Saccharification of Mango peel wastes by using microwave assisted alkali pretreatment to enhance its potential for bioethanol production

Garima Tiwari*, Abhishek Sharma and Satyawati Sharma

Centre for Rural Development & Technology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi-110016, India

***Corresponding author:**

Research scholar

Centre for Rural Development and Technology, Indian Institute of Technology Delhi

Hauz Khas, New Delhi- 110016, India

Email: tiwarigarima21@yahoo.com

Abstract

India ranks first among world's mango producing countries accounting for about 50% of the world's mango production. India's share is around 52% of world production i.e. 12 million tonnes as against world's production of 23 million tonnes .The current study presents a systematic exploration of the influences of microwave assisted alkali pretreatment (including power and irradiating time) on enzymatic hydrolysis and reducing sugar content of mango peel wastes (FPW). It was observed that higher reducing sugar concentration was observed at 450 W. The enzymatic hydrolysis of microwave assisted alkali pretreated mango peel waste (1 % NaOH) under optimal conditions (450W for 2min), gave maximum reducing sugar i.e. 0.704 g/g dry biomass. Atomic force microscopy revealed that surface of all FPWs got changed noticeably when microwave assisted alkali pretreatment was employed. X-ray diffraction also showed the prominent role of microwave heating in the disruption of recalcitrant structures and improving the enzymatic digestibility of MPWs. We believe that microwave assisted alkali pretreatment could be an energy-saving technology aimed to valorize the waste in a cost effective way.

Keywords: Microwave assisted alkali pretreatment; Mango peel wastes; Bioethanol; Enzymatic hydrolysis; Reducing sugar yield

INTRODUCION

Almost 60% of processed fruits are converted into waste that includes peel, seeds and membrane residues. According to Zentek *et al*. (2014), despite the usefulness of fruit peel wastes (FPWs) as animal feed, they are now disfavored due to decline in livestock farming in industrialized countries. Furthermore, FPWs when dumped into the landfills create serious environmental problems (Choi *et al*. 2015). The utilization of FPWs as promising feedstock for green energy production particularly bioethanol, could lead to conversion of potential problem into a sustainable solution.

After banana the largest production in India is of mango. The processing of mango fruit generates high quality of solid and liquid wastes. Pulp, peel and fibrous materials are obtained during the preparation of raw material. This contributes about 40 to 50% of total fruit waste. Annually utilization of this mango waste 0.4 to 0.6 million tons of mango peel is generated in India (Anonymous, 2004).

Depolymerization of structural carbohydrate polymer into monomeric sugars and their subsequent conversion to ethanol as biofuel these are the two main steps of bioethanol production (Gupta *et al*. 2010). In this context, microwave irradiation is found to be a potent alternative to conventional heating in acid/alkali pretreatment in improving enzymatic hydrolysis efficiency. Studies have shown that microwave irradiation can uniformly alter the ultrastructure of cellulose in short span of time without generating any waste product (Gong *et al*. 2010).

In present study, we have explored the efficacy of microwave-assisted alkali pretreatment of mango alone and in combination of all. The present approach was assessed by evaluating the reducing sugar (RS) from mango peel waste (MPW) at different microwave power and irradiation time. The structural differences of raw and pretreated MPW were also examined using

X-ray diffraction (XRD) to correlated the yield of reducing sugar with the availability of desired polysaccharides particularly cellulose and hemicelluloses.

MATERIALS AND METHODS

Raw materials

Peel wastes of mango (MPW) were collected from the local fruit juice shops at Indian Institute of Technology Delhi campus. All peel wastes were cut dried and ground to the fine powder. The grounded biomass was sieved through 20-40 mesh size to obtain a particle size of 1-3 mm and the sieved material was stored at room temperature till further usage.

Chemical analysis of the feedstocks

Moisture content of fruit peels was determined by heating them in an oven at 105 $^{\circ}$C overnight, until they reached a constant weight. The ash content was determined by incineration at 550 $^{\circ}$C ± 5 $^{\circ}$C for 24 h in a muffle furnace according to AOAC methods. Cellulose content in the fruit peels was estimated by the method of Updegroff (1969), while the content of hemicelluloses was calculated by the difference between NDF and ADF (Goering and Van Soest P J 1975). The calculation for lignin was based on the difference between ADF and cellulose. Carbon and nitrogen contents were estimated using a CHN analyzer (CHNOS Elementar, Vario EL III model). Crude protein was calculated by multiplying the nitrogen content by a factor of 6.25 (Gothwal *et al*. 2012). RS estimation was done by DNS method (Miller 1959).

