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APPLICATION OF FTIR-ATR SPECTROSCOPY FOR DETERMINATION OF GLUCOSE IN HYDROLYSATES OF SELECTED STARCHES

Alica BARTOŠOVÁ, Maroš SOLDÁN, Maroš SIROTIAK,
Lenka BLINOVÁ, Anna MICHALIKOVÁ

ABSTRACT

Fourier transform infrared spectroscopy was evaluated as an easy and simple analytical method for determination of starch residues after enzymatic hydrolysis. Different starch sources were liquefaction by α-amylase enzyme Termamyl SC for 25 minutes in autoclave. In the next step were starches solutions enzymatically hydrolysed by enzyme pollulanase Promozyme® for 24 hours to 60°C water bath. Total glucose in starch hydrolysate was determined using Fourier Transformed Infrared Spectroscopy (FTIR) with ATR accessory with diamante crystal by recording the absorption of different carbohydrate in spectral range from 700 – 4000 cm^{-1}. Based on calibration curves of glucose the release of total glucose in hydrolysates was calculated.

KEY WORDS

starch, enzymatic hydrolyse, sugars, FTIR-ATR

INTRODUCTION

Biomass has become an alternative source for production of fuels because it is renewable and could reduce greenhouse gas emissions by replacing petroleum sources. Bioethanol as a biofuel is produced from sugar, starch or lignocellulose biomass. Current research has been focused on using starch biomass. In pre-treatment process starches are enzymatic hydrolysed to sugars and in next step are these sugars converted to ethanol.

The aim of this paper was to determinate sugars in hydrolysates of potato, corn and rice starches. These starches were enzymatic hydrolysed by α-amylase enzyme Termamyl used for liquefaction of starches and by enzyme pollulanase Promozyme® used for saccharification of starches solutions. The hydrolysis of corn starch may be considered as a first and key step in corn processing for bioethanol production. The main role of this step is to effectively provide the conversion of two major starch polymer components: amylose, a mostly linear a-D-(1–4)-

Alica BARTOŠOVÁ, Maroš SOLDÁN, Maroš SIROTIAK, Lenka BLINOVÁ, Anna MICHALIKOVÁ - Institute of Safety and Environmental Engineering, Department of Environmental Engineering, Faculty of Materials Science and Technology, Slovak University of Technology, Botanická 49, 917 24 Trnava, Slovak Republic, E-mail: alica.bartosova@stuba.sk

glucan and branched amylopectin, a α-D-(1–4)-glucan, which has α-D-(1–6) linkages at the branch points, to fermentable sugars that could subsequently be converted to ethanol by yeasts or bacteria. Recent advances in the developing of termostable α-amylases, the starch liquefying enzymes which catalyse the hydrolysis of internal α-D-(1–4)-glucosidic linkages in starch in a random manner [1,2] and effective glucoamylases [3,4], the starch saccharifying enzymes which catalyse the hydrolysis of α-D-(1–4) and α-D-(1–6)-glucosidic bonds of starch from the non-reducing ends giving glucose as the final product, have led to commercial establishment of the so called 'two enzyme cold process'.

PRINCIPLES OF FTIR-ATR SPECTROSCOPY

Attenuated total reflection (ATR) has grown into the most widely practiced technique in infrared spectrometry. The reasons for this are fairly straightforward: the technique requires little or no sample preparation, and consistent results can be obtained with relatively little care or expertise. ATR is a technique whereby the sample is placed in contact with a sensing element, and a spectrum is recorded as a result of that contact. Unlike many other sampling techniques used in infrared spectrometry, radiation is not transmitted though the sample; consequently, the sample does not have to be thin enough to allow transmission of the incident radiation, with no band having an absorbance greater than 2.0 AU [5]. When radiation passes from one transparent medium to another and the two media have different refractive indices, the angle at which the radiation is described by Snell´s low; that is,

$$n_1 sin\theta_1 = n_{21} sin\theta_2$$

where n_1 and n_2 are the refractive indices of the two transparent media, and θ_1 and θ_2 are, respectively, the angle of incidence and refraction with respect to the normal to the interface [5].

