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OPTIMIZATION OF THE ALCOHOLIC AND ACETIC FERMENTATION PROCESS OF VINEGAR FROM APPLE 'DELICIOUS DE VISCAS'

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ABSTRACT

Worldwide, vinegar is a fermented condiment which has been used as a beverage, preservative and antioxidant for many centuries. Vinegar can be produced by using by-products or second quality products without compromising the quality of the final product due to its acetic nature which offers the vinegar a unique flavor. Vinegar has been of interest to the scientific community due to its chemical composition rich in antioxidants and organic acids which are also responsible for its organoleptic properties. Vinegar is commonly obtained by alcoholic fermentation and acetic acid fermentation from various carbohydrate-rich sources, including rice, wheat, pears, apples, by-products among others. Alcoholic fermentation involves the addition of yeast *Saccharomyces cerevisiae* and the consecutive addition of *Acetobacter aceti* for the acetic fermentation. In the valley of Mala (Peru), farmers used a modified version of the Orleans method to produce vinegar from second quality apples of the "Delicious de Viscas" variety, obtaining different results. This study aimed to optimize both alcoholic and acetic fermentation times in apple vinegar production, it used two experimental designs focused on each fermentation process. First, alcoholic fermentation was carried out starting by the standardization of apple must by the addition of brown sugar. Then, for the acetic acid fermentation, a grid was used to enhance contact of the vinegar mother and the product. The results indicated that the total soluble solid (TSS or ^{o}Bx) in the apple must, and the concentration of the yeast inoculum, significantly influenced the duration of alcoholic fermentation at a 95% confidence level. In addition, it was determined that neither the vinegar mother grid arrangement nor the initial acidity percentage significantly affected the acetic fermentation time, also at a 95% confidence level. The experimental designs identified the optimal conditions for the factors studied in both alcoholic and acetic fermentation, keeping most temperature and pH constant. Consequently, optimization reduced fermentation times from 672 to 38 hours for alcoholic fermentation and from 2856 to 42 hours for acetic fermentation.

Key words: Apple, must, vinegar, acetic fermentation, alcoholic fermentation, optimization

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INTRODUCTION

Vinegar is a traditional acid condiment derived from the double fermentation of carbohydrate-rich sources, mainly fruits such as pears, grapes, berries, pineapple, apples [1] and cereals including rice, wheat, barley and other starch-rich grains [2]. Vinegar is considered as natural GRAS (*Generally recognized as safe*) food [3] and has been used for centuries as a seasoning, food preservative and cleaning agent [4]. Additionally, several studies relate the composition of vinegar and the presence of functional molecules such as organic acids and polyphenols [5] with antioxidant, antihyperglycemic, antihyperlipidemic properties, among others, which may play an important role in disease prevention [6]. Moreover, the global vinegar market increased by 74% from 2018 to 2022, while projections indicate a further 15% growth by 2027 [1]. This positions vinegar as a promising alternative to revalue fruits typically discarded due to imperfections that do not meet the characteristics demanded by retailers and consumers, thereby serving as raw material for vinegar production [7]. Additionally, second quality raw materials can be used to produce acidified products without compromising quality of the final product [8].

The production of vinegar requires a two-stage fermentation process that involves the initial production of ethanol and followed by the production of acetic acid [9]. Both steps generate different metabolites such as organic acids, amino acids and volatile compounds which contribute to the taste, flavor and functional properties [10]. The first stage, alcoholic fermentation, is an anaerobic biochemical process in which sugar or starches found in fruit and cereals are converted into ethanol through the action of yeasts [11]. *Saccharomyces cerevisiae* strains are commonly employed in this stage [12] due to their ability to produce products of uniform and standard quality [13]. The use of this yeast also offers several other industrial advantages, including rapid growth, efficient metabolism and high ethanol productivity [14]. However, the production of alcohol is influenced by factors such as total soluble solids (TSS), the quality and quantity of yeast strains and fermentation conditions [11].

Once ethanol has been produced, acetic acid fermentation ensues, leading to the obtaining of vinegar [8]. This process is aerobic, based on the oxidation of ethanol to acetic acid by acetic acid bacteria (AAB) [15]. *Acetobacter* is one of the most commonly used AAB in acetic fermentation, mainly due to its acid resistance and robust reaction capabilities [16]. Furthermore, vinegar production often involves the inoculation of “mother of vinegar”. The “vinegar plant” or “mother of vinegar” refers to a film of AAB (bacterial cellulose) synthesized by microorganisms that form a gelatinous layer on the surface [17]. This layer serves to ensure bacterial survival by acting as a protective membrane or by enhancing oxygen availability to facilitate fermentation [18]. Complete aeration of the system is fundamental for maintaining

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bacterial viability and maximizing yields [15]. Overall, many technological strategies in this field should be studied to optimize vinegar production.

