Environmental Sustainability (2019) 2:33–41
https://doi.org/10.1007/s42398-019-00048-2

ORIGINAL ARTICLE

Valorisation of orange peel: supplement in fermentation media for ethanol production and source of limonene

Pamela Jha[1] · Surabhi Singh[1] · Madathil Raghuram[1] · Gokul Nair[1] · Renitta Jobby[1] · Anand Gupta[1] · Nitin Desai[1]

Received: 7 May 2018 / Revised: 31 January 2019 / Accepted: 2 February 2019 / Published online: 20 February 2019

Abstract

The usage of the citrus wastes for strategic valorization is a concept for the production of various commercial products. Wastes from citrus fruits have a considerate amount of sugar and limonene. In the present work, partially removed limonene from hydrolysed orange peel was used for supplementation as carbon source in minimal media for the production of ethanol. A novel yeast strain, *Saccharomyces cerevisiae* strain BT1 (MK373758) was isolated from Toddy, obtained from *Borassus flabellifer* (toddy palm), Bhatan, Mumbai. This strain was used in the formulated fermentation media for the production of bioethanol. Optimization of critical parameters was done for pH, temperature and time of fermentation vs production of bioethanol. The Brix, pH, sugar content and ethanol content were measured during the fermentation process. The ethanol content was estimated by dichromate method and high performance liquid chromatography (HPLC) and the yield obtained was 0.95%. In addition, limonene was also extracted from hydrolysed orange peel and estimated by gas chromatography-flame ionization detection (GC-FID) and was found to be 0.4% (v/v). The two valuable products, bioethanol, and D-limonene, obtained from orange peel waste, promises its valorisation at a larger scale.

Keywords Bioethanol · Yeast · Fermentation · Orange waste · Limonene

Introduction

The valorization of the plentifully available lignocellulosic materials has been gaining increased attention for the production of a wide range of applications as a zero-waste approach (Arevalo-Gallegos et al. 2017; Bilal et al. 2017; Iqbal et al. 2017). These applications generate products, which include biochemicals, biofuels, animal feed, enzymes, and biocomposites (Asgher et al. 2017; Ahmad et al. 2017).

The current concept of valorization of waste has led to an increased importance of different fruit wastes, being studied for the production of various commercial products including bioethanol. Among the citrus fruits, orange is the most produced fruit worldwide and its global production has increased to 47.8 million in 2017/2018 (USDA 2018). India ranks ninth among top orange producing countries in the world with a contribution of 4% to the world's total production (The Daily Records 2018). The waste generated after the consumption of the oranges is of major concern due to its complexity in disposal and additional economic burdens on production.

This environmental problem can be assisted to some extent by utilization of fruit waste as a renewable resource that can play a significant part in the forthcoming bio-economy era as its chemical complexity fits perfectly to the concept of biorefinery development for the production of energy, chemicals and bio-based polymers (Lin et al. 2013; Mirabella et al. 2014). Orange peel belongs to this group of valuable biomass wastes (Mrudula and Anitharai 2011), from which ethanol and D-limonene can be obtained, which could contribute substantially to a bio-based economy. Ethanol is an efficient fuel and can be used to enhance octane content as well as to replace lead as an anti-knocking agent (Calabro et al. 2017). The current global economic demand of bioethanol is 14 million gallons per year (GPY). As per energy Independence and Security Act, 2007, the bioethanol demand will go up to 36 billion GPS per year by 2022. Bioethanol can be mixed with gasoline to any percentage, most common being E10, E20 among other blends like E85, E95, E100, etc. (Wysocka et al. 2015) to be used as a fuel.

✉ Pamela Jha
pamelajha@gmail.com

[1] Amity Institute of Biotechnology, Amity University, Mumbai-Pune Expressway, Bhatan, Post-Somathne, Panvel, Mumbai, Maharashtra 410206, India

Currently, corn is majorly being used to make ethanol but it has several disadvantages like high Carbon footprint. As corn is a water-intensive crop, the ethanol produced from it is limited to 17 billion GPY. Hence, there is a huge opportunity to meet the gap between the production and demand of bioethanol by non-corn sources.

Also, essential oil from orange peel contains a considerable amount of D-limonene (approximately 90%). It has numerous applications as combustion in engines, a powerful degreaser in cleaning applications, as a natural pesticide, as an additive in food products and fragrances (Karr and Coats 1988). D-Limonene has also been studied for its anti-carcinogenic and anti-microbial properties (Erasto and Viljoen 2008). As per John et al. (2017), global D-limonene demand is expected to cross 65-kilotons by 2023, which was 45-kilotons in 2015. India imported 5.92-kilotons of D limonene worth US$ 22.24 Mn. D-Limonene market is expected to reach over US$ 451.8 Mn by 2022. A United States Department of Agriculture report predicts "green chemicals" produced using biomass will represent 22 percent of the chemical market by 2025 (USDA 2008).

Ethanol can be made from oil or biomass conversion by microbes through a fermentation process (Ohgren et al. 2006). Along with the production of ethanol, orange peel can also be used as a good source of D-Limonene, which gives oranges its characteristic citrus scent.

Citrus peel waste is a valuable lignocellulosic feedstock for bioethanol production due to its richness in fermentable sugars and low lignin content. The major composition (%) of orange peels is pectin (23.02 ± 2.12), lignin (7.52 ± 0.59), cellulose (37.08 ± 3.1) and hemicellulose (11.04 ± 1.05), which make it suitable as fermentation substrate when hydrolyzed (Marín et al. 2007).

