phosphates (P-O-C stretch) grouped under simple hetero-oxy compounds	Lott *et al.*, (2000)

Table.1B FTIR spectroscopy results of thin shell variety

Thin Shell Almonds				
Sl No.	**Spectral frequency range**	**Peak values**	**Origin of Peak**	**Reference**
1.	3200-3400	3278	Normal "Polymeric" OH group(Alcohol and hydroxyl compounds group frequencies)	Thygesen *et al.*, (2003), Subramanian *et al.*, (2009)
2.	2800-3000	2923	Methylene C-H asymmetric/symmetric stretch(Saturated aliphatic alkane/alkyl group frequencies)	Hernandez and Zacconi ´ (2009), Maqsood and Benjakul (2010)
3.	2800-3000	2854	Methylene C-H asymmetric/symmetric stretch(Saturated aliphatic alkane/alkyl group frequencies)	Hernandez and Zacconi ´ (2009), Maqsood and Benjakul (2010)
4.	1600-1800	1632	Amide	Thygesen *et al.*, (2003), Subramanian *et al.*, (2009)
5.	1600-1800	1743	Ester (Carbonyl compound group frequencies)	Sanahuja *et al.*, (2009)
6.	1400-1600	1540	Aliphatic Nitro compounds (Simple hetero-oxy compounds)	Martinez *et al.*, (2003)
7.	1200-1400	1397	Carboxylates (Carboxylic acid salts) Carbonyl compound group frequencies	Zahm *et al.*, (2011)
8.	1200-1400	1237	Aromatic ethers (Oxy compounds group frequencies)	Dimick *et al.*, (1983)
9.	1000-1200	1040	Primary amine	Maga and Katz. (2012)
10.	800-1000	993	Aliphatic phosphates (P-O-C stretch) grouped under simple hetero-oxy compounds	Lott *et al.*, (2000)

Table.1C FTIR spectroscopy results of Dhebar variety

Dhebar Almonds				
Sl No.	**Spectral frequency range**	**Peak values**	**Origin of Peak**	**Reference**
1.	3200-3400	3279	Normal "Polymeric" OH group (Alcohol and hydroxyl compounds group frequencies)	Thygesen *et al.*, (2003), Subramanian *et al.*, (2009)
2.	2800-3000	2923	Methylene C-H asymmetric/symmetric stretch(Saturated aliphatic alkane/alkyl group frequencies)	Hernandez and Zacconi ´ (2009), Maqsood and Benjakul (2010)
3.	2800-3000	2854	Methylene C-H asymmetric/symmetric stretch (Saturated aliphatic alkane/alkyl group frequencies)	Hernandez and Zacconi ´ (2009), Maqsood and Benjakul (2010)
4.	1600-1800	1631	Amide	Thygesen *et al.*, (2003), Subramanian *et al.*, (2009)
5.	1600-1800	1743	Ester (Carbonyl compound group frequencies)	Sanahuja *et al.*, (2009)
6.	1400-1600	1536	Aromatic nitro compounds (Simple hetero-oxy compounds)	Martinez *et al.*, (2003)
7.	1400-1600	1400	Phenol (hydroxyl compounds)	Bolling *et al.*, (2011)
8.	1400-1200	1237	Aromatic ethers (Oxy compounds group frequencies)	Dimick *et al.*, (1983)
9.	1000-1200	1043	Primary amine (CN stretch)	Maga and Katz. (2012)

