

Rapid Method Analysis for Quantification of Novel Oligosaccharides by Thin Layer Chromatography

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Abstract:

A novel oligosaccharide containing cashew fruit crush fermentation medium v separated by thin-layer natural action (TLC) technique. The magnitude of oligo sugar was measured employing a spectrophotometer technique. The results showed that the weight unit of trisaccharide content was comparable to the gr of the samples. The total saccharide content varied among the cashew fruit crush fermentative medium varieties; The SII-6 strains (228 ±0.01mg/ contain a lot of oligosaccharide content as compared to SII-16 strains and SI- strains.

Keywords: cashew apple juice, oligosaccharide, raffinose, TLC.

1. INTRODUCTION

Oligosaccharides are comparatively new useful food elements with the nice potential to enhance the standard of the many foods. Not solely are they useful changes within the style and Physic-chemical properties of food, however, they even have properties useful to the health of customers. The assembly and use of organic process oligosaccharides are increasing and their main uses are in bread, beverages, baby milk, yogurt, confectionery, and milk desserts.

Oligosaccharides of various varieties are found as innate elements in many acquainted foods together with fruits, vegetables, milk, and honey. The main claim created for these merchandises is that they're 'foods designed to assist maintain an honest channel setting and increase enteric bifidobacteria'. Together with fiber, oligosaccharides are the foremost in style ingredient employed in healthy foods [4].

Oligosaccharides are usually outlined as an occasional super molecule level of a chemical process (DP) and low relative molecular mass.

Therefore The notion of indigestible oligosaccharides is derived from observing that the carbon anatomic atoms (C 1 or C 2) of the oligosaccharide monosaccharide units some polysaccharides are set. Cashew tree grows in a fairly large area and is grown in 32 countries worldwide. India, Brazil, Nigeria, and Vietnam are the main producers of pulp.

The cultivation of cashew haywire in Brazil Associate in Nursing in several Asian countries is an agricultural activity aimed at the main at the assembly of around the bend, which represents only 100% of the burden of the fruit. Large quantities of cashew apple units of measurements discarded inside the sphere once the haywire space unit removed. Consistent with official information, Brazil's Northeast has Associate in Nursing annual production of regarding a pair of million tonnes of cashew apples and ninetieth of this production is underutilized. As such, apple cashew is taken into account a remnant of agriculture and its nutrient juice could also be an acceptable low-cost substrate for microbic growth.

The cashew apple is made in vitamins, minerals, reducing sugars (fructose and glucose), and a few

amino acids [18,14, 1]. Though cashew potable may be consumed within the type of juice, ice cream, and different foods, farming of cashew is associate in nursing agricultural activity aimed chiefly at manufacturing haywire. The utilization of potable is attention-grabbing as a result of it's an inexpensive supply of carbohydrates and minerals.

Cashew juice contains 219 mg of water-soluble vitamin per one hundred cubic centimeter serving, which is twelve times quite pineapple and five times quite orange fruit. This juice additionally contains a lot of atomic number 12 (260 mg) than oranges, bananas or pineapples. The metals content (565 mg) is double that of orange, four times that of mango and five times that of pineapple [19].

Many studies have reportable the purposeful properties of cashew drink, like anticarcinogen[9], and medicament action against redness that causes bacterium known as *Helicobacter pylori* [10,2] and additionally its inhibitor properties [8]. With the problems mentioned on top of associated with cashew fruit unfold and widespread use of some organic acids and oligosaccharides, this analysis aims to deal with the employment of cashew fruits as a substrate for the assembly of oligosaccharides exploitation autochthonous microorganisms

2. MATERIALS AND METHODS

2.1. Sample collection

Cashew apples were collected from four different places in Cuddalore District (Tamil Nadu, India). The cashew apple samples were taken to a laboratory where the apples were separated from the nuts. Apples were carefully washed with clean water. The apples were then sliced and ground using a grinder in a blender. The juices obtained by pressing the porridge were filtered through a 0.5 mm sieve. It was then stored and frozen at -80°C for analyses. Cashew fruit crush contains a high level of phenol that was removed with the employment of processed gelatin.

