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An Ecological and Economical Strategies of Pretreatment to Explore Biovalorization Potential of Lignocellulosic Agro-Wastes for Ethanol Production

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Authors' contributions

This work was carried out in collaboration between all authors. Author LB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MAG managed the analyses of the study. Author SJ managed the literature searches.

Original Research Article

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ABSTRACT

Recent breakthrough in industrial biotechnology offer important opportunities for the utilization of agro-industrial residues like wheat straw, bagasse, peels of Pineapple (*Ananas cosmosus*), Sweet orange (*Citrus sinensis var mosambi*) and Litchi (*Litchi chinensis*). Pretreatment becomes the pivotal step to effectively harness the sugars entrapped within the shield of lignin, which will further make these substrates capable as an ideal substrate for microbial process to produce bioethanol. The major objective of the present investigation was to evaluate the effect of steam explosion, microwave and solar pretreatment on the above lignocellulosic agro-wastes using the chemicals in less concentration for improved yield of reducing sugars. The optimization criterion was the fermentable sugar yields, which were analyzed by HPLC. The best hydrolysis was obtained from the steam explosion pretreatment with 1% HNO₃ for bagasse, wheat straw and peels of *Litchi chinensis*, and with 1% H₂SO₄ for peels of *Ananas cosmosus*. A comprehensive account of the above result indicate that 78.2%, 59.56%, 52.15%

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saccharification (maximum) was obtained when *L. chinensis* peels, wheat straw and bagasse were steam exploded with 1% HNO₃. 75.52% saccharification (maximum) was obtained when *A. cosmosus* peels were steam exploded with 1% H₂SO₄. With 1% NaOH steam explosion of *Citrus sinensis* var *mosambi*, saccharification was 90.32%. Microwave and solar pretreatment was found suited to only bagasse, as some percentage of glucose, fructose and arabinose was detected in filtrates of 1% HNO₃ and distilled water treated bagasse. These results show that an integrated exploitation of these lignocellulosic wastes from agricultural production is economically possible and highly advantageous for ethanol production utilizing suitable microorganism. Results indicate that only a mild pretreatment is necessary in an industrial, economically feasible system.

Keywords: Pretreatment; acid hydrolysis; steam explosion; solar energy; TLC; HPLC.

1. INTRODUCTION

In USA, transportation accounts for 30% of the total energy consumption. Burning fossil fuels such as coal and oil releases CO₂, which is a major cause of global warming. With only 4.5% of the world's population, the US is responsible for about 25% of global energy consumption and 25% of global CO₂ emissions [1]. With respect to fossil fuels, biomass-based fuels have the advantage of decreasing greenhouse gas (GHG) emissions. In this context, ethanol produced from biomass, "bioethanol", has become a major energy carrier for a sustainable transportation sector. Bioethanol is an oxygenate fuel with a high octane number [2].

Currently, United States and Brazil are the largest bioethanol producers in the world from corn and sugarcane respectively. However, in some countries with low availability of agricultural lands, the production of biofuels from dedicated crops could lead to direct conflict with food productions. Lignocellulosic materials like agro-forest residues, could, offer a great potential as biomass source for bioethanol production. In fact, they are virtually abundant and low cost [3-5].

Lignocellulosic feedstocks are composed primarily of carbohydrate polymers (cellulose and hemicellulose) and phenolic polymers (lignin). Cellulose (C₆H₁₀O₅)_n is a linear polysaccharide polymer of glucose made of cellobiose units that are packed by hydrogen bonds. The structure of this polymer is rigid and compact, so the biomass needs pretreatment that breaks its structure to facilitate the action of the enzymes. The individual cellulose chains are packed and organized into crystalline microfibrils. Within these microfibrils, cellulose is found in two forms, namely amorphous and crystalline. The crystalline form of cellulose is very difficult to degrade. Hemicellulose such as xylan (C₅H₈O₄)_n is a short polymer of pentoses and hexoses sugars. The dominant sugars in hemicelluloses are mannose (six-carbon sugar) in softwoods and xylose (five carbon sugar) in hardwoods and agriculture residues [6]. Hemicellulose contains also, galactose, glucose and arabinose. This polymer is amorphous and easier to hydrolyse than cellulose. Lignin [(C₉H₁₀O₃) (OCH₃)]_n is a phenyl propane polymer that contains many functional groups such as hydroxyl, methoxyl and carbonyl. Unlike cellulose and hemicellulose, lignin cannot be utilized in the fermentation process. In fact, it has high resistance to chemical and enzymatic degradation.

The main technological barrier that impedes widespread utilization of this resource for production of fuels and other commodity products is the lack of low-cost technologies to

overcome the recalcitrance of lignocellulose. The conversion of lignocellulosic biomass into ethanol requires a pretreatment step to change the physical and chemical structure of biomass and to enhance the hydrolysis rate [7].

There are several pretreatment strategies, all aimed at opening the structure of the cell biomass and allow the enzymes to access the internal polysaccharides. The available pretreatments can be grouped in chemical, biological, physical and physico-chemical processes. Biological pretreatment uses microorganisms such as brown, white and soft-rot fungi which degrade lignin and solubilize hemicelluloses. The biological process is interesting for its low energy requirement, however, the rate of hydrolysis process is very low. Bioconversion of wheat straw residue to fuel ethanol would provide an attractive possibility to boost the development of biofuels in a sustainable way [8].

Among the investigated pretreatment, the steam explosion (SE) appears one of the most promising approach since it limits the use of chemicals mostly to the use of saturated steam [9]. The process employs high pressure steam with temperature typically ranging from 160° to 260°C for few minutes. This is followed by explosive decompression of biomass [10-12]. Zhu et al. [13] examined three microwave/chemical processes for pretreatment of rice straw viz. microwave/alkali, microwave/acid/alkali and microwave/acid/alkali/H₂O₂ for its enzymatic hydrolysis and for xylose recovery from the pretreatment liquid. In microwave-assisted heating, unlike the conventional methods, the heat is generated within the material, thus rapid heating occurs. As a result of this rapid heating, many microwave-assisted organic reactions are accelerated, incomparable with those obtained using the conventional methods. Thus, higher yields and selectivity of target compounds can be obtained at shorter reaction times. In addition, many reactions not possible using the conventional heating methods, had been reported to occur under microwave heating [14].

