

PHILIPPINE SCIENCE HIGH SCHOOL WESTERN VISAYAS

Doña Lawaan H. Lopez Campus
Iloilo City

QUALITY VINEGAR FROM CACAO (*Theobroma cacao*) SEED COATING

A Research Paper Presented to the
Faculty of the Philippine Science High School Western Visayas
Iloilo City

in Partial Fulfillment
of the Requirements in
Technology Research II

by

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February 2000

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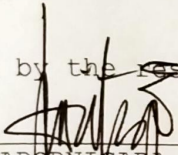
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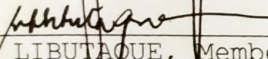
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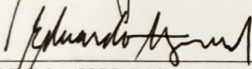
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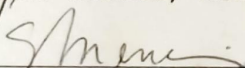
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ABSTRACT

This study aimed to produce quality vinegar from the seed coatings of ripe cacao. The independent variable in this study was the ripe cacao seed coatings. The dependent variables were pH, percentage acetic acid and density. The mean and standard deviation will be employed as descriptive statistical tools. The One-Way Analysis of Variance (ANOVA) and Scheffe test, all set at 0.05 alpha, were used as inferential statistical tools. The study produced a vinegar from ripe cacao seed coating and was compared to two other commercial vinegar. Significant differences were observed in the pHs and percentage acetic acid of the product and two other commercial vinegar. There was also a significant difference in the density of the product and the cane vinegar. However, there was no significant difference in the densities of the product and palm vinegar. These differences imply that the vinegar produced was inferior to commercial ones.

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QUALITY VINEGAR FROM CACAO (*Theobroma cacao*) SEED-COATING

Chapter 1

Introduction to the Study

Background of the Study

It is known that the major source of vinegar in the Philippines is the coconut tree. Since the coconut tree also has other uses the researchers thought up to find an alternative source for making vinegar.

It has come to the attention of the researchers that the chocolate making industry yields the seed coating of the Cacao fruits (*Theobroma cacao*) as mere by-products. The researchers thought of using these seed coatings from the Cacao fruits as the alternative raw material for making vinegar.

The success of this study might provide boost to the vinegar industry and ultimately contribute to the improvement of the economy.

The relationship between the independent and dependent variables in the study is presented in Figure 1.

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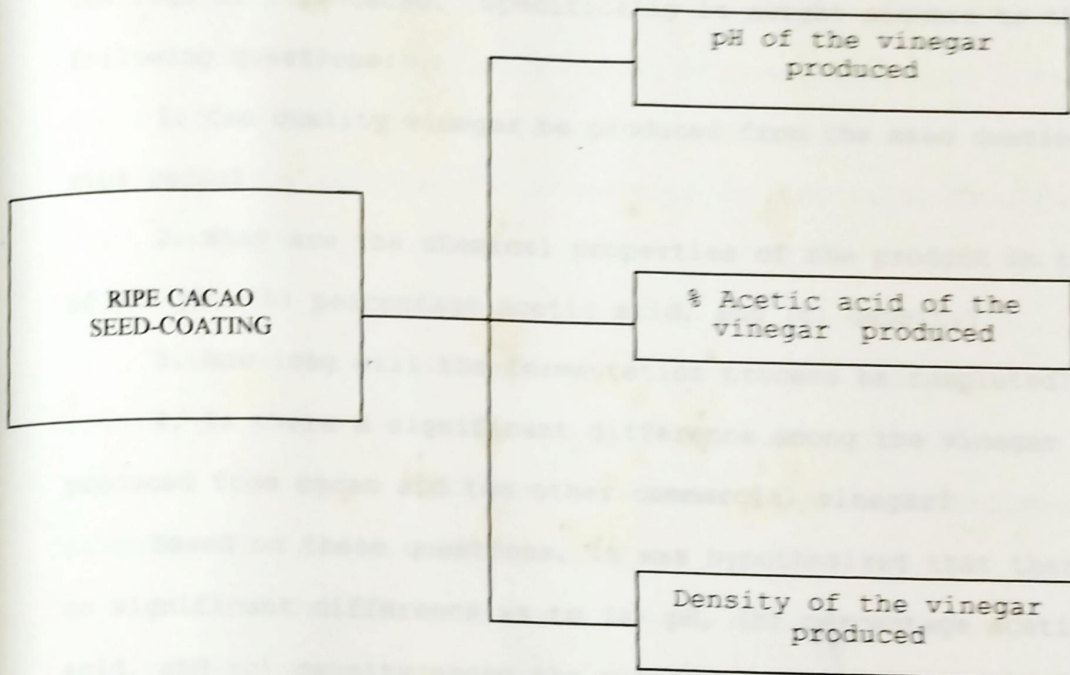


