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Artículo Científico

Submerged fermentation with fruit and vegetable waste on organic acid production and enzymatic activity

Fermentación sostenible con desechos de frutas y verduras para la producción de ácidos orgánicos y actividad enzimática

Oscar Tello-Pérez[1], David Chávez-Flores[1], María Aurora Martínez-Trujillo[2], Francisco Javier Zavala-Díaz de la Serna[1], Beatriz A. Rocha-Gutiérrez[1], Lourdes Ballinas-Casarrubias[1], Héctor Rubio-Arias[3] and María del Rosario Peralta-Pérez[1]*

[1] Universidad Autónoma de Chihuahua, Facultad de Ciencias Químicas, Campus Universitario 2. Circuito Universitario s/n, Chihuahua, Chihuahua. C.P. 31125, México.

[2] Tecnológico de Estudios Superiores de Ecatepec. Av. Tecnológico S/N, Col. Valle de Anáhuac, CP 55210, Ecatepec de Morelos, Estado de México, México.

[3] Universidad Autónoma de Chihuahua, Facultad de Zootecnia y Ecología, Periférico. R. Almada. km. 1. Chihuahua, Chihuahua C.P. 31453, Mexico.

*Correspondencia: Correo electrónico: mperalta@uach.mx (María del Rosario Peralta-Pérez)
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Abstract

Fermentations often require the addition of nutrients or inoculants, along with the control of specific parameters. This leads to increased operational costs and complicates implementation, especially in areas with limited interest in organic waste treatment. The objective was to conduct submerged fermentation without adding of inoculum, using solely fruit and vegetable waste, to evaluate organic acid levels and enzymatic activities as an alternative for producing high-value-added products. To achieve this, waste from oranges, bananas, apples, carrots, papayas, and pineapples was utilized; these were placed in a 6 L plastic container with 4 L of distilled water and 400 g of piloncillo (jaggery). Fermentation progress was monitored over 49 days, with a 30 mL sample analyzed every 7 days. Organic acids were quantified via HPLC-UV; reducing and total sugars, proteins, and enzymatic activities were determined using spectrophotometry. A significant concentration of lactic acid (16.53 g/L), comparable to that observed in cultures featuring specialized microorganisms, was noted. The

highest pectinase activity was recorded on day 28 (55 U/L). These findings highlight the potential for generating high-value-added products through submerged fermentations.

Keywords: **bioproducts, food waste, lactic acid, submerged fermentation, waste valorization**

Resumen

Las fermentaciones frecuentemente requieren la adición de nutrientes o inóculos, así como el control de ciertos parámetros. Esto aumenta los costos operativos y dificulta su implementación, especialmente en lugares donde no hay interés en tratar residuos orgánicos. El objetivo fue realizar una fermentación sumergida sin la adición de inóculo y utilizando solo residuos de frutas y verduras para evaluar la cantidad de ácidos orgánicos y actividades enzimáticas como alternativa para obtener productos de alto valor agregado. Para ello, desechos de naranja, plátano, manzana, zanahoria, papaya y piña se utilizaron y colocaron en un recipiente de plástico de 6 L con 4 L de agua destilada y 400 g de piloncillo. La fermentación se monitoreó durante 49 días analizando una muestra de 30 mL cada 7 días. Ácidos órgánicos se cuantificaron por cromatografía de líquidos, mientras que azúcares reductores y totales, proteínas y actividades enzimáticas se determinaron por espectrofotometría. La concentración de ácido láctico (16.53 g/L) fue similar a la obtenida con microorganismos especializados. La mayor actividad de pectinasa (55 U/L) se registró al día 28. Los resultados demuestran que se pueden obtener productos de alto potencial biológico mediante fermentaciones sumergidas.

Palabras clave: **bioproductos, residuos alimentarios, ácido láctico, fermentación sumergida, valorización de residuos.**

1. Introduction

About 1.3 billion metric tons of food are wasted in the world annually. It is estimated that about nearly one third of all cereals, and half of all fruits and vegetables are wasted. In some developing countries with hot and humid climates, the unsuitable post-harvest treatment of fruit and vegetables within the storage and transport infrastructure leads to the deterioration of these products. The food processing and manufacturing industry produces food losses due to damage during transportation, unsuitable transport systems, issues during storage, losses during processing, raw material contamination, improper packaging, and others (Papargyropoulou *et al*., 2014). The retail and wholesale system also generate food waste due to problems in storage and handling, and lack of refrigeration. The generation of food waste by the end consumer is caused by excessive or inappropriate purchasing, poor storage conditions, improper preparation or cooking, excessive portions that will not be eaten, and due to confusion between expiration and use by dates (Dou and Toth, 2021).