From a technological point of view, vinegar manufacturing can be performed using three different ways: the Orleans method, the fast Generator method and the Submerged method [19]. The Orleans or French method, also known as the slow traditional process, typically takes between 8 and 14 weeks to complete [20]. Initially, a small amount of "mother of vinegar" is added to the alcohol, this is where AAB oxidizes ethanol into acetic acid. Once the system reaches an acidity of at least 4%, a portion of the vinegar is removed from the top, while a new alcoholic medium is periodically added to sustain the process, which may take several months [21]. However, in contrast with the other methods, Orleans method allows obtaining a final product with high sensory quality, owing to the balanced development of organic acids and amino acids during long-term fermentation [22].

Consequently, to standardize vinegar production technology, it is essential to optimize the fermentation times for both alcoholic and acetic fermentation stages from apples of the "Delicious de Viscas" variety, grown in the Mala Valley, Lima, Peru. These apples, which are often deemed unsuitable for sale due to their size and the presence of minor blemishes and bruises, can be effectively utilized in vinegar production.

MATERIALS AND METHODS

Materials

Raw materials used to make the vinegar were apples of the "Delicious de Viscas" variety of second quality, from the Azpitia hamlet, located in the northeastern area of the province of Mala, Lima region, Peru. For the fermentations, the strain *Saccharomyces cerevisiae* [var. Bayanus], strain "Prise de Mousse" from the Champagne region (France) was used for the alcoholic fermentation, while the strain for the acetic fermentation was *Acetobacter aceti* of spontaneous formation. Besides that, brown sugar was used to adjust the TSS.

Proximate analysis of Delicia's apple

Carbohydrates (calculated), ash (AOAC 940.26, 22nd. Ed. (2023).), total energy (calculated), fat (AOAC 930.09, 22nd. Ed. (2023).), moisture (AOAC 920.151B, 22nd. Ed. (2023).) and protein (AOAC 920.152, 22nd. Ed. (2023).) were determined for Delicia's apple.

Experimental design

The alcoholic fermentation process used initial TSS measured in degrees Brix (°Bx) and the amount of inoculated yeast (g) as independent variables through a factorial design. The initial TSS values (14, 15, and 16 °Bx) were selected based on the

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typical range found in Delicia's apple must, which varies from 14 to 16 °Brix. Additionally, vinegar made from fruits such as apple, grape, mango, passion fruit, pineapple, and pear typically falls within a range of 11.2 to 15.9 °Brix. For yeast concentration, 1, 2, and 3 g were chosen based on previous studies. Other authors have used 1 to 2 grams of yeast per liter of liquid, as this amount is sufficient to initiate fermentation effectively [23]. The inverse of time was used as the response variable to maximize it. Optimization was performed using the response surface method. Finding the lowest fermentation time through two independent variables, TSS and amount of yeast, was the optimization criterion in alcoholic fermentation.

In the acetic fermentation process, the initial acidity percentage and the grid arrangement were considered as independent variables and a randomized complete block design (RCBD) was applied to evaluate their effects. Finding the lowest fermentation time through a quantitative variable (acidity percentage) and a categorical variable (grid arrangement presence) was the optimization criterion in acetic fermentation.

Preparation of apple must

First, the apples were washed by immersion in water, then cut, crushed and pressed using a homemade fruit extractor to separate the solid components. The TSS of the must was adjusted with sucrose to ensure repeatability of the experiment. It was assumed that to achieve an additional degree of alcohol during fermentation, 17 grams of sugar per liter of must should be added [24].

Alcoholic fermentation

The *Saccharomyces cerevisiae* wine yeast strain was inoculated into the apple must to accelerate the alcoholic fermentation process. *Table 1* presents the variables used to evaluate and reduce the fermentation time. The independent variables included the initial total soluble solids (TSS) measured in degrees Brix (°Bx) and the amount of inoculated yeast (g), as determined by a factorial design. Additionally, the experimental conditions were maintained at an environmental temperature of 25–28°C and a pH range of 3.0–4.0.

Acetic fermentation by modification of the Orleans method

After alcoholic fermentation, the product obtained was filtered using cloth filters to remove solid interferents generated in the bioprocess. As previously mentioned, the initial acidity of the alcoholic fermentation product was an experimental variable. This acidity level was established by the addition of unpasteurized vinegar with 6% acidity, which also contained acetic bacteria (fermentative agent), as indicated in *Table 2*.

For the fermentation, a spontaneously formed *Acetobacter aceti* strain or "mother of vinegar" was used to act as a catalyst for ethanol oxidation. Fermentation systems

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with air inlets, consisting of plastic containers equipped with an outlet valve, were employed to monitor the acidity during the fermentation process (*Figure 1A*). As shown in (*Figure 1B*), a modification of the Orleans method was carried out where the mother of vinegar was supported by a grid that allowed increasing the contact of the AAB with the air. This process was carried out at room temperature (25 - 30 °C) with a pH range between 2.7 and 3.0.

