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Detection of changes in the water, blackcurrant- and raspberry juice infrared spectrum in the range 2,500−4,000 cm^{-1}

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Abstract. This research was conducted to develop a method for the establishment of changes in absorbance that occurs in the OH stretching vibration region 2,500−4,000 cm^{-1} of FT-IR transmission spectra of water and juices. With the methodology described in this paper, a signal-to-noise ratio was obtained, which allows to measure the reproducibly of the FT-IR transmission spectra of water and juices. The article points out some important aspects of how the spectra of ultrapure water, blackcurrant- (Ribes nigrum L.) and raspberry (Rubus idaeus) juices were measured in the OH stretching vibration region 2,500−4,000 cm^{-1}. The graphical comparison of raw spectra illustrates that the differences which occur in the spectra of water and juices are hard to differentiate. For the clear distinction of differences in spectra, there are four hidden peaks identified in the analysis of spectra by using the deconvolution method. There are clear differences identified in the comparison of percentages of the hidden peaks of spectra in the areas of ultrapure water, blackcurrant and raspberry juice. Repeated measurements and analysis of spectra provide reproducible results. It was established that the developed method can be used for the detection of changes in the FT-IR transmission spectra of water, juices and aqueous solutions with low molar concentrations of additives. The distinction of changes in the spectra is a precondition for research of the clustered structures of water.

Key words: water structures; clustered water; FTIR; hidden peaks; raspberry juice; blackcurrant juice.

INTRODUCTION

The study of water structure in biological systems has been a research topic for more than half a century (Plumridge & Waigh, 2002; Laurson & Mäeorg, 2015). Scientists have used different spectroscopic methods to reveal water structure: NIR (Bharmoria et al., 2014), Raman (Walrafen, 1967), IR (Falk & Ford, 1966) NMR (Dillon & Dougherty, 2003; Conte, 2015). When water structure changes under the different factors affecting it, the number of hydrogen bonding may increase or decrease. The changes in water structure also change the infrared radiation interaction with water and will, therefore, cause changes in the infrared spectra. The strength of hydrogen bonds decreases with the increase of bonds in length which depends on the geometry of water clusters and is easily affected by the environment (Jeffrey & Saenger, 1991; Calhorda, 2000). In order to investigate water structure, any technique to prevent its alteration is needed. Infrared spectroscopy is well suited for this kind of research in biological systems. Walrafen studied the structure of pure water by infrared spectroscopy and in his model described that the broad OH vibration maxima in the range of 3,000–3,800 cm^{-1} could give four deconvoluted peaks located around ~ 3,245, ~ 3,420, ~ 3,520, ~ 3,620 cm^{-1} and weak fifth centred near 3,060 cm^{-1}(Walrafen, 1972). These peaks were assigned as non-hydrogen-bonded OH stretching peaks 3625–3645 cm^{-1}; 3,540–3,555 cm^{-1}, hydrogen-bonded OH stretching peaks 3,395–3,420 cm^{-1}; 3,215–3,220 cm^{-1} and fifth component 3,050–3,090 cm^{-1} arising from Fermi resonance between the overtone of the bending vibration at ~1,650 cm^{-1} and coupling main component of 3,215–3,220 cm^{-1} correspondingly (Walrafen, 1972). The researchers have thereafter used changes in the absorption spectra in the aforementioned regions in order to describe changes in the clustered structure of water. The high molar concentration of additives (inorganic salts, urea, sugars etc.) in aqueous solutions causes clearly distinctive changes in spectra (Afrin et al., 2014; Bharmoria et al., 2014; Mafy et al., 2015). In biological systems the concentration of different dissolved substances is a few percent (Despa, 2005), therefore, differences in the infrared spectra of the fundamental vibration region of pure water are low as compared to the spectra of the biological system. The aim of the paper is to identify the reproducibility of small changes by eliminating all the factors affecting the measurements.

