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FTIR – METHOD FOR THE RAPID DETECTION OF GLUTEN IN FOOD PRODUCTS

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Abstract: *Fourier transform infrared spectroscopy (FTIR) is a method that identifies functional groups and the structure of different molecules. The IR spectrum is an image of the absorbed light, as a function of wavelength. It usually contains a large number of peaks. The ATR technique is one of the most commonly used techniques in infrared spectroscopy. Infrared spectroscopy (IR) enables the identification of proteins (gluten) based on the position and therefore the aim of this work is to determine the presence of gluten in different food products as soon as possible. The IR spectrum of molecules from wheat flour samples, gluten-free flour and food product purchased on the market of the territory of the Republic of Srpska/Bosnia and Herzegovina. Samples were recorded in solid state, on an Agilent Cary 630 FTIR instrument, in the range of 4000 to 650 cm^{-1}. Spectra were processed in Agilent MicroLab software. Using the GRAMS/AI software, the samples are presented at the dependence of the wave number and transmittance. Based on the recorded spectra, it can be seen that the functional groups absorbed at approximately the same frequency, regardless of the samples in which they are located. On the wave number 1641 cm^{-1} (amide I) the peak area in wheat flour is Xav=1248.33 (SD=73.29, CV=5.87%), in gluten-free flour Xav=729.03 (SD=33.98, CV=4.66%) and in food product Xav=715.16 (SD=78.29, CV=10.95%). Considering that the number of people who are allergic to gluten is increasing every day, this method can quickly and easily identify gluten in food products.*

Key words: *FTIR, gluten, wheat flour, gluten-free flour, food product*

Introduction

Wheat flour is a complex natural system which contains carbohydrates, amino acids, proteins, dietary fiber, fat, water, minerals and vitamins (Shewry and Hey, 2015; Baniwal et al., 2021). It is a basic ingredient in a variety of baked goods and flour dishes (Wilderjans et al., 2013; Witczak et al., 2016). Its properties are related mainly to the amount of gluten. Some people are intolerant to gluten (Biesiekierski, 2017; Gobbetti et al., 2018).

Gluten is the composite of two storage proteins, gliadin and a glutenin (Biesiekierski, 2017). Glutenins are water insoluble and gliadins are water soluble (Rasheed et al., 2014). People with gluten sensitive enteropathy, the severe form of which is celiac

disease are sensitive to gliadins. The only effective form of treatment for these disorders is a gluten-free diet (Pietzak and Kerner, 2012; Scherf, 2019).

In industry, mostly classical methods are used for gluten determination (Ortolan and Steel, 2017). In recent years, a number of gluten analytical detection methods have been developed, based on techniques such as enzyme linked immunosorbent assay (ELISA), polymerase chain reaction and liquid chromatography coupled with mass spectrometry (Osorio et al., 2019). Both types of methods are rather expensive and often require appropriate sample preparation (Osorio et al., 2019; Xhaferaj et al., 2020).

The use of mid-infrared (IR) and near-infrared (NIR) spectroscopy is well established in the literature as a rapid technique for grain control, flour, dough and bread analysis (Kaddour and Cuq, 2011).

FTIR spectroscopy could be used to non-destructively, requiring minimal or no sample preparation, no waste production and the ability to assess several components simultaneously from a single spectrum and rapidly determine biochemical fingerprints that give required information about molecular structure and chemical characteristics of the sample (Santos et al., 2019; Dzurendova et al., 2020).

The region of the IR spectrum is divided into near (12 500-4 000 cm^{-1}), medium (4 000-400 cm^{-1}), far (400-10 cm^{-1}) and *fingerprint* is area between 500 and 1 500 cm^{-1} (Harper et al., 2017). Functional groups are usually absorbed at approximately the same frequency, regardless of the molecules in which they are located (Dzurendova et al., 2020).

This method is extremely important when analyzing the secondary structure (amide I and amide II) of gluten (Bradford et al., 2008; Sujka et al., 2017). Amide I band consists of C=O stretching, and amide II N-H bending vibrations. The amide I band is used as a useful indicator of the secondary structure of the gluten (Sujka et al., 2017).

Considering that the number of people who are allergic to gluten is increasing every day, this method can quickly and easily identify gluten in food products.

Material and method

Fourier transform infrared spectroscopy (ATR-FTIR spectroscopy) recorded IR spectra of wheat flour samples (protein content 9.8 g/100 g, ash content: max 0.55%, moisture max: 15%, acidity: max 3), gluten-free flour (protein content 3.6 g/100 g) and food product based on wheat flour (protein content 7.6 g/100 g). Samples were purchased on the market of the territory of the Republic of Srpska/Bosnia and Herzegovina.