XRD analysis

Powder X-ray diffraction (XRD) patterns of the analysis of raw and pretreated MPW were carried out on a Bruker D8 Advance diffractometer with Ni-filtered Cu Ka radiation (k = 0.15406 nm) with a scan speed of 2° min^{-1} and a scan range of 10 –60° for wide angle diffraction at 30 kV and 15 mA. Peaks were fitted by using Origin 8.5. Crystallinity index (CrI) was calculated by using the following formula (Segal *et al*. 1959).

CrI (%) = $(I_{002}-I_{18.0°}) / I_{002} \times 100$

where I_{002} is the maximum intensity of the (002) lattice diffraction at 2Θ = 22.4° and $I_{18.0°}$ is the intensity diffraction of the background scatter at 2 Θ =18.0°. Data were analyzed by peak deconvolution method (Park *et al*. 2010). The crystallinity degree (CrD) was calculated according to following formula.

CrD = $F_c / (F_a + F_c) \times 100\%$

where Fc and Fa are the area of crystal and amorphous regions, respectively.

Microwave-assisted alkali pretreatment

A domestic microwave (Samsung M183DN, Korea) with an operating frequency of 2450 MHz and output power of 100–600 W was used to study the effect of microwave pretreatment on FPW. 10% peel waste was loaded to 1% NaOH solution in a 500 ml stoppered flask at the center of a rotating circular plate in the microwave oven. For power optimization MPW was irradiated at 100-600 W for 5 min. For optimizing the irradiating time, the experiments with variable irradiating time (1-6 min) were carried out at an already optimized microwave power of a particular FPW. The pretreated MPW were obtained by pump filter and kept for drying for compositional and other structural analysis. The dried pretreated MPW were further used for enzymatic hydrolysis. Experiments were carried out in triplicates and average values were recorded.

Enzymatic hydrolysis

Enzymatic hydrolysis of MPW (200 mg) was carried in 50 ml capped tube containing 20 ml 0.1M sodium acetate buffer (pH 5.0) and enzyme (30 FPU/g DS) from *Aspergillus niger* (P Code 101273533, Sigma, USA; 0.8 U/mg) incubated at 50 $^{\circ}$C and 150 rpm for 48 h.

Statistical analyses

All experiments were done in triplicates and all values are mean.

RESULTS AND DISCUSSION

Compositional analysis of fruit peel wastes

Proximate analysis (Table 1a) revealed that the MPW had highest moisture content of 78.34% while the lignin and protein content was 8.38 % and 2.36% respectively. The ash content of MPW was 4%. A perusal of the results showed that MPWs was rich in insoluble sugars (cellulose and hemicelluloses) justifying their selection as a promising feedstock for bioethanol production (Table 1b). Cellulose (34.22%) and hemicellulose (26.17%) content was high. In accordance to our findings, compositional analysis of dried MPW by Reddy *et al*. (2011) revealed a higher amount of reducing sugars. Likewise, Choi *et al*. (2015) reported high levels of fermentable sugars (viz., glucose, sucrose and fructose) in the peel wastes of banana, apple, pear and citrus fruits.

Table 1a Compositional analysis of raw mango peel wastes (results expressed as weight percent, oven dry weight basis)

Parameters (%)	MPW
Moisture	78.34 ± 0.16
Ash	4 ± 0.11
Insoluble dietary fibre	55.43 ± 1.84

Protein	2.36 ± 0.01

*Abbreviations used: MPW: Mango peel waste

*The data are displayed as the mean ± SD, and are derived from three independent experiments

Table 1b Lignocellulosic changes of mango peel wastes before and after microwave assisted pretreatment (results expressed as weight percent, oven dry weight basis)

	Before Pretreatment (%)			After Pretreatment (%)		
Fruit wastes	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
MPW	34.22 ± 2.09	17.88 ± 2.01	8.38 ± 1.52	44.1 ± 3.00	26.17 ± 2.01	3.11 ± 1.44

*Abbreviations used: MPW: Mango peel waste

Effect of microwave assisted alkali pretreatment of MPWs

Fig 1 illustrates the effect of microwave assisted alkali pretreatment for 5 min on reducing sugar content of selected MPWs. MPW gave maximum RS yield (0.510 g/g) at 450 W. In an another experiment, the time course profiles studied for maximum RS yield from MPW at 450 W were studied (Fig 2). Maximum RS yield was obtained from MPW (0.704 g/g) with 4 min treatment. It was observed that at specific higher microwave power, shorter irradiating time was required to attain maximum RS yield after enzymatic hydrolysis. At 450 W, microwave pretreatment of MPW for 6 min resulted in less RS yield compared to yield observed at 4 min treatment time. It is believed that with increase in time at a given power, the water content of the substrate becomes too low for microwave irradiation to efficiently execute the hydrolysis of complex sugars (Xuebin *et al*. 2011).