MATERIALS AND METHODS

High-quality ultrapure water was obtained by the Milli-Q® Advantage A 10 Water Purification system (Merck Millipore). The resistivity of ultrapure water at 25 °C was 18.2 MΩ·cm and TOC (total organic carbon) 3 μg l^{-1}. The water sample was held in a closed glass flask. The blackcurrant *Ribes nigrum L.* (Pamjati Vavilova) and raspberry *Rubus idaeus* (Novokitaivska) juices, which were employed in the studies, were pressed from defrosted berries of the 2015 year harvest by a belt press Voran EBP 500, pasteurized at Polli Horticultural Research Centre PlantValor of the Institute of Agricultural and Environmental Sciences, the Estonian University of Life Sciences, and the samples were preserved in closed polypropylene tubes at 7 °C. The yield of blackcurrant juice (without previous enzyme treatment) was 53.8% and the yield of raspberry juice 64.0%. The content rated values of total sugar and content of soluble solids were based on the published research data of the four year average of raspberry (Novokitaivska) 10 ± 2°Brix (Arus et al., 2008) and five year average of blackcurrant (Pamjati Vavilova) 15 ± 1°Brix (Kaldmaee et al., 2013). The content of soluble solids in the homogenised juice samples was measured by a Pocket Refractometer 'ATAGO' PAL- α at 22 °C.

Infrared spectra were measured at a resolution of 4 cm^{-1} with the Perkin Elmer Spectrum BXII FT-IR system equipped with a DTGS detector in the absorbance mode with 32 scans for each sample in the range 2,500–4,000 cm^{-1}. The homemade reduced sample compartment was flushed continuously with N_2. Spectra acquisition started after 3 minutes from the cleaning process of the sample compartment with N_2, i.e. after having invariably checked the background noise. The spectra were acquired by the application of CaF_2 windows (thickness 4 mm; diameter 32 mm). A 1.2 μl proportion of the sample was applied on the CaF_2 window by using a 10 μl Hamilton syringe. The sample was spread on the window with an even layer and covered with another window. The edge of the windows was tightly framed with two layers of Parafilm® tape to stabilise the layers of the fluid. The thickness of the layer between the windows was 1.5 μm which was calculated by applying the volume formula for a cylinder. All the measurements were performed at a room temperature in the range of 22–24 °C. Good reproducibility reveals that 2 °C temperature variations of the measurement interval do not affect the result of the measured FT-IR spectra. At least three measurements of each sample were carried out for the reproducibility of the measurement. For the deconvolution of spectra OriginPro 2016 Sr1 b9.3.1 273 (OriginLab Corporation) a software peak analyser tool (Goal: Fit Peaks Pro; Anchor Points Finding Method: 2nd Derivative; Fitting Function: Line; Current number of Peaks 4; baseline parameters and peak centres are released) was used. All the spectra before the deconvolution were normalised to 0–1.

For precise measurements and the detection of small changes in infrared spectra, all the sources of noise and interferences should be detected and eliminated. One of the serious sources of noise is the background spectrum which contains signals from water, carbon dioxide and alkane. The above background spectrum is generally subtracted from the spectrum of the sample. Since the atmosphere in the laboratory is likely to change, the subtraction of the background spectrum does not prove extremely precise and some noise will still be registered.

The noise disturbs the detection of small changes in spectra and the correct deconvolution. In order to decrease noise, the sample compartment should be flushed with dry nitrogen. Nevertheless, flushing as it is recommended by spectrometer companies fails to appear sufficiently efficient. Therefore, a special N_2 purgeable tube was manufactured and tightly installed in the sample compartment of the spectrometer. It may be followed in the comparison of the background spectrum measured in the common laboratory environment versus the background spectrum measured in the environment purged with N_2 (using installed tube), that in the water vapour absorption region 3,400–4,000 cm^{-1} the peaks decrease up to 80–90%, as shown in Fig. 1. This level of noise is acceptable for the measurements of small spectral changes in the OH stretching vibration region and thus is there no need for an expensive spectrometer with closed and inert gas filled optical system.

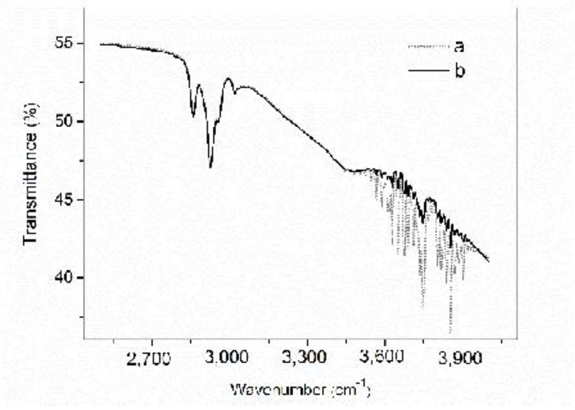


Figure 1. Comparison of the background spectra: a) ordinary air in room, b) N_2 flushed special sample compartment.

RESULTS AND DISCUSSION

Fig. 2 shows that the infrared spectra of ultrapure water, blackcurrant- and raspberry juice have a joint peak absorption maximum at 3,404 cm^{-1}. Moreover, all the spectra reveal an isosbestic point (the i.e. common point where spectral bands cross) at 3,590 cm^{-1} which suggests the existence of differences in the spectra (Afrin et al., 2014; Walrafen et al., 1986). To the opposite, when the crossing points between the spectra are missing, this would mean that the absorption spectral band profiles are the same, and no changes occur in the spectra.

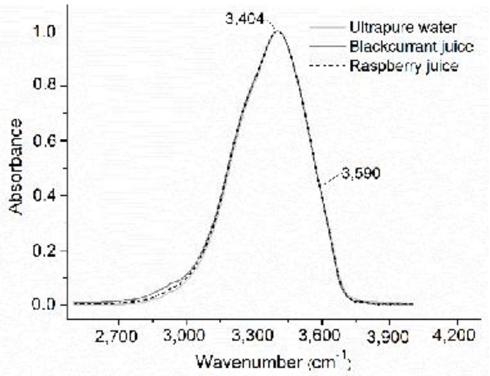


Figure 2. FT-IR spectra of ultrapure water, blackcurrant juice and raspberry juice in the range 2,500–4,000 cm^{-1}.

Fig. 3 shows that the observed lines of spectra do not overlap at the beginning and the end of spectra, but the order of the lines changes at 3,590 cm^{-1}, thus indicating the presence of different interactions between juice components and water molecules. These changes would be difficult to detect should the spectra stand separately. It is common knowledge that the strong absorption bands that belong to the water stretching mode at 3,404 cm^{-1} consist of four individual maxima (Venyaminov & Prendergast, 1997) which are visualised after the deconvolution of spectra.

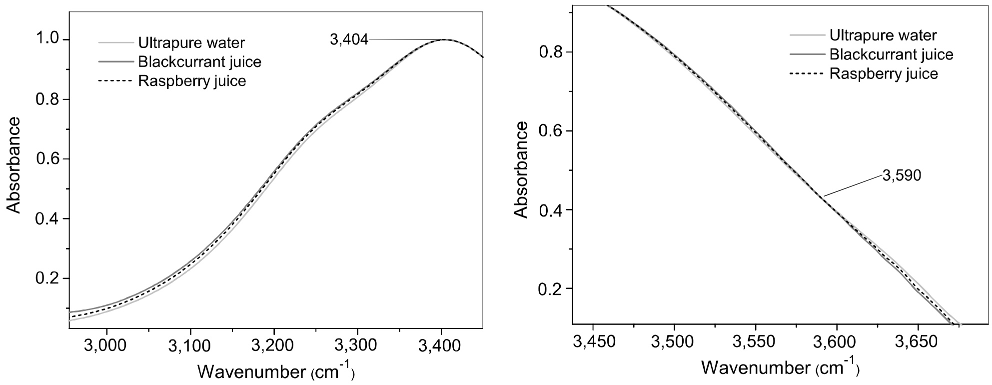


Figure 3. The joint peak absorption maximum at 3,404 cm^{-1} and isosbestic point in the 3,590 cm^{-1} of FT-IR spectra of ultrapure water, blackcurrant juice and raspberry juice in the range 3,000–3,700 cm^{-1}.

The deconvoluted FT-IR spectra of ultrapure water and juices are shown in Fig. 4, the maximum of bands corresponds to area A1: 3,100−3,220 cm^{-1}, A2: 3,240−3,265 cm^{-1}, A3: 3,430−3,440 cm^{-1} and A4: 3,580−3,590 cm^{-1}. As deconvolution was performed without restriction, it mean baseline parameters and peak centres was released, as expected sifhts in the maximum of bands locations and result is accordance with Walrafen research. (Walrafen, 1972). Fig. 5 shows that the researched substances are compared to each other whereas the integrated hidden peaks (A1, A2, A3, A4) area of the deconvoluted FT-IR spectra are all different. The hidden peaks areas A2, A4 of juices are higher and A1, A3 lower than ultrapure water in the same peak areas. The error bars in Fig. 5 show that deviations in the measurements of spectra are very low and fail to pose any impact on the results.

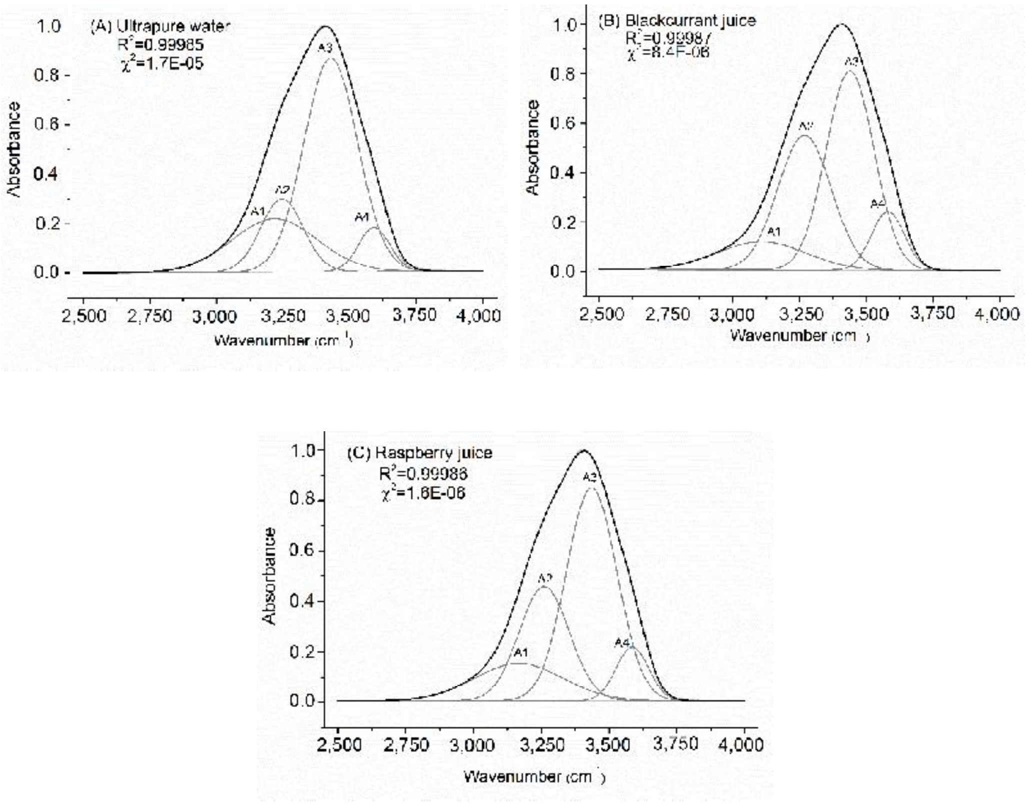
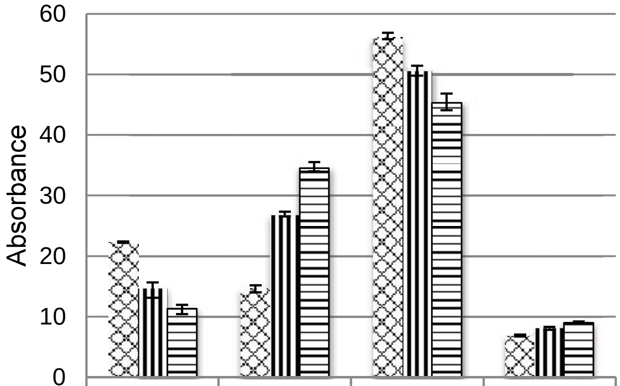


Figure 4. Comparison of deconvoluted FT-IR spectra: a) ultrapure water, b) blackcurrant juice and c) raspberry juice in the range 2,500−4,000 cm^{-1}.



	A1	A2	A3	A4
▨ Ultrapure water	22	15	56	7
❚❚ Raspberry juice	15	27	50	8
□ Blackgurrant juice	11	35	45	9

Figure 5. Comparison of integrated hidden peaks (A1, A2, A3, A4) area in percent of the total area of the deconvoluted FT-IR spectra ultrapure water, blackcurrant juice and raspberry juice in the range 2,500–4,000 cm^{-1}.

CONCLUSIONS

It has been demonstrated that by using the modified sample compartment, which has been purged with N_2, it is possible to measure reproducibly the infrared spectra of water and juices in the OH stretching region 2,500–4,000 cm^{-1}. The deviations that occur in the spectra of water and juices are hard to discern. By using the deconvolution method in analysing the spectra, a clear distinction may be observed in the deviations within the spectra. There are clear deviations in the hidden peaks areas of ultrapure water, blackcurrant and raspberry juice whereas it refers to the deviation in the structure of water in juices. The method developed can be used for the detection of changes in the FT-IR transmission spectra of water, juices and other aqueous solutions with low molar concentrations of additives.

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