Chemical pretreatments employ different chemical agents like acids and alkalis [15]. Dilute-acid hydrolysis can be used either as a pretreatment of lignocellulose for enzymatic hydrolysis, or as the actual method of hydrolyzing to fermentable sugars [16]. Another pretreatment method used a mixture of H₂SO₄ and acetic acid with an efficiency of 90% saccharification [17].

Alkali pretreatment is based on the use of alkaline solutions such as NaOH, Ca(OH)₂ or NH₃ to remove lignin and part of the hemicellulose, and increase the enzymes accessibility to the biopolymers. The wet oxidation is an approach in which, the material are treated with water and air or oxygen at temperatures above 120°C for a period of e.g. 30 min. The process represents an effective method in separating the cellulosic fraction from lignin and hemicellulose [15]. Pretreatment of lignocellulosic materials with acidified organic solvents (mixture of ethylene glycol, water and HCl at 178°C for 90 min) has also been successfully used [18]. Moreover, further pretreatment optimization of lignocellulosic materials to remove lignin can significantly enhance the hydrolysis of cellulose improving the bioethanol yield and in addition the recovered lignin can also be converted into valuable compounds. Converting waste agricultural residues to a high quality fuel may provide an economic opportunity for India.

The present study aims to evaluate an economic, ecofriendly and an effective aspects of process of physico-chemical pretreatments, for which various forms of heat energies viz. solar, microwave and steam explosion were tested. Chemicals used for hydrolysis included sodium hydroxide, crude slacked lime, sulphuric acid, nitric acid. Distilled water without any chemical to perform wet oxidation was used. Utilization of peels of *Ananas cosmosus*, *Litchi*

chinensis and *Citrus sinensis* var *mosambi* for ethanol production is an innovative approach of utilization of agro-wastes along with wheat straw and bagasse.

2. MATERIALS AND METHODS

2.1 Materials

All chemicals (except crude slacked lime) used in the study were of analytical grade and were purchased from Qualigens, Himedia & Merck.

2.2 Methods

2.2.1 Collection and processing of lignocellulosic substrates

The ligno-cellulosic agro wastes viz. wheat straw, bagasse, peels of fruits like Litchi (*Litchi chinensis*), Pineapple (*Ananas cosmosus*) and Sweet lemon (*Citrus sinensis* var *mosambi*) were collected either from local farms (wheat straw) or from local fruit juice vendors (bagasse, peels of Litchi, Pineapple and Sweet orange) of Gwalior (26°13'N 78°11'E /26.22°N 78.18°E), Madhya Pradesh, India. Wheat straw was dried in a forced-air oven at 55.8°C for 24h. The milled wheat straw was stored at room temperature. It was then reduced to powdered form. Peels of *Citrus sinensis* var *mosambi*, *Litchi chinensis* and *Ananas cosmosus* were air dried for 4-5 days. All dried substrates were ground into fine powder by use of laboratory blender at 3000 rpm. Samples were preserved in sealed plastic bags at 4°C to prevent any possible degradation or spoilage.

2.2.2 Pretreatment of substrates

Chemical treatment was done with H₂SO₄ (1% v/v), H₂SO₄ (10% v/v), NaOH (1% v/v), HNO₃ (1% v/v), 1% Ca(OH)₂ (w/v) and with distilled water (to perform wet oxidation). In all these sets of experiments, 1g of substrate was mixed with 10ml of above mentioned chemicals/10ml distilled water. Three major physico-chemical pretreatments viz. solar treatment, microwave treatment and steam explosion were given to all the above chemically treated substrates.

Solar pretreatment was performed in open petri dishes exposed to sunlight for about 6 hrs (from 10 am to 4 pm), at temperature of 34±3°C. Microwave pretreatment was given to the substrates for 5.0 min in a microwave oven (LG Electronics Tianjin Appliances Co., Ltd., Tianjin, PR China). The oven's irradiation power of 700 W was used in all pretreatment processes. Prior to the microwave treatment, soaking time of 90 min was given to all substrates with their respective chemicals. Steam explosion of raw material was carried out at flask level. 1g of raw material along with its chemical was taken in the 100mL flask, and was subjected to sudden steam depressurization in an autoclave (15 psi, 20 min, and 121°C) by fully opening the steam exhaust valve.

After pretreatment, the supernatant was filtered and collected for further biochemical tests. The residue left behind was thoroughly washed twice with distilled water to remove all associated chemicals and were dried in an oven at 70°C for 24h, after which they were reweighed.

2.2.3 Analytical methods

Determination of total carbohydrate was done by anthrone method [19]. The filtrate was also used to determine the concentration of reducing sugar based on the method using 3,5-dinitrosalicylic acid reagent (DNS method) [20]. Further identification of liberated sugars in filtrates was done using thin layer chromatography [21].

Thin layer chromatography was performed using Silica gel G. The filtrates obtained were subjected to one dimensional chromatogram on a Silica gel G layer plate. Each plate was activated at 105°C prior to use for 10 min. The solvent system used was ethyl acetate: isopropanol: water: pyridine (26:14:7:3, v/v/v/v). The plate was sprayed with Aniline-diphenylamine (5 volume of 10g/l aniline and 5 volumes of 10g/l diphenylamine in acetone with 1 volume of 85% phosphoric acid). The R_f values relative to the solvent are reported here (Table 2).

2.2.4 Analysis of sugars by high performance liquid chromatography

The sugars in the samples were analyzed using high performance liquid chromatography (HPLC, Waters) with sugar pak column (Waters USA) and a system composed of a 510 pump, a refraction index differential detector (RI 2414 USA) and a data processor with register (Waters, USA). The samples were filtered through membrane filters 0.45µm (Millipore) before injection. The temperature of the column was maintained at 70°C by column oven (Dyna, Mumbai) with injection valve of 20µl. The RI detector was operated at 30°C and the solvent systems used were water as mobile phase at flow rate of 0.2ml/min. Calculations and analysis were performed using Empower 2 software Build 2154 (Waters).