In the present study, the valorisation of orange waste has been attempted for the production of various products of commercial importance. The waste obtained from orange (*Citrus sinensis*) peel was used as the substrate for the production of biofuel and D-limonene. The waste was hydrolysed and essential oil was removed. The hydrolysate without essential oil was used as carbon source supplement for the ethanol production using *Saccharomyces cerevisiae* BT1, isolated from toddy. The fraction containing essential oil was quantified for D-limonene.

Materials and methods

Materials

All media were procured from Himedia. Tribasic dihydrate sodium citrate, citric acid monohydrate, starch, 3,5-dinitro salicylic acid and sodium potassium tartrate tetrahydrate were procured from SRL Pvt Ltd. Mumbai. Sulfuric acid and sodium hydroxide were procured from Thomas Baker Chemicals Pvt Ltd., Mumbai. Standard limonene was purchased from Sigma Aldrich (St. Louis, MO, USA). Ethanol was procured from Changshu Hongsheng Fine Chemicals Co. Ltd., China.

Collection of wastes

Fresh orange peels were collected from local juice vendors in Panvel, Navi Mumbai. The orange rinds were separated from the piths and were stored at − 20 °C, until use.

Pretreatment of wastes

Orange rinds (200 g) were taken and homogenized using a blender. The slurry of crushed rinds and distilled water was prepared in the ratio of 1:5 and was subjected to a Rotary Evaporator (Superfit, PBU-6D), set at 70 °C, 40 rpm and 750 mm of Hg for 1 h. The slurry for which limonene was removed and treated with dilute sulphuric acid (5% v/v) for an hour (Oberoi et al. 2010). The acid hydrolysate was filtered through Whatman Filter no 42. The filtrate and residue, thus obtained, were used separately in the fermentation media.

Limonene analysis

Extraction of limonene

The rinds were cut into small pieces and mass of approximately 0.1 g was taken, which was mixed with methanol in the ratio of 1:50. After shaking vigorously for 5 min, the samples were allowed to stand for 10 min. 0.5 mL of the aliquot from the mixture was taken, diluted to 1:10 times and used for further analysis (Davidowski and DiMarco 2009).

Analysis and quantification of limonene

Gas chromatography-flame ionization detection (GC-FID) analyses were performed on a Shimadzu system composed of GC-17A (ver. 3) equipped with a split/splitless injector, autosampler AOC-20i, and FID (Shimadzu, Milan, Italy). Separations were performed on a MDN5S (Supelco, Bellefonte, PA) 30-m × 0.25-mm i.d. × 0.25-μm film thickness column. The temperature of the program was 50 °C to 250 °C at 3 °C/min. The injection volume was 1.0 μL, the pressure was 102 kPa (constant pressure) and carrier gas was Helium at 30 cm/s of average linear velocity. The split ratio was 1:100 and the detector was set at 280 °C. The data were acquired by Class-VP 4.3 software (Shimadzu) (Mondello et al. 2004).

Isolation and identification of yeast

A sugary sap was collected from *Borassus flabellifer*, commonly known as Toddy palm, by palm tappers located in Bhatan village, Navi Mumbai. The sap was then immediately streaked onto Yeast Peptone Dextrose (YPD) plates and incubated at 30 °C for 48 h.

The colonies obtained were further restreaked on YPD plates till pure culture was obtained. Glycerol stock of the isolate was maintained at − 80 °C. Monochrome staining was performed for the pure culture. Identification of the isolate was done by amplification and sequencing of variable regions within the large subunit rDNA (LSU-rDNA) using DF and DR primers similar to LROR and NL4 (DF-5′ ACC CGCTGAACTTAAGC 3′ and DR-5′ GGTCCGTGTTTC AAGACGG 3′). Sequences were further used for taxa identification using the NCBI BLAST program and compared to the GenBank database and a phylogenetic tree was generated by Neighbour Joining (NJ) method.

Growth profile and cell count

A growth curve analysis of isolated yeast was carried out by inoculation of toddy yeast into YPD broth. These broths were incubated at 30 °C, under shaking conditions (130 rpm). The absorbance (610 nm) was taken for 25 h at an interval of 2 h. The cells in the mid log phase were counted by Hemocytometer (Absher and Marlene 1973) and their viability was checked by Trypan blue exclusion test (Strober 2015).

Media preparation

Growth media

The growth media was prepared containing (g/L): yeast extract 10.0, peptone 20.0, and glucose 50.0. These flasks were incubated at 30 °C, under shaking conditions (130 rpm) (Wilkins et al. 2007).

Fermentation media

The yeast fermentation medium (YFM), prepared contained 50 mM citrate buffer (pH 4.8) with different sugar sources (Table 1). The first set of fermentation media contained sugar mixture (SM) containing 1.4 g/L sucrose, 57.4 g/L glucose, 8.6 g/L galactose and 33.2 g/L fructose (Wilkins et al. 2007). The second, third and fourth media contained orange residue (R), filtrate (F) and a combination of residue and sugar mixture (R + SM), respectively. The second media contained orange residue (R), obtained after filtration of the slurry from which limonene was removed. Similarly, the third media contained the filtrate (F), obtained after filtration of the same slurry. The pH of all the YFMs ranged from 3.5 to 4.5 for all the flasks.

Table 1 Composition of fermentation media

Media no.	Media components	C (mL)	SM (mL)	R (g)	F (mL)
First	I + C + SM	21.5	21.5	–	–
Second	I + C + R	43	–	6	–
Third	I + C + F	21.5	–	–	21.5
Fourth	I + C + SM + R	21.5	21.5	3	–

I inoculum (2%), *C* citrate buffer, *SM* sugar mixture, *R* residue, *F* filtrate

Fermentation

The yeast was grown in growth media (2.5.1) and mid log phase (15–17 h old) culture (2% v/v) was inoculated into different YFM for 3–4 days, 30 °C at 130 rpm (Wilkins et al. 2007). During the fermentation, pH, sugar content (brix and DNSA), were analysed by the following methods.