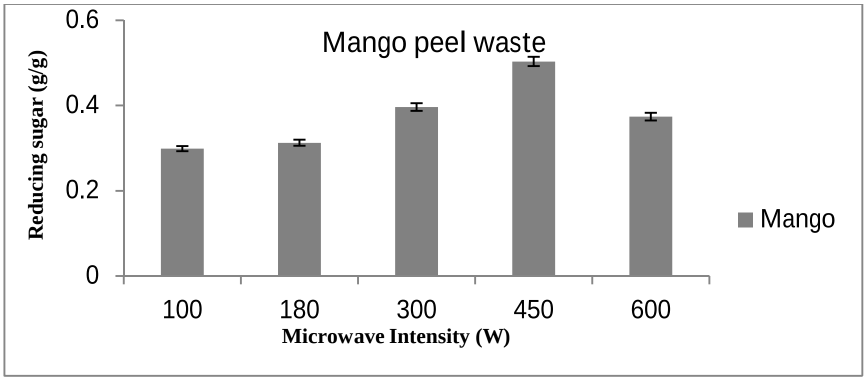

Fig 1 Maximum reducing sugar yield of microwave assisted alkali pretreated mango peel waste at various microwave power

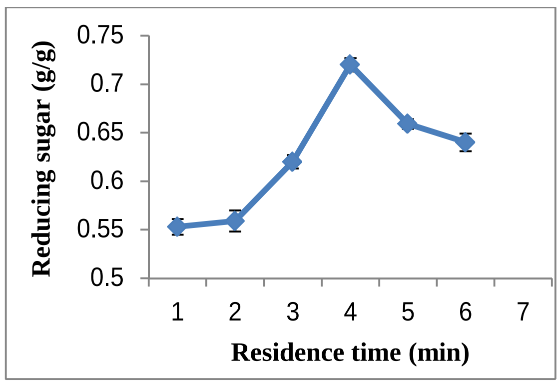

Fig 2 Reducing sugar yield of mango peel wastes (MPW) during time profiling by microwave assisted alkali pretreatment at best identified microwave power (450W)

Microwave irradiation is known to degrade hemicelluloses and lignin, thereby increasing the accessibility of hydrolytic enzymes (Binod *et al*. 2012). The disruption is caused through dielectric polarization by microwave energy and breakage of ester bonds among cross-linking lignin and xylan hemicelluloses by aqueous NaOH (Nomanbhay *et al*. 2013). This combined effect might have caused the loosening of biomass and a better exposure of cellulose for enzymatic hydrolysis.

XRD analysis

XRD profile of raw and microwave assisted alkali pretreated MPWs are shown in Fig 4. The CrI and CrD of MPWs before and after pretreatment are given in Table 2. In general, the CrI and CrD values were higher in pretreated samples compared to raw ones. The enhancement of CrI value after microwave pretreatment could be due to removal of amorphous part in the form of lignin and hemicelluloses from the treated MPWs. The results proved the efficacy of microwave irradiation in removal of amorphous portion and increasing crystalline cellulose content in the MPWs (Binod *et al*. 2012).

