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Tea Wastes as An Alternative Sustainable Raw Material for Ethanol Production

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INTEGRATING sustainability in life became an urgent necessity. Currently, tea sectors play important role in adopting new technologies in order to reduce tea wastes. Collection of these wastes for production of ethanol will contribute to the improvement of life and the environment. Simple technique was proposed for saccharification of spent tea leaves either by cellulase or fermentation with baker's yeast. Scanning electron microscope analysis showed certain modification for the morphology of both green and black tea leaves. Brunauer Emmett Teller analysis confirmed the increment in the surface area of both tea leaves. X- Ray Diffraction analysis proved the amorphous nature of their cellulose with little crystallinity. This nature eases their saccharification because cellulose is usually susceptible to degradation in its amorphous form. The percentage of produced ethanol was 74.4% from spent green tea leaves and 80.7% from spent black tea leaves after 24 h at mild temperature. The habit of drinking tea in Egypt shed the light on a promising approach relies on the production of ethanol from tea wastes especially spent tea leaves.

Keywords: Tea wastes, Ethanol, Cellulose, Amorphous, Saccharification, Bakers' yeast.

Introduction

Tea is the second beverage after water worldwide. Billions tons of tea wastes are produced in nearly all parts of the world. Their disposal is costly and not devoid of environmental impact [1]. These wastes dumped into landfills or burned. Currently tea sectors play an important role in adopting new technologies for changing consumer behaviors in order to reduce wastes. As well as exploring sustainable ways and new innovations for such purpose [2] .

Tea is the official hot drink in Egypt. Tea is the favorite beverage for millions of Egyptians. They relied on tea to set the rhythm of the day; it is a traditional habit in the morning and after meals. Because of this passion Egypt came as the fifth country in the world for the highest tea consumption and is ranked third on the basis of per capita consumption among the Middle East. Consumption of tea in Egypt is about 100,000 metric tons annually [3].

Due to the role of cafe as public places for meeting in Egyptian society, different categories of people go to the cafe especially categories of workers to middle-income. Number of visitors of cafes varies from day to day according to the daily events. In personal interview, owners of famous café in Maadi-Cairo and Khan El-Khalili region said that at the end of the day about 1kg to 1.5 Kg of spent tea leaves could be collected from one cafe. There are street side cafes all over the city, either traditional or westernized. In poor districts, you may find 10 cafes in the same area (area of 20 Kilometer^2). Which means that you can get about 15 kilogram or may be more of spent tea leaves per day from 10 cafes in the same area. That means that about 4500 Kg spent tea leaves may be collected per month from one area.

According to unofficial estimation the number of coffee shops and cafes on the level of the governorates of Egypt reached to around 1.5 million cafes [4]. From theoretical point of view,

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the habit of drinking tea in Egypt shed the light on a promising approach relies on the production of ethanol from tea wastes especially spent tea leaves. It is possible to collect these wastes, but it requires study of feasibility, requires convincing the owners of cafes and also to find the necessary labor. Recently, the largest tea buyer (Unilever) participated in a programme with Essex town councils and the united kingdom entitled" Waste and Resource Action Programme (WRAP)" in order to improve the recycle of tea bags and to keep about 370000 tones of tea wastes from dumping in landfills. Moreover, the programme of Kerbside collection points for wasted food is in line with Unilever plan. Therefore it launched campaign in 2007 to grow tea wastes recycling awareness [5].

India, Sri Lanka, and Kenya suffer from enormous quantities of tea wastes generated from production, processing or tea canning. Recently tea waste is sold with reasonable prices in electronic auctions and the tea sector in India has been leading the event [6]. However, very few Indian companies buy small quantities of tea waste. This generally does not solve the tea waste management crisis, but opens the way for other countries such as Egypt to buy it at acceptable prices. Profit margin for selling one kg of tea wastes is about 25-30 rupees in some regions in India [7]. Tea Board of India encourages selling by-product of tea industry which includes tender stem, discarded tea leaves and buds [8].

The current crisis of huge quantities of tea waste has made many researchers finding ways to exploit these wastes [9]. Different studies have demonstrated that tea wastes able to remove synthetic dyes [10], turbidity [11] and also remove some types of heavy metal ions from water [12]. Recently porus carbon has been drived from tea wastes[13]

To the authors' knowledge few studies have demonstrated the utilization of tea wastes for ethanol production [14-18].But the mentioned studies deals with techniques that did not fit within the concept of green chemistry and have their own limitations.

Lignocellulosic wastes can be used for ethanol production but with difficulty [19]. This difficulty is due to their chemical structures as they consist of cellulose, hemicellulose and lignin. Moreover the crystallinity of cellulose also responsible for making the lignocellulosic wastes non- effective [20]. Therefore pretreatment process is required as a first step. Different methods were used for such purpose. Structure disruption of cellulosic materials by physical pretreatment is the easier process. In which surface area of the cellulosic materials is increased by reduction of their size into powder. Then they followed by chemical pretreatment as a second step [21]. Recently attention has been focused on utilization of biomass residue. From an economical and technical point of view it is very important to select the source of biomass. The source is preferred to characterize by its structural and chemical simplicity. Spent tea leaves meet these criteria. As well as the applied technique in the present study fit within the concept of green chemistry. It is environmentally friendly and more economically viable since there is no need for the expensive reagents and sophisticated equipment. The objective of the present study is to utiliz tea wastes as an alternative sustainable raw materials for ethanol production with simple tehniquqe.

Materials and Methods

Materials

The dry bakers' yeast, *Saccharomyces cerevisiae* was brought from Egyptian Sugar Company of integrated industries. Baker's yeast used as it is without further purification .Tea samples both green and black tea leaves were obtained either from local market in the form of small beads. Cellulase enzymes were dedicated from Microbial Chemistry Department at National Research Centre of Egypt. Sodium acetate was obtained from Fluka. Glucose was obtained from Merck. Carboxy methyl cellulose (CMC) and the other chemicals were purchased from Sigma.