Sugar content

Determination of sugar concentration by refractometer

The concentration of sugar in a sample can be determined by measuring the refractive index of the sample and the index of refraction of a solution containing sugar is proportional to its concentration and is expressed in brix.

Determination of reducing sugars by 3,5-dinitrosalicylic acid (DNSA)

This method involves the oxidation of the aldehyde functional group present in sugar, for example, glucose and the ketone functional group in fructose, with simultaneous, reduction of 3,5-dinitrosalicylic acid (DNS) under alkaline conditions (Miller 1959).

Analysis and quantification of ethanol by HPLC

The ethanol concentration of the fermentation product was estimated using high performance liquid chromatography (HPLC). These fermentation products obtained, with and without delimonization using four different media (Table 1) were analysed. Standard ethanol for HPLC was purchased from Sigma Aldrich (St. Louis, MO, USA). Purified water was used for sample preparation and dilution; which was dispensed from Barnstead Water Purifier system of APS Water Service Corporation (USA). Mobile phase and standard samples for HPLC analysis

were filtered using PVDF, Durapore® GV 0.22 µm membrane disc. HPLC columns used for analysis was Aminex® HPX-87P, 300 × 7.8 mm. Purified water (18.2 mΩ/cm) was used as a mobile phase which was filtered through PVDF, Durapore® GV 0.22 µm membrane disc and sonication was done for 30 min in order to degas mobile phase prior to using for HPLC analysis. All analytical experiments were conducted using Agilent Technologies-1100 series HPLC, comprising of a system controller, a column compartment, pumps, a degasser, one auto injector with a 100 µL sample loop and Refractive Index Detector (RID) (Bio-Rad Laboratories 2012; McBee and Maness 1983).

The percentage of theoretical yield (%) TY for ethanol production was calculated by the following equation (Wilkins et al. 2007):

$$\% \text{ TY} = 100 \times (\text{Et} - \text{E0})/[0.511 \times (\text{Fr} + \text{Gl} + \text{Ga}) + 0.538 \times (\text{Su})],$$

Where Et is the concentration of ethanol at time t, E0 is the initial concentration of ethanol at time 0, Fr is the initial concentration of fructose, Gl is the initial concentration of glucose, Ga is the initial concentration of galactose, Su is the initial concentration of sucrose (concentrations expressed as g/L).

Results and discussion

Limonene analysis

Limonene is a common naturally occurring compound with a citrus scent. It is used as an additive in food products and fragrances, and is classified by the United States Food and Drug Administration (USFDA) as Generally Recognized as Safe (GRAS). It has also been approved by the United States Environmental Protection Agency (USEPA) for usage as a natural insect repellent and personal care products. In this study, the quantification of limonene by GC indicated that limonene was partially removed from the peels. The GC chromatogram of standard limonene (0.8 mg/mL) was used to calculate the limonene content in the methanolic extract of the orange peels (Fig. 1a). The methanol extract of the peels showed D-limonene with a value of 4.642 mg/mL (Fig. 1b). The yield of D-limonene with the current extraction process was found to be 0.4%. The basic parameters affecting the quality of an extract and thereby, the yield are the solvent used for extraction, the manufacturing process used, with the type of equipments employed. Giwa et al. (2018) have reported the maximum yield of essential oil from the orange peels by the methods steam distillation, water distillation and solvent extraction were 4.4%, 3.47% and 2.536%, respectively.

Isolation and identification of yeast

The colony morphology of the pure cultures, obtained onto YPD plates, were round, white, opaque and mucoid with inconsistency. The monochrome staining showed budding oval cells, typically seen for *S. cerevisiae*. The BLAST analysis for the amplified sequence obtained using primers DF and DR for the variable region of LSU-rDNA done showed 99% similarity to *S. cerevisiae*. The sequence was submitted to NCBI with the accession number MK373758. The isolate was named as *S. cerevisiae* strain BT1 and was further used for fermentation studies.

Growth profile and cell count

The growth curve of the isolate followed the typical sigmoidal curve, with exponential phase from 8th to 22nd hours, after inoculation. The viability and the cell count of the cells from the mid-log phase culture were determined as 95% and cell count was found to be $11{,}776 \times 10^4$ cells/mL. The mid-log phase culture and 10% inoculum size was used into the fermentation media.