Spectroscopic analysis

Spectra of wheat flour, gluten-free flour and food product based on wheat flour samples were collected at room temperature using Attenuated Total Reflection (ATR) method. A small amount of solid sample was placed on the crystal. The nozzle, which allows contact with the solid sample, is closed. The spectra were collected six times for each sample with 32 scans at a resolution of 8 cm^{-1} over a wave number ranging from 4000 to 650 cm^{-1}. A background air spectrum was scanned before each measurement and subtracted from spectra of all samples. ATR crystal was carefully cleaned with 70% (v/v) ethanol and dried with an appropriate tissue before measurement of the next sample. Infrared spectra were recorded on an Agilent Cary 630 FTIR instrument. Spectra were processed in Agilent MicroLab software. Using the GRAMS/AI software, the samples are represented by the dependence of the wave number and transmittance. The average value, standard deviation and coefficient of variation of wave number, width and peak area was calculated using this software.

Results and discussion

Figure 1 shows the spectrum of gliadin standard (Wheat Gluten Gliadin, MP Biomedicals, LCC, France, degree of purity meets the standard requirement) obtained during ATR-FTIR recording.

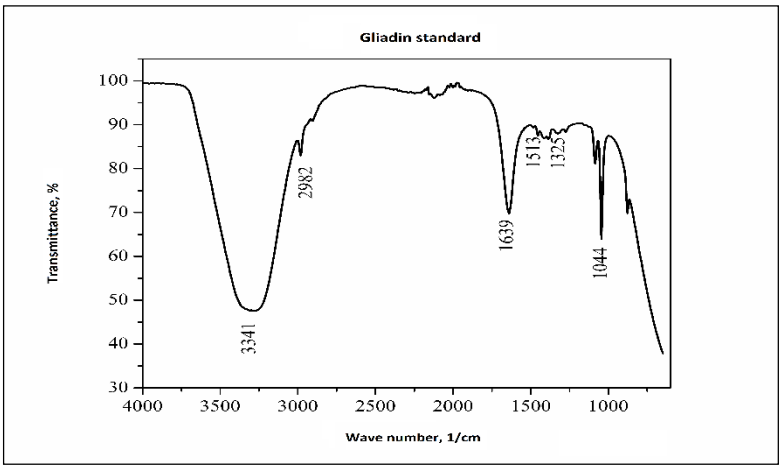

Figure 1. ATR-FTIR spectrum of gliadin standard

Intense band in the range of 3600-3200 cm^{-1} (the most intense band at 3341 cm^{-1} in the recorded gliadin standard) originates from stretching (OH) and bending (NH) vibrations (Robertson et al., 2006). Bands in the range of 3000-2800 cm^{-1} (the most intense band at 2982 cm^{-1} in gliadin standard) originate from stretching C-H bonds. The band in the area of 1600-1700 cm^{-1} (most intense at 1639 cm^{-1} in gliadin standard) originates from amide I band, in the area of 1500-1600 cm^{-1} consisting of

N-H bending and C-H stretching vibrations) (the most intense at 1513 cm^{-1} in the gliadin standard originates from amide II band) and in the range from 1350-1200 cm^{-1} (the most intense at 1325 cm^{-1} in the gliadin standard) is amide III band. The area between 1500 and 900 cm^{-1} (the most intense band at 1044 cm^{-1} in gliadin standard) is called the "*fingerprint*" area (Robertson et al., 2006; Sujka et al., 2017).

Table 1 shows the peak width and area at a certain wave number obtained after recording wheat flour samples by ATR-FTIR technique.

Table 1. Peak width and area at a certain wave number obtained after recording wheat flour samples by ATR-FTIR technique

Wave number (cm^1)	Peak width	Peak area
3275	Xav=667.56 (SD=17.19, CV=2.57%)	Xav=9135.91 (SD=80.73, CV=0.88%)
2925	Xav=385.91 (SD=61.62, CV=15.97%)	Xav=1067.74 (SD=59.13, CV=5.54%)
1641	Xav=177.97 (SD=14.01, CV=7.87%)	Xav=1248.33 (SD=73.29, CV=5.87%)
1541	Xav=88.99 (SD=2.43, CV=2.73%)	Xav=241.90 (SD=16.61, CV=6.87%)
1242	Xav=85.88 (SD=2.18, CV=2.54%)	Xav=116.88 (SD=9.84, CV=8.42%)
993	Xav=67.96 (SD=3.51, CV=5.16%)	Xav=151.72 (SD=4.43, CV=2.92%)

Xav=average value, SD= standard deviation, CV=coefficient of variation

Based on the obtained results, it can be seen that the highest width and area was obtained at the wave number 3275 cm^{-1} and is Xav=667.56 (SD=17.19, CV=2.57%) and Xav=9135.91 (SD=80.73, CV=0.88%), and the lowest at the wave number 993 cm^{-1} and is Xav=67.96 (SD=3.51, CV=5.16%) and Xav=151.72 (SD=4.43, CV=2.92%) (Table 1).

