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Potential Use of Citrus (Peel) Waste to obtain Bioethanol

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A B S T R A C T

In the present study, a specialized and updated bibliographic review has been carried out, in order to have the most recent technologies that allow the development of efficient processes for the conversion of waste into biofuels, the same ones that contribute to reducing the environmental impact. An in-depth bibliographical research was carried out to know the processes of obtaining bioethanol from its shells. The results show that the success of obtaining the final product depends on an optimal fermentation, however it can be achieved in a short time of 24 hours, in addition the released results show that the mandarin peels present a better yield of ethanol, followed by the orange with a considerable proportion and finally the lemon peel with a minimal contribution.

Introduction

The environmental issue is a matter of concern, both for the State and for economic sectors and the community in general, which is why awareness of the importance of protecting the environment is promoted every day through education programs and the implementation of public policies and the legislation of regulations and fiscal incentives (Jiménez, Mestre, & Márquez, 2016).

Citrus juice producing industries generate large amounts of waste worldwide, so if their waste is not properly managed, it can generate contamination in water, soil, and air (Behzad and Keikhorso, 2018). It states that the main problem of orange solid waste is high fermentability, due to its high carbohydrate content, which accelerates its degradation and generates a bad smell.

For this reason, on a global scale, the scientific community has become important for the use of waste in the industrial sector because during the transformation or industrialization of the raw material it generates a large amount of waste, which according to Anwar, *et al*., (2008); Pérez, (2019) by arranging them in the environment they generate negative effects on it. However, they can be useful for industrial processes in other activities; but these residues have not been used by Ecuadorian industries, perhaps due to ignorance of the value of the products that have undergone transformation.

It is important to take into account that during the industrialization of the products, a large amount of waste is lost, as is the case of the citrus industry, especially oranges. Researchers (Anwar, et al., 2008) in the investigation on the Physicochemical Characteristics of citrus seeds and seed oils in Pakistan, estimate that 50% by weight of this fruit is wasted as residue consisting of the peels, seeds and the pulp.

Therefore, from the environmental point of view, renewable energies are a viable alternative for the productive sectors, which must be projected to work on the saving and efficient use of energy, in order to contribute to the reduction of gas emissions. Greenhouse effect (Alvarado & Hernández, 2018).

The table shows a summary of the most significant experiments carried out in recent years.

Table.1 Experiments to obtain bioethanol

Materia prima	Pretratamiento	Hidrólisis	Fermentación	Etanol obtenido
Rastrojos de maíz	Explosión de vapor	Enzimática usando enzimas termo-activas	*S. cerevisiae* al 11,50%, 35 °C, 96 h y 1,8 g de inóculo/ 100 mL	33,8 $g \cdot L^{-1}$
Desechos de tallo de Yuca	-	Ácida con ácido clorhídrico (HCl)	*S. cerevisiae* entre 40 y 50 °C, 8 h y 0,2 g de inóculo/100 mL	2,7 g por cada 15 g de materia prima. 32,4% de pureza
Caña de azúcar	Deslignificación usando NaOH 1M durante 2 h	Enzimática mediante celulasas	*S. cerevisiae*. 20 mL de medio por cada 1 g de materia prima. 5 días.	11,8 $g \cdot L^{-1}$
Bagazo de Caña de azúcar egipcia	Triturado y esterilización en autoclave 20 min a 121 °C	Enzimática con *Aspergillus terreus*	*S. cerevisiae* a 30 °C, 48 h y 10% en volumen de inóculo	0,249 g de etanol por 1 g de bagazo
Pulpa de remolacha de azúcar	Calentado con vapor entre 152 y 175 °C y a 4 -8 bar de presión	Enzimática usando celulasa comercial a 50 °C 24 h	*S. cerevisiae* a 30°C durante 24 h	0,5 g por 1 g de glucosa
Bagazo de Sorgo dulce	Calentamiento a 200 °C de la muestra impregnada en SO_2 al 2,5% durante 7,5 min	Enzimática usando celulasa comercial a 50 °C durante 72 h	*S. cerevisiae* a 30 °C durante 48 h con 3 – 5 $g \cdot L^{-1}$ de inóculo	15,3 g por cada 100 g de bagazo
Paja de arroz	Pretratamiento con Na_2CO_3 0,5M a 100 °C durante 3 h	Enzimática usando celulasa comercial	*Mucor hiemalis* a 37 °C durante 72 h y con 1 g de inóculo por 1 L de biomasa	12,8 $g \cdot L^{-1}$. 15,4 g de etanol/ 100 de paja de arroz
Fruta de palma	Deslignificación en NaOH 0,1M a 100 °C durante 60 min	Enzimática con celulasa a 50 °C durante 72 h	*S. cerevisiae* a 30 °C, 72 h y con el 10% en volumen de inóculo	12,1 $g \cdot L^{-1}$
Pulpa de café	-	Ácida con H_2SO_4	*S. cerevisiae*, 30 °C durante 24 h	7,4 $g \cdot L^{-1}$
Cáscara de naranja	Calentamiento con vapor a 200 °C	Ácida con H_2SO_4, 5% en peso, 115 °C y 15 min	*S. cerevisiae* durante 24 h	11,9 $g \cdot L^{-1}$
Cáscara de naranja	Triturado. Deslignificación con NaOH 2% en volumen. Esterilizado a 121 °C	Enzimática con celulasa comercial	*S. cerevisiae* durante 24 h y a 75 rpm	16,3% de pureza
Cáscara de naranja	Esterilizado a 121 °C durante 15 min	Ácida con H_2SO_4 al 5%	*S. cerevisiae* durante 15 h a 40 °C	12,97 $g \cdot L^{-1}$
Cáscara de naranja	Triturado en partículas de 2 mm x 2 mm	Ácida con 100 mL de H_2SO_4 al 5%	*S. cerevisiae* durante 24 h a 30 °C	0,112 g por 1 g de materia prima. 10,7% de pureza
Cáscara de naranja	Triturado en partículas de 2 mm x 2 mm	Enzimática con mezcla de diferentes enzimas	*S. cerevisiae* durante 24 h a 30 °C	0,187 g por 1 g de materia prima. 17,9% de pureza

Table.2 Characterization of Citrus Peel

Biomass	Humidity (%)	Reducing sugars (%)	Cellulose (%)	Hemi-cellulosa (%)	Lignin (%)
Lemon	70,0	1,8	21,6 (23,1a; 22,8b)	6,0 (8,1a, 22,4b)	8.9 (7.6a; 8.3b)
Mandarin	77,5	3,4	20.2 (22.5a; 20,8b)	7,8 (6,0a; 17,2b)	9.1 (8.6a; 8.9b)
Orange	69,4	2,8	23,5 (37,1a; 22,0b)	10.4 (11.0a; 19.9b)	7,6 (7,5a; 8,4b)

Source: (Boluda & López, 2013)(Marín, Benavente, CastilloJ, Soles, & Pérez, 2007)

Characterization of citrus waste from agroindustry

The characterization obtained by researchers (Tejeda, Marimóm, & Medina, 2014) of had given peels resulted in a higher moisture content for lemon peels and a higher content of reducing sugars, cellulose, hemicellulose and lignin in mandarin peels, as seen in the following table 2.

It can compare the results of the research carried out by the authors (Boluda & López, 2013; Marín, Benavente, CastilloJ, Soles, & Pérez, 2007; Tejeda, Marimóm, & Medina, 2014) can be compared with those reported in other investigations in which they have worked with citrus residues. Table.3 below shows the lignin removal reached between 45.5% and 70.1%.

Table.3 Characterization of Delignified Biomass

Biomass	Lignin (%)	Performance (%)
Lemon	4,87	45,5
Mandarin	2,72	70,1
Orange	3,50	53,7

The table 4 (Tejeda, Marimóm, & Medina, 2014) present the yields obtained in the production of reducing sugars, which were between 13.88 and 21.10 g/100 g of dry biomass, being higher that of the shells of tangerine.

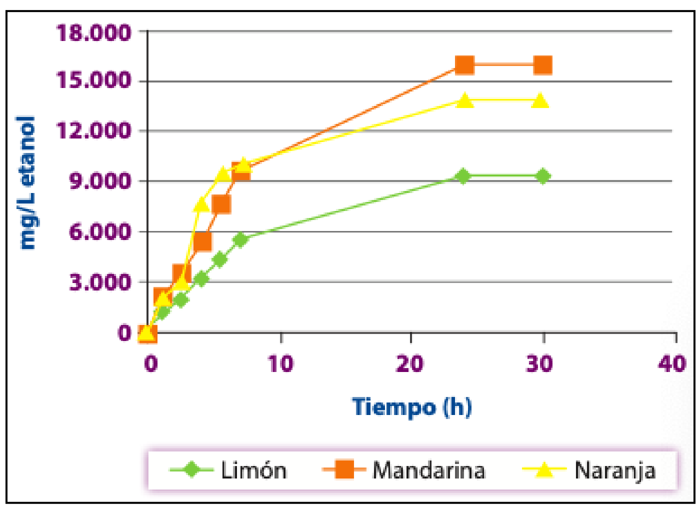
The optimal fermentation time achieved in 24 hours. Ethanol concentrations reached 9.32, 15.96, and 13.90 mg/L for lemon, orange, and tangerine peels.

Table.4 Characterization of glucose syrups

Biomass	Reducing sugars (g/L)	Dextrose syrup volume (mL)	Yield (g/100)
Lemon	84,5	21,5	17,29
Mandarin	93,7	25,3	21,11
Orange	89,4	23,8	13,88

The results described in table 4 are presented in the following figure 1.

Figure.1 Fermentation Progress



The inadequate treatment of these residues and many of which are deposited in the field, under normal conditions, spontaneous fermentation occurs with the consequent production of greenhouse gases such as: methane, carbon dioxide, as well as other effects, including the mantles of Water.

According to several studies, there are adequate technologies that allow these residues to be processed into biofuels.

Biofuel

Renewable fuels of biological origin that can be obtained from herbaceous and woody plants, manure, biomass to different types of agricultural and livestock waste, etc. are understood (Hazell and 2006; Litran, 2017).

Bioethanol is realize as a renewable fuel, complementary or substitute for gasoline, they are produced from sugars, starch or cellulose, contained in different vegetable materials, (United Nations, 2008), but in this work the studies made in citrus residues such as oranges, which contain sugars from which bioethanol is obtained through the process of fermentation and distillation.

The government of many countries have taken the initiative for years with public policies in an effort to replace gasoline with bioethanol and diesel with biodiesel.

Box.1 Policy goals set for biofuels

País / Región	Bioetanol	Biodiesel
América del Norte		
USA	Renewable Fuels Standard y Alternative Fuels Standard: 28,000 millones de lts. de combustibles renovables en el 2012; 132,000 millones de lts. de combustibles renovables y alternativos en 2017 (15% del uso proyectado de gasolinas al 2017).	
Canadá	5% en 2010	2% de contenido renovable en diesel oil y fuel oil en 2012
Europa		
Unión Europea	5.75% al 2010, 8% al 2015 y 10% al 2020 para biocombustibles en sustitución de diesel oil y gasolinas para transporte (computado sobre base energética)	
Asia		
Japón	Sustitución de 500,000m3 de gasolinas para transporte por año al 2010 (1.8 millones de lt/año de bioetanol en el corto plazo, 6 millones de m3 de bioetanol producido localmente , al 2030 que representa el 10% de la demanda actual de gasolinas)	
China	15% del consumo para transporte al 2020	
India	5% al 2012, 10% al 2017	
Oceanía		
	liesel+bioetanol al 2010	
Argentina	5% sobre el producto final al 2010	5% sobre el producto final al 2010
Bolivia		2,5% a partir del 2007 hasta llegar a un 20% en el 2015.
Brasil	22% desde el 2001	2% al 2008 y 5% desde el 2013 y 20% al 2020
Colombia	10% a partir del 2006, por regiones	5% a partir del 2008
Paraguay	18% mínimo	1% en 2007, 3% en 2008, 5% en 2009
Perú	7,8% a partir del 2006 y en forma progresiva por regiones	5% a partir del 2008 y en forma progresiva por regiones

Fermentation

To obtain biomass, (Tejeda, Marimóm and Medina, 2014) states that researchers follow the same process; First, the collection and adaptation of the raw material, then the lignin is eliminated, then it proceeds with hydrolysis and fermentation to finally carry out distillation. However, (Sánchez, Gutiérrez, Muñoz and Rivera, 2010) proposes that the role of industries and the scientific community to obtain a quality product should seek to improve techniques or venture with novel pretreatments in order to degrade the solid lignin-cellulose-hemicellulose structure to mono and disaccharide sugars without producing inhibitors, in addition to aspects of economic and environmental interest such as low energy consumption, low investment costs, use of cheap, efficient, recyclable and applicable and equally effective reagents in different kinds of substrate.

Laboratory studies show that to produce bioethanol they make use of various equipment and materials Autoclave, oven, laminar flow chamber, stereoscope, semi-micro vacuum distillation equipment, refractometer, microwave, chromatograph, vacuum pump, analytical balance, digital balance, magnetic stirrer, photographic camera, petri dishes, 150ml glass jars, test tubes, pipettes, beakers, burner, tweezers, dissection needle, platinum loop, thermal blanket, Erlenmeyer flasks, parafilm paper, watch glass, sieve, scissors, knife, paper towel, aluminum foil and electric oven.
Reagents such as Kovacs, 30% methylene blue hydrogen peroxide, gentian violet and lugol.

Mineral salts: Potassium chloride, Ferrous sulfate heptahydrate, Sodium nitrate and Magnesium sulfate heptahydrate, Dipotassium phosphate, Tetracycline, Potato Dextrose Agar and Bactopeptone.

The biomasses used in the laboratory have been the peels of lemon (*Citrus limon CPW* L.), tangerine (*Citrus nobilis*) and orange (*Citrus sinensis*). Collection of hotel waste in the city of Cartagena. A 50 g sample of each biomass was washed with distilled water at 60°C and 70% ethyl alcohol. Moisture, reducing sugars were determined by the Fehling method, cellulose and hemicellulose by near infrared spectroscopy (NIRS), and lignin by UV spectroscopy. Size reduced in a blade mill and lignin removal in 0.1N NaOH solution for 15 minutes and calcium sulfate at rest for 3 hours. The particulate material separated from the solution by decantation. Acid-soluble lignin has been determined in the filtrate by UV spectroscopy.

The delignified material has been subjected to acid hydrolysis with 25 mL of 5% sulfuric acid per 50 grams of fruit peel at 125°C and 15 psi for 15 minutes. The syrups obtained were separated by centrifugation and the sugar content was determined.

Technological processes for the production of bioethanol from citrus waste.

Researchers (Ernandes, *et al.*, 2010) have proposed *Zymomonas mobilis* and *Saccharomyces cereviseae* as the most efficient strains of microorganisms for the production of bioethanol from lignocellulosic feedstock due to their high conversion rates of sugars into ethanol and carbon dioxide, cited by (Tejeda, Marimóm, & Medina, 2014) .

Hydrolysis

Laboratory studies carried out by (Tejeda, *et al.*, 2010) hydrolysis (chemical process for the degradation of cellulose into sugars to later ferment them) has been carried out, adding 50 ml of 5% sulfuric acid for every 100 grams of fruit peel, at a temperature of 125°C and 15 psi, regulated by means of an autoclave, for 15 minutes, as reported by (Sun and Cheng, 2002). Then the syrups obtained have been separated from the components that precipitated, by centrifugation. They have determined the sugar content in the syrups obtained.
Isolation of microorganisms. Isolation of *Zymomonas mobilis*. The *Z. mobilis* strain must be isolated in a medium based on apple juice, in 40 ml flasks at room temperature for 7 days under microaerophilic conditions.

After hydrolysis, they have adjusted the pH to 4.5 - 5.0 with NaOH 5 N, and as nutrients they have used 0.25% phosphate $(NH4)_3PO_4$, nitrogen inoculated with 0.1% P/V yeast. commercial dry active (*Saccharomyces cerevisiae*) dissolved in a little syrup as reported (Cuadrado and Vélez, 2006) .

Fermentation

Fermentation has been carried out in 250 ml Erlenmeyer flasks, with an effective working volume of approximately 50 ml in anaerobic conditions at 30°C and 200 rpm, in Nouva II orbital shakers, for 5 hours, according to (Ruiz & Arias, 1997). Controlling the pH and temperature of alcoholic fermentation. The researchers have taken samples every 90 minutes for 7 hours and later at 24 hours, that is, according to (Litrán, 2017) at this stage, the mother liquor has been transformed through the fermentation process into ethanol and carbon dioxide.

Distillation

The distillation process carried out by the researchers (Tejeda, et al., 2010) involves heating the solution contained in a pycnometer connected to a condenser to 100°C in a water bath. The following graphic describes the process.

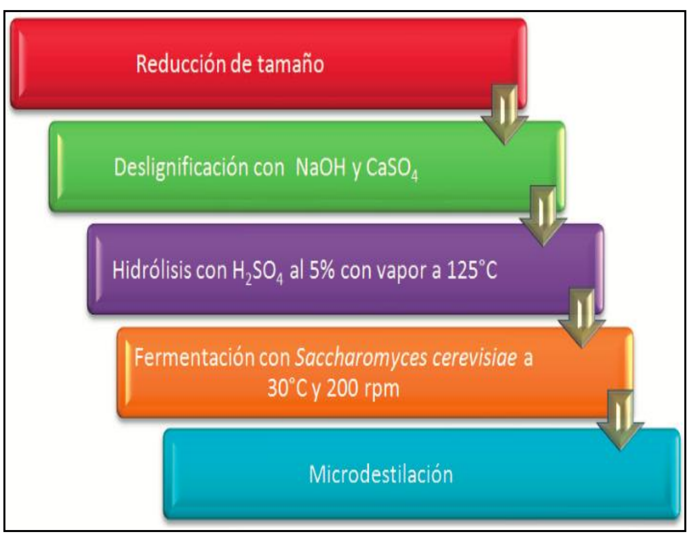


Figure.2 Diagram of the Distillation Procedure

Once the liquor has fermented, the product obtained after fermentation must be distilled to finally obtain bioethanol.

Use of Bioethanol as Biofuel or Biofuel.

Bioethanol can be used as an additive in gasoline and as a fuel in cars with a gasoline engine (explosion or Otto type). (Aguilar, 2010). The most common is that it is used mixed with gasoline in different quantities. Therefore, bioethanol contains very similar properties to the use of fuels that are burned to obtain another type of energy, in this way bioethanol can be used for transportation or for electricity and heat generation.

If the percentage of bioethanol is low, between 5 and 10% (E5 and E10), almost any modern gasoline engine can run on it (care should be taken with old engines over 20 years old because they may not admit more of 5%).

If the percentage of bioethanol is high, normally E85 (85% bioethanol and only 15% gasoline), adapted engines are required: gasoline models called Flexifuel.

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For concentrations of 100% (E100) special motors are normally required.

Bioethanol has a higher octane number than gasoline, so the engine gives a little more power. However, it has a lower calorific value and more fuel is needed to cover the same kilometres, so the engine consumption increases (in liters per 100 km) (Motorpassion, 2018)

Additionally, among the uses and applications of bioethanol as a biofuel, it can be adapted in land transport, in aviation, in heating systems, solvent for industrial use, in cooking systems, as a lubricant for engines, to generate electricity, power devices, as a solvent for domestic paint and among others (Maldonado, 2020).

International regulations to consider and use bioethanol as a biofuel

Characterization of the bioethanol obtained

Obtained bioethanol from the cellulose resident in the orange peel, it is essential to perform the evaluation of certain parameters in order to check the quality of the biofuel (Litrán, 2017).

Not only for the result, which is bioethanol, evaluated, but also some characteristics such as density, acidity, turbidity and coloration as part of the current regulations on biofuels.

According to the ASTM 4806 standard, the density of bioethanol cannot be greater than 0.99 g/mL. Its density must be measured at a temperature of 15 oC, otherwise it will affect the performance of the motor.

The UNE-EN 15491 standard states that the acidity index must not exceed the maximum values regulated by this standard, since the acidity index can produce greater corrosion, as well as wear and deposits in the engine that uses it.

Turbidity and coloration as the degree of opacity of a fluid and the absence of turbidity define the UNE-EN 15769 standard.

In conclusión, the present work, an investigation of the process of obtaining bioethanol from citrus waste, especially orange peel as raw material, has been carried out and an alternative solution for the use of environmentally friendly fuels has been found. In addition, avoiding the environmental contamination of the greenhouse effect produces citrus residues when exposed to the open field.

Fruit residues should not be wasted, on the contrary, they should be converted into products that are extremely useful, such as bioethanol, which serves as an alternative energy source.

The results of the different experimental studies showed that bioethanol meets the appropriate conditions such as density, acidity, and turbidity according to current regulations, however it is important to continue with research and experiments to improve performance, lower production costs, as well as the reduction of the time to obtain it.

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