Table.1D FTIR spectroscopy results of Telangi selection variety

Telangi Selection Almonds				
Sl No.	**Spectral frequency range**	**Peak values**	**Origin of Peak**	**Reference**
1.	3200-3400	3279	Normal "Polymeric" OH group (Alcohol and hydroxyl compounds group frequencies)	Thygesen *et al.*, (2003), Subramanian *et al.*, (2009).
2.	2800-3000	2855	Methylene C-H asymmetric/symmetric stretch (Saturated aliphatic alkane/alkyl group frequencies)	Hernandez and Zacconi ´ (2009), Maqsood and Benjakul (2010)
3.	2800-3000	2923	Methylene C-H asymmetric/symmetric stretch (Saturated aliphatic alkane/alkyl group frequencies)	Hernandez and Zacconi ´ (2009), Maqsood and Benjakul (2010)
4.	1600-1800	1743	Ester (Carbonyl compound group frequencies)	Sanahuja *et al.*, (2009)
5.	1600-1800	1633	Amide (Carbonyl compound group frequencies)	Thygesen *et al.*, (2003), Subramanian *et al.*, (2011)
6.	1400-1600	1402	Carboxylates (Carboxylic acid salts), Carbonyl compound group frequencies	Zahm *et al.*, (2011)
7.	1400-1600	1541	Aliphatic Nitro compounds (Simple hetero-oxy compounds)	Martinez *et al.*, (2003)
8.	1200-1400	1237	Aromatic ethers (Oxy compounds group frequencies)	Dimick *et al.*, (1983)
9.	1000-1200	1038	Primary amine	Maga and Katz. (2012)
10.	800-1000	992	Aliphatic or Aromatic phosphates (P-O-C stretch) Simple hetero-oxy compounds	Lott *et al.*, (2000)

