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PHYSICOCHEMICAL PROPERTIES OF FRUCTAN INULIN PRODUCED FROM *ACETOBACTER* SPECIES

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ABSTRACT

Physicochemical properties of fructan inulin produced from *Acetobacter* species and natural inulin were investigated. From cryscopic method, the molecular weights have been found 5010 $gmol^{-1}$. for bacterial inulin and 4700 $gmol^{-1}$. for natural inulin. The determination of branches for both kinds of inulin indicates that both polymers are beta-(2→1) linked polyfructan with 6.0% and 4.4% beta (2→6) branches for bacterial inulin and natural one, respectively. Moreover, the spin-lattice relaxation times (T_1) for bacterial inulin was lower than that of natural one. This result indicates a great order of protons in bacterial inulin.

Keywords: Fructan inulin, *Acetobacter* species, physicho-chemical properties.

INTRODUCTION

Fructans are oligomeric or polymeric carbohydrates that are synthesized from sucrose and consist of a fructose chain, which may contain a terminal glucose molecule. Fructan synthesis is widespread among bacteria, occurring in gram-positive as well as gram-negative families and has been demonstrated for some fungal species (Hendry *et al.,* 1993). Fructan synthesized by bacteria as a component of the exopolysaccharide are high molar mass polymer, which are in almost all cases of the levan type, characterized by the beta-2→6- linkage type of fructose monomers (Cote et al, 1993). The only bacterial species known so far that produces an inulin-type fructan consisting of beta-(2→1) linked fructose molecules and 5% beta (2→6) linked branches is *Streptocococcus mutans* (Loesche, 1986). Recently, fructan inulin was extracted from *Acetobacter* species with high purity and crystalinity (Keshk, 2003). Inulin is a polysaccharide contained in chicory, dahlia and other plants. The chemical structure of inulin is a beta-2→1 linked fructose polymer terminated with sucrose residue (Capita *et al.,* 1989). Inulin is the reserve carbohydrate present in the roots and tubers of many composites and other plat families, and which by a single enzyme –inulinase can be hydrolysed to fructose and glucose, its components. Inulin and inulin oligomers have a growing interest in the food and petfood industries (Gupta *et al.,* 1993). Since the polydispersity and degree of polymerization of inulin depend on the species, the harvest periods and preparation methods. In order to know the difference between the bacterial inulin and natural one, the inulin produced from *Acetobacter* species and inulin from chicory were investigated using physicochemical studies and ^{1}H-NMR pulse relaxation time (T_1 value).

MATERIAL AND METHODS

Chicory inulin is sigma product. The other chemicals used throughout this work were purchased from Sigma and Aldrich Chemical Co.

Culture medium

The *Acetobacter* species was submerged grown on sterile synthetic medium containing (g/l): Sucrose 7.5; Peptone 10.0; beef extract 3.0; Yeast extract 3.0 and sodium chloride 5.0 and was incubated at 37°C for 14 days.

Inulin Extraction and Purification

The fermentation culture was centrifuged (6000 rpm at 4°C) for 20 min to remove cells. Acetone was added (1:2) to a clear solution. The precipitate is washed by ethanol then petroleum ether. Then the pure precipitate is kept under vacuum till complete dryness.

Molecular Weight Measurements

Both bacterial inulin and natural inulin were

dried in a vacuum oven at 60°C over silica gel for 24 h. The water used for solution studied was HPLC grade. Solutions were made up by weight and sound velocity and density measurements were carried out at 20°C. Cryoscopic measurements were determined using an advanced milk cryoscope 4L2 from Advanced Instruments Inc. Molecular weight were calculated from cryoscopic values using the following equation:

$$M = 1860c / T(100-c)$$

Where M is the molecular weight of the solute (g mol^{-1}), 1860 is the factor used for milk freezing point, c is the concentration of the solution (g/ $100ml^{-1}$) and T is the depression of freezing point.