Fermentation

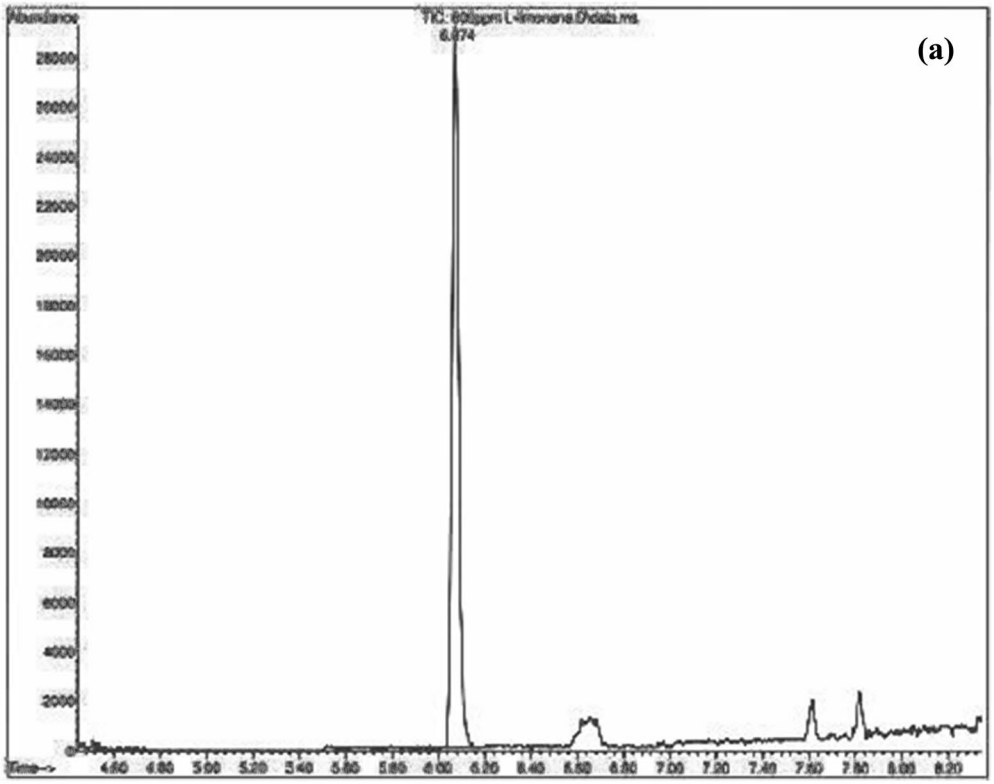
Sugar content

A decrease in sugar content was observed after 3 days of fermentation. Different sugar sources showed a different degree of utilization. It was observed that in fermentation samples with SM, R, F, and R + SM, decreased to 23%, 9%, 12.5% and 50%, respectively. Whereas, after 4 days of fermentation, there was no decrease in sugar content in the sample containing SM, but further decrease to 32% was observed in samples containing R. In case of the sample with F, only 3% additional decrease was obtained after 4 days of fermentation. The sample with R + SM was further decreased by 10%, after 4 days of fermentation. The trend and percentage decrease in sugar content measured by both brix DNSA methods were comparable (Table 2). The sugars released after treatment with acid, alkali or enzyme treatment is one of the major critical parameters in the determination of ethanol yield (Irfan et al. 2014).

Ethanol estimation

The HPLC chromatogram of standard ethanol (0.78 mg/mL) showed a retention time of 23 min (Fig. 2) and titre values of the samples were tabulated accordingly. The ethanol estimated was negligible in all four media, wherein limonene was not extracted. Thereafter, limonene was extracted as mentioned in section "Pretreatment of wastes" wherein 0.4% of limonene was estimated by GC ("Extraction of

Standard Limonene : 800 ppm



Sample : Limonene content (4.642mg/mL)

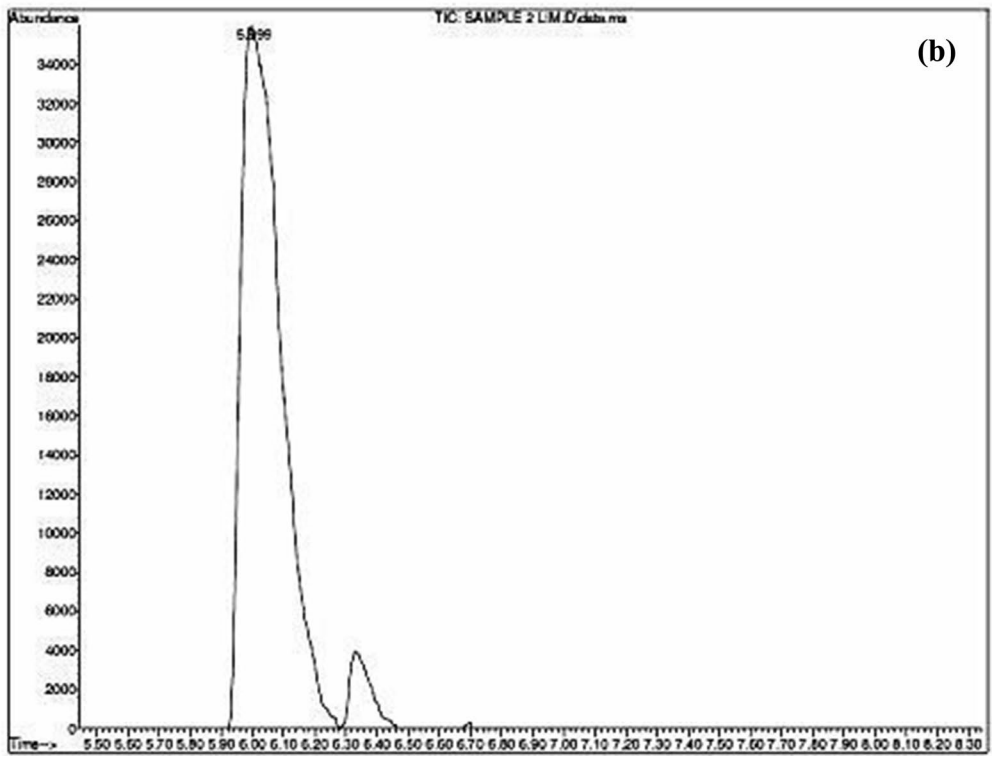


Fig. 1 GC chromatogram **a** standard limonene (0.8 mg/mL), **b** orange extract (4.6 mg/mL)