2.2. Isolation and cultivation of oligosaccharide-producing microorganism strains.

To isolate a microorganism culture that produces novel oligosaccharides, samples of raw cashew juice like yellow and red varieties were collected. The cashew juice was serially diluted and placed on a medium plate. Morphologically

apparent colonies were transferred to the recent medium and mature at 37°C for twenty-four h. associate degree overall of seventy-three strains were differentiated singly and keep as frozen stock stains at -20°C within the nutrient broth medium. All microorganism strains were on an individual basis cultivated within the nutrient broth medium for 2-3 days at 37°C

2.3. Oligosaccharides production

A strain of SI-10 (*Bacillus safensis*), SII-6 (*Bacillus paralicheniformis*), SII-16 (*Bacillus subtilis* subsp. *inaquosorum*) was optimized synthetic medium composed of sucrose 50 g/L (food grade), yeast extract 20 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 20g/L, $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ 0.01g/L; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01g/L; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.02g/L; NaCl 0.01 g/L and K_2HPO_4 20 g/L. Then the medium was sterilized at 121°C for fifteen min in an autoclave. The oligosaccharides manufacturing native strains were inoculated with the artificial medium. The medium incubated at 30°C in a very orbital shaker at one hundred fifty revolutions per minute for twelve hrs in a rotary shaker [10].

2.4. Production of oligosaccharides in cashew fruit crush (CAJ) medium:

Regarding 500ml of the sterilized CAJ sample red and yellow were taken in separate one thousand milliliter flask. The media was ready by diluted processed cashew fruit crush 25g/L to adding disaccharide 25g/L, yeast extract 2g/L; ammonia sulfate 2g/L and K_2HPO_4 10g/L. The preliminary pH was adjusted to 6.5 and also the substance was sterilized in an autoclave for fifteen min at 121° C. fifty milliliters (10%) of this cell culture was used as a matter for cashew fruit crush fermentation medium. The fermentation medium was maintained in a very rotary shaker for twenty-four h at 30°C and one hundred fifty revolutions per minute. Cells were then removed by natural action at 7000 revolutions per minute for fifteen min. The supernatant of the fermentation medium was additionally analyzed for the presence of oligosaccharides then the media was kept in a refrigerator.

2.5. Estimation of reducing sugar

The quantifying the initial rate of the discharge of reducing the sugar by exploitation the DNS technique [13]. The crude soured broth contains 1ml was created up to 3ml with H_2O . After adding 2ml of the DNS chemical agent, it had been

heated at 80°C for fifteen min during a boiling water tub. When cooling, the reducing sugars were liberated and it had been measured spectrophotometrically at 580 nm and expressed as an aldohexose equivalent. aldohexose was taken as a regular.

2.6. *Oligosaccharides Detection*

The oligosaccharides obtained throughout fermentation of cashew fruit juice were known and characterized by thin-layer natural action on silicon dioxide plates of 250 µm thickness. Samples of ten µL were absorbed onto the plates on a line regarding one.5 cm higher than the lower plate edge. When drying with a hairdryer, the plate was irrigated for 2 ascents during a solvent mixture composed of n- butanol: acetone: liquid (50:40:10 v/v). To render sugars visible, plates were sprayed to saturation with an answer containing zero.3 g/100 ml of N* (1-naphthyl) ethylamine dihydrochloride during a solvent system composed of methanol: vitriol (95:5 v/v) [14]. Raffinose was used as a reference normal. The plates were then heated in an oven at 120° C till spots were visible (approximately 8-10 min)[20].

2.7. *Estimation of oligosaccharides*

The amount of 20 µl of the concentrated sugar extract was applied on an Eastman Chromagram sheet developed by ascending chromatography using a mixture of n-butanol:acetone:water (5:4:1 v/v). The modified 5ml of H₂SO₄ and 45ml of methanol were mixed together and used assay reagent. The guide-strip technique was accustomed to verify the amounts of a trisaccharide. The sugar stain within the recording was removed and also the sugar was extracted with two millimeter H₂O in an exceedingly tubing long at temperature. One millimeter of the sample was mixed with one milliliter of 0.02 M thiobarbituric acid and one milliliter of targeted acid. The mixture is heated in an exceedingly boiling water bathtub for six minutes and then cooled in running water [17]. The yellow color made was browse at 440nm in an exceeding spectrophotometer. The concentration of sugar was measured from working standards, ranging from 100 mg of sugar per g. III.

3. **RESULT AND DISCUSSION**

In the present study investigated novel microorganism strains from cashew juices made new saccharide victimization sucrose because of the sole supply of carbon. Several microorganism

strains were isolated from cashew fruit crush. The sucrose linear impact was positive on oligosaccharides yield and negative on residual sugars. The rise in sucrose concentration improved oligosaccharide formation.