Figure 1: Modification of Orleans method A) System of fermentation B) Grid of mother of vinegar

Fermentation was considered finished when the acidity reached 4 %. The product was then filtered and pasteurized at 80 °C for 20 min in a water bath to stop fermentation as recommended by other authors [25]. Finally, the vinegar was bottled in 215 ml plastic bottles.

Data analysis

The results obtained were processed with the statistical package "STATGRAPHICS Centurion XV version 15.1.02" for both experimental designs.

RESULTS AND DISCUSSION

Apple cider vinegar is traditionally produced from ripe apples, which are processed into a must that undergoes both alcoholic and acetic fermentation. According to studies, mature apples are the most desirable for fermentation, since, at this stage, they have the maximum sugar concentration. This high sugar content is advantageous for improving the biochemical conversion of sugar into ethanol.

The proximate composition of Delicia's apple fruit is as follows: of 15.98 g/100g of carbohydrates, 0.00 g/100g of ash, 66.05 g/100g of total energy, 0.13 g/100g of fat, 83.65 g/100g of moisture and 0.24 g/100g of protein. These values differ significantly from those reported by Dakuyo [26] for Cascade apples. Specifically, Cascade apples contain 88.6 g/100 g of carbohydrates, a value markedly higher than that of

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Delicia apples. Similarly, the levels of ash (3.05 g/100 g), fat (2.64 g/100 g), and protein (5.65 g/100 g) in Cascade apples are considerably higher than those of Delicia apples. Additionally, the energy content of Cascade apples is nearly four times greater than that of the Delicia variety. In contrast, the moisture content is comparable between the two apple varieties. Notably, the proximate composition of this specific variety has not been reported in the literature, making this study a valuable contribution to the scientific community. This not only fills a gap in the existing knowledge but also highlights the potential applications of second quality apples of the "Delicious de Viscas" variety, contributing to reduced greenhouse gas emissions (GHG) through decreased food waste and sustainable resource use.

The alcoholic fermentation is an anaerobic bioprocess which is a critical stage that significantly influences vinegar quality. Moreover, it involves the role of sugar as electron donor, electron acceptor and carbon source. As shown in ***Figure 2***, alcoholic fermentation mechanism requires two molecules of pyruvate formed by glycolysis (degradation of glucose), which is a ten-step process that occurs under absence of oxygen conditions [27]. Then, the two molecules of pyruvate are decarboxylated to two molecules of acetaldehyde and finally they are reduced to two molecules of ethanol by NADH [28]. Furthermore, metabolites and by-products that contribute to sensory characteristics are also generated by the metabolic action of yeast [29].

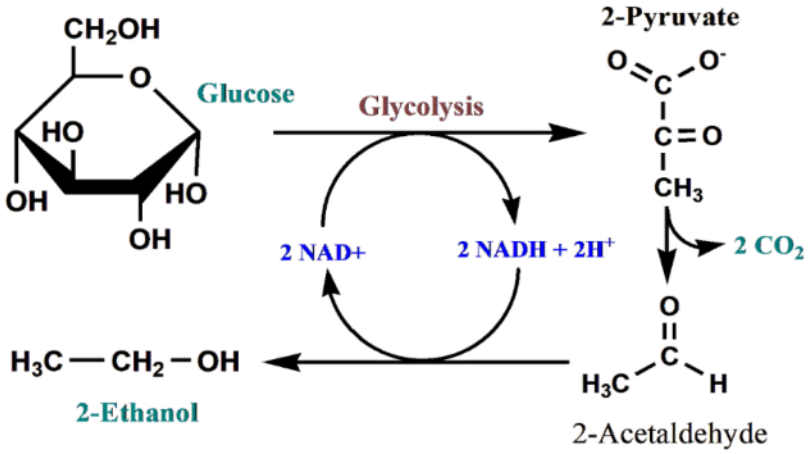

Figure 2: Schematic pathway of alcoholic fermentation
Adapted from Abbaspour [27]

For many years, in the traditional vinegar production process with apples from Azpitia orchard, density and acidity percentage were the parameters which determined the end of alcoholic and acetic fermentation, respectively. Some local vinegar producers did not have a standardized process, structure, or methodology to follow. Therefore, one of the main disadvantages of the traditional process in Azpitia is the lengthy time it requires. According to previous data collection, the

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alcoholic fermentation took 672 hours, while acetic fermentation, 2856 hours generating economic losses.

Table 3 shows the results of the alcoholic fermentation times obtained from the experiments. It should be noted that the end of alcoholic fermentation was defined when TSS (°Bx) remained constant, as shown in *Figure 3*.

Figure 3A shows the follow-up of the °Bx of alcoholic fermentation with 0 g of yeast. From initial values of 14, 15 and 16 °Bx (shown in blue, red and green), values close to 5 °Bx were reached after 131, 139 and 158 h, respectively. Similarly, *Figure 3B* and *3C* show the alcoholic fermentation values with 1 and 2 g of yeast respectively, with initial values of 14, 15 and 16 °Bx and reaching values close to 5 °Bx after 57, 70 and 84 h respectively, while for 2 g of yeast, values close to 6 °Bx were reached after 33, 58 and 62 h. In contrast to the traditional method of alcoholic fermentation (672 h), there was a notable reduction in time (33 to 158 h).