3. RESULTS AND DISCUSSION

3.1 Evaluation of Pretreatment Techniques and Biochemical Tests

The first assessment after any stage of lignocellulosic materials treatment was the yield measurement, which is a parameter for the process classification and crucially dependent on raw material and on the conditions employed in the treatment. The total yield is inversely proportional to the weight loss of substrate and might be measured as the ratio between the mass of material obtained after the treatment stage and the initial mass used to perform the same. Industrially it is more appropriate to assess the sorted or cleaned yield, i.e., the yield of the material after the removal of wastes (material not shredded) and impurities of vegetable origin (sand, stones and metals). However, in this work, due mainly to the small amount used in the reactions only total yields of the treatments were determined.

It has long been recognized that some form of pretreatment is necessary to achieve reasonable rates and yields in the enzymatic hydrolysis of biomass. This has generally been attributed to the crystallinity of cellulose, the lignin hemicellulose sheath that surrounds the cellulose, and the lack of available surface area for enzymes to attack. A number of different pretreatment methods, such as dilute sulphuric acid (1% v/v), concentrated sulphuric acid (10% v/v), sodium hydroxide (1% w/v), nitric acid (1% v/v), 1% calcium hydroxide (w/v) and distilled water (10ml/1g substrate) in steam autoclaving, microwave and solar treatment have been extensively investigated. Table 1 (a-c) shows the comparison of the percentage reduction in weight of substrates after three treatments. The amount of weight lost following chemical pretreatment of residue was due to lignin removal. Greater weight loss equals

more lignin loss and this percent weight lost was used to compare pretreatment effects on lignin removal.

Table 1(a). % Weight loss of substrates after steam explosion pretreatment

Chemical	<i>Ananas cosmosus</i> peel	<i>Litchi chinensis</i> peel	<i>Citrus sinensis</i> var <i>mosambi</i> peel	Wheat straw	Bagasse
1% NaOH	50.4	43.1	41.2	39.4	49.4
1% HNO ₃	52.0	48.5	64.9	46.5	44.9
1% H ₂ SO ₄	56.4	42.0	66.0	40.3	41.7
10% H ₂ SO ₄	52.2	32.7	61.5	47.0	28.3
Slacked lime	33.3	30.2	36.1	21.7	22.3
DW	21.5	31.5	38.8	21.4	21.7

The values are mean of three replicates. Standard deviation was within 10%

Table 1(b). % Weight loss of substrates after microwave pretreatment

Chemical	<i>Ananas cosmosus</i> peel	<i>Litchi chinensis</i> peel	<i>Citrus sinensis</i> var <i>mosambi</i> peel	Wheat straw	Bagasse
1% NaOH	24.3	39.0	35.8	29.6	23.0
1% HNO ₃	26.4	32.9	47.1	23.4	22.5
1% H ₂ SO ₄	23.3	36.1	35.5	22.1	20.0
10% H ₂ SO ₄	19.3	26.9	13.8	26.8	14.0
Slacked lime	24.4	18.3	15.3	16.0	21.6
DW	28.7	22.3	29.6	16.8	13.4

Table 1(c). % Weight loss of substrates after solar pretreatment

Chemical	<i>Ananas cosmosus</i> peel	<i>Litchi chinensis</i> peel	<i>Citrus sinensis</i> var <i>mosambi</i> peel	Wheat straw	Bagasse
1% NaOH	20.1	17.3	26.4	14.2	10.7
1% HNO ₃	8.6	32.3	31.1	14.6	23.6
1% H ₂ SO ₄	12.7	21.0	19.2	14.2	16.6
10% H ₂ SO ₄	17.6	9.8	22.9	25.6	19
Slacked lime	15.2	17.4	23.8	7.9	16.1
DW	11.6	21.8	14.5	4.6	11.5

Considerable amount of carbohydrate was liberated in all three major pretreatment studies (i.e. steam explosion, microwave and solar) with 10% H₂SO₄, followed by 1% H₂SO₄ and 1% HNO₃, but however reducing sugars were not detected after 10% H₂SO₄ pretreatment(except in *Ananas cosmosus* peel filtrate of steam explosion)as presented in Fig. 1(a-c). The decrease of sugar content in acid treated samples with increasing of acid concentration might be due to the degradation of monomeric sugars (xylose, glucose) in furfural and hydroxymethyl furfural. The results in Figs. 2(a-c) showed that, the treatment with 1% H₂SO₄ in steam explosion pretreatment was the most suitable for highest level of reducing sugar. As presented in Fig. 2b, Performance of 1% NaOH was produced the highest level of reducing sugars in microwave pretreatment. It seems that the synergic action existed between the microwave irradiation and alkali treatment during the pretreatment. It has also been proved that microwave irradiation could enhance the pentosans and lignin degradation reaction in NaOH aqueous solution [22]. The mechanism probably is that microwave irradiation enhances the saponification of intermolecular ester bonds crosslinking

xylan hemicelluloses and other components, for example, lignin and other hemicelluloses [23].

3.2 Thin Layer Chromatographic Analysis Report

Rf value for the analytical grade samples are given in Table 2. The spots obtained were found to be matching with the standard sugars and found to be arabinose, rhamnose, xylose, galactose, glucose, fructose, sucrose, are given in Table 3(a-c). Thus TLC analysis gives accurate confirmation for the presence of arabinose, rhamnose, xylose, galactose, glucose, fructose, sucrose in various sets of pretreatment. This was the first assay of the sugar profile of filtrate of the five substrates, which was further developed for characterization and evaluation of their quality with regards to their sugar composition.

Table 2. Rf values matching of the analytical standard samples and the separated samples

Sugars	Rf (Scale of Rf =1)
Lactose	0.03
Maltose	0.10
Sucrose	0.26
Galactose	0.22
Glucose	0.28
Mannose	0.31
Sorbose	0.38
Fructose	0.35
Arabinose	0.37
Xylose	0.43
Ribose	0.47
Rhamnose	0.53

Table 3(a). Sugars detected in Thin Layer Chromatography after steam explosion pretreatment

Chemical	<i>A. cosmosus</i> peel	<i>L. chinensis</i> peel	<i>C. sinensis mosambi</i> peel	Wheat straw	Bagasse
1% NaOH	Gl, Fr	ND	Gl	ND	Su
1% HNO ₃	Ar, Gl	Ar, Xy	Xy, Gl	Xy, Gl	Xy, Gl
1% H ₂ SO ₄	Gl, Xy, Fr	Ar, Xy	Gl	Gl, Fr	Xy
10% H ₂ SO ₄	Xy	Ar	ND	ND	Xy
1%Ca(OH) ₂	ND	ND	ND	ND	ND
DH ₂ O	ND	ND	Gl, Fr	ND	Gl, Fr

