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Microwave assisted alkali pretreatment of fruit peel wastes for enzymatic hydrolysis

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ABSTRACT

The present study proved the potential of generating reducing sugars from various fruit peel wastes (pineapple, mango, orange) using microwave assisted alkali pretreatment. The results showed that enzymatic hydrolysis of pretreated pineapple peel waste produced maximum reducing sugar, i.e. 0.774 g/g dry biomass. X-ray diffraction study revealed the prominent role of microwave heating in the disruption of recalcitrant lignocellulosic structures and improving the enzymatic digestibility of FPWs. We believe that microwave assisted alkali pretreatment of FPWs could be an energy-saving technology aimed to valorize the agro-industrial wastes in a cost effective way.

Key words: Enzymatic hydrolysis, Fruit peel wastes, Microwave assisted alkali pretreatment, Reducing sugars

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.

Bioethanol production process comprises two main steps: depolymerization of structural carbohydrate polymer into monomeric sugars and their subsequent conversion to ethanol as biofuel (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 ultra structure 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 FPWs (pineapple, orange and mango) alone and in combination of all. The present approach was assessed by evaluating the reducing sugar (RS) from FPWs at different microwave power and irradiation time. The structural differences of raw and pretreated FPWs 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

Peel wastes of pineapple (PPW), orange (OPW) and mango (MPW) were collected from the local fruit juice shops at Indian Institute of Technology Delhi campus. Mixed fruit peel waste (MFPW) was prepared by combining all peel wastes in equal ratios. 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.

Moisture content of fruit peels was determined by heating them in an oven at 105°C overnight, until they reached a constant weight. The ash content was determined by incineration at 550°C ± 5°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 1975). The calculation for lignin was based on the difference between ADF and cellulose. Carbon and nitrogen contents were estimated using a CHN analyzer. Crude protein was calculated by multiplying the nitrogen content by a factor of 6.25 (Gothwal *et al*. 2012). RS estimation was done by

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DNS method (Miller 1959).

Powder X-ray diffraction (XRD) patterns of the analysis of raw and pretreated FPWs were carried out on a Bruker D8 Advance diffract meter with Ni-filtered Cu Ka radiation (k = 0.15406 nm) with a scan speed of 2° min 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.

A domestic microwave 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. The 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, individual FPW and their mixture were 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 FPWs were obtained by pump filter and kept for drying for compositional and other structural analysis. The dried pretreated FPWs were further used for enzymatic hydrolysis. Experiments were carried out in triplicates and average values were recorded.

Enzymatic hydrolysis of PPW, OPW, MPW and MFPW (200 mg) was carried in 50ml capped tube containing 20 ml 0.1 M sodium acetate buffer (pH 5.0) and enzyme (30 FPU/g DS) from *Aspergillus niger* (0.8 U/mg) incubated at 50°C and 150 rpm for 48 h.

All experiments were conducted in triplicates and each values represents a mean ± standard deviation.

RESULTS AND DISCUSSION

Compositional analysis of fruit peel wastes

Proximate analysis (Table 1) revealed that the OPW had highest moisture content of 83.23%, while the MPW lowest, i.e. 78.34%. MPW also recorded the lowest lignin (8.38%) and protein content (2.36%). The ash content of PPW, OPW, MPW and MFPW was 3.5, 4.6, 4 and 3.9% respectively. A perusal of the results showed that all peel wastes were rich in insoluble sugars (cellulose and hemicelluloses) justifying their selection as a promising feedstock for bioethanol production (Table 2). Cellulose content was highest in PPW (36.27%) followed by MFPW (34.64%), MPW (34.22%) and OPW (32.20%). Hemicelluloses content ranged from 12.4 to 22.5% and was highest in PPW. High hemicelluloses content in FPWs indicated the presence of significant amount of C5 and C6 sugars to be converted into ethanol. 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)

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

Parameters (%)	Fruit peel wastes			
	PPW	OPW	MPW	MFPW
Moisture	77.81 ± 0.12	83.23 ± 0.15	78.34 ± 0.16	75.56 ± 0.20
Ash	3.5 ± 0.13	4.6 ± 0.09	4 ± 0.11	3.9 ± 0.18
Insoluble dietary fibre	63.5 ± 1.88	48.11 ± 1.91	55.43 ± 1.84	52.1 ± 1.89
Protein	6.31 ± 0.02	7.06 ± 0.03	2.36 ± 0.01	4.13 ± 0.02

PPW: Pineapple peel waste; OPW: Orange peel waste; MPW: Mango peel waste; MFPW: Mix fruit peel waste. *Each value represents a mean ± standard deviation