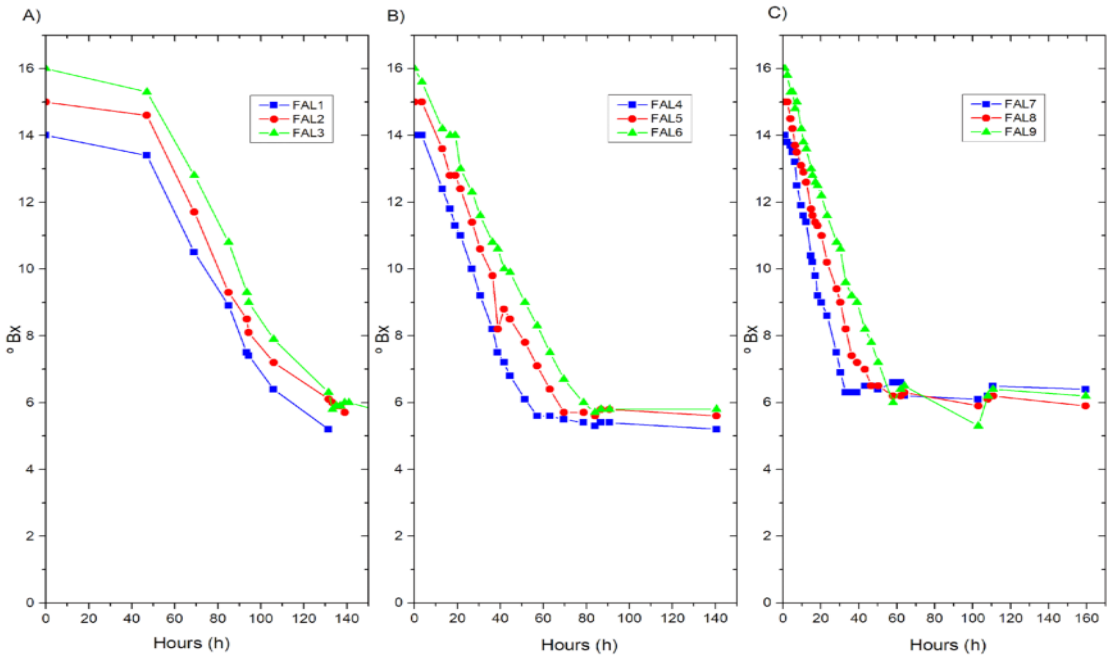

Figure 3: Alcoholic fermentation using A) 0 g, B) 1 g and C) 2 g of Saccharomyces cerevisiae yeast

Alcoholic fermentation results were statistically analyzed in order to identify the factors affecting the time of this process. The factors evaluated were: Factor A (yeast) and Factor B (TSS) while the response evaluated was the inverse of the fermentation time. *Figure 4* shows the Pareto diagram at a confidence level of 95%, it was observed that both, yeast and TSS, directly influence the alcoholic fermentation time since they exceed the critical statistical limit of the standardized effect. Similarly, in the study conducted by Liu [30], it was reported that the initial sugar concentration (%) presents significant effect on alcohol % because a p-value

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<0.0001 was obtained in the analysis of variance, while the fermentation temperature in the range of 20 to 32 ^{o}C did not present significant effect on alcohol content (p-value = 0.7732). On the other hand, both the interaction of these two factors and the effect of each factor squared had no significant effect on the dependent variable.