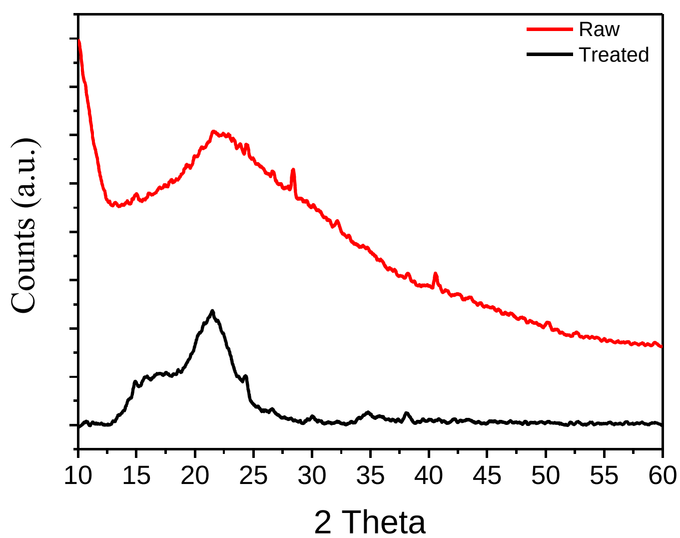

Fig 3 X-ray diffraction pattern of raw and treated mango peel wastes

Table 2 Crystallinity index and degree of raw and microwave pre-treated mango peel waste (results expressed as weight percent, oven dry weight basis)

Parameters	Raw MPW	Pretreated MPW
Crystallinity index	10.02± 0.009	53.14 ± 0.005
Degree of crystallinity	9.95± 0.012	69.08 ± 0.008

Pretreated MPW: 450W for 5min

*Abbreviations used: MPW: Mango peel waste

* The data are displayed as the mean ± SD, and are derived from three independent experiments

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REFERENCES

1. Anonymous. 2004. By-products and waste utilization (value addition of mango processing waste). Food Digest. 27: 14-17.
2. Binod P, Satyanagalakshmi K, Sindhu R, Janu K U, Sukumaran R K and Pandey A.2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. *Renewable Energy* **37**: 109-116.
3. Choi I S, Lee Y G, Khanal S K, Park B J and Bae H J.2015. A low-energy, cost-effective approach to fruit and citrus peel waste processing for bioethanol production. *Applied Energy* **140**: 65–74.
4. Goering H K and Van Soest P J.1975. Forage fiber analysis. *Agriculture Research Series Handbook*: 379.
5. Gong G, Liu D and Huang Y.2010. Microwave-assisted organic acid pretreatment for enzymatic hydrolysis of rice straw. *Biosystem Engineering* **107**: 67-73.
6. Gupta R, Sharma K K and Kuhad R C.2010.Separate hydrolysis and fermentation (SHF) of *Prosopis juliflora*, a woody substrate, for the production of cellulosic ethanol by *Saccharomyces cerevisiae* and *Pichia stipitis*-NCIM 3498. *Bioresource Technology* **100**: 1214-1220.
7. Gothwal R, Gupta A, Kumar A, Sharma S and Alappat B J.2012. Feasibility of dairy waste water (DWW) and distillery spent wash (DSW) effluents in increasing the yield potential of *Pleurotus flabellatus* (PF 1832) and *Pleurotus sajor-caju* (PS 1610) on bagasse. *3 Biotech* **2**: 249–257.
8. Miller G M.1959.Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* **31**: 426-8.

9. Nomanbhay S M, Hussain R and Palanisamy K.2013.Microwave-Assisted Alkaline Pretreatment and Microwave Assisted Enzymatic Saccharification of Oil Palm Empty Fruit Bunch Fiber for Enhanced Fermentable Sugar Yield. *Journal of Seismology and Earthquake Engineering* **3**: 7-17.

10. Park S, Baker J O, Himmel M E, Parilla P A and Johnson D K.2010. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnology for Biofuels* **3**: 1-10.

11. Reddy L V, Reddy O V S, Wee Y J.2011. Production of ethanol from mango (*Mangifera indica* L.) peel by *Saccharomyces cerevisiae* CFTRI101. *African Journal of Biotechnology* **10**: 4183-4189.

12. Segal L, Greely J J, Martin A E J and Conrad L M.1959.An empirical method for estimating the degree of crystallinity of native cellulose using X-ray diffractometer. *Textile Research Journal* **29**: 780-94.

13. Sharma A, Sharma S and Dalela M.2014. Nematicidal activity of *Paecilomyces lilacinus* 6029 cultured on Karanja cake medium. *Microbial Pathogenesis* **75**: 16-20.

14. Updegraff D M.1969. Semi micro determination of cellulose in biological materials. *Analytical Biochemistry* **32**: 420-424.

15. Xuebin L, Bo X, Yimin Z and Irini A.2011. Microwave pretreatment of rape straw for bioethanol production: Focus on energy efficiency. *Bioresource Technology* **102**: 7937–7940.

16. Zentek J, Knorr F and Mader A.2014.Reducing waste in fresh produce processing and households through use of waste as animal feed. Global Safety of Fresh Produce: A Handbook of Best Practice, Innovative Commercial Solutions and Case Studies: 140-152.