

Obtaining pectinases by solid-state fermentation with different carbon sources

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ABSTRACT

Introduction: The use of agroindustrial waste, such as fruit peels, in the production of wines and ciders constitutes an innovative strategy to reduce food waste and promote the circular economy.

Objective: To obtain pectinases through solid-state fermentation using different carbon sources, with the aim of applying them as clarifying agents in the liquor industry.

Materials and Methods: A completely randomized block design (CRBD) with an ABC factorial arrangement was used, where factor A corresponded to the carbon sources (passion fruit, pineapple, and orange peels), factor B to the type of cider (orange and plum), and factor C to the enzyme concentrations (2% and 4%). Data were analyzed using ANOVA with a significance level of $p < 0.05$, evaluating physico-chemical parameters such as pH, acidity, °Brix, absorbance, and turbidity.

Results: Values ranged from pH (2.60–3.93), acidity (0.54–0.62%), °Brix (6.75–7.03), absorbance (0.60–1.82), and turbidity (19.60–121.65). Passion fruit peel exhibited the highest pectin esterase activity (22,000 U/mL) and the greatest absorbance reduction (2.45 to 2.25), achieving an overall viscosity decrease of 93.92%, demonstrating its high efficacy

as a clarifier. Microbiological analyses showed that mold and yeast counts remained within established limits, and no mesophilic aerobes, total coliforms, or enterobacteria were detected, ensuring the safety of the final product.

Conclusion: Passion fruit peel stood out as the best carbon source due to its high enzymatic activity and clarification efficiency, demonstrating the potential of agroindustrial waste to generate added value and promote sustainable processes in the liquor industry.

KEYWORDS

Clarification, waste, tropical fruits, cider.

INTRODUCTION

In Ecuador, the agroindustrial sector plays a fundamental role in economic development and improving the population's quality of life. However, the increasing demand for food and the need to diversify the product offering drive industrial processes that, in turn, generate a significant increase in waste production¹. According to the Food and Agriculture Organization of the United Nations (FAO), approximately 30% of the food produced worldwide is wasted throughout the supply chain, from production to consumption².

This figure not only represents a significant loss of natural, economic, and human resources, but also generates considerable environmental impacts, such as greenhouse gas emissions and water waste³. Thus, food waste, which in many cases still retains nutritional and functional value, is why the

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idea of a second chance was born, converting underutilized raw material with great potential to be used in new food or industrial applications, thus promoting more sustainable and circular production models⁴.

Due to this, research into the use of pectinases or pectinolytics was born, being an enzyme of great industrial importance, which is naturally produced by the peels of fruits and vegetables or in turn by microorganisms such as bacteria, yeasts and fungi, the latter being the most used and among them stand out fungal strains such as *Aspergillus niger* and *Aspergillus awamori*⁵. It is also emphasized that the enzyme pectinase degrades the plant polysaccharides present in the primary cell wall of plant cells, obtaining an increase in yield and a reduction in turbidity and viscosity in both juices and liquors⁶.

The selection of the appropriate substrate for solid-state fermentation is important, since its composition in sugars, fiber and pectin directly influences the production yield of pectinase⁷. Recent studies have shown that the use of fruit peels as a substrate in solid-state fermentation (SSF) processes has become popular due to their high content of organic compounds such as sugars, fiber, cellulose and pectin⁸. Furthermore, these peels have high availability and represent a common waste in the agri-food industries⁹. Among the different peels generated by the agroindustry, passion fruit, pineapple and orange peels stand out for their high biotechnological potential.

Passion fruit peel (*Passiflora edulis*) is one of the most representative residues of the tropical fruit agroindustry, this by-product can constitute up to 50% of the total weight of the fruit and has a high content of dietary fiber, soluble sugars and pectins¹⁰. It also contains bioactive compounds such as polyphenols, flavonoids and vitamin C that, although not directly involved in enzyme production, can promote microbial growth by acting as metabolic cofactors^{11,12}. Several studies have reported the use of passion fruit peel in fermentations to obtain pectinases due to its high proportion of pectin¹³.

Another waste obtained within the food industry is the pineapple peel (*Ananas comosus*) this residue, in addition to containing cellulose and hemicellulose, is rich in fermentable sugars such as glucose, fructose and sucrose, which makes it ideal as a source of assimilable carbon for enzyme-producing microorganisms¹⁴. Furthermore, its seasonal availability and low cost make this residue a viable alternative for the sustainable production of enzymes for industrial processes¹⁵. On the other hand, orange peel (*Citrus sinensis*) is known for its high pectin content, which gives it particular value in biotechnological processes aimed at the production of pectinases (Ramos-Alvarado et al. 2020).

On the other hand, the production of crystalline alcoholic beverages such as wines, piscos and fruit liqueurs requires clarification processes to eliminate pectins, polymers present in the

cell walls of fruits that generate unwanted turbidity¹⁶. The use of pectinases obtained by solid-state fermentation constitutes an effective and economical alternative, also reducing the need for chemical additives. The application of these enzymes allows obtaining beverages with improved physicochemical and sensory properties and stability during storage⁵.

Incorporating the use of residues such as passion fruit, pineapple, and orange peels into the production of pectinases for the liquor industry not only represents an opportunity for technological innovation but also a step toward meeting environmental sustainability goals. Therefore, the objective of this research was to obtain pectinases by solid-state fermentation, using different carbon sources for application as a clarifier in the liquor industry.

MATERIALS AND METHODS

This research was developed in the Industrial Biotechnology, Bromatology and Biosciences

laboratories of the IASA II Campus of the University of the Armed Forces "ESPE", located in the Luz de América parish, Hacienda Zoila Luz, via Quevedo, km 24. This project was carried out under an experimental approach, which included the activation of the fungus *Aspergillus oryzae* and its subsequent solid-state fermentation, as well as the performance of physicochemical and microbiological tests.

Experimental Design

For the evaluation of pectinases derived from solid-state fermentations using various carbon sources from agro-industrial waste (peels) and their application at different concentrations, a completely randomized block design (CRBD) was used with an A*B*C factorial arrangement, where factor A = Carbon sources (passion fruit, pineapple, and orange peel), factor B = Type of cider (orange and plum cider), and factor C = Concentrations (2% and 4%). In the present investigation, an ANOVA ($p < 0.05$) was applied. Statistical processing of the data and the generation of graphics were performed with the Statgraphics program.

Experimental management

Preparation of cider or wine-type drinks

To produce alcoholic beverages such as cider or fruit wine, fresh fruits were used, previously washed and disinfected to ensure aseptic conditions. From these fruits, 600 ml of plum and orange juice were extracted, initially recording the Brix and pH values. The juices obtained were pasteurized (DARITECH) at 70 °C, while 30 mL of each extract was separately set aside at 30 °C for the activation of commercial yeast (*Saccharomyces cerevisiae*), using a dose of 1 g per 1000 mL of substrate, leaving it to stand for 15 to 20 minutes to ensure its activation. Subsequently, once the pasteurized ex-

Table 1. Treatments for obtaining pectinases from solid-state fermentation (SSF)

Treatments	Description
T1	Passion fruit + orange + 2%
T2	Passion fruit + orange + 4%
T3	Passion fruit + plum + 2%
T4	Passion fruit + plum + 4%
T5	Pineapple + orange + 2%
T6	Pineapple + orange + 4%
T7	Pineapple + plum + 2%
T8	Pineapple + plum + 4%
T9	Orange + orange + 2%
T10	Orange + orange + 4%
T11	Orange + plum + 2%
T12	Orange + plum + 4%

tracts reached a temperature of approximately 40 °C, sugar was added until a soluble solids concentration of 19 °Brix was obtained, a favorable condition for alcoholic fermentation. Finally, the activated yeast was incorporated into the total volume of the extracts and the mixture was transferred to a sealed bioreactor to promote anaerobic conditions. The fermentation carried out over a period of 19 days, after which the ciders or fruit wines were filtered and packaged in glass bottles for subsequent storage and analysis.

Obtaining pectinase

For the conditioning of the pectinase-producing microorganism, *Aspergillus oryzae* was used, previously activated by preparing an inoculum in potato dextrose agar (PDA) medium. The culture medium was prepared using 3.9 g of potato dextrose agar dissolved in 100 ml of distilled water, followed by sterilization. Subsequently, the medium was dispensed in sterile Petri dishes and inoculated with *A. oryzae* spores, incubated for 48 h at 37 °C, the process continued for an additional 72 h at room temperature to promote optimal mycelial development and sporulation. Once fungal growth was obtained, the spores were resuspended in 0.9% sterile saline solution, using a ratio of 100 ml per 10 g of spores. The resulting suspension was homogenized and the appropriate inoculum concentration was determined, establishing it at 1×10^6 spores/mL, an amount that was used for the inoculation of the substrate in the solid-state fermentation¹⁷.

Obtaining pectinase enzymes by fermentation on solid substrate (SSF)

Orange, pineapple, and passion fruit peels were used as substrates, selecting only those in good condition, free of signs of rot or contamination. The samples were washed with distilled water, dried (orange: 25 °C/24 h; pineapple and passion fruit: 28 °C/42 h), weighed (100 g per treatment), and packaged in sterile glass jars. Sterilization was carried out in an autoclave for 25 min at 121 °C. Inoculation was carried out with *Aspergillus oryzae* (1×10^6 spores/mL) using 37.5 mL of suspension per treatment, incubating for 4 days at temperatures between 25–28 °C. A second inoculation was performed with the same volume to optimize enzyme production, extending the fermentation to a total of 7 days. The enzyme extract was obtained by washing with saline solution (0.9%), vacuum filtration, and centrifugation (2000 rpm/15 min). Purification was carried out by selective precipitation with sodium hydrogen phosphate (4.5%) and calcium chloride (5%), generating a gel that allowed the removal of contaminating proteins. The purified supernatant was concentrated by ethanol precipitation (3:1 v/v) and stored for 12–24 h at 4 °C. Finally, the precipitate was lyophilized to evaluate its capacity as a clarifying agent.

Evaluation of the physicochemical parameters of ciders with pectinases

The physicochemical characterization of the cider samples was carried out following standardized procedures. Regarding the pH determination, it was carried out using a potentiometer previously calibrated with buffer solutions of pH 4 and 7, in accordance with the provisions of the Ecuadorian Technical Standard NTE INEN 2 325 (2002). For the analysis of titratable acidity, 5 ml of each sample was used with 0.02 N NaOH using phenolphthalein as an indicator, until reaching the pale pink turn, and the acidity expressed as acetic acid was calculated using the official formula established following the methodology described in the Ecuadorian Technical Standard NTE INEN 374 (2016)¹⁸. On the other hand, the obtaining of total soluble solids (°Brix) was carried out using a digital refractometer calibrated with distilled water, following the methodology recommended by the Ecuadorian Technical Standard NTE INEN 380 (1985)¹⁹. Determination of soluble solids. For absorbance measurements, a spectrophotometer set to a wavelength of 530 nm was used to evaluate phenolic compounds and color stability in fermented beverages²⁰. Finally, turbidity was determined using 20 ml of sample in a previously calibrated turbidimeter, in accordance with the international standard ISO 7027-1:2016²¹.

Determination of the enzymatic activity of pectinases

Pectin esterase activity

The sodium hydroxide (NaOH) titration method was used. 4 mL of the lyophilized 10% enzyme extract (pectinase) were

placed in a 20 ml solution of 1% citrus pectin, previously dissolved in 0.15 M NaCl. The samples were incubated for 72 hours at 25 °C. After this time, the samples were titrated with 0.02 N NaOH²². Pectin esterase activity was quantified using the following equation:

$$APE = \frac{(V_S - V_b)(Normalidad\ de\ NaOH)}{V_t}$$

Where:

V_S = Volume of NaOH consumed per sample titration [mL].

V_b = Volume of NaOH consumed per titration of the blank [mL].

V_t = Volume of sample used [mL].

Pectate lyase activity

It was evaluated by the increase in absorbance between 225 and 232 nm. 4 mL of the 10% lyophilized enzyme extract were mixed with 2.5 ml of 0.5% citrus pectin dissolved in 50 mM Tris-HCl buffer and 1 mM CaCl₂. The samples were incubated for 72 hours at 25 °C, using the uninoculated lyophilized precipitate as a control. An increase of 0.1 absorbance units per minute at 232 nm in 1 mL corresponds to one unit of enzyme activity²³. The activity was calculated using the following equation:

$$APL = M_1 - M_0 \text{ Ecu2}$$

Where:

M_1 = Abs of the incubated sample [A].

M_0 = Abs of the control sample [A].

Endo activity polymethyl galactose

The sampling solutions contained 10 mL of 2% pectin, 2 mL of Acetate Buffer pH 4.5 and 5 ml of 10% lyophilized pectinase solution, which were incubated at 25°C for 72 hours. The viscosity of the following samples was determined: Blank; Pectin solution without pectinase, Control; Pectin solution with pectinase without incubation and Test: Pectin solution with incubated pectinase²². The measurements were performed using rotor #6. Polymethylgalacturonase activity was determined using the following equation:

$$RV (\%) = \frac{(V_b - V_m)}{V_b} \times 100$$

Where:

V_b = Viscosity of the target [Cp].

V_m = Viscosity of the incubated sample [Cp].

Microbiological analysis of ciders enzymatically treated with pectinases

Inside the laminar flow chamber, 2 ml of the best treatment previously established by physicochemical analysis were ex-

tracted and serial dilutions (1:10) in peptone water were made using a micropipette. Cultures were performed on Petrifilm TM plates to count enterobacteria, aerobes, coliforms, molds and yeasts, taking small aliquots of the diluted samples and transferring them to the Petrifilm sheets. It was ensured that the samples were evenly distributed after gentle pressure on their exterior. Finally, the Petrifilm of enterobacteria, aerobes and coliforms were incubated in the oven for 48 hours at 37 °C, while the Petrifilm of molds and yeast were incubated at room temperature for 72 hours²⁴,

RESULTS

The pH values obtained in the different treatments show a clear influence of the three factors evaluated (carbon source, type of cider and concentration), evidencing significant differences ($p < 0.05$) between some groups, as can be seen in Table 2. T6 (Pineapple + orange + 4%) with 3.93 presented a higher content, demonstrating a lower acidification of the system, attributed to the combination of fruits with lower acid content and higher sugar content. Unlike T12 (Orange + plum + 4%) with 2.60 obtained a lower content, indicating a higher production of organic acids during the fermentation process, favored by the presence of plum, a fruit that promoted acidification in all its combinations.

Regarding acidity content, significant differences were observed between treatments, although these were relatively narrow. Thus demonstrating that treatments T3 (0.62), T4 (0.61), T7 (0.62±0.01), T8 (0.62), T11 (0.62) and T12 (0.60) recorded the highest values; it is emphasized that these treatments included plum cider in their formulations, reaffirming the higher degree of fermentation and production of organic acids. On the other hand, the lowest acidity value (0.54) was obtained by T10 (Orange + orange + 4%), associated with the treatments with the lowest general acidification.

Regarding the °Brix content, T8 (7.03) showed the most representative value, corresponding to the combination of Pineapple + plum + 4%. This result is attributed to a higher contribution of initial sugars or lower consumption of these during fermentation. Meanwhile, the other treatments remained relatively stable, oscillating between 6.75 and 6.90 °Brix, reflecting a slight variation in the content of residual sugars.

Values peaked at T3 (1.82), associated with passion fruit + plum + 2%. This indicates a higher presence of colored or cloudy compounds in this treatment, likely derived from both the fermentation process and the characteristics of the fruits used, such as plum, known for its phenolic pigment content. T2 (0.51) showed the lowest absorbance, indicating greater clarification or a lower presence of pigments and suspended solids.

On the other hand, turbidity contents were recorded in T8 (121.65), with Pineapple + Plum + 4% as the most representa-

Table 2. Physicochemical results of the study of pectinases obtained from three different substrates when interacting with two wines and two concentrations, in wine clarification

Treatment	pH	Acidity	°Brix	Absorbance	Turbidity
T1	3.90±0.03 ^{bc}	0.55±0.02 ^{sd}	6.85±0.10 ^a	0.64±0.05 ^a	19.60±1.2 ^c
T2	3.86±0.02 ^{bc}	0.57±0.02 ^{be}	6.85±0.08 ^a	0.51±0.05 ^c	20.30±1.1 ^c
T3	2.70±0.04 ^a	0.62±0.02 ^a	6.90±0.02 ^{ab}	1.82±0.01 ^d	117.95±2.5 ^a
T4	2.71±0.03 ^a	0.61±0.02 ^a	6.85±0.10 ^a	1.53±0.02 ^b	119.29±2.3 ^{ab}
T5	3.81±0.02 ^b	0.58±0.01 ^{bc}	6.90±0.02 ^{ab}	0.63±0.05 ^a	35.85±1.5 ^d
T6	3.93±0.03 ^c	0.58±0.03 ^{bc}	6.83±0.06 ^a	0.60±0.03 ^{ac}	36.50±1.6 ^d
T7	2.66±0.02 ^a	0.62±0.01 ^a	6.80±0.09 ^a	1.54±0.06 ^b	119.75±2.4 ^{ab}
T8	2.70±0.02 ^a	0.62±0.04 ^a	7.03±0.02 ^b	1.50±0.07 ^b	121.65±2.8 ^b
T9	3.81±0.03 ^b	0.58±0.03 ^{bc}	6.75±0.05 ^a	0.65±0.05 ^a	22.15±1.3 ^c
T10	3.79±0.02 ^b	0.54±0.01 ^d	6.80±0.02 ^a	0.61±0.04 ^{ac}	22.40±1.4 ^c
T11	2.61±0.03 ^a	0.62±0.02 ^a	6.90±0.01 ^{ab}	1.54±0.07 ^b	119.35±2.3 ^{ab}
T12	2.60±0.01 ^a	0.60±0.02 ^{ac}	6.87±0.02 ^{ab}	1.54±0.06 ^b	119.92±2.5 ^{ab}

tive value. It is worth mentioning that fermentation can disrupt the cellular matrix of fruits, facilitating the release of these components and increasing turbidity. Unlike T1 (19.60), which consisted of combinations with passion fruit + orange + 2%, this reflects greater visual stability and possibly better clarification.

Enzymatic activity tests for pectinase enzymes

Figure 1 shows the average pectin esterase activity obtained from different carbon sources used in solid-state fermentation. The results indicate that passion fruit peel produced the highest enzyme activity, with a value of 22,000 U/mL, followed by orange with 19,000 U/mL, while pineapple had the lowest production (7,000 U/mL). Therefore, it is demonstrated that the passion fruit substrate constitutes a more favorable carbon source for the development of pectinase-producing microorganisms.

Regarding the Pecto Lyase Activity values (Figure 2) obtained in the control and after 72 h of fermentation, it is observed that in all cases the absorbance values decreased after the fermentation process, demonstrating a clarifying effect of the enzymatic treatment. The greatest difference between the control and the 72-hour period was recorded for the passion fruit carbon source, increasing from approximately 2.45 to 2.25, indicating greater efficiency in the degradation of pectins or compounds responsible for turbidity. In the case of pineapple, the values were the lowest, both in the control and after fermentation (2.20 - 2.12), while orange showed an intermediate

trend (2.38 - 2.18). These results demonstrate the potential of the fermentation system used as a substrate with passion fruit.

Regarding the average viscosity values obtained with and without incubation, based on the different carbon sources used during fermentation (Figure 3), it is observed that in all treatments, viscosity decreased significantly after the incubation process with *endopolymethylgalacturonase*. A reduction in viscosity (93.92%) was observed in the incubated samples compared to the non-incubated ones

Microbiological analysis of ciders enzymatically treated with pectinases

Table 3 shows the microbiological results obtained for ciders made with 2% and 4% orange concentrations. In both

Table 3. Microbiological analysis of *Citrus sinensis* (L.) ciders

Parameters	Orange cider (2%)	Orange cider (4%)
Molds and Yeasts	10 UFC	10 UFC
Aerobic	Absence	Absence
Coliforms	Absence	Absence
Enterobacteriaceae	Absence	Absence

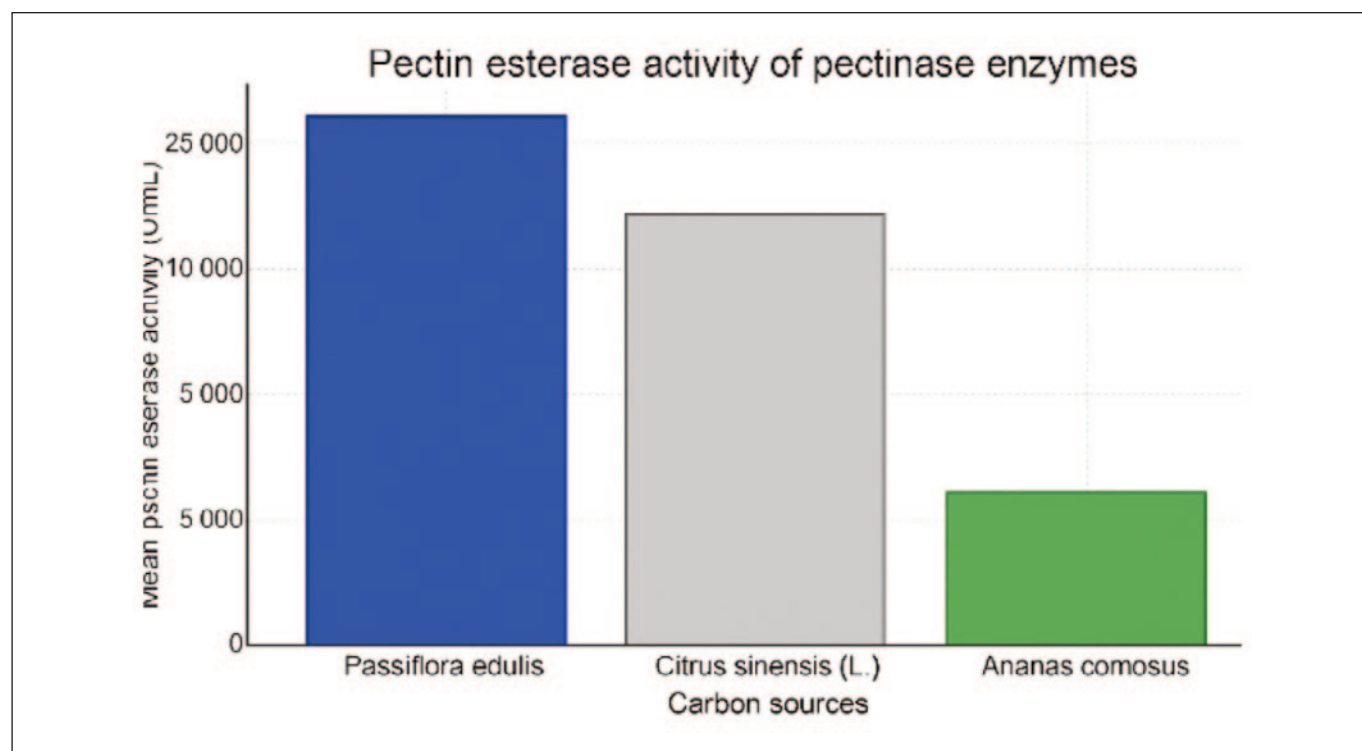


Figure 1. Pectin esterase activity of pectinase enzymes from different carbon sources

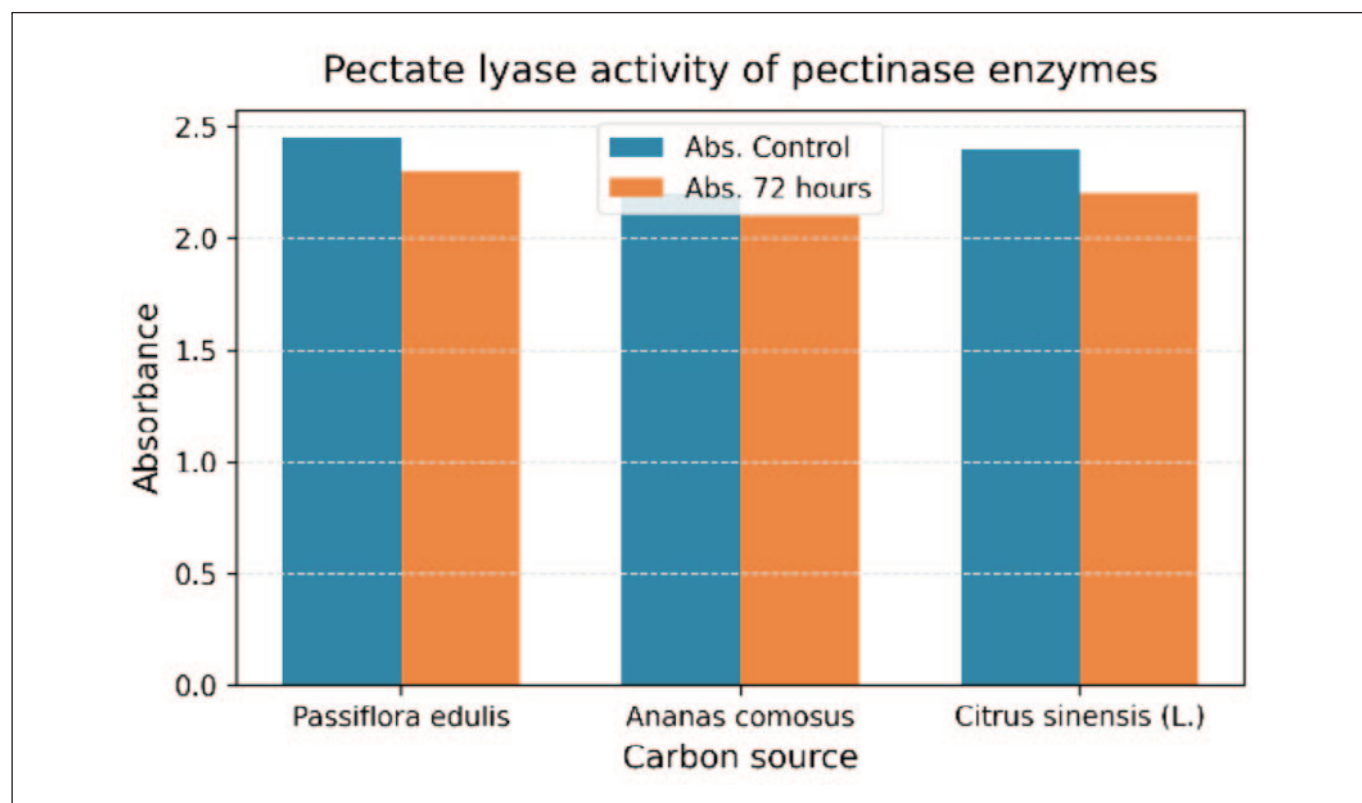


Figure 2. Pectate lyase activity of pectinase enzymes

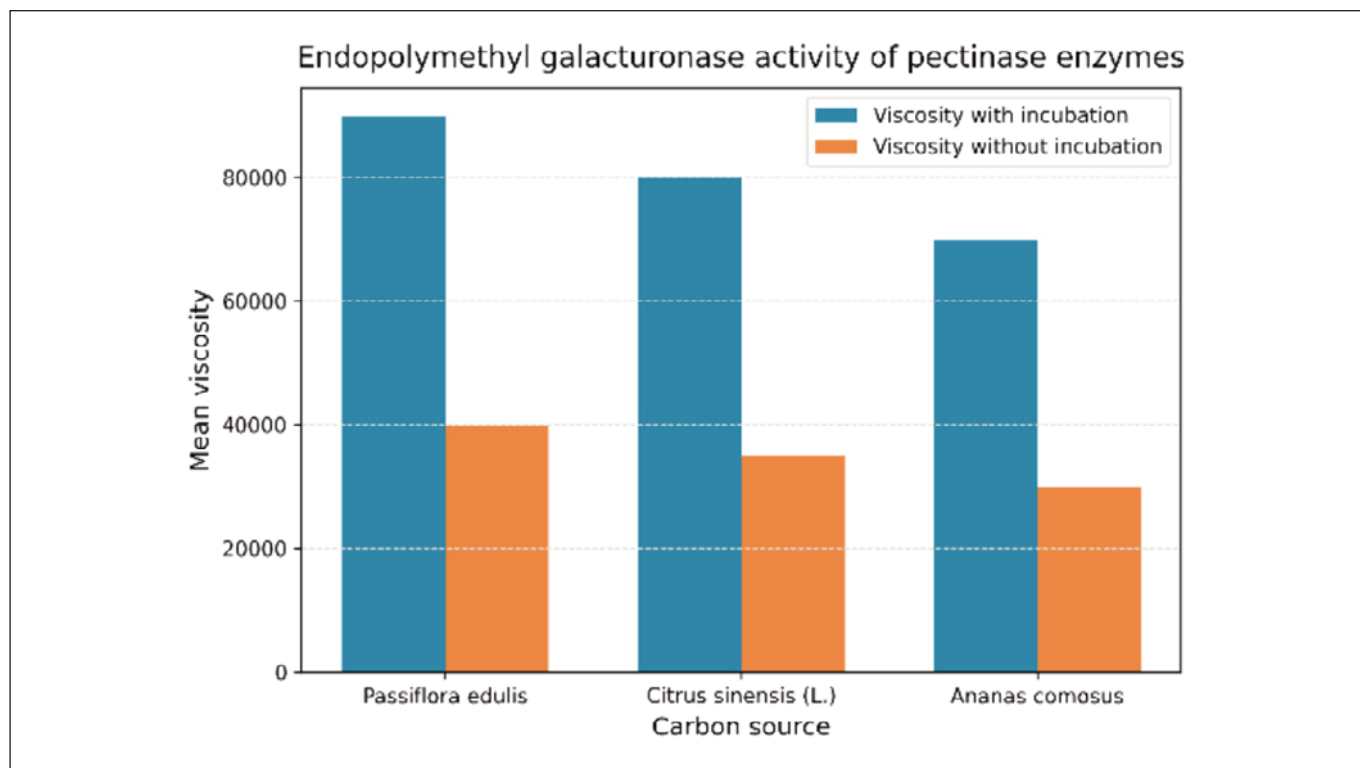


Figure 3. Endopolymethyl galacturonase activity of pectinase enzymes

treatments, the mold and yeast load was minimal, with values of 10 CFU, indicating adequate hygiene and control of the fermentation process. Furthermore, the absence of mesophilic aerobes, coliforms, and enterobacteria was recorded. These results demonstrate that the combination of raw materials and the treatment applied during the fermentation process were effective in inhibiting the growth of undesirable microorganisms, ensuring a final product that is microbiologically stable and suitable for consumption.

DISCUSSION

Physicochemical results of the study of pectinases obtained from three different substrates when interacting with two wines and two concentrations, in wine clarification

The pH values obtained in the present study show similarity with those reported in research on the extraction of pectin from orange peel waste by microwave-assisted acid hydrolysis (MHA), where pH values between 2.17 and 2.55 were observed during the extraction process¹⁷. Similarly, studies related to the extraction of pectinases from passion fruit peel reported pH ranges between 2.0 and 4.0, values that are adequate to optimize enzymatic activity¹³. In general, combinations with plum tended to generate higher acidity levels (low pH), while mixes with orange and without plum maintained higher pH values.

Regarding acidity contents, they are related to studies that evaluated the quality characteristics of apple wine in different must pretreatments, where they reported an acidity value of 0.94%²⁵. Furthermore, it is emphasized that the use of fungal pectinases, commonly used in wine and cider clarification processes, not only favors the breakdown of pectins and improves the filterability of the final product but can also influence the increase in acidity. This is due to the release of galacturonic acids during the pectin hydrolysis process¹⁶.

The soluble solids content behaviors are consistent when making pineapple and mango peel wines, reporting a value of 6.2 °Brix²⁶. Similarly, in a study conducted on the quality of apple wine subjected to different must pretreatments, soluble solids contents were recorded ranging from 5.57 to 9.47 °Brix²⁵. These results support that the °Brix levels obtained in the present study are within the expected range for fermented beverages made from fruits or agro-industrial by-products.

On the other hand, the absorbance results are similar to those obtained when making grape wine using papaya latex (*Papain*) and yausabara gel (*Pavonia sepium*) presenting values between 0.80 – 1.8²⁷. In studies carried out on rosé must, they indicate that the application of pectinase, both free and immobilized, allowed to significantly reduce the absorbance, thus confirming its effectiveness and significant reductions in the absorbance of rosé must, confirming the effectiveness of

this enzyme in the elimination of compounds responsible for turbidity²⁸.

Enzymatic activity tests for pectinase enzymes

In relation to obtaining the pectin esterase enzyme, they are comparable in research on the use of *Endopolygalacturonase* and pectin esterase enzymes from *Aspergillus Niger*. reported an activity of up to 17 U/ml, 40 U/ml and 150 U/ml. Demonstrating that the pectinase enzymes obtained disintegrate pectin into reducing sugars and also reduce intracellular adhesiveness, degree of esterification and its rigidity²². Meanwhile, when studying the pectinase enzyme in the enzymatic degradation and physical and chemical characteristics of peach bagasse pectin, they reported absorbance values between 1.46 and 5.6, comparable to those obtained in this research²⁹. It is worth mentioning that the addition of pectinase during maceration prior to alcoholic fermentation favors the release of phenolic and aromatic compounds by breaking down the cell walls of the fruit, thus improving juice yield, filterability and color intensity in wines³⁰. On the other hand, the use of the endopolymethyl enzyme galacturonase. In enzymatic processes in juices it has been shown to significantly reduce viscosity by 90%. For example, in a study with guava pulp treated with commercial pectinase, viscosity decreased from 15.90 to 1.32 mPa s in just 3 minutes, that is, a reduction greater than 90%⁸. Likewise, when producing wines, reductions of 93.92% in viscosity were observed after use, demonstrating the effectiveness of these enzymes in improving fluidity and oenological performance³⁰.

Microbiological analysis

Microbiological analyses of the treatments for molds and yeasts were within the range permitted by Ecuadorian Technical Standard 2801:2015²⁵, which establishes a value of 10 CFU. It is worth mentioning that the mold and yeast count observed in this study is positive, since the yeasts present generally correspond to those involved in the fermentation process, such as *Saccharomyces cerevisiae*, a microorganism widely used for its ability to produce ethanol and improve the sensory profile of wines²². Furthermore, the absence of aerobes, coliforms, and enterobacteria reinforces the microbiological quality of the product, avoiding risks to the consumer³⁰.

CONCLUSION

The study demonstrated that obtaining pectinases through solid-state fermentation, using fruit peels as carbon sources, represents an efficient alternative for use as a clarifying agent in the liquor industry. Among the sources evaluated, passion fruit peel proved to be the most favorable due to its high enzymatic activity and ability to improve the clarity of the final product. Furthermore, the microbiological safety of the ciders produced was confirmed, ensuring their suitability for con-

sumption. These results reinforce the potential of utilizing agro-industrial waste as a sustainable strategy for developing innovative products in the food and liquor sectors.

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