Figure 1. Chemical properties of the vinegar produced from ripe cacao seed-coating.

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Although we are producing vinegar, we only produce a certain percent of what we totally consume. This is because we only have few sources.

Coconut is our major source of vinegar. But, as stated earlier, it has too many uses. What if one day we only have a few coconuts left for our vinegar production? What we need is another source material for vinegar production aside from the over used coconut to help increase and stabilize our vinegar production.

Other alternatives aside from coconut for vinegar production should be considered. We the researchers believe that the seed coating of ripe cacao fruits could be extracted and fermented to produce quality vinegar.

Since there are many cacao trees in the Philippines, the seed coating may serve a good alternative for vinegar making. If the study will be successful, it may help a lot in improving the economy of the country.

Definition of Terms

For the purpose of clarity and single mindedness, the following terms are given their conceptual and operational meaning.

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Cacao - is a tree of the American tropics whose seeds are the source of chocolate and cocoa (Grolier Encyclopedia, 1993).

In this study the term "cacao" meant as defined.

Fermentation - is defined as the foaming that occurs during the manufacturing of wine and beers (McHenry, 1993).

In this study the term "fermentation" meant the process of turning sugar into acetic acid in order to produce vinegar.

Vinegar - is an acidic liquid obtained from fermentation of alcohol and used either as a condiment or preservative (Grolier Encyclopedia, 1993).

In this study the term "vinegar" meant the substance produced from the seed coating of cacao through fermentation.

Product - a thing produced; a substance obtained from another by chemical change (Microsoft Bookshelf Basics, 1994).

In this study, the term meant the vinegar produced from the fermentation of extract from cacao seed coating.

Scope and Delimitation

The preparation of the vinegar was conducted at the house of one of the researchers. The remaining part was conducted at the Philippine Science High School Western Visayas Research Laboratory. The cacao seed-coatings were obtained from Janiuay,

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Iloilo. The results of the study were obtained after one and a half months. Due to uncontrollable conditions such as weather, impurities, lack of time, inaccuracy in measurements and amateur procedures followed by the researchers the results of the study cannot be considered that accurate.

There are several ways in which a dilute alcohol solution may be converted to vinegar. The oldest consists in allowing a partially filled barrel of wine to stand until its liquor is vinegar. This procedure has been modified in the slow and continuous Orleans process by adding and drawing off vinegar as fermentation proceeds. Air is admitted into the barrel, which rests on its sides, through holes bored in the ends above the liquid level. The bacteria will form a thin film on the surface of the liquid which gradually becomes thicker and more gelatinous. This film, known as the Mother of Vinegar, must not be disturbed during fermentation or when wine is added and vinegar withdrawn, since it may sink to the bottom of the barrel, where in the absence of air, it will ferment without producing acetic acid. Sometimes a framework of "rafts" is provided to support the bacterial film.

The Orleans process, though producing vinegar of high quality, is slow and costly. For this reason the quick vinegar process is more widely used. Essentially this process involves

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Chapter 2

Review of Related Literature

Process in Vinegar Making

There are several ways in which a dilute alcohol solution may be converted to vinegar. The oldest consists in allowing a partially filled barrel of wine to stand until it turns to vinegar. This procedure has been modified in the slow and continuous Orleans process by adding and drawing off vinegar as fermentation proceeds. Air is admitted into the barrel, which rests on its sides, through holes bored in the ends above the liquid level. The bacteria will form a thin film on the surface of the liquid which gradually becomes thicker and more gelatinous. This film, known as the Mother of Vinegar, must not be disturbed during fermentation or when wine is added and vinegar withdrawn, since it may sink to the bottom of the barrel, where in the absence of air, it will ferment without producing acetic acid. Sometimes a framework or "raft" is provided to support the bacterial film.