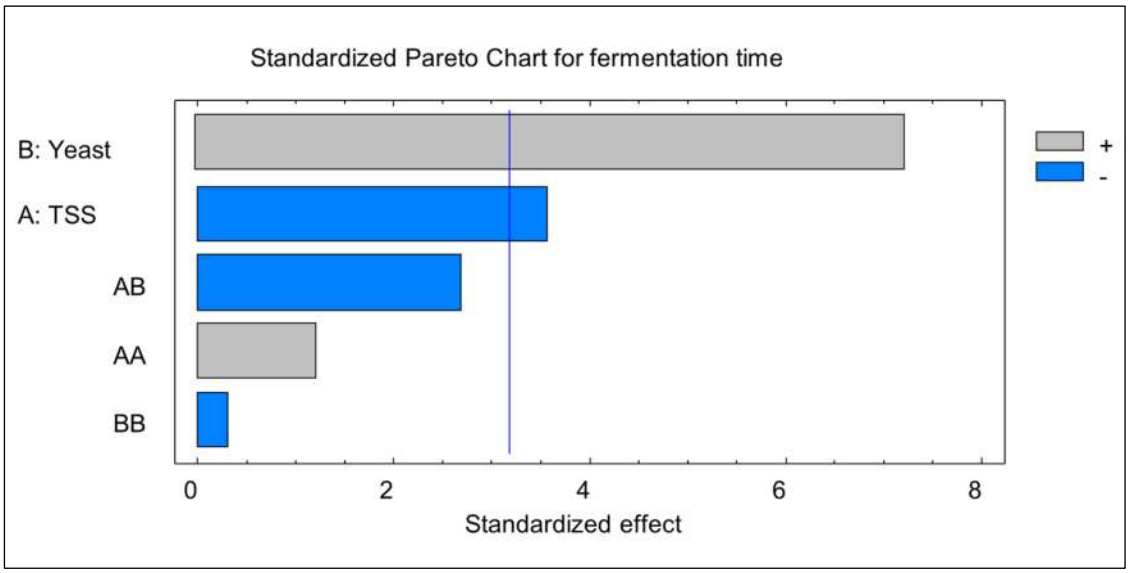

Figure 4: Pareto plot of the significance level of the factors in the response variable

According to Chelladurai [31], the response surface method allows optimizing a response influenced by independent variables. In addition, a second-order regression equation is obtained to predict the response considering the input parameters.

Figure 5 presents the response surface plots (*Figure 5A*) and contour lines (*Figure 5B*) to understand the effect of TSS and amount of yeast on fermentation time. In addition, equation 1 presents the regression equation to fit, understand and represent, both mathematically and statistically, the effect of TSS and amount of yeast inoculated on the inverse of alcoholic fermentation time. The response surface method allows optimizing a response influenced by independent variables [31]. In addition, a second-order regression equation is obtained to predict the response considering the input parameters. The regression parameters are shown in Table 7 and Table 8.

Fermentation time $(h)^{-1}$ = 0.47 - 0.06*(TSS) + 0.06*yeast (g) + 0.01* $(TSS)^{2}$ – 0.01* (TSS) *yeast (g) - 0.001*yeast $(g)^{2}$ (Eq. 1)

A) **B)**

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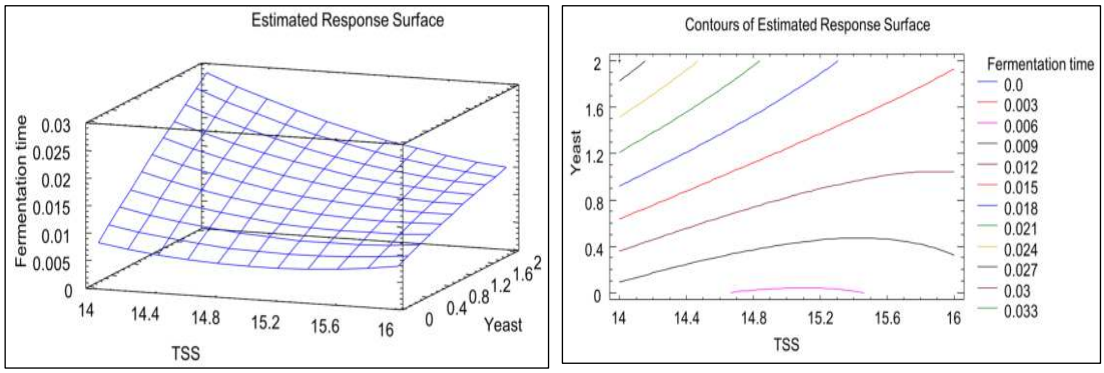

Figure 5: Estimated response surface of alcoholic fermentation

In *Table 4*, the values of TSS and mass of inoculated yeast (g) obtained from the optimization are reported. Minimum alcoholic fermentation time with initial apple must with 14 °Bx and 2 g of inoculated yeast, reaches an optimum alcoholic fermentation time of 33 hours.

In treatments FAL1, FAL2 and FAL3, alcoholic fermentation occurred spontaneously due to the action of microflora from apple, but also from the winery equipment and environment. In addition, microflora diversity depends on several factors such as crop variety, climatic conditions and fermentation practices [32]. According to the results obtained (*Table 3*), the fermentation times of these treatments were longer than the other treatments (FAL4, FAL5, FAL6, FAL7, FAL8 and FAL9) inoculated with *Saccharomyces cerevisiae* yeast. However, according to Daccache [29], natural alcoholic fermentation processes present fruity characteristics, which could influence sensory acceptability. In contrast, fermented products in which additives were added, or other modern treatments are performed, a higher yield in alcoholic content is obtained.

On the other hand, Bonassa [33], in his research to optimize alcoholic fermentation time of sugarcane, TSS content in the initial must was greater than 15 ºBx using 0.1 g of inoculum, the fermentation process amounted to 46 hours. In contrast, times greater than 58 hours were obtained when working with more than 15 ºBx in the apple must and using between 1 and 2 g of yeast. From what was reported by the authors, it can be inferred that the source of sugars also influences fermentation times. Additionally, according to Kara [3], it has been shown that even different apple varieties influence the quality of the product and therefore the fermentation processes.

Acetic fermentation was carried out by the Orleans method. Traditionally, a culture in the form of a floating cellulose mat made by *Acetobacter aceti* strains is known as “mother of vinegar” [34], it is relevant to keep this biofilm suspended close to the surface, as oxygen is essential for its existence [1]. One of the risks associated with

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this method is that the culture layer will be dragged to the bottom of the fermentation tank, which will cause the fermentation to stop until the layer forms again [15]. Briefly, as shown in *Figure 6*, ethanol is oxidized by alcohol dehydrogenase (ADH) to produce acetaldehyde, and then, by the action of aldehyde dehydrogenase (ALDH) is oxidized to acetic acid [34].

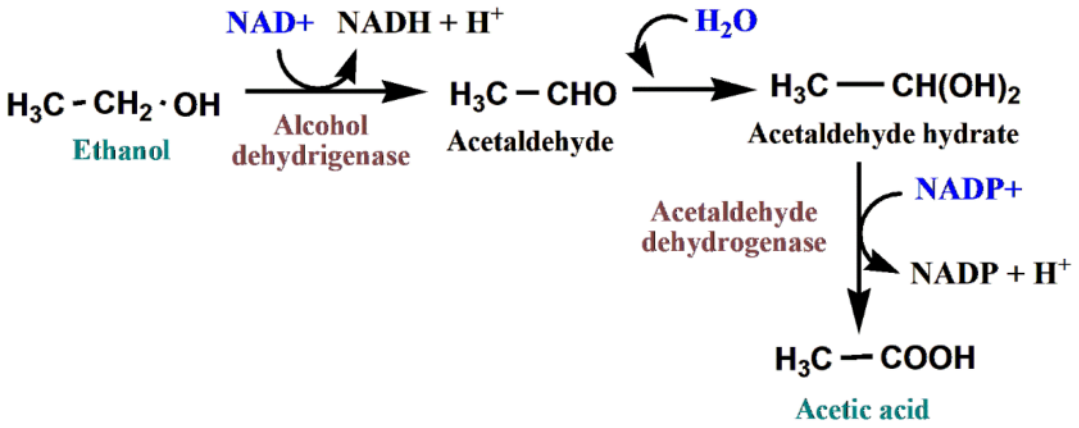

Figure 6: Schematic pathway of acetic acid fermentation. Adapted from Mota [35]

In the present investigation, a technological modification was made in which the use of surface grids was implemented to keep the "mother of vinegar" in contact with oxygen to favor aerobic fermentation. Similarly, Merli [15], highlighted the importance of ensuring complete aeration and oxygen control during the acetic fermentation process to increase yield and keep bacteria viable.

In this case, temperature was maintained between 25 and 30 °C due to environmental conditions. In addition, several studies consider this temperature range as optimal for AAB growth, and pH between 2.7 and 3.0. To emphasize, this pH value was determined by a trial-and-error process until favorable results were achieved after the fermentation process. In general, AAB are resistant to low pH, even when the recommended pH for their growth is between 5.5 and 6.3 [15]. However, according to studies conducted by Soumahoro [36], the optimum pH conditions for cocoa fermentation using AAB was between 4 and 5.

As shown in *Table 5*, acetic fermentation process times are reported from the experimentation. According to several research, most vinegars usually have between 4 to 9% acidity [37]. Therefore, acetic fermentation time was determined on 4% acidity. For instance, in a recent study by Sulieman & Yousif [38], vinegar was obtained from the edible part of Nabag fruit (*Ziziphys-spina christa*) with 6.24% acidity.

Figure 7 shows the monitoring of the acidity percentage of the various acetic fermentation treatments with a grid (FAC1, FAC2 and FAC3) and without a grid

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(FAC4, FAC5 and FAC6) at 2, 2.5 and 3% initial acidity. Initial acidity percentage of 3% (FAC3 and FAC6) obtained a final acidity percentage of 4% after 40 h of fermentation, while those with 2.5% initial acidity (FAC2 and FAC5) reached the value of 4% final acidity after 56 and 76 h, and those treatments with 2% initial acidity (FAC1 and FAC4) reached the desired acidity value to be considered as vinegar after 90 h.

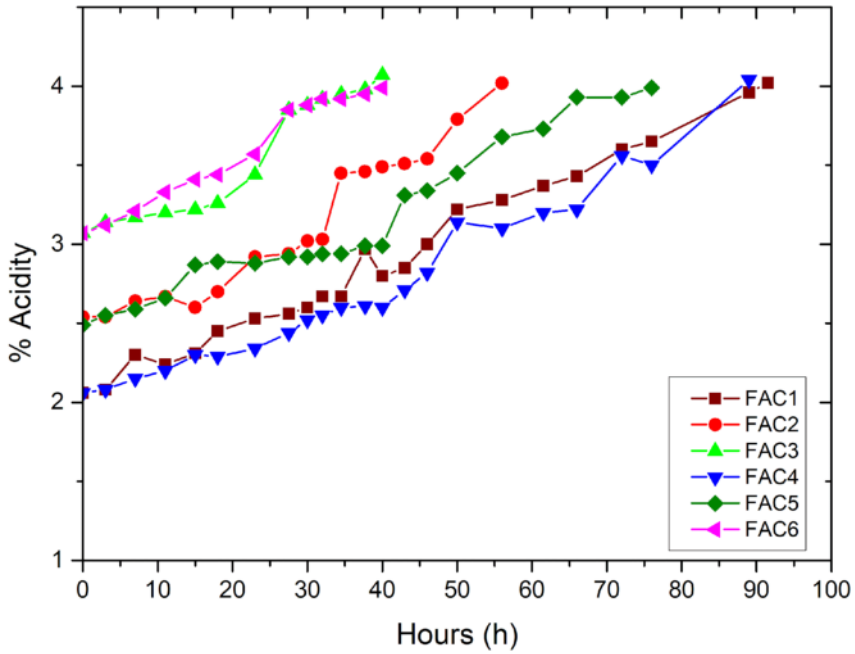

Figure 7: Acetic fermentation with and without grid at 2, 2.5 and 3% initial acidity

As shown in Table 6, p-value of the variables: acidity percentage and grid arrangement are greater than 0.05, so the factors have no significant effect on fermentation time at a confidence level of 95.0%. However, according to Zhang [39], adjusting the raw material composition, fermentation temperature and oxygen flow increases the concentration of alcohol and secondary metabolites such as acetic acid, polyphenols and esters allowing to obtain a better flavor in the product.

Through statistical treatment of data, the optimal parameters for the acetic fermentation process were determined with an initial acidity percentage of 3% (expressed as acetic acid) and with the use of a grid at the liquid/gas interface as a support for the vinegar mother, apple cider vinegar was obtained in an optimal acetic fermentation time of 39.2 hours, which is less than the time reported by various references. In the research done by Soumahoro [36] based on the acetic fermentation of cocoa with different strains of AAB at 30, 35, 40 and 45 ^{o}C, initial acidity percentage was approximately 3.7% and after acetic fermentation with the *Acetobacter okinawensis* strain, vinegar obtained 6.2% acidity at 30 ^{o}C after 144 h.

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On the other hand, with the *Acetobacter tropicalis* strain, vinegar was obtained after 96 h. The article concludes by reporting that the production of acetic acid depends on the fermentation conditions, at a pH between 4 - 5 and with temperatures less than or equal to 30 °C. However, Matsumoto [40] carried out an investigation of the effect of temperature (reaching up to more than 40 °C) with the incubation of various AAB adapted for vinegar production and reported a time of 80 h for acetic fermentation. It should be noted that the author started with an approximate acidity of 0.5% and defined the end of fermentation at 5% acidity.

As validation of the experimental part, the optimum values obtained were experimentally verified to evaluate the proximity between them, which is necessary for the standardization of the process. This verification was performed in triplicate. Figure 8 shows, for alcoholic fermentation (Figure 8A) and acetic fermentation (Figure 8B), the values that demonstrate an acceptable proximity of the optimum process parameters. From these tests, alcoholic fermentation times of 38 hours and acetic fermentation times of 42 hours were obtained.

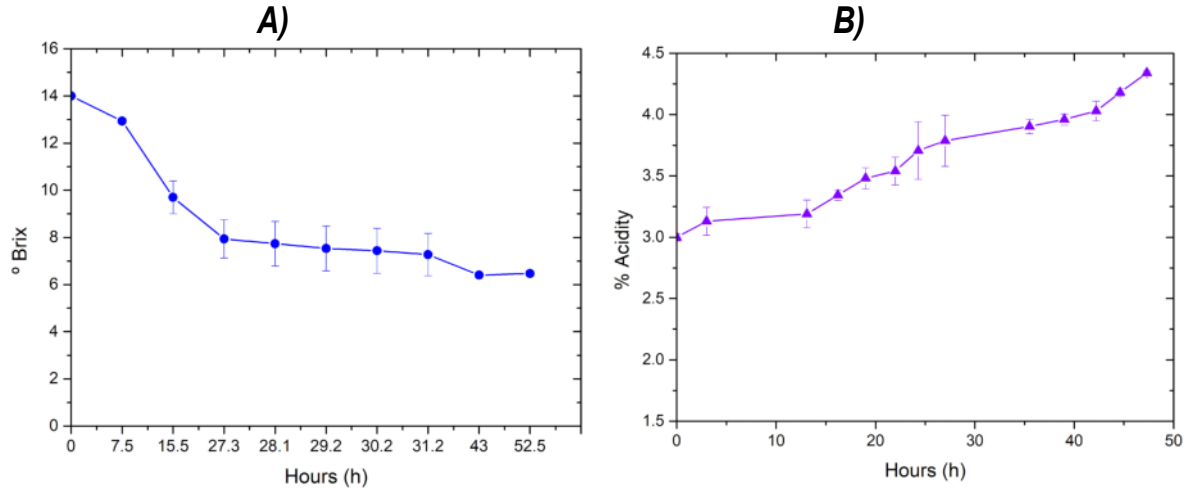

Figure 8: Alcoholic and acetic acid fermentation carried with optimal conditions

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CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The purpose of this study was to optimize the time of the fermentation process, both alcoholic and acetic fermentation, during the production of apple cider vinegar. Based on the results of the research, it can be concluded that it was possible to reduce the alcoholic fermentation time from 672 to 38 hours, representing a reduction of up to 94% compared to the traditional method. The optimum operating conditions were must at an initial 14 °Bx and 2 g of inoculated yeast. Similarly, the optimization of the acetic fermentation process was possible reducing the time from 2856 to 42 hours, which is a 99% decrease from the traditional method. The optimal conditions that allowed this reduction were: initial total acidity of 3% and with a grid as a support for the vinegar mother. Overall, the total fermentation process has gone from 3528 to 80 hours, demonstrating a substantial improvement in the efficiency of the vinegar production process.

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Contribution of the authors

Fernando De la Cruz: Conceptualization, methodology, research. **Katherina Changanaqui**: Original draft, editing, **Angie Castillo**: Original draft, writing, **Jonnatan Bañon:** Research, Data Curation, validation, **María García**: Conceptualization, research, **Tarsila Tuesta**: research, project management.

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Table 1: Treatments to evaluate alcoholic fermentation times according to factorial design

Treatments	TSS (°Bx)	Yeast (g)
FAL1	14	0
FAL2	15	0
FAL3	16	0
FAL4	14	1
FAL5	15	1
FAL6	16	1
FAL7	14	2
FAL8	15	2
FAL9	16	2

Note: FAL refers to alcoholic fermentation

Table 2: *Treatments to evaluate acetic fermentation times according to a RCBD*

Treatment	Acidity (%)	Grid arrangement
FAC1	2	With grid
FAC2	2.5	With grid
FAC3	3	With grid
FAC4	2	Without grid
FAC5	2.5	Without grid
FAC6	3	Without grid

Note: FAC refers to acetic fermentation

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Table 3: ***Alcoholic fermentation times reported during the experimental trials***

Treatments	TSS (°Bx)	Inoculated yeast (g)	Hours (h) of alcoholic fermentation
FAL1	14	0	131.4
FAL2	15	0	139
FAL3	16	0	158
FAL4	14	1	57.1
FAL5	15	1	69.5
FAL6	16	1	83.0
FAL7	14	2	33.0
FAL8	15	2	58.0
FAL9	16	2	62.0

Note. The table shows the reduction of alcoholic fermentation times without yeast and with yeast. It is observed that keeping the initial °Bx value constant, if the amount of yeast is higher, then the fermentation time is shorter

Table 4: Results obtained for the optimum formulation

Factor	Minimum	Maximum	Optimum
TSS	14.0	16.0	14.0
Yeast	0.0	2.0	2.0

Note. Prepared with STATGRAPHICS Centurion XV Version 15.1.02

Table 5: ***Acetic fermentation times reported by experimental trials***

Treatments	Initial acidity (w/v)	Grid arrangement	Hours (h) of acetic acid fermentation
FAC1	2	With grid	91.5
FAC2	2.5	With grid	57.5
FAC3	3	With grid	39.2
FAC4	2	Without grid	87.3
FAC5	2.5	Without grid	75.5
FAC6	3	Without grid	40.5

Note. The table shows the change in acetic fermentation time by grid arrangement. For initial values of 2.5 and 3.0% acidity, shorter fermentation times were obtained using the grid as a support for the “mother of vinegar”.

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Table 6: Analysis of variance time of acetic fermentation

Main effects	SC	GL	CM	F-Ratio	p-value
A: initial % acidity	2459.89	2	1229.94	18.40	0.0515
B: grid	38.0017	1	38.0017	0.57	0.5295
Residual	133.663	2	66.8317		
Total (corrected)	2631.55	5			

Table 7: Model Summary of the regression equation

S	R-sq	R-sq(adj)	R-sq(pred)
0.0023984	96.10%	89.60%	55.38%

Table 8: Analysis of variance of alcoholic fermentation

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	0.000425	0.000085	14.78	0.025
Linear	2	0.000375	0.000187	32.56	0.009
yeast	1	0.000302	0.000302	52.43	0.005
TSS	1	0.000073	0.000073	12.68	0.038
Square	2	0.000009	0.000004	0.77	0.535
yeast*yeast	1	0.000001	0.000001	0.09	0.781
TSS*TSS	1	0.000008	0.000008	1.46	0.314
2-Way Interaction	1	0.000042	0.000042	7.22	0.075
yeast*TSS	1	0.000042	0.000042	7.22	0.075
Error	3	0.000017	0.000006		
Total	8	0.000442			

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