FT-IR SPECTROSCOPIC ANALYSIS OF FUNGAL CHITOSAN-GLUCAN COMPLEXES AND CHITOSAN CONTAINED FOOD SUPPLEMENTS

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Abstract

The aim of this study was FT-IR spectroscopic characterization of commercial chitosan lactates originated from mycelium *Aspergillus niger*, chitosan contained food supplements, chitin-glucan complexes from mycelium *A. niger* and mushrooms of genera *Pleurotus* and *Agaricus*. Samples of fungal polysaccharides and food supplements were compared with pure chitin and chitosan standards. The degrees of deacetylation (*DD*) of chitosan standards, commercial chitosan lactates and food supplements were obtained by 1D UV method. The samples were also analyzed by organic elemental analysis to obtain the N/C ratios. It was found that this ratio is indicative for the chitosan content in food supplements as well as for the *DD* values of pure chitosans and chitosan lactates in case of no organic nitrogen impurity. FT-IR spectra of the samples were interpreted and processed by cluster and PCA analysis. Specific IR marker bands were assigned to various forms of chitosan units (*N*-acetylated, free base and cationic). According to FT-IR spectra chitosans from mycelium and crab shells have the same structure. Some chitosan lactate samples, however, contain another polysaccharide component that could be β-glucan. In all chitosan lactates only part of non-acetylated amino groups is in cationic form bound to lactate, the others are in free base form. It was confirmed that all analyzed food supplements contained chitosan as the major polysaccharide component. The polysaccharide complexes isolated from fungi contained chitin in smaller quantities. The cluster and PCA analysis evaluated the structural differences between the samples and separated them into several subgroups dependently on *DD* and other structural specificities.

Introduction

Cell wall polysaccharides of fungi include β-glucans, chitin and mannans and many finding support the hypothesis that chitin is covalently linked with β-glucan [1, 2]. Chitin-chitosan polymers are the major constituents of shells of arthropods such as crabs, shrimps, lobster and insects and are also produced extracellularly by brown algae [3, 4]. A beneficial effect of chitin-chitosan as a food supplement is the reduction of plasma cholesterol and triacylglycerols due its ability to bind dietary lipids, thereby reducing intestinal lipid absorption [5]. (1→3)(1→6)-β-D-glucans, constituent of fungal and certain bacterial cell walls, belong to the naturally occurring agents with stimulating effects on the defence mechanism of the living organism [6].

The aim of this study was the structural characterization of fungal chitin-glucan complexes and chitosan lactates as well as chitosan containing food supplements. The structural differences between the samples were evaluated by statistic comparison with standards of mono- and polysaccharides.

Material and Methods

Sample preparation

Pulverized samples of dry mycelium of *Aspergillus niger* and food supplements was subsequently washed with acidic ethanol, ethanol and acetone and then dried. Samples of purified mycelium were deproteinised and then deacetylated respectively with 2 % and 50% NaOH. Products were neutralised by washing with distilled water, then washed with ethanol and acetone and dried. Isolation of polysaccharides from fruit bodies of genera *Agaricus* and *Pleurotus* was adapted according to Feimund et al. [7] (see Fig. 1).

Analytical methods

Elemental analyses were performed using PerkinElmer 2400 Series II CHNS/O Elemental Analyzer. The ration N/C was calculated on the basis of data of elemental analysis. Degrees of deacetylation (*DD*) of chitosan samples were estimated by 1st derivation of UV spectra [8] using UNICAM UV-Vis spectrometer UV4 (GB) in spectral range 190–200 nm and slit 2 nm. Sample solutions were measured in diluted acetic acid. The zero cross-point for different concentrations of acetic acid was obtained and the effect of GlcN was eliminated. The calibration was carried out with GlcNAc solutions. The content of β-glucan in mycelium and mushrooms was determined by Megazyme enzymatic set K-YBGL 10/2005. Carbohydrates were released from the isolated polysaccharides by Saeman hydrolysis [9] and analyzed as their alditol acetates by gas chromatography [10, 11] using a Hewlett-Packard 5890 gas chromatograph with DB-225 capillary column (length 30 m, internal diameter 0.25 mm, film thickness 0.15 μm) and FID detector.

