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Effect of two enzyme systems on the removal of mucilage from coffee cherry beans (*Coffea arabica* L.)

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ABSTRACT

Objective: To evaluate and compare the percentage of mucilage removal from coffee (*Coffea arabica* L.) in pulping using two enzyme systems, Celuzyme and Macerex PM.

Design/methodology/approach: Seven treatments combinations were evaluated (type of enzyme and concentration level, plus a control) at 30 min intervals for 3 h. The experimental unit was 0.2 kg of pulped coffee.

Results: Results showed when using these enzyme systems (Macerex PM and Celuzyme) the percentage of mucilage removal increased and time was significantly reduced by 3 to 4 h compared to the natural fermentation time of 15 to 20 h.

Limitations on study/implications: Effect of two enzyme systems, Macerex PM and Celuzyme, at different concentrations (mg L^{-1}).

Findings/conclusions: The Macerex PM and Celuzyme enzyme systems showed 95% and 84.5% removed mucilage compared to 35% of the control.

Keywords: Degumming process, pectinase, cellulase, hemicellulase, fermentation time.

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INTRODUCTION

The process of transforming cherries coffee into parchment coffee consists of separating the seed from the pulp. This process can be carried out by two methods; the wet method begins with collecting and sorting coffee beans at optimum ripeness for pulping, mucilage removal and drying, whereas the dry method is based on drying coffee cherries in the sun for a prolonged time (Correa *et al.*, 2014).

Currently, Mexico ranks eleventh in terms of volume of coffee production after Brazil, Vietnam, Colombia, Indonesia, Honduras, Ethiopia, India, and Uganda, with a production volume of between 3 and 4 million sacks (60 kg per sack) per year (SIAP, 2019; ICO, 2019). Countries like Colombia and Ecuador have applied pectolytic enzyme systems and specific

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products to reduce the fermentation time in degumming coffee cherries, which they have achieved. These enzyme systems contain polygalacturonase and pectinesterase. Pectinase was developed especially for the degumming of coffee beans.

Users who use wet processing in Mexico have not paid much attention to automation and/or modernization. As it is well-known, water is the main element in the wet processing method since it lubricates, transports, washes and facilitates the processing operations. The traditional fully washed technique without recycling uses 4 to 5 m^3 of water per quintal of processed coffee cherry beans (1 quintal of coffee cherry beans=250 kg) whereas modern mechanical mucilage removal machines producing semi-washed coffee use only about 0.25 to 0.5 m^3 of water per quintal of processed coffee cherry beans (Enden & Calvert, 2010).

Thus, in the state of Veracruz, processing 1.2 million quintals of coffee cherry beans demands about 6 million cubic meters of water, which is used and returned to the water bodies with a high degree of contamination of organic origin (SIAP, 2019). The objectives of this research were: 1) To evaluate and compare the percentage of mucilage removal from coffee (*Coffea arabica* L.) in pulping using two enzyme systems, Celuzyme and Macerex PM and 2) Select the best enzyme system for removing mucilage from coffee beans.

MATERIALS AND METHODS

Analyzes were conducted in the coffee section of the food analysis laboratory at the Colegio de Postgraduados Campus Córdoba, located in Amatlán, Veracruz, Mexico at 650 masl, 18° 50' NL and 96° 51' WL.

Coffee cherries (31 kg) of the variety Colombia were harvested at optimum ripeness in the municipality of Ixhuatlán del Café, Veracruz, Mexico at 1180 masl, 19° 03' 01.95" N and 96° 54' 24.45" W, in the autumn of 2015. To preserve the quality of the coffee berries, prolonged exposure to high temperatures and humidity levels before starting the pulping and fermentation of the mucilage was avoided. The pulp was removed from 31 kg of coffee berries using a mechanical pulping machine (Mod. DV 255 CM, Penagos Brand, Santander, Colombia), obtaining 13.6 kg of pulp, 17 kg of coffee beans and a 0.7 kg reduction in juice and grain.

Enzyme systems

In this study, two enzyme complexes were assessed: Macerex PM (Enmex, México) and Celuzyme (Enmex, México). Macerex PM is a standardized enzyme system containing pectinase and cellulase obtained by controlled fermentation of *Aspergillus niger* and *Trichoderma reesei*. Macerex is a product designed to maximize the extraction of juice and solids in fruit maceration or liquefaction. On the other hand, Celuzyme is an enzyme system designed for degrading cellulose and other structural polysaccharides of plant cells, and this enzyme complex is produced by controlled fermentation of a strain of *Trichoderma longribrachiantum*. It contains cellulase, hemicellulase and beta-glucanase activities.

Treatments and experimental design

Two types of enzyme systems, Celuzyme and Macerex PM, at three different concentration levels (200, 300, and 400 mg L^{-1}) and a control were evaluated (seven

treatments). The control was allowed to demucilaginate until it reached 97% mucilage removal but without reaching an alcoholic phase. The experimental unit was 0.2 kg of pulped coffee, to which the enzyme was added and mixed in with a stainless-steel spatula. A completely randomized experimental design with repeated measures, as described below, was used (Equation 1):

$$y_{ijk} = \mu + \alpha_i + rep(\alpha)_{k(i)} + \tau_j + (\alpha * \tau)_{ij} + \varepsilon_{ijk} \quad (1)$$

where y_{ijk} is the response variable observed in the treatment i; time j in the replicate k; μ is the overall mean; α_i is the effect of the treatment i; $rep(\alpha)_{k(i)}$ is the random effect of the repetition k within treatment i; τ_j is the effect of the time j; $(\alpha * \tau)_{ij}$ is the effect of the interaction between the treatment i and time j; and ε_{ijk} is the experimental error independent and identically normal distributed with mean 0 and constant variance $\sigma^2\left(\varepsilon_{ijk} IIDN\left(0,\sigma^2\right)\right)$. For data analysis, the GLIMMIX procedure of SAS (SAS, version 9.3) was used. The autoregressive is of order 1. Mean comparisons were made using Fisher's least significant difference (LSD) test ($P \leq 0.05$).

Variables evaluated

Measurements of percentage of mucilage removed, pH and temperature were taken in 20 g samples at 30 min intervals for 3 h of fermentation in each of the treatments. A mercury thermometer (Mod. 360, LAUKA Brand, Import) was used to measure temperature and a potentiometer (Conductronic, Modelo pH120) for pH.

The technique of the four rinses was used for mucilage removal (López-Blanco, 2017). To determine the amount of mucilage removed water was added to the sample and kept stirring until the mucilage was removed, this water with coffee beans was weighed and by weight difference the percentage of mucilage in the coffee sample was estimated (Equation 2):

$$\%MS = \left(\frac{W_s - W_{bwm}}{W_s}\right) * 100 \quad (2)$$

where $\%MS$ is the percentage of mucilage present in the sample; W_s is the amount in grams of the coffee beans sample; and W_{bwm} is the amount in grams of coffee beans without mucilage.

Then the amount of mucilage that was removed in each of the six sampling times was evaluated using the following formula (Equation 3):

$$\%MR_t = \left(1 - \frac{SPR_t - SWM_t}{MS_t}\right) \quad (3)$$

where $\%MR_t$ is the percentage of mucilage removed at time t; SPR_t is the weight of the coffee beans sample with mucilage partially removed at time t; SWM_t is the total weight of

the coffee beans sample without mucilage at time *t*; and MS_t is the weight of mucilage of the sample at time *t* and this was estimated with the following equation (Equation 4):

$$MS_t = MR_0 * SWM_t \quad (4)$$

where MR_0 is the initial mucilage removed, estimated using the formula (Equation 5):

$$MR_0 = \frac{SPC - SWM_0}{SWM_0} \quad (5)$$

where *SPC* is the weight in the pulped coffee sample; and SWM_0 is the coffee sample without mucilage at time zero.

Water with the sample was weighed to calculate the water expenditure and this was estimated with the following equation (Equation 6):

$$W_w = W_{w+s} - W_s \quad (6)$$

where W_w is the water weight used in mucilage removal; W_{w+s} is the weight of the water with the sample; and W_s is the weight of the sample.

RESULTS AND DISCUSSION

Removal of coffee mucilage

Analysis of variance showed that there was a highly significant difference between treatments and the interaction between treatments and time in the percentage of mucilage removed. In Figure 1, Macerex PM enzyme at 400 mg L^{-1} can be seen to have obtained the highest mucilage removal percentage (65%) followed by Celuzyme enzyme at 400 mg L^{-1}

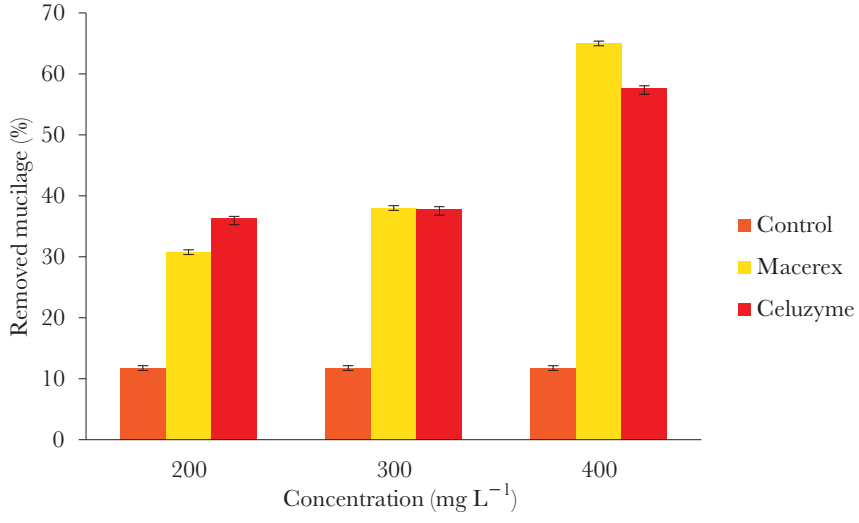

Figure 1. Average values of % of mucilage removed as a function of enzyme type and concentration (mg L^{-1}).

with 57.66% mucilage removed. Both treatments had the highest enzyme concentration compared to the rest of treatments and were statistically different from each other and with other treatments.

The use of enzymes (Macerex PM and Celuzyme) and the concentration added to the pulped coffee had a significant effect on the amount of mucilage removed relative to the control; for example, at concentrations of 200 and 300 mg L^{-1} of enzyme, average mucilage removal percentages were 22 and 26.2% higher than the control treatment (natural fermentation), respectively.

Coffee mucilage removal time

Analysis of variance results showed that there was a highly significant difference in mucilage removal time among treatments. In general, the time required to remove the largest amount of mucilage was lower in enzyme-added treatments compared to the control treatment (Figure 2). The time required to remove at least 90% of the mucilage adhering to the coffee beans under the traditional method (without enzyme) was greater than 10 h, while using 400 mg L^{-1} of the Macerex PM and Celuzyme enzymes resulted in removing 95 and 84.5% of the mucilage, respectively, in a 3 h period. Treatments with concentrations of 200 and 300 mg L^{-1} of enzymes (both enzymes) required more than 3 h to remove 80% of mucilage.

To study the mucilage removal response curve as a function of the enzyme type and concentration in the treatments, an orthogonal polynomial contrast was performed. Results indicate that the enzyme concentration has a quadratic effect on the average percentage of mucilage removed (P=0.0001). It can also be seen that for each added unit of enzyme in mg L^{-1}, the percentage of mucilage removed is higher in the Macerex than the Celuzyme enzyme (Figure 3).

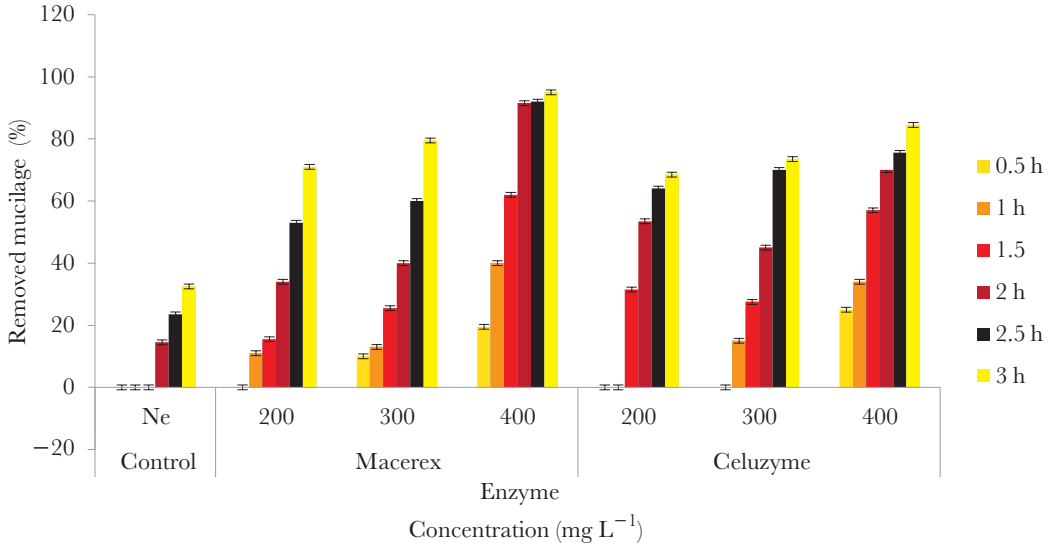

Figure 2. Average mucilage removal percentages based on enzyme type, concentration (mg L^{-1}) and time. Ne stands for no enzyme.

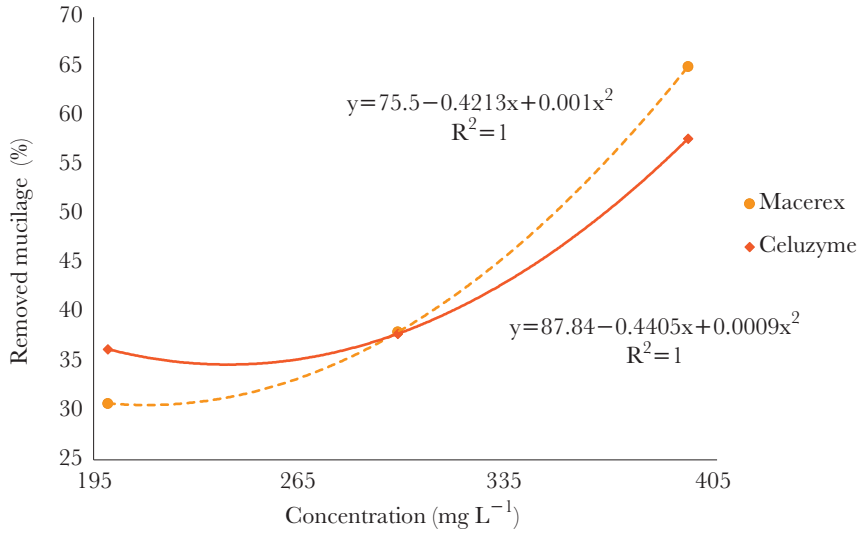

Figure 3. Mucilage removal response curve as a function of enzyme type and concentration (mg L^{-1}).

Enzymes have been used more frequently in industrial processes to accelerate juice extraction and substrate digestion processes. Macerex PM contains pectinase and cellulase, and Celuzyme contains cellulase, hemicellulase and beta-glucanase. Escalante-Minakata *et al.* (2013) showed that Macerex PM improved by 2.5 times the yield of juice extraction in banana while Glucozyme-400 had no a positive effect on the extraction of banana liquids at any stage of ripeness; thus, it seems that the effectiveness of the enzymes will depend on the chemical nature of the substrate.

In recent years the coffee agro-industry has been introducing the use of enzymes in wet processing (Peñuela-Martínez *et al.*, 2010); for example, the pectinase from *Bacillus subtilis* strain Btk27 (Oumer & Abate, 2017), and the pectinase produced by fermentation in solid state using coffee pulp with *Aspergillus niger* CFR 305 (Murthy & Naidu, 2011). It has been reported that the fresh mucilage has between 85 to 91% water and between 7.50 to 9.82% carbohydrates, the latter comprising 47.9% of reducing sugars, 29.8% of non-reducing sugars such as sucrose, 7.3% fiber and about 15.0% non-fibrous substances, such as pectic substances (Puerta-Quintero & Ríos-Arias, 2011).

The degradation speed of mucilage depends of variety coffee, for instance, coffee arabica to hydrolyze the mucilage requires more time than robusta (*Coffea canephora*) as well as depending on the inherent concentration of pectinolytic enzymes ambient temperature and pH. Murthy & Naidu (2011) reported that the duration for digestion in conventional coffee demucilage varies from 48 to 72 h depending on temperature and thickness of mucilage, while in treatments with the enzyme system in the wet fermentation process 50 and 76% pectin was degraded in about 1 and 2 h respectively depending upon the type of enzyme compared to 8% degraded pectin in about 1 h and continued up to 48 h with 100% pectin decomposition in natural fermentation for remove coffee fruit skin, mucilage and the parchment in robusta coffee.

Puerta-Quintero (2009) showed that by using pectin concentrates (enzymes), mucilage removal time (fermentation) was reduced from 20 h (traditional method) to 2 h depending

upon the type of enzyme and concentration; on the other hand, En-Sheng *et al.* (2014) found that when applying extracts of crude enzymes of *Aspergillus tubingensis* they eliminated the mucilage of cherries coffee in 3 h at 30 °C at pH 6.

Quite often the mucilage breakdown is not complete even after 72 h of fermentation. Haile & Kang (2019) mention that if the degumming time is extended, the sugars (present in coffee pulp) can degrade into acids while other enzymes that can cause deterioration to the grain are synthesized, producing a heterogeneous product and sometimes one of poor quality. Peñuela-Martínez *et al.* (2011) reported that the use of enzymes allows a greater control in the beneficiary reducing the risks of deterioration of quality due to prolonged or incomplete fermentations.

pH behavior in coffee mucilage removal

Analysis of variance results showed a highly significant difference between treatments, time, and the interaction between both treatment factors on pH (P=0.0001). A significant statistical difference was found in the average pH level between the enzyme-added treatments and the control. However, treatments with the same enzyme concentration level were not statistically different. In general, a decrease in pH over time was observed in all treatments and these average pH levels over time were higher in the control treatment compared to the treatments with enzymes added (Figure 4).

According to the literature, during coffee fermentation, the pH of the substrate decreases in the first 20 h due to the formation and dissociation of acids, mainly due to the effect of lactic acid (Puerta-Quintero, 2012). Considering the above, the results in this study demonstrate an accelerated decrease in pH in treatments with enzymatic fermentations, reaching below 4.5 in just 3 h, so an important effect of pH on the degradation of the mucilage in enzyme treatments. These results agree with those obtained by Puerta-Quintero & Ríos-Arias (2011).

Temperature during mucilage removal

The temperature of the cherry coffee beans varied between 22.6 and 25 °C during mucilage removal. In Figure 5 we can see that the treatments with the Celuzyme enzyme

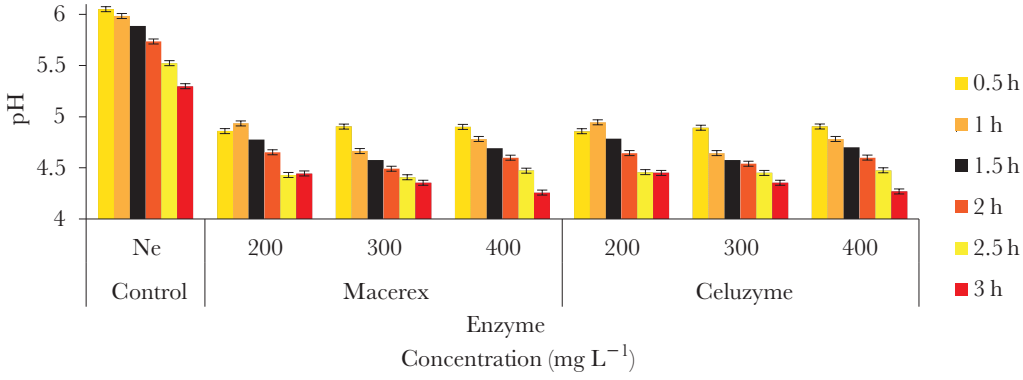

Figure 4. Average pH values of the wash water in coffee degumming based on enzyme type, concentration (mg L^{-1}) and time. Ne stands for no enzyme.

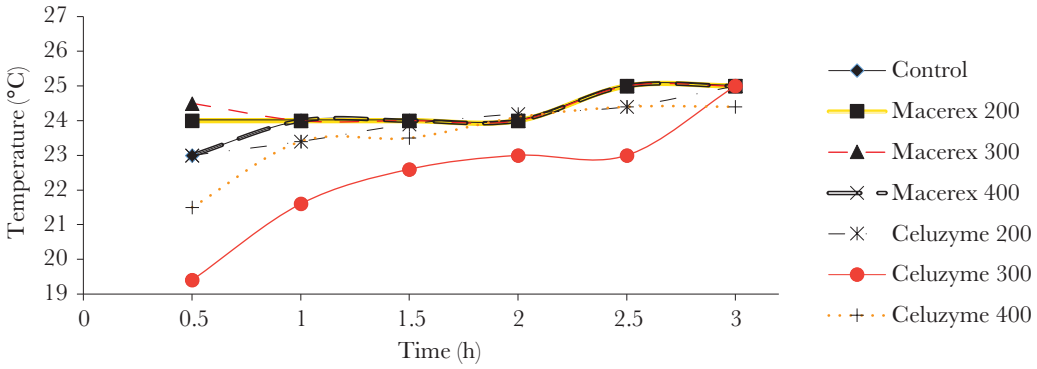

Figure 5. Average temperature during mucilage removal from coffee.

showed lower temperatures in the initial phase of the experiment, but as the experiment progressed the temperature was very similar in all treatments, while the Macerex PM enzyme showed higher temperatures throughout the process. This may be because the added enzymes and natural bacteria and enzymes from coffee tissues need a certain time to activate, but once stabilized in the medium they begin to act on the substrate causing the temperature to increase; that is, greater microbial and enzyme activity was observed over time.

Murthy & Naidu (2011) and En-Sheng *et al.* (2014) found that the use of commercial enzyme preparations in cherry coffee beans requires a certain temperature at which these enzymes accelerate the mucilage removal process through fermentation. On the other hand, Peñuela-Martínez *et al.* (2010) determined that the removal of mucilage using the TPL Rohapect® enzyme does not depend on the interaction of temperature and concentration. In this regard, the type of enzyme used to accelerate mucilage removal is very important since its activity influences factors affecting the formation of odors and flavors in the coffee.

Water consumption during mucilage removal

The traditional coffee beneficiary consists of a manual pulper and a fermentation tank where the process takes approximately 12 h and consumes 25 to 30 L of water per kg of cherry coffee. Using the technique of the four rinses to remove the mucilage from the grain 4.2 L of water are required per kg of dry parchment coffee obtained at the end of the process (Peñuela-Martínez *et al.*, 2010; Puerta-Quintero, 2012).

Innovations in the process of beneficiary allow to reduce water expenditure during washing, example of this we have to López-Blanco (2017) used a modified tank with discontinuous washing with a water expenditure of 5.3 L per kg of dry parchment coffee, reporting a water saving of 84% compared to conventional scrubbing channel washing which spent 33.8 L per kg of dry parchment coffee. On the other hand, modern mechanical mucilage removal machines that produce semi-washed coffee use only approximately 4.5 L of water per kg of dry parchment coffee (Enden & Calvert, 2010).

In the present study, the technique of the four rinses was used for mucilage removal. The total water consumption during mucilage removal of 31 kg of processed coffee cherry

beans was 28.9 L of water, the equivalent of 0.93 L per kg of coffee cherry beans or 4.2 L per kg of dry parchment coffee. This water expense represents a saving of 87.6% compared to conventional scrubbing channel washing (López-Blanco, 2017).

CONCLUSIONS

The use of pectic enzyme systems, such as Macerex PM and Celuzyme, significantly reduces the time for removing mucilage from coffee compared to the natural fermentation time. The Macerex PM enzyme showed a higher mucilage removal percentage. The use of commercial enzyme preparations is a viable technological alternative in the coffee agro-industry since it allows better pH, temperature and fermentation control in wet coffee processing; also, water consumption is significantly lower than in traditional coffee processing. These results have important implications in the wet method to help coffee industry in optimizing its process to be more sustainability without compromise the quality of coffee beans, and finally improve its revenues.

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