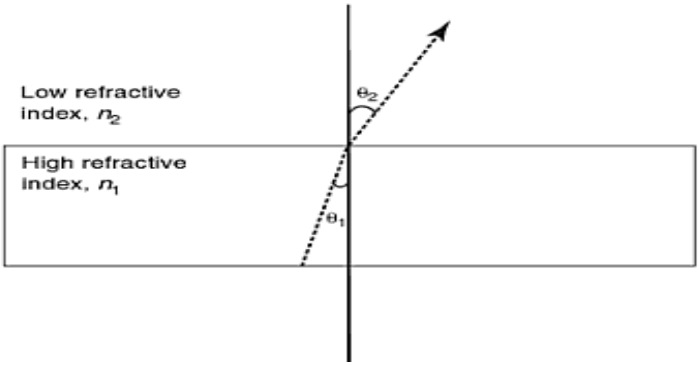

Fig. 1 *Shell´s law where the beam originates in the optically dense medium of refractive index n_1 and travels to the optically rare medium of refractive index n_2. The angles are measured with respect to the normal to the surface [5]*

MATERIAL AND METHODS

Samples of starches and enzymes

There are three samples of starch: corn starch and potato starch (Dr. Oetker) were obtained from a local grocery shop rice starch was earned from milling rice.

Termamyl 120L, a heat-stable α-amylase from *Bacillus licheniformiswas* used for corn meal liquefaction. Promozyme® is heat-stable debranching enzyme obtained from a selected

strain of Bacillus acidopullulyticus, and belongs to the group of debranching enzymes known as pullulanases. The enzymes were gift from Novozymes, Denmark.

Enzymatic hydrolyse

Solutions of starch were prepared; 15 g of each kind of starch were dissoluble in 50 mL of distilled water and adjusted by acetate buffer to pH 5.3 (2 mol/L CH_3COOH, 0.2 mol/L $C_3H_3O_2Na$). In the first step was adding 3 mL of α-amylase enzyme Termamyl for liquefaction in autoclave in temperature 119°C for 10 minutes. After that, another 3 mL of enzyme Termamyl were add, solutions were autoclaved in temperature 119 for 8 minutes and bring to room temperature. After cooling down, in the second step, solutions were adjusted by acetate buffer to pH 4.2 and 7.5 mL [6] of enzyme pollulanase Promozyme® were added. Process of saccharification was taken 24 hours in temperature 60°C in water bath. The spectra were recorded using a Varian Resolutions Pro and samples of starches were measured in the region 4000-700 cm^{-1}; each spectrum was measured 40 times. Resulting spectra of hydrolysates were corrected for background buffer absorbance.

Determination of infrared spectra by FTIR-ATR

Identification of starches and their hydrolysates were performed using an Infrared Fourier Transform Spectroscopy with ATR technique - Attenuated Total Reflectance (Varian 660 Dual MidIR MCT / DTGS Bundle) (Fig.2). Samples were directly applied to a diamante crystal of ATR and resulting spectra of them were corrected for background air absorbance. The spectra were recorded using a Varian Resolutions Pro and samples of starches were measured in the region 4000-700 cm^{-1}; each spectrum was measured 40 times.

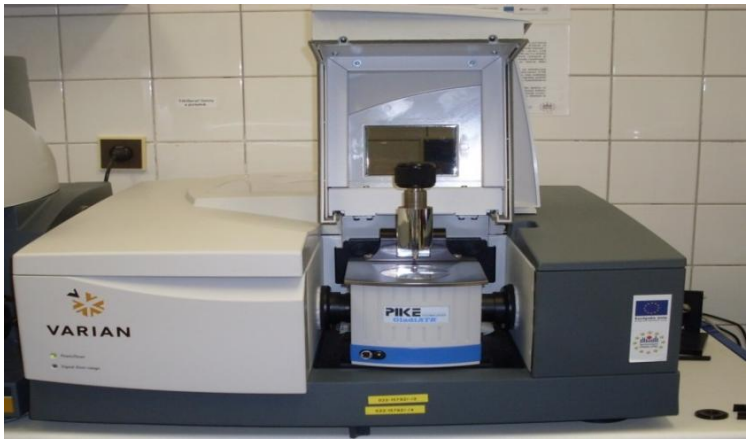

Fig. 2 *Infrared spectrometer Varian 660 MidIR Dual MCT/DTGS Bundle with ATR*

RESULTS

Determination of starches

In Fig. 3 are showed IR spectra of three samples of starches. The IR spectra obtained from native potato spectra showed at 1149 cm^{-1} C-O, C-C stretching with some C-OH contributions; 1076, 1077, 1049, 998, 991 , and 925 cm^{-1} (COH bending and CH_2-related modes) and 854,855, and 843 cm^{-1} (C-O-C symmetrical stretching and C-H deformation). A band near 946 cm^{-1} is interpreted as a skeletal mode indicating the α-1,4linkage of glucose molecules in starch amylose. The absorption band 855 cm^{-1} formerly identified in potato.

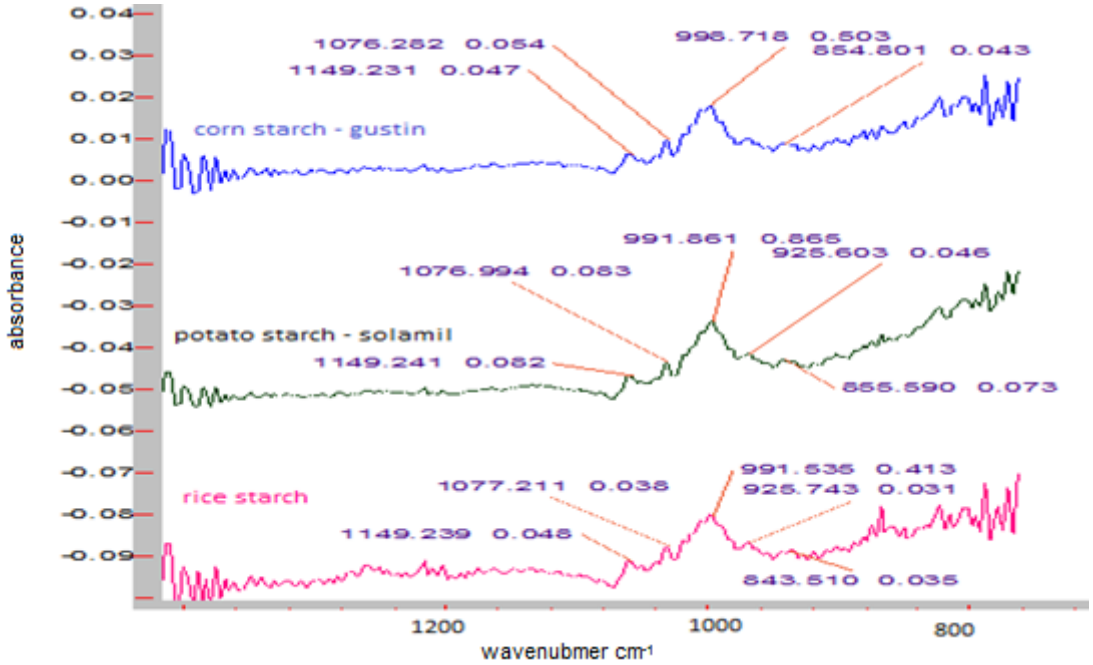

Fig. 3 *Spectrum of corn, potato and rice starch*

Determination of starch/glucose

The aim was hydrolysed starch to glucose and then quantitatively determined it by a calibration curve prepared from aqueous solutions of D-glucose pa concentrations of 2.5%, 5.0% 7.5% 10.0% 12.5%,15.0% 17.5%, 20.0%, 22.5% and 25%.

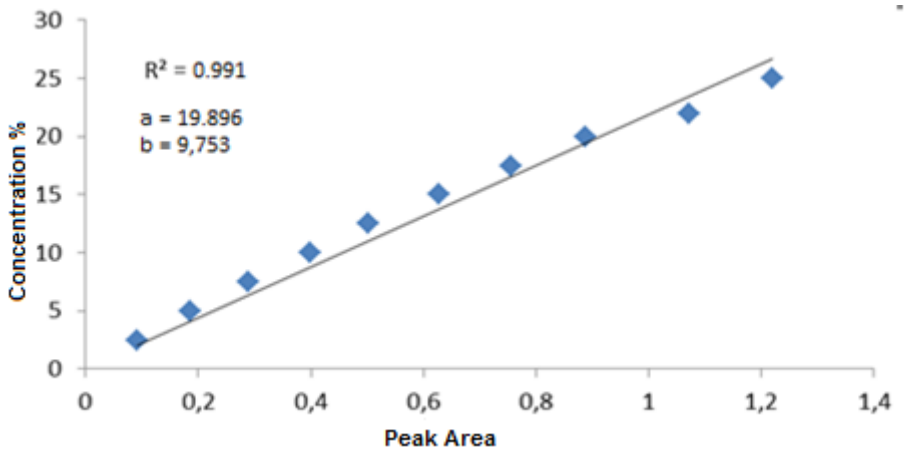

Fig. 4 *Concentration curve of glucose*

Calibration curve represents the dependence of the peak area on the concentration of glucose (Fig.4). Each sample was measured on an IR spectrophotometer of 4000-700 cm^{-1} and each spectrum was measured 40 times.

As can be seen in Fig. 5 the FTIR spectra of starch hydrolysates are well defined. There are showed intense fingerprint bands in the wavenumber range 1150-900 cm^{-1}. In this region, the specific absorption for each carbohydrate was identified. The characteristic bands of glucose have specific maxima at 1150, 1106, 1076 cm^{-1}, and the peak at 1029 cm^{-1} having the highest absorption. The most intense peak of glucose (1033 cm^{-1}) is characteristic to the C-O stretch vibration.