Table 3(b). Sugars detected in Thin Layer Chromatography after microwave pretreatment

Chemical	<i>A. cosmosus</i> peel	<i>L. chinensis</i> peel	<i>C. sinensis mosambi</i> peel	Wheat straw	Bagasse
1% NaOH	ND	ND	Gl, Fr	Gl, Fr	Gl, Fr
1% HNO ₃	Xy	Gl, Xy	ND	Gl, Xy	Xy
1% H ₂ SO ₄	ND	ND	ND	ND	ND
10% H ₂ SO ₄	Xy	Xy	Gl	Gl	Fr
1%Ca(OH) ₂	ND	ND	ND	ND	ND
DH ₂ O	ND	ND	ND	ND	ND

Table 3(c). Sugars detected in Thin Layer Chromatography after solar pretreatment

Chemical	<i>A. cosmosus</i> peel	<i>L. chinensis</i> peel	<i>C. sinensis</i> <i>mosambi</i> peel	Wheat straw	Bagasse
1% NaOH	Ar	Ar	Ar	Ar	Ar
1% HNO ₃	Ar, Xy	Gl, Xy	Gl	Gl, Fr	Gl, Fr
1% H ₂ SO ₄	Gl, Ar	Xy	Xy	Ar	Ar, Gl
10% H ₂ SO ₄	Xy	Xy, Gal	Xy, Gal	Xy	Xy
1%Ca(OH) ₂	ND	ND	Rh	ND	Rh
DH ₂ O	ND	Xy	Gl	ND	Gl, Ar

(Ar- Arabinose, Rh – Rhamnose, Xy- Xylose, Gal – Galactose, Gl - Glucose, Fr -Fructose, Su – Sucrose, ND- Not detected)

3.3 High-Performance Liquid Chromatography Report

HPLC was performed on 50 samples, out of which 30 samples were the filtrates of 30 experiments of steam explosion. 10 prospectus samples each from microwave and solar pretreatment were also tested by HPLC. Table 4 depicts that 12.42% xylose and 46.71% arabinose was found in filtrate of *Litchi chinensis* peels when it was treated with HNO₃ (1% v/v) in steam explosion. In the similar pre-treatment strategy, wheat straw and bagasse filtrate consisted 11.54% and 11.58% xylose respectively. The maximum yield of monomeric sugars from wheat straw (7.83%, w/v, DS) by dilute H₂SO₄ (0.75%, v/v) pretreatment was reported by Saha et al. [24]. These results depict that mostly hemicellulose are hydrolyzed to simple sugars. Comparative to these substrates, the filtrate of *Ananas cosmosus* peel was rich in sugars when treated with H₂SO₄(1% v/v), consisting of 18.89% glucose, 38.81% xylose and 29.31% fructose which shows that the more cellulose polymers and hemicellulose are converted into monomer of sugars. Alkaline treatment with NaOH (1% v/v) was found best suited for *Citrus sinensis var mosambi* peel which shows that the polymers are converted into monomer and dimers of sugars. Dilute NaOH treatment of lignocellulosic materials has been found to cause swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure [25]. Peaks and area covered by different sugars representing the above percentages can be seen in Fig. 3 (a-e). Unhydrolysed starch also constituted a major fraction in almost all the filtrates. Microwave and solar pretreatment was found suited to only bagasse, as some percentage of glucose, fructose and arabinose was detected in filtrates of HNO₃(1% v/v) and distilled water treated bagasse. These results indicate that only a mild pretreatment is necessary in an industrial, economically feasible system.

The sugars liberated in the filtrate are mostly hemicelluloses sugars. It is because of amorphous nature of hemicelluloses that they are easily degraded. The cellulose has a majority crystalline character and so its structure is not so easily broken by diluted mineral acid as in this work. The concentration of xylose in the hydrolysates was always higher than that of other sugars, showing the increase loss of hemicellulose during various treatments (Table 4). Taking into account that the arabinose is also generated by the breakdown of hemicelluloses chains, this difference is even greater. 1% HNO₃ and 1% H₂SO₄ steam explosion pretreatment have been interpreted as representing the hydrolysis of the easy to hydrolysis fraction of the xylan. Other treatments have been interpreted as representing the hydrolysis of hard to hydrolysis fraction of xylan.

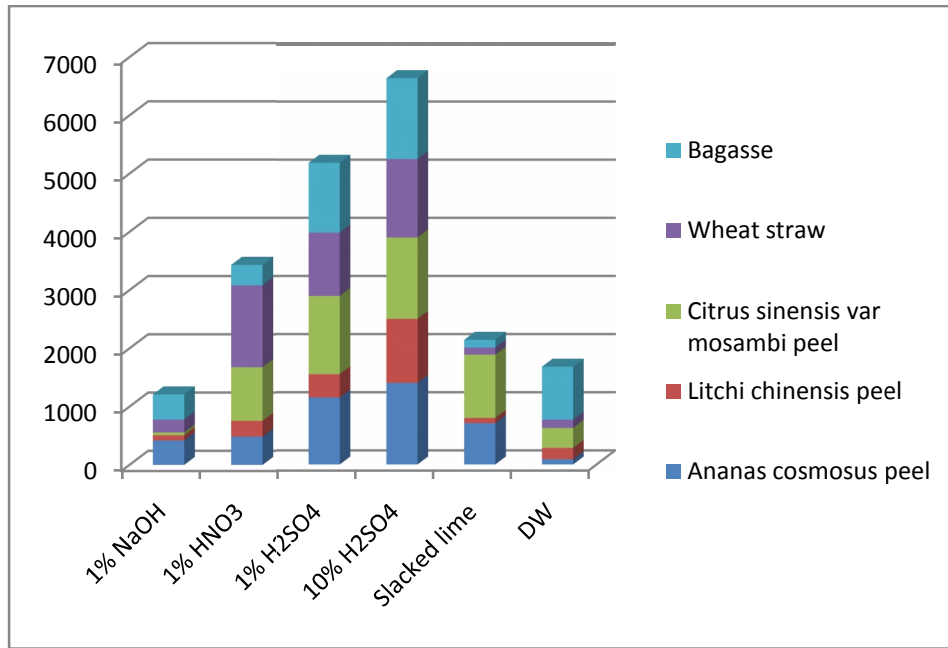


Fig. 1a. Total carbohydrate (mg/ml) in filtrates after steam explosion pretreatment

