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Enrique Salazar José Llorente * , Fernando Javier Cobos Mora , Aurelio Amaiquema , Brayan Torres

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Keywords: inhibition; preservative; interaction; residues

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Evaluation of the Minimum Inhibitory Concentration of Polyphenols Combined with *Saccharomyces cerevisiae* as Antimicrobials for Growth of *Listeria monocytogenes*

Enrique José Salazar Llorente *, Fernando Javier Cobos Mora, Aurelio Esteban Amaiquema Carrillo and Brayan Fernando Torres Salvatierra

Technical University of Babahoyo / Babahoyo / Av. Universitaria km 21/2 Av. Montalvo Babahoyo, Los Ríos; fcobos@utb.edu.ec (F.J.C.M.); esteban3carrillo@outlook.com (A.E.A.C.); torressalvatierra@faciag.utb.edu.ec (B.F.T.S.)

* Correspondence: ejsalazar@utb.edu.ec; Tel.: 0987128999

Abstract: This study used a completely randomized design with a bifactorial arrangement in triplicate to evaluate dif-ferent concentrations of flavonoid extracts from orange (Citrus sinensis), onion (Allium cepa), tamarillo (So-lanum betaceum) and cocoa (Theobroma cacao) peels in combination with the yeast Saccharomyces cerevisiae to inhibit the growth of Listeria monocytogenes in fruit juices. The shells were dehydrated at 50-57 °C for 8 hours and pulverized. The dehydration yields were: orange (32 %), onion (13 %), tamarillo (14 %) and cocoa (34 %). Flavonoids were extracted with Soxhlet using 70% ethanol for 5 hours, concentrating with a rotary evaporator. They were quantified by UV spectrophotometry and the Folin-Ciocalteu method, being the highest in orange (300,75-372,60 mg Q/L) and onion (205,70-534,96 mg Q/L), while tamarillo presented the lowest, with possible errors in the measurement. Soluble solids losses ranged from 2,30 g (cocoa) to 5,27 g (orange). Cocoa and orange extracts showed greater potential as natural preservatives, inhibiting the growth of Listeria monocytogenes in fruit juices, with a minimum inhibitory concentration of 300 µg/mL and 400 µg/mL, re-spectively.

Keywords: inhibition 1; preservative 2; interaction 3; residues 4

1. Introduction

Agroindustry produces every day a large amount of waste, especially husks, seeds, pomace and bagasse, which, if not properly managed, can be a source of various environmental problems, so an alternative is sought for the treatment of these wastes for their valorization and utilization, largely based on agroindustrial waste. Within these lines of action, the extraction of bioactive compounds has a great potential that may be of interest for its application in the food industry [1].

One of the most important problems in the food industry is that of antimicrobial resistance, fundamental in an industry were achieving control in the elimination of pathogenic microorganisms such as, for example, Listeria monocytogenes, is key to maintaining the hygienic-sanitary safety of foodstuffs. Listeria monocytogenes, a microorganism that stands out for its resistance to adverse conditions, is a very high risk: especially in foods for direct consumption, since its power of multiplication is very high, posing significant health problems in food safety [2].

Listeriosis is a rare but potentially serious infection caused by Listeria monocytogenes. The main mode of transmission is through the consumption of contaminated food. Although it mainly affects the elderly, pregnant women and people with weakened immune systems, it can also occur in healthy adults and children. Listeria monocytogenes is a small, facultative anaerobic, non-sporulating, motile, gram-positive bacillus that generates mild hemolysis on blood agar. As a facultative intracellular pathogen, it has a complex pathogenesis and is able to cross intestinal, placental and

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hematoencephalic barriers, causing gastroenteritis, maternal-fetal infections and meningoencephalitis [3].

Polyphenols are bioactive compounds that can be found in vegetables, fruit and vegetable residues, and have been characterized by their antimicrobial properties. They are natural metabolites that provide food safety, as they act as natural antimicrobials in food, inhibiting pathogenic microorganisms from developing and causing foodborne diseases [4].

Phenolic compounds (PC) are biomolecules that plants synthesize in response to various types of stress, whether biotic or abiotic. These compounds are predominantly divided into two groups: flavonoid polyphenols (which include flavones, flavonols, flavan-3-ols, flavanones, flavanones, anthocyanidins, isoflavones and also proanthocyanidins) and non-flavonoids (such as phenolic acids, stilbenes, tannins, coumarins or neolignans). Many of these FCs basically act as antimicrobial barriers in plants when faced with infections, which makes them a source of interest as effective antimicrobial agents against a wide range of undesirable microorganisms in food [5].

In addition, the species *Saccharomyces cerevisiae* is a yeast that is widely used for the fermentation process of food and beverages, and has demonstrated that it may have potential not only as a fermenting agent, but also as an antimicrobial agent. In the union of *Saccharomyces cerevisiae* with polyphenols, it can provide a synergistic approach, so that the antimicrobial effect will be increased, and therefore the concentration of polyphenols will be lower, and at the same time, the organoleptic properties of the food will be maintained [6].

The cell wall of *Saccharomyces cerevisiae* is composed of mannan-oligosaccharides and β-glucans, which play a fundamental role in protecting against the colonization of pathogenic bacteria and favor macrophage growth. It also contains a good amount of proteins and peptides and has a profile of amino acids with a high biological value, which have beneficial effects that enhance the activity of the immune system [7].

In the Bringas et al. (2020) study shows that the minimum concentration analysis to inhibit *Listeria monocytogenes* ATCC 19115 was 20 μg/mL, while for Pseudomonas aeruginosa ATCC 27853 it was 40 μg/mL. The polyphenols present in the essential oils of *Citrus sinensis L.* Osbeck peel showed remarkable antibacterial activity, especially against Gram-positive bacteria such as Listeria. This is due to the action of compounds such as flavonoids and other polyphenols, which act as natural antimicrobial agents, offering significant potential as food preservatives [8].

Listeria monocytogenes is a microorganism present in various environments, capable of surviving adverse conditions and can cause serious infections in both humans and animals. It is transmitted mainly through the consumption of contaminated food and has a remarkable ability to adapt to extreme conditions, which allows it to persist in the production of different foods. This resistance can generate significant economic losses due to the mandatory recall of contaminated products from the market [9].

The main objective of this study was to determine the minimum inhibitory concentration (MIC) of polyphenols combined with *Saccharomyces cerevisiae* as antimicrobials for the control of *Listeria monocytogenes* in food.

2. Materials and Methods

The present research was carried out at the Escuela Polytechnical del Litoral (ESPOL), at the Centro de Investigations Biotechnological del Ecuador (CIBE) in the Biosafety Level 2 laboratory.

Two types of completely randomized experimental designs were used with a bifactorial arrangement with 12 treatments and 3 replicates each, giving a total of 72 objects of study.

Experimental Design 1

Dependent variable: Growth of Listeria monocytogenes (E).

Independent variable: Flavonoid extract concentration (Orange, (A) Onion, (B) Cocoa, (C) Tamarillo (D)).

Concentrations: 200 ug/mL, 300 ug/mL, 400 ug/mL.

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Each combination of concentration and extract type was tested with the same amount of *Listeria monocytogenes* inoculum. The combinations are listed below:

"Table 1" presents different concentrations of extracts for four types of samples: Orange (A), Onion (B), Cocoa (C) and Tamarillo (D). Each sample is tested at three specific concentrations: 200 µg/mL (E1), 300 µg/mL (E2) and 400 µg/mL (E3).

Table 1. Combination of concentration and types of extracts.

