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Obtención de bioetanol y caracterización química de la cáscara de naranja Valencia cultivada en Ríoverde, San Luis Potosí-México

Chemical characterization and bioethanol extraction from peel of 'Valencia' oranges grown in Rioverde, San Luis Potosí-Mexico

Obtenção de Bioetanol e Caracterização Química da Casca de Laranja Valência Cultivada em Ríoverde, San Luis Potosi-México

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Resumen

La obtención de bioetanol es uno de los retos más importantes en la generación de biocombustibles amigables con el medio ambiente, la búsqueda de matrices que produzcan niveles altos de este combustible representa un área de estudio de innovación tecnológica, en donde el desperdicio agroindustrial puede considerarse una alternativa para la producción de bioetanol de segunda generación que a corto plazo evita el incremento de desperdicios en vertederos y proporciona un valor agregado a los subproductos agroindustriales. En este trabajo evaluamos la composición química de la naranja de la región de Rioverde San Luis Potosí, la cual contiene en la cáscara por cada 100 g; 2.4 mg de fenoles totales equivalente de ácido de gálico cuantificados por Folin-Ciocalteu, 0.038 de flavonoides totales equivalentes de quercetina cuantificados por acetato de potasio, 2.9 g glucosa, 2.3 g fructosa, 0.9 g sacarosa y 14.25 mg de ácido ascórbico cuantificado por CLAR. Finalmente, se obtuvieron 20 mL de etanol al 90 % por fermentación anaeróbica de 2 kg de cáscara empleando Saccharomyces cerevisiae. El etanol obtenido permitió activar un termo-motor, por lo que la factibilidad para usar la cáscara de naranja para la producción de biocombustible o como aditivo alimenticio es viable.

Palabras clave : Biocombustibles; Fenoles totales; Flavonoides totales

Abstract

Bioethanol extraction is one of the most important challenges in the generation of environmentally friendly biofuels. The search for matrices to allow producing high levels of these fuels represents an area of study in technological innovation, where agro-industrial waste can be considered an alternative for the production of second-generation bioethanol, thus avoiding the increase of waste in landfills and providing added value to agro-industrial byproducts. In

this work, we evaluated the chemical composition of oranges grown in the Rioverde, San Luis Potosí region. The values obtained per 100 g of peel were: 2.4 mg total phenols (expressed as Gallic acid equivalent, quantified by Folin-Ciocalteu); 0.038 mg of total flavonoids (expressed as quercetin equivalent, quantified by potassium acetate); 2.9 g glucose; 2.3 g fructose; 0.9 g saccharose; and 14.25 mg ascorbic acid, quantified by HPLC. Finally, the 20 mL of 90 % ethanol obtained by anaerobic fermentation of 2 kg peel using Saccharomyces cerevisiae produced enough energy to power a thermomotor. These results provide important evidence supporting the feasibility of using orange peel to produce biofuel or food additives.

Keywords : Biofuels; Total phenols; Total flavonoids.

Resumo

A obtenção de bioetanol é um dos desafios mais importantes na geração de biocombustíveis amigáveis com o meio ambiente, a procura de matrizes que produzam níveis elevados de este combustível representa uma área de estudo de inovação tecnológica, onde os resíduos agroindustriais podem ser considerados uma alternativa para a produção de bioetanol de segunda geração que, a curto prazo evita o incremento de resíduos em aterros e oferece um valor agregado aos subprodutos agroindustriais. Neste trabalho avaliamos a composição química da laranja da região de Ríoverde San Luis Potosí, a qual contem na casca por cada 100g; 2,4 mg de fenóis totais equivalente de ácido de gálico quantificados por Folin-Cicolteu, 0,038 de flavonóides totais de quercetina quantificados por acetato de potássio, 2,9 g glicose, 2,3 g frutose, 0,9 g sacarose e 14,25 mg de ácido ascórbico quantificado por CLAR. Finalmente, 20 ml de etanol a 90% foram obtidos por fermentação anaeróbica de 2 kg de casca utilizando Saccharomyces cerevisiae. O etanol obtido permitiu ativar um termo-motor, pelo que a viabilidade dos usos da casca de laranja para a produção de biocombustível ou como aditivo alimentar é viável.

Palavras-chave: Biocombustíveis; Fenóis totais; Flavonóides totais

Introduction

For many decades the nonrenewable sources or fossils have covered the energetic demand. Nevertheless, due to this the production of these fuels is decreasing, and its use contributes a lot to global warming, the worldwide trend is the production of renewable energy (Willem *et al.*, 2009).

Furthermore, one of the biggest problems of the current century is related to the management of solid waste, which also contributes to global warming. An example of this is the amount of organic material that according to data from SAGARPA (Agriculture, Livestock, Rural Development, Fishery, and Food Department), orange peel only contributes up to 4,896,045 tons (SAGARPA, 2018).

The research in the management of this kind of resources has extended to many areas, being one of the most promising the second-generation bioethanol extraction, because this product doesn't compete with the food field, which means that the cost of raw material doesn't increase.

Based on the peel considered as waste, it could be used as a high value sub product. For this, it is necessary to perform a chemical characterization of orange peel to evaluate its potential and in a parallel way evaluate too, the possibility of using it to obtain ethanol.

Materials and methods

Biological material

The biological material (Valencia orange, *Citrus sinensis* Osbeck) was bought at Mexico City's Central de Abastos, cultivated in Rioverde, San Luis Potosí, located between the parallels 22° 25' and 21° 33' of North latitude; the meridians 99° 44' and 100° 25' of West longitude; altitude between 100 y 2600 m. Borders on the North with the municipalities of Villa Juárez, Alaquines, Cárdenas, Rayón y San Ciro de Acosta; on the South with the municipality of San Ciro de Acosta, Guanajuato state and the municipality of Santa María del Río; on the West with Santa María del Río, Ciudad Fernández, San Nicolás Tolentino, and Villa Juárez municipalities.

The fruits were washed with tap water and soap, sanitized with sodium hypochlorite 2% (v/v) and were left for ten minutes before drying them. The orange's peel, pulp, and seeds were separated by hand; the peels and the seeds were dehydrated in a Labconco Model 6 lyophilizer (Labonco, MO, USA) at 0.04 mbar, and 50 ºC for 48 hours. Finally, the lyophilized peels were pulverized and stored in a glass container for subsequent analysis.

Obtention of extract

The extraction was done by maceration. 5 g of lyophilized peels were homogenized in 50 mL of HPLC grade methanol and left for 24 hours on an incubator Labnet Model 211DS at 160 rpm at room temperature. Each extract was filtered with Whatman No. 2 paper. Afterwards, the solvent was evaporated in a vacuum at 40°C, using a (Büchi model R-3) rotary evaporator. The dried extracts were stored at -20 ºC until subsequent analysis (Chel-Guerrero *et al*., 2018; Can-Cauich *et al*., 2017).

Chemical characterization

Ashes' percentage

The organic substance/matter was calcinated at 400 °C for 3 hours in a crucible. The ashes' percentage was calculated as follows: 100X (crucible with ashes (g) - crucible (g)) / (crucible with sample (g) - crucible (g)).

Moisture content

This method is based on weight lost due to the water evaporation; for this, the sample was left at 60 °C, until a constant weight was observed. The moisture content was calculated as follows: 100X [(sample's initial weight (g) - final weight dry sample (g)) / (Initial weight sample (g))].

Determination of reducing sugars

The presence of reducing sugars was determined by two methods: a) Fehling test using a commercial kit (Golden Bell Reactivos^{MB}), and b) Benedict test.

a) 25 mg of orange peel methanol extract was deposited in four test tubes containing star apple dissolved in 1 mL of Fehling A solution (copper solution), and 1 mL of Fehling B (alkaline solution). The tubes were shaken and incubated in hot water at 90 °C for ten minutes. A brick red

color precipitate indicates the presence of reducing sugars. Fructose and saccharose were used as positive and negative controls, respectively.

b) Benedict. 1 g of each dried orange peel extract, glucose, and fructose as a positive control, and saccharose as a negative control were dissolved in different test tubes with 12 mL of distilled water and 5 mL of the Benedict reagent. The presence of reducing sugars was indicated by red, yellow or green characteristic colorations (Aruna, 2016).

Total flavonoids content

The total flavonoids qualitative analysis was made with the adapted Aarland method (Aarland *et al*., 2017), 1 mg of orange peel lyophilized extract was suspended in 1.5 mL of methanol, 0.1 mL of 10 % aluminum chloride (w/v), 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes and the absorbance at 415 nm was determined (Aarland *et al*., 2017). A calibration curve of quercetin of 10-100 µg mL-1 in methanol was prepared. The results were expressed as mg equivalent of quercetin equivalent per 100 g.

Total phenols content

The total phenolic compounds were determined using the Folin-Ciocalteu's reagent. 50 mg of orange peel lyophilized extract were diluted in 5 mL of methanol, then, 0.2 mL of the previously diluted extract was mixed with 1 mL of the reactive Folin-Ciocalteu (2 N), (previously dissolved in distilled water 1:10), the mix was incubated for 1 minute before adding 0.8 mL of a solution of sodium carbonate 7.5 % (p/v) in distilled water. Phenols were evaluated at 765 nm using a spectrophotometer (JENWAY 6705 UV/VIS). The standard curve was prepared with gallic acid (Chel-Guerrero *et al*., 2018; Zarza *et al*., 2021).

Quantification of organic acids

To determine the organic acids (citric, malic, oxalic, tartaric, ascorbic): 1 mg of each dried extract was dissolved in 1 mL of HPLC grade water and was filtered with nylon filters of 0.45 µm (Millex, Millipore, Bedford, USA.). 20 µL of the filtered extracts and the acid standards were injected in a high-performance liquids chromatograph (Agilent Technology, model 1260, equipped with a quaternary pump, degasser, automatic injector and a thermo stable body for column), with a multiple wavelength detector adjusted to 250 and 210 nm. The used column was X-Terra MS C_{18}, 5µm (4.6 x 250 nm), and the mobile phase consisted of phosphate buffer (50 mM, pH 2.8) in an isocratic way. The flow was adjusted to 0.7 mL/min. The results were registered at 210 nm, and were interpolated in a standard curve of citric, malic, oxalic, tartaric and ascorbic acids, respectively. The results were expressed as mg of each acid/100 g of lyophilized peel extract (Aarland *et al*., 2017; Gómez-Covarrubias *et al*., 2020).

Quantification of sugars by HPLC

The determination of sugars (glucose, fructose and saccharose) was performed by HPLC Agilent 1260 (chromatograph mentioned above) using a refractive index detector. The column used was a Hi-Plex Ca^{+2} (8 % crosslinked, 7.7x300 mm, 8µm) using as a mobile phase HPLC grade water in an isocratic way. The flow was set at 0.6 mL/min and the column temperature was fixed at 80 °C. 0.5 g of lyophilized peels was macerated in 15 mL of HPLC grade methanol for 24 hours. Afterwards, 2 mL of the extract were filtered with nylon filters of 0.2 µm and transferred to vials. The injection volume was 20 µL. Saccharose, glucose and fructose were used as standards, all the measurement were made in triplicate (Cervantes-Arista *et al*., 2020; Gómez-Covarrubias *et al*., 2020).

Infrared spectrum

An infrared spectrum was taken from the lyophilized samples before and after fermentation, the infrared equipment used was an Agilent IFT-Cary 360.

Fermentation

0.575 g of yeast were added to 0.5 kg of orange peel and was activated with 20 g dextrose and let resting by 2 weeks. The reactor system (Figure 2a) was adapted to allow the gases to escape, but not to allow oxygen in. Afterwards, it was put in the distillation equipment (Figure 2b).

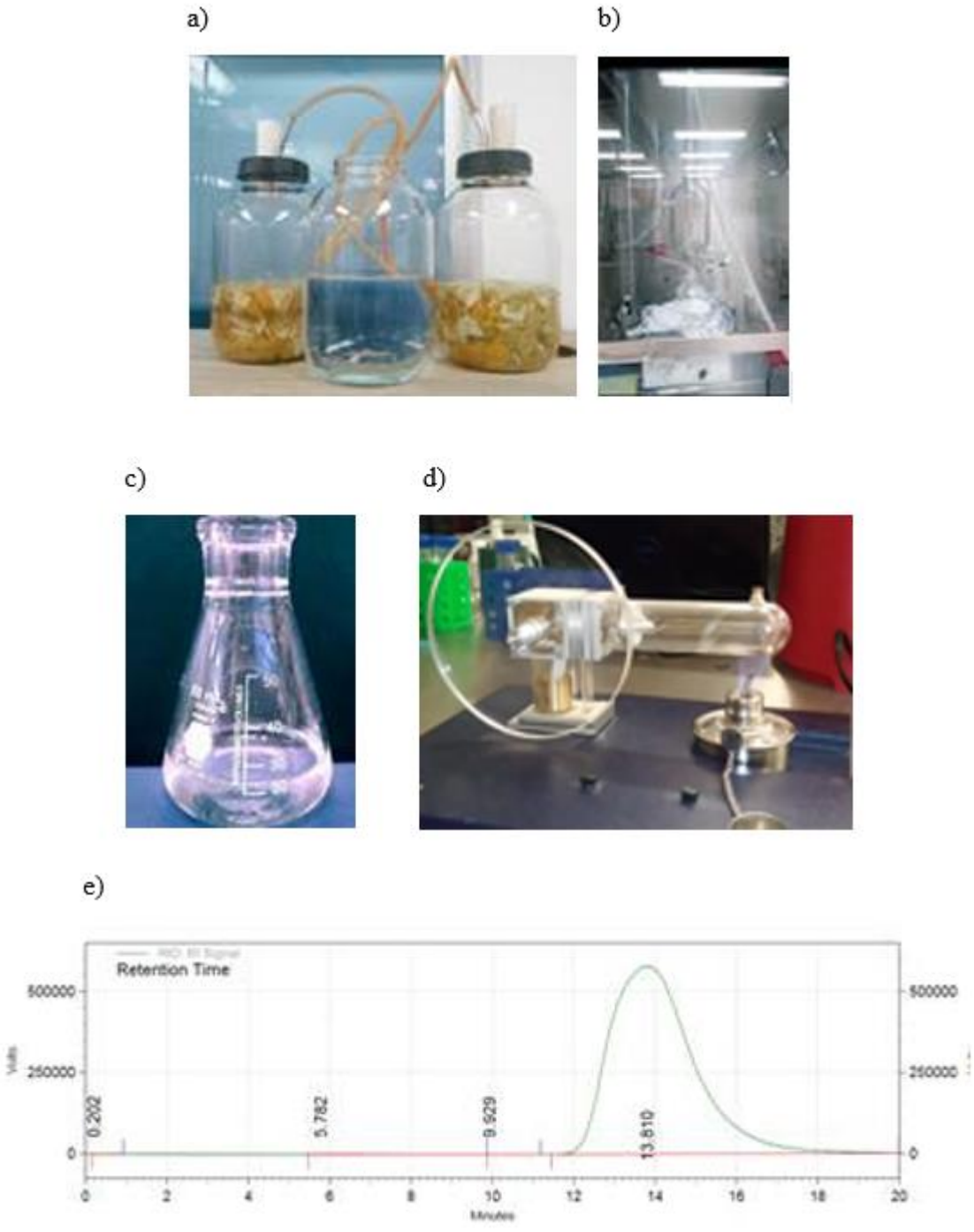


Figure 2. a) Reactor system, b) distillation equipment, c) Physical aspect of the sample, d) Stirling motor and color of the flame, e) alcohol purity determination by HPLC.

Distillate properties and alcohol determination by HPLC

The determination of alcohol content was made by HPLC using the chromatograph mentioned above, using a refractive index detector. The column used was Hi-Plex Ca^{+2} (8 % crosslinked, 7.7 x 3000 mm, 8 µm) using as mobile phase HPLC grade water in an isocratic way. The flow was of 0.6 mL/min, and the column temperature was fixed at 80 °C. 2 µL of the distillate were filtered with nylon filters of 0.2 µL and were transferred to the injection vials. The injection volume was 20 µL. Ethanol dilutions were used to prepare the calibration curve.

The flame transparent test was made; the distillate smell was determined by the senses, and a Stirlingmotor Hot Air Engine (phywe 04372.00) was used.

Results and discussion

Raw material extraction and chemical characterization

The average weight of the 100 oranges used in this study was 138 g. The relation between the peel and the pulp is shown in Table 1, 21.50 % peel and fruit proportion. If we multiply by the reported orange production by SAGARPA (4'896,035) we would get a total of available raw material of 1'052,647.552 tons.

Table1. Fruit bromatological analysis, peel composition, and evaluation of the distillate obtained from the peel

a) Fruit composition

	Composition	Moisture	Dry Material	Ashes
Peel	21.50%	99%	1%	0.04%
Seeds	0.80%	12.50%	87.50%	0.41%
Pulp	77.70%	99.90%	0.10%	0.38%

b) Peel Composition

Glucose g/100g	2.94 ± 0.163
Fructose g/100g	2.274 ± 0.1866
Saccharose g/100g	0.907 ± 0.097
Total Flavonoids mg EAQ/100g	0.038 ±0.0005599
Total Phenols mg EAG/100g	2.396 ± 0.014

c) Evaluation of the distillate of the bioethanol extraction from peels

Alcohol degrees	90°
Color	Colorless, transparent
Odor	Ethanol characteristic with orange notes
Flame	Bright white
Flavor	Strong, slightly citric

In the proximal composition of the orange peel, we found 99 % of humidity, so the amount reported by SAGARPA is reduced to 10,526.48 ton. The chemical study indicated that the main organic acid was ascorbic acid, we did not find malic, oxalic, tartaric, and citric acids (Figure 1a). It is important to note that due to the amount of ascorbic acid (14.25 mg/100g), it could be thought of as food additive, because this acid has a high antioxidant power.

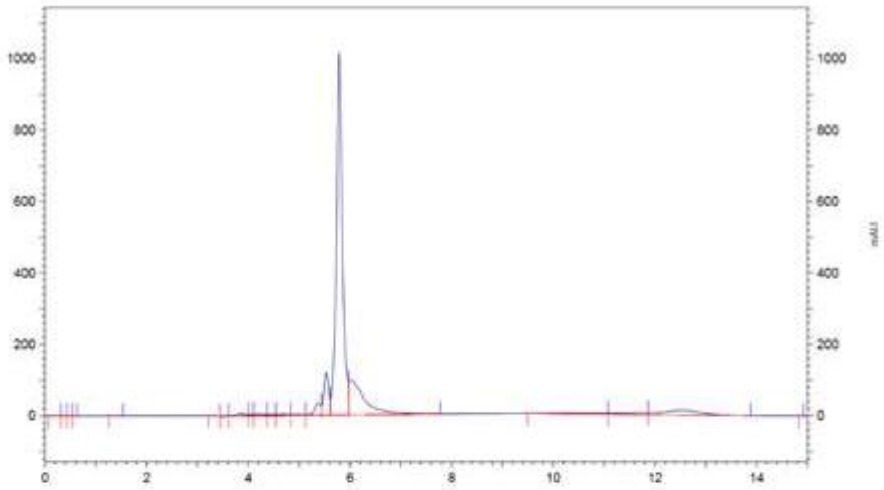
Regarding the sugar content, both Fehling and Benedict test were positive (Table 1). Due to this, sugars were quantified by HPLC, finding the total sugar content of glucose, fructose, and saccharose fluctuating between 6.11 ± 0.148 g/100 g of sugar in 100 g of lyophilized peel; the glucose´ presence was higher 2.94 ± 0.163, followed by fructose 2.274 ± 0.1866 and in lesser amounts saccharose 0.907 ± 0.097. The chemical profile of the peel obtained by infrared spectrum shows the characteristic bands of the alcohols that correlate with the phenols and sugars (Figure 1b).

Bioethanol

After fermentation we obtained an average distillate production of real ethanol of 20 mL for 2 kg of fermented peel, the distillate presented a characteristic ethanol odor, but with the presence of drops of orange essence (Figure 2c). The flame test indicated that it was ethanol, and it was possible to operate the Stirlingmotor (Figure 2d). If the theoretical yield for 1 g of glucose is 0.51 g of ethanol, this experiment produced 15.78 g (20 mL) of ethanol per 122.2 g of total sugar (see sugar quantification), this represents a 25 % yield.

The chemical profile of the orange peel waste was determined again after maceration but it was observed that the bands correspondent to the hydroxyl's groups decreased (Figure 1), nevertheless, they did not disappear completely, with which we can contemplate complex sugars deterioration to increase the alcohol efficiency production (Figure 1).

a)



b)

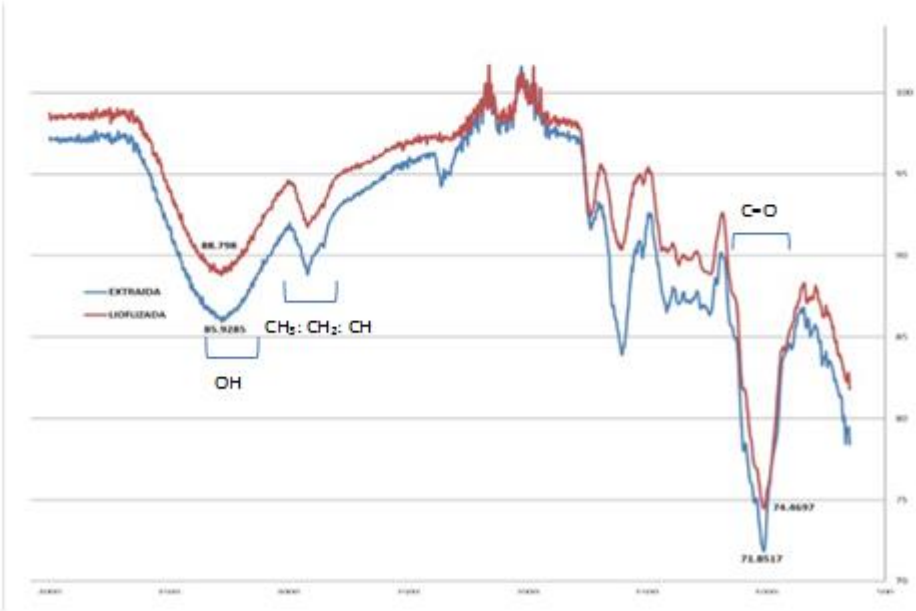


Figure 1. a) Chromatogram of the organic acids of the lyophilized peels, and b) IR spectrum of the lyophilized peels before and after the fermentation

The distillate purity was high as we can see in Figure 2e, where we only got a peak that corresponds to the ethanol retention time and corresponds as well to the odor and the flame, the degree percentage of alcohol was 90°, calculated in the relation weight-volume of the elution mix injected to the HPLC equipment, which is a high degree for a first distillation (Table 1), and the kind of distillation, as well, that could be used for the communities to generate light with a Bunsen burner, or to sterilize medical supplies, having with this an energy saving. It is also possible to adapt the

operation of the Hot Air Stirling Engine (Phywe 04372.00), to a process for obtaining ethanol, since the ethanol produced in this process was capable of activating the engine for approximately one minute. Motor that in turn can be adapted to a power generator.

If we get a production of 4'896,035 oranges tons, the biomass content available would be 1'052,467.525 tons, from which, based in this study, we could obtain 10'526,475.52 L of ethanol. If we consider taking advantage of the 10 % of the sub product, we will get 1052,648 L.

The energy costs are by hour, 0.793 Mexican pesos per hour. In a nine hours distillation process the cost would be 3.43 Mexican pesos about to get 20 mL of ethanol, which would give us a price of 0.357 Mexican pesos / mL. At this moment, it is considered that the peel as waste has not purchase value, only transportation costs which we are not considered here.

The price for the ethanol of oranges peel is lower than the corn bioethanol: 0.653 Mexican pesos/mL. So, the use of this matrix to get this biofuel is viable, and in addition we are contributing to the proper management of the residual biologic material to reduce the CO_2 indexes that contribute to global warming.

On the other hand, it is important to point out that being waste material will not affect the local market price, as happens with others fermented materials like corn and sugar cane. From another perspective it is possible to consider the bioethanol not only as an energetic component, but it is also possible to use as an antiseptic.

Conclusions

Due to the above mentioned, we consider that the bioethanol production from orange peels could be viable for the local market, in a parallel way because of the ascorbic acid contents and phenols we can suggest the peel like a food additive.

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References

AARLAND, C.R., BAÑUELOS-HERNÁNDEZ, A.E., FRAGOSO-SERRANO, C.M., SIERRA-PALACIOS, E., DÍAZ DE LEÓN-SÁNCHEZ, F., PÉREZ-FLORES, L.J., RIVERA-CABRERA, F. y MENDOZA-ESPINOZA, J.A. (2017) Studies on Phytochemical, Antioxidant, Anti-inflammatory, Hypoglycemic and Cytotoxic Activities of Echinacea Extracts. *Pharm. Biol*. 55 (1), 649–656.

CAN-CAUICH, C.A, SAURI-DUCH, E., BETANCUR-ANCONA, D., CHEL-GUERRERO, L., GONZÁLEZ-AGUILAR, G.A., CUEVAS-GLORY, L.F., PÉREZ-PACHECO, E., MOO-HUCHIN, V.M. (2017). Tropical fruit peel powders as functional ingredients: Evaluation of their bioactive compounds and antioxidant activity. *Journal of Functional Foods*, 37, 501-506. https://doi.org/10.1016/j.jff.2017.08.028.

ARUNA, S.S. (2016) Studies on biochemical analysis and antioxidant property in cuinary fruit peels colected from Kannamagalam town of Vellore districts. *Der Pharmacia Lettre*, 8(4), 381-388.

CERVANTES-ARISTA, C., ROMAN-GUERRERO, A., OIDOR CHAN, V.H., DÍAZ DE LEÓN–SÁNCHEZ, F., ÁLVAREZ-RAMÍREZ, E.L., PELAYO-ZALDÍVAR, C., SIERRA-PALACIOS, E. y MENDOZA-ESPINOZA, J.A. (2020) Classification, chemical characterization, antioxidant capacity and anti-hyperglycemic effect of fruits of the *Stenocereus stellatus* species collected in an arid region of Mexico. *Food Chem*. 328, 127076. https://doi.org/10.1016/j.foodchem.2020.127076

CHEL-GUERRERO, L.D., SAURI-DUCH, E., FRAGOSO-SERRANO, M., PÉREZ-FLORES, L.J., GÓMEZ-OLIVARES, J.L., SALINAS-ARREORTUA, N., SIERRA-PALACIOS, E. y MENDOZA-ESPINOZA, J.A. (2018) Phytochemical Profile, Toxicity and Pharmacological Properties of Tropical Fruit Peels using In Vivo e In Vitro Models. *J. Med. Food*. 21(7):734-743. doi: 10.1089/jmf.2017.0124

GÓMEZ-COVARRUBIAS, S.I., RIVERA-CABRERA, F., MENDOZA-GASTELUM, J.I., OIDOR-CHAN, V.H., AARLAND, R.C., CRUZ-SOSA, F., DÍAZ DE LEÓN-SÁNCHEZ, F. y MENDOZA-ESPINOZA, J.A. (2020) Effect of Pasteurization on Chemical and Functional Properties of Juice of Xoconostle (*Opuntia joconostle*). *J. Food Qual. Hazards Control*. 7: 11-17. DOI: 10.18502/jfqhc.7.1.2447

SAGARPA. 2018. *Resumen Nacional Intención de Cosechas 2018*. México.

WILLEM P.N. y COOPER C.J. (2009) Implications of fossil fuel constraints on economic growth and global warming. *Energy Policy*. 37 (1). 166-180. ISSN 0301-4215. https://doi.org/10.1016/j.enpol.2008.08.013.

ZARZA-GARCÍA, A.L., MOO-HUCHÍN, V.M., TOLEDO-LÓPEZ, V.M., GODOY-HERNÁNDEZ, G., RIVERA-CABRERA, F., CLARENC AARLAND, R., SAURI-DUCH y E. MENDOZA-ESPINOZA J.A. (2021) Chemical, Nutritional, and Biological Composition of Three Seed Morphotypes of *Bixa Orellana* L. Bixaceae (Achiote) In The Yucatan Peninsula, México. *Pak. J. Bot*. 53(6): 2199-2205

MENDOZA-ESPINOZA, J. A.; RAMÍREZ RAMÍREZ, Z. F.; SIERRA-PALACIOS, E.; CHÁVEZ-BAEZA, C.; GÓMEZ-CANSINO, R.; BUSTAMANTE-CAMILO, P.; DÍAZ DE LEÓN SÁNCHEZ, F. (2022). Obtención de bioetanol y caracterización química de la cáscara de naranja Valencia cultivada en Ríoverde, San Luis Potosí-México. *Revista Ciencias Agronómicas*, (39), e017. https://doi.org/10.35305/agro39.e017