Methods

Tea leaves preparation

Samples of green and black tea leaves were placed in boiled water for 5 min and repeatedly washed with tap water (brewed tea). Then rinsed and air dried overnight. Finally, the dried leaves were stored in dried container at room temperature. These dried leaves (spent tea leaves) considered as untreated. Thermal treatment of dried tea leaves was carried out in domestic microwave (Micromaxx) model mm 2854 made in Germany as a kind of pretreatment. Tea leaves were soaked in water at medium temperature of microwave 300 ˚F for 10 min. These leaves were referred to as microwave treated.

Saccharification of spent tea leaves by baker's yeast

Untreated and microwave treated tea leaves were incubated separately with dry baker's yeast in aqueous solution for both green and black tea. The experiment was maintained at 40 ˚C for 24 h under shaking conditions at150 rpm. Then aqueous phase was separated with cloth sheath and centrifuged at 6000 rpm for 15 min to be analyzed by gas chromatography. Measurement of reducing sugar in the aqueous phase demonstrated according to Somogyi [22]. The solid phase was washed repeatedly with extensive distilled water and rinsed for enzymatic saccharification.

Enzymatic saccharification of spent tea leaves

The residual tea leaves (solid phase) were used as substrate for cellulase enzyme. The cellulase enzyme had an activity of 1.85 U/ml of filter paper enzyme, 3.7 U/ml of carboxy methyl cellulase enzyme, 1 U/ml of glucosidase, i.e. 6.55U/ml multi cellulase enzyme. The reaction mixture contained 0.5g of substrate with 10ml cellulase (total activity 65.5 U) in citrate buffer and incubated at 40˚C under agitation (150 rpm) for 24 h. Reducing sugar and ethanol librated had been estimated.

The saccharification percentage was calculated according to Abdel-Halim [23] as follows:
Saccharification percentage = Reducing sugar (mg/ml) x 0.9 x 100 / initial substrate (mg/ml)

Effect of different concentrations of dry baker's yeast and different pH on saccharification

Examination was planned to investigate the potency of different concentration of baker's yeast for production of reducing sugars by fermenting the microwave spent tea leaves. According to the fact sheet of Egyptian Sugar Company of integrated industries, 1 g baker's yeast contains 20 $x10^6$ yeast cells. The different concentrations of baker's yeast were range from 0.5 g to 3 g.

Effect of pH value was assessed in different pH range; 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0.

Estimation of cellulosic content of spent tea leaves

It was worthwhile to analyze the cellulosic contents of all types of tea leaves (untreated, microwave treated). Hemi-cellulose, cellulose and lignin content were estimated according to the modified method of Ghose [24].Certain weight of spent green and black tea leaves (10 g) were extracted with 5% NaOH with ratio of 1:20 at 90˚C for 3 h. The mixture was filtered after cooling. The residue was dried and weighed as crude cellulose. The filtrate then precipitated with 3 volumes of ethanol. The formed precipitate was filtrated and weighed as hemicellulose after drying. The remaining filtrate represents water soluble content (lignin, phenols& other extractives)

Ethanol production and Gas chromatography analysis

Series of experiments were conducted. Microwave treated and untreated tea leaves were investigated for ethanol production. They were first incubated with dry baker's yeast in aqueous solution without any addition for 24 h. At the end of incubation period ethanol concentration was determined. Then the exhausted tea leaves were incubated with cellulase enzyme for another 24 h. At the end of incubation time ethanol concentration was determined. Analysis of ethanol yield was performed according to Seo et al [25] by gas chromatography (Hewlett-Packard19091J-413, Agilent USA).The flame ionization detector were used with temperature 250 ˚C. Hydrogen was the carrier gas with flow rate of 30 ml/min.

Tea leaves characterization

The morphology of untreated, microwave treated and saccharified tea leaves were investigated by scanning electron microscope (SEM, Quanta FEG 250, FEI), where, the samples were coated with a thin layer of Au by sputtering coating device.

The infrared spectra of all types of spent tea leaves were recorded with Fourier Transform Infrared (FTIR) Spectroscopy (JASCO FT-IR 6100, made in Japan) using the KBr disk sampling method. The FTIR spectra were collected in the wave number range from 4000 to 400 cm^{-1} at a number of 32 and a resolution of $4cm^{-1}$[26].

X- Ray Diffraction (XRD) analysis was used for investigation of cellulose crystallinity of spent tea leaves. X-ray diffraction (XRD) was carried out using a Philips PW1390 X-ray diffractometer (U.S.D.). XRD patterns were recorded at room temperature in the 2θ range from 10 to 70º in 0.02º steps counting for 19 seconds per step using CuKα radiation (1.5418 Å) [27].

Brunauer–Emmett–Teller (BET) analysis

Surface area of tea leaves were measured by BET (Brunauer–Emmett–Teller) analysis. The mechanism for the estimation of surface area of any solid materials is by adsorption of gas molecules physically on their surface .Therefore, the surface area of spent tea leaves was measured according to Brunauer et al [28] using Nitrogen gas adsorbents.

Results

Estimation of cellulosic content of spent tea leaves

The percentages of cellulosic, hemicelluosic and water soluble contents of green and black tea leaves were determined. The results **Table 1** reveled that cellulose and hemicellulose percentages of microwave treated green tea leaves decreased about 22 % and 11 %, respectively in comparison to untreated green tea leaves. With regard to black tea leaves, the percentage of cellulosic content of microwave treated leaves was decreased by 32% less than that of untreated one. The percentage of hemicellulose of microwave treated black tea leaves was decreased by 25 % less than that of untreated one.

In general it could be concluded that lignocellulosic contents of green tea leaves were higher than those of black tealeaves. The reductions in the lignocellulosic components were due to the removal of complex polysaccharides [29]. It was reported that the amount of cellulose, hemicellulose and lignin of tea wastes after microwave treatment was 17.5 % cellulose, 41.3 % hemicellulose and 41.2 % lignin [30].