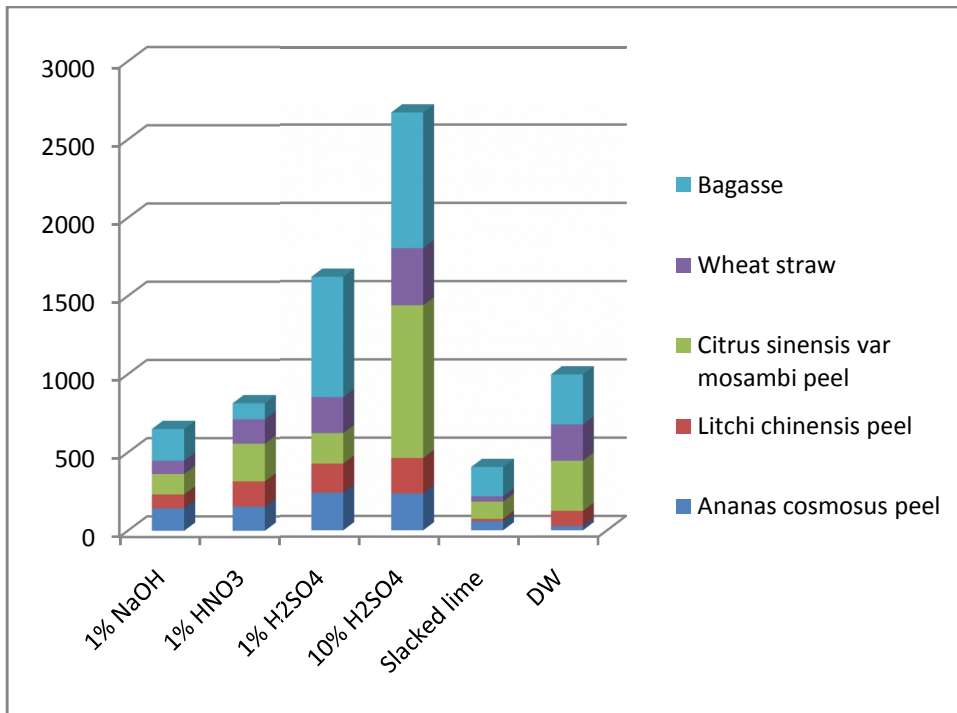


Fig. 1b. Total carbohydrate (mg/ml) in filtrates after microwave pretreatment

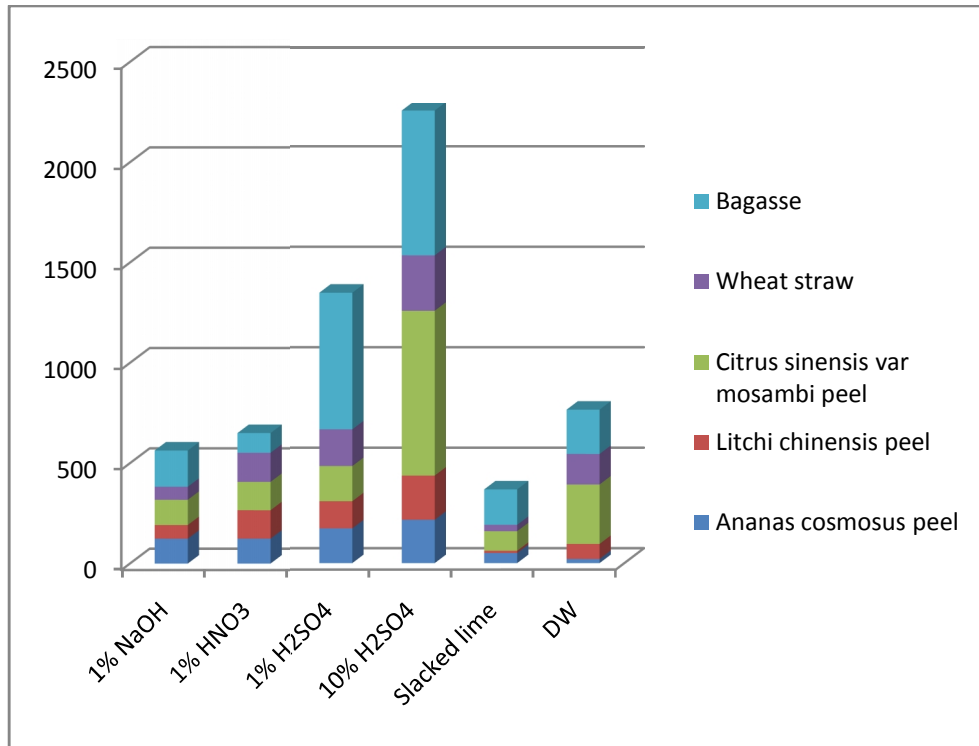


Fig. 1c. Total carbohydrate (mg/ml) in filtrates after solar pretreatment

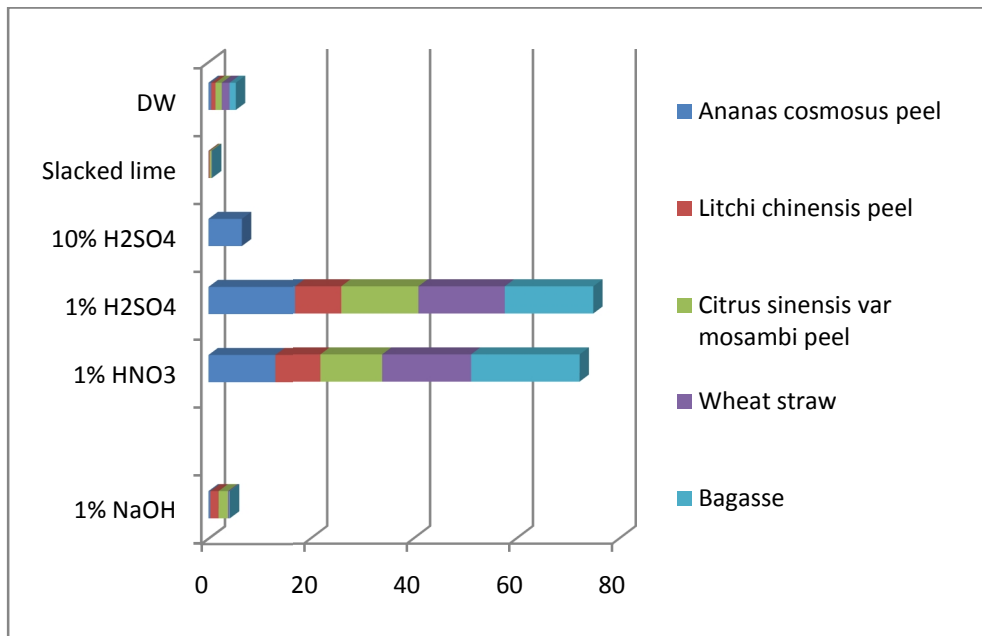


Fig. 2a. Reducing sugars (mg/ml) in filtrates after steam explosion pretreatment

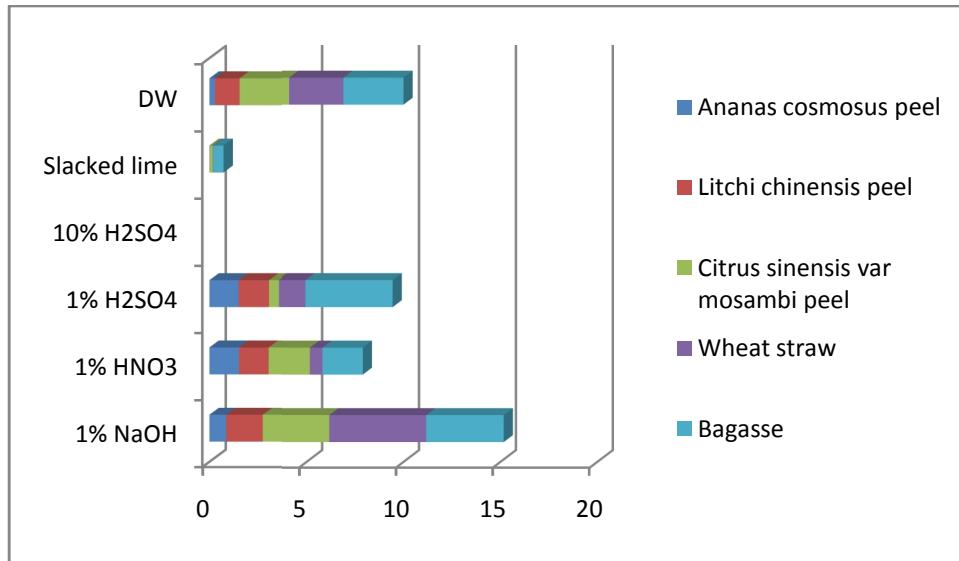


Fig. 2b. Reducing sugars (mg/ml) in filtrates after microwave pretreatment

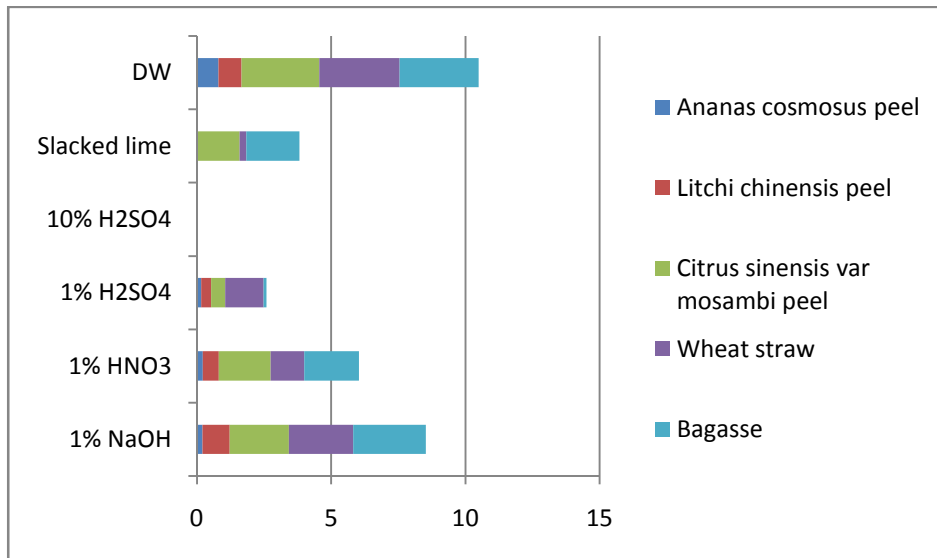


Fig. 2c. Reducing sugars (mg/ml) in filtrates after solar pretreatment

One aspect that should be emphasized is that high concentration achieved, especially for xylose was due to the scale used. The average volume of hydrolysate was 10.0ml, making the sugar stay more concentrated. At larger scale, the concentration value would be lower because the volume of hydrolyzed would be greater.

A comprehensive account of all the above results indicate that 90.32% saccharification was obtained when *Citrus sinensis var mosambi* peels were steam exploded with 1% NaOH.

Table 4. HPLC profile of various steam explosion, microwave and solar pretreatments