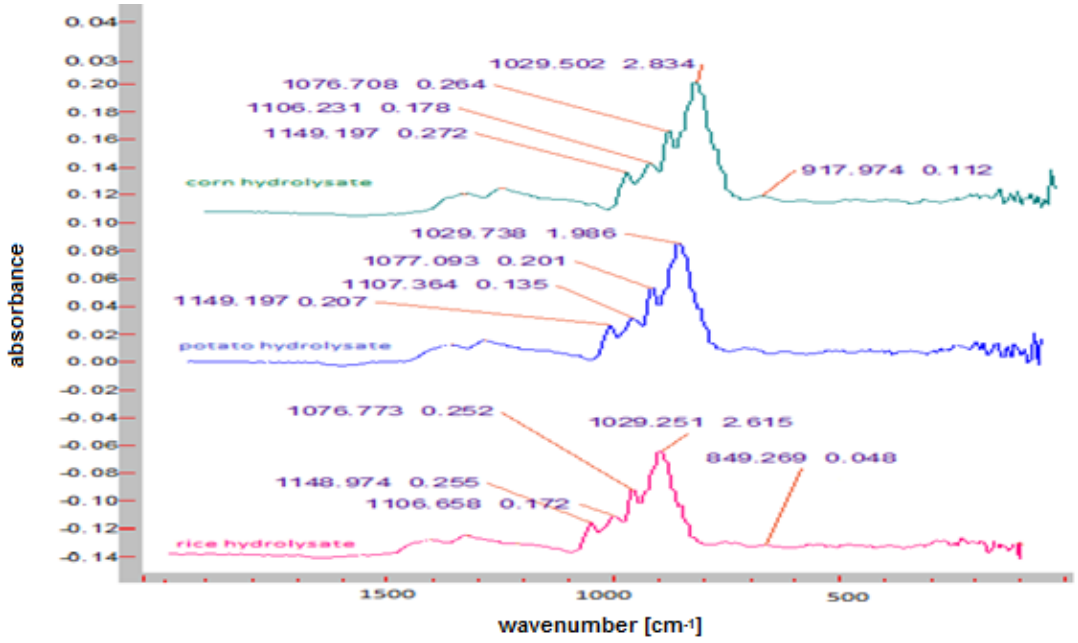

Fig. 5 *Spectra of corn, potato and rice hydrolysates*

PEAK AREAS OF STARCH HYDROLYSATES AND CONCENTRATION OF THEIR GLUCOSE Table 1

	Peak area	Concentration of glucose [%]
Potato starch$_{(n=3)}$	1.0565	22.78
Corn starch $_{(n=3)}$	0.9315	20.29
Rice starch$_{(n=3)}$	0.7315	16.31

Determination of concentration of glucose after enzymatic hydrolyse of starches was calculated from calibration curve conforms to equation in the form

$$c = 19.896 \times Peak\ area + 1.153.$$

Concentration of glucose of potato hydrolysate was 22.8%, corn hydrolysate was 20.29% and finally concentration in rice hydrolysate was 16.31%.

In Fig. 6 can by seen simple prove of hydrolyses of starch to glucose. Reaction of starch with Lugol´s iodine reagent yields a blue-black colour. A negative test is the brown-yellow colour. It means there are no more starches after enzymatic hydrolysates in these samples.

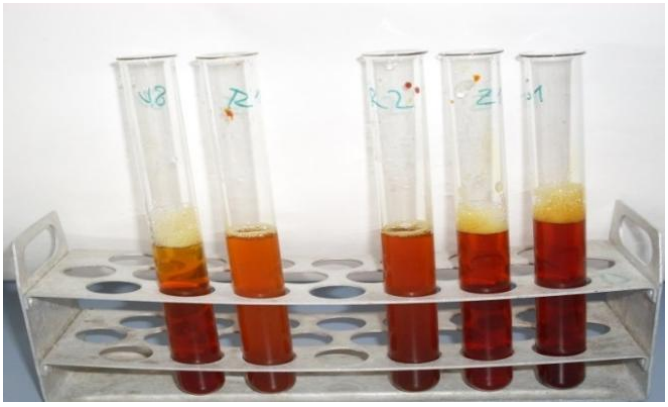

Fig. 6 *Lugol´s test of starches*

CONCLUSIONS

In this paper the FTIR spectroscopy as a non-destructive tool and useful method to determine glucose after hydrolyses of starch was applied. The time of FTIR analysis is considerably reduced compared to the classical methods. These results are promising and have a real analytical potential to supervise in routine procedure for determination sugars in natural carbohydrate polymers.

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REFERENCES

1. KONSOULA, Z., LIAKOPOULOU-KYRIAKIDES, M. 2004. Hydrolysis of starches by the action of ana-amylase fromBacillus subtilis. Process Biochem, 39: 1745–9.
2. HENKE, J.M., KLEPPER, G, SHMITZ, N. 2005. Tax exemption for biofuels in Germany: is bio-ethanol really an option for climate policy? Energy 2005; 30:2617–35.
3. TANRISEVEN, A., ULUDAG, Y.B., DOGAN, S. 2002. A novel method for the immobilization of glucoamylase to produce glucose from maltodextrin.Enzyme Microb Technol 30:406–9.
4. KEARSLEV, M. W., DZIEDZIC, S.Z. 1995. Starch Hydrolysis Products and Their Derivatives. Netherlands, Kluwer Academic / Plenum Publishers. ISBN:0751402699
5. GRIFFITHS, P.R. HASETH, J.A. *Fourier Transform Infrared Spectrometry*. Second Edition. Canada, Wiley. ISBN 978-0-471-19404-0
6. QUIGLEY, T.A., KELLY, C.T., DOYLE, E.M., FOAGARTY, F.M. Patterns of raw starch digestion by the glucoamylase of Cladosporium gossypiicolaATCC38026. Process Biochem 998;35:677–81.