Infrared spectra of some fructans

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Abstract. The FT-IR spectra of fructan – inulin (RAFTILINE), widely applied in the food industry and crystalline fructose as the main component of fructans, were studied. Special interest was to study the spectra of the levan precipitate and fructan syrup – produced by *Zymomonas mobilis* during the fermentation on sucrose-based medium.

It was shown that levan precipitate and fructose syrup does not contain lipids and nucleic acids. Levan precipitate consists of ∼93% of fructose and admixture of glucose, mannan and enzyme – levansucrase. Fructan polymer inulin consists principally of linear chains of fructosyl units linked by a β(2-1) bonds ended by a glycosyl unit. The links between the molecules are of a very special type: the β(2-1) form (2) [8]. The bacterial fructans of the levan type are high molecular weight polymers, i.e., they are composed of β-(2,6)-fructosyl-fructose linked molecules and side chains [17]. The FT-IR spectra of levan, apart from inulin's, in the carbohydrate region 900–1200 cm^{-1}, shows overlapping broad band with maximum at ∼1030 cm^{-1} and stronger absorption at ∼940 cm^{-1}. The differences in both spectra could be caused by different structure and glucose, sucrose and mannan influence.

Keywords: IR-spectroscopy, levan, fructose syrup, inulin

1. Introduction

Fructose or fruit sugar, is a simple sugar found in honey, fruit and different plants. It is sweeter than glucose and sucrose. Chemically it is a monosaccharide with the empirical formula $C_6H_{12}O_6$ – the same as glucose but differs from it in structure. It is best obtained by hydrolysis of polyfructose – inulin that is carbohydrate of plant origin naturally occuring in significant amounts [8].

Inulin is a polymer of fructans and consists principally of linear chains of fructosyl units linked by β(2-1) bonds ended by a glycosyl unit [9]. Native inulin is a mixture of poly- and oligosaccharides which almost all have the chemical structure GFn (G = glucose, F = fructose, and n = number of fructose units linked to one another). The links between the molecules are of a very special type: the β(2-1) form (2), which makes these molecules indigestible for all higher animals [8] and has significant "dietary fiber effects" often comparable to pectins. The concept of dietary fiber, first described in the context of the hypothesis developed by Burkitt and Trowell in 1975, covers actually a series of complex compounds which are, according to the current most widely accepted physiological definition, "the sum of polysaccharides and lignins that are not hydrolyzed by the endogenous secretion of the human digestive tract" [1].

Fructans are a diverse group of polysaccharides that contains two or more β-linked fructose units. Fructans containing from 2 to 9 fructose units are named fructooligosaccharides (FOS). In the most prominent structural polymer types, inulin and levan, the fructose chain emerges from the fructose part of a sucrose molecule, proceeding via $\beta2 \rightarrow 1$ and $\beta2 \rightarrow 6$ linkages, respectively [10]. Fructans are naturally found not only in plants, but also in bacteria and fungi, probably serving very different functions. Most bacterial fructans are high molecular weight polymers of the levan type, i.e., they are composed

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of β-(2,6)-fructosyl-fructose linked molecules and side chains [17]. Levans are part of the exopolysaccharide that protects the cells from desiccation, helps in surface attachment, and is – in some plant pathogenic species – involved in preventing the invading bacteria from being recognized by the host defense system [13,14]. Levan is extracellulary produced by different bacteria *Bacillus subtilis*, *Erwinia herbicola* [7] and *Zymomonas mobilis* [20]. *Z. mobilis* is a unique bacterium among the microbial evolutionary world and it's taxonomic position has not been fully established. In a sucrose-based medium this gram-negative, ethanol-producing bacterium produces various by-products: levan of high molecular mass [4], sorbitol, gluconic acid, and FOS [19]. Levan is viscous, biologically active, non-toxic and can be used as thickener or stabilizer in the food, pharmaceutical and cosmetic industries and is a good raw material for fructose production, acts as immunomodulator, and is applied as blood plasma substitute, prolongator of medicine, and a cholesterol lowering agent [22].

FOS are known as sweeteners with reduced energetic value, stimulators of probiotics [23]. In our laboratory has been developed a new product – fructan syrup [3] obtained from *Z. mobilis* sucrose fermentation. Fructan syrup has a pleasant honey-like taste, serves as a source of prebiotics, and soluble fiber – levan, and is of interest as potential ingredient in foods because of their effects on intestinal flora, functionality, and reduced caloric value.

The aim of our work was to study the IR spectra of our products: levan precipitate and fructan syrup as well as in the food industry widely applied inulin – RAFTILINE, and fructose as the main component of fructans.

2. Materials and methods

2.1. Microorganisms and preparation of the samples

The levan-producing strain *Zymomonas mobilis 113 "S"* and a two-stage fermentation process was used as described previously [5]. The culture liquid, containing ethanol, levan, and gluconic acid, was centrifuged at 28 600 g, after the second stage of fermentation. The cell-free supernatant (100 ml) was treated with ethanol (1 : 2.5) and the obtained sediment is a crude levan suitable for application in the food industry.

Fructan syrup was prepared from sucrose syrup (65%) using as biocatalyst 5 g/100 g levan-levansucrase sediment at incubation temperature 45°C during 48 h.

Inulin was obtained from ORAFTI (Belgium) as a commercial food additive RAFTILINE.

2.2. Analytical methods

The cell mass in the culture liquid was determined after centrifugation for 15 min (at 5000 g) and drying at 105°C. The optical density of a 10 time diluted culture was measured at 590 nm. The ethanol concentration was determined by GLC (Chrom 4, the column Inerton AW-HHDS + 5% PEG, $T_t = 80°C$, $T_S = 200°C$). The gluconic acid concentration was determined by HPLC (Shimadzu LC-4A, the column Shimadzu SCR 101 (H), spectrophotometer SPD-2AS, $\lambda = 210$ nm, the mobile phase 0.01 N H_2SO_4, the flow 1.2 ml/min at 40°C). Levan was precipitated by ethanol (65 v%) and determined as fructose after hydrolysis of polysaccharide [20]. The glucose, fructose, and sucrose concentrations were determined by HPLC (the column Pinacle Amino 5 μm, 250 × 4.6 with a mobile phase, acetonitryle : water 75 : 25, refractive index detector). The yield of FOS was calculated from the estimated sugar content in the levan-free solution before and after hydrolysis.

Fructan syrup and levan precipitate for IR spectral analyses were dried in thermostat at 45°C, 1.5–3.2 mg of sample were mixed with 1 g KBr, milled and pelleted. 1.0–2.5 mg of RAFTILINE – inulin or fructose (Aldrich) was mixed with 1 g KBr, milled and pelleted.

The prepared KBr pellets of fructose, RAFTILINE (inulin), levane precipitate, and fructan syrup were registered on the FT-IR spectrometer Perkin Elmer Spectrum RXIFT-IR, absorption mode between 700 and 3500 cm^{-1}, resolution 4 cm^{-1}, 16 scans.

3. Results

3.1. Interpretation of the IR spectra of carbohydrates

Crystalline mono- and oligosaccharides give nice infrared spectra with several absorption lines and carbohydrates are a class of bio-molecules well investigated by vibrational spectroscopy. This has to do with the fact that unlike the nucleic acids, the proteins, or the lipids these biomolecules lack prominent polar, infrared-activated functional groups with heteroatoms and multiple bonds. The predominance of C–C and C–O bonds in carbohydrates and the similar mechanical properties of these bonds give rise to broad, unresolved infrared absorption bands. The specificity of carbohydrates arises from the geometry of the many O–H groups and the configuration of the C–O, C–C, and C–H bonds in the skeletal base configuration [15]. The IR-absorption a spectrum of crystalline carbohydrates and absorption band interpretation has been widely reported [2,6,16,21].

The IR spectra of carbohydrates can be divided in three specific spectral regions: 1200–900 cm^{-1}, 3000–2700 cm^{-1} and 900–600 cm^{-1}.

The spectral region between 1200 and 900 cm^{-1} is generally dominated by a complex sequence of intensive peaks due mainly to strongly coupled C–C, C–O stretching and C–O–H, C–O–C deformation modes of various oligo- and polysaccharides [18]. In all carbohydrates the most intensive is a broad band at ∼1080 cm^{-1} caused by ν (CC) and ν(CO) vibrations. This band serves as characteristic for IR quantitative analysis of microbial biomass, as is not overlapped by absorption of proteins, lipids or nucleic acids [12]. It has been established that the most intensive absorption bands of carbohydrates in the IR-spectra are: strong complex absorption at 1080 cm^{-1}, and 1170 cm^{-1}, 1030 cm^{-1} (valent stretching vibrations of COC groups and ring vibrational modes in the composition of cyclic structures) [16].

In the 3000–2700 cm^{-1} region carbohydrates show few sharp absorption bands: 2930 cm^{-1} assigned to C–H streching (assym) of CH_2, 2870 cm^{-1} assigned to C–H stretching (sym) of CH_3, and C–H stretching bands in the range of 2700–3100 cm^{-1}.

The region between 900 and 600 cm^{-1} exhibits a variety of weak but extremely characteristic features superimposed on an underlying broad spectral contour. This region may contain weakly expressed bands arising from aromatic ring vibrations of phenylalanine, tyrosine, tryptophane, and the various nucleotides. With the exception of only a few peaks (e.g., band near 720 cm^{-1}, resulting from the >CH_2 rocking modes of the fatty-acid chains), valid assignments can hardly be achieved. Therefore this region can be referred as "fingerprint region" [18]. Bands at 960 cm^{-1} and 830 cm^{-1} are not so intensive, but useful for conformational studies of carbohydrates. In order to identify carbohydrate in a mixture it is necessary to select the characteristic absorption bands.

In the region below 1500 cm^{-1}, most of the normal modes are highly coupled vibrations rather than characteristic group frequencies and is predicted to be a complex ring-mode in each case, nevertheless, the vibrational spectra of carbohydrates in this region are very similar [16].
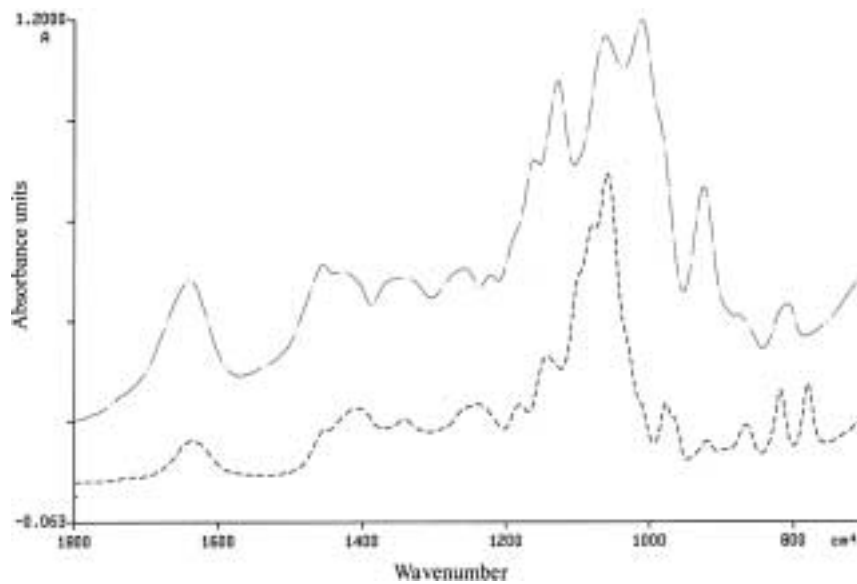
Fig. 1. IR absorption spectra of: fructose - - -, levan precipitate -·--·.

3.2. IR spectra of fructose and inulin

The most intensive in the IR spectra of fructose (Fig. 1) is a broad band with maximum at $\sim$1050 cm^{-1} and a shoulder at 1140 cm^{-1}. In 3000–2700 cm^{-1} region appears one broad band with maximum at 2930 cm^{-1} and shoulder at 2870 cm^{-1}. The most interesting are two sharp and of similar intensity lines in the "fingerprint region" at 835 and 770 cm^{-1}. These lines could serve as characteristic in studies of mixtures.

RAFTILINE (inulin) contains 6.1% of other carbohydrates (Table 1). In the IR spectra of inulin (Fig. 2) the most intensive is a broad band with maximum at $\sim$1050 cm^{-1} with two shoulders at 940 and 1130 cm^{-1}. Broad band with low intensity arises at 1640 cm^{-1}, but can't be used as specific for inulin in biological mixture, as usually in this region is a strong absorption of amides. Like in all carbohydrates two overlapped bands are at 2930 cm^{-1} and 2870 cm^{-1}.