Pretreatment done & chemical used	<i>Ananas cosmosus</i> peel	<i>Litchi chinensis</i> peel	<i>Citrus sinensis</i> var <i>mosambi</i> peel	Wheat straw	Bagasse
SE/ 1% HNO ₃	Glucose 1.91% Fructose 22.90% Arabinose 25.28%	Arabinose 12.42% Xylose 46.71%	Glucose 3.04% Xylose 2.16%	Glucose 1.54% Xylose 11.54%	Glucose 6.39% Xylose 11.58% Fructose 4.56 %
SE/1%NaOH	Sucrose 0.28% Glucose 3.28% Fructose 4.75%	Xylose 0.56% Unknown sugar-RT 25.535	Raffinose 4.46% Glucose 4.98% Fructose 7.67%	Raffinose 5.86% Glucose 0.50% Fructose 0.78%	Sucrose 0.61% Glucose 0.29% Fructose 0.21%
SE/ 1%H ₂ SO ₄	Glucose 18.89% Xylose 38.81% Fructose 29.31%	Xylose 8.42% Arabinose 15.71%	Glucose 4.11% Xylose 1.30%	Glucose 2.13% Xylose 1.35% Fructose 8.09%	Xylose 6.18%
SE/10% H ₂ SO ₄	Xylose 16.7%	Arabinose 29.6% Unknown sugar-RT 25.73	Glucose 1.04%	Glucose 0.863% Fructose 6.08%	Xylose 5.76%
SE/Slacked lime	Sucrose 0.81% Glucose 0.016%	Glucose 0.02% Xylose 0.76%	Fructose 0.53%	Sucrose 0.89%	Sucrose 0.79% Arabinose 2.04%
SE/DW	Starch 10.98%	Unknown sugar-RT 25.627	Glucose 1.51% Fructose 2.02%	Glucose 0.88% Fructose 0.08%	Glucose 1.68% Fructose 2.21%
Microwave/1% HNO ₃	Starch 26.6%	Unknown sugar-RT 25.572	Glucose 0.25% Fructose 0.44%	Glucose 0.425% Fructose 1.46%	Glucose 3.8% Fructose 4.06%
Microwave/DW	Starch 6.48%	Unknown sugar-RT 25.559	Fructose 0.519%	Fructose 1.06%	Fructose 1.38%
Solar/ 1% HNO ₃	Starch 24.8%	Unknown sugar-RT 25.627	Glucose 2.38% Fructose 2.68%	Glucose 0.616% Fructose 2.0%	Glucose 4.74% Fructose 2.90%
Solar/DW	Starch 5.45%	Unknown sugar-RT 25.566	Glucose 0.566% Fructose 1.49%	Starch 5.72%	Sucrose 0.12% Glucose 0.22% Arabinose 14.34%

(SE- Steam explosion, DW- Distill water)

75.52% saccharification was obtained when *Ananas cosmosus* peels were steam exploded with 1% H₂SO₄ 78.2% saccharification was obtained when *Litchi chinensis* peel were steam exploded with 1% HNO₃. 59.56% and 52.15% saccharification was obtained when wheat straw and baggase were respectively treated with 1% HNO₃ in steam explosion.

Negligible amount of sugars were obtained after steam explosion with lime. Lime pretreatment removes amorphous substances (e.g., lignin and hemicellulose), which increases the crystallinity index. Calcium hydroxide (slake lime) is the least expensive and it is possible to recover. Pietrobon et al. [26] reported that use of 3% calcium hydroxide (w/v) for pretreatment of baggase at 121°C, 30 minutes showed the presence of glucose (0.013mg/ml) and xylose (0.010mg/ml), but no arabinose and galactose were observed. Moreover, in our experiment using lower concentration of (1%) Calcium hydroxide obtained 2.04% arabinose.

It has been reported by Chandel et al. [27] that in acid hydrolysis of sugarcane bagasse at 140°C for 30 min and initial solid: liquid ratio (1:10), there was a two fold increase (g/l) in total furans, total phenolics and acetic acid with the mere change in the concentration of acid from 1% to 3%. These toxic component may lead to stop the growth of microorganism by affecting the rate of sugar uptake with simultaneous decay in product formation, hence should be removed from the filtrates before proceeding for its fermentation. Lesser the concentration of these inhibitors, less and cheap efforts will be needed to detoxify these filtrates for the further use. Hence in this work less concentration of various acids is not only cost effective in terms of cost of acids used or in terms of cost of their detoxification, but is also environment friendly as the effluents liberated after the thorough wash of the substrates (left after the pretreatment), will contain even mild acid concentrates. Microwave is energy intensive but time saving. On the contrary solar treatment is energy extensive but time taking procedure. Solar energy can be harnessed in a better way for the pretreatment procedures for the bioethanol production in future. It can be proved as one of the options for economic and ecological bioethanol production from cheap residues like the peels of *Litchi chinensis*, *Ananas cosmosus* and *Citrus sinensis var mosambi*. This will not only solve the energy crisis but there will also be the best waste management of these peels, once they get accumulated especially by the fruit juice industries. Use of crude slacked lime as a source of chemical will further reduce the cost of pretreatment.

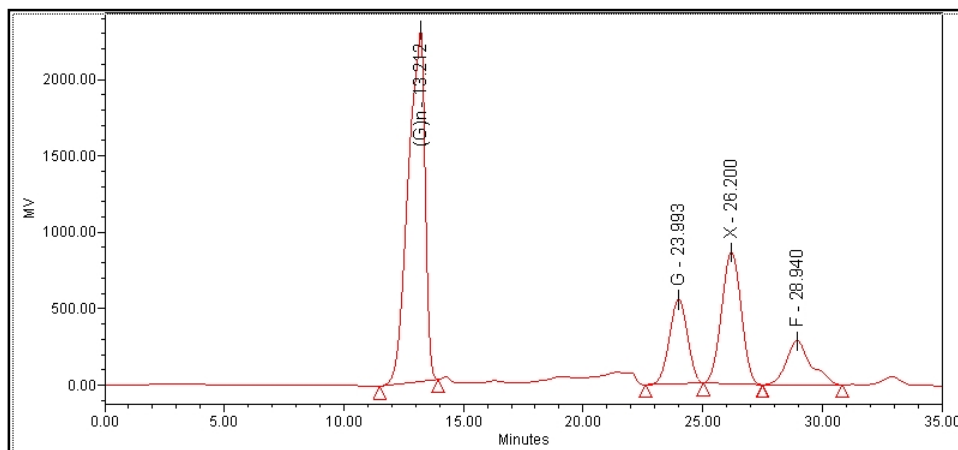


Fig. 3a. HPLC chromatogram of sugars liberated from baggase after 1%HNO₃ steam explosion

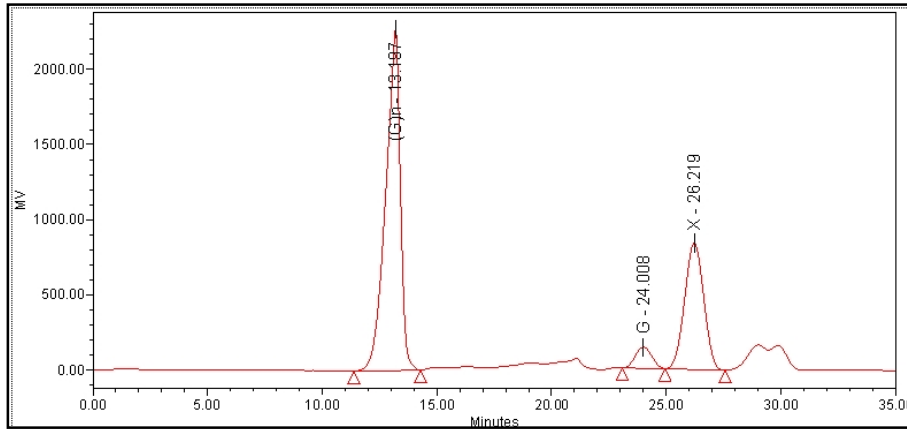


Fig. 3b. HPLC chromatogram of sugars liberated from wheat straw after 1% HNO₃ steam explosion

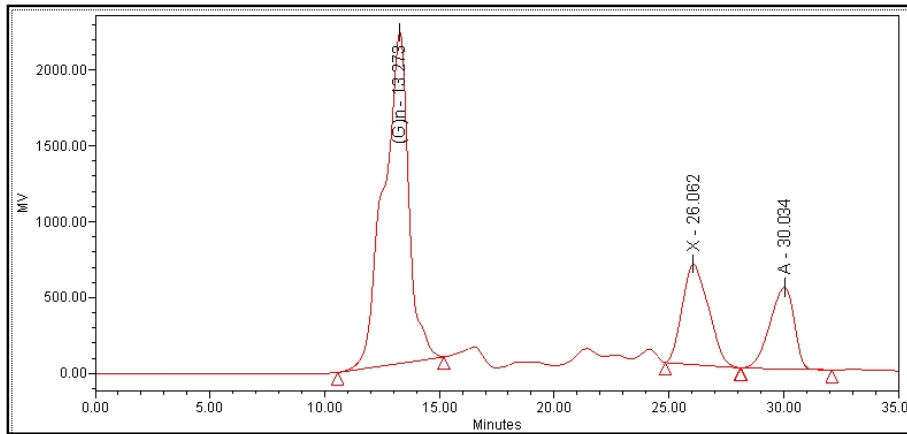


Fig. 3c. HPLC chromatogram of sugars liberated from *Litchi chinensis* peel after 1% HNO₃ steam explosion

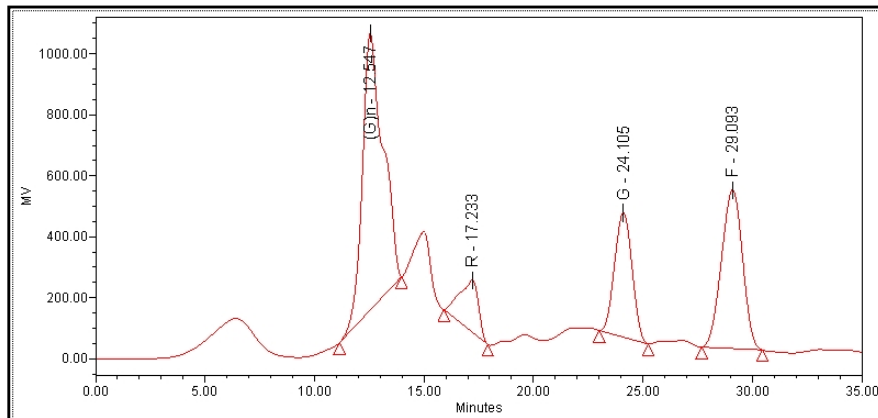


Fig. 3d. HPLC chromatogram of sugars liberated from *Citrus sinensis* var *mosambi* peel after 1% NaOH steam explosion

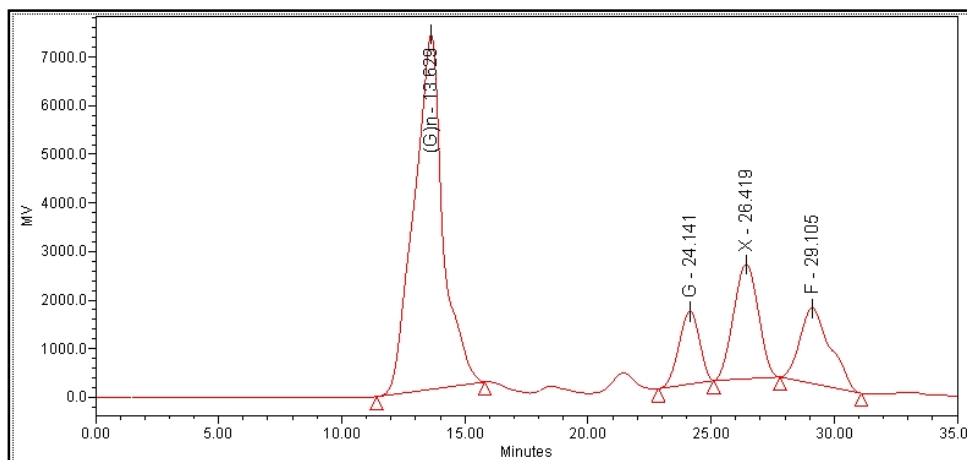


Fig. 3e. HPLC chromatogram of sugars liberated from *Ananas cosmosus* peel after 1% H₂SO₄ steam explosion

4. CONCLUSION

Steam explosion with 1% HNO₃ is proved to be the best pretreatment strategy for *Litchi chinensis* peel, bagasse and wheat straw. Looking at the high yield of sugars, it can also be concluded that bioethanol can be extracted from the peelings of pineapple (*Ananas cosmosus*) when treated with 1% H₂SO₄. Though bitter in taste, peels of *Citrus sinensis var mosambi* are rich in sugars, which can be harvested when treated with 1% NaOH in steam explosion. Our results reveal that an integrated exploitation of these lignocellulosic wastes from agricultural production is economically possible and highly advantageous for ethanol production utilizing suitable microorganism. It will indeed be a great help to the economy and the environment if ever this research becomes successful. It will benefit the economy as this will use only wasted peelings; it will not be in any way detrimental to the environment-friendly energy source and this will consequently lessen the pollution worldwide. This research will also answer the crisis of looking for a clean, alternative source of energy. The researchers would recommend the people to not throw away these fruit peelings whenever they consume the fruit, and also these fruits-made product manufacturers to store the peelings of the fruits they make use of. The researchers would also recommend local waste management committees to religiously collect these fruits peelings from citizens and submit them to laboratories.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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