Food spoilage and waste have serious environmental, social, and economic impacts. Food waste has a negative impact on climate change due to great water consumption during food production, along with the emission of considerable amounts of greenhouse gasses such as methane and CO_2. Some authors estimated than in US, food waste exceeds 55 million metric tons annually (Conrad, 2020).

This amount is almost 29 % of annual production, generating at least 113 million metric tons of CO_2, representing 2 % of national emissions, and costing the US $198 billion.

The most common way to dispose of food waste is via landfill. In many countries, food waste is mixed with household waste and dumped in landfills. Therefore, food waste is considered part of municipal solid waste (Girotto *et al.*, 2015). This waste generates greenhouse gases like methane and carbon dioxide and no value-added product or benefit can be obtained from landfilled waste. Furthermore, leachate is another problem related to the presence of food waste in landfills. Landfilling and incineration represent the most undesirable options for food waste management (Mian *et al*, 2017). However, these practices are increasingly used because food waste has a very complex chemical composition, as it is rich in carbohydrates, proteins, and lipids depending on the type and origin of the wasted food, and this affects the speed and feasibility of its treatment.

Some countries like Germany recycles 64 % of organic waste, Austria 58 %, and USA 34.5 %. Although food waste decreased during the COVID-19 pandemic, México is the Latin American country that generates the highest amount of food waste, approximately 39 %, consisting of fruits, vegetables, and bread (Vargas-López *et al*., 2022). In the particular case of Mexico, investment in food waste treatment is low, so economical and efficient alternatives need to be proposed to demonstrate the benefits and profitability of food waste treatment.

Due to the chemical composition of food waste, several treatment methods can be developed and used to obtain value-added products such as biofuels, organic acids, enzymes, and other important biocomposites from food waste. Some of the applications that are creating more value in the market are: animal feed (selling price: $70-200/ton of biomass), electricity generation ($60-150/ton of biomass), biofuels ($200-400/ton of biomass) and biocomposites like organic acids, enzymes, and biodegradable plastics ($1000/ton of biomass) (Kiran *et al*., 2014; Mujtaba *et al*., 2023).

There is extensive research focused on the production of high-value-added biocomposites. This research work focuses on the evaluation of the conditions needed to produce value-added biocomposites from waste, coupled with the principle of zero waste, and has given rise to the industrial symbiosis where the waste produced by a company is processed immediately using additional treatment. However, to apply the concept of industrial symbiosis, it is necessary to identify, characterize and quantify the waste to know its potential for producing biocomposites (Mirabella *et al*., 2014). Fermentation of food waste has been successfully used to produce several organic products like volatile fatty acids (Lee *et al*., 2014) or L-lactic acid and both products are used in the production of biodegradable plastics and bioenergy.

Furthermore, large amounts of some types of enzymes are commonly used in industrial processes. Knowledge about enzymes has led researchers to develop new technologies for their production using cheaper substrates. Several kinds of food waste have been used to produce enzymes such as lipases, proteases, cellulases, amylases and pectinases, particularly from fruit and vegetable waste (Panda *et al*., 2016; Selvakumar and Sivashanmugam, 2017).

Organic acids have been reported as one of the largest categories among bioproducts and are compounds with weak acidic properties that do not dissociate totally in the presence of water. They are important due to their use as building block chemicals and application in a broad range of industries. Lactic acid has received considerable attention because of its use in the food, cosmetic, pharmaceutical, and chemical industries. It contains two reactive functional groups (carboxylic and

hydroxyl) and therefore can be subjected to a variety of chemical conversions to form other useful chemicals. Interest in this acid has increased because it can be used as a monomer to produce polylactic acid (a biodegradable plastic) (Li *et al*., 2020).

Although research in food waste has increased in recent years, further investigation is needed to demonstrate that the treatment and use of this kind of waste is a feasible idea that provides added value and profit and is also an environmentally friendly process (Valta *et al*., 2017). The literature reports that it is possible to obtain enzymes and organic acids from the fermentation of food waste (Merrylin *et al*., 2020). It is important to note that the mixture used in this study is typically produced in local juice stands in México, so the implementation of a simple and economical process for waste can be an additional source of income for local stalls or it could be an incentive to treat this type of waste.

The objective of this work was to assess an anaerobic fermentation process using fruit and vegetable waste without controlling the experimental parameters like pH and temperature or adding inoculum and characterize this fermentation through the quantification of specific metabolites (e.g. organic acids and enzymes) to obtain high value-added products. This could provide an alternative to a growing generation of food waste treatments and provide green solutions to excessive food waste.

To our knowledge, there are very few studies reporting these conditions that obtain good amounts of bioproducts like organic acids and enzymes.

2. Materials y methods

Given the prevalence of fruit and juice sales sites in México, fruit waste was utilized for the study. The fruit waste, including the peals of orange, banana, apple, papaya, pineapple, and carrot bagasse were collected from a natural juice store located in downtown Chihuahua City, Mexico. The waste was not inoculated with any fungal or bacterial strain. Furthermore, no optimization was done for pH conditions, temperature, solid-liquid relation or other parameters. Fermentation occurred due to the presence of the endogenous microorganisms of the fruit and vegetables used. All the analyses were performed in duplicate.

2.1. Materials and Reagents

The food wastes were processed and fermented on the same day of their collection. Jaggery and purified water from a commercial establishment were used. All reagents employed were analytical and HPLC grade (brand J.T. Baker and Sigma Aldrich; Waltham, U.S.A.).

2.2. Fermentation preparation

The following amounts of wastes were weighed for the fermentation experiment and the duplicate: 600 g of orange peel, 200 g of banana peel, 200 g of mixed apple and carrot bagasse, 100 g of papaya peel and 100 g of pineapple bagasse. The total 1,200 g were cut into small pieces and blended in a food processor (brand Black+Decker, model FP4200B-LA, Edo.Mex, México) along with water and 400 g of jaggery (Arun and Sivashanmugam, 2015a.). The average temperature during the

fermentation time was 19.1°C and the pH was 5.0. Both containers were closed and stored in a dark and dry area in the laboratory. The fermentation was monitored for 49 d, taking a 30 mL sample from each container every 7 days, and shaking the container to homogenize the mixture before taking each sample. The samples were centrifuged in Falcon tubes at 1,000 *g* for 30 min. The obtained supernatant (fermentation extract) was aliquoted and stored in Eppendorf tubes at -20 °C for further analysis. Organic acids (malic, oxalic, lactic, acetic, and citric) were quantified from the fermentation extracts by means of HPLC-UV (DIONEX UltiMate 3000, brand Thermo Scientific, Waltham, U.S.A.; coupled to a UV detector). Additionally, reducing sugars, total sugars, and proteins concentrations, as well as lipase, amylase, protease and pectinase activities were quantified using spectrophotometric methods. A dialysis was done using dialysis cassettes (Slide-A-Lyzer Dialysis Cassette G2, brand Thermo Fisher Scientific; Waltham, U.S.A.) before the quantification of proteins and enzymatic activities.

2.3. Reducing and total sugars quantification

The measurements for reducing sugars were performed using the modified 3-5 dinitrosalycilic acid method (DNS method) written by Miller (1959). A dextrose calibration curve was plotted at 100-2,000 mg/L. To quantify reducing sugars, appropriate dilutions were prepared with distilled water ranging from 1:5 to 1:100.

The total sugars were determined using the phenol-sulfuric method (Dubois *et al*., 1956). A dextrose calibration curve was plotted at 10-100 mg/L. Different dilutions were prepared with distilled water from 1:100 to 1:1,000 depending on the sugar concentrations in each of the samples.

2.4. Organic acids quantification by HPLC

Individual 10 mg/mL stock solutions were prepared of malic, lactic, citric, oxalic, and acetic acid. Different stock solution volumes were injected by the autosampler in the HPLC equipment (DIONEX UltiMate 3000, brand Thermo Scientific, Waltham, U.S.A.; coupled to a UV detector) to reach concentrations ranging from 0.156 mg m/L to 10 mg/mL. In this manner, the elution times, the UV spectrum, and the calibration curves were obtained for each acid. Fifty mM monopotassium phosphate (KH_2PO_4) was employed as the mobile phase, with a pH of 2.7 and at a flow rate of 0.7 mg/mL. A C30 column (ProntoSIL Bischoff, Leonberg, Alemania) was used at 28 °C following the modified approach by Arun & Sivashanmugam (2015b.). To quantify the organic acids in the fermentation extract, the samples were allowed to reach room temperature and filtered through 0.2 μm nylon filters (Whatman). Then, 1 mL of sample was added into a 2 mL glass vial. A volume of 2 μL was injected to perform the HPLC analysis by using the same mobile phase and parameters implemented in the quantification of stock acid solutions.

2.5. Dialysis protocol

The samples were allowed to reach room temperature and dialyzed using cassettes Slide-A-Lyzer Dialysis Cassette G2, molecular weight cutoff was 10K (brand Thermo Fisher Scientific; Waltham, U.S.A.). This was done due to the high sugar concentrations and as a way to prevent spectrophotometric interference during the measurements for proteins and enzymatic activities. The

dialysis membrane was hydrated by submerging the cassette in distilled water for 2 min. The plastic piece of the cassette was carefully placed on a paper towel to eliminate any excess humidity, avoiding any contact, drying or damage to the membrane. The cap of the cassette was removed and the sample was placed in the cassette. After filling up the cassette, the membrane surface was gently squeezed to remove the air inside, and then closed and submerged in 1 L of distilled water. The sample was dialyzed for 1 h at room temperature and slightly shaken in the process. Subsequently, the distilled water was changed and dialyzed for an additional 1 h. This procedure was followed two more times, letting it dialyze for 2 h. Lastly, the sample was left dialyzing overnight. The cassette and the cap were then removed to recover the sample for further analysis.

2.6. Proteins and enzymatic activities quantification

Proteins were measured through the Bradford method (Bradford, 1976) by using bovine serum albumin (BSA Sigma-Aldrich, St. Louis, U.S.A.) at concentrations of 10-100 mg/L.

The lipase activity assay was performed using the methodology described in Margesin *et al*. (2002). One mL of fermentation extract was added to a test tube with 5 mL of 0.1 M NaH_2PO_4/NaOH buffer (pH 7.25) and pre-incubated at 30 °C in a water bath for 10 min. Fifty μL of a 0.1 M p-nitrophenyl butyrate (pNPB) solution was added, diluted in 2-propanol and previously stored at 20 °C. The tube contents were mixed and incubated at 30 °C for 10 min. To stop the reaction, the tube was placed in an ice bath for another 10 min. The p-nitrophenol (pNP) release was measured by reading the samples in a spectrophotometer at 400 nm. The obtained absorbance was compared to a p-nitrophenol (pNP) calibration curve at 0-15 ppm. One unit of lipase activity was defined as the amount of enzyme that released 1 μmol of p-nitrophenol under reaction conditions.

The protease activity was assayed by a modified methodology posed by Haddar *et al*. (2009). A 0.5 mL aliquot of the fermentation extract was pre-incubated at 60 °C and mixed with 0.5 mL of a casein solution dissolved at 1 % in a 0.1 M NaH_2PO_4/NaOH buffer (pH 7.0). The mixture was incubated for 15 min at 60 °C, and the reaction was stopped by adding 0.5 mL of 20 % w/v trichloroacetic acid. The mixture was let cool for 5 min and then centrifuged – at room temperature for 15 min and at 1,000 G – with the purpose of removing the precipitate. 5 mL of distilled water was added to the supernatant and the absorbance was measured at 280 nm using quartz cuvettes. Since the fermentation extract contained molecules with absorbance at 280 nm, a blank was used for each of the samples. The obtained absorbance for the sample was compared to a tyrosine calibration curve at 10-220 mg/L, while zero was established with a blank. One unit of protease activity was defined as the required amount of enzyme to release 1 μg of tyrosine per minute under reaction conditions.

The gluco-amylase activity was determined based on the procedure described by Silva *et al*. (2013). Pre-incubation was performed at 45 °C for 10 min on a starch solution dissolved at 1 % in a 0.2M acetates solution (pH 5.0). An amount of 0.5 mL of enzymatic extract was added and then incubated for 20 min at 45 °C. Afterwards, 1 mL of 3-5 dinitrosalicylic acid reagent was added. A blank was prepared for each sample. The samples were boiled for 5 min and 5 mL of distilled water was placed in each of them. The absorbance was measured at 575 nm, adjusting to zero with a blank. The released sugars concentration was estimated by using a dextrose calibration curve at 100-2,000 mg/L. One unit of gluco-amylase activity was defined as the amount of enzyme that was needed to release 1 μmol of dextrose under reaction conditions.

The pectinase activity was assayed in accordance with the proposed methodology by Martinez-Trujillo *et al*. (2009). A 0.5 mL aliquot from a pectin solution dissolved at 0.5 % in a 0.2M acetates buffer (pH 5.0) was pre-incubated at 45 °C to be mixed with 0.5 mL of fermentation extraction. The mixture was incubated for 20 min at 45 °C, and the reaction stopped when 1 mL of 3,5 dinitrosalicylic acid was added. Once again, a blank was prepared for each of the samples. The samples were boiled for 5 min and 5 mL of distilled water was placed in each of them. The absorbance read was recorded at 575 nm by adjusting the spectrophotometer with a blank. The released sugars concentration was calculated using a galacturonic acid curve at 50-1,000 mg/L. One unit of pectinase activity was defined as the required amount of enzyme to release 1 μmol of galacturonic acid under specific reaction conditions.

3. Results and discussion

Fig. 1 shows the development of reducing and total sugars over fermentation time. The initial concentrations were 27.2 and 81.4 g/L, respectively. At the beginning of the fermentation time, most of the sugars were not reducing sugars; however, the analysis performed on day 7 suggested that total sugars were converted into reducing sugars. On day 7, the total sugars concentration was 63.5 g/L, while the reducing sugars concentration was 61.8 g/L. By day 14, both concentrations drastically decreased and stabilized. These results helped identify the sugar consumption profile during the fermentation process.

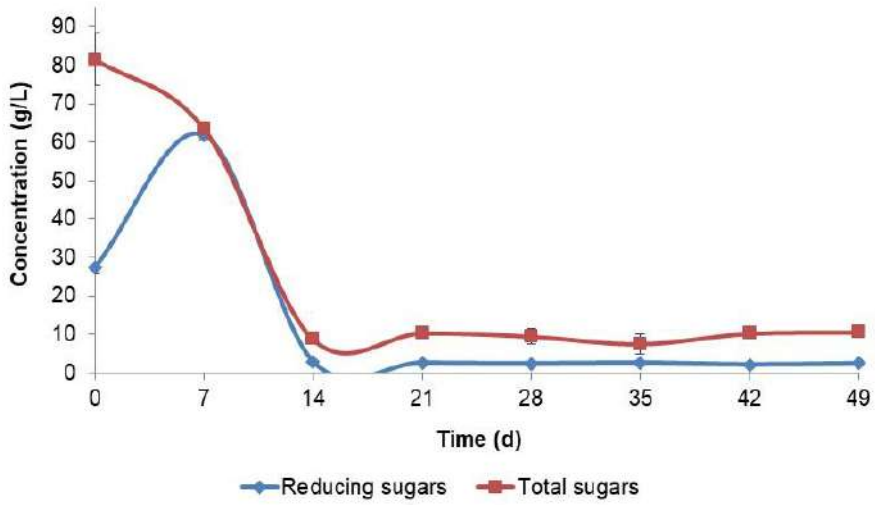

Figure 1. Comparison of concentration reducing sugars and total sugars over fermentation time**.** The average of three repetitions and their standard deviation are indicated in the graph.
Figura 1. Comparación de la concentración de azúcares reductores y azúcares totales durante el tiempo de la fermentación. Se indica en la gráfica el promedio de tres repeticiones y su desviación estándar.

Due to the fact that the experiment was performed on a complex material mixture containing various polysaccharides, the increase in the reducing sugars concentration after 7 days may have been caused by their hydrolysis. The hydrolysis was catalyzed by enzymes and/or the endogenous microorganisms present in the bulk (Shrestha *et al*., 2017). In the present study, the sugars concentration after day 14 of fermentation remained constant, the consumption of the reduced sugars occurred at the same velocity as the consumption of the total sugars. Despite this fact, it was decided to continue fermentation in order to give time for the production or accumulation of biocomposites.

All acids were able to be quantified in a single injection since their peaks had distinct elution times and could be easily identified under the conditions implemented. These chromatograms were useful to determine the UV absorption spectrum and elution time for each of the acids. By injecting different volumes of the standard solutions, it was possible to create a calibration curve that could quantify the acid concentrations in the fermentation extracts.

The quantification of organic acids over time is plotted in Fig. 2. Low oxalic acid and malic acid concentrations were observed at the beginning of the fermentation (0.3 g/L and 6.1 g/L respectively). The oxalic acid concentration remained unchanged, while the malic acid showed values close to zero after 7 days. At time zero, the citric acid was found to have a concentration of 0.4 g/L. After that, no citric acid was detected. Evidently, no citric, malic or oxalic acid were produced during the fermentation.

On the other hand, lactic acid production throughout the fermentation showed increasing behavior from day 7 and reached its maximum (16.53 g/L) on day 42. Such level had no significant variation after 21 days of fermentation. Additionally, a smaller production of acetic acid was found, which reached 2.60 g/L in almost of the observations.

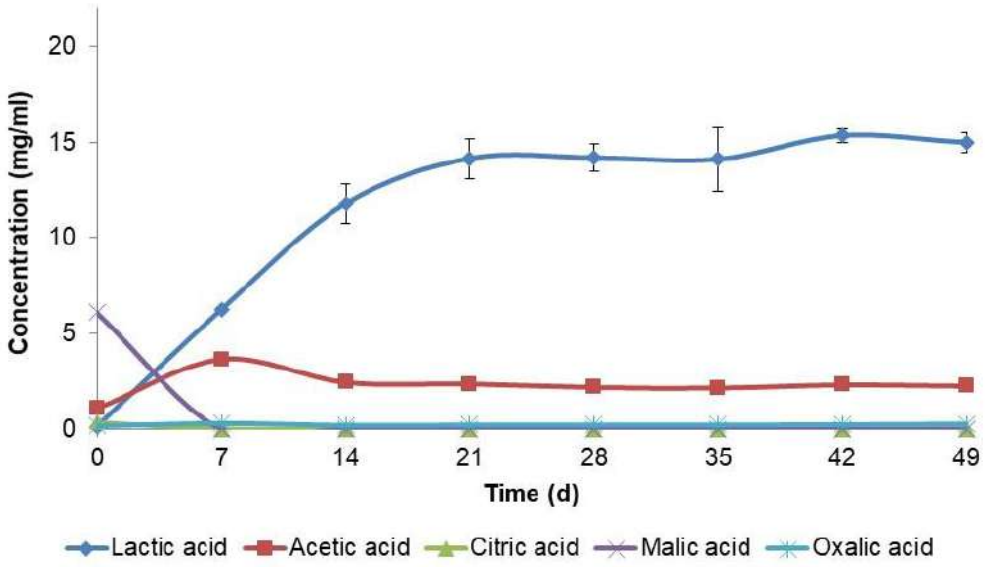

Figure 2. Organic acids quantification (lactic, acetic, citric, malic, oxalic acid) by HPLC. The average of three repetitions and their standard deviation are indicated in the graph.
Figura 2. Cuantificación de ácidos orgánicos (láctico, acético, cítrico, málico y oxálico) por HPLC. Se indica en la gráfica el promedio de tres repeticiones y su desviación estándar.

No citric acid, malic acid or oxalic acid production was observed, which suggests that fermentation occurred under anaerobic conditions. This can be supported by the high lactic acid concentration (16.53 g/L) obtained from day 21. Furthermore, it can be suggested that the fermentation was a lactic anaerobic system. It is known that lactic acid bacteria (LAB) are present in plant tissues (Sharma *et al*., 2020; Yu *et al*., 2020).

A low acetic acid concentration was also detected after the 7th day. Lactic acid and acetic acid have been produced by a wide variety of food wastes through different anaerobic fermentation systems. Li *et al*. (2015) studied acetic acid production from cafeteria food waste by using yeast (Saccharomyces cerevisiae) and acetobacteria (lyophilized beer vinegar) in lab-scale batch fermenters for 10 days. The bioreactor containing both yeast and acetobacteria had the highest acetic acid production (25.88 g/L). The bioreactor in which only yeast was used produced 16.18 g/L of acetic acid, while the one with acetobacteria produced 20.51 g/L. These production results were similar to the ones obtained in this study. However, it must be highlighted that the investigation was carried out with native microbiota which could decrease production costs as it eliminates the need to prepare an inoculum or provide control for the fermentation process. This is especially important in countries such as Mexico, where there is little capacity for economic investment in the treatment of waste. In future studies, it would be relevant to determine the microbial population composition throughout the fermentation.

Furthermore, lactic acid is the most available organic acid on the planet and can be produced through either fermentation or chemical synthesis, yet it has been documented that 90 % of its production is done by bacterial fermentation (Panda *et al*., 2016). The lactic acid concentration achieved in this study was lower than that reported in other works (Yuan *et al*., 2018; Abedi and Hashemi, 2020). However, it is important to emphasize that we attained this production in just one week without strict control of experimental conditions, highlighting the efficiency of the process employed. For instance, Song *et al*., 2022a. investigated lactic acid production with mango skin using a submerged spontaneous anaerobic fermentation process. The results showed a maximum production around of 26.8 g/L obtained with an initial pH of 6.8, an incubation time of 30 d and at 37 °C.

The amount of lactic acid produced during fermentation with food waste highly depends on variables such as type of food, sugars concentration, treatment prior to fermentation, type of fermentation, conditions (pH, agitation, aeration, temperature, and others), equipment used and inoculum type (Song *et al*., 2022b.).

Wang *et al*. (2016) measured lactic acid production through food waste fermentation (fruit, vegetables, chicken, fish and rice) without pH adjustments, sterilization or inoculum addition. Their results determined that amylase addition increased lactic acid production up to 22 g/L after 72 h, and 26 g/L after 192 h. The process was brought to a pilot plant scale in a fermenter with 20 kg of food waste, 20 kg of water and 25 U of amylase for every gram of dry material. After 48 h, a lactic acid production of 21.5 g/L was achieved. This quantity is around 5 g/L larger than the one obtained in this study. This is an encouraging fact since this study was performed without any type of control, thus production expenses were lower. Additionally, sugars consumption ended after 14 d and the highest lactic acid production was registered at day 21, even though the fermentation was performed for 49 d. This indicates that fermentation time can be reduced.

Fig. 3 presents the proteins concentration throughout the fermentation process. The maximum concentration measurement (59.7 g/L) was obtained after 14 d of fermentation. From day 28 on, the proteins concentration remained almost at a constant value of 25 g/L. The maximum protein

concentration coinciding with the peak lactic acid production and the greatest in reducing sugars concentration. Although it was not possible to determine microbial growth due to the system's complexity, these coincidences may be attributed to the culture being in the exponential growth phase, fully adapted to the system, and thus capable of consuming the carbon source (sugars) without requiring further changes in proteins production.

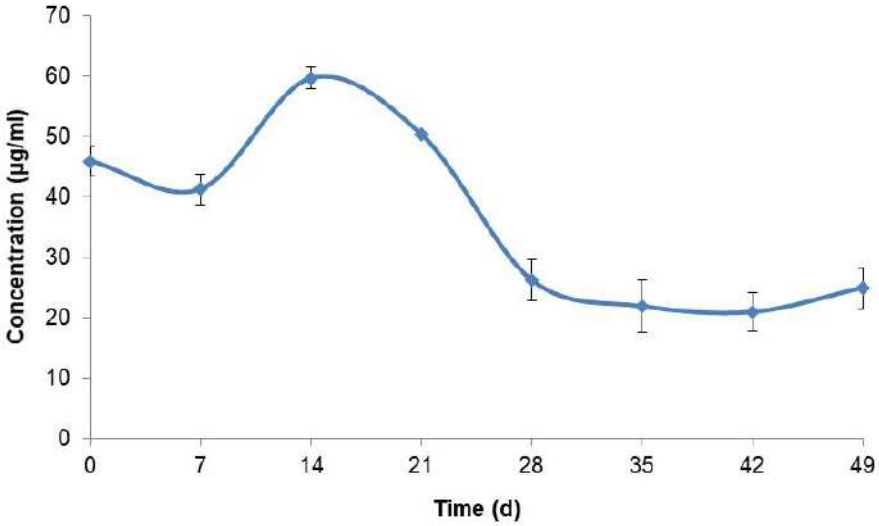

Figure 3. Total proteins quantification during fermentation. The average of three repetitions and their standard deviation are indicated in the graph.
Figura 3. Cuantificación de proteínas totales durante la fermentación. Se indica en la gráfica el promedio de tres repeticiones y su desviación estándar.

A study that used a similar culture medium composition to this work quantified the amount of proteins over 3 months of fermentation, and the only difference was the type of food wastes. The results determined that the proteins concentration was 42 mg/L after the fermentation was completed (Arun and Sivashanmugam, 2015a.). This production was 30 % larger than the proteins concentration obtained on day 49 of fermentation in this study (29.5 mg/L). This result may differ because the food waste used and the methodology followed were not the same, and this supports the hypothesis that the biocomposites obtained depend on the waste food source.

Fig. 4 plots the quantification for lipase activity and pectinase activity during fermentation. Lipase activity increased and reached its highest value on day 49 (7.7 U/L), while the pectinase activity was detected after the 14th day of fermentation and its maximum measurement was obtained after 28 days (55 U/L). From that day on, a small decline in the pectinase activity in the fermentation extracts was observed in 35 d and 42 d, increasing again on day 49 d. No amylase or protease activity was found.

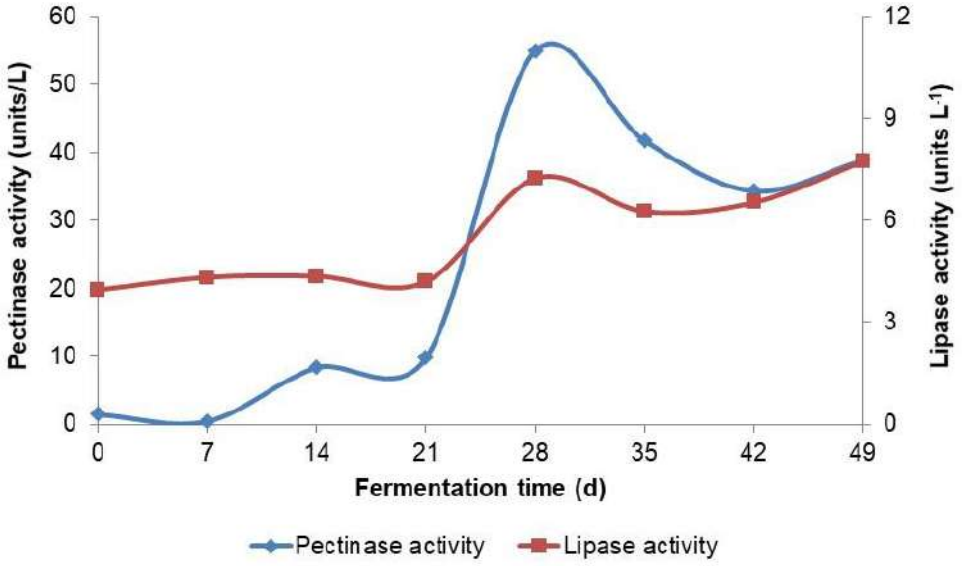

Figure 4. Lipase activity and pectinase activity quantification during fermentation. The average of three repetitions and their standard deviation are indicated in the graph.
Figura 4. Determinación de la actividad lipase y actividad pectinase durante la fermentación. Se indica en la gráfica el promedio de tres repeticiones y su desviación estándar.

As for enzymatic activities, amylase activity was not found at any point in the fermentation process, whereas protease activity was detected slightly from the beginning of the process. This result can be attributed to the fact that not enough enzymes were present due to the low protein and starch concentrations in the food waste. It is worth noting that no references were found regarding lipase production by using orange waste as raw material and p-Nitrophenol esters for enzymatic activity quantification. At time zero of the fermentation, an activity of 0.0039 U/mL was detected. This may be a result from the chemical equilibrium in the reaction (p-nitrophenyl butyrate ↔ p-nitrophenol + butyrate), where the reaction occurs in both directions. It is well understood that only a few reactions move in one direction, most are reversible to some degree. The lipase activity may be due the endogenous enzymatic activity of the fruit wastes. This could be attributed to the fact that papaya wastes were fermented and it is known that they present lipolictic activity (Rivera *et al*., 2017). In a study developed by Okino-Delgado and Fleuri (2014), the presence of lipases in wastes from the orange juice processing industry was proven for the first time. Three of the assayed wastes – referred to as skin, core and fruit – presented lipolytic activity.

Inducible lipases production from food waste, mainly from lipids-rich wastes like vegetable oils, has been described in the past. Fibriana *et al*. (2020) documented large-scale lipase production employing microorganisms such as fungi and bacteria in the presence of oil or fat in the substrates, the lipase activity was in the range from 20 to 460 U/mL depending on the conditions employed.

At days 0 and 7, pectinase activity was not registered, most likely because at high reducing sugars concentrations, microorganisms do not have the need to use polysaccharides as an energy source. The decrease in pectinase activity after the 28th day could have resulted from the pectin reduction, a polysaccharide that may have been present during the fermentation. Another reason to explain the

decrease in the activity can be attributed to the lactic acid production which decreases the pH. The optimal pH for most pectinases has been reported to be around 5.8 (KC *et al*., 2020). The pectinase activity observed in this study (0.055 U/mL) can be attributed to the high pectin content of the orange skin.

Food waste represents a major global issue with ethical, moral and social implications, as well as economic, environmental and health impacts. Therefore, the study of this topic should not be taken lightly, and relevant information to help reduce food waste should reach all strata of society.

4. Conclusiones

The results evidenced that value-added products can be obtained through submerged spontaneous fermentations. Significant quantities of lactic acid and pectinase activity were produced, both occurred in just 14 days of fermentation. These findings highlight the potential for generating high-value-added products through submerged fermentations. The products obtained in this fermentation could be tested in the future as a household cleaning solution or as a raw material to produce biofertilizers and biodegradable plastics.

Author Contributions

Conceptualization, David Chávez-Flores and Ma. Rosario Peralta-Pérez; methodology, Beatriz A. Rocha-Gutiérrez; validation, María Aurora Martínez-Trujillo; formal analysis, Lourdes Ballinas-Casarrubias; research, Óscar Tello-Pérez.; data retention, Francisco Javier Zavala-Díaz de la Serna; writing of the original draft, Óscar Tello-Pérez.; writing-proofreading and editing, Héctor Rubio-Arias.; supervision, Ma. Rosario Peralta-Pérez.

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Conflicts of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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