Table 2 Decrease in sugar content during fermentation

S. no	Days	Fermentation media	Sugar content (Brix)		Sugar content (mg/mL)	
			Initial	Final	Initial	Final
1	3	SM	2.6 ± 0.0.1	2 ± 0.1	1.45 ± 0.07	0.78 ± 0.04
2		R	2.2 ± 0.2	2 ± 0.1	0.80 ± 0.06	0.73 ± 0.06
3		F	1.6 ± 0.1	1.4 ± 0.2	1.40 ± 0.09	0.80 ± 0.05
4		R + SM	6.4 ± 0.5	3 ± 0.1	1.46 ± 0.11	1.12 ± 0.90
5	4	SM	2.6 ± 0.2	2 ± 0.1	1.45 ± 0.08	0.70 ± 0.07
6		R	2.2 ± 0.1	1.5 ± 0.2	1.00 ± 0.05	0.75 ± 0.05
7		F	1.6 ± 0.3	1.2 ± 0.7	1.40 ± 0.08	0.75 ± 0.08
8		R + SM	6.4 ± 0.3	2.5 ± 0.5	1.46 ± 0.13	0.95 ± 0.06

SM sugar mixture, *R* residue of treated orange waste, *F* filtrate of treated orange waste

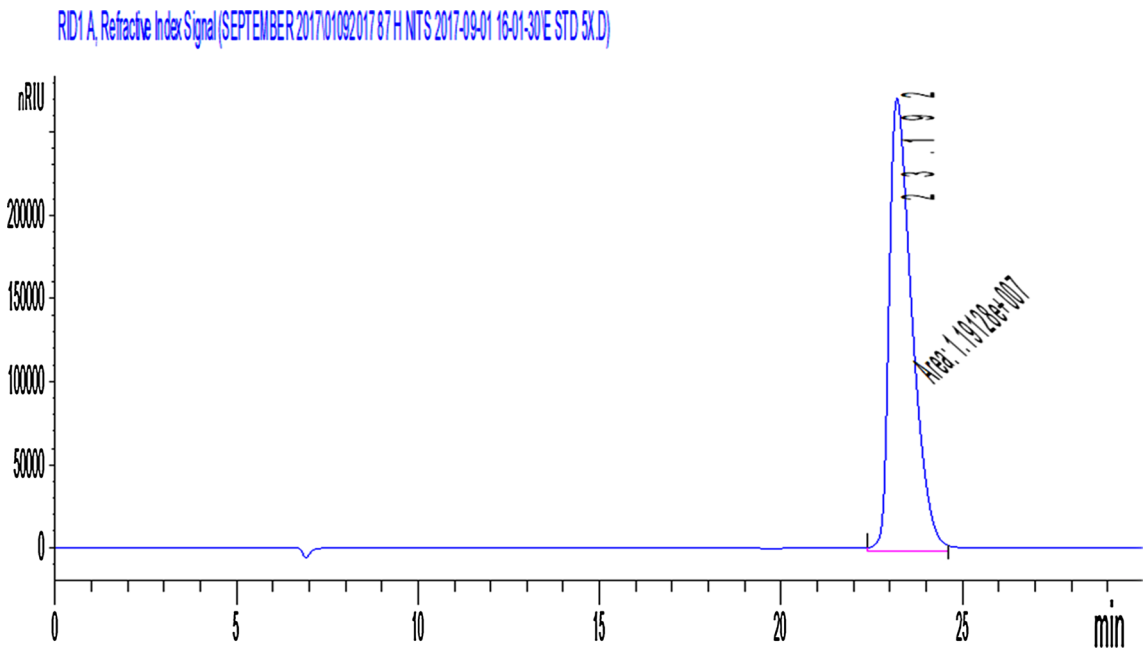


Fig. 2 HPLC chromatogram of standard ethanol

limonene"). After limonene extraction, the slurry from which limonene was removed, was used in second, third and fourth fermentation media ("Fermentation media") and ethanol content was estimated. The negative control containing SM, which was not inoculated with yeast showed negligible titre (Fig. 3). The presence of R in R + SM showed a titre value of 8.7 g/L after 3 days (Fig. 4), whereas a higher titre value of ethanol (9.5 g/L) was obtained, after 4 days of fermentation (Fig. 5). The TY calculated for ethanol obtained after 3 and 4 days of fermentation is 16.9% and 18.5%, respectively.

In a similar report (Mishra et al. 2012), 9.8 g/L of ethanol was reported by grinding, enzymatic hydrolysis simultaneous saccharification of orange peel, after 3 days of fermentation. In another study, Santi et al. (2014) have reported 15 g/L of ethanol yield by using acid catalyzed steam explosion and enzymatic hydrolysis using separate hydrolysis and fermentation. In this study, the reason for low titre might be the presence of residual limonene and dilute acid hydrolysis pretreatment strategy. The studies based on the impact of D-limonene on the ethanol production from orange peel shows that removal of limonene is very essential for the production of ethanol as it acts as a major inhibitor for downstream processing and therefore, reduce the yield (Wilkins 2009).

All standard alcohols such as ethanol can be analysed by using a different analytical technique such as GC and HPLC for precise assay or titre value estimation, which provides the insight of the fermentation process. The content of limonene plays a very significant role for the titre value development of the ethanol; as traces of limonene may hinder the process for the productivity and yield of the ethanol production