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in allowing a 10 to 13 percent alcohol solution to trickle slowly in contact with air through a tower filled with some inert material which supports a film of acetic acid bacteria. The towers or generators are 8 to 20 feet high and 4 to 15 feet in diameter. They are usually constructed of wood and have a perforated bottom to admit in air. The generator is packed with an inert material such as beechwood shavings or coke, which supports the bacterial film and permits air to pass upward through the generator freely. The nutrient solution is distributed evenly over the top surface of the supporting material and allowed to trickle down through the generator. A nutrient mixture to support bacterial growth is added to the alcohol solution before it is run into the generator. Some vinegar is also added to prevent the growth of undesirable bacteria and to supply seed for the development of desirable types (Johnston, 1995).

Fermentation

Fermentation, originally, is the foaming that occurs during the manufacture of wine and beer, a process at least 10,000 years old. That the frothing results from the evolution of carbon dioxide gas was not recognized until the 17th century. Louis Pasteur in the 19th century used the term fermentation in a narrow

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sense to describe the changes brought about by yeast and other microorganisms in the absence of air (anaerobically); he also recognized that the ethyl alcohol and carbon dioxide are not the only products of fermentation.

In the 1920's, it was discovered that the extracts of muscle catalyze, in the absence of air, the formation of lactate from glucose and that the same intermediate compounds formed in the fermentation of grain are produced by muscle. An important generalization thus emerged: that fermentation reactions are not peculiar to the action of yeast but also occur in the many instances of glucose utilization.

Glycolysis, which means dissolution of sugar, was originally defined around 1930 as a splitting of sugar into lactate. It can be further defined as fermentation, in which the six-carbon sugar glucose is broken down into two molecules of the three-carbon organic acid, pyruvic acid (of its ionized form pyruvate), coupled with the transfer of chemical energy to the synthesis of ATP. The pyruvate may then be oxidized, in the presence of oxygen, be reduced to lactic acid, alcohol or other products (McHenry, 1993).

Fermentation is a process of decomposition of organic substances, catalyzed by enzymes, and giving off energy and gas. It is well known that fruit juices gradually alter in character

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with the evolution of bubbles and gas; that milk sours without special treatment; and that all dead organic matters purifies or changes its chemical composition with time. These changes are brought about by the activity of various minute organisms. Which contain enzymes. Enzymes are the active agents of fermentation process. In many cases they can be extracted from the organisms or from the tissue without losing their effectiveness, showing that the presence of living cells is not essential to the fermentation. Enzymes facilitate such chemical changes in somewhat the manner as catalysts speed up certain inorganic reactions. Perhaps the most important fermentation process is that by which commercial alcoholic liquors are produced. This process is due to a fungus called yeast. When this is introduced into solutions of sugars at the appropriate temperature, the cells multiply. These yeast cells contain enzymes which produce the changing characteristics of fermentation. The chief enzymes present in yeast are zymase, which causes the breaking-up of glucose (grape-sugar) and fructose (fruit-sugar); invertase, which converts cane-sugar into invert-sugar, a mixture of glucose and fructose; and maltase, which converts maltose into glucose. In the manufacture of beer, the action is begun by another enzyme, diastase, which is formed in the grains of barley during malting. The diastase converts the starch in the malt into

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dextrin and maltose. After the introduction of yeast into the wort, the maltose is converted into glucose by the enzyme maltase, and the enzyme zymase proceeds to set up alcoholic fermentation. The effect of this fermentation is to brake up the sugar into alcohol and carbon dioxide, thus: $C_6H_{12}O_6$ (glucose)
 $2 C_2H_5O$ (ALCOHOL) + $2 CO_2$ (carbon dioxide).

Acetic fermentation is brought about by *Mycoderma aceti* which finds its way into weak alcoholic solutions from the air, and living upon the nitrogenous matter in the solution, causes the alcohol to combine with the oxygen of the air, thus:
 $C_2H_5O + O_2 \rightarrow C_2H_3O_2$ (acetic acid) + H_2O . The formation of acetic acid in this way accounts for the souring of beer and light wines when exposed to the air, and is the basis of the manufacture of vinegar.