FT-IR spectroscopy

FT-IR spectra (4000–400 cm^{-1}) of the samples were measured by Brucker IFS-55 FT-IR spectrometer equipped with Golden Gate single reflection diamond ATR system. The spectra were recorded at the absorbance mode from (mid infrared region). Five replicate spectra (128 co-added scans) were collected for each sample at the resolution of 8 cm^{-1}.

Multivariant analysis

Obtained FT-IR spectra were transferred via a JCAMP.DX format into the data analysis software package for PCA and each spectrum, within the 1200–800 cm^{-1} region, was auto-scaled (centred and divided by the standard deviation). The spectra were also compared using the cluster analysis (Statistica7, Statsoft CZ) on single windows 1720–1220 cm^{-1}. Spectra were baseline corrected and normalized at 1026 cm^{-1} (CC,CO vibration of polysaccharide).

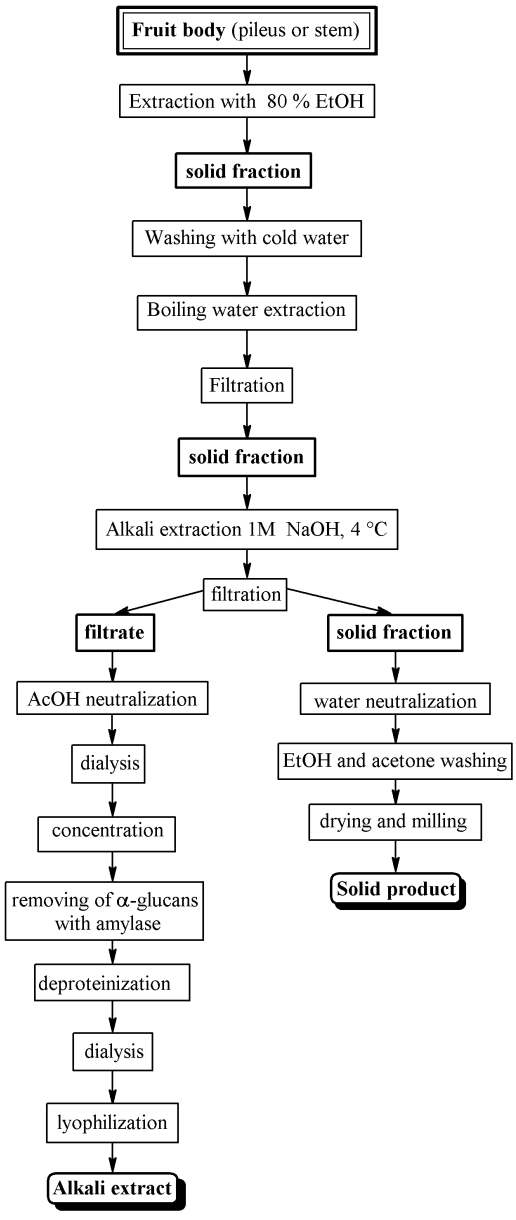


Figure 1 : Scheme of isolation of alkali soluble and insoluble polysaccharide fractions from mushroom fruit bodies

Results and Discussion

Elemental analysis

The value of N/C calculated on the basis of the elemental analysis data can be applied in the structural characterization of polysaccharide standards, chitosan lactates and food supplements.

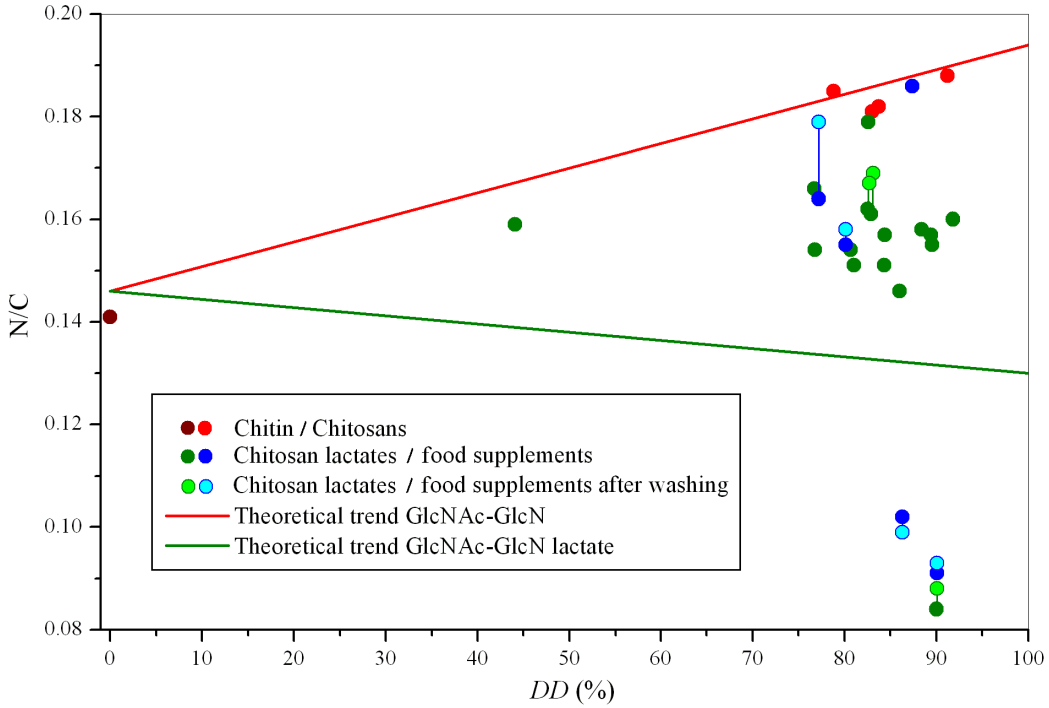


Figure 2 : The correlation between ratio N/C and the degree of deacetylation DD of chitin→chitosan.

The samples of chitosan lactate and food supplements are placed between the theoretical trends GlcNAc→GlcN and GlcNAc→GlcN lactate according to their composition (Fig. 2). Obtained N/C values ranged from 0.13 (chitosan lactate) to 0.194 (chitosan NH_2). It was found that this ratio is indicative for the chitosan content in food supplements as well as for the *DD* values of pure chitosans and chitosan lactates in case of no organic nitrogen impurity. The large deviation of the N/C values of chitosan lactate from the GlcNAc→GlcN lactate line could be explained by partial conversion of GlcN units to free base form. Several has relatively small nitrogen content (N/C <0.11) that may be caused by the presence of other polysaccharides (β-glucans, cellulose).

FT-IR spectra

FT-IR spectra of chitosan lactates are shown in Fig. 3a. The spectral region 1800–1500 cm^{-1} is sensitive to the form of amino groups: 1660 [amide I], 1630 [$\delta_{as}(NH_3^+)$], 1584–1587 [$\delta(NH_2)$], 1550 (amide II) and 1530 cm^{-1} [$\delta_s(NH_3^+)$]. The bands at ~1644 and ~1566 cm^{-1} were assigned respectively to δ (H_2O) and $\nu_{as}(COO^-)$ of lactate. In spite of complicated analysis the evaluation of the prevailing form of chitosan (free base NH_2, cationic NH_3^+ and *N*-acetamide -$NHCOCH_3$) based on 2nd derivation of spectra is successful (Fig. 3b). Spectral differences between the chitosan lactates reflect the presence of free base, cationic and *N*-acetamide forms of monosaccharide units at various relationships.

Statistic processing of spectral data

The distribution of the samples in the PC1×PC3 axes was done according to their composition in GlcNAc (PC1 negative), GlcN (PC1 positive) and Glc (PC3 positive) (Fig. 4a, b). It is evident from the PCA scores scatter plot (Fig. 4b) that the most of chitosan lactates showed spectral difference from chitosan standards due to the presence of lactate. Exceptions were chitosan lactates 1, 2 (free base form, 2 is highly acetylated) and 12 (presence of ß-glucans). The food supplements are observed according to their composition. Food supplement 1 is carboxylic salt of chitosan that corresponds with shifting into PC1 positive and PC3 negative; food supplement 2 contained dietary fibres

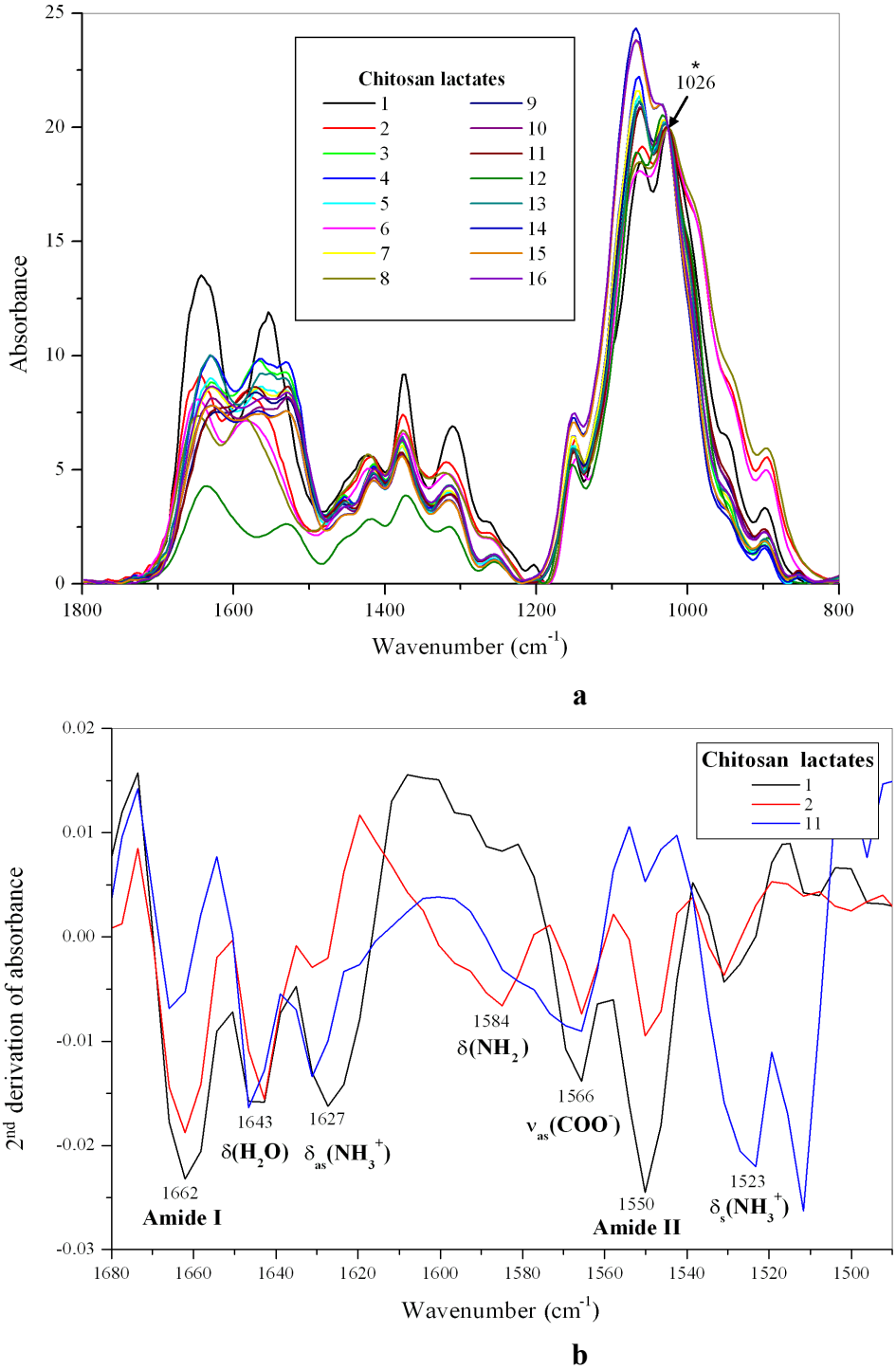
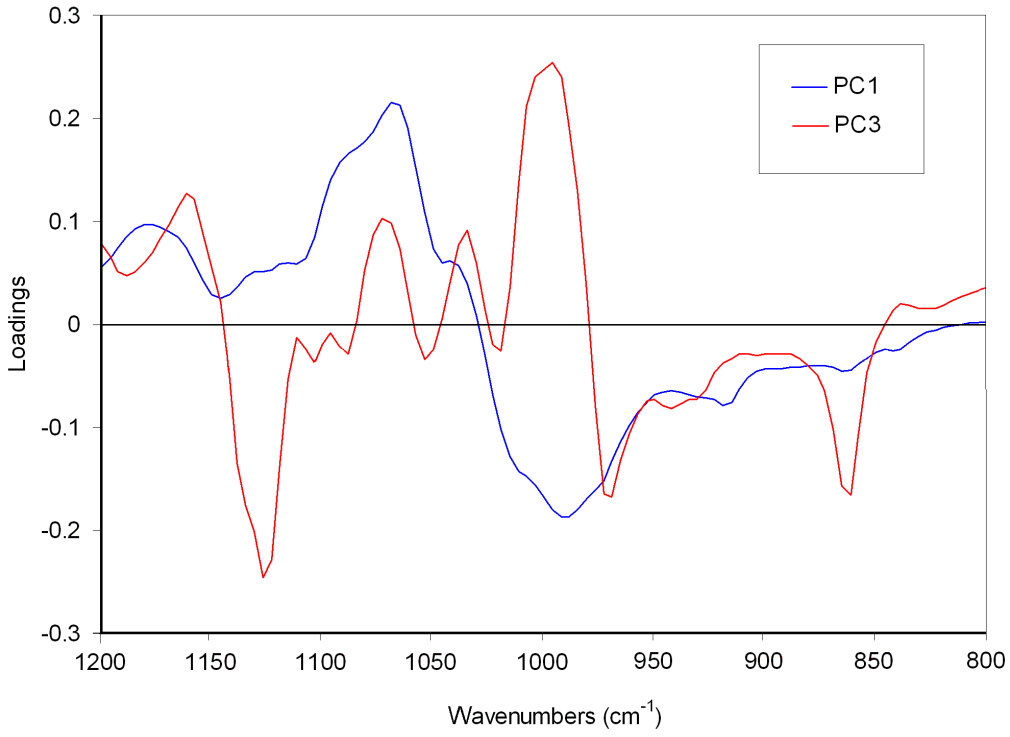


Figure 3 : FT-IR spectra of chitosan lactates (a) and 2nd derivation of FT-IR spectra of chitosan lactates 1, 2 and 11 in the region of 1680–1490 cm^{-1} (b)

(PC1 negative, PC3 positive – like ß-glucan and cellulose); food supplements 3, 4 and 5 contained chitosan as major component (PC1 and PC3 negative – like chitosan standards). Lyophilised alkali extracts from mushroom fruit bodies rather contain ß-glucan than chitin or chitosan (PC1 negative, PC3 positive). By contrast, the solid products definitely contained chitin and they are in PC1/PC3 positive region like D-GlcN. Cluster analysis of the spectra of chitin, chitosans, chitosan lactates and food supplements is illustrated in Fig. 5. Spectra of most chitosan lactates were incorporated into an own cluster. An exception was chitosan lactate 1, 2 (cluster of chitin, chitosan standards food supplements 3–5) and 12, which, together with food supplements 1 and 2, did not below to any



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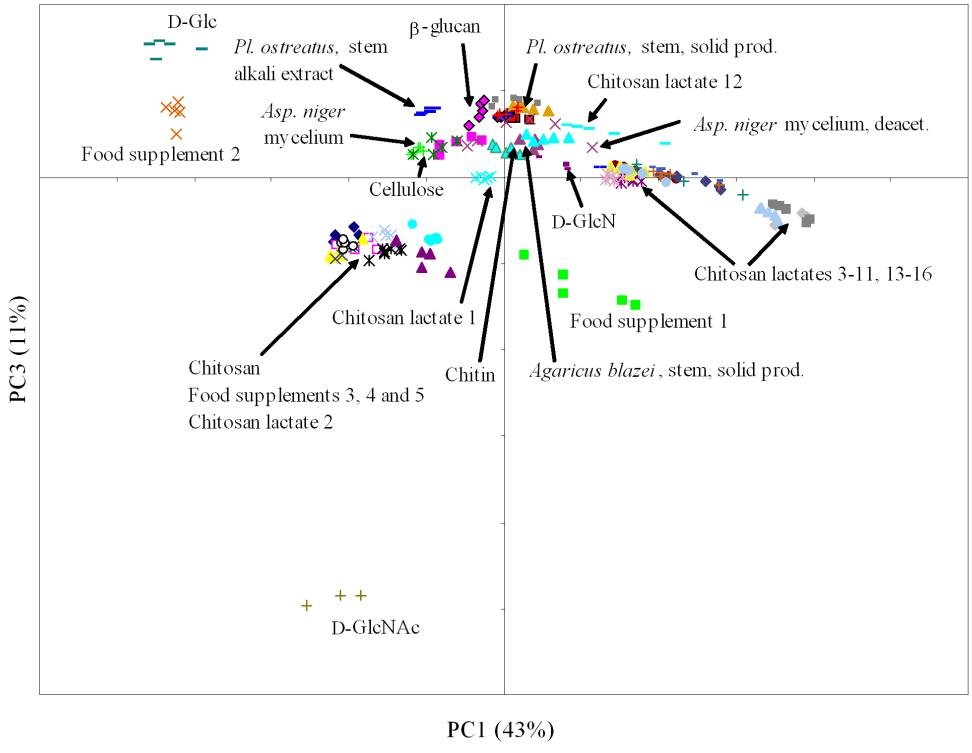

b

Figure 4 : PCA of FT-IR spectra in the wavenumber region of 1200–800 cm^{-1}: (a) loading plot, (b) Scores scatter plot (PC1 x PC3 - axes cross each other at the origin)

cluster. Obtained results of PCA and cluster analysis agree with the data of elemental analysis and interpretation of FT-IR spectra.

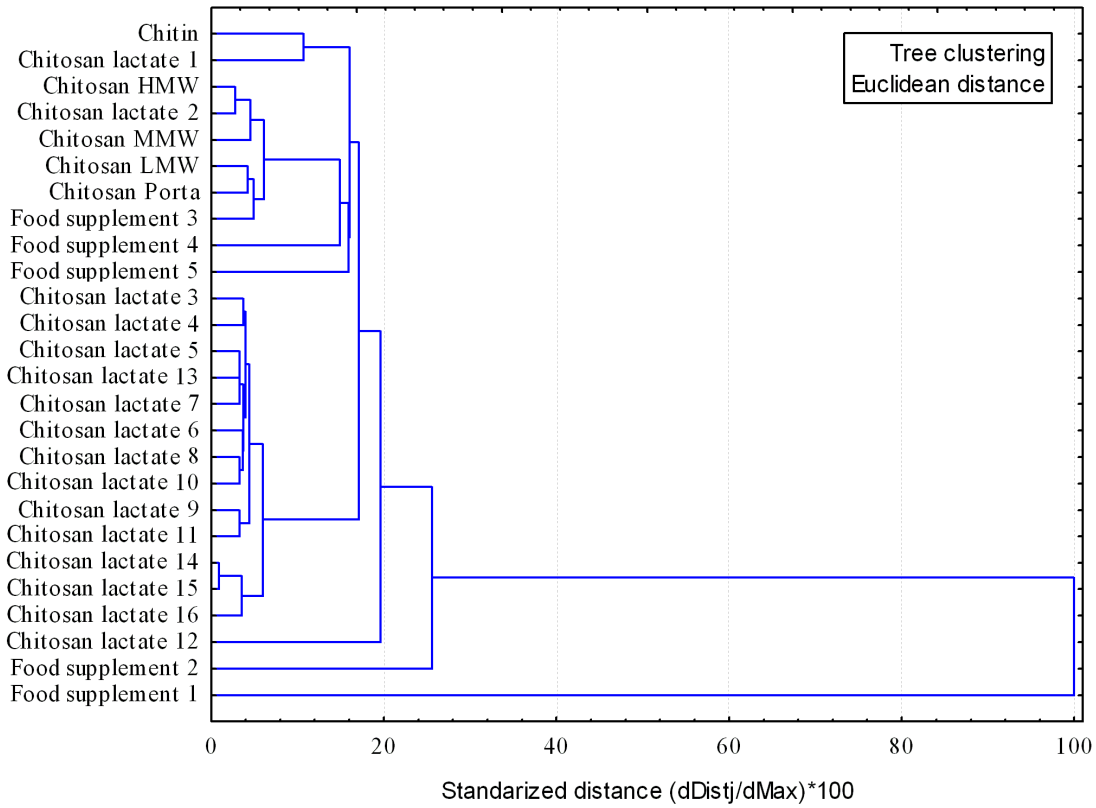

Figure 5 : Cluster analysis of FT-IR spectra in region 1720-1220 cm^{-1}, ATR

Acknowledgements

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References

[1] A. Amnuaykanjanasin, L. Epstein, J. M. Labavitch, Physiol Molecular Plant Pathol, 63 (2003) 107-115.

[2] J. Wu, P. C. K. Cheung, K. Wong N. Huang, Food Chem, 85 (2004) 101-105.

[3] I. Furda, Amer Chem Soc Symp, 214 (1983) 105-122.

[4] R. Kawasaki, M. Tanaka, M. Fujie, S. Usami, K. Sakai, T. Yamada, Virology, 302 (2002) 123-131.

[5] S. S. Koide, Nutr Res, 18 (1998) 1091-1101.

[6] D. Slameňová, J. Lábaj, L. Križková, G. Kogan, J. Šandula, N. Bresgen, P. Eckl, Cancer Letters, 198 (2003) 153-160.

[7] S. Freimund, M Sauter, O Kappeli, H. Dutler, Carbohydr Polym 54 (2003) 159-171.

[8] S. C. Tan, T. K. Tan, S. M. Wong S, E. Khor, Carbohydr Polym 30 (1996) 239-242.

[9] R. R. Selvendran, J. F. March, S. G. Ring, Analyt Biochem 96 (1979) 282-292.

[10] A. B. Blakeney, P. J. Harris, R. J. Henry, B. A. Stone, Carbohydr Res, 113 (1983) 291-299.

[11] P. J. Harris, A. B. Blakeney, R. J. Henry, B.A.Stone, J. Assoc. Anal Chem, 71 (1988) 272-275.