ORIGINAL ARTICLE

Application of Fourier transform infrared (FTIR) spectroscopy for the identification of wheat varieties

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Abstract Quality characteristics of wheat are determined by different physiochemical and rheological analysis by using different AACC methods. AACC methods are expensive, time consuming and cause destruction of samples. Fourier transforms infrared (FTIR) spectroscopy is one of the most important and emerging tool used for analyzing wheat for different quality parameters. This technique is rapid and sensitive with a great variety of sampling techniques. In the present study different wheat varieties were analyzed for quality assessment and were also characterized by using AACC methods and FTIR technique. The straight grade flour was analyzed for physical, chemical and rheological properties by standard methods and results were obtained. FTIR works on the basis of functional groups and provide information in the form of peaks. On basis of peaks the value of moisture, protein, fat, ash, carbohydrates and hardness of grain were determined. Peaks for water were observed in the range $1{,}640~\text{cm}^{-1}$ and $3{,}300~\text{cm}^{-1}$ on the basis of functional group H and OH. Protein was observed in the range from $1{,}600~\text{cm}^{-1}$ to $1{,}700~\text{cm}^{-1}$ and $1{,}550~\text{cm}^{-1}$ to $1{,}570~\text{cm}^{-1}$ on the basis of bond amide I and amide II respectively. Fat was also observed within these ranges but on the basis of C-H bond and also starch was observed in the range from $2{,}800$ and $3{,}000~\text{cm}^{-1}$ (C-H stretch region) and in the range $3{,}000$ and $3{,}600~\text{cm}^{-1}$ (O-H stretch region). As FTIR is a fast tool it can be easily emplyed for wheat varieties identification according to a set criterion.

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Introduction

Wheat is a major cereal crop and an important component of human diet particularly in developing countries. Wheat is considered as unique among the cereals largely due to the fact that its grain contains unique chemical and physical properties. Besides being a rich source of carbohydrates, wheat contains other valuable components such as protein, minerals, phytochemicals and vitamins (Anjum et al. 2008; Harold et al. 2005).

Several techniques are used to check the differences in grains of different wheat varieties. These techniques include the FTIR spectroscopic analysis (Jamme et al. 2008; Mills et al. 2005), SDS-PAGE, spectroscopic analysis by using atomic absorption spectroscopy and some other standard methods (Cubadda et al. 2004). Chemical mapping using Fourier transform IR (FTIR) spectroscopy has been successfully applied to a wide range of agricultural products e.g. flax stem, wheat, maize, oat, rye, grains, soybean seed (Wetzel and Reffner 1993; Himmelsbach et al. 1998). Fourier transform infrared spectroscopy is a powerful tool for compositional analysis and has been widely used to analyze cell wall of cereal grains and composition of hard and soft milling wheat using FTIR imaging in conjunction with multivariate statistical analysis and identified spectral differences which were confined to specific cell types within the starchy endosperm tissue (Barron et al. 2005).

FTIR is a rapid, nondestructive, time saving method that can detect a range of functional groups and is sensitive to changes in molecular structure. FTIR provide information on the basis of chemical composition and physical state of

the whole sample (Cocchi et al. 2004). Fourier Transform-Infrared spectroscopy (FTIR) has been used as a rapid, accurate and nondestructive technique for measuring many wheat quality parameters. Fourier-transform Raman spectrometry is a powerful tool for the investigation of primary cell wall characteristics at a molecular level and providing complementary information to that obtained by Fourier transform infrared micro-spectroscopy. In addition the sensitivity and accuracy of FTIR detectors along with wide variety of software algorithms have dramatically increased the practical use of infrared for quantitative analysis (Dowell et al. 2006).

The mandate of the present study was to assess the physical, chemical and rheological properties of different wheat varieties by standard method and advance technique FTIR and their advantages over standard methods.

Materials and methods

Procurement of raw materials

Four commercially available wheat varieties were procured from Wheat Research Institute, Faisalabad Pakistan. The grains of each wheat variety was cleaned manually to remove damaged seeds, dust particles, seeds of other grains/crops and other impurities like metals and weeds. All the chemicals and reagents used in the study were purchased from sigma company USA.

Physical tests

The wheat grains of different varieties were analyzed for different physical characteristics like thousand kernel weight by counting one thousand wheat kernels of each variety randomly and weighing them separately and for test weight in kilograms per hectoliter (Kg/hl) according to procedure described in AACC (2000).

Wheat milling

The tempered wheat was milled through Brabender Quadrumate Senior Mill (C.W. Brabender Instruments, Inc.) to get break, reduction and straight grade flour according to the method No. 26–95 described in AACC (2000).

Dough rheological properties

The flour samples of each wheat variety was evaluated for farinographic characteristics by using Brabander Farinograph equipped with 50 g bowl according to procedure prescribed in AACC (2000) method No 54–21. The properties of dough were obtained by Farinograph. Dough characteristics such as water absorption, dough stability, dough development time, tolerance index and softening of dough were interpreted from Frinogram generated by Frinograph. Mixographic characteristic of straight grade flour was also estimated using mixograph according to the procedure prescribed in AACC (2000).

Chemical tests by standard methods

The flour samples of each wheat variety was analyzed for the moisture, ash, fat and protein contents according to their respective method Nos. 44- 15A, 08–01, 30–10, 46–10 and 32–10, given in AACC (2000).

Fourier transform infrared (FTIR) spectrometric analysis

(a) Sample preparation

Flour of each wheat variety was collected. These flour samples were converted into pellets and pellets were cut into thin sections. These thin sections were oven dried and their spectra were recorded on FTIR (Tensor 27, Bruker Optics, Germany).

(b) FTIR measurements

FTIR spectra were recorded for moisture content, crude fat, crude protein, ash content and starch contents

(i) Moisture content

Moisture content was determined by FTIR in mid infrared range. Peaks for water was observed in the $1,640 \text{ cm}^{-1}$ to $3,300 \text{ cm}^{-1}$ as mentioned by Manley et al. (2002)

(ii) Crude fat

Crude fat of flour samples was observed having peaks at $1,600 \text{ cm}^{-1}$ to $1,700 \text{ cm}^{-1}$ and $1,550 \text{ cm}^{-1}$ to $1,570 \text{ cm}^{-1}$ as mentioned by Che-Man and Setiowaty (1999).

(iii) Crude protein

Crude protein will be observed having peaks of amide I band at $1,600 \text{ cm}^{-1}$ to $1,700 \text{ cm}^{-1}$ and amide II at $1,550 \text{ cm}^{-1}$ to $1,570 \text{ cm}^{-1}$ observed by Manley et al. (2002).

(iv) Ash contents

Ash content was determined by FTIR using method mentioned by Armstrong et al. (2006).

(v) Starch contents

Spectra of starch were recorded using FT-IR, key bands. Spectra were recorded in regions below 800 cm^{-1}, $800–1,500 \text{ cm}^{-1}$ (the fingerprint region), the region between 2,800 and $3,000 \text{ cm}^{-1}$ (C-H stretch region), and finally the region between 3,000 and $3,600 \text{ cm}^{-1}$ (O-H stretch region) by method as mentioned by Belton et al. (1991).

Statistical analysis

All the analyses were carried out in triplicates. The data obtained for all parameters was statistically analyzed by performing analysis of variance (ANOVA) with DMR using STATISTIX 8.1 software according to the methods as described by Steel et al. (1997).

Results and discussion

Physical characteristics

The results regarding physical characteristics of wheat grains are presented in Table 1. The mean values of grain length ranged from 6.37 to 7.07 mm in different wheat varieties. The maximum grain length (7.07 mm) was found in the wheat variety of Pasban-90 followed by Seher (6.67 mm), Fsd-08 (6.57 mm) and Lasani-08 (6.37 mm). The lowest grain length (6.37 mm) was found in wheat variety Lasani-08. The highest grain width (3.60 mm) was found in the wheat variety Pasban-90 followed by Seher-06 (3.47 mm). The lowest grain width was observed in Lasani-08 (3.17 mm). The thousand-kernel weight was found to be maximum (49.34 g) in wheat variety Seher-06 followed by Fsd-08 (46.15 g), Lasani-08 (40.80 g) and pasban-90 (32.35 g). The lowest thousand-kernel weight (32.35 g) was found in wheat variety Pasban-90. The highest test weight (77.73%) was found in wheat variety Lasani-08 followed by Pasban-90 (76.89%) and Seher (75.05%). The lowest test weight was found (74.87%) in wheat variety Fsd-08.

Dough rheological properties

Water absorption was found to be highest (61.93%) in wheat variety Pasban-90 followed by Fsd-08 (60.33%) and Lasani-08 (59.5%). The lowest water absorption (59.4%) was found in wheat variety Seher-06. Mean value for dough development time was found to be higher (5.47 min) in wheat variety Fsd-08 followed by Lasani-08 with time (5.0 min).The lowest dough development time(4.33 min) was found in wheat variety Pasban-90. The highest mean value for dough stability (5.73 min) was found in wheat variety of seher-06 and the lowest dough stability (4.26 min) was found in the wheat variety Pasban-90. Mean value for softening of dough was higher in wheat variety Fsd-08 (123.33 BU) followed by Seher-06 (106.00 BU) and then Pasban-90 (102.33 BU). The lowest softening of dough was found in wheat variety Lasani-08 (91.67 BU). The highest peak height (44.17%) was found in wheat variety Seher-06 and the lowest peak height (36.33%) was found in wheat variety Pasban-90. The results explicated that the highest mixing time (9.00 min) was found in the wheat variety Lasani-08, followed by Fsd-08 (8.17 min) and lowest mixing time (5.83 min) was found in wheat variety Pasban-90.

Chemical composition by AACC methods and FT-IR spectral representation

The results regarding chemical composition of wheat varieties as determined by AACC methods are presented in Table 1. The results showed that maximum moisture content (10.50%) was found in Seher-06 and lowest moisture content (9.17%) was found in wheat variety pasban-90. The protein content varied from 10% to 11.77% among different wheat varieties. It was observed that wheat variety Fsd-08 contain significantly highest (11.77%) protein content as compared to other wheat varieties and the wheat variety Seher-06 (10%) exhibited the lowest protein content. The fat content was found to be significantly highest (1.83%) in wheat variety fsd-08, followed by Lasani-08 (1.62%). The fat content was significantly lowest (1.24%) in wheat variety Pasban-90. The ash content was found highest (1.35%) in wheat variety Fsd-08 followed by Lasani-08 (1.21%). The

Table 1 Physical and chemical characteristics of different wheat varieties

	Lasani-08	Fsd-08	Seher-06	Pasban-90
Physical characteristics				
Grain length (mm)	6.4b	6.6b	6.7ab	7.1a
Grain width (mm)	3.2b	3.3ab	3.5a	3.6a
Kernel weight (g)	40.8c	46.2b	49.3a	32.4d
Test weight (Kg/hl)	77.7a	74.9b	75.1b	76.9a
Chemical characteristics				
Moisture content (%)	10.0ab	9.6bc	10.5a	9.2c
Protein content (%)	11.2a	11.8a	10.0b	11.3a
Fat content (%)	1.6ab	1.8a	1.4bc	1.2c
Ash Content (%)	1.2ab	1.4a	1.1b	1.1b

Values sharing the same letter are not significantly different from each other

Each value is a mean of 3 replicates i.e. *n*=3

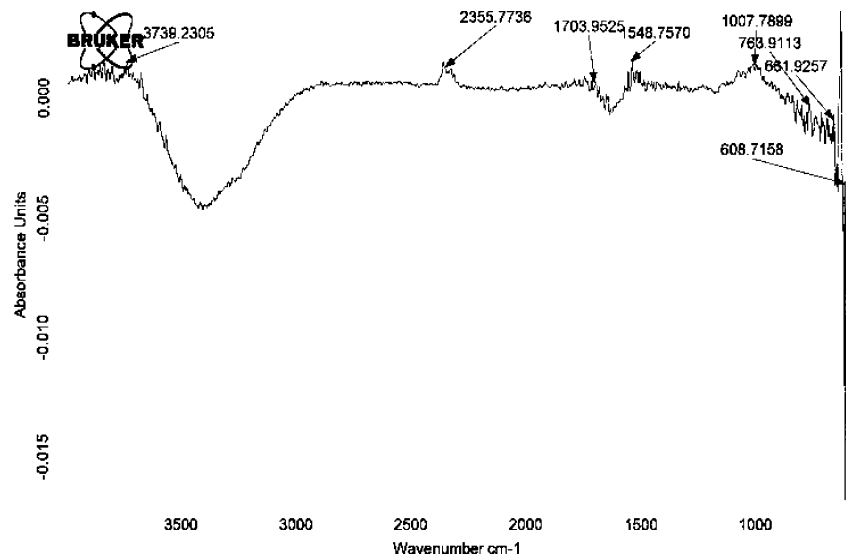**Fig. 1** FTIR spectra of wheat variety Lasani-08
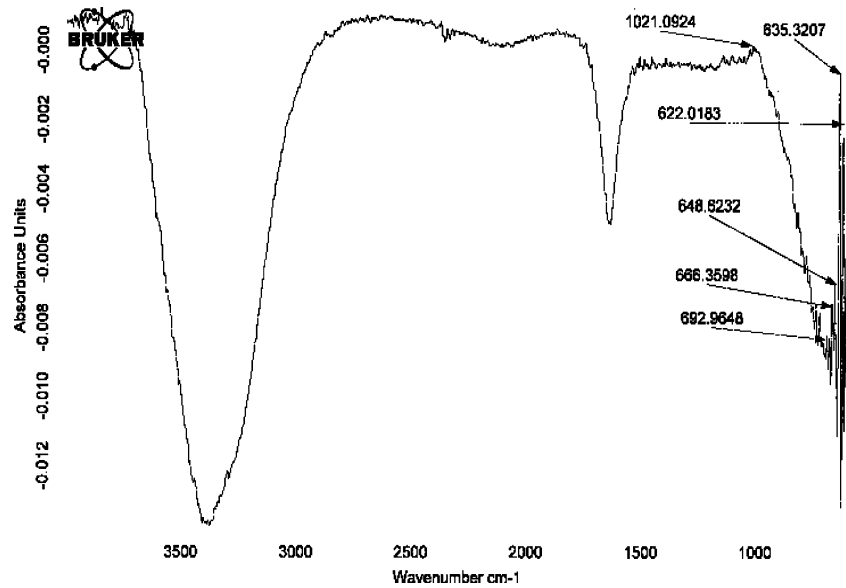
lowest ash content (1.11%) was found in flour of wheat variety Seher-06.

Moisture contents were determined by FTIR on mid infrared range. Peaks for water were observed on 1,640 cm^{-1} and 3,300 cm^{-1} (Manley et al. 2002). The absorption is done on the base of functional groups H and OH. The absorption spectra of wheat flour taken also show strong peaks in the same region represent presence of moisture in flour. FTIR is one of the most elusive methods for the analysis of moisture. Water absorbs strongly in the infrared region of the spectrum due to its OH stretching and H bending vibrations however its quantization is frequently complicated by spectral interferences from other OH containing constituents such as alcohols, phenols and hydro peroxides and confounded further by hydrogen bonding

effects (Dong et al. 2000). The spectra of the four wheat varieties were taken which showed peaks on the same wavelengths. The absorption peaks in protein is done on the basis of amide group I and amide group II. Here protein spectra were also taken on the low molecular weight and high molecular weight. The peaks are representative of the chemical groups of the components present in the samples. By the extraction protein was separated from the four flour samples and spectra were taken. Absorption bands that are two primary features of the protein, amide I and amide II bands were observed at approximately 1,660 cm^{-1} and 1,550 cm^{-1} respectively. Amide I arises from the stretch of C=O of the peptide group in the protein. The amide II band is mostly from the NH bending and secondarily from the effect of CN stretch. The strong absorbance of the NH

Fig. 2 FTIR spectra of wheat variety Fsd-08

Fig. 3 FTIR spectra of wheat
variety Seher-06
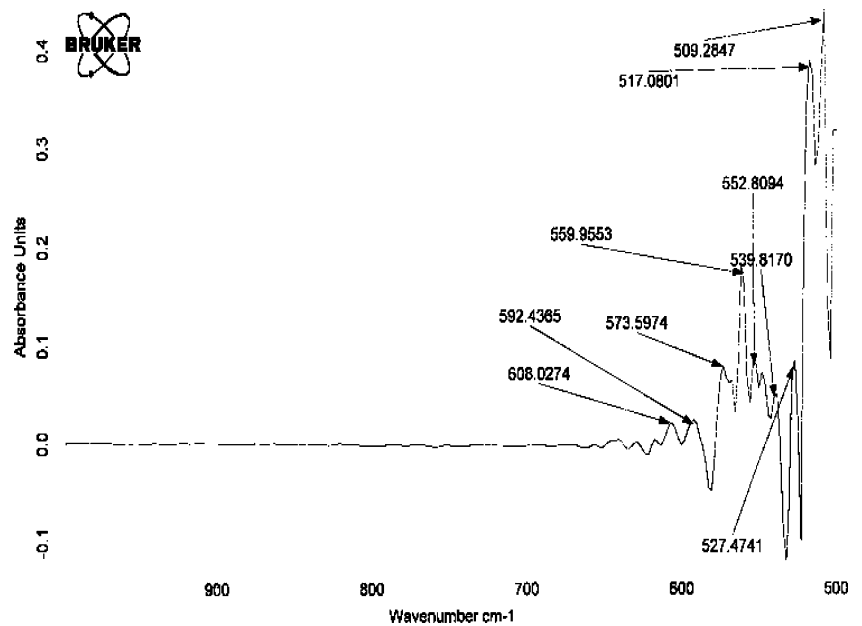
stretch in the spectrum indicates that the wheat variety is rich in protein. Crude protein was observed having peaks of amide I band at 1,600 cm^{-1} to 1,700 cm^{-1} and amide II at 1,550 cm^{-1} to 1,570 cm^{-1} (Manley et al. 2002). FTIR is one of the most elusive methods for the analysis of fat. Crude fat of flour samples was observed having peaks at 1,600 cm^{-1} to 1,700 cm^{-1} and 1,550 cm^{-1} to 1,570 cm^{-1}. The absorption peaks are determined on the basis of C-H bonds. The absorption spectra of wheat flour taken also show strong peaks in the same region represent presence of fat in flour. It has been reported that FTIR spectroscopy could be utilized as a quality control method for fat and moisture determination in butter and high-fat products (Van de Voort et al. 1992). Similar results were also reported by Che-Man and Setiowaty (1999). The ash contents are more

accurately determined by FTIR spectra which were determined at the peak values 835- to 2,502 nm with 8 cm^{-1} wave number resolution and 200 scans/ spectrum.

Starch contents

The major constituent of the wheat flour is starch contents. Starch contents were also estimated with FTIR only for wheat quality assessment with objectives of FTIR application for starch content determination. Spectra of starches bands originate mainly from the vibrational modes of amylose and amylopectin because these are the main components of starch. The peaks was observed in the regions below 800 cm^{-1}, 800 to 1,500 cm^{-1} (the fingerprint region), the region between

Fig. 4 FTIR spectra of wheat
variety Pasban-90
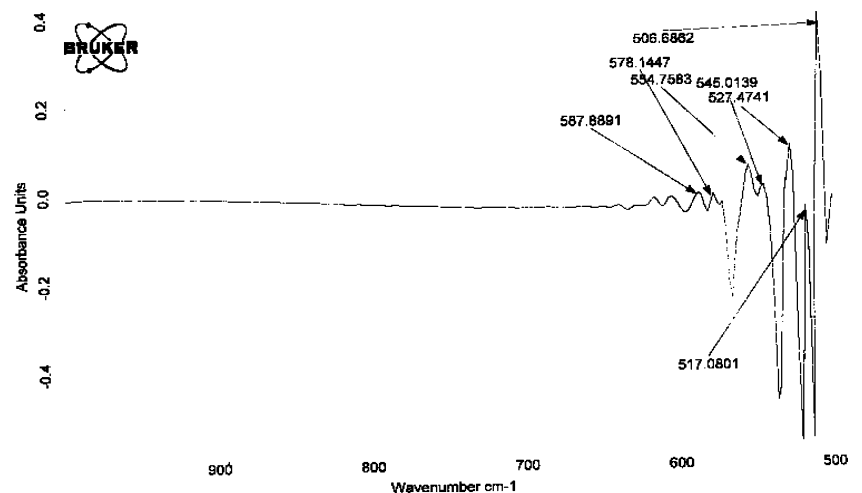
2,800 and 3,000 cm^{-1} (C-H stretch region), and finally the region between 3,000 and 3,600 cm^{-1} (O-H stretch region) The infrared spectra of mono- and oligosaccharides were shown to be important in the structure elucidation of small molecules and also in polymer analysis where they represent structural units. The most frequently used IR spectral range in carbohydrate analysis was the anomeric region at 950 to 750 cm^{-1} where it is possible to distinguish bands characteristic for a and b conformers or pyranoid and furanoid ring vibrations of mono- and polysaccharides. Determination of the esterification degree of carbonyl groups of pectin by means of infrared spectroscopy was carried out at 1,745 and 1,605–1,630 cm^{-1} for ester vs. carboxylate regions respectively. The present study is well supported by previous studies of Kizil et al. (2002); Chatjigakis et al. (1998) (Figs. 1, 2, 3 and 4).

Conclusion

The present study clearly indicates that the physical properties showed significant differences in different wheat varieties. In the case of chemical parameters there are also large variation existed among different wheat varieties. As the mandate of the present study was to assess the physical, chemical and rheological properties of different wheat varieties by standard method and advance technique FTIR and their advantages over standard methods. On the basis of results it is concluded that these methods are expensive, time consuming and cause destruction of samples but Fourier transforms infrared (FTIR) spectroscopy is one of the most important tools used for analyzing wheat for different tests. FTIR provides an excellent means to visualize the chemical composition of different wheat varieties and it is very quick, reliable and cheaper analytical technique which can effectively be used for estimation of different wheat quality parameters. The values determined by this technique are very close to the values determined by the standard procedures and preferred over dispersive due to following reasons. It is non destructive technique, provides precise measurement which requires no external calibration, increases speed, collecting a scan every second has minimum sample preparation.

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