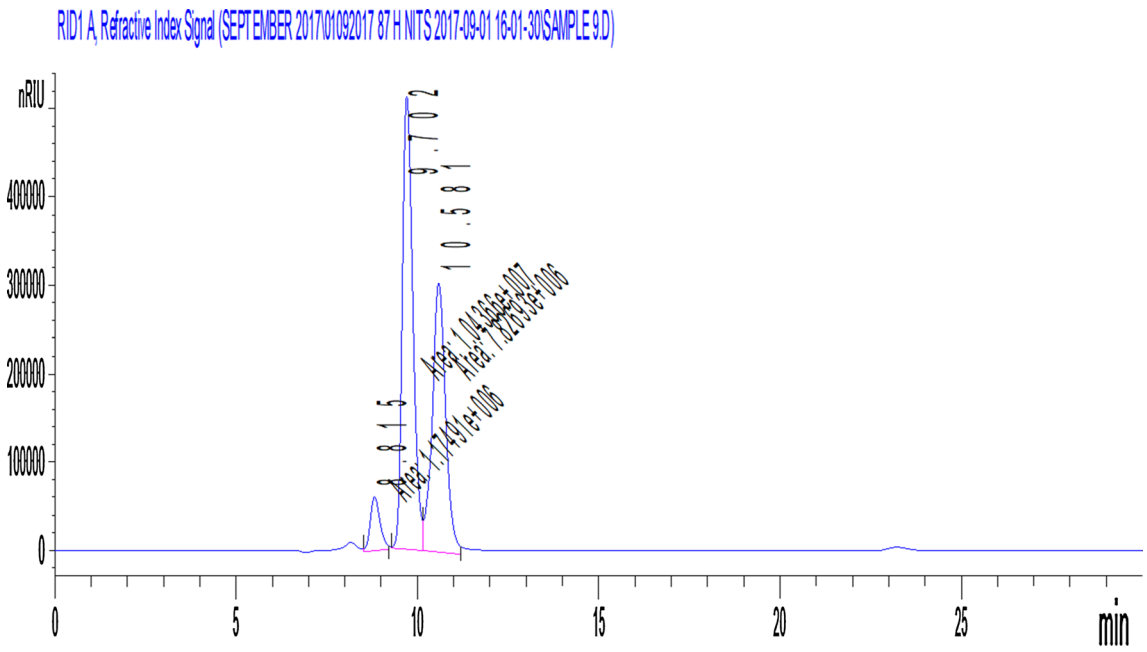


Fig. 3 HPLC chromatogram of sample after 4 days of fermentation using sugar mixture (SM)

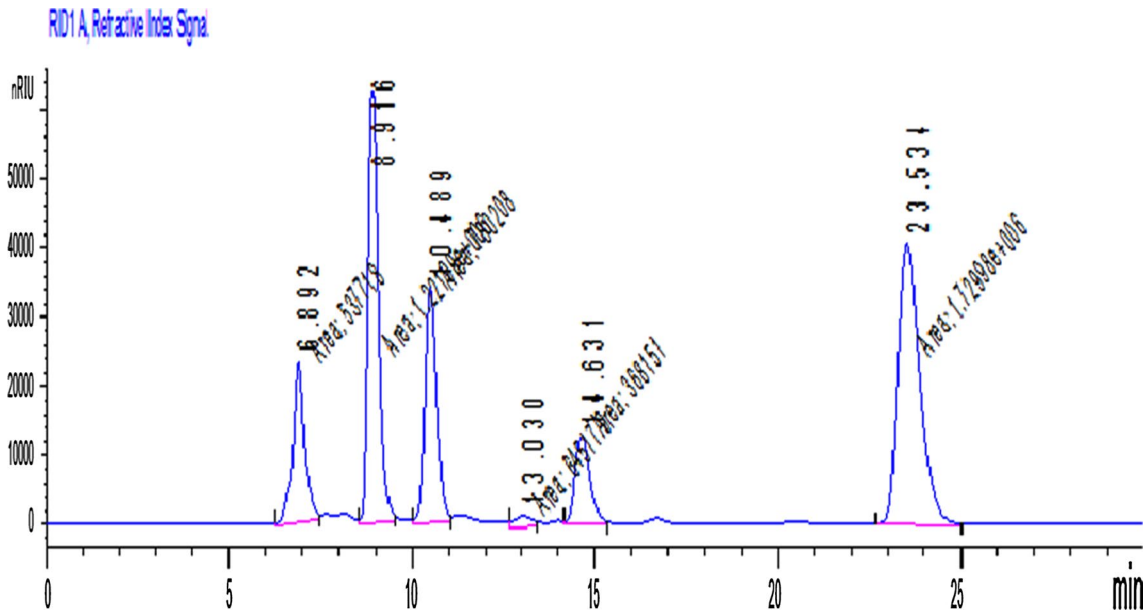


Fig. 4 HPLC chromatogram of sample after 3 days with sugar mixture(SM) and residue (R)

(McBee and Maness 1983). The accuracy of the analytical technique for the detection of the content of ethanol plays a very significant impact on the process development and its design; also with due consideration for the limonene analysis with equivalent importance and significance in the productivity (Brokl et al. 2011). In the citrus waste biorefinery, compounds with high value such as D-limonene (Bousbia et al. 2009), pectin, and flavonoids contained in citrus waste should be extracted, leaving the lignocellulosic fraction to be used as a substrate in the fermentation process for the production of biofuels or value-added chemicals (Vlysidis et al. 2017). Increase in productivity of ethanol in the current study can be achieved by optimizing various factors. The first approach can be an additional removal of limonene, to attain its residual concentration of 0.1% (Wilkins et al. 2007). This can be achieved by optimizing the parameters