Vinegar

Vinegar was probably the first acid recognized and used by humans. It is produced by the action of air in the alcohol in wine. Ancient people knew that vinegar's sour taste and odor were far different from wine. It was also learned that wine could be kept from going sour by keeping it in a close container.

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Although chemists knew about acid they did not know why acids acted as they did. One chemist reasoned that the particles that make up acids have short spikes. He claimed that these spikes were the reason for its pinching feeling on the skin.

Anton Lavoisier thought that oxygen was a necessary part of all acids. Oxygen means Former (or in Greek: to make sour). In 1810, a group of chemists though found out that it was hydrogen, not oxygen, which gave acids their characteristic pinching feeling.

Vinegar is an acidic liquid obtained from the fermentation of alcohol and used either as a condiment or a preservative. Vinegar may be sharp, rich, or mellow. It is made by combining sugary materials (or materials produced by hydrolysis of starches) with vinegar or acetic acid bacteria and air. The sugars or starches are converted to alcohol by yeast of the genus *Saccharomyces*, and the bacteria makes enzymes that cause oxidation of the alcohol (Johnston, 1997).

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Different Sources of Vinegar

Vinegar may be produced from almost any raw material which can be fermented to alcohol. Materials in common use include malt, wine, dilute alcohol, and fruit juices, particularly cider. Each of these raw materials gives the vinegar it produces its distinctive flavor, because the characteristic flavoring materials originally present are retained or modified during fermentation. Some acetic acid is made for industrial use by the fermentation of dilute alcohol. This process is economical only when a dilute acetic acid solution can be used without concentration, because there are cheaper chemical methods for producing concentrated acetic acid. Therefore when fruit juices or other sugar-containing materials are used, the sugar must first be fermented to alcohol. Usually there are wild yeast in the fruit juice which will bring about this change, but this yeast vary in type and each of them produces different flavoring materials. For this reason, in commercial practice the yeast to be added is selected for the agreeable flavor it imparts to the vinegar (Johnston, 1995).

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Cacao

Cacao is a tree of the American tropics whose seeds are the source of chocolates and cocoa (Grolier Encyclopedia, 1993). Chocolate was also reported to be obtained by grinding roasted cacao beans that have been removed from the shell. Cocoa is chocolate with much of the fat removed. The Spanish word cacao is a variation of the Nahuatl (an Aztec language) Cacahuatl, meaning "The seed". "Cocoa" is an English language corruption of cacao.

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Chapter 3

Research Design and Methodology

The study aimed to produce quality vinegar from the seed coating of ripe cacao. Specifically it determined whether or not quality vinegar can be produced from the seed coating of ripe cacao. It also determined the chemical properties of the vinegar produced in terms of pH, percentage acetic acid and density. It was hypothesized that there exists no significant difference as to pH, percentage acetic acid and density among the product and the two other commercial vinegar.

The One Way Analysis of Variants (ANOVA) set at .05 alpha level of significance was employed to test this null hypothesis.

To answer these problems and to test the hypothesis, the study followed a scheme consisting of four parts namely:

- (a) Preparation, (b) Fermentation, (c) Testing, and
- (d) Comparison.

Preparation consisted of the gathering of materials, extraction of the seed coating and pre-treatment to insure that microorganisms do not contaminate the seed coating.

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The second part consisted of the fermentation process of the seed coating and post-treatment of the substance produced.

Finding the chemical properties of the vinegar produced, such as pH, percentage acetic acid and density, fell under testing.

When testing if the vinegar produced had no significant difference from two other commercial vinegar, we compared their chemical properties using the One-way Analysis of Variance (ANOVA) and the Scheffe Test. This fell under comparison.

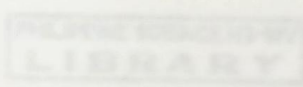
When performing this study we needed the following materials and equipment, as shown in Tables 1 and 2.

Methodology

Preparation

This part consisted of the gathering of materials, extraction of the seed coating and pre-treatment to insure that microorