Acta Agrophysica, 2008, 11(1), 7-21

APPLICATION OF NIR SPECTROSCOPY FOR THE ANALYSIS OF WATER-CARBOHYDRATE INTERACTIONS IN WATER SOLUTIONS[*]

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Abstract. The paper shows how NIR spectroscopy can be used to describe water-glucose and water-fructose interactions that occur in water solutions. Using the technique of spectra subtraction, it was possible to identify isosbestic points that were assigned for glucose and fructose in water solutions by NIR spectra. Average values of the points with their standard deviations were calculated. A detailed analysis of the difference spectra made it possible to determine an additional absorbance extreme of glucose at ν = 4303 cm^{-1} which has not been, as yet, described in the world literature on the subject. The paper also compares water bonding forces of both glucose and fructose and relates the results to the measurements of water activity. Finally, the results obtained by the author are compared with the ones available in the literature.

Keywords: Near-infrared spectroscopy, fructose, glucose, carbohydrate solutions, water activity, water properties

INTRODUCTION

Water state in food products has always been investigated by a large number of research units (Mathlouthi 2003). In spite of employing advanced research methods and modern technological equipment, our knowledge on the subject is still incomplete (Lewicki 2004). Every now and then we learn about original developments or new details supplementing earlier works on water behaviour in food (Obsborn and Fearn 1988, Giangiacomo 2006). A substantial group of food products studied are carbohydrate water solutions, i.e. solutions of glucose, fructose and sucrose (Rambla *et al.* 1997). There are strong interactions which occur between water and saccharides

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[*] The study was accomplished within the State Committee for Scientific Research in years 2007-2008 as the research project No.0755/P01/2007/32.

which, in turn, affect not only the quality of food products but also the structural changes of water itself, particularly observable at water concentrations above 30% (Giangiacomo 2006, Mathlouthi *et al.* 1980, Starzak and Mathlouthi 2003).

To study the structural changes of water in food products NIR spectroscopy has been commonly used (Blanco and Villarroya 2002, Franks 1983, Luck 1974). The NIR spectra are created by vibrations cased by OH groups. These water molecule vibrations consist mainly of three different types, i.e. symmetric and asymmetric tensile and bending vibrations (Franks 1983, Czarniak-Matusewicz *et al.* 2005). NIR water spectra are characterised by four principal maximum values that occur at λ_1 = 970 nm ((ν_3 = 10309 cm^{-1}), λ_2 = 1190 nm ((ν_3 = 8403 cm^{-1}) λ_3 = 1450 nm (ν_3 = 6898 cm^{-1}) and λ_4 = 1940 nm ((ν_3 = 5154 cm^{-1}) (Büning-Pfaue 2003). In particular, there is a high intensity of radiation absorption at λ_4 = 1940 nm (Osborn and Fearn 1988). NIR spectra of carbohydrate water solutions are characterised by peak values occurring, for glucose, at the following wavelengths: 1587 nm ($\nu \approx$ 6301 cm^{-1}), 2121 nm ($\nu \approx$ 4715 cm^{-1}), 2271 nm ($\nu \approx$ 4403 cm^{-1}); and for fructose at 1583 nm ($\nu \approx$ 6317 cm^{-1}), 2123 nm ($\nu \approx$ 4710 cm^{-1}), 2271 nm ($\nu \approx$ 4403 cm^{-1}) (Rambla *et al.* 1997, Borensten *et al.* 1997). Considering the above, it should be noted that the spectral analysis of the absorbance of NIR carbohydrate water solutions proves to be particularly effective in the range of $\lambda \in (1800;2200)$ nm, which corresponds to wave number of $\nu \in (5555; 4545)$ cm^{-1}. As a result, in this spectral range it is possible to conduct very exact analysis of the changes that occur in the water structure (Czarnik-Matusewicz *et al.* 2005; Czarnik-Matusewicz and Pilorz 2006, Giangiacomo 2006). The spectral area in the range of $\nu \in (5500;4700)$ cm^{-1} was used by Czarnik-Matusewicz *et al.* to investigate the impact of temperature on water structure (Czarnik-Matusewicz *et al.* 2005, Czarnik-Matusewicz and Pilorz 2006).The same band was also employed by Giangiacomo to analyse the influence of carbohydrates on water structure (Giangiacomo 2006). He found that the higher the increase of sugar concentration in water the more symmetrical the water absorbance peak becomes, accompanied by its shift and simultaneous lowering of the absorbance minimum value towards greater wavelengths (lower values of wave numbers (Giangiacomo 2006). Giangiacomo succeeded in identifying the two extreme values at λ_1 = 1900 nm and λ_2 = 1960 nm by calculating the differences between pure water spectra and the carbohydrate solutions investigated. As a result, he was able to state that the spectral changes caused by the increase of sugar concentration are analogous to the drop of water temperature. The above is the effect of increase of the stability of bound water particles formed by water molecules due to an increase of the spaces between water molecules (Giangiacomo 2006). By the same token, he confirmed a structure-maker effect of carbohydrates on water.

The spectra of carbohydrate water solutions provide a coded set of information on the state of water bonds, water structure and carbohydrates found in it. To conduct a detailed analysis and to obtain more information on the nature of the phenomenon a number of spectral research methods have been used. The simplest include direct analysis by classifying and comparing the changes in the analysed spectra, which makes it possible to identify, for example, isosbestic points (Luck, 1974). An alternative and most commonly used method is an indirect method which involves calculations of the first and second derivative of the spectrum (Czarniak-Matusewicz *et al.* 2005, Ramla *et al.* 1997). Another indirect method involves spectral subtraction (Giangiacomo 2006). This method enables us to identify a specific interaction of some definite components of the solution, e.g. various salts (Frost and Molt 1997, Li *et al.* 2003, Nørgaard *et al.* 2005). Still another indirect method is the division of the spectrum into individual components characteristic of specific types of vibrations (Li *et al.* 2003, Czarnik-Matusewicz and Pilorz 2006).

The purpose of this paper is to characterize water and carbohydrates interactions in water solutions using NIR spectrum analysis in the range of $\nu \in (11000;4000)\,\mathrm{cm}^{-1}$. Particular attention is paid to the spectral bands of $\nu \in (5500;4300)\,\mathrm{cm}^{-1}$. The interactions investigated were concerned with water and monosaccharides (i.e. glucose and fructose) in water solutions at concentrations of up to 66.7%. Detailed analysis was focused on the effect of the carbohydrate concentrations on the changes occurring in the spectra. The results were compared with the data obtained by other researchers. Special attention was paid to the author's original observations followed by a discussion of the results obtained.

MATERIALS AND RESEARCH METHODOLOGY

In the investigations both crystalline glucose (produced by Chempur Poland) and fructose (produced by Sigma-Aldrich Laborchemikalien GmbH Germany) were used. The water used in the investigations was distilled twice. Mass concentration of the carbohydrates (glucose and fructose) in the solutions was identical and amounted to 20%, 33.3%, 42.9%, 50%, 55.6%, 60%, 63.6% and 66.7%. Since the glucose solutions at concentrations above 50% were over-saturated, they were obtained by heating in hermetically sealed glass containers at 55°C.

All the measurements were performed at constant temperature of 20±1°C. The solution spectra were obtained using a Nexus FT-IR spectrometer (Thermo Nicolet Corporation, USA). The device, equipped with helium and neon laser light source and Michelson interferometer, made it possible to obtain continuous transmittance and absorbance spectra in the range of the wave number $\nu \in \langle 12000; 4000\rangle\ \mathrm{cm}^{-1}$ using the Fourier transform. Both InGaAs detector and XT-KBr beam splitter were used. The spectroscope was controlled by OMNIC ESP 5.1 software installed on a PC Pentium II. In order to obtain a uniform spectrum each sample was scanned 50 times. The specimens were placed between two cylindrical quartz cells with a 0.2 mm optical path (thickness of samples).

The spectroscopic measurements were performed at the same set background and light intensity. The analysis was carried out within the whole NIR spectrum range, i.e. within the wave number $\nu \in (12000;4000)$. Special attention was paid to the following wave number: $\nu \in (5500;4300)\,\mathrm{cm}^{-1}$.The spectra of pure water, glucose and fructose water solutions were compared using the subtraction technique. In particular, the following wave numbers were studied (wavelengths): $\nu_1 \approx 5260\,\mathrm{cm}^{-1}$ ($\lambda_1 \approx$ 1900 nm), $\nu_2 \approx$ 5102 cm^{-1} ($\lambda_2 \approx$ 1960 nm), $\nu_3 \approx$ 5000 cm^{-1} ($\lambda_1 \approx$ 2000 nm). These are the frequencies that are characterized by extremes caused by OH water group vibrations (Czarnik-Matusewicz *et al,* 2005; Giangiacomo 2006).

RESULTS AND DISCUSSION

Figure 1 shows a set of primary spectra obtained for pure water an glucose solutions with rising concentrations in the range of wave number $\nu \in (12000; 4000)$. Analogous results for pure water and fructose water solutions are presented in Figure 5.

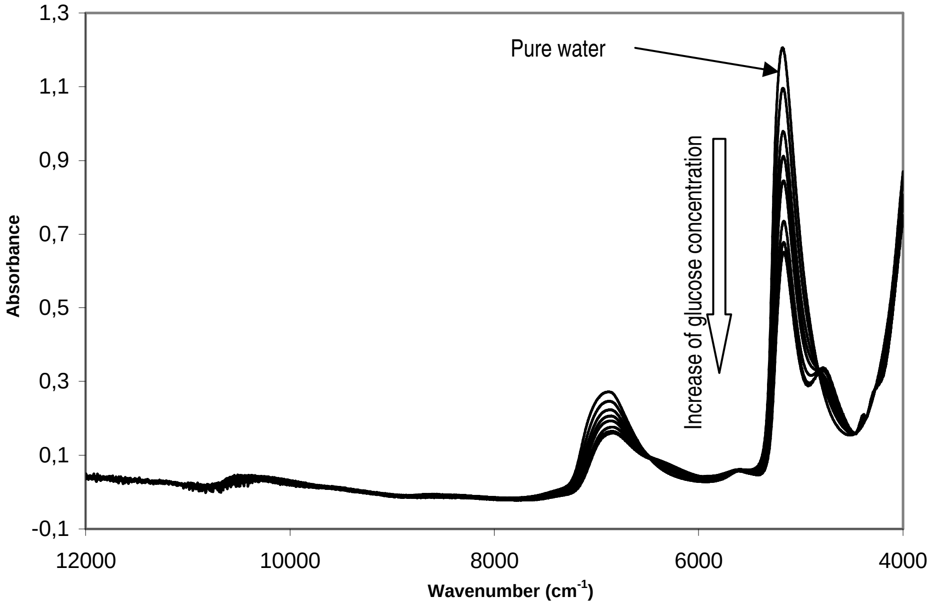

Fig. 1. Spectra of pure water and glucose water solutions of increasing concentration

The spectra shown in Figure 1 are characterized by two distinct peaks with maxima at $\nu = 6880$ and $\nu = 5177$ cm^{-1} for pure water. These spectra are typical for radiation absorbance by pure water (Czarnik-Matusewicz *et al.* 2005). As the glucose concentration increases there is a drop of the maximum value of absorbance of both peaks, accompanied by their narrowing on either side. A detailed analysis of the spectral changes in the range $\nu \in (5300;4800)$ cm^{-1} makes it possible to draw similar conclusions to those obtained by Giangiaqcomo (Giangiacomo, 2006). An increase of glucose concentration in this spectral range causes a decrease of solution absorbance and a shift of the maximum towards lower values of the wave number. Figure 2 shows the changes of the maximum absorbance value dependent on the glucose concentration for the peak in the spectral range $\nu \in (5300;4800)$ cm^{-1}. Figure 3 presents the changes of the position (location) of the maximum value in the function of glucose concentration. As a result of regression analysis, the linear decrease of the maximum absorbance value and maximum location was found to be dependent on the solution concentration in both figures (Figure 2 and Figure 3).

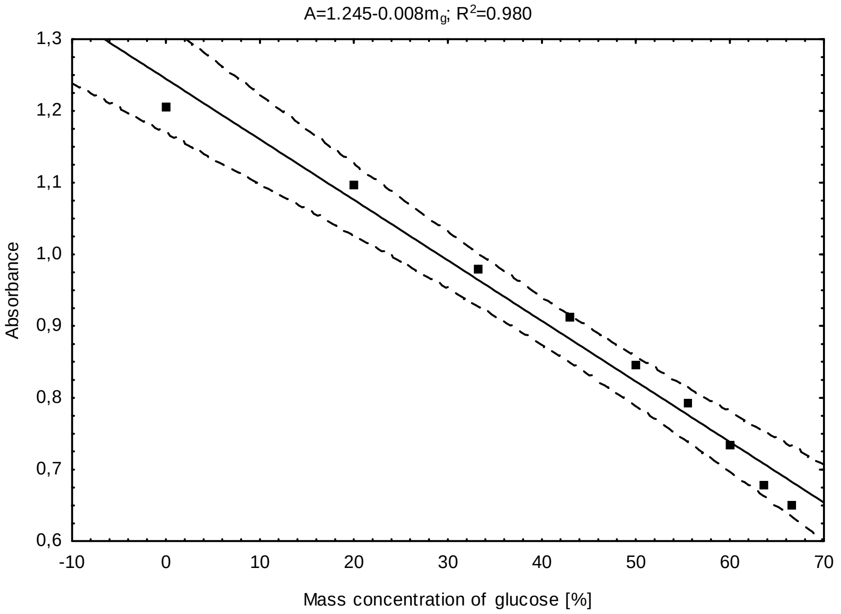

Fig. 2. Changes of peak absorbance value in the spectral range $\nu \in (5500;4800)$cm^{-1} depending on the mass concentration of glucose solution

Figure 4 shows the difference spectra of secondary absorbance resulting from the subtraction of the spectra of increasing concentration of glucose solutions from the pure water spectra. The spectra are characterized by maxima close to the peaks typical of vibrations of free molecules of water (Giangiacomo 2006, Czarnik-Matusewicz 2006). This effect shows unequivocally that an increase of glucose concentration in the solution results, first of all, in a decrease of free water particles. At the same time the spectra show ranges of the wave number in which the difference spectra have negative values. This aspect of the analysis was not dealt with by Giangiacomo in his paper (Giangiacomo 2006). These areas show higher absorbance values for glucose solutions than pure water. A characteristic phenomenon observed here is the change of the absorbance sign that occurs in all the spectra independently of glucose concentration at one single point called the isosbestic point (Luck 1974). There are several such points for glucose water solutions in the whole NIR range.

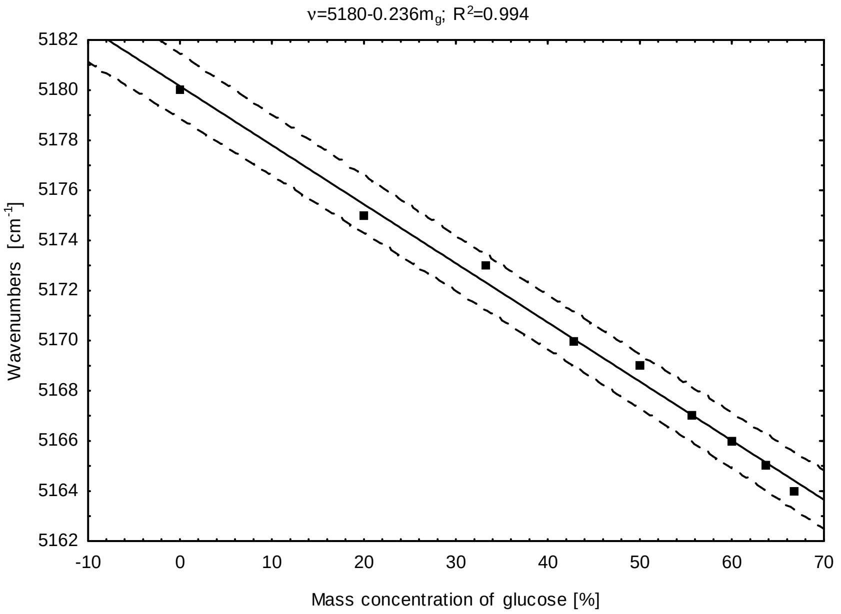

Fig. 3. Changes of maximum absorbance location in the spectral range $\nu \in (5500;4800)\,\mathrm{cm}^{-1}$ depending on the mass concentration of glucose solution

It is possible to determine exact values of the wave number at which isosbestic points appear by showing two differences spectra in one figure: first between water and glucose spectrum at a given concentration and the other a reverse one, i.e. between the glucose solution at the same concentration and pure water. Figure 5 presents two such difference spectra obtained from the primary spectra of pure water and the glucose solution at the concentration of 60%. It can be noticed that water solutions of glucose have four isosbestic points in the whole NIR range at which spectra intersect, i.e. for $\nu \in \{6475;5624;4825;4258\}\mathrm{cm}^{-1}$. Additionally, there are two characteristic points at which the differences spectra are equal to zero, i.e. the absorbance values in the pure water spectra and glucose concentration are constant and equal to zero, which occurs for $\nu \in \{4463;4351\}\mathrm{cm}^{-1}$. There is also another point where the zeroing of differences spectra is blurred and occurs in a wide range of wave number $\nu \in \{9749;8854\}\mathrm{cm}^{-1}$. As a result, the range was neglected in our consideration. At all these points the glucose concentration does not affect the absorbance value of the solution and is equal to the absorbance value of pure water. It is essential to be able to determine the exact location of these points since it will allow us to analyse the composition of multi-component solutions (including glucose as one of the components). Using an analogous procedure to the one that enabled us to obtain the spectra shown in Figure 5, Table 1 shows the location of the isosbestic points determined independently using the spectra of the investigated glucose concentrations. This made it possible to calculate both the average value und standard deviation of the identified isosbestic points. Special attention should be paid to two isosbestic points – IP_3 and IP_6 – at which all the spectra intersect and the standard deviations of the wave number values assume the smallest value.

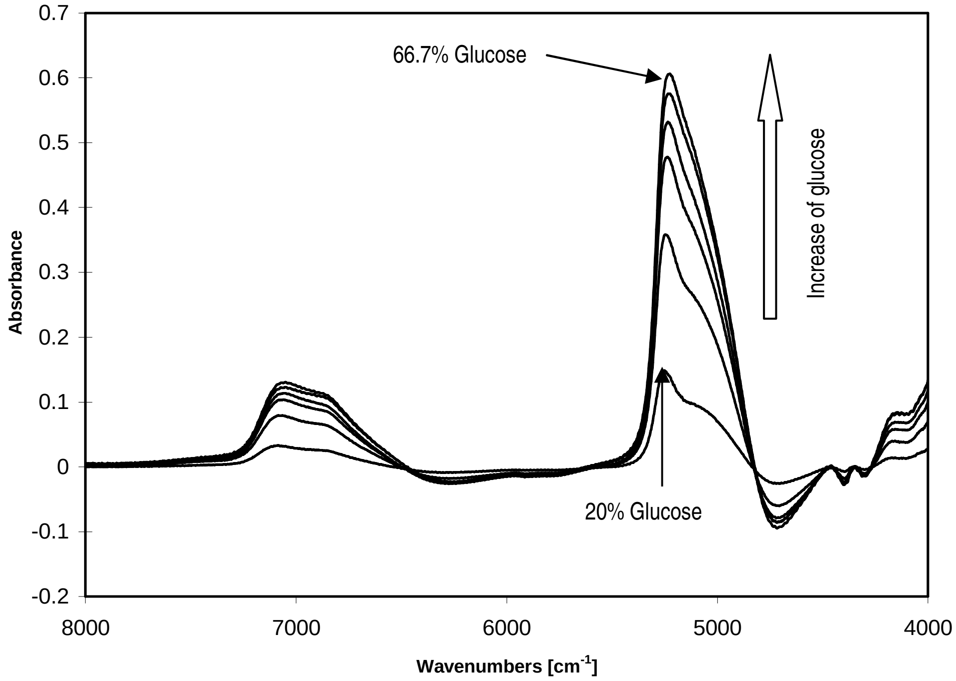

Fig. 4. Difference spectra resulting from the subtraction of glucose spectra from pure water spectra at increasing glucose mass concentration

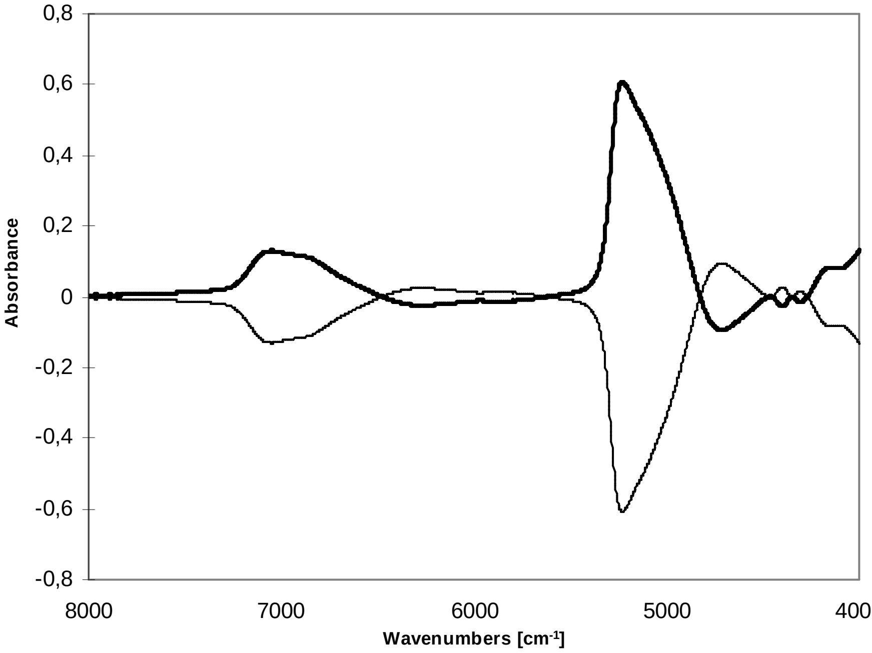

Fig. 5. Two difference spectra obtained from primary spectra of pure water and glucose water solution at the concentration of 60%

Table 1. Wave number values with isosbestic points (IP) dependent on glucose mass concentration

No	Glucose mass concentration in the solution (%)	IP_1	IP_2	IP_3	IP_4	IP_5	IP_6
		(cm^{-1})					
1	20	6536	5520	4835	4461	4351	4265
2	33,3	6516	5538	4829	4461	4351	4272
3	42,9	6503	5561	4830	4463	4351	4265
4	50	6493	5591	4827	4472	4355	4270
5	55,6	6498	5569	4829	4467	4350	4266
6	60	6498	5571	4827	4466	4351	4268
7	63,6	6471	5618	4823	4472	4354	4272
8	66,7	6471	5622	4826	4469	4349	4269
Average Value		6498.3	5573.8	4828.3	4466.4	4351.5	4268.4
Standard Deviation		21.6	35.7	3.5	4.5	2.0	2.9

Using Figure 5 we can also identify the ranges of wave number values where glucose is responsible for the absorbance radiation in water solutions. The global extreme for glucose absorbance occurs for $\nu = 4715$ cm^{-1}, and the local extreme for $\nu \in$ {6300; 4400; 4303} cm^{-1}. Disregarding some minor errors, these values are in good agreement with the data available in literature (Rambla *et al.* 1997). The last peak at $\nu = 4303$ cm^{-1}, existence of which was impossible to identify in literature, constitutes an exception. The peak can be distinctly identified in the differences spectra obtained by Giangiacomo. However, it was not analysed by the author (Giangiacomo 2006).

Further parts of the paper present the results obtained for fructose water solutions at the same concentration values and using analogous procedures as for glucose. Figure 6 shows primary spectra of fructose solutions and pure water. The fructose spectral patterns are very close to the glucose ones, so it is difficult to assess the differences between these two monosaccharides.

Figure 7 shows the results of subtracting the investigated fructose solutions from pure water spectra. The difference spectra obtained in this way show, when compared to the analogous glucose spectra, both quantitative and qualitative differences. Apart from the ranges typical of radiation absorption by water, the main qualitative differences can be observed, first of all, in the range of $\nu \in$ (4480;4250) cm^{-1}. There is only one single peak and a local minimum at ν = 4403 cm^{-1}. The minimum, similarly to the one occurring at ν = 6317 cm^{-1} and the global at ν = 4715 cm^{-1}, exactly overlap with the values for fructose described in literature (Rambla *et al.*, 1997). Fructose is also characterized by some other values of isosbestic points except for one point IP$_3$ at $\nu \approx 4830$cm^{-1}.

Using an analogous procedure to determine isosbestic points as earlier for glucose, Table 2 presents the coordinates of the points determined for each concentration separately. It also gives their average values with standard deviations. Additionally, to highlight glucose and fructose differences concerning the isosbestic points, Figure 8 shows difference spectra of pure water compared with 60% fructose concentrations and inverse to it. It can be clearly observed that in the range of $\nu \in$ (7500;4000) cm^{-1}cm$^{-1,}$ there are only four isosbestic points, unlike in the case of glucose that has six such points. The last two points show a relatively small deviation of the wave number. Thus it can be assumed that their location is very stable, similarly to the maxima related to the radiation absorption by fructose.

The differences in the “strength” of water bonds between glucose and fructose are particularly visible in the range of $\nu \in (5300; 4800)$cm^{-1}. This phenomenon is illustrated in Figure 9 which shows the qualitative changes of radiation between both glucose and fructose spectra at the same concentration c = 50%. Fructose produces stronger bonds, especially of free water particles, which can be seen in higher values of the extreme in the difference spectra at $\nu = 5174$ cm^{-1}. The results of the absorbance dependence at this wave number in the function of the concentration of the investigated monosaccharide solutions are shown in Figure 10.

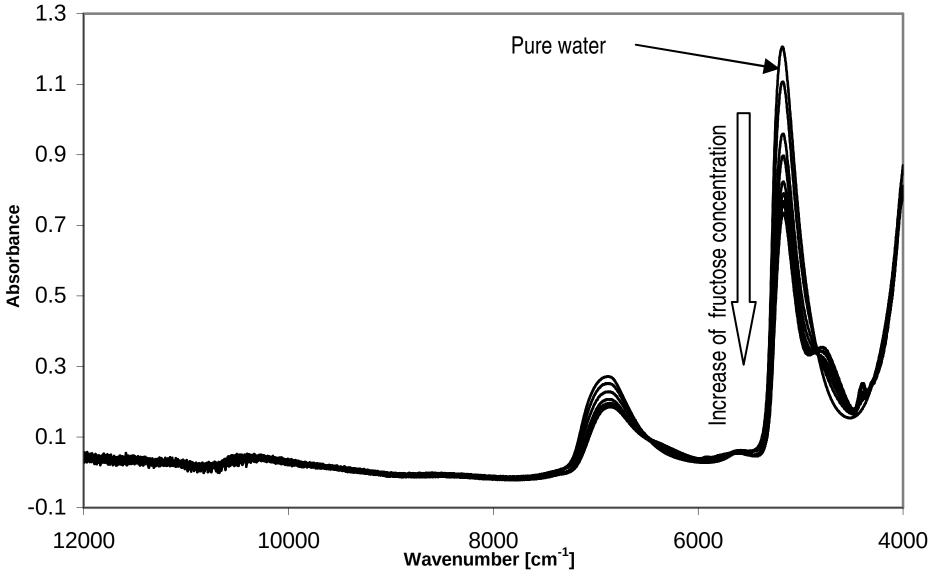

Fig. 6. Primary spectra of pure water and fructose solutions

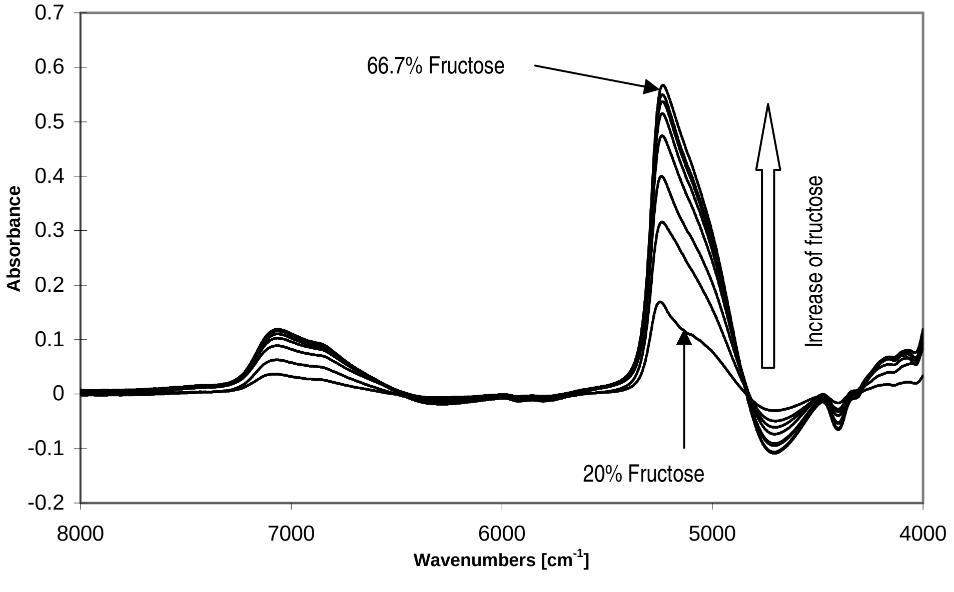

Fig. 7. Difference spectra of fructose solutions at concentrations of 20%; 33.3%; 42.9%; 50%; 55.6%; 60%; 63.6%; 66.7%

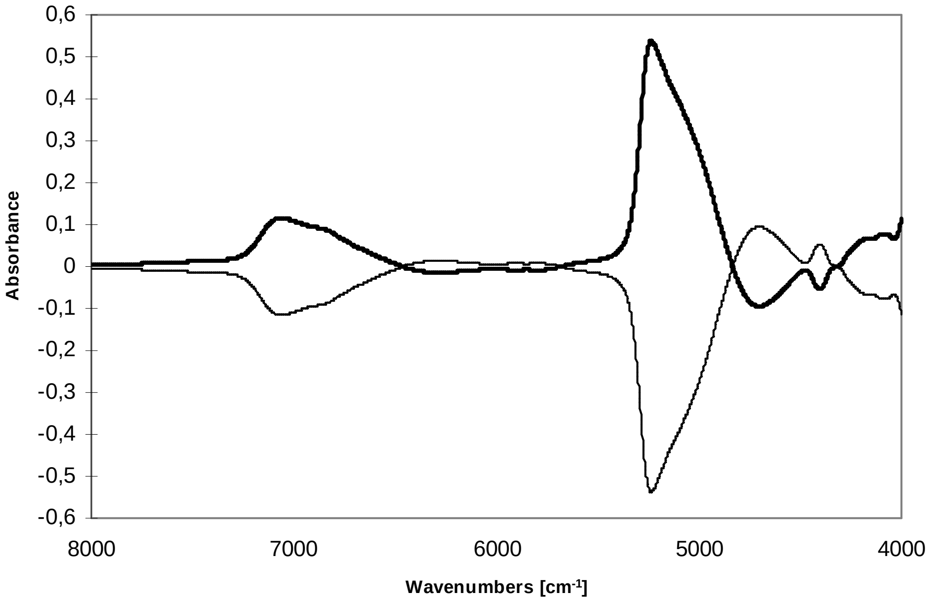

Fig. 8. Two difference spectra obtained from the primary spectra of pure water and fructose water solutions at the concentration of 60%

Table 2. Wave number values with isosbestic points (IP) depending on fructose concentrations

No	Fructose concentration in the solution %	IP_1	IP_2	IP_3	IP_4
		cm^{-1}			
1	20	6507	5638	4839	4309
2	33.3	6502	5614	4830	4314
3	42.9	6442	5703	4827	4324
4	50	6450	5692	4827	4321
5	55.6	6475	5667	4834	4311
6	60	6462	5683	4834	4313
7	63.6	6470	5676	4836	4303
8	66.7	6482	5656	4836	4298
Average Value		6473.8	5666.1	4832.9	4311.6
Standard Deviation		23.0	29.3	4.4	8.6

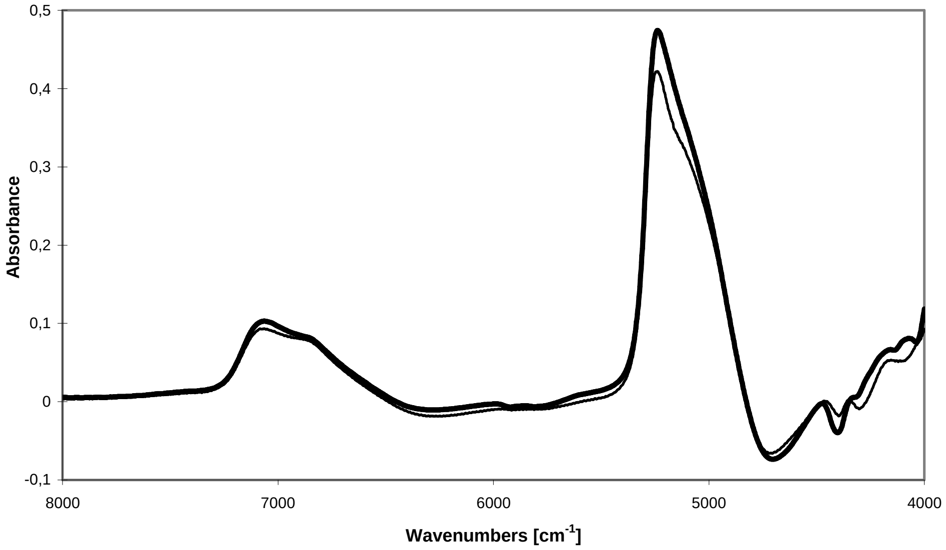

Fig. 9. Difference spectra of pure water and glucose solution (thin spectrum) and fructose solution (bold spectrum) at the concentration of 50%

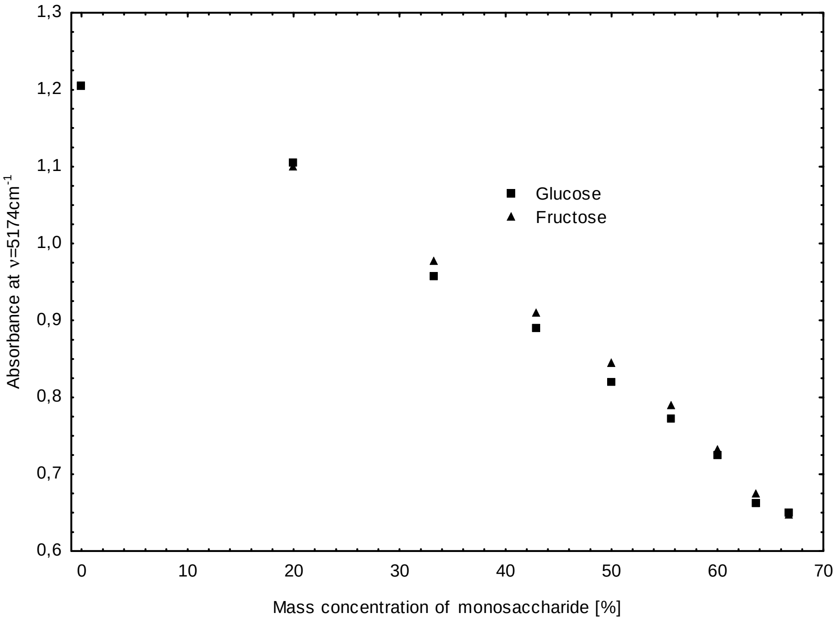

Fig. 10. Absorbance changes of water solutions of the investigated monosaccharides (■ – glucose and ▲ – fructose) at $\nu = 5174$ cm^{-1} in the function of their mass concentration

Comparing the results shown in Figure 10 with the changes of water activity under the influence of glucose and fructose concentrations, we can state that the dependence patterns, as far as the quality is concerned, are similar (Gaida *et al.* 2006). Fructose tends to produce stronger water bonds at concentrations of 50%, which can be clearly observed both in water activity measurements (Gaida *et al.* 2006) as well as at absorbance value for ν = 5174 cm^{-1}. However, there are, so far, no full quantitative representations of the dependence between water activity and absorbance value on monosaccharide concentration.

CONCLUSSIONS

The analyses carried out in the work made it possible to:

- verify the correctness of the results by referring them to the literature reports,
- identify the location of the isosbestic points that occur in the glucose and fructose water solution spectra,
- determine the absorbance maximum for glucose at ν = 4303 cm^{-1} by using the difference spectra. The maximum does not appear in fructose water solutions and its existence has not been mentioned in literature,
- indicate the difference between the bonding force in both glucose and fructose solutions by making comparisons of the absorbance values in the range of $\nu \in (5300; 4800)$ cm^{-1}.

A specific effect of the investigations has been an exact identification of isosbestic points for both glucose and fructose water solutions in NIR spectra. One of these points occurs for glucose at the average wave number value of $\nu_{a\nu}$ = 4828.4 cm^{-1} and is characterized by standard deviation SD = 3.5cm^{-1} and, respectively, for fructose $\nu_{a\nu}$ = 4832.9 cm^{-1} and SD = 4.4 cm^{-1}. In both cases these points practically overlap. So it can be concluded that by making use of the data together it is possible to determine both the concentration of the carbohydrates in the solution and the glucose content in multi-component solutions. Additionally, the identified absorbance differences existing between glucose and fructose in the range $\nu \in (5300; 4800)$ cm^{-1} allow us to analyse the phenomenon of water bonding that takes place between these two monosaccharides. However, some more investigations are needed to describe the phenomena quantitatively.

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WYKORZYSTANIE SPEKTROSKOPII W BLISKIEJ PODCZERWIENI DO ANALIZY ODDZIAŁYWAŃ POMIĘDZY WODĄ I WĘGLOWODANAMI W ROZTWORACH WODNYCH

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Streszczenie. W pracy wykorzystano spektroskopię w bliskiej podczerwieni do charakterystyki oddziaływań zachodzących pomiędzy wodą a glukozą oraz wodą i fruktoza w roztworach wodnych. Opierają się na analizie polegającej głównie na odejmowaniu widm zidentyfikowano w oryginalny sposób punkty izobestyczne, występujące w roztworach wodnych fruktozy i glukozy na widmach NIR. Obliczono średnie wartości liczb falowych, przy których one występują wraz z ich odchyleniem standardowym. Szczegółowa analiza widm różnicowych umożliwiła identyfikację dodatkowego ekstremum absorbancji glukozy przy $\nu = 4303$ cm^{-1}, na temat, którego nie istnieją doniesienia w literaturze światowej. Porównano również siłę wiązania wody przez glukozę i fruktozę oraz odniesiono uzyskane wyniki do pomiarów aktywności wody. Otrzymane rezultaty badań skonfrontowano z dostępnymi w literaturze przedmiotu.

Słowa kluczowe: spektroskopia NIR, fruktoza, glukoza, roztwory węglowodanowe, aktywność wodna