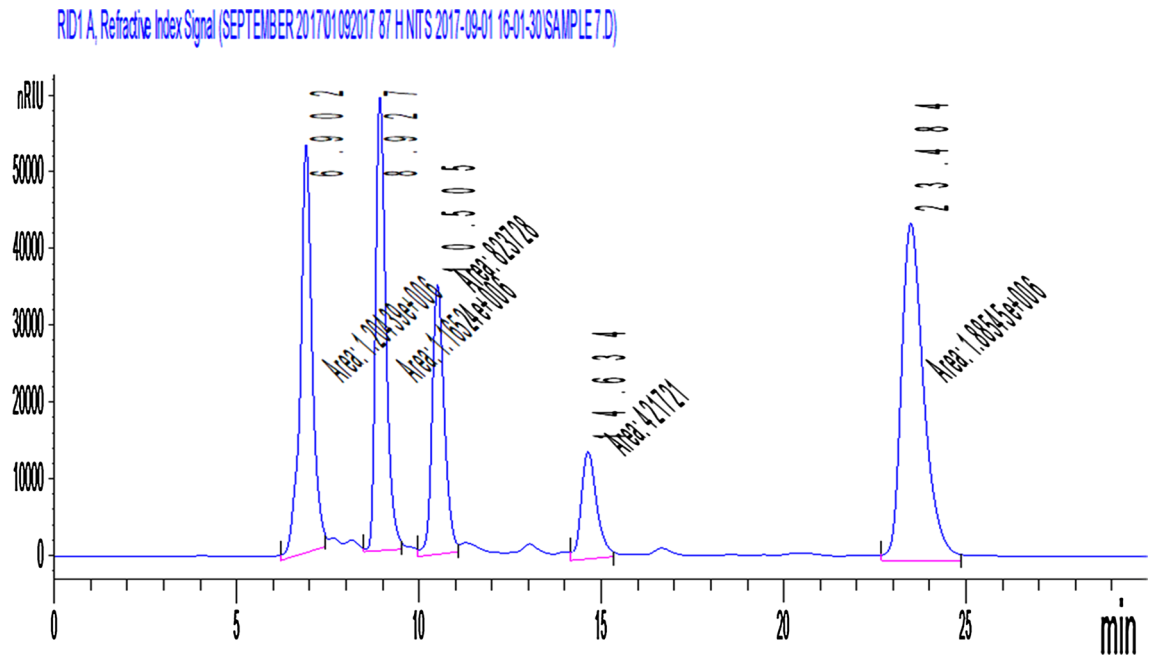


Fig. 5 HPLC chromatogram of sample after 4 days of fermentation in sugar mixture (SM) and residue (R)

of the rotary evaporator. Limonene acts as a suppressor for ethanol production by yeast and further processing of limonene for various applications (Wilkins 2009). The second approach can be to ensure complete hydrolysis of complex sugars like pectin, cellulose, hemicellulose, and lignin, present in orange peel in order for the microorganism to utilize the simple sugars and convert into ethanol (Marín et al. 2007). The third approach can be the optimization of inoculum size, as reported by Joshi et al. (2015), wherein 20% (v/v) inoculum gave a higher yield of ethanol. The fourth approach is the removal of pectin since it increases the viscosity of the fermentation media and interferes with the process of fermentation (John et al. 2017).

Valorisation of orange peel

The processing of orange peel in this manner and combining the approaches mentioned in section "Ethanol estimation", not only enables us to minimize waste disposal but also adds further value through the production of other high-value products: limonene and pectin. The recovery of limonene reduces the cost of production of bioethanol from orange peel waste by 30% (Zhou et al. 2007). Of late, the limonene is being studied as aviation fuel (Tracy et al. 2009), apart from being used as degreaser, pesticides, and fragrances (Karr and Coats 1988). The separated pectin can be used in the food industry as a thickening, gelling or stabilizing agent (Ezejiofor et al. 2011). This utilization aims for zero waste economy which can help to tackle waste management, economic and environmental problems (Mirabella et al. 2014).

Conclusion

With further optimization and validation, the present method can be used for large scale production of bioethanol along with D-limonene from orange peel. The process exploits waste management through valorization of orange peels, which is readily available. This will help to alleviate energy crisis with avoidance of virgin land and water resources helping to establish a sustainable renewable resource. Fermented drinks from toddy have been used traditionally and hence, the strain would not be harmful even if present in residual concentrations in the fermented product. Small citrus fruit units can get rid of their waste with low investment as an economically feasible approach which can be sold to the government for a subsidized rate to achieve the valorization of orange peels. Industries that produce and sell packaged citrus juices can get rid of the on-site waste by utilizing it for the production of alcohol with limonene and pectin as byproducts.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

Absher M (1973) Hemocytometer counting. In: Kruse PF Jr, Patterson MK Jr (eds) Tissue culture: methods and applications. Academic Press, New York, pp 395–397

Ahmad Z, Asgher M, Iqbal HMN (2017) Enzyme treated wheat straw based PVOH: development and characterization. BioResources 12(2):2830–2845

Arevalo-Gallegos A, Ahmad Z, Asgher M, Parra-Saldivar R, Iqbal HMN (2017) Lignocellulose: a sustainable material to produce value-added products with a zero waste approach—a review. Int J Biol Macromol 99:308–318

Asgher M, Ahmad Z, Iqbal HMN (2017) Bacterial cellulose assisted de-lignified wheat straw-PVA based biocomposites with novel characteristics. Carbohyd Polym 161:244–252

Bilal M, Asgher M, Iqbal HMN, Hu H, Zhang X (2017) Biotransformation of lignocellulosic materials into value-added products—a review. Int J Biol Macromol 98:447–458

Bio-Rad Laboratories Inc. (2012) Chromatography Aminex HPLC columns. http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6333.pdf

Bousbia N, Vian MA, Ferhat MA, Meklati BY, Chemat F (2009) A new process for extraction of essential oil from citrus peels: microwave hydrodiffusion and gravity. J Food Eng 90:409–413

Brokl M, Hernández-Hernández O, Soria AC, Sanz ML (2011) Evaluation of different operation modes of high performance liquid chromatography for the analysis of complex mixtures of neutral oligosaccharides. J Chromatogr A 1218(42):7697–7703

Calabro PS, Folino A, Tamburino V, Zappia G, Zema DA, Zimbome SM (2017) Valorisation of citrus processing waste: review. Proceedings Sardinia 2017/16th international waste management and landfill symposium. S. Margherita di Pula, Cagliari, pp 1–42