Saccharification of spent tea leaves for sugar production

As a kind of starvation response no sugars were added to the saccharification medium. The only source of energy was spent tea leaves. Yeast consumed tea leaves and produced reducing sugars.

As shown in **Table 2** reducing sugar produced from black tea leaves was found to be greater than green tea leaves although both of them are belong to the same genus and species *Camellia sinensis* [31]. This result may be attributed to the fact that black tea produced from oxidation of green tea and contain greater amounts of arabinose, galactose and rhamnose compared to green tea. These sugars might be fermented [32]. The presence of caffeine, catechins, theaflavins and others in untreated black and green tea leaves may inhibit yeast activity, therefore, reducing sugars produced in smaller amount [33].

The production of reducing sugars from microwave treated cellulosic materials of tea leaves was higher than those produced without pretreatment. This result in agreement with what reported by Zhang, et al. [34].The data of **Table 2** reveled that microwave treatment technique enhanced the hydrolysis rate of cellulose and increased the yield of reducing sugars by about 49 % and 100 % compared with untreated black and green tea leaves respectively.

The glucose yields from microwave treated black or green tea leaves are higher compared with glucose yield of 18 % from SC-CO2 pretreatment of corn stover as reported by Naveen, et al. [35]. The microwave pretreatment technique of the present study has the advantage that there is no discharge of toxic chemicals. Microwave-treatment was found to be helpful in improving the fermentable sugar yield from cassava residues through saccharification [36].

TABLE 1. Structural composition of different types of spent tea leaves.

Type of Tea leaves	Cellulose (%)	Hemicellulose (%)	Water soluble content (Lignin, phenols& other extractives) (%)
Untreated green tea	14.26	33.33	52.44
Microwave treated green tea	12.76	26.03	61.22
Untreated black tea	28.13	10.53	61.34
Microwave treated black tea	19.23	7.9	73.17

TABLE 2. Reducing sugar and saccharification percentage of spent tea leaves by baker's yeast.

Treatments	Green Tea		Black Tea	
	Reducing sugars (mg/0.1g waste)	Saccharification %	Reducing sugars (mg/0.1g waste)	Saccharification %
Untreated tea leaves	10.5	9.45	33.2	29.88
Microwave treated tea leaves	21.0	18.90	49.6	44.64

Effect of different concentrations of dry baker's yeast on saccharification

A promising enhancement in saccharification was achieved by increasing baker's yeast concentrations from 0.5 to 2.5 % for green tea leaves and from 0.5 to 2.0 % for black tea leaves as shown in **Table 3**. Yeast biomass can be considered a key factor for enhancing cellulosic saccharification [37].

The saccharification% of green tea was the maximum (42.57 %) at 2.5 % baker's yeast. For black tea the saccharification % was 73 % at 2.0 % equivalent to $40×10^6$ yeast cells in 24 h. It was reported that, approximately $500×10^6$ yeast cells were required for complete wheat mash saccharification after 66 h [38].

Effect of different pH values on saccharification efficiency

The effect of pH on saccharification was assessed in different pH range from 4.5 to 7.0. The data **Table 4** suggested that, saccharification of tea leaves by baker's yeast was pH dependent. The highest saccharification for black tea leaves was preferable at pH 4.5. *Saccharomyces crevice* in general has optimum pH around 4.5 [39]. As the pH increased from 5.0 to 7.0 there was a reduction in reducing sugar production [40]. Highest saccharification for green tea leaves was preferably at pH 5.5. Higher pH value is undesirable for saccharification of green tea leaves both tea leaves are chemically differs in their structures as reported by Pawel, et al. [41].

TABLE 3. Effect of baker's yeast concentrations on reducing sugar and saccharification percentage.

	Green tea		Black tea	
Baker's yeast conc. (%)	**Reducing sugars (mg/0.1g waste)**	**Saccharification (%)**	**Reducing sugars (mg/0.1g waste)**	**Saccharification (%)**
0.5	12.0	10.8	37.7	33.93
1.0 (control)	39.6	17.82	49.5	44.55
1.5	25.5	22.95	76.5	68.85
2.0	41.0	36.9	81.1	73.00
2.5	47.3	42.57	67.8	61.02
3.0	33.0	29.7	61.2	55.08

TABLE 4. Effect of initial pH on reducing sugar and saccharification percentage of spent tea leaves .

	Green tea		Black tea	
Initial pH range	**Reducing sugars (mg/0.1g waste)**	**Saccharification (%)**	**Reducing sugars (mg/0.1g waste)**	**Saccharification (%)**
4.5	40.1	36.09	93.3	83.97
5 (control)	47.3	42.57	81.1	73.00
5.5	62.0	55.80	80.5	72.45
6.0	43.4	39.09	72.5	65.25
7.0	24.0	21.60	70.0	63.00

Saccharification of spent tea leaves for ethanol production

The profile of ethanol production from black and green spent tea leaves is shown in **Figure 1.** The highest ethanol production was 80 % and 74 % respectively. The hydrolysis of lignocellulosic material of untreated tea leaves was effective. It was concluded that the hydrolysis of lignocellulosic material of untreated tea leaves was effective for production of reducing sugars. However it was gave small quantity of ethanol. This could be due to the inhibitory effect of polyphenols of untreated tea leaves on yeast. These observations are similar to what reported for plant polyphenols against yeast [42]. Moreover microwave assisted in extraction of chemicals and phenols from tea waste before fermentation which effectively alleviated the inhibitory action according to some literatures [43 & 44].These results strongly imply that phenolic compounds of tea leaves is responsible for low ethanol production from untreated tea leaves. To obtain high ethanol production with low coast; pretreatment process was optimized. Acid and alkaline pretreatment usually utilized for this purpose [45]. The exhausted treated tea leaves were subjected for another 24h with cellulase enzyme. The ethanol production was 37.63 % for black tea leaves and 34.43 % for green tea leaves. No big difference could be noted between green and black tea leaves for ethanol production.

An examination was planned to address the effect of invertase that had been automatically immobilized on tea leaves [46]. An experiment was performed by subjection exhausted tea leaves to boiling for15 min to inhibit invertase. Ethanol production decreased by 45.77% for green tea leaves and decreased by 50.9 % for black tea leaves in absence of invertase. All results together suggest that significantly high ethanol was obtained after incubation of tea leaves with baker's yeast as compared with enzymatic hydrolysis of tea leaves. Accordingly, it can be considered new promising technique for ethanol production compared to Mustafa et al, [47] who reported the fermentation of acid-pretreated tea waste for ethanol production using *Saccharomyces cerevisiae*.

Morphological characterization of spent tea leaves (SEM)

A scanning electron microscope was used to examine the surface morphology of spent green tea leaves before and after modification as shown in **Figure 2**A. The leaf surface of untreated green tea leaves and their stomata without any changes (Figure **2**B) shows that there were changes in tea leaves surface as they started to degrade due to microwave and hence increased the surface area. **Figure 2**C shows that there were completely changes in tea leave surface as they degraded and lost the fibers due to saccharification which increased the surface area. Antonio and Roberto [48] obtained the same results through the elimination of lignin from rice straw.

Figure 3 shows the scanning electron micrographs of the surface of spent black tea leaves before and after modification. **Figure 3**A shows the leaf surface of untreated black tea leaves. **Figure 3**B shows obvious disruption in the epidermal surface and the structure of microwave treated black tea leaves. **Figure 3**C shows that the surface became irregular and shrank in the treated tea leaves. The same result was obtained by Flórez et al [49]. This result suggested that treated tea leaves were more appropriate for production of ethanol than untreated one.

Brunauer–Emmett–Teller (BET) Analysis

As shown in **Table 5** black tea leaves showed distributed micro-pores of 25.6 Å diameter, while green tea leaves was 26 Å. The pore size did not change after or before treatment. The results also indicated that the surface area was 31, 94 and 102 m^2/g for untreated, microwave treated and baker's yeast treated spent black tea leaves respectively indicating the surface modification of the leaves. In the same trend the surface area increased from 145 to 318 to 340 m^2 / g for untreated, microwave treated and baker's yeast treated spent green tea leaves respectively.

It was concluded that the tea surface area that might be accessible to cellulase hydrolysis increased due to cellulosic disruption. The results revealed that thermal activation by microwave and the incubation of tea leaves with baker's yeast is effective for cellulose hydrolysis. It was reported that CO_2 released during fermentation facilitates the entrance of CO_2 into the cellulosic structure and caused more swelling which led to cellulosic disruption [50].

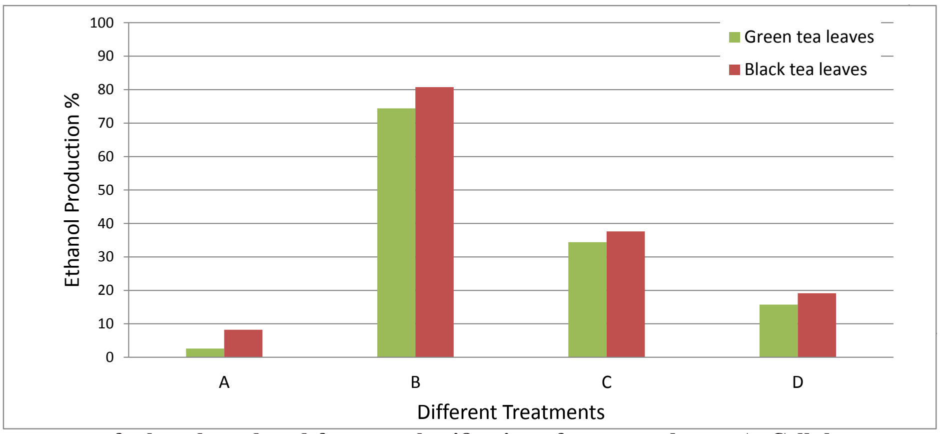
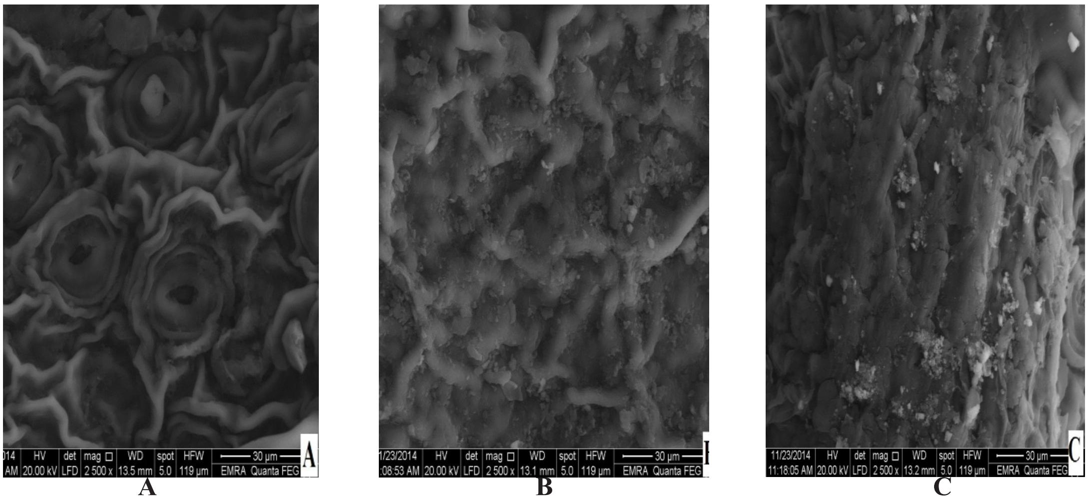
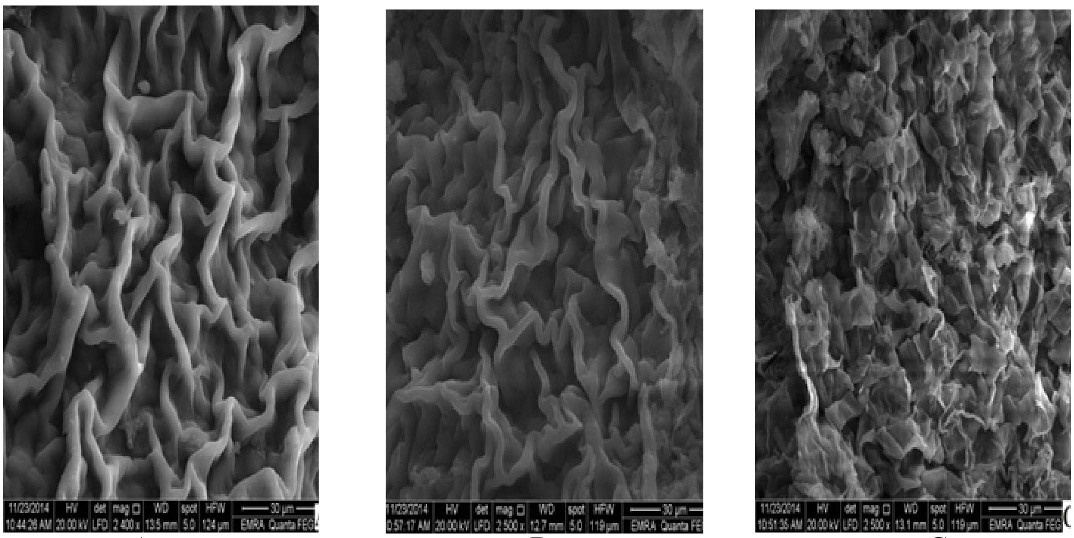