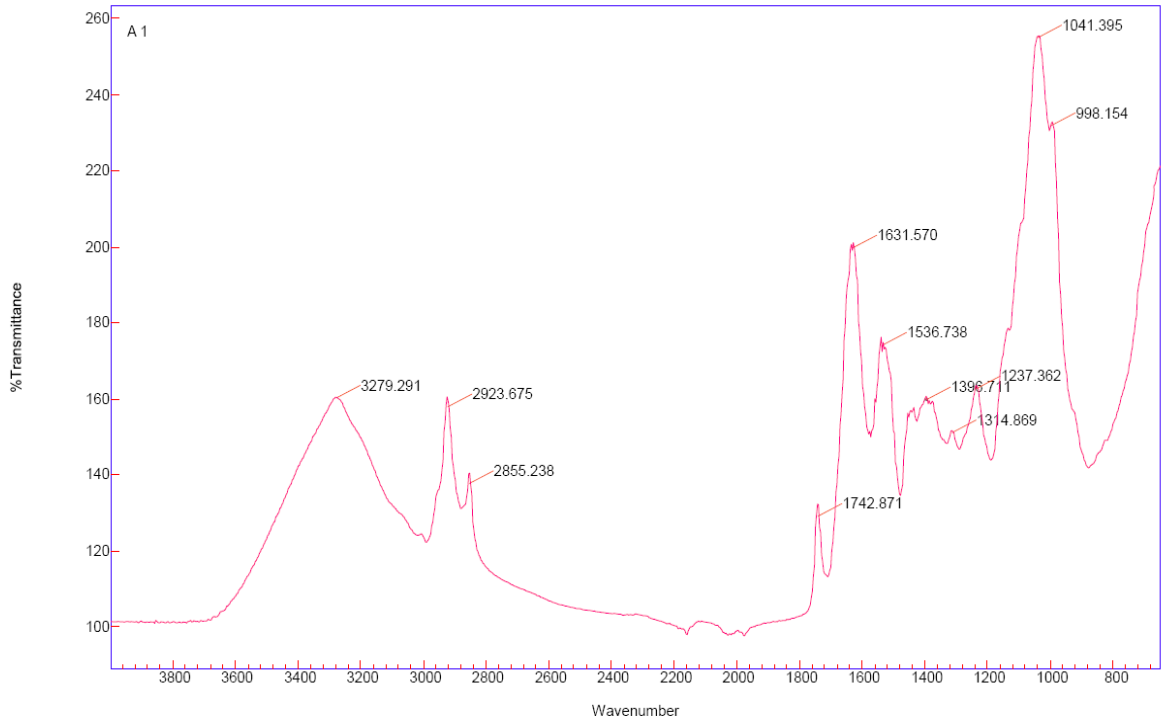

Fig.1 FTIR spectrum of Katha variety

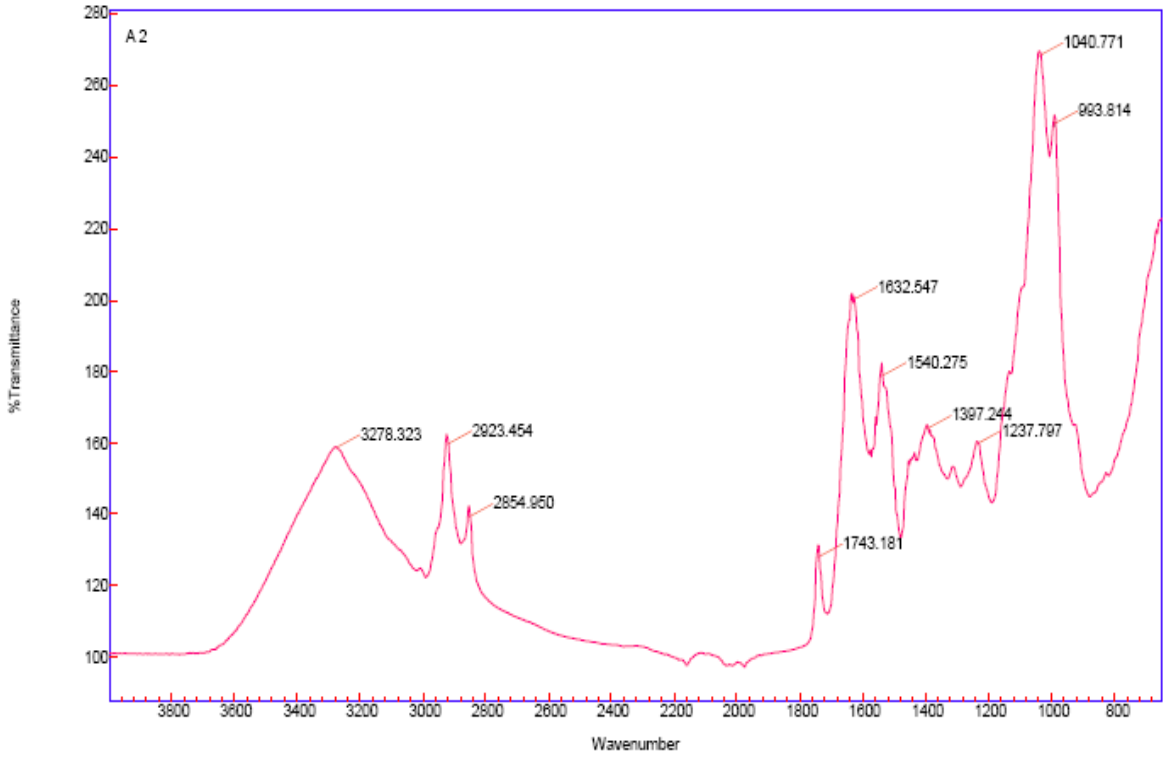

Fig.2 FTIR spectrum of thin shell

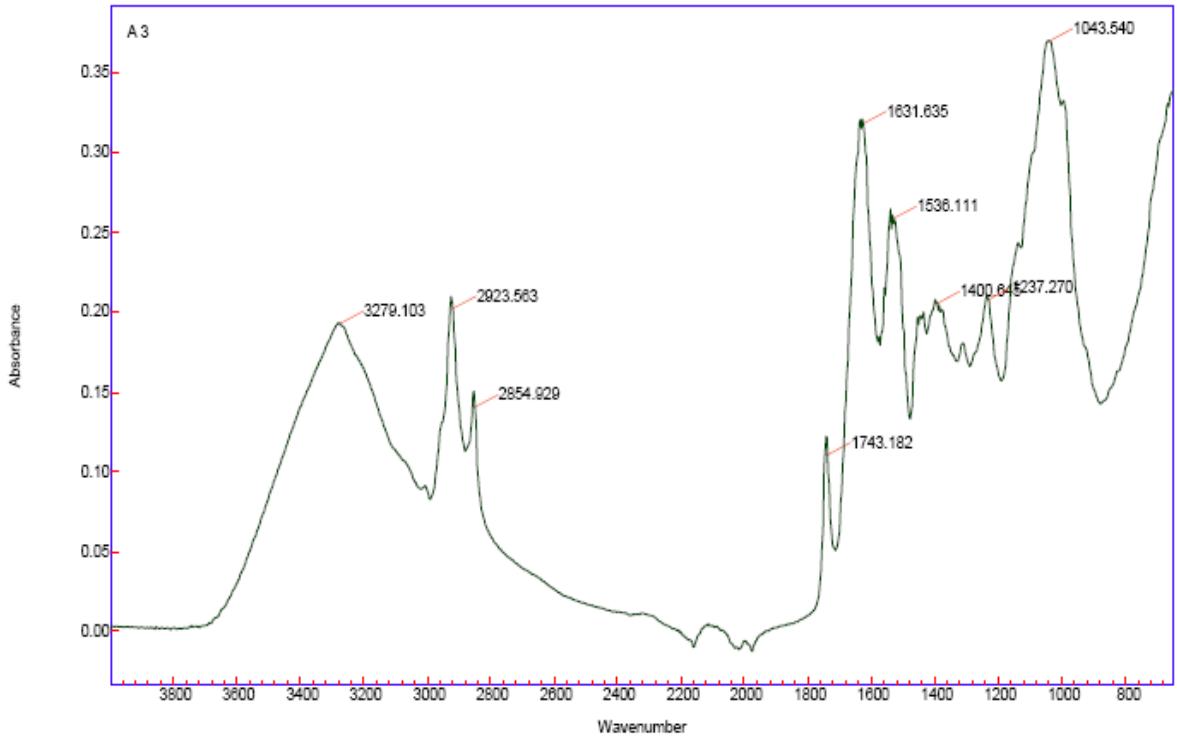

Fig.3 FTIR spectrum of Dhebar variety

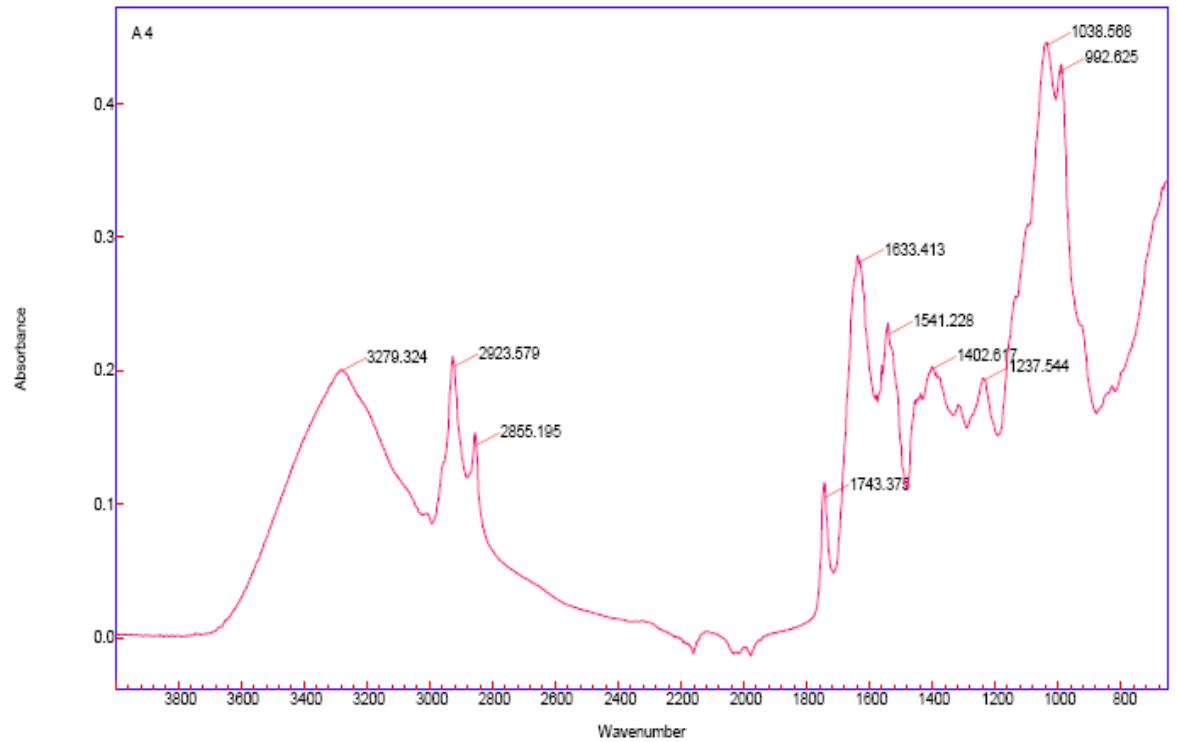

Fig.4 FTIR spectrum of Telangi selection variety

Plate.1 (A) Almond varieties under study – (Without Shell)

Plate.1 (B) Almond varieties under study – (With Shell)

In thin shell variety (Table 1B and Fig. 2) a distinct peaks at 3278cm^{-1} was observed in the spectrum range of 3200-3400cm^{-1} indicating the presence of Normal "Polymeric" OH group (alcohol and hydroxyl compounds group frequencies) as previously reported in the studies of Thygesen *et al.,* (2003) and Subramanian *et al.,* (2009).

The two peaks viz. 2923 cm^{-1} and 2854cm^{-1} falling in the spectrum range of 2800-3000cm^{-1} indicated the presence of Methylene C-H asymmetric/symmetric stretch (Saturated aliphatic alkane/alkyl group frequencies). Such peaks has also been reported in studies conducted by Hernandez and Zacconi´ (2009), Maqsood and Benjakul (2010).

A peak having frequency 1743cm^{-1} indicated the presence of Carbonyl functional group frequencies of esters and the result is supported with finding of Sanahuja *et al.,* (2009) between the frequency range of 1600-1800cm^{-1} wherein the peak at 1632cm^{-1} indicated the presence of amides which is supported by the findings of Thygesen *et al.,* (2003) and Subramanian *et al.,* (2009).

The studies of Martinez *et al.,* (2003) indicates the association of nitro compounds with flavours of bitter almonds and in our study we found the presence of a peak 1540cm-^{1} in spectral frequency range of 1400-1600cm^{-1} which is largely associated with Aliphatic Nitro compounds (Simple hetero-oxy compounds).

The frequency range 1200-1400cm^{-1} showed two peaks wherein peak at 1397cm^{-1} indicated the presence of Carboxylates (Carboxylic acid salts) and Carbonyl compound group frequencies whereas peak at 1237cm^{-1} is related to the presence of Aromatic ethers (Oxy compounds group frequencies). The studies conducted by Zahm *et al.,* (2011) and Dimick *et al.,* (1983) also indicate the presence of such compounds in nuts and fruits, respectively.

Another frequency band of 1040cm^{-1} was found between the frequency ranges 1000-1200cm^{-1} which indicated the presence of Primary amine followed by presence of aliphatic phosphates (P-O-C stretch) grouped under simple hetero-oxy compounds as indicated by peak at 993cm^{-1} in the spectral frequency range of 800-1000 cm^{-1}. Presence of such compounds has also been reported by Maga and Katz (2012) in food and Lott *et al.,* (2000) in seeds and fruits, respectively.

FTIR Analysis of Dhebar variety (Table 1C Fig. 3) shows that spectrum range of 3200-3400cm^{-1} is marked with presence of wave no. 3279cm^{-1} which indicates polymeric O-H stretch and the same has also been reported by Subramanian *et al.,* (2009) and Thygesen *et al.,* (2003).

In range of 2800-3000cm^{-1} lies two wave numbers viz., 2923cm^{-1} 2854cm^{-1} which indicated the presence of methylene C-H asymmetric/symmetric stretch (Saturated aliphatic alkane/alkyl group frequencies) and is supported by results of studies done in past by Hern´andez and Zacconi (2009) and Maqsood and Benjakul (2010).

In case of peaks lying in the range 1600-1800cm^{-1} there are two main peaks 1743 cm^{-1} and 1631 cm^{-1}. The peak 1631cm^{-1} indicates presence of amides while 1743cm^{-1} indicates the presence of ester compounds which forms the major components (like oil) in the almonds. The results are supported by the work of Thygesen *et al.,* (2003), Subramanian *et al.,* (2009) and Sanahuja *et al.,* (2009).

The spectrum range of 1400-1600cm^{-1} showed presence of 1536cm^{-1} wave no. indicating presence of aromatic nitro compounds whereas 1400cm^{-1} wave no. indicates the presence of phenols. Aromatic nitro compounds associated with bitter flavour of almond has been mentioned by Martinez *et al.,* (2003) in his work on acute nitrobenzene poisoning. The presence of phenols and its related compounds have also been reported by Bolling *et al.,* (2011).

The spectra range of 1400-1200 cm^{-1} showed

peak 1237cm^{-1} indicating presence of aromatic ethers (oxy compounds group frequencies). Dimick *et al.,* (1983) also reported presence of such aromatic compounds in his studies on apple flavours. The spectrum range of 1200-1000cm^{-1} showed a peak 1043 cm^{-1} which indicated the presence of primary amine compounds (C-N Stretch). Maga and Katz (2012) have also reported the presence of such compounds (amines) in food.

The FTIR analysis of Telangi selection, (Table 1D Fig. 4), revealed that the first range of spectra 3200-3400cm^{-1} with the peak value 3279cm^{-1} and indicated the presence of presence of hydroxyl group (O-H) and the same has been reported in past by Thygesen *et al.,* (2003) and Subramanian *et al.,* (2009).

The other spectrum range of 2800-3000cm^{-1} showed peaks viz., 2855cm^{-1} and 2923cm^{-1}, indicative of the presence of methylene (C-H) asymmetric/symmetric stretch. Similar results have been reported by Hern´andez and Zacconi (2009) and Maqsood and Benjakul (2010).

In the spectral range 1600-1800cm^{-1}, the presence of peaks 1743cm^{-1} and 1633 cm^{-1} are indicative of the presence of Carbonyl compound group frequencies which are supported by the results obtained by Sanahuja *et al.,* (2009), Thygesen *et al.,* (2003) and Subramanian *et al.,* (2011).

The spectral range of 1400-1600cm^{-1} shows a peak as 1402cm^{-1} indicating the presence Carboxylates (Carboxylic acid salts) and the other peak at 1541cm^{-1} is indicative of the presence of Aliphatic Nitro compounds (Simple hetero-oxy compounds).

In the spectral range of 1200-1400cm^{-1}, the peak at 1237cm^{-1} indicated the presence of aromatic ethers. The studies on apple flavours also find the mention of such aromatic compounds in Rosaceae family (Dimick *et al.,* (1983).

The spectral range of 1000-1200cm^{-1} showed a peak values as 1038cm^{-1} indicating the presence of primary amines and amines has been reported as part of building blocks of amino acids (Maga and Katz, 2012).

The peak at 992cm^{-1} in the spectral range of 800-1000 cm^{-1} indicated the presence of indicating the presence of aliphatic or aromatic phosphates (P-O-C stretch) grouped under simple hetero-oxy compounds. Presence of such phosphorus related compounds has also been studied by Lott *et al.,* (2000) crop seeds and fruits.

The four varieties considered for the current study *viz.* Katha, Thin shell, Dhebar and Telangi selection are the local varieties found in the Kinnaur region of Himachal Pradesh. These varieties are considered native to this area and have been traditionally used in the local areas only. Generally most of the compounds in the almonds are similar but the deviations could be there due to the different geographical areas, growth environments and also due to agronomic practices followed in that specific location. Therefore, in order to highlight the nutritional importance of these traditional varieties the FTIR spectroscopic study was conducted to explore the functional groups of biological importance. The results showed characteristic peak values referring the presence of diverse class of functional groups like esters, amines, carboxylic acids, aromatics, phenols and amides.

Acknowledgement

We are grateful to Regional Horticultural Research and Training Station (Dr Y.S. Parmar University of Horticulture and Forestry, Solan), Sharbo, Distt. Kinnaur, Himachal Pradesh for providing us the samples of these four varieties of almonds to carry out this study.

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How to cite this article:

Sunil Sharma and Neeraj. 2018. FTIR Spectroscopic Characterization of Almond Varieties (*Prunus dulcis*) from Himachal Pradesh (India). *Int.J.Curr.Microbiol.App.Sci.* 7(05): 887-898.
doi: https://doi.org/10.20546/ijcmas.2018.705.108