Davidowski S, DiMarco B (2009) The extraction and quantification of limonene from citrus rinds using GC/MS, 1st edn [ebook]. Perkinelmer, Waltham, pp 1–3

Erasto P, Viljoen AM (2008) Limonene: biosynthetic, ecological and pharmacological relevance. Nat Prod Commun 3:1193–1202

Ezejiofor TLN, Eke NV, Okechukwu RI, Nwoguikpe RN, Duru CM (2011) Waste to wealth: industrial raw materials potential of peels of Nigerian sweet orange (*Citrus sinensis*). Afr J Biotechnol 10(33):6257–6264

Giwa SO, Muhammad M, Giwa A (2018) Utilizing orange peels for essential oil production. J Eng Appl Sci 13(1):17–27

Iqbal HMN, Kyazze G, Keshavarz T (2017) Advances in the valorization of lignocellulosic materials by biotechnology: an overview. BioResources 8(2):3157–3176

Irfan M, Nadeem M, Syed Q (2014) Ethanol production from agricultural wastes using *Saccharomyces cerevisiae*. Braz J Microbiol 45(2):457–465

John I, Muthukumar K, Arunagiri A (2017) A review on the potential of citrus waste for D-limonene, pectin, and bioethanol production. Int J Green Energy 14(7):599–612

Joshi SM, Waghmare JS, Sonawane KD, Waghmare SR (2015) Bioethanol and bio-butanol production from orange peel waste. Biofuels 6(1–2):55–61

Karr LL, Coats JR (1988) Insecticidal properties of D-limonene. J Pesticide Sci 13:287–290

Lin CSK, Pfaltzgraff LA, Herrero-Davila L, Mubofu EB, Abderrahim S, Clark JH, Koutinas AA, Kopsahelis N, Stamatelatou K, Dickson F, Thankappan S, Mohamed Z, Brocklesby R, Luque R (2013) Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. Energy Environ Sci 6(2):426–464

Marín FR, Soler-Rivas C, Benavente-García O, Castillo J, Pérez-Alvarez JA (2007) By-products from different citrus processes as a source of customized functional fibres. Food Chem 100(2):736–741

McBee GC, Maness NO (1983) Determination of sucrose, glucose, and fructose in plant tissue by high-performance liquid chromatography. J Chromatogr 264:474

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426

Mirabella N, Castellani V, Sala S (2014) Current options for the valorization of food manufacturing waste: a review. J Clean Prod 65:28–41

Mishra J, Kumar D, Samanta S, Vishwakarma KM (2012) A comparative study of ethanol production from various agro residues by using *Saccharomyces cerevisiae* and *Candida albicans*. J Yeast Fungal Res 3(2):12–17

Mondello L, Casilli A, Tranchida PQ, Costa R, Dugo P, Dugo G (2004) Fast GC for the analysis of citrus oils. J Chromatogr Sci 42(8):410–416

Mrudula S, Anitharai R (2011) Pectinase production in solid state fermentation by *Aspergillus niger* using orange peel as substrate. Glob J Biotechnol Biochem 6:64–71

Oberoi HS, Vadlani PV, Madl RL, Saida L, Abeykoon JP (2010) Ethanol production from orange peels: two-stage hydrolysis and fermentation studies using optimized parameters through experimental design. J Agric Food Chem 58(6):3422–3429

Ohgren K, Bengtsson O, Gorwa-Grauslund MF, Galbe M, Hahn-Hägerdal B, Zacchi G (2006) Simultaneous saccharification and cofermentation of glucose and xylose in steam-pretreated corn stover at high fiber content with *Saccharomyces cerevisiae* TMB3400. J Biotechnol 126(4):488–498

Santi G, Crognale S, D'Annibale A, Petruccioli M, Ruzzi M, Valentini R, Moresi M (2014) Orange peel pretreatment in a novel lab-scale direct steam-injection apparatus for ethanol production. Biomass Bioenerg 61:146–156

Strober W (2015) Trypan blue exclusion test of cell viability. Curr Protoc Immunol 2:111 A3.B.1-3

The Daily Records (2018) Top 10 largest citrus producing countries in the world. http://www.thedailyrecords.com/2018-2019-2020-2021/world-famous-top-10-list/world/largest-citrus-producing-countries-world-statistics-states/6867/

Tracy NI, Chen D, Crunkleton DW, Price GL (2009) Hydrogenated monoterpenes as diesel fuel additives. Fuel 88(11):2238–2240

USDA (2008) "US. Biobased Products: Market Potential and Projections Through 2025". Office of the chief Economist, Office of Energy Policy and New Uses, U.S. Department of Agriculture

USDA (2018) Citrus: world markets and trade, pp 1–2. https://www.fas.usda.gov/data/citrus-world-markets-and-trade

Vlysidis A, Koutinas A, Kookos I (2017) Techno-economic evaluation of refining of food supply chain wastes for the production of chemicals and biopolymers. In: Morone P, Papendiek F, Tartiu VE (eds) Food waste reduction and valorisation :sustainability assessment and policy analysis. Springer, New York, pp 166–167

Wilkins MR (2009) Effect of orange peel oil on ethanol production by *Zymomonas mobilis*. Biomass Bioenerg 3(3):538–541

Wilkins MR, Suryawati L, Maness NO et al (2007) Ethanol production by *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* in the presence of orange-peel oil. World J Microbiol Biotechnol 23:1161

Wysocka J, Doskocz J, Haller P, Kardasz P, Jaworska E (2015) The use of alcohols and their compounds as biofuel and gasoline blends. J Civ Environ Eng 5:187

Zhou W, Widmer W, Grohmann K (2007) Economic analysis of ethanol production from citrus peel waste. In: Proceedings of the Florida State Horticultural Society, vol 120, pp 310–315