Table 2 Lignocellulosic changes of fruit peel wastes before and after microwave assisted pretreatment (results expressed as weight percent, oven dry weight basis)

Fruit wastes	Before pretreatment (%)			After pretreatment (%)		
	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
PPW	36.27 ± 2.00	22.5 ± 1.84	14.68 ± 1.58	48.41 ± 3.01	30.09 ± 1.98	10.34 ± 1.24
OPW	32.2 ± 2.11	15.01 ± 1.99	11.14 ± 1.49	39.73 ± 2.99	23.09 ± 1.66	8.39 ± 1.49
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
MFPW	34.64 ± 2.19	12.4 ± 1.88	9.13 ± 2.00	42.88 ± 2.98	21.11 ± 2.04	5.42 ± 1.51

PPW: Pineapple peel waste; OPW: Orange peel waste; MPW: Mango peel waste; MFPW: Mix fruit peel waste. *Each value represents a mean ± standard deviation

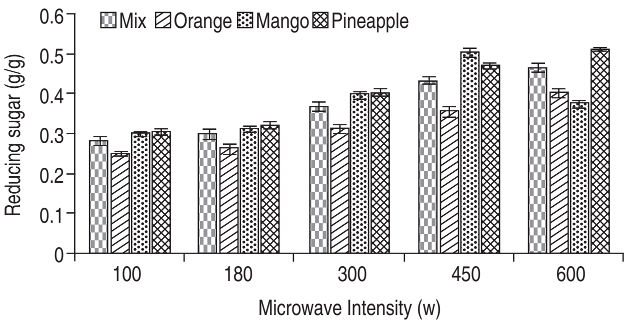

Fig 1 Maximum reducing sugar yield of microwave assisted alkali pretreated fruit peel waste at various microwave power. PPW: Pineapple peel wastes; OPW: Orange peel wastes; MPW: Mango peel wastes; MFPW: Mixed fruit peel wastes.

in the peel wastes of banana, apple, pear and citrus fruits.

Effect of microwave assisted alkali pretreatment of FPWs

Fig 1 illustrates the effect of microwave assisted alkali pretreatment for 5 min on reducing sugar content of selected FPWs. Among all the four different feed stocks, PPW gave maximum RS yield (0.610 g/g) at 600 W. In another experiment, the time course profiles studied for maximum RS yield from PPW, OPW, MFPW at 600 W and MPW at 450 W were studied (Fig 2a-d). Maximum RS yield was obtained from PPW (0.774 g/g) with 2 min treatment. It was observed that at specific high microwave power, shorter irradiating time was required to attain maximum RS yield after enzymatic hydrolysis. At 600 W, microwave pretreatment of PPW for 6 min resulted in 9.81% less RS yield compared to yield observed at 2 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).