Fig. 1. Percentage of ethanol produced from saccharification of spent tea leaves A, Cellulase+ treated tea leaves (denatured invertase); B, Cellulase+ treated tea leaves (active invertase); C, microwave treated tea leaves incubated with baker's yeast; D, Untreated tea leaves+ baker's yeast.



A B C

Fig. 2. Scanning Electron micrographs of different samples of spent green tea leaves A, untreated green tea leaves; B, microwave treated green tea leaves; C, baker's yeast treated green tea leaves.



A B C

Fig. 3. Scanning Electron micrographs of different samples of spent black tea leaves A, untreated black tea leaves; B, microwave treated black tea leaves; C, baker's yeast treated spent tea leaves.

TABLE 5. Effect of CO_2 on surface area of spent tea leaves.

Surface area and pore size	Untreated tea leaves	Microwave treated tea leaves	Baker's yeast treated tea leaves
Surface area (m²/g)			
Black tea	31	94	102
Green tea	145	318	340
Pore size(diameter (Å)			
Black tea	25.6	25.6	25.6
Green tea	26	26	26

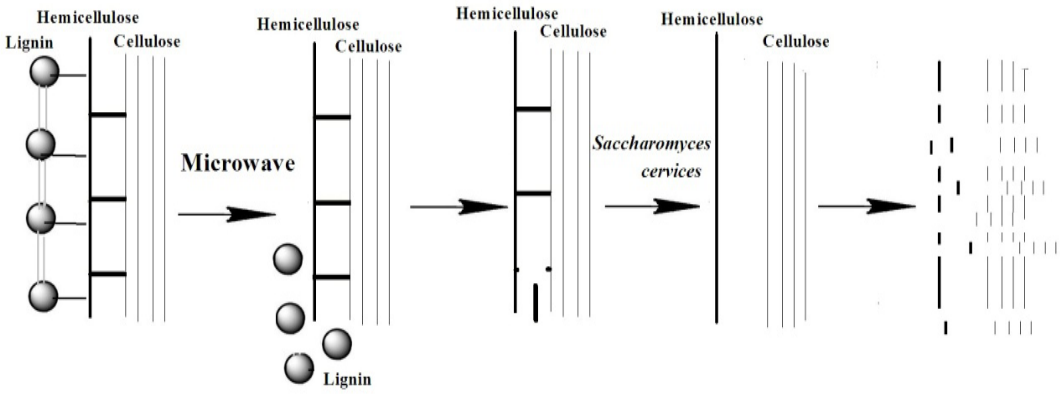


Fig.4. Author's conceptual illustration for saccharification mechanism.

Conceptual Illustration for Saccharification Mechanism

It is worthy to note that the appropriate treatment can noticeably improve the loosing of the fiber. **Figure (4)** illustrated the proposed saccharification mechanism and explains why the lignocellulosic contents decreased in all stages. It was reported that lignocellulosic material that pretreated by microwave irradiation led to removal of lignin and cleavage of some bonds between cellulose and hemicellulose [51]. This step enhanced the susceptibility of cellulosic and hemi cellulosic contents to *Saccharomyces cervices*. The pressure caused by the production of CO_2 in a closed bottle is enormous and can form carbonic acid in presence of water. Carbonic acid can assist the hydrolysis of hemicellulose and dissociate the bond between cellulose and hemicellulose as reported by Azuma et al [52].

X-ray diffraction (XRD) analysis

The X-ray diffraction (XRD) analysis was carried out to identify the cellulose structure of green and black tea leaves. The samples of tea leaves were used as a whole without extraction of cellulose. The XRD patterns (diffractograms) of the green spent tea leaves and black tea leaves for the untreated, microwave treated and baker's yeast treated samples are shown in **Figure 5 & 6** the structure of all samples were amorphous with little crystallinity. This result ensured that the structure of both tea leaves cellulose is amorphous which is in agreement with [53]. Beguin and Aubert [54] reported that XRD of green spent tea leaves have crystalline region. The diffractograms showed that the intensities of the peaks increased in treated tea leaves and also the peaks became narrower which may be evident for improvement of cellulose solubility. Where increase in peaks intensity of green and black tea leaves after microwave treatment and yeast treatment indicated the increase in porosity [55]

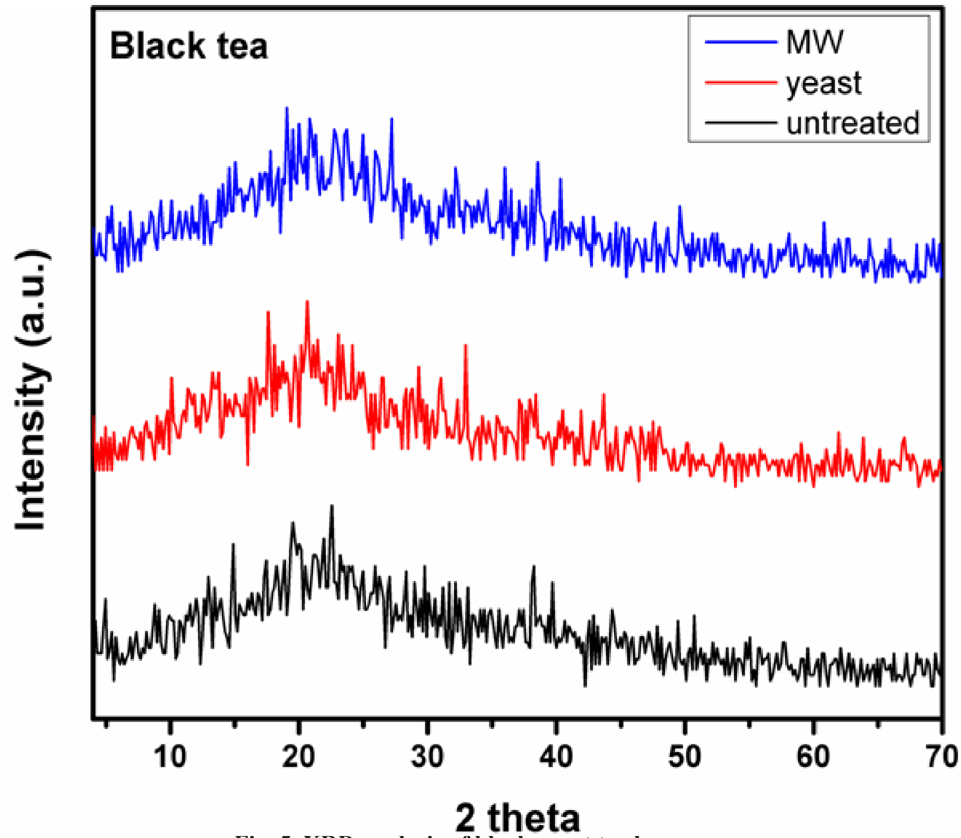


Fig. 5. XRD analysis of black spent tea leaves.

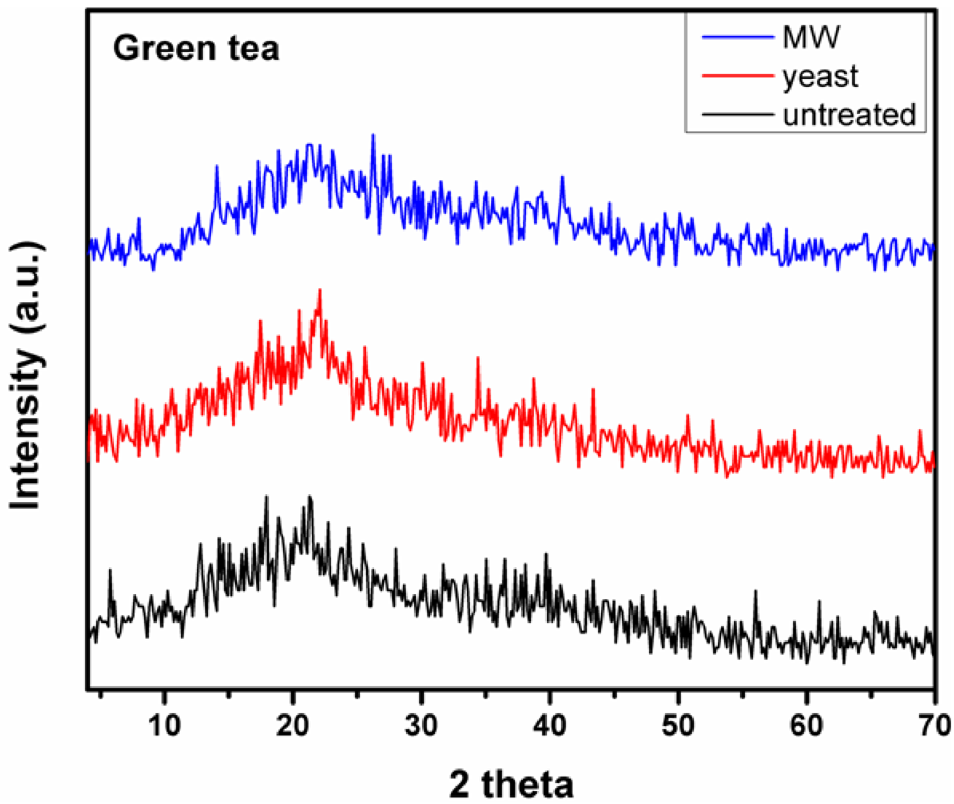


Fig. 6. XRD analysis of green spent tea leaves.

Fourier Transform Infrared Spectroscopy (FTIR)

The modification of cellulosic surface was examined using FTIR (Fourier Transform Infrared).The objective of this experiment was to investigate the effect of thermal treatment by microwave on the properties of tea leaves and also the effect of saccharification produced by baker's yeast.

(FTIR) for spent black tea leaves

Figures (7a &7b) display the FTIR spectra of the untreated black tea leaves as compared to the same leaves after treatment with microwave and baker's yeast, respectively. As shown from this **Figure 7a**, the spectrum of the untreated sample exhibits the main characteristic absorption bands of cellulose and lignin [56, 57, and 58]. The small peak at 3755 cm^{-1} is assigned for a very small amount of crystalline OH, which is in accordance with very little crystallinity found in the XRD pattern obtained for the same samples. The broad and strong band at 3413 cm^{-1} is attributed to the stretching vibration of the bond O-H. The stretching vibrations of C-H appeared at 2926 and 2856 cm^{-1}. In addition, the aromatic skeletal vibrations including C=C vibration are displayed at 1523 and 1448 cm^{-1}[56, 58]. The peaks located at 1641 cm^{-1} and 1077 cm^{-1} are ascribed to the vibrations of water molecules adsorbed in cellulose and C-O, respectively [59]. On the other hand, this figure shows some visible and measurable differences in the FTIR spectra of the treated tea leaves. These differences involved appearance of a new shoulder at 2270 cm^{-1} in the OH and appearance of a new peak at 1725 cm^{-1}, assigned for C=O stretching vibration, in the spectrum of the sample treated with baker's yeast. Also, one can easily notice the overall spectral shift towards lower waveumbers, which refers to weakness and/or loss of cellulose and lignin bonds in the tea leaves to hydrolysis after treatment with either microwave and/or baker's yeast. The spectral shift together with the appearance of new peaks confirmed the hydrolysis of the tea leaves after baker's yeast treatment. These findings are in good accordance with the literature [56, 58]

(FTIR) for spent green tea leaves

Figure 8 shows the FTIR spectra of microwave and baker's yeast treated green tea leaves as compared to the spectrum of baker's yeast treated black tea leaves. As it can be noticed from this figure, nearly the same spectral features are obtained for the green tea leaves as in case of black tea leaves those of black tea samples [56, 58]. However, spectral shift to lower wave umbers in case of black tea is slightly higher than that of green tea leaves. This implies a little better enhancement in hydrolysis for the treated black tea leaves, which is in accordance with the other measurements found for the same samples in this work.

Coast of ethanol

Harvesting of lignocellosic crops is available seasonally and not throughout the year, this is difficult for biomass suppliers [60].However tea wastes can be an alternative for continues biomass supply because it is available for long-term storage, and ensures that the bio-refinery works continuously throughout the year. Tea plant is evergreen shrub and has more than three harvesting seasons or may be more in the same year [61]. Tea wastes can be considered collectable for saccharification.

One kilogram wastes leads to production of 17.6 L ethanol. The cost of 1kg tea wastes for sale in India is 25 rupees. By simple calculation, the cost of 1 L ethanol will coast 4.54 LE as shown in **Table 6**

Conclusion

It is concluded that spent tea leaves are promising alternative raw materials for production of ethanol. Saccharification of tea leaves is baker's yeast dependent and the amorphous nature of tea leaves eases their saccharification. The production of ethanol of the study is cost effective.

Acknowledgment

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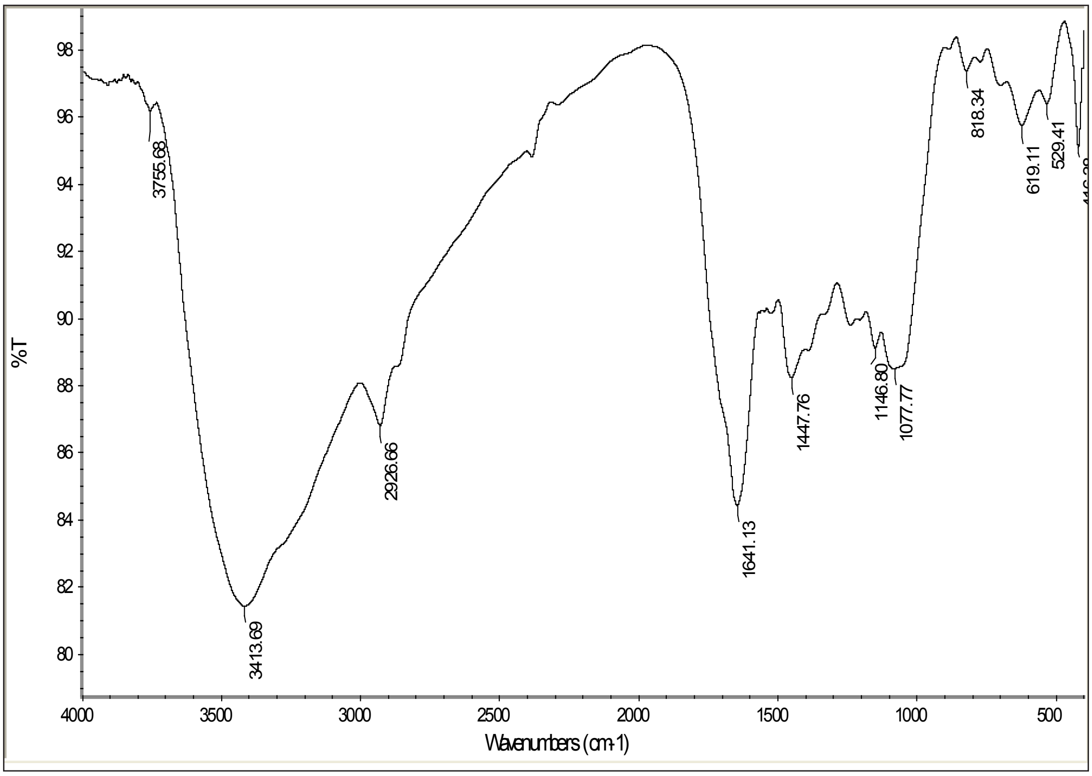

Fig. (7a). FTIR spectrum of untreated spent black tea leaves.

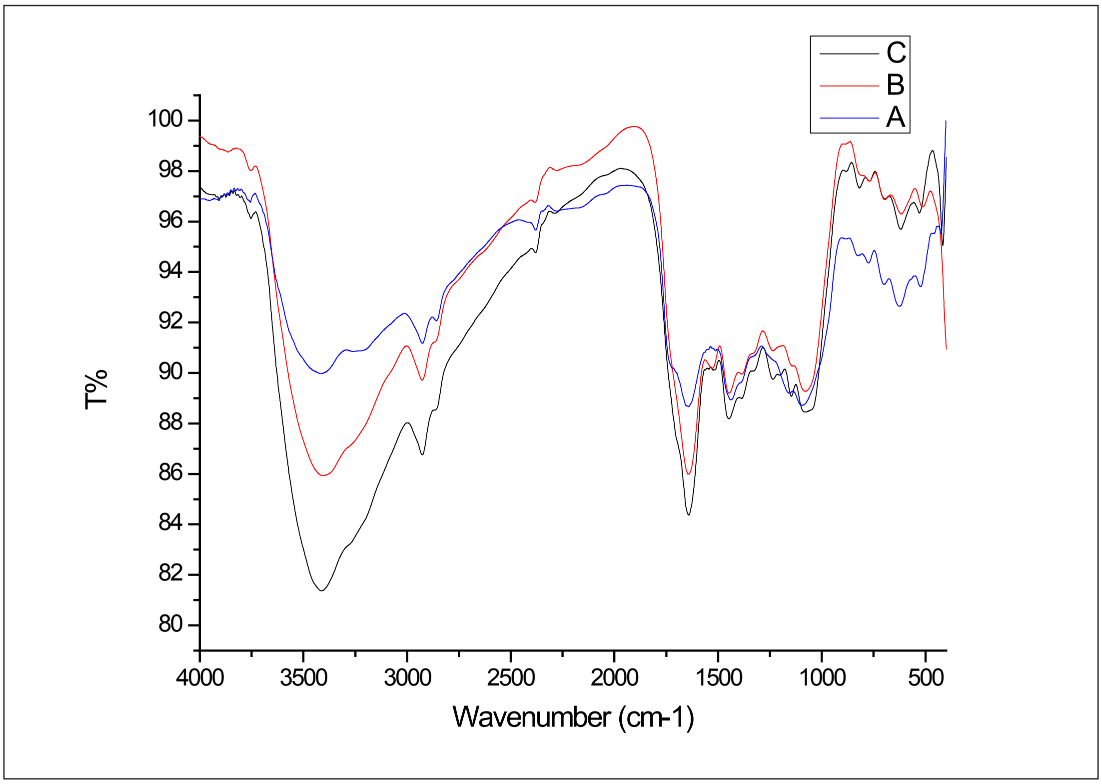

Fig. (7b). FTIR spectra of untreated spent tea leaves (C) as compared to microwave treated spent tea leaves (B) and baker`s yeast treated (A) spent tea leaves.

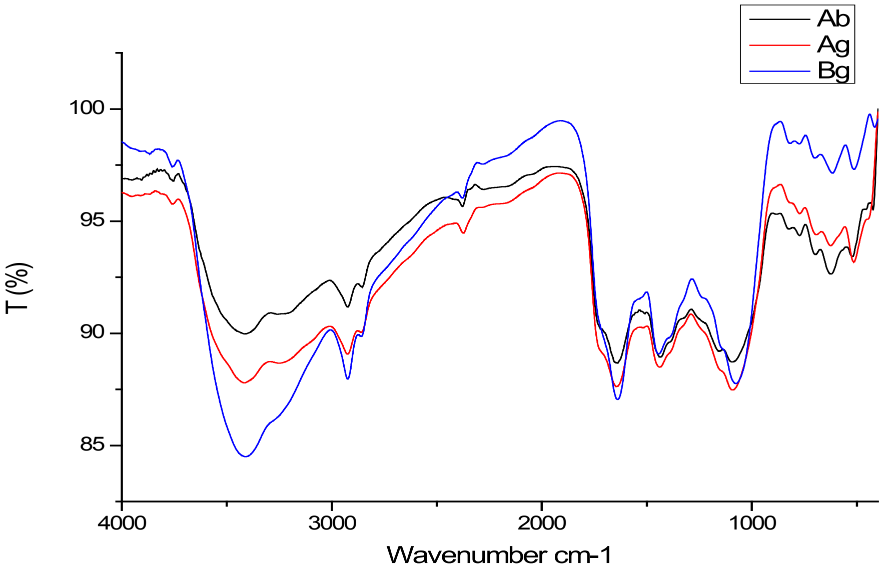

Fig. 8. FTIR spectra of baker's yeast treated spent green leaves (Ag) as compared to microwave treated spent green leaves (Bg) and baker's yeast treated black spent tea leaves (Ab).

TABLE 6. Cost of production of 1L ethanol.

Ingredients	Amount	Cost LE
Tea wastes	1 kg	5.75
Baker's yeast	2 kg	70
Other expanse	--------	4.25
Ethanol produced	17.6 L	80
1 L Ethanol	---------	4.54 LE

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مخلفات الشاي كمواد خام مستدامة بديلة لإنتاج الإيثانول

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أصبح دمج الاستدامة في الحياة ضرورة ملحة. تلعب قطاعات الشاي حاليًا دورًا مهمًا في تبني تقنيات جديدة للحد من مخلفات الشاي. وسيساهم تجمع هذه النفايات لإنتاج الإيثانول في تحسين الحياة والبيئة. تم اقتراح تقنية بسيطة لتسكر بقايا مخلفات أوراق الشاي إما عن طريق انزيم السليلولاز أو التخمير مع خميرة الخباز. أظهر تحليل المجهر الماسح الإلكتروني تغيرات لمورفولوجيا أوراق الشاي الأخضر والأسود. أكد تحليل Brunauer Emmett Teller الزيادة في مساحة سطح كل من أوراق الشاي. أثبت تحليل حيود الأشعة السينية أن الطبيعة سليلوز أوراق الشاي ن النوع غير المتبلورة مع وجود أجزاء قليلة التبلور. تسهل هذه الطبيعة عملية التسكر لأن السليلوز عادة ما يكون عرضة للتحلل في شكله غير المتبلور. كانت نسبة الإيثانول المنتج ٧٤،٤ ٪ من أوراق الشاي الأخضر و ٨٠،٧ ٪ من أوراق الشاي الأسود بعد ٢٤ ساعة في درجة حرارة معتدلة. عادة شرب الشاي في مصر تسلط الضوء على نهج واعد يعتمد على إنتاج الإيثانول من مخلفات الشاي خاصة تفل أوراق الشاي .