Table 2 shows the peak width and area at a certain wave number obtained after recording gluten-free flour samples by ATR-FTIR technique.

Table 2. Peak width and area at a certain wave number obtained after recording samples of gluten-free flour by ATR-FTIR technique

Wave number (cm^1)	Peak width	Peak area
3275	Xav=687.43 (SD=6.25, CV=0.91%)	Xav=9139.59 (SD=151.11, CV=1.65%)
2924	Xav= 349.25 (SD=27.77, CV=7.95%)	Xav=730.73 (SD=28.25, CV=3.87%)
1638	Xav=395.15 (SD=9.87, CV=2.50%)	Xav=729.03 (SD=33.98, CV=4.66%)
1542	Xav=100.64 (SD=0.00, CV=0.00%)	Xav=206.11 (SD=14.82, CV=7.19%)
1242	Xav=57.04	Xav=99.44

	(SD=4.92, CV=8.62%)	(SD=3.66, CV=3.68%)
993	Xav=119.27 (SD=0.00, CV=0.00%)	Xav=1915.64 (SD=53.94, CV=2.82%)

Xav=average value, SD= standard deviation, CV=coefficient of variation

Based on the obtained results, it can be seen that the highest width and area was obtained at the wave number 3275 cm^{-1} and is Xav=687.43 (SD=6.25, CV=0.91%) and Xav=9139.59 (SD=151.11, CV=1.65%), and the lowest at the wave number 1242 cm^{-1} and is Xav=57.04 (SD=4.92, CV=8.62%) and Xav=99.44 (SD=3.66, CV=3.68%) (Table 2).

Table 3 shows the peak width and area at a certain wave number obtained after recording samples of food product based on wheat flour samples by ATR-FTIR technique.

Table 3. Peak width and area at a certain wave number obtained after recording food product based on wheat flour samples by ATR-FTIR technique

Wave number (cm^{-1})	Peak width	Peak area
3296	Xav=627.43 (SD=24.78, CV=3.95%)	Xav=6975.14 (SD=100.95, CV=1.45%)
2924	Xav=113.06 (SD=3.52, CV=3.11%)	Xav=900.02 (SD=42.08, CV=4.67%)
1694	Xav=264.64 (SD=5.27, CV=1.99%)	Xav=715.16 (SD=78.29, CV=10.95%)
1590	Xav=75.79 (SD=4.65, CV=6.13%)	Xav=75.42 (SD=6.78, CV=8.99%)
1237	Xav=73.55 (SD=1.41, CV=1.92%)	Xav=124.98 (SD=8.62, CV=6.90%)
986	Xav=125.49 (SD=11.52, CV=9.18%)	Xav=1521.59 (SD=38.33, CV=2.52%)

Xav=average value, SD= standard deviation, CV=coefficient of variation

Based on the obtained results, it can be seen that the highest width and area was obtained at the wave number 3296 cm^{-1} and is Xav=627.43 (SD=24.78, CV=3.95%) and Xav=6975.14 (SD=100.95, CV=1.45%), and the lowest at the wave number 1237 and 1590 cm^{-1} and is Xav=73.55 (SD=1.41, CV=1.92%) and Xav=75.42 (SD=6.78, CV=8.99%) (Table 3).

Figure 2. shows comparative IR spectra of wheat flour, gluten free flour and food product based on wheat flour.