Orange (A)	Onion (B)	Cocoa (C)
A1E1 (200 µg/mL)	B1E1 (200 µg/mL)	C1E1 (200 µg/mL)
A2E1 (300 µg/mL)	B2E1 (300 µg/mL)	C2E1 (300 µg/mL)
A3E1 (400 µg/mL)	B3E1 (400 µg/mL)	C3E1 (400 µg/mL)

"Table 2" presents concentration data for extracts from four samples: Orange, Onion, Cocoa and Tamarillo each tested at three different concentrations (200, 300 and 400 µg/mL). For each concentration, three labeled replicates (E1 to E36) are included for each sample, allowing accurate and repeated evaluation of the properties of the extracts at each concentration level.

Table 2. Inoculum of Listeria monocytogenes with the different extracts and concentrations.

Extract	Concentration (µg/ml)	Replica 1	Replica 2	Replica 3
Orange	200	E1	E2	E3
Orange	300	E4	E5	E6
Orange	400	E7	E8	E9
Onion	200	E10	E11	E12
Onion	300	E13	E14	E15
Onion	400	E16	E17	E18
Cocoa	200	E19	E20	E21
Cocoa	300	E22	E23	E24
Cocoa	400	E25	E26	E27
Tamarillo	200	E28	E29	E30
Tamarillo	300	E31	E32	E33
Tamarillo	400	E34	E35	E36

Experimental Design 2

Dependent variable: Listeria monocytogenes growth (E).

Independent variable: Yeast colonies (3 units/1mL H_2Odistilled) H.

Independent variable: Flavonoid concentration (Orange, (A) Onion, (B) Cocoa, (C) Tamarillo (D)).

Concentrations: 200 ug/mL, 300 ug/mL, 400 ug/mL.

"Table 3" shows the concentrations of Orange (A), Onion (B), Cocoa (C) and Tamarillo (D) extracts at different levels: 200 µg/mL, 300 µg/mL and 400 µg/mL. Each sample is presented with 3 units per 1 mL of water (H_2O), and each concentration has a specific label (E1, E2, E3) for each type of sample.

Table 3. Combination of concentration and types of extracts.

Orange (A)	Onion (B)	Cocoa (C)	Tamarillo (D)
A1E1 (200 µg/mL)	B1E1 (200 µg/mL)	C1E1 (200 µg/mL)	D1E1 (200 µg/mL)
(3 units /1mL H_2O)	(3 units /1mL H_2O)	(3 units /1mL H_2O)	(3 units /1mL H_2O)
A2E1 (300 µg/mL)	B2E1 (300 µg/ml)	C2E1 (300 µg/mL)	D2E1 (300 µg/mL)
(3 units /1mL H_2O)	(3 units /1mL H_2O)	(3 units /1mL H_2O)	(3 units /1mL H_2O)
A3E1 (400 µg/mL)	B3E1 (400 µg/ml)	C3E1 (400 µg/mL)	D3E1 (400 µg/mL)

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(3 units /1mL H_2O)	(3 units /1mL H_2O)	(3 units /1mL H_2O)	(3 units /1mL H_2O)

"Table 4" details the evaluation of yeast colonies for Orange, Onion, Cocoa and Tamarillo extracts at concentrations of 200, 300 and 400 µg/mL, with 3 units per mL in each case. Each concentration has three labeled replicates (E1 to E36) for each sample type, facilitating comparison of the number of colonies formed as a function of extract concentration.

Table 4. Inoculum of Listeria monocytogenes with the different extracts and concentrations.

Extract	Concentration (µg/ml)	Yeast Colonies	Replica 1	Replica 2	Replica 3
Orange	200	3 units/mL	E1	E2	E3
Orange	300	3 units/mL	E4	E5	E6
Orange	400	3 units/mL	E7	E8	E9
Onion	200	3 units/mL	E10	E11	E12
Onion	300	3 units/mL	E13	E14	E15
Onion	400	3 units/mL	E16	E17	E18
Cocoa	200	3 units/mL	E19	E20	E21
Cocoa	300	3 units/mL	E22	E23	E24
Cocoa	400	3 units/mL	E25	E26	E27
Tamarillo	200		E28	E29	E30
Tamarillo	300		E31	E32	E33
Tamarillo	400		E34	E35	E36

Raw material dehydration process

For the flavonoid extraction process, the following procedure was followed. First, raw materials were selected, including orange peels (*Citrus sinensis*), onion peels (*Allium cepa var. viviparum*), Tamarillo (*Solanum betaceum*), and cocoa (*Theobroma cacao*). After reception, the wet shells were weighed in grams and then subjected to a controlled dehydration process. This process was carried out at a temperature of 50 to 57 °C for a time of 8 hours.

Subsequently, the percentage of dry sample was calculated using the formula:

$$\% \text{ Sample} = \frac{\% \textit{ Grams of dry sample}}{\% \textit{ rams of wet sample}} \boldsymbol{x\ 100}\% \quad (1)$$

After the dehydration process, the samples were crushed in a mortar until a fine powder was obtained. The same procedure was applied for each of the selected raw materials. Finally, the dehydrated samples were weighed, vacuum sealed and stored in an environment at -4 °C for preservation, ensuring the stability of the flavonoids for subsequent extraction [10].

Flavonoids extraction process

Sample Reception

Samples of orange peels, onion peels, cocoa and Tamarillo pulp were received and weighed. These raw materials were prepared and placed in buckets for further processing for flavonoid extraction.

Soxhlet

The samples were subjected to the extraction process using Soxhlet equipment, where 70 % ethanol was used as solvent. The process was carried out at a temperature of 78 °C for a period of 5 hours, allowing continuous extraction of the flavonoids present in the samples [11].

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Cooling

After extraction in the Soxhlet, the extracts obtained were allowed to cool for 15 minutes. This cooling is essential to stabilize the extracted compounds and prepare the extract for the next concentration step.

Rotary evaporator

The cooled extract was concentrated using a rotary evaporator, operating at a temperature of 60 °C for 15 minutes. This stage allowed the elimination of excess solvent, concentrating the flavonoids in a reduced volume of extract [12].

Weighing and Storage

The concentrated extract was collected in centrifuge tubes and weighed to determine the final amount of extracted flavonoids. Finally, the tubes were sealed and stored at a temperature of -4 °C, ensuring the preservation of the compounds for future analysis or applications.

Application of the Soxhlet method

For the preparation of the solvent to be used in the Soxhlet method the following formula was applied:

$$\frac{C2 \ X \ V2}{C1} = V1 \qquad (2)$$

C2 = Represents the percentage of purity of the reagent to be used.
V2 = The desired volume of the reagent.
C 1= Concentration of the solvent.

Rotavaporator

After 5 hours in the soxhlet the samples are removed and taken to rotavaporation in which this helps us to eliminate the ethanol present in the sample so that it is eliminated correctly the temperature should be 60 ° C at 90 rpm for a time of 15 minutes after that time the sample is removed and stored in centrifuge tubes and stored in the refrigerator at a temperature of - 4 ° C in which it was evidenced that when being correctly eliminated the ethanol the liquid samples were frozen facilitating the process for the following stage that is the lyophilization.

Lyophilization

Sample reception

This is the initial phase of the process where the sample is received and prepared for the following steps. During this stage, it is ensured that the sample is in suitable conditions to be subjected to ultrafreezing and subsequent lyophilization.

Ultrafreezing

In this stage, the sample is frozen at extremely low temperatures, approximately -80 °C, for a time of 1 hour. This step is crucial to preserve the integrity of the sample before subjecting it to the freeze-drying process, minimizing the formation of large ice crystals that could damage the cell structure.

Lyophilization

After freezing, the sample undergoes the lyophilization process itself, which lasts approximately 24 hours. In this step, the water present in the frozen sample is removed by sublimation, which means

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that the ice passes directly from solid to vapor without passing through the liquid state. This process is essential to dehydrate the sample without altering its chemical composition [13].

Storage

Once freeze-drying is completed, the sample is stored at a temperature of -4 °C. This storage temperature is low enough to avoid degradation of the sample, keeping it in optimal conditions until it is needed for use or analysis.

To determine the initial and final weight of the freeze-dried product and to obtain the amount of solid extract obtained, the following formula was used:

$$\boldsymbol{Ext = t1 - T1L} \quad (3)$$

Ext: total extract.
T1L: centrifuge tube with the lyophilized sample (knowing that the assay is performed in triplicate, i.e. T1, T2, T3).
t1: empty centrifuge tube (knowing that the test is performed in triplicate, i.e. t1, t2, t3).

In order to obtain data that favor the development of this research, the loss of soluble solids (water and ethanol) in the extraction processes was determined, i.e. how much was lost in the rotary evaporator plus what was lost in the freeze-drying by means of the following formula:

$$\boldsymbol{Ps = M + t1 - T1L} \quad (4)$$

Ps: total loss of soluble solids.

M: Weight of the sample (M1, M2, M3 will be replaced in the formula).
t1: Empty centrifuge tube (knowing that the test is performed in triplicate i.e. t1, t2, t3).
T1L: centrifuge tube with lyophilized sample (knowing that the assay is performed in triplicate i.e. T1, T2, T3).

Quantification of total flavonoids by ultraviolet light spectrophotometer

The sample of both onion, Tamarillo and orange, is 25 mg this process was carried out in an eppendorf

The sample was moistened with 200 ul of 50 % ethanol and with the help of the magnetic stirrer the sample was mixed.

The previously moistened sample was placed in the sonicator for 30 minutes.

After this process the samples were dried and placed in the centrifuge for 10 minutes at 1000 rpm (it was recommended that the samples should face the front).

The obtained liquid was stored in another container and the supernatant that was left in the eppendorf was again subjected to a similar process.

In the supernatant 10 ul of 70 % acetone is added.

This is also mixed with the help of the magnetic stirrer until a single mixture is made.

This new mixture is taken to the sonifier for 30 minutes.

After this process the samples were dried and placed in the centrifuge for 10 minutes at 1000rpm (it was recommended that the samples should face the front) [14].

Reagent Preparation

A volume of 10000 ul of methanol was mixed with 10 mg of quercetin.

Potassium acetate was prepared in a diluted ratio of 0,98 g reagent+10 ml distilled water.

A mixture of potassium acetate (2,5 mL), absolute methanol (15 mL), aluminum chloride (2,5 mL), distilled water (30 mL) was made.

Dissolutions 1:2 and 1:10 were performed in triplicate.

The samples were stored in a rack

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Sample in plates

20ul of each sample was placed in 96 inoculation plates where each sample was correctly distributed with the dilutions that had been made previously.

The calibration curve was made adding to the sample the mixture of reagents so that it reacts.

The plate was left to rest for 30 minutes and covered with aluminum foil.

The plate with the samples was taken to a UV light spectrophotometer [15].

Quantification of total flavonoids with the Folin-Ciocalteu method

The sample of both onion, Tamarillo and orange, is 25 mg this process was performed in an eppendorf.

The sample was moistened with 200 ul of 50 % ethanol and with the help of the magnetic stirrer the sample was mixed.

The previously moistened sample was placed in the sonicator for 30 minutes.

After this process the samples were dried and placed in the centrifuge for 10 minutes at 1000 rpm (it was recommended that the samples should face the front).

The obtained liquid was stored in another container and the supernatant that was left in the eppendorf was again subjected to a similar process.

In the supernatant was added 10ul of acetone 70 %.

This is also mixed with the help of the magnetic stirrer until a single mixture is made.

This new mixture was taken to the sonicator for 30 minutes.

After this process the samples were dried and placed in the centrifuge for 10 minutes at 1000 rpm (it was recommended that the samples should be facing forward).

Sample in plates

20 ul of each sample was placed in 96-plate plates where each sample was correctly distributed with the dilutions that had been previously performed.

The 20 ul of sample were mixed with 100 ul of FC reagents and 80 ul of Na_2CO_3 solution, incubated for 60 minutes at room temperature.

The calibration curve was made by adding to the sample the mixture of reagents to react.

The plate with the samples was taken to a UV light spectrophotometer [16].

Procedure for Inoculating *Saccharomyces cerevisiae* in Papa Dextrose Agar (PDA)

Preparation of Papa Dextrose Agar (PDA)

PDA powder was dissolved in distilled water at a concentration of 39 g/L, following the manufacturer's instructions.

The medium was autoclaved at 121 °C for 15-20 minutes.

Subsequently, the medium was allowed to cool to approximately 50 °C before being poured into sterile Petri dishes.

The plates were allowed to solidify at room temperature.

Medium Inoculation

The *Saccharomyces cerevisiae* strain was in liquid culture and well shaken prior to inoculation.

A sterile pipette was used to transfer a small amount of the *Saccharomyces cerevisiae* liquid culture to the center of each PDA plate.

The plates were incubated in an incubator at 30 °C for 48 hours in an inverted position to prevent condensation [17].

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Measurement with the Spectrophotometer

Cuvette Filling

An aliquot of the sample is placed in a spectrophotometer cuvette. The cuvette is transparent at the wavelength used (usually 600 nm for estimating cell concentration).

Absorbance Reading: The spectrophotometer measures the amount of light passing through the sample at a specific wavelength (in this case, 600 nm) and calculates the absorbance or optical density.

Interpretation of Optical Density

Relationship to Cell Concentration: Optical density is directly related to the concentration of cells in the sample. As the number of cells increases, the optical density increases because more cells absorb and scatter the light passing through the sample.

Scales of Measurement

Low OD (e.g., < 0.1): May indicate a low concentration of cells.

Medium OD (e.g., 0.2 - 0.8): Represents exponential or logarithmic phase growth.

High OD (e.g., > 1.0): May indicate high cell concentration and possible arrival at the stationary or saturation phase of growth. This same procedure was performed on Listeria Monocytogenes bacteria [18].

DNA Extraction

Preparation for Extraction

Saccharomyces cerevisiae colonies were sampled from PDA plates with a sterile spatula and transferred to a microcentrifuge tube.

Sodium hydroxide (NaOH) solution was added to the tube to perform cell lysis. The concentration used was 0,1 M.

The tube was incubated at 50 °C for 30 minutes to allow complete cell lysis.

Neutralization and Purification

After NaOH treatment, the solution was neutralized by adding a neutralization buffer, typically with acetic acid solution or a specific buffer to neutralize NaOH.

The mixture was centrifuged at 13,000 rpm for 5 min to sediment the cell debris.

The supernatant containing DNA was transferred to a new microcentrifuge tube.

DNA was precipitated using ethanol or isopropanol and washed with 70 % ethanol for purification.

Preparation for PCR

The PCR reaction was prepared using primers specific for *Saccharomyces cerevisiae* and a standard PCR kit.

The reaction was performed in a thermal cycler following the appropriate amplification program.

PCR products were analyzed by agarose gel electrophoresis to verify DNA amplification.

Positive PCR products were sent to a sequencing laboratory to obtain the sequence of the amplified DNA [19].

Procedure for Inoculating *Listeria monocytogenes* in Tryptic Soy Agar (TSA)

Preparation of Tryptic Soy Agar (TSA)

TSA powder was dissolved in distilled water, following the recommended ratio (usually 30 g/L).

The medium was autoclaved at 121 °C for 15-20 minutes.

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After sterilization, the medium was allowed to cool to approximately 50 °C before pouring into sterile Petri dishes.

The plates were allowed to solidify at room temperature.

Medium Inoculation

A sterile pipette was used to transfer a small amount of the liquid culture of Listeria monocytogenes to the center of each TSA plate.

If a solid strain was used, a small portion of the colony was picked up with a sterile spatula and gently spread over the agar surface in the Petri dish.

The sample was spread over the agar surface with zig-zag motions to ensure even distribution.

Plates were incubated in an incubator at 37 °C for 24-48 hours in inverted position to avoid condensation.

After the incubation period, growth of colonies characteristic of *Listeria monocytogenes* was observed, appearing as small, smooth, gray to white colonies. Subsequently, it was taken to a spectrophotometer to see the growth curve, following the procedures described above [20].

DNA extraction

Listeria monocytogenes colonies were sampled from TSA plates with a sterile spatula and transferred to a microcentrifuge tube.

Sodium hydroxide (NaOH) solution was added to the tube to perform cell lysis. The concentration used was 0,1 M.

The tube was incubated at 50 °C for 30 minutes to allow complete cell lysis.

Neutralization and Purification

After treatment with NaOH, the solution was neutralized by adding a suitable neutralization buffer.

The mixture was centrifuged at 13,000 rpm for 5 minutes to sediment cell debris.

The supernatant containing DNA was transferred to a new microcentrifuge tube.

DNA was precipitated using ethanol or isopropanol and washed with 70 % ethanol for purification.

Preparation for PCR

The PCR reaction was prepared using primers specific for *Listeria monocytogenes* and a standard PCR kit.

The reaction was performed in a thermal cycler following the appropriate amplification program.

PCR products were analyzed by agarose gel electrophoresis to verify DNA amplification.

Positive PCR products were sent to a sequencing laboratory to obtain the sequence of the amplified DNA [21].

Minimum Inhibitory Concentration (MIC) of extracts in fruit juices

To determine the Minimum Inhibitory Concentration (MIC) of flavonoid extracts in fruit juices, extract solutions with an initial concentration of 4 mg/mL were prepared and diluted seriously in double concentrated BHI broth in test tubes, achieving final concentrations of 3200 to 1.25 µg/mL. 0.4 mL of bacterial inoculum (1.5 × 10^8 CFU/mL) was inoculated into each tube and incubated at 37 °C for 24 hours. The negative control contained only BHI broth and inoculum. The MIC was determined as the lowest concentration that completely inhibited bacterial growth, as assessed by the absence of turbidity or growth in Petri dishes. The results showed that the MIC for the extracts was 300 µg/mL for orange, 400 µg/mL for onion, 400 µg/mL for cocoa, and 400 µg/mL for Tamarillo with three independent replicates for each extract to ensure accuracy [22].

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3. Results

The present study employed a completely randomized design with a triplicate bifactorial arrangement of different concentrations of flavonoid extracts (Orange, Onion, Cocoa and Tamarillo in combination with the yeast *Saccahromyces Cerevisiae* which functions as a natural preservative inhibiting the growth of Listeria monocytones in fruit juice samples. The results are shown belowworks as a natural preservative by inhibiting the growth of *Listeria monocytones* in fruit juice samples. The results are shown below:

Dehydration of Orange peel (*Citrus sinensis*), Onion peel (*Allium cepa*), Tamarillo (*Solanum betaceum*) and Cocoa (*Theobroma cacao*).

Orange peel

For the dehydration process of the orange peel, 280,79 gr of wet peel were weighed and dehydrated for a period of 8 hours at a temperature of 50 to 57 °C. After completing the dehydration process, the samples were reduced in a mortar until a powder was obtained. Then we proceeded to weigh the dehydrated sample and obtained a weight of 89,96 gr of dehydrated orange peel. To obtain the sample percentage we applied the formula already stipulated.

$$\frac{89{,}96\ gr}{280{,}79\ gr} \text{ x } 100\% = 0{,}32\ \%$$

This estimation resulted in 32 % of orange peel samples, which were vacuum-sealed and stored at -4 °C.

Onion peel

For the onion peel dehydration process, 138,10 gr of wet peel were weighed and dehydrated for a period of 8 hours at a temperature of 50 to 57 °C. Once the dehydration process was completed, the samples were reduced in a mortar until a powder was obtained. The dehydrated sample was then weighed and a weight of 18,63 gr of dehydrated onion peel was obtained. To obtain the percentage of the sample we applied the formula already stipulated.

$$\frac{18{,}63\ gr}{138{,}10\ gr} \text{x } 100\% = 0{,}13\ \%$$

This estimation resulted in, although we obtained 13 % of onion peel samples, which were vacuum sealed and stored in an environment of -4 ºC.

Tamarillo

For the dehydration process of the Tamarillo peel, 728,55 gr of wet peel were weighed and dehydrated for a period of 8 hours at a temperature of 50 to 57 °C. Once the dehydration process was completed, the samples were reduced in a mortar until a powder was obtained. The dehydrated sample was then weighed and a weight of 104,10 gr of dehydrated Tamarillo peel was obtained. To obtain the percentage of the sample we applied the formula already stipulated.

$$\frac{104{,}10gr}{728{,}55\ gr} \text{x } 100\% = 0{,}14\ \%$$

This estimation resulted in a 14 % sample of Tamarilloes, which were vacuum sealed and stored at -4 °C.

Cocoa

For the cocoa dehydration process, 320,40 gr of wet shells were weighed and dehydrated for a period of 8 hours at a temperature of 50 to 57 °C. Once the dehydration process was completed, the samples were reduced in a mortar until a powder was obtained. The dehydrated sample was then

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weighed and a weight of 110,10 gr of dehydrated cocoa was obtained. To obtain the percentage of the sample we applied the formula already stipulated.

$\frac{110{,}10gr}{320{,}40\ gr}$x 100% = 0,34 %

This estimate resulted in a 34 % sample of cocoa, which was vacuum packed and stored at -4 °C.

"Table 5" shows the weight loss of samples after an extraction process. Cocoa decreased from 320,40 gr to 110,10 g, Onion from 138,10 gr to 18,63 gr, Orange from 280,74 gr to 89,96 gr, and Tamarillo from 728,55 gr to 104,10 gr. These reductions indicate the amount of material removed or lost during processing.

Table 5. Initial weight and final weight of the residues to obtain the flour.

M.Raw	Initial Weight	Final Weight
Cocoa	320,40 gr	110,10 gr
C. Onion	138,10 gr	18,63 gr
C. Orange	280,74 gr	89,96 gr
C. Tamarillo	728,55 gr	104,10 gr

Extraction of flavonoids

Samples of orange, onion, cocoa and Tamarillo peels were received and weighed to extract flavonoids. These samples were processed using a Soxhlet apparatus with 70 % ethanol at 78 °C for 5 hours. The extracts were then cooled for 15 min to stabilize them. The cooled extract was concentrated with a rotary evaporator at 60 °C for 15 min to remove excess solvent. Finally, the concentrated extract was weighed, stored in centrifuge tubes and kept at -4 °C to preserve the flavonoids.

"Table 6" shows the sample weights of four materials: cocoa, onion, orange and Tamarillo. For each material, three measurements of boll weight (m1, m2, m3) and three measurements of centrifuge tube weight (t1, t2, t3) are recorded.

Table 6. Initial weight and final weight of the residues to obtain the flour.

Samples	Weight Balls	Weight of Centrifuge tubes
Cocoa	m1: 115,3200 gr	t1: 10,5000 gr
	m2: 123,4500 gr	t2: 10,6000 gr
	m3: 121,7890 gr	t3: 10,4500 gr
C. Onion	m1: 109,0225gr	t1:10,1162 gr
	m2: 97,5165gr	t2: 9,9466 gr
	m3: 132,4163gr	t3: 9,9649 gr
C. Orange	m1: 169,7243 gr	t1:10,7882 gr
	m2:168,1958 gr	t2:10,1897 gr
	m3: 180,4852g	t3: 10,2107gr
C. Tamarillo	m1: 109,0127g	t1: 9,7225gr
	m2: 97,5113g	t2: 9,6373gr
	m3: 132,4039g	t3: 10,0843 gr

Application of the Soxhlet method

250 mL of solvent was prepared using 175 mL of ethanol and 75 mL of distilled water, dividing 83,33 mL per balloon of the Soxhlet equipment. After 5 h of Soxhlet extraction, the samples were taken to the rotary evaporator to remove ethanol at 60 °C and 90 rpm for 15 min. The samples were then stored in centrifuge tubes in the refrigerator at -4 °C, where they were frozen, facilitating the next stage of lyophilization.

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"Table 7" shows the values obtained in the weighing of the empty centrifuge tubes, and the weight of the empty centrifuge tubes with the sample after being lyophilized, i.e. initial weight and final weight, making a difference of values to obtain the amount of solid extract obtained.

Table 7. Values obtained in the weighing of centrifuge tubes with samples and empty centrifuge tubes.

Samples	Weight Centrifuge Tubes (Vacuum)	Weight of Lyophilized Centrifuge Tubes	Total Extract
Cocoa	t1: 10,2000 gr	T1L: 12,5000 gr	Ext 1: 2,3000 gr
	t2: 10,3000 gr	T2L: 12,6000 gr	Ext 2: 2,4000 gr
	t3: 10,1500 gr	T3L: 12,5500 gr	Ext 3: 2,3500 gr
C. Onion	t1: 10,1162 gr	T1L: 12,8867 gr	Ext 1: 2,7705 gr
	t2: 9,9466 gr	T2L: 12,7436 gr	Ext 2: 2,7970 gr
	t3: 9,9649 gr	T3L: 12,7429 gr	Ext 3: 2,7780 gr
C. Orange	t1: 10,7882 gr	T1L: 12,4329 gr	Ext 1: 2,2547 gr
	t2: 10,1897 gr	T2L: 12,3501 gr	Ext 2: 2,1604 gr
	t3: 10,2107 gr	T3L: 11,9363 gr	Ext 3: 1,7256 gr
C. Tamarillo	t1: 9,7225 gr	T1L: 12,7195 gr	Ext 1: 2,9970 gr
	t2: 9,6373 gr	T2L: 12,7456 gr	Ext 2: 3,1083 gr
	t3: 10,0843 gr	T3L: 13,1384 gr	Ext 3: 3,0541 gr

Loss of soluble solids

"Table 8" shows the loss of soluble solids for various samples. Cocoa has losses of 2,3000 g, 2,4000 g and 2,3500 g. The onion has losses of 3,2295 g, 3,2030 g and 3,2220 g. Orange has the highest losses, with 4,7453 g, 4,8396 g and 5,2744 g. Finally, Tamarillo shows losses of 4 g, 3,8917 g and 3,9459 g.

Table 8. Loss of soluble solids by weight of centrifuge tubes (vacuum) and weight of lyophilized centrifuge tubes.

Samples	Weight Centrifuge Tubes (Vacuum)	Weight of Lyophilized Centrifuge Tubes	Soluble Solids Loss
Cocoa	t1: 10,2000 gr	T1L: 12,5000 gr	Ps 1: 2,3000 gr
	t2: 10,3000 gr	T2L: 12,6000 gr	Ps 2: 2,4000 gr
	t3: 10,1500 gr	T3L: 12,5500 gr	Ps 3: 2,3500 gr
C. Onion	t1: 10,1162 gr	T1L: 12,8867 gr	Ps 1: 3,2295 gr
	t2: 9,9466 gr	T2L: 12,7436 gr	Ps 2: 3,203 gr
	t3: 9,9649 gr	T3L: 12,7429 gr	Ps 3: 3.222 gr
C. Orange	t1: 10,7882 gr	T1L: 12,4329 gr	Ps 1: 4,7453gr
	t2: 10,1897 gr	T2L: 12,3501 gr	Ps 2: 4,8396 gr
	t3: 10,2107 gr	T3L: 11,9363 gr	Ps 3: 5,2744gr
C. Tamarillo	t1: 9,7225 gr	T1L: 12,7195 gr	Ps 1: 4 gr
	t2: 9,6373 gr	T2L: 12,7456 gr	Ps 2: 3,8917 gr
	t3: 10,0843 gr	T3L: 13,1384 gr	Ps 3: 3,9459 gr

Quantification of total flavonoids by UV light spectrophotometer

10 mg of quercetin was mixed in 10,000 µL of methanol and a dilute potassium acetate solution was prepared. Potassium acetate, absolute methanol, aluminum chloride and distilled water were combined into a mixture. Triplicate 1:2 and 1:10 dilutions of the samples were made and stored in a rack. Twenty µL of each sample was placed in 96-well plates, a calibration curve was made with

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reagents, and allowed to stand for 30 minutes before measuring in a UV spectrophotometer. In the first assay, the results were negative for Tamarillo and high for onion and orange. Described in table 9 and 10.

Table 9. Tamarillo samples for flavonoid identification.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0,151	0,161	0,155	0,151	1,077	1,14	1,161	1,178	0,195	0,183	0,205	0,204
B	0,278	0,304	0,294	0,28	1,174	1,099	1,191	1,244	0,213	0,233	0,215	0,203
C	0,388	0,399	0,396	0,406	1,042	1,075	1,062	1,049				
D	0,497	0,487	0,479	0,506	0,925	0,891	0,896	0,916				
E	0,624	0,634	0,627	0,656	0,819	0,823	0,755	0,821				
F	0,287	0,74	0,753	0,77	0,589	0,621	0,637	0,618				
G	0,878	0,87	0,871	0,893	0,057	0,054	0,054	0,054				
H	1,051	0,896	0,883	0,898	0,221	0,229	0,233	0,239				

Table 10. Tamarillo samples for flavonoid identification.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0,096	0,106	0,1	0,096	1,022	1,085	1,106	1,123	0,14	0,128	0,15	0,149
B	0,223	0,249	0,239	0,225	1,119	1,044	1,136	1,189	0,158	0,178	0,16	0,148
C	0,333	0,344	0,341	0,351	0,987	1,02	1,007	0,994				
D	0,442	0,432	0,424	0,451	0,87	0,836	0,841	0,861				
E	0,569	0,579	0,572	0,601	0,764	0,768	0,7	0,766				
F	0,232	0,685	0,698	0,715	0,534	0,566	0,582	0,563				
G	0,823	0,815	0,816	0,838	0,002	-0,001	-0,001	-0,001				
H	0,996	0,841	0,828	0,843	0,166	0,174	0,178	0,184				

Quantification of total flavonoids with the Folin-Ciocalteu method

Samples of onion, Tamarillo and orange (25 mg each) were prepared in an eppendorf, wetted with 200 µL of 50% ethanol and mixed with a magnetic stirrer. After sonication for 30 min, the samples were centrifuged and the supernatant was treated with 10 µL of 70% acetone, mixed and sonicated again. They were then centrifuged and dried. 20 µL of each sample was placed in 96-well plates, mixed with 100 µL of FC reagents and 80 µL of Na2CO3, and incubated for 60 min at room temperature. The plate was measured in a UV spectrophotometer to evaluate the presence of flavonoids, finally checking if flavonoids were present in the Tamarillo peel.

"Table 11" shows that cocoa shows flavonoid concentrations ranging from 60,94 to 88,53 mg Q/L, with Cocoa 3 having the lowest values. Oranges show significantly higher concentrations, between 300,75 and 372,60 mg Q/L, with minor variations between samples. Onion has intermediate concentrations, from 205,70 to 534,96 mg Q/L, with Onion 2 showing the highest values. Tamarillo shows low values, with Tamarillo 3 showing negative results, indicating possible problems in measurement or sample preparation.

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Table 11. Flavonoid concentration of the different samples.

	Spectrophotometer reading					Average		Concentration total flavonoids	
Sample	ABS 1	ABS 2	ABS 3	ABS 4	ABS average	(mg Q/L) 1	(mg Q /L) 2	(mg Q /L) 3	(mg Q/L) 4
Cocoa 1	0,987	1,02	1,007	0,994	1,002	85,68	88,53	87,41	86,28
Cocoa 2	0,87	0,836	0,841	0,861	0,852	75,59	72,66	73,09	74,82
Cocoa 3	0,764	0,768	0,7	0,766	0,750	66,46	66,80	60,94	66,63
Orange 1	1,031	0,993	1,094	1,039	349,27	335,19	372,60	0,250	0,011
Orange 2	0,919	0,942	0,900	0,920	307,79	316,31	300,75	0,250	0,011
Orange 3	0,964	0,985	1,010	0,986	324,45	332,23	341,49	0,250	0,011
Onion 1	0,873	0,875	0,889	0,879	290,89	291,63	296,81	0,250	0,011
Onion 2	1,442	1,503	1,532	1,492	501,63	524,22	534,96	0,250	0,011
Onion 3	0,643	0,678	0,669	0,663	205,70	218,67	215,33	0,250	0,011
Tamarillo 1	0,701	0,646	0,641	0,663	227,19	206,81	204,96	0,250	0,011
Tamarillo 2	0,352	0,331	0,365	0,349	97,93	90,15	102,74	0,250	0,011
Tamarillo 3	0,026	0,028	0,027	0,027	-22,81	-22,07	-22,44	0,250	0,011

Table 12. Flavonoid concentration of the different samples with standard deviation.

Extract preparation		Dilution factor	Concentration total flavonoids			Average		Standard deviation
g	L	FD	(mg Q /g) 1	(mg Q /g) 2	(mg Q /g) 3	(mg Q /g) 4	(mg Q /g)	SD
8	0,05	1	0,54	0,55	0,55	0,54	0,00	0,01
8	0,05	1	0,47	0,45	0,46	0,47	0,46	0,01
8	0,05	1	0,42	0,42	0,38	0,42	0,41	0,02
8	0,08	1	0,47	0,49	0,51	0,49	0,49	0,02
3	46,103	44,246	49,183	46,511	2,49	0,52	24,68	26,79
3	40,628	41,752	39,699	40,693	1,03	0,45	20,47	22,79
3	42,828	43,855	45,077	43,920	1,13	0,51	22,66	25,23
3	38,397	38,495	39,180	38,691	0,43	0,43	19,68	22,23
3	66,215	69,197	70,615	68,676	2,25	0,81	35,59	39,34
3	27,153	28,864	28,424	28,147	0,89	0,33	14,45	15,98
3	29,988	27,300	27,055	28,114	1,63	0,27	14,27	15,39
3	12,926	11,900	13,562	12,796	0,84	0,14	6,83	7,34
3	-3,012	-2,914	-2,963	-2,963	0,05	-0,03	-1,48	1,72

The spectrophotometry results in Table 10 and 11 indicate that flavonoid concentrations vary significantly among samples. For cocoa, flavonoid concentrations range from 0,41 to 0,55 mg/g, with relatively constant values and a low standard deviation (0,01), suggesting a moderate presence of

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flavonoids. Oranges show the highest concentrations, ranging from 20,47 to 26,79 mg/g, with a standard deviation of 0,52, indicating high variability but, in general, a higher amount of flavonoids compared to other samples. Onion shows intermediate concentrations, from 14,45 to 39,34 mg/g, with a standard deviation of up to 2,25, reflecting considerable variability and a significant presence of flavonoids. In contrast, Tamarillo shows unusually low or negative values, especially in Tamarillo 3, with negative values for flavonoid concentration suggesting process errors or interferences, resulting in a standard deviation of 1,72, which may indicate problems in measurement or sample preparation.

"Figure 1" shows the relationship between the concentration of orange, tamarillo and onion polyphenols (in mg/L) and their average absorbance (Abb). As the concentration increases, the absorbance also increases linearly, indicating a good correlation between the two variables. The equation of the line is y = 0,0027x + 0,0876, with a coefficient of determination R^2 of 0,9955, suggesting a very accurate fit of the model to the data. This implies that the concentration of polyphenols in these extracts is proportional to the measured absorbance.

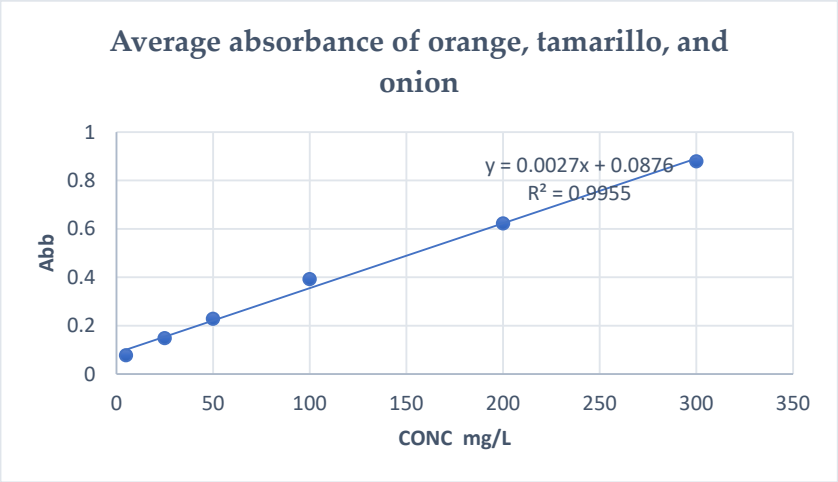

Figure 1. Average absorbance of orange, tamarillo, and onion.

"Figure 2" shows the calibration curve for cocoa, where the linear relationship between concentration and absorbance is observed. As the concentration increases, the absorbance also increases proportionally, as indicated by the equation of the line y = 0,0116x + 0,0069. The coefficient of determination R^2 = 0,9945 reflects a very strong fit, meaning that the model explains almost all of the variability in the data. This suggests a direct and reliable relationship between cocoa concentration and measured absorbance.

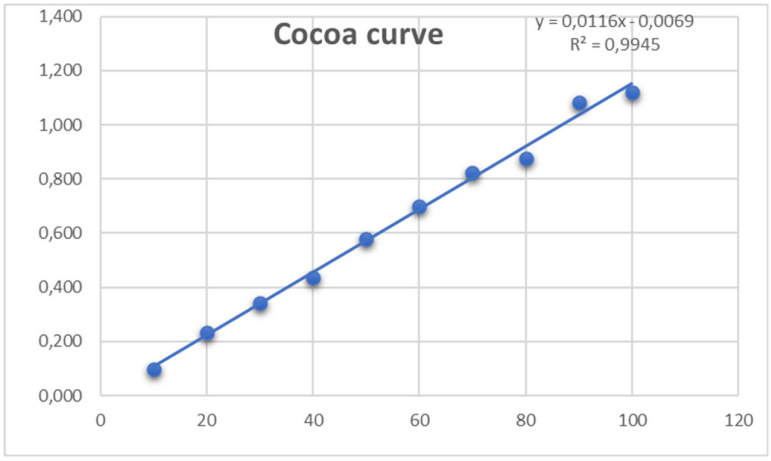

Figure 2. Cocoa Curve.

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Isolation, multiplication and DNA extraction of the yeast *Saccharomyces cerevisiae*

PDA medium was prepared by dissolving 39 g/L of powder in distilled water, autoclaved and poured onto sterile plates. Once solidified, it was inoculated with Saccharomyces cerevisiae and incubated at 30 °C for 48 hours. The yeast culture was prepared under controlled conditions of temperature and agitation, and samples were taken at specific intervals. Then, an aliquot of each sample was placed in a spectrophotometer cuvette, measuring absorbance at 600 nm to estimate cell concentration. The spectrophotometer calculated the optical density based on the amount of light that passed through the sample. For DNA extraction, *Saccharomyces cerevisiae* colonies were taken, lysed with 0,1 M NaOH at 50 °C, and the solution was neutralized. The mixture was centrifuged to obtain DNA in the supernatant, which was then precipitated with ethanol or isopropanol and washed with 70 % ethanol. DNA was prepared for PCR using specific primers and verified by agarose gel electrophoresis. Positive PCR products were sent to the laboratory for sequencing.

"Figure 3" shows the lag phase (0-2 hours), the optical density (OD) is low at the beginning (0,05) and slowly increases to 0,25, indicating that *Saccharomyces cerevisiae* cells are adapting to the new medium and starting to divide. During the exponential phase (2-8 hours), OD increases rapidly, reaching 1,00 at 8 hours, reflecting a high cell growth rate with a steep slope in the graph. In the stationary phase (8-12 hours), OD stabilizes, slowly increasing to 1,10, signaling that the culture has reached the stationary phase; here, cell growth is balanced by cell death due to medium saturation or debris accumulation.

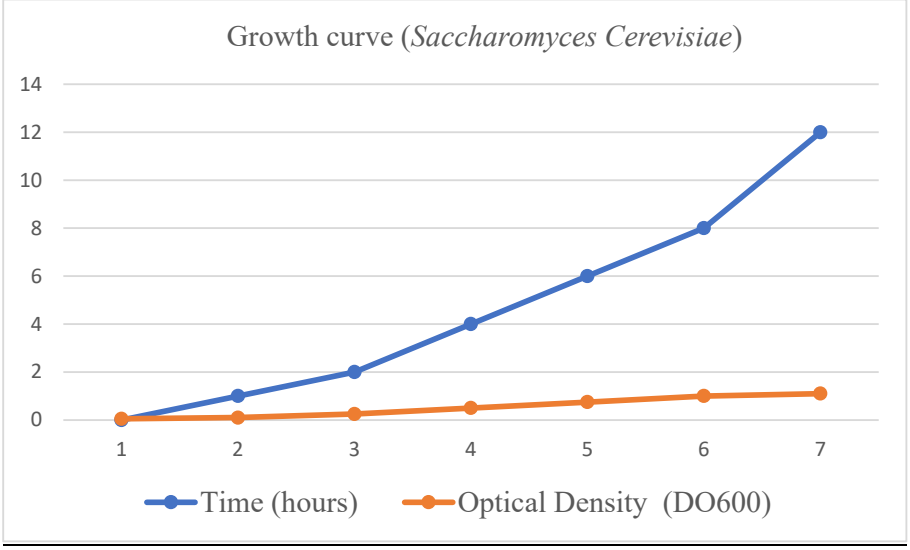

Figure 3. Growth curve (*Saccharomyces Cerevisiae*).

Isolation, multiplication and DNA extraction of the yeast *Listeria Monocytogenes*

TSA medium was prepared by dissolving the powder in distilled water (30 g/L) and sterilizing at 121 °C for 15-20 minutes, then poured onto sterile plates and allowed to solidify. For inoculation, a sample of *Listeria monocytogenes* was transferred to TSA plates, which were incubated at 37 °C for 24-48 hours. An aliquot of each sample was then placed in the cuvette of a spectrophotometer, measuring absorbance at 600 nm to estimate cell concentration. The spectrophotometer calculated the optical density based on the amount of light passing through the sample. After growth, DNA extractions were performed using NaOH for cell lysis, followed by neutralization and precipitation with ethanol. Finally, a PCR reaction was prepared with specific primers, DNA was amplified and the products were analyzed by gel electrophoresis and sent for sequencing to obtain the sequence of the amplified DNA.

"Figure 4" shows that during the Lag phase (0-2 hours), the optical density (OD) starts low (0,04), indicating that Listeria monocytogenes is adapting to the new medium. In this period, the OD slowly

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increases to 0,20, suggesting an adjustment and the beginning of cell division. In the Exponential phase (2-8 hours), the OD increases more rapidly, reaching 0,95 at 8 hours, reflecting accelerated growth of the bacterial population. In the Stationary phase (8-12 hours), OD stabilizes and slowly increases to 1,05, indicating that cell growth is balanced by cell death and that nutrients are beginning to be depleted, resulting in a slowdown of growth. DNA and products were analyzed by gel electrophoresis and sent for sequencing to obtain the amplified DNA sequence.

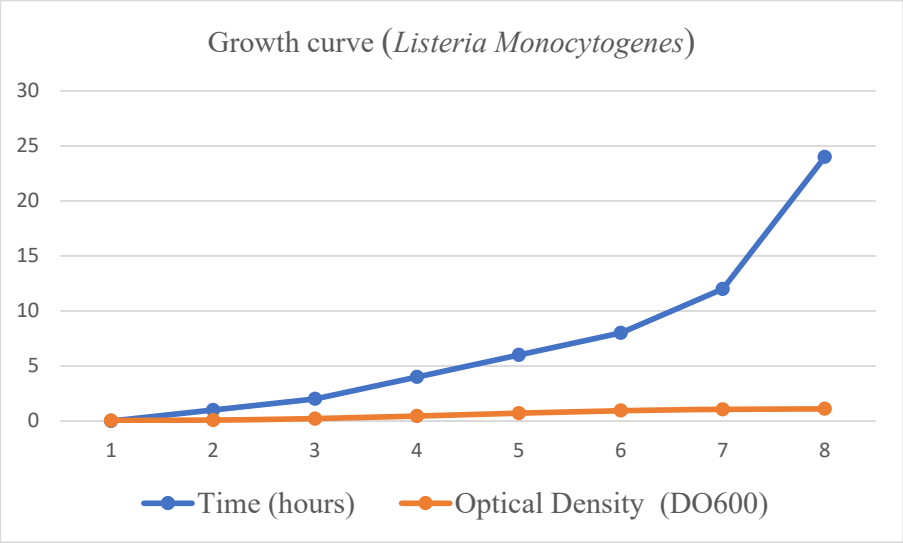

Figure 4. Growth curve (*Listeria Monocytogenes*).

"Table 13" shows that orange extract (A) demonstrates the greatest reduction in the number of yeast colonies at concentrations of 200 µg/mL and 300 µg/mL, indicating its high capacity to inhibit the growth of Listeria monocytogenes. Cocoa extract (C) also shows a significant reduction in the number of colonies at concentrations of 300 µg/mL and 400 µg/mL, although it does not reach the full efficacy of orange extract. Onion extract (B), despite showing reduction in colony numbers, is not as effective as orange and cocoa extracts. Finally, Tamarillo extract (D) shows a lower reduction compared to orange and cocoa extracts, especially at lower concentrations.

Table 13. Number of Yeast Colonies and Listeria monocytogenes Growth.

Extract	Concentration (µg/ml)	Replica 1	Replica 2	Replica 3	Colony Average
Orange	200	2	2	1	1,67
Orange	300	0	0	0	0,00
Orange	400	0	0	0	0,00
Onion	200	4	3	3	3,33
Onion	300	2	1	2	1,67
Onion	400	0	0	0	0,00
Cocoa	200	3	3	2	2,67
Cocoa	300	1	1	1	1,00
Cocoa	400	0	0	0	0,00
Tamarillo	200	5	4	5	4,67
Tamarillo	300	3	2	3	2,67
Tamarillo	400	1	1	1	1,00

"Table 14" shows that the orange extract has an MIC of 300 µg/mL, indicating that this concentration is sufficient to completely inhibit the growth of Listeria monocytogenes. Onion, cocoa and Tamarillo extracts have an MIC of 400 µg/mL, showing that this concentration is necessary to

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achieve complete inhibition of bacterial growth. Among them, the Tamarillo extract is the least effective, requiring the same concentration as the other three extracts but not exceeding their efficacy.

Table 14. Number of Yeast Colonies and Listeria monocytogenes Growth.

Extract	Minimum Inhibitory Concentration (MIC) (µg/mL)
Orange	300
Onion	400
Cocoa	400
Tamarillo	400

"Figure 3" shows that the orange extract has an MIC of 300 µg/mL, indicating that this concentration is sufficient to completely inhibit the growth of *Listeria monocytogenes*. Onion, cocoa and Tamarillo extracts have an MIC of 400 µg/mL, showing that this concentration is necessary to achieve complete inhibition of bacterial growth. Among them, the Tamarillo extract is the least effective, requiring the same concentration as the other three extracts but not exceeding their efficacy.

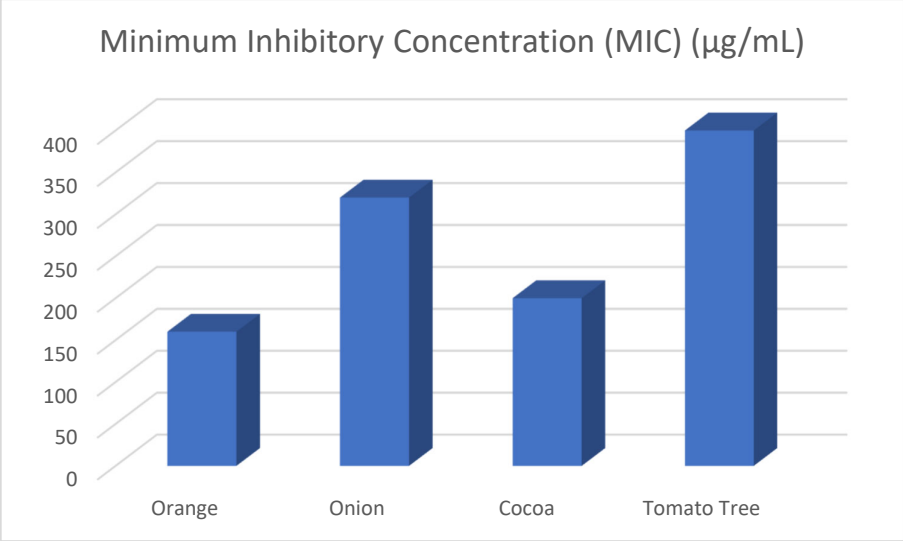

Figure 3. Concentration is sufficient to completely inhibit the growth of *Listeria monocytogenes*.

4. Discussion

The results obtained in our research show significant variability in the weight reduction of fruit and seed peels after dehydration. For example, orange and cocoa peel presented a weight reduction of 32 % and 34 %, respectively. These values are in line with those reported by other studies such as those of Stechina et al. (2017) [23], who found similar reductions in orange peels when using similar dehydration methods. However, onion peel and Tamarillo showed reductions of 13 % and 14 %, respectively, which are lower compared to other studies such as Tinoco et al. (2010) [24], who reported higher reductions of 20 % to 25 % in onion peels using more advanced dehydration techniques.

Regarding flavonoid extraction, the values obtained for total flavonoid concentration in orange (300,75 – 372,60 mg Q/L) and onion (205,70 – 534,96 mg Q/L) peels are consistent with previous studies such as Aguilar et al. (2015) [25], who found similar ranges in citrus and onion peels. However, our results for Tamarillo show very low or even negative concentrations, which differs markedly from the findings of Vega et al. (2021) [26], who reported significant concentrations in

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Tamarillo extracts. This could indicate problems in the extraction method or variability in the quality of the plant material used.

Evaluation of Saccharomyces cerevisiae and Listeria monocytogenes growth on PDA and TSA media shows growth phases consistent with existing literature, as described in the study by Pilco et al. (2023) [27]. The observed lag, exponential and stationary phase agrees with typical growth patterns described for these microorganisms. However, the results of inhibition of Listeria monocytogenes growth by plant extracts reveal that both cocoa and orange peel have a significant inhibitory effect at concentrations of 300 and 400 µg/mL, which is in agreement with the results of Jones et al. (2013) [28] who obtained 290 and 420 µg/mL. However, onion extract showed lower efficacy compared to previous studies, such as Tönz et al. (2024) [29], who documented a more pronounced inhibition with onion extracts.

Despite the consistency in the antimicrobial activity of the cocoa and orange peel extracts, the onion extract showed lower efficacy compared to the study by Álvarez et al. (2021) [30]. In their research, onion extracts showed more prominent inhibition, suggesting that our extract preparation or the extraction technique used might have affected its antimicrobial potency.

Furthermore, our results for Tamarillo peel in terms of antimicrobial activity do not agree with those reported by Tam et al. (2021) [31], who found considerable inhibition in Tamarillo extracts. This discrepancy could be due to variations in the quality of the plant material or differences in extraction and analysis methods.

5. Conclusions

The present study concluded that dehydration and flavonoid extraction of orange, onion, Tamarillo and cocoa peels showed significant variations in flavonoid concentration and inhibition efficacy against Listeria Monocytogenes. Orange peel showed the highest concentration of flavonoids, between 300,75 and 372,60 mg Q/L, and presented a minimum inhibitory concentration (MIC) of 300 µg/mL, sufficient to completely inhibit the growth of Listeria monocytogenes. On the other hand, onion, cocoa and Tamarillo extracts have an MIC of 400 µg/mL, indicating that this concentration is necessary to achieve complete inhibition of bacterial growth. Among them, the Tamarillo extract is the least effective, requiring the same concentration as the other three extracts but not exceeding their efficacy. These results suggest that orange peel is the most promising for Listeria inhibition in combination with Saccharomyces in fruit juices, thanks to its high concentration of flavonoids and lower MIC, thus offering greater protection against Listeria contamination in liquid products.

Author Contributions: Conceptualization, Brayan Torres; methodology, Enrique Salazar; software, Fernando Cobos; validation, Aurelio Amaiquema; formal analysis, Enrique Salazar; research, Aurelio Amaiquema; resources, Brayan Torres, Brayan Torres and Fernando Cobos; formal analysis, Enrique Salazar; research, Aurelio Amaiquema; resources, Brayan Torres; data curation, Brayan Torres; original drafting, Fernando Cobos; writing, revising and editing, Enrique Salazar; visualization, Aurelio Amaiquema; supervision, Enrique Salazar; project administration, Fernando Cobos; obtaining funding; Institutional.

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Conflicts of Interest: No conflict of interest.

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