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

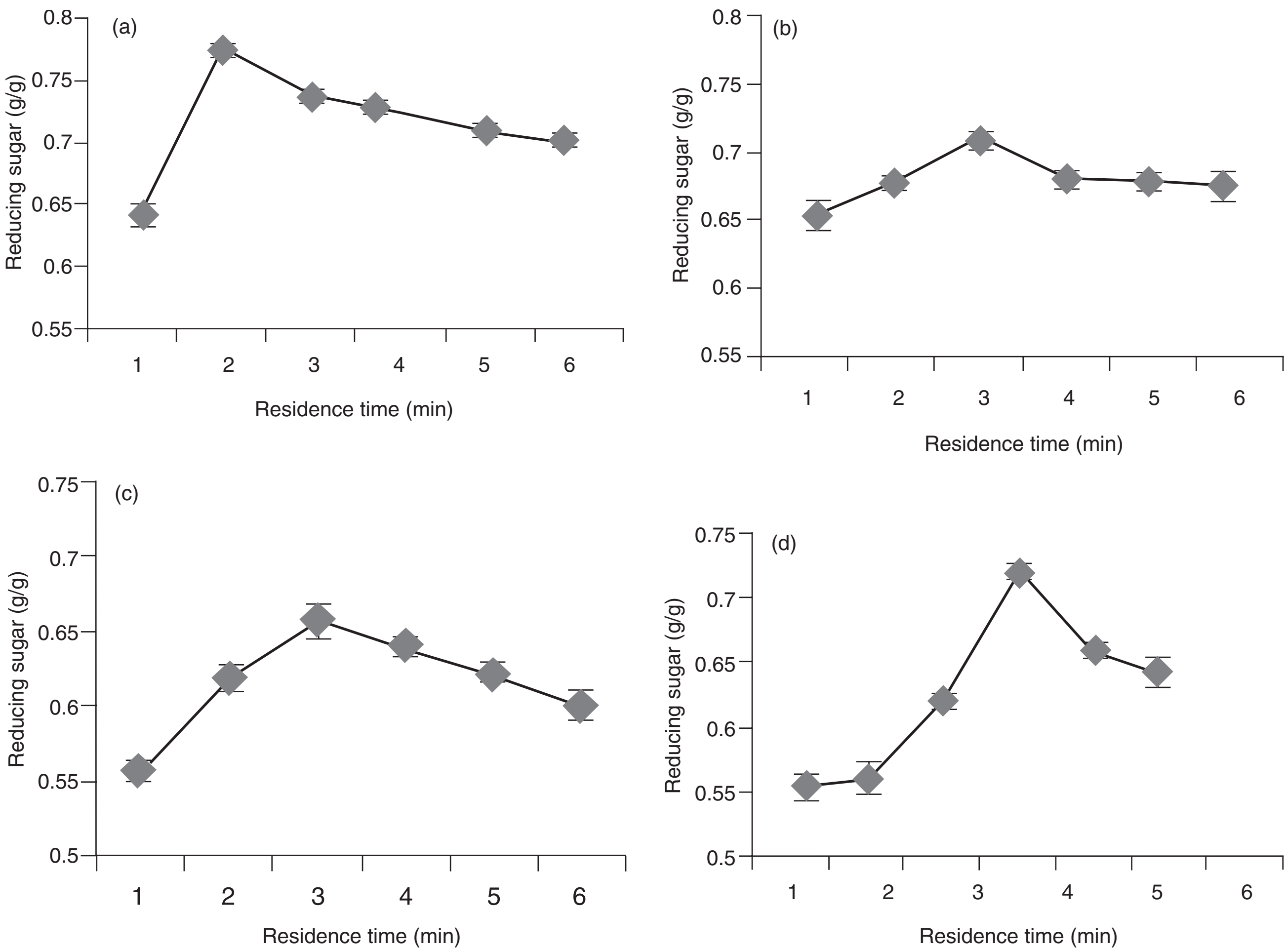

Fig 2 Reducing sugar yield of fruit peel wastes during time profiling by microwave assisted alkali pretreatment at best identified microwave power – (a) PPW at 600 W; (b) MFPW at 600 W; (c) OPW at 600 W; (d) MPW at 450 W. PPW: Pineapple peel wastes; OPW: Orange peel wastes; MPW: Mango peel wastes; MFPW: Mixed fruit peel wastes

Table 3 Crystallinity index and degree of raw and microwave pre-treated fruit peel waste (results expressed as weight percent, oven dry weight basis)

Parameters (%)	Before pretreatment				After pretreatment			
	PPW	OPW	MPW	MFPW	PPW[1]	OPW[2]	MPW[3]	MFPW[4]
Crystallinity index	11.12± 0.008	12.77± 0.011	10.02± 0.009	15.89± 0.009	58.11± 0.011	43.09± 0.009	53.14 ± 0.005	49.36 ± 0.002
Degree of crystallinity	9.22 ± 0.006	10.09± 0.009	9.95± 0.012	14.89± 0.007	72.06 ± 0.009	58.25 ± 0.010	69.08 ± 0.008	65.72 ± 0.010

PPW1: 600W for 2min; OPW2: 600W for 3min; MPW3: 450W for 5min; MFPW4: 600W for 3min. PPW: Pineapple peel waste; OPW: Orange peel waste; MPW: Mango peel waste; MFPW: Mix fruit peel waste. *Each value represents a mean ± standard deviation.

exposure of cellulose for enzymatic hydrolysis. Overall, the differential response of all FPWs in terms of RS yield necessitates an optimal microwave power and irradiating time for a particular feedstock.

XRD analysis

The CrI and CrD of FPWs 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 CrI of raw and pretreated samples ranged from 10.02 to 15.89% and 43.09 to 58.11% respectively. 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 FPWs. Among the pretreated FPWs, highest CrD was recorded for PPW (72.06%) followed by MPW (69.08%), MFPW (65.72%) and OPW (58.25%). The results proved the efficacy of microwave irradiation in removal of amorphous portion and increasing crystalline cellulose content in the FPWs (Binod *et al*. 2012).

With this study we can conclude that microwave assisted alkali pretreatment of FPWs could be an energy-saving technology for the valorization of the waste in a cost effective way.

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