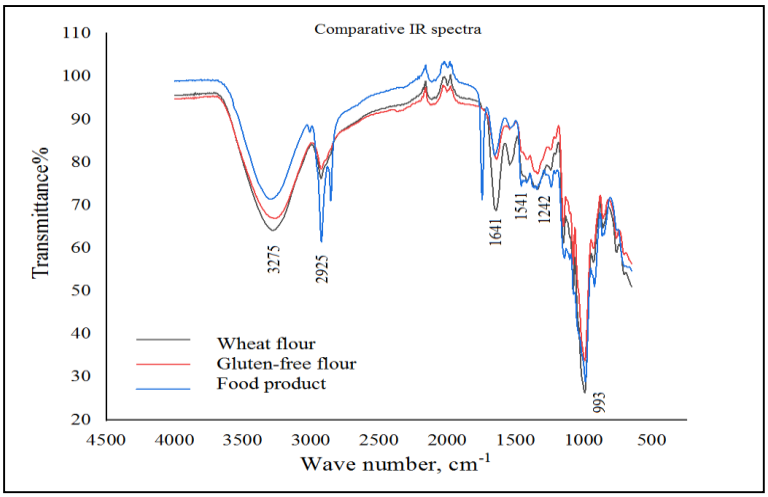

Figure 2. Comparative IR spectra of different samples

Based on the recorded and overlapping IR spectra from wheat flour, gluten-free flour and food product based on wheat flour samples, it can be seen that the functional groups adsorbed at approximatelly the same frequency, regardless of the molecules in which they are located.

Czaja et al. (2016) used FT-Raman spectroscopy for gluten quantification in wheat flour. Results of similar quality were found using a PLS model derived from NIR (near infrared)) spectra obtained in DRIFTS (diffuse reflectance infrared Fourier transform spectroscopy) mode. Based on the obtained results, this method can be used as a simple, fast and precise method for quantitative gluten analysis in flour.

Arslan et al. (2020) used FTIR spectroscopy for rapid detection of wheat flour adulteration with barley flour. 20 pure cereal flours and 120 flour blends were analyzed using FTIR spectroscopy. The spectra were collected in the region of 4000-450 cm^{-1}. Based on the obtained results adulteration could be detected by this method.

Comparing the results obtained in this paper with authors Czaja et al. (2016) and Arslan et al. (2020), it can be seen that they are in agreement.

Conclusions

By investigating FTIR as a method for the rapid detection of gluten in food products, the following conclusions were reached.
Based on the recorded spectra, it can be seen that the functional gropus absorbed at approximatelly the same frequency, regardless of the samples in which they are located.

On the wave number 1641 cm^{-1} (amide I) the peak area in wheat flour was Xav=1248.33 (SD=73.29, CV=5.87%), in gluten-free flour Xav=729.03 (SD=33.98, CV=4.66%) and in food product Xav=715.16 (SD=78.29, CV=10.95%).

Taking into that the number of people who are allergic to gluten is increasing every day, this method can quickly and easily identify gluten in food products.

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FTIR – METODA ZA BRZU DETEKCIJU GLUTENA U PREHRAMBENIM PROIZVODIMA

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Sažetak: *Furijeova infracrvena spektroskopija (FTIR) je metoda koja identifikuje funkcionalne grupe i strukturu različitih molekula. IR spektar predstavlja sliku apsorbovane svjetlosti u funkciji talasne dužine. Sadrži veliki broj pikova. ATR tehnika je jedna od najčešće korištenih tehnika u infracrvenoj spektroskopiji. Infracrvena spektroskopija (IR) omogućava identifikaciju proteina (glutena) na osnovu položaja i stoga cilj ovog rada je određivanje glutena u različitim prehrambenim proizvodima što je moguće brže. IR spektri molekula su snimljeni iz uzoraka pšeničnog brašna, bezglutenskog brašna i prehrambenog proizvoda nabavljenih na tržištu Republike Srpske/Bosne i Hercegovine. Uzorci su snimljeni u čvrstom stanju, na Agilent Cary 630 FTIR instrumentu, u rasponu od 4000 do 650 cm^{-1}. Spektri su obrađeni u softveru Agilent MicroLab. Koristeći GRAMS/AL softver, uzorci su predstavljeni kao zavisnost talasnog broja i transmitancije. Na osnovu snimljenih spektara uočeno je da su funkcionalne grupe apsorbovale na približno istoj frekvenciji, bez obzira na uzorke u kojima se nalaze. Na talasnom broju 1641 cm^{-1} (amid I) površina pika kod pšeničnog brašna iznosi Xav=1248,33 (SD=73,23, CV=5,87%), kod bezglutenskog brašna Xav=729,03 (SD=33,98, CV=4,66%) i kod prehrambenog proizvoda Xav=715,16 (SD=78,29, CV=10,95%). Uzimajući u obzir da se svakim danom povećava broj ljudi koji su alergični na gluten, ovom metodom se može brzo i jednostavno identifikovati gluten u prehrambenim proizvodima.*

Ključne riječi: *FTIR, gluten, pšenično brašno, bezglutensko brašno, prehrambeni proizvod*