3.3. IR spectra of fructan syrup and levan

Fructan syrup was obtained from *Z. mobilis* sucrose fermentation by "extracellular-levansucrase" sediment as biocatalyst. Fructan syrup differs from the known FOS products by levan additive (Table 1).

The FT-IR spectra of fructan syrup (Fig. 3) showed very intensive band with maximum at $\sim$1050 cm^{-1} and small, but separate one at 940 cm^{-1} that could indicate glucose. Absorbance band in 2800–3000 cm^{-1} is similar as in the spectra of inulin and fructose.

The chemical composition of levan precipitate was studied by chromatography and the results are showed in Table 1. It must be mentioned that levan precipitate from *Z. mobilis* contains some protein admixture – enzyme-levansucrase, in an active complex with levan in small amount [20].

Comparison of levan precipitate and fructose spectra (Fig. 1) proved fructose as the dominating component, but the shape changes of the broad carbohydrate absorption band with maximum at 1080 cm^{-1}, testifies the presence of other carbohydrates and in general fructose polymer form. The main differences

Table 1

Chemical composition of RAFTILINE, levan precipitate, fructan syrup

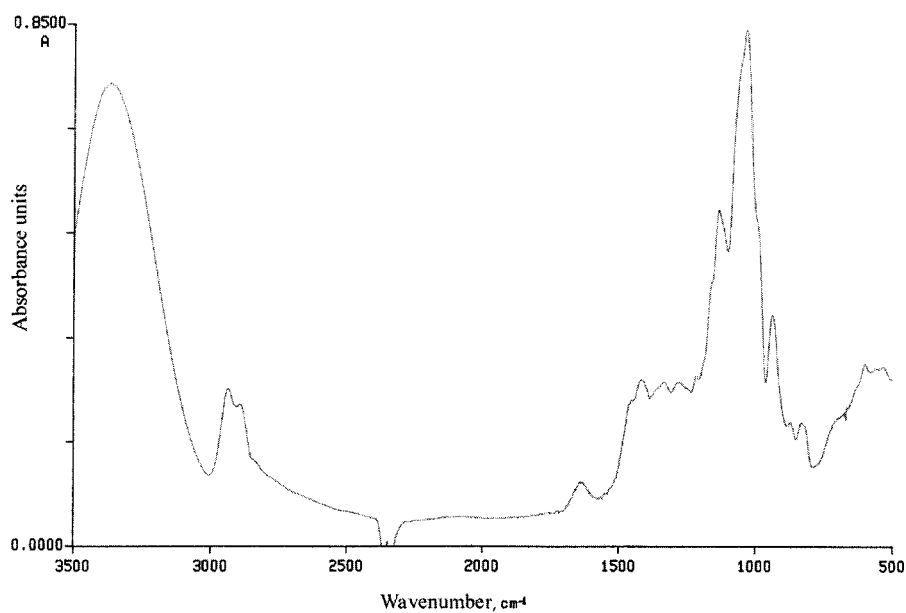| Component	Concentration, %
RAFTILINE ST	
Inulin	93.9
Glucose + fructose	1.1
Sucrose	5.0
Levan precipitate	
Fructose	93.0
Glucose	3.0
Mannan	2.0
Fructan syrup	
Carbohydrates, total	65.0
FOS	27.0
Levan	7.0
Sucrose	6.0
Glucose + fructose	22.0
Water	35.0
The energetic value of 100 g fructan syrup	186 kcal or 776 kJ

Fig. 2. IR absorption spectra of RAFTILINE.

in fructose and levan precipitate spectra appeared in 800–600 cm^{-1} region that indicates the presence of other carbohydrates and amino acids.

