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BIOCONVERSION OF ORANGE PEEL WASTE BY *ESCHERICHIA COLI* AND *SACCHAROMYCES CEREVISIAE* TO ETHANOL

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ABSTRACT: An alternative energy sources using the sweet orange (*Citrus sinensis* (L.) waste as the feedstock was investigated; this is an approach to environmental protection. The extraction of pectin from sweet orange peel waste (pith) and the production of ethanol from the resultant liquid pectin with the aid of *Escherichia coli* (*E. coli*) and *Saccharomyces cerevisiae* (yeast) were carried out. Dried pith was separated using various particle sizes ranging from 0.075, 0.5, 1.0 and 5 mm. It was observed that pith with a particle size of 1.0 mm produced a larger volume of pectin while the pith with a particle size of 0.075 mm produced the least volume of pectin. 1,770 ml pectin was obtained from 802 g of pith; this shows that citrus fruit (especially orange) contains a high amount of pectin. *E. coli* (bacteria), yeast (fungus) and a mixture of both were added to the produced pectin which was fermented to ethanol. It was however noticed that sample pectin + *E. coli* + yeast, and sample pectin + *E. coli* produced a good volume of ethanol, but sample pectin only and sample pectin + yeast did not produce ethanol. The energy content of the total produced ethanol is 1526.6 Btu which can be mixed with pure gasoline to obtain an optimum energy content that can be used to power a citrus processing plant in Nigeria. The purpose of this paper is to obtain an alternative energy source for citrus plants using the waste generated by them such as orange peel.

INTRODUCTION: Pectin is a polysaccharide complex carbohydrate found naturally occurring substance present in all cell walls of plant tissue. Pectin is a polysaccharide, naturally occurring substance present in all plant tissue. Pectin exists in varying amounts in fruit cell walls and has important nutritional and technological properties [1]. In the cell walls, they serve as one of the main agents cementing the cellulose fibrils and may be linked covalently to other polymers. Intracellular pectin provides the channels for passage of nutrients and water.

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Pectin has been used for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer with its applications extend to fruit products for pharmaceuticals [2, 3, 4]. The main sources of pectin are pears, carrot, apples, grapes, guavas, gooseberries, and oranges. Its main application can be found in the cosmetic, food and pharmaceutical industries. The recent adoption of pectin in the energy industry is borne on the need to convert waste to bio-ethanol. This action is intended to mitigate climate change, land competition and cost of feedstock. Energy demand from fuels have risen from 16 billion liters in 2000 and projected to increase to about 140 billion towards 2020 [5, 6]. Hence, it beholds on modern researchers to think on energy options that would not negatively impact on life-forms [7]. The bio-fuel option is very viable with a recent growing demand [8, 9].

More specifically, is the bio-ethanol option whose average bulk purchase price has risen in Europe from 0.55 € per litter in 2011 [10] and it is projected to attain an average bulk purchase price of 0.65 € per litter in the future [11].

Recent studies have shown that bio-ethanol can be derived from cellulosic materials [8], algae-based, sugarcane [12, 13], starch, lignocellulosic biomass [14] cotton [15] wheat straw [16], *etc*. In Nigeria, the wastes from citrus peels are enormous because of the large consumption. Hence, the objective of this paper is to seek better ways of maximizing bio-ethanol production from orange peels. There are studies on improving bio-ethanol yield from orange [17, 18, 19].

In the fruit processing plant, the management system is challenged by the waste they generate such as peels and skins. Orange peel is a major waste that has a substantial burden on the environment; hence it is necessary to find a feasible way to dispose of the peels to have a positive environmental impact or turn them into useful products [20]. Its recent discovery in industries has helped to curb environmental pollution, as these papers can be recycled so that they can be re-used instead of being wasted [6, 21]. This work is carried out to explore the potential of orange waste as a means of producing ethanol, which is used for energy generation: this is sustainable, renewable and environmentally friendly.

This project aims to investigate the possibility of using and transforming orange waste into something valuable (*e.g*., ethanol and pectin). In the worldwide economy, much focus has been laid on the rising oil price which has become a hot topic. The rising oil price has increased the interest of finding other possible ways to produce fuel, and the production of bio-ethanol has grown steadily during the last 25 years.

MATERIALS AND METHODS:

Sample Collection and Preparation: The inocula used (bacteria and fungi) were freshly prepared and cultured in the Microbiology laboratory of Covenant University.

Preparation of Samples: The pith was blanched with boiling water for 5 min to inactivate the enzyme and was cut into pieces by Tiffiny fruit processor (model: Mini food processor No MC 9, Tiffiny). The pith was oven dried at temperature 80 ºC until constant weight was attained to ensure proper dryness. The dried pith was ground and stored in an airtight closed container coated with aluminum foil at room temperature until needed. Various sizes of mesh ranging from 0.0075, 0.5, 1.0, 5.0 mm were used to vary the particle size before the extraction of pectin.

GC-MS Analysis: The GC-MS analysis of pectin + *E. coli* and pectin + *E. coli* + yeast was performed using Clarus 500 Perkin Elmer Gas Chromatography equipped with an Elote-5 capillary column (5% Diphenyl 95% dimethyl polysiloxane) (30mm*0.25mmID*0.25μmdf) and mass detector turbo mass gold of the company which was operated in EI mode. Helium was the carrier gas at a flow rate of 1 ml/min. The injector was operated at 200 ºC, and the oven temperature was programmed. The identification of components was based on a comparison of their mass spectra with those of Wiley and NBS libraries.

Extraction of Pectin: Simple distillation was used for removal of essential oil and pectin from orange peel. Water was added to the various quantity of obtained pith after sieving with different sizes of the sieve, and allowed to stand for an hour. The resultant mixture was boiled gently for 15 min, and it was allowed to cool to room temperature. The distillate resulted in two phases, oil, and water. The contents were filtered overnight using separating funnel. The filtrate (liquid pectin) was stored in a refrigerator till needed.

Preparation of Inocula: *S. cerevisiae* (yeast) and *E. coli* were prepared according to the methods described by [22, 23, 24, 25, 26]. The production of ethanol from obtained pectin was carried out using different conditions, *viz*:

Experimental Run 1: Production of ethanol using pectin + yeast only. 350 ml of liquid pectin was put in a flask and yeast (*S. cerevisiae*) was mixed with the liquid pectin. The resultant mixture was allowed to ferment by anaerobic respiration under room temperature for 14 days. The resulting mixture was filtered for a simple distillation process.

Experimental Run 2: Production of ethanol using pectin + *E. coli* only. 350 ml of liquid pectin was put in a flask, and *E. coli* was mixed with the liquid pectin. The resultant mixture was allowed to ferment by anaerobic respiration under room temperature for 14 days. The resulting mixture was filtered for a simple distillation process.

Experimental Run 3: Production of ethanol using pectin + *E. coli* + yeast. 350 ml of liquid pectin was put in a flask, bacteria (*E. coli*) and yeast (*S. cerevisiae*) were added to the mixture. The resultant mixture was allowed to ferment by anaerobic respiration under room temperature for 14 days. The resulting mixture was filtered for a simple distillation process.

Experimental Run 4: Control (pectin only). 350ml of liquid pectin was allowed to ferment anaerobically for 14 days at room temperature.

RESULTS AND DISCUSSION: Table 1 shows the quantity of pith obtained using various sieve sizes while **Table 2** gives the volume of liquid pectin extracted from each particle size.

TABLE 1: MASS OF THE VARYING PARTICLE SIZE AFTER SIEVE ANALYSIS

Particle size (mm)	Mass of pith (g)
0.075	78
0.5	94
1.0	490
5.0	140

TABLE 2: VOLUME OF LIQUID PECTIN EXTRACTED FROM EACH PARTICLE SIZE

Particle size (mm)	The volume of liquid pectin extracted (ml)
0.075	280
0.5	320
1.0	760
5	410
Total volume extracted	1,770

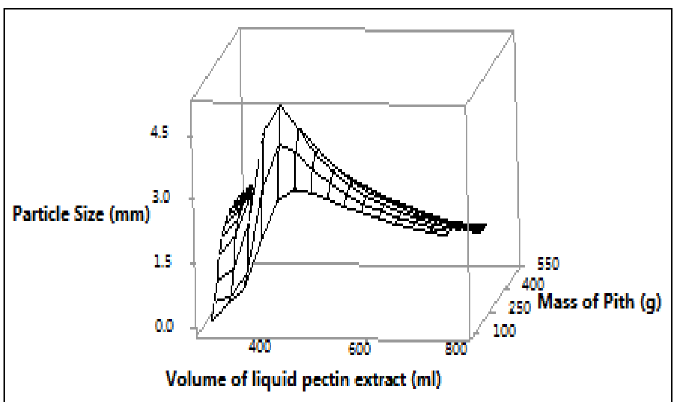

FIG. 1: SURFACE PLOT OF PARTICLE SIZE (mm) *vs.* MASS OF PITH (g), VOLUME OF LIQUID (ml)

Fig. 1 shows the effect of different sizes on the mass of the pith and the volume of pectin obtained. Particle size 1 mm with the pith mass of 0.49 gave the highest yield of pectin while **Fig. 2** shows the impact of particle size on pectin yield.

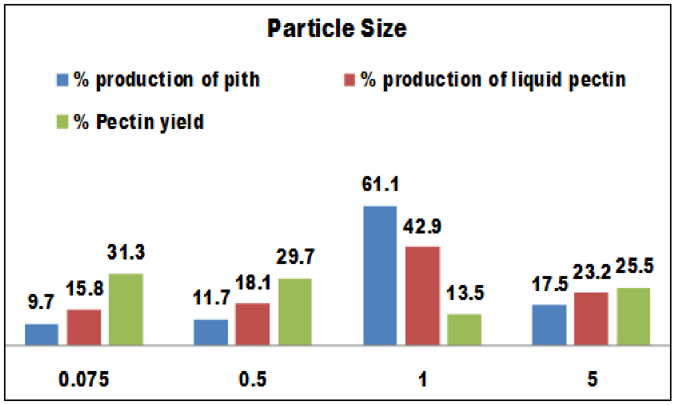

FIG. 2: PARTICLE SIZE IMPACT ON PECTIN YIELD

Y_{pec} (%) = P / Bi × 100 1

Where Y_{pec} (%) is the extracted pectin yield in percent (%), P is the amount of extracted pectin in (g), Bi is the initial amount of orange peel.

Table 3 shows the percentage of pectin obtained from the pith. The equations 2, 3, 4 and 5 for the particle size 0.075, 0.5, 1 and 5 are written as shown respectively below:

$y = 10.8x - 2.6667$ 2

$y = 9x + 1.8333$ 3

$y = -23.8x + 86.767$ 4

$y = 4x + 14.067$ 5

The particle size x = 0.075 has the highest relationship of production pitch, pectin yield, and liquid pectin. The particle size x = 1 had the highest variance in its relationship of production pitch, pectin yield, and liquid pectin. The total yield of 22 and 40 ml was obtained from both pectin + *E. coli* and pectin + *E. coli* + yeast respectively, **Table 4**.

From the experiment carried out, two distinct observations were made:

It was noticed that the thickness of the liquid pectin was inversely proportional to the surface area of the pith. Hence, blended pith with the particle size of 0.075 mm produced a thicker liquid than others.

It was also noticed that the mass of pith was directly proportional to the absorption rate of water.

TABLE 3: PERCENTAGE AMOUNT OF PECTIN YIELD

Particle size (mm)	Mass of pith (g)	% production of pith	The volume of liquid pectin extracted (ml)	% production of liquid pectin	Pectin yield	% Pectin yield
0.075	78	9.7	280	15.8	358.97	31.3
0.5	94	11.7	320	18.1	340.43	29.7
1	490	61.1	760	42.9	155.10	13.5
5	140	17.5	410	23.2	292.86	25.5

TABLE 4: PERCENTAGE YIELD OF ETHANOL UNDER DIFFERENT CONDITIONS AT VARIOUS TIMES

Days	Pectin only (ml)	Pectin + *E. coli* (ml)	(%) Pectin + *E. coli* (ml)	Pectin + yeast (ml)	Pectin + *E. coli* + yeast (ml)	(%) Pectin + *E. coli* + yeast
0	0.0	0.0	0	0.0	0.0	0.0
5	0.0	3.0	13.6	0.0	3.3	8.2
10	0.0	7.4	33.6	0.0	14.7	36.8
12	0.0	6.6	30.0	0.0	12.2	30.5
14	0.0	5.0	22.7	0.0	9.8	24.5
Total Yield		22			40	

Thus, blended pith with the particle size of 1.0 mm absorbed.

Production of Ethanol: The extracted pectin in liquid form was collected and fermented to produce bio-ethanol, using:

Pectin only, pectin + *E. coli*, pectin + yeast and pectin + *E. coli* + yeast.

From the data in **Table 4**, it was discovered that: Pectin + *E. coli* and pectin + *E. coli* + yeast produced a good volume of ethanol. This is because pectin is a polysaccharide which therefore contains complex sugar. Fermenting pectin only did not produce a significant amount of ethanol because the complex sugar present could not be broken down to enhance fermentation. However, during distillation, a little amount of ethanol was noticed to have vaporized but couldn't pass through the Liebig condenser.

This shows that liquid pectin can ferment on its own under anaerobic conditions, but the reaction will be a slow process and hence will require more days or even months to produce a significant volume of ethanol. Due to the vaporization of ethanol during production, a decrease in the percentage of ethanol yield was noticed. Pectin + *E. coli* had the highest ethanol yield on the 10th day of fermentation (33%) while pectin + *E. coli* + yeast also had the highest production of ethanol on the 10th day of fermentation (36%) both yield decreases as fermentation progresses.

Pectin + yeast did not produce alcohol, the fungi (yeast) which are an important microorganism in brewing industries was not able to perform because the sugars present were still in its complex form. However, during distillation, a little amount of ethanol was noticed to have vaporized but couldn't pass through the Liebig condenser. This also shows that it can ferment on its own under anaerobic conditions with the help of the fungi, but the reaction will be a slow process and hence will require more days or even months to produce a significant volume of ethanol.

Pectin + *E. coli* + yeast produced more ethanol, compared to pectin + *E. coli*. This was possible because the bacteria *E. coli* was added to the liquid pectin which broke down the complex sugars present to simple sugars and after 5 days, the fungi yeast was added to convert the broken sugars to ethanol.

These experiments show that pectin which is present in orange waste is a good raw material for producing bio-ethanol and hence, industries (especially fruit juice processing industries) should see it as an alternative energy source. This will help them to reduce their operating cost, dependence on fossil fuels and create a cleaner and greener environment.

During the experiment, 100 ml sample of the producing agent (Pectin + *E. coli* and pectin + *E. coli* + yeast) was taken at different time intervals to measure the volume of ethanol produced.

This shows that the ethanol production of the two agents (Pectin + *E. coli* and pectin + *E. coli* + yeast) reached their peaks on the 10th day after which, the production began to decline.

Energy Content of the Produced Ethanol: 40 ml of ethanol produced from pectin + *E. coli* + yeast is equal to 0.011 gal and has an energy content of 839.63 Btu of energy. Using a typical citrus processing plant as a basis in the calculations, which has the capacity of consuming 180 MW of energy (30,709,274.4 Btu = 402.3 gallons of ethanol) annually, the plant will need 36,572 times of the produced ethanol to power the plant for a year.

Also, 22 ml of ethanol produced from pectin + *E. coli* is equal to 0.006 gal and has an energy content of 457.98 Btu of energy. Thus, the plant will require 67,050 times of the produced ethanol to power the plant for a year.

Considering the total produced ethanol (62 ml of ethanol), which is equal to 0.02 gal and has an energy content of 1526.6 Btu of energy. The plant will require 20,115 times of the total produced ethanol to power the plant for the year.

TABLE 5: GAS CHROMATOGRAPHY FOR PECTIN + *E.COLI* + YEAST

Retention time	Compound	Area %
3.848	Ethylbenzene	0.79
4.363	Benzene, 1, 3-dimethyl	4.14
6.234	Benzene, 1,2,4-trimethyl	3.76
6.612	Benzene, 1-methyl 1-3-propyl	0.38
6.703	Benzene, 4-ethyl,1,2-dimethl	0.46
6.938	Benzene,1-methyl-4-(1-methylethyl)	0.69
7.413	Benzene 1,2,3,5-tetramethyl	2.03
10.073	Cyclopropane	25.72
11.761	Diethyl phthalate	7.68
15.137	*n*-hexadecanoic acid	1.52
20.499	Cholesterol	10.42
25.082	3-Eicosene	0.77

TABLE 6: GAS CHROMATOGRAPHY FOR PECTIN + *E. COLI*

Retention time	Compound	Area %
3.854	Ethylbenzene	1.66
3.991	P-xylene	6.21
4.369	Benzene, 1,3-dimethyl	2.8
5.53	Benzene, 1,2,3-dimethyl	1.23
5.862	Benzene, 1,2,4-trimethyl	4.94
6.24	Benzene, 1,2,3-trimethyl	1.93
6.703	Benzene,1-methyl-2-(1-methylethyl)	0.97
6.938	Benzene,4-ethyl-1,2 dimethyl	1.23
7.018	Benzene,4-ethyl-1,2 dimethyl	1.21
7.413	Benzene, 1,2,4,5-trimethyl	2.84
10.073	Cyclopropane	52.82
11.761	Diethyl phthalate	8.36

Table 5 and **6** shows the components detected using GC-MS for pectin + *E. coli* + yeast and pectin + *E. coil* samples with cyclopropane having the highest % area of 20.499 and 52.82 in samples with pectin + *E. coli* + yeast and pectin + *E. coil* respectively. It is also known as trimethylene. Cyclopropane is a colorless flammable gaseous hydrocarbon. It is a cycloalkane with molecules containing rings of three carbon atoms. It has a chemical formula C_3H_6 and Molecular weight: 42.0797. The structure of the cyclopropane was shown in **Fig. 3**. **Fig. 4** revealed the Mass Spectrum cyclopropane.

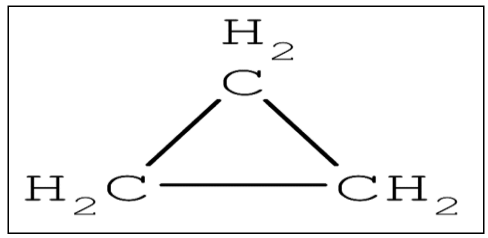

FIG. 3: STRUCTURE OF CYCLOPROPANE

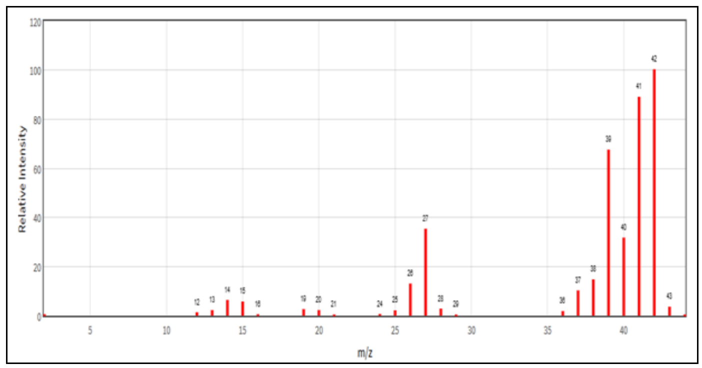

FIG. 4: CYCLOPROPANE MASS SPECTRUM

CONCLUSION: This work proved that that citrus waste (pith) used as feedstock is capable of producing ethanol which could power a plant. It is often mixed with gasoline at different ratios to obtain good energy content. Hence, the project is feasible.

From 90 oranges a total of 802 g of pith was obtained, which was then used as raw materials (pith) to extract liquid pectin considering the varying particle sizes and time of boiling. The total volume of 1,770 ml of liquid pectin was extracted which was further used to produce bio-ethanol. This shows that citrus fruit (especially orange) contains a high amount of pectin which can be used by food and drink producing companies as stabilizers in fruit juice and in making jam. Pharmaceutical industries can also use it in making drugs capable of stooping diarrhea in human beings. The Federal Government and manufacturing industries should invest massively in the generation of energy from citrus waste, as this project has proved the fact that it is highly productive, environmentally friendly and it meets the standard of the clean air act emissions. The world today is looking for various alternatives to fossil fuels and is highly concerned about its negative impacts on the environment (*e.g.*, environmental degradation, global warming, etc.). This project is, therefore, the solution to the challenge of fossil fuels and is highly recommended for use by industries and the Nation.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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