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Solid-State Fermentation for Phenolic Compounds Recovery from Mexican Oregano (*Lippia graveolens* Kunth) Residual Leaves Applying a Lactic Acid Bacteria (*Leuconostoc mesenteroides*)

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Abstract: The Mexican oregano by-products are a source of bioactive molecules (polyphenols) that could be extracted using solid-state fermentation (SSF). This study fermented the by-products via SSF (120 h) with a lactic acid bacteria (LAB) *Leuconostoc mesenteroides*. Sequentially, a bioactive and chemical determination was made according to the phenolic content, antioxidant activity (DPPH•/FRAP), bioactive properties (α -amylase inhibition and antimicrobial activity against *Escherichia coli*), and chemical composition (HPLC-MS). The results showed that the total phenolics and flavonoid content, as well as the antioxidant activity, increased (0.60, 2.55, and 3.01 times, respectively) during the SSF process compared with unfermented material. Also, the extracts showed antimicrobial activity against *E. coli* and α -amylase inhibition. These inhibitory results could be attributed to bioactive compounds identified via HPLC, such as gardenin B, trachelogenin, ferulic acid, and resveratrol 3-O-glucoside. Therefore, the application of *L. mesenteroides* under SSF on oregano by-products comprises an eco-friendly strategy for their valorization as raw materials for the recovery of phenolic compounds that could be natural alternatives against synthetic antioxidant and antimicrobial agents, promoting a more circular and sustainable supply system within the oregano industry.

Keywords: circular economy; valorization; bioactive properties; Mexican oregano; sustainable production; antimicrobial; α -amylase inhibition

1. Introduction

Nowadays, conventional production lines must change for sustainable modernization according to the new trends through the implementation of a “circular economy” and “sustainable intensification” concepts accompanied by principles such as “elimination of residues”, “zero-waste”, “regeneration of natural environments”, and pollution prevention [1,2]. Under this new perspective, the rural activities developed in the north of Mexico comprise a potential target for modernization, especially the exploitation of native oregano plants (*Lippia graveolens* kunth), the cultivation of which entails cooperative production

(5 or more communities) and has been reported to reach up to 6500 tons oregano/year [3]; mainly, its economic income is due to the sale of dry biomass.

However, the growing interest in essential oils (EOs) has promoted the cultivation of oregano as a source of EOs; in the last decade, it has been reported that there are 50 cultivated hectares for EO extraction (Chihuahua state) with a potential production of 104.5 tons, which could produce a range of spent oregano biomass between 104.51 and 101.88 tons according to the extraction efficiency (0.36–2.5%) of the equipment [4–6]. Additionally, the economic analysis made by the Mexican Secretariat of Economy showed in March 2024 that the financial sector in Mexico related to EO, perfumery, and cosmetics, among others, has reached USD 3.18 billion (Exports) [7]; a growing value for the EO sector. Unfortunately, the EO extraction techniques include mainly conventional methods (e.g., hydro-distillation) with lower extraction yields with vast quantities of unexplored residual production (50 and 90 kg of solid wastes per essential oil litter), principally aromatic water and spent oregano leaves that remain without any potential application and end up in landfills, causing environmental and economic issue [8–11].

However, the spent biomass encompasses a potential valuable bioactive source due to previous research projects that have reported the rich chemical composition of oregano by-products: flavonoid (14.3–54.7 mg/dry g), anthocyanins (0.38–10.2 mg/100 g), or tannins (12.4–510.3 mg/g) content [12–14]. Also, Cid-Peréz et al. [15] reported antioxidant and antimicrobial compounds as bioactive non-volatile compounds (caffeic and rosmarinic acid) in polar subfractions, and thymoquinone, thymol, and carvacrol, among others (non-polar solvents), highlighting that it is possible to extract valuable molecules from oregano by-products. Thus, the potential recovery of bioactive compounds from oregano by-products comprises an attractive alternative for revalorizing these residual materials.

A novel technology for recovering bioactive compounds is SSF, which is defined as the growth of microorganisms on solid lignocellulosic substrates that act as a nutrient source in the near absence of water (with little or no added water) [16]. This technology has advantages such as low energy and water requirements, agro-industrial residues as a carbon source, and simple equipment in comparison with emerging technologies [17–21]. These characteristics are fascinating for a potential future integration by the local communities in the north of Mexico.

Regarding SSF, the main applied microorganisms involve fungal strains (e.g., *Aspergillus* spp.) due to their efficient enzymatic system and low moisture requirements [22–24]. However, its application could be limited by the potential production of mycotoxins, which could have a negative impact on human health. Thus, the search for novel GRAS (Generally Recognized As Safe) strains has been a novel topic to implement safer bioprocessing technologies. Recently, lactic acid bacteria (LAB) have gained attention as an attractive group for SSF with advantages such as being GRAS microorganisms, the production of secondary metabolites (bacteriocins, organic acids, volatile compounds, etc.), sensorial property boosting, and functioning as a means of potential integral valorization of soluble extracts and residual biomass (fermented after SSF) [25]. Previously, their application has been explored in soybean and vegetable sources for the recovery of bioactive compounds (polyphenols) via hydrolytic machinery (feruloyl esterase, β -glucosidase, and tannase) [26–28] that release the bond phenolics linked to the cell wall [29].

Among LAB strains, *Leuconostoc* comprises a novel genus with the ability to produce aromatic compounds, exopolysaccharides, and bacteriocins [30], with a reported application for plant-fermented products (Kimchi and sauerkraut) and a successful application to increase the phenolic content in ginseng [31,32]. Thus, *Leuconostoc* spp. could be an excellent microorganism to develop an SSF valorization technique for the extraction and production of bioactive compounds (extracts) and produce fermented biomass similar to current food products (e.g., kimchi and sauerkraut).

To our knowledge, this article illustrates the first approach to applying SSF technology with a LAB strain to extract bioactive components from oregano by-products. Additionally, this study aimed to investigate the influence on phenolic content and profile as well as

potential bioactivities (antioxidant capacity and enzymatic and microbial inhibitions) of extracts from *L. graveolens* by-products after SSF by *L. mesenteroides*.

2. Materials and Methods

2.1. Chemicals and Reagents

The present study applied chemical reagents (analytical grade) such as Ethanol (absolute), distilled water, gallic acid (>98.5%), quercetin ($\geq 95\%$), 2,2-Diphenyl-1-picrylhydrazyl (DPPH[•]), 2,4,6-Tripyridyl-s-triazine (TPTZ, $\geq 98\%$), Tween 80, Folin–Ciocalteu reagent, sodium carbonate anhydrous (Na₂CO₃, $\geq 99.5\%$), sodium hydroxide (NaOH, $\geq 99.7\%$), sodium nitrite (NaNO₂, $\geq 97.0\%$), aluminum chloride (AlCl₃, 98%), hydrochloric acid (HCl, 37%), and ammonia ferric sulfate dodecahydrate ([FeNH₄(SO₄)₂ · 12 H₂O], $\geq 99\%$) and biological reagents as dextrose (>97.5%), α -amylase (A3306), and MRS agar. All the reagents were purchased from Sigma Aldrich (Toluca, México). The *L. mesenteroides* (18C6) strain was provided by the Food Research Department collection from the Autonomous University of Coahuila.

2.2. Plant Material

The mature leaves from oregano plants were harvested (August–October 2020) from “Ejido 4 de Marzo” (Latitude, 25°33′49.5380″ N, Longitude, 102°33′59.0388″ O in Parras de la Fuente, Coahuila, Mexico). A cleaning step (distilled water) was applied to remove dirt, followed by a drying step in the oven (50 °C, 24 h).

2.3. EOs Extraction Process

The hydro-distillation system involves putting 100 g of cleaned oregano leaves and 1000 mL of water into a flat-bottom ball flask of 1 L (Extraction time = 1 h); the spent leaves (by-product) are dried in the oven (50 °C, 24 h) for stabilization and stored in dark bags until the SSF process.

2.4. Solid-State Fermentation Process

The bioprocessing was carried out in a glass flask functioning as a bioreactor (250 mL), the fermentation substrate was the spent leaves of *L. graveolens* using a unique inoculum size (1×10^7 cells g⁻¹ of the substrate), with a 90% moisture level, and a temperature of 37 °C. The fermentation was carried out for 120 h (sampling time: 24 h); the recovery of fermented extracts was accomplished via mechanical extraction (10 mL distilled water) using a press system (syringe 20 mL, BD Plastipak™, Becton Dickinson, CDMX, Mexico), the recovered extracts were filtered (0.22 μ m, Millex[®]-GS, Merck, Darmstadt, Germany) and stored in the dark at –20 °C temperature until use.

2.5. Polyphenolic Compounds Evaluation in the Solid-State Fermentation Process (SSF)

2.5.1. Phenolic Compounds Determination

Total Polyphenolic Compounds (TPC)

The total polyphenolic compounds in the fermented extracts from SSF were determined using the methodology proposed by Georgé et al. [33] for hydrolyzed tannins and the condensed tannins methodology, according to Amaya-Chantaca et al. [34]. The results were reported as total polyphenolic compounds in milligrams per oregano by-product gram (mg TPC/g oregano).

Hydrolyzed Tannins (Folin–Ciocalteu)

The Folin–Ciocalteu assay determined the total polyphenol concentration (electron transfer method). In a 96-well microplate, 25 μ L of the sample, 25 μ L of the Folin–Ciocalteu reagent, and 25 μ L of sodium carbonate (0.7 M) were added, followed by a homogenization and incubation step (40 °C, 30 min). After that, 200 μ L of distilled H₂O was added, and the absorbance was measured at 750 nm (UV-visible Epoch™ Microplate Spectrophotometer).

The results were expressed as gallic acid milligram equivalent per oregano gram (mg GAE/g) according to linear regression (calibration curve).

Condensed Tannins (HCl–Butanol)

The HCl–butanol method was applied to the quantification of condensed tannins, it was carried out according to Swain and Hillis [35] with slight modifications. A sample volume of 500 μL was mixed with 3 mL of HCl:butanol (1:9) and 1 mL of ferric reagent. The reaction mix was incubated at 100 $^{\circ}\text{C}$ (1 h), and the final absorbance was measured at 460 nm (UV-visible Epoch™ Microplate Spectrophotometer). The results were expressed as the catechin milligram equivalent per gram (mg CE/g) according to the calibration curve.

Total Flavonoid Compounds (TFC)

The quantification was determined according to De la Rosa et al. [36]. An initial volume of 31 μL (sample) was mixed with 9.3 μL of sodium nitrite (5%, *w/v*) and 9.3 μL of distilled water. The solution was mixed and incubated at 40 $^{\circ}\text{C}$ (3 min). Then, 9.3 μL of 10% (*w/v*) aluminum chloride was added and incubated (3 min). Finally, 125 μL of sodium hydroxide (0.5 M) was added and incubated at 40 $^{\circ}\text{C}$ (30 min) in the dark. The final absorbance was measured at 510 nm (UV-visible Epoch™ Microplate Spectrophotometer). The results were reported as the milligram equivalent of catechin per oregano gram (mg CE/g) according to a calibration curve prepared with the same standard.

2.6. Antioxidant Activity in the Solid-State Fermentation Process (SSF)

2.6.1. DPPH• Radical Scavenging Assay

The methodology was carried out according to Brand-Williams et al. [37]; a working solution of DPPH• radical (60 mM) was diluted in methanol. A working volume of 295 μL (DPPH• radical) was added to each 5 μL of the sample, followed by incubation (30 min) at room temperature. The final absorbance was determined at 517 nm (UV-visible Epoch™ Microplate Spectrophotometer), and the results were expressed as the milligram equivalent of gallic acid per gram of sample (mg GAE/g oregano).

2.6.2. Ferric Reducing Capacity (FRAP)

The FRAP assay was conducted according to Delgado-Andrade et al. [38] with some modifications. The FRAP reagent was prepared by mixing 2 mL of TPTZ (10 mM) diluted in HCl (40 mM) + 2 mL (FeCl_3 , 20 mM) + 20 mL of acetate buffer (0.3 M, pH 3.6).

The FRAP reagent was mixed with 10 μL of the sample or standard (Trolox). The reaction mix was incubated (15 min) at room temperature in darkness. The absorbance was read at 593 nm (UV-visible Epoch™ Microplate Spectrophotometer); the results were expressed as the milligram equivalent of Trolox per gram of oregano (mg Trolox/g oregano).

2.7. Inhibitory Activity of Extracts against α -Amylase

The α -amylase inhibition was tested according to Chen et al. [39] with slight modifications, using acarbose (1 mg/mL) (control +) and buffer (control –).

A volume of 20 μL of the extracts was mixed and incubated with 20 μL α -amylase (10 U/mL in 0.02M pH 6.9, phosphate buffer), and the mix was incubated for 45 min (37 $^{\circ}\text{C}$). Then, 40 μL of starch (2%, *w/v*) was added and incubated for 5 min. Finally, 200 μL of DNS reagent was added to stop the reaction and boiling (10 min). The absorbance was determined at 540 nm (UV-visible Epoch™ Microplate Spectrophotometer).

The inhibition of α -amylase was calculated according to the following Equation (1):

$$\text{Inhibition } \alpha\text{-amylase (\%)} = [(A_{Ca} - A_c) - (A_{Sa} - A_s)] / (A_{Ca} - A_c) \times 100 \quad (1)$$

where A_{Ca} and A_{Sa} were the absorbances of buffer and samples mixed with α -amylase, and A_c and A_s were the absorbances without α -amylase.

2.8. Antimicrobial Activity via Agar Diffusion Assay (ADA)

The antimicrobial activity was determined against a relevant bacterial strain in the food industry (*Escherichia coli*, Food Research Department, Chemistry School, Autonomous University of Coahuila) via the Agar Diffusion Assay (ADA), following the methodology proposed by Avaiyarasi et al. [40], with some modifications. A TSAYE agar (Tryptone Soy Yeast Extract Agar) was seeded with 1×10^8 cells/mL. Once the agar was solidified, 8 mm holes were punched; 50 μ L of each fermented extract was added to each well. The experiments were incubated (37 °C, 24 h), and clear zones around the wells were measured to calculate the antimicrobial activity, which was expressed as inhibition diameter (mm).

2.9. RP-HPLC-ESI-MS Analysis of Extracts

The analysis using high-performance liquid chromatography was carried out using a Varian HPLC system, which includes an autosampler (Varian ProStar 410, Varian Inc., Palo Alto, CA, USA), a ternary pump (Varian ProStar 230I, Varian Inc., Palo Alto, CA, USA), and a PDA detector (Varian ProStar 330, Varian Inc., Palo Alto, CA, USA), as well as a liquid chromatograph ion trap mass spectrometer (Varian 500-MS IT Mass Spectrometer, Varian Inc., Palo Alto, CA, USA) equipped with an electrospray ion source.

The analytical method was applied according to Ascacio-Valdés et al. [41]. A 5 μ L sample was injected into a Denali C18 column (150 mm \times 2.1 mm, 3 μ m, Grace, Columbia, MD, USA) at 30 °C. The mobile phases were formic acid (0.2%, *v/v*; solvent A) and acetonitrile (solvent B). All MS experiments were carried out in the negative mode $[M - H]^{-1}$ in a full scan mode acquired in the *m/z* range 50–2000.

The data were collected (MS Workstation software, V 6.9), and phenolic compounds were identified by comparing the retention time of the HPLC analysis with MS with the literature and the database of the Food Research Department from Autonomous University of Coahuila (DIA-UAdEC).

2.10. Statistical Analysis

The experiments were conducted in triplicate, and results were reported as mean \pm standard deviation (SD). SPSS Software (IBM® SPSS Statistics) was used as the statistical program to develop a one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. Figure 1 provides a general diagram of the analytical process.

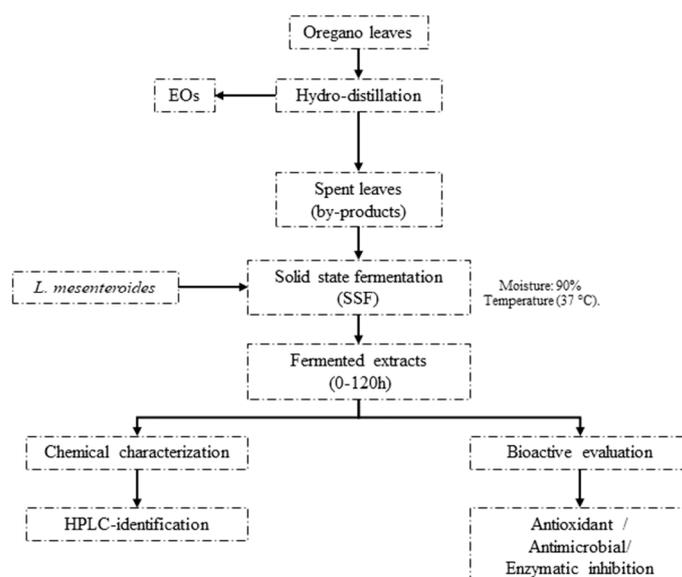


Figure 1. General process diagram of bioactive activity and chemical evaluation of *Lippia graveolens* by-product valorization through SSF process.

3. Results and Discussion

3.1. Phenolic Compounds

The results showed a statistical difference in the total polyphenolic content through the SSF process, the greater release of polyphenolic compounds after the application of *L. mesenteroides* was shown at 72 h with an increase of 0.60 times compared to time zero (Figure 2A). The increment is within the ranges of polyphenol increases reported using (0.25 to 2 times) LAB strains in other matrices (e.g., cereals) [42,43]. Similarly, the increase in total flavonoids was quantified at 2.55-times greater than at the initial time (Figure 2B) at 96 h of the SSF process; our result is higher than the values reported by Hwang et al. [31] with a strain of *L. mesenteroides* in ginseng (2.17 times). The increment in phenolic compounds concentration by *L. mesenteroides* strains has been previously observed in a garlic–*Cirsium setidens* Nakai blend with remarkable findings with an increase in total phenolic compounds (27.81 ± 0.34 gGAE/g) and flavonoids (33.80 ± 0.44 gQE/g) related mainly with the LAB enzymes [38]. It has been reported that LAB produces enzymes capable of releasing bioactive compounds such as tannase, β -glucosidase, and feruloyl esterase [32,44]. A study carried out by Lee et al. [45] reported that *L. mesenteroides* was capable of producing β -glucosidase that changes the flavonoid composition and the liberation of aglycones forms with major bioactive activity.

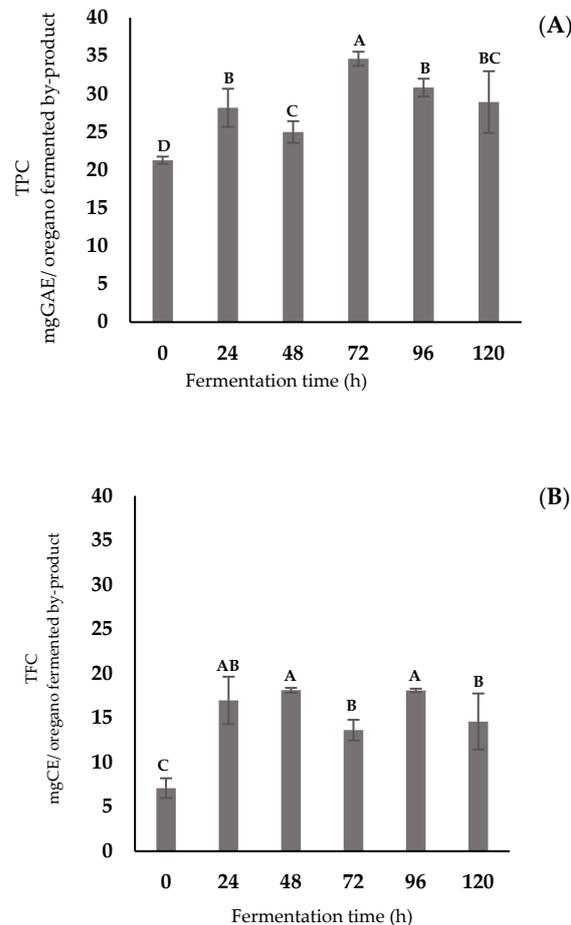


Figure 2. Polyphenolic compounds concentration in fermentative extracts obtained from SSF process using *L. mesenteroides*. (A) Total polyphenolic content (TPC) and (B) total flavonoid content (TFC). Different letters show significant differences ($\alpha = 0.05$).

Also, the enzymes may be capable of hydrolyzing the β -glucosidic linkages and liberating the biomolecules from cell wax with the combination of other enzymes with the capacity to hydrolyze other plant cell wall components (esterase). Otherwise, there are

changes in flavonoid structures from the metabolism of *L. mesenteroides*; it has been reported that *L. mesenteroides* has the potential to express glucan sucrose enzymes involved in the glycosylation of flavonoids in the fermentation process (ex., glucosides from quercetin, luteolin, myricetin, and fisetin, among others) [46–48]. Thus, glycosylation may promote the solubility of aglycone forms of flavonoids and benefit the extraction of higher quantities of bioactive molecules in water.

However, higher concentrations of phenolic compounds could have a negative impact on microorganisms (LAB). As a response, some mechanism for detoxification of the environment could be promoted [49]. This behavior has been previously reported in LAB-fermented Chinese chives (*Allium tuberosum*) [50] and, recently, in avocado leaves fermented by *L. mesenteroides* 21, where the concentration of Folin–Ciocalteu reacting substances (e.g., polyphenols) suffered a reduction after 72 h that could be explained by a possible degradation of biotransformation (phenolic acid decarboxylase and reductase, among others) into reduced forms [51].

3.2. Antioxidant Activity

The antioxidant activity (Figure 3) is related to the content of polyphenolic compounds; the data showed an increment through fermentation; the DPPH• radical assay showed the potential of the extract to inhibit an oxidative radical, and the highest activity against the radical was recorded at 96 h up to 2.51-times higher than at the initial time point. The iron-reducing power assay (FRAP) showed a similar behavior where, in the first hours of fermentation, the antioxidant activity was lower than after the SSF process. The highest value was obtained at 120 h (3.01-times higher), but it is statistically similar to the extract recovered at 96 h. The increment in antioxidant activity can be related to the extraction of polyphenolic compounds via the SSF process and the enzymes involved in it. Also, in literature, *L. mesenteroides* has been associated with the production of secondary metabolites with antioxidant properties; Lee et al. [52] reported the production of four amino acid derivatives with the level of antioxidant activity that could be produced in our study. Similarly, Zhang et al. [53] reported the production of an exopolysaccharide with antioxidant activity that could be produced in the SSF process.

3.3. Antimicrobial Activity

The fermentation extracts showed antimicrobial activity (Table 1) against *E. coli*; the greatest level of inhibition belonged to the 96 h sample of the SSF process with an inhibition diameter of 12.06 ± 2.66 mm, which may correspond to the time point with a high quantity of polyphenolic compounds. In addition, not only the polyphenol release may explain the activity, but because the production of antimicrobial peptides in LAB has been reported, the bacteriocins could interact negatively with the membrane of pathogenic bacteria, generating disruption, hindering protein transit, and leading the cell to death [54]. Specifically, in the literature, the expression of genes of two antimicrobial peptides (hymenoptaecin and apidaecin) has been reported for *L. mesenteroides* (TBE-8) that could provide antimicrobial activity [55]. Otherwise, the fermented extract obtained at 120 h of fermentation showed a strong reduction in the antimicrobial activity, a possible explanation could be related to the absence of a chemical antimicrobial component (see Section 3.5, Ferulic acid), and, also, the enzymatic process applied in the SSF may produce an extract with higher sugar content that could reduce the antimicrobial potential of the extract. In the literature, there is a precedent reported by Molet-Rodríguez et al. [56] that the integration of bioactive molecules (e.g., essential oils) into food products (complex matrix) involves a complex balance, due to the presence of nutrients (fructose, sucrose, and glucose) that could help bacteria to reduce the negative impacts of natural antimicrobial components.

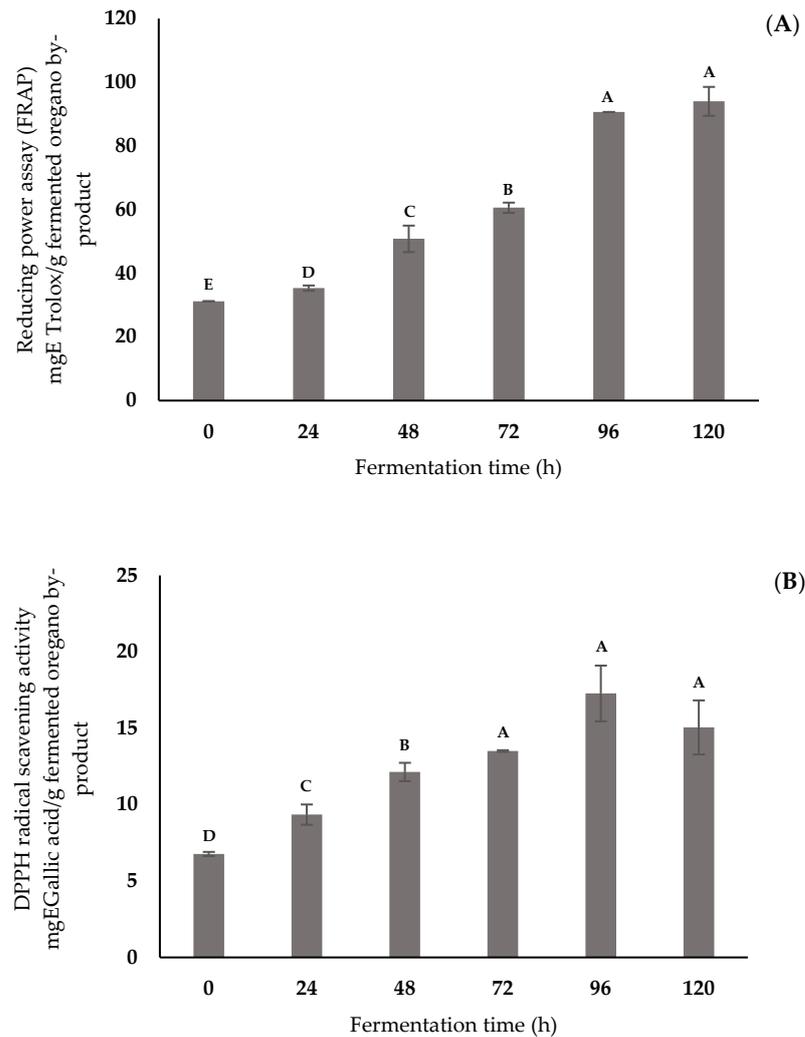


Figure 3. Antioxidant activity of fermentative extracts via the SSF process using *L. mesenteroides*; (A) FRAP assay and (B) DPPH[•] assay. The different letters show significant differences ($\alpha = 0.05$).

Table 1. Antimicrobial activity against *E. coli* and α -amylase inhibition of fermented extracts under SSF process using *L. mesenteroides*.

Fermentation Time (h)	Bioactivities	
	Antimicrobial Activity (<i>E. coli</i>) Inhibition mm	α -Amylase Inhibition (%)
0	3.3 ± 0.56^C	3.24 ± 1.28^C
24	4.73 ± 1.3^C	2.17 ± 1.28^C
48	2.96 ± 1.72^C	4.22 ± 1.03^C
72	4.03 ± 0.45^C	4.61 ± 1.27^C
96	12.06 ± 2.66^B	11.32 ± 3.7^B
120	2.8 ± 0.1^C	14.10 ± 0.45^B
Tetracycline (1 mg/mL)	22.36 ± 0.55^A	—
Acarbose (1 mg/mL)	—	94.07 ± 2.6^A

The letters show significant differences according to the comparison of means of all experiments ($\alpha = 0.05$).

It is important to note that none of the extracts showed activity greater than the positive control with tetracycline at a standard concentration of 1 mg/mL (22.36 ± 0.55 mM). On the other hand, it could be possible to increase the antimicrobial activity by concentrating the crude extract of the fermentation.

3.4. Potential α -Amylase Inhibition

The findings reported that the extract from fermentation has a light inhibition of α -amylase (Table 1). The highest value was reported for the 120 h ($14.10 \pm 0.45\%$) sample and correlated with the time at which there was the highest concentration of flavonoids. Flavonoids have been reported as molecules with potential as inhibitors of α -amylase by binding to proteins via hydrogen bonds [57–59].

The inhibition values were lower than the positive control (acarbose) with a value of $94.07 \pm 2.6\%$; but it is reported that higher inhibition of α -amylase might cause diarrhea, flatulence, and abdominal pain due to bacterial fermentation by intestinal bacteria [60,61]. Our data suggest that oregano by-products could be a source of compounds with the capacity for α -amylase inhibition.

3.5. RP-HPLC-ESI-MS Analysis

The HPLC-MS analysis of extracts before the solid-state fermentation process (0 h) revealed six compounds (Table 2); a total of two identified compounds have been previously reported in other works for oregano varieties. For example, caffeic acid and medioresinol have been reported in oregano varieties [62,63]. Caffeic acid has been reported as an antioxidant component with antimicrobial activity against pathogens (*Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, among others) [64]. Also, medioresinol was associated with the capacity to act synergistically with antibiotics in bacterial infection and the capacity to act as an auxiliary to prevent pyroptosis of endothelial cells in ischemic stroke [65,66]. Four compounds have not been related to oregano species but contain metabolites with potential bioactivities present in plants that could be synthesized by oregano plants (5-nonadecylresorcinol, sinensetin, phlorin, and petunidin 3-O-(6''-acetyl-glucoside)): for example, sinensetin is a novel compound with numerous bioactivities (anticancer, anti-inflammatory, antioxidant, antimicrobial, anti-obesity, anti-dementia, vasorelaxant, and antitrypanosomal activities) that could be exploited by the food and pharmaceutical industry [67].

Table 2. Identified compounds via RP-HPLC-ESI-MS from the SSF of Mexican oregano by-products using *L. mesenteroides*.

ID	Tentative Assignment	[M – H] [–] (m/z)	Class	Fermentation Time (h)					
				0	24	48	72	96	120
1	Caffeic acid 4-O-glucoside	341.1	Hydroxycinnamic acids	X	X	X	X		
2	5-nonadecylresorcinol	375.1	Alkylphenols	X	X	X			
3	Medioresinol	387.2	Lignans	X	X	X	X	X	X
4	Sinensetin	371.1	Methoxyflavones	X	X	X	X	X	X
5	Phlorin	287.1	Other polyphenols	X					
6	Petunidin 3-O-(6''-acetyl-glucoside)	520.4	Anthocyanins	X					
7	Kaempferol 3-O-(2''-rhamnosyl-6''-acetyl- galactoside) 7-O-rhamnoside	783.2	Flavonols		X				
8	Resveratrol 3-O-glucoside	389.1	Stilbenes		X	X	X	X	X
9	Gardenin B	357.3	Methoxyflavones		X				
10	3,4-DHPEA-EA	377.1	Tyrosols		X				
11	Trachelogenin	387.2	Lignans			X			
12	Quercetin 3-O-glucuronide	447.1	Flavonols			X			
13	Phloretin	273	Dihydrochalcones			X	X		
14	5-O-galloylquinic acid	343.1	Hydroxybenzoic acids				X		
15	p-coumaroyl tyrosine	327.2	Hydroxycinnamic acids				X		
16	Ferulic acid	193.1	Methoxycinnamic acids					X	
17	Hydroxycaffeic acid	195.1	Hydroxycinnamic acids						X
18	Feruloyl glucose	355.1	Methoxycinnamic acids						X
19	Tangeretin	1075	Methoxyflavones						X

The X represents the presence of the identified compounds at that time of fermentation. Blanks indicate the absence of the compounds at that time of fermentation.

The SSF process enriched the fermentation extracts with the release of 13 compounds (7–19) shown in Table 2; the liberation of different polyphenols through fermentation may be the result of LAB enzymes capable of degrading the cell wall (tannase, β -glucosidase and feruloyl esterase) [32,44,52]. Among the released bioactive compounds, the enriched extracts highlight their interesting bioactivities with potential in industry; for example, the SSF releases gardenin B (24 h), a methoxylated flavonoid with novel health benefits, that showed a cytotoxic effect as an apoptotic inducer against human leukemia cell lines (HL-60 and U-937) [68]. Recently, gardenin B was reported as a promising antiviral auxiliary with the capacity to attach with the SARS-CoV-2 main protease (Mpro) [69]. Another novel compound is trachelogenin (48 h), a lignan with relevant health activities; for example, Moura et al. [70] associated it with in vitro antitumor capacity via induction of cell death, with the formation of autophagosomes and cytoplasmic vacuolization. Also, an inhibited interaction between trachelogenin and hepatitis C virus glycoprotein and the host entry factor (CD81) [71] has been demonstrated. The other compounds (kaempferol, quercetin, ferulic acid, and tangeretin, among others) have been associated with beneficial activities such as antioxidant, antimicrobial, antiviral, etc. [72–74]. Thus, the bioactive compounds released in the extracts comprise bioactive molecules with highlighted bioactivities for the food and pharmaceutical industries.

The data shown in Table 2 could be used for relating the bioactivities obtained with the compounds found in the extracts; the ability to inhibit α -amylase could rest in the presence of sinensetin and medioresinol, the potential of which was determined in previous works [75,76]. Furthermore, the antimicrobial potential against *E. coli* could be related to the presence of chemical molecules like caffeic acid 4-*O*-glucoside, resveratrol 3-*O*-glucoside, sinensetin, and ferulic acid, which have been related with this activity in the literature [77–79]. Also, the potential synergetic effect between the chemical compounds could be responsible for the higher antimicrobial activity reported at 96 h; for example, Skroza et al. [80] demonstrated positive interactions between resveratrol and ferulic acid, showing a decrease in the MIC (Minimum inhibitory concentration) value from 2500 micromolar to 1250 micromolar (molar ratio 1:1); both compounds could be found in the SSF extracts.

4. Conclusions

This research article highlights important insights concerning evidence of the hidden bioactive compounds and potential bioactivity of oregano by-products subjected to SSF technology. Our findings from bioprocessing using *L. mesenteroides* increase the current efforts looking for novel sustainable processing lines under circular economy trends. Also, the application of SSF with *L. mesenteroides* increases the extraction of total polyphenolic compounds (0.60 times) and flavonoids (2.55) as compared to the initial time point. The extracts showed a potential inhibitory capacity against α -amylase ($14.10 \pm 0.45\%$) that could be explored as a possible auxiliary in glucose control. Also, the extracts showed antibacterial capacity against *E. coli* (12.06 ± 2.66 , 96 h) that could be explored for product development. The HPLC-MS analysis determined that SSF releases compounds of interest not found before fermentation (0 h) such as gardenin B, trachelogenin, ferulic acid, and resveratrol 3-*O*-glucoside.

Finally, applying LAB in an SSF process comprises a potential alternative for recovering bioactive extracts from Mexican oregano residues, which may trigger their use as raw materials to develop industrial bioproducts. Additionally, the following studies will be guided in evaluating residual fermented biomass, which could be a novel fermented vegetable food product.

Also, exploiting Mexican resources will bring value to the region while minimizing environmental impact by applying bioprocessing tools. However, more studies are needed to understand the bacterial mechanism involved in the liberation of polyphenols and the possible biotransformation that biomolecules may suffer via LAB metabolism.

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