The utilization of a part sublimate catalyst permits the elongation of the saccharide chain [16]. The strains (SII-16, SII-6, and SI-10) were selected within the initial screening victimization TLC analysis as a result of they made novel spots expecting a brand new saccharide throughout twenty-four hours incubation within the medium (figure-1).

The results show that SII-6 strain from spot showed the presence of 2 isomers, R_f 0.29 and 0.44 and SII-16 strains from spots showed the presence of R_f zero.29 and 0.50, SI-10 strain from spots was showed 2 isomers of R_f zero.29 and 0.56 severally. R_f worth of 0.27, a price clone of that for saccharose, the saccharide of the medium. These values and therefore the color of the spots once treatment with amine diphenylamine indicated that they were in all probability aldohexose (R_f lactose.39) and disaccharide (R_f 0.40). Neither acid nor disaccharidase treatment modified the movement of those materials intending; however, treatment with aldohexose enzyme yielded solely fruit sugar.

When amounts of sugars commonly used paper was on our TLC plates, it was impossible to obtain separations. Because such small sugars used in these TLC experiments, some of the common paper chromatography sprays were not sensitive enough to locate the individual components[12]. Carbohydrate dispersion and filtration are significantly necessary once exploitation carbon sources for fermentation operation as a result of in terms of flows it's doable to get knowledge associated with production and production.

On the opposite hand, particularly once carbohydrates are originally used, though they're purchased commercially, their synthesis is usually reportable as a share and exhibits high levels of heterogeneousness from one sample to a different. For this reason, the precise identification of the composite components will be of nice importance, considering that the particles have specific growth rates that supported the chemical characteristics of the carbon supply, like relative molecular mass or chemical complexness.

Even once the separation has been improved, distinguishing the best sites or definitions also because of the chance of communication between the highest sites and also the chemical structure of the elements ought to be thought-about. the dearth of normal ingredients in GOS, XOS, FOS over DP5, and polysaccharide stressed the necessity for various approaches to the event of the tactic.

Several studies on molecular characterization are dispensed within the past [6,7] and have unconcealed an instantaneous link between chemical structure and retention (Martin's equilibrium relationship) [21].The chemical structure of any given style of GOS, XOS and FOS/inulin will be taken as an easy chain of chemical compound (galactose, xylose, and fructose) connected to a aldohexose cell (except XOS, wherever the top is carbohydrate also) and relative to the moving distance (MD) measured in millimeters.

The moving distance represents the space between the tip and therefore the starting (placing) of the moving purpose on the plates. The reducing sugar content was drastically reduced when fermentation with inoculated isolates. The most reducing sugar was detected in CAJR-P selection mistreatment the autochthonous isolates *Bacillus paralicheniformis* (SII-6) was 0.242 ± 0.01 mg/ml. Then this was followed by cashew fruit crush *Bacilli subtilis* subsp. *inaquosorum* SII-16 (0.178 ± 0.02 mg/ml), *Bacillus safensis* (0.223 ± 0.01 mg/ml) severally (table-1). Therefore, they were monitored for the assembly of a unique sugar. When every strain was separately incubated with the basal medium containing the culture supernatants were noticed and developed on tender loving care plates (table-1).



Figure -1: Detection of TLC plate

Table -1: Estimation of reducing sugar content in fermented cashew apple juice

S.No	Isolate	Reducing sugar(mg/ml)
1	SII-6	0.242 ± 0.01
2	SII-16	0.178 ± 0.02
3	SI-10	0.223 ± 0.01

SII- Cashew apple juice red variety; SI- Cashew apple juice yellow variety

This result shows that the sugar spots developed in the TLC plates were scraped off and the oligosaccharide content of fermented broth was calculated as milligram equivalent to raffinose content. The maximum oligosaccharides production was noticed in CAJR-P variety using the indigenous isolates *Bacillus paralicheniformis* (SII-6) was 228 ± 0.01 mg/g. Then this was followed by *Bacillus subtilis* subsp. *inaquosorum* SII-16 (151 ± 0.01 mg/g), and *Bacillus safensis* SI-10 (155 ± 0.01 mg/g) (table-2).

Table-2: Oligosaccharides content presented in the fermented cashew apple juice supplemented medium

S.No	Isolates	mg Raffinose equivalent/g of sample
1	SII-6	228 ± 0.01
2	SII-16	151 ± 0.01
3	SI-10	155 ± 0.01

SII- Cashew apple juice red variety; SI- Cashew apple juice yellow variety

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