In the spectra of levan fructose does not show two separate sharp lines at 835 and 770 cm^{-1}, but in this region appears one broad band at 810 cm^{-1} that could result from overlapping of fructose, glucose and mannan lines [11,16].
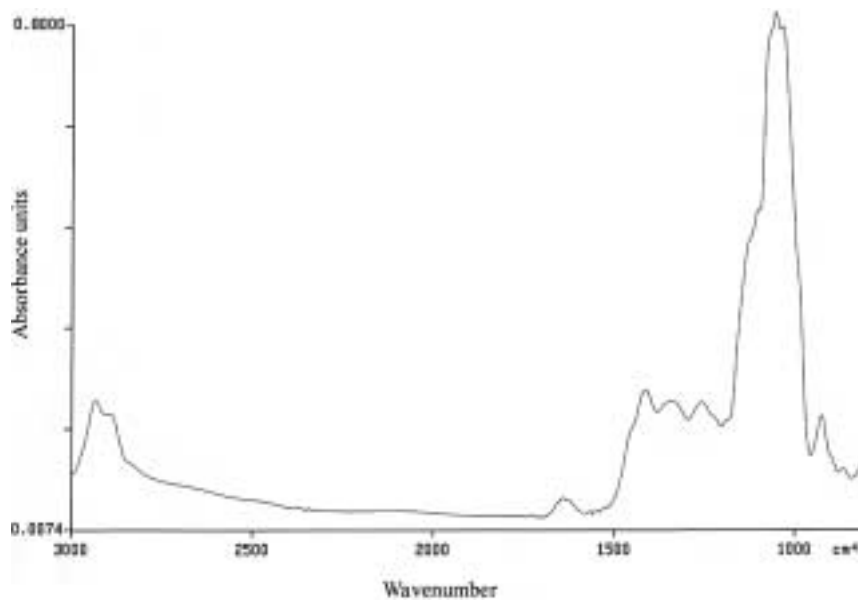
Fig. 3. IR absorption spectra of fructan syrup.

Levan precipitate IR-absorption spectra, showed an increase of broad absorption band at 1600 cm^{-1}. This was interpreted as degradation of proteins, accompanied by an increase of absorption in 1600 cm^{-1} area caused by free amino acids, simultaneously causing decrease in the intensity of "ammonium" band in 1410 cm^{-1} area and changing its shape [16]. A characteristic weak feature is often observed around 1400 cm^{-1}, which may be attributed to the symmetric stretching vibrations of COO^{-} functional groups of amino acid side chains, free fatty acids, or other derivatives [18]. Often the precipitates of extracellular polysaccharides contain negligible concentrations of nucleic acids, proteins, and free amino acids (0.3–3.0%). For evaluation of the purity of our levan precipitate the spectra was studied to find out the presence of main biochemical components of the microbial cell. Previously we have shown that as the characteristic absorption bands could be used: 1080 cm^{-1} for carbohydrates, 1250 cm^{-1} for nucleic acids, 1550 cm^{-1} for proteins and 2930 cm^{-1} for lipids [12]. Assessment of levan precipitate spectra showed that levan precipitate did not contain lipids, nucleic acids, but absorption bands in the principal component characteristic band regions in fructose and levan precipitate spectra are of carbohydrate origin, proving that the preparation is free of bacterial cells or their fractions. This investigation proved that the applied levan precipitation method allows obtaining preparation mainly consisting of fructose, with small amounts of amino acids and some other bacterial carbohydrates.

4. Conclusions

In the FT-IR spectra of crystalline fructose the most interesting are two sharp lines of a similar intensity in the "fingerprint region" at 835 and 770 cm^{-1}. These lines could serve as characteristic in studies of mixtures with fructan or identification of fructans in microbial biomass.

Fructan polymer inulin consists principally of linear chains of fructosyl units linked by a β(2-1) bonds ended by a glycosyl unit. The links between the molecules are of a very special type: the β(2-1)-form (2), which makes these molecules indigestible for all higher animals [8] and possibly also bacteria. The

bacterial fructans of the levan type are high molecular weight polymers, i.e., they are composed of β-(2,6)-fructosyl-fructose linked molecules and side chains [17]. Bacteria can utilize levan apart from inulin. The FT-IR spectra of levan, apart from inulin's, in the carbohydrate region 900–1200 cm^{-1}, shows overlapping broad band with maximum at $\sim$1030 cm^{-1} and stronger absorption at $\sim$940 cm^{-1}. The differences in both spectra could be caused by the structure and influence of glucose, sucrose and mannan.

It was shown that levan precipitate and fructose syrup does not contain lipids and nucleic acids and the applied precipitation method allows obtaining a preparation mainly consisting of fructose. It was shown that levan precipitate consists of 93% of fructose and admixture of glucose, mannan and enzyme-levansucrase. This admixture does not influence the value of the product and applicability in the functional foods.

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