Determination of Branching

i. Methylation analysis of inulin samples:

Premethylation was carried out according to the method of Ciucanu and Kerek (1984). Inulin (24.6 mg, corresponding to 0.456 mmol OH) was added. After 20 min.stirring at room temperature MeI (100µl, 1.62 mmol, 3.5 equi. /OH) was added and the suspension was stirred overnight at room temperature. The reaction mixture was poured into water and extracted with dichloromethane two times. The organic extracts were washed with water four times and then dried over calcium chloride. After evaporation of the solvent, the methylated inulin was obtained as a colorless clear film. Portions of about 2mg of the permethylated inulin were submitted to acid hydrolysis with 0.5 M trifluoroacetic acid at 100°C for 1hr. after careful evaporation of the acid and co distillation with toluene to remove the trace of acid. The reduction was performed with sodium borohydrid-d_4 in 0.5 M ammonia (0.5M, 0.5 ml) for 1hr at 60°C. Excess of the reagent was destroyed with acetic acid, and then the borate was removed by evaporation with methanol/acetic acid for five times. The residue was acetylated with acetic anhydride (150µl) and pyridine (25ml) at 90°C for 2.5 hr. The acetic anhydride was destroyed with $NaHCO_3$ solution, the products extracted with dichloromethane and thoroughly washed with aq. $NaHCO_3$ and subsequently with water. The dried fraction phase was used for gas-liquid chromatography (GLC) and gas-liquid chromatography-mass-spectrometry (GLC-MS) analysis.

ii. Reductive Cleavage

It was performed according to Rolf and Gray (1984). The permethylated inulin (2.0mg) was dissolved in dichloromethane (0.025 mmol solution). Triethylsilane (14.1µl) and $Me_3SiOSO_2CF_3$ (7.8ml) were added. To the reaction mixture acetic anhydride (15µl) was added after 2.5 hr at room temperature. After further 2hr the reaction was quenched with $NaHCO_3$ solution. The organic phase was washed with aq. $NaHCO_3$ and water, dried and used for GLC and GLC-MS analysis.

Spain-Lattice Relaxation Times

Spain lattice relaxation time (T_1 value) of the protons were measured using a Bruker PC 120 NMR process analyzer, a low-resolution pulsed nuclear magnetic resonance spectrometer operating at a resonance frequency of 20 MHz and at temperature of 36°C.

RESULTS AND DISCUSSION

From molecular weight determination, bacterial inulin has higher molecular weigh (5010) than that of natural inulin (4700); this data can be related to the higher degree of polymerization of bacterial inulin. From methylation analysis and reductive cleavage, it is evident that both bacterial inulin and natural one are 2→1 linked fructan with about 6% and 4.4% of 2→1,6 branched residue for bacterial inulin and natural inulin respectively. After methylation analysis, the corresponding partially

Table - 1: Change in H-NMR spin lattice relaxation times of both kinds of inulin with different concentrations (g/100g of water)

	Spin lattice relaxation times T_1 (s)					
Concentration	3	6	10	14	18	20
Natural inulin	3.50	2.62	2.30	2.00	-	-
Bacterial inulin	3.15	2.25	1.99	1.00	-	-

Concentration (g of inulin in 100 g of water)

methylated and acetylated mannitol and glucitol derivatives were identified by GLC and GLC-MS. In addition, small amounts of presumably undermethylated components were also detected. For reductive cleavage, slight demethylation cannot be excluded. Reductive cleavage gave the expected 2,5-anhydroglucitol and –mannitol derivatives. These results matches with the reported results of Loesche (1986).

^{1}H-NMR pulse relaxation studies provide another method of analyzing the state of order of the inulin fraction of the solution protons. Spin-lattice relaxation is a process, which allows nuclear spins to return to equilibrium following some disturbance of Proton Magnetic Resonance. As the nuclei approach equilibrium, the energy released is dissipated in the lattice. Bacterial inulin has shorter relaxation time than that of natural inulin. Shorter relaxation times mean greater degree of order, probably due to greater hydrogen-bond energy, creating hydration sites and loss of motional freedom. Table -1 lists the difference between the spin-lattice relaxation times (T_1 values) of bacterial inulin and natural one. From Table -1, the increase in the concentration of solute entails decease in T_1 was observed, it may be due to the order order solute protons constitute an increasing proportion of the total protons. The T-values represent an average of all protons. In view of the concentrations of the two kinds of inulin, most protons are water protons. Therefore, the T-values mostly reflect average mobility of water protons and they change with concentration as well as nature of inulin.

Conclusion

Bacterial inulin has higher molecular weight than that of natural inulin indicating that bacterial inulin has higher degree of polymerization than that of natural inulin. Moreover the number of branching of bacterial inulin is higher than that of natural one. On the other hand the relaxation time of bacterial inulin solution is lower than that of natural one due to bacterial inulin are arranged in order form, which give highly crystalinity than that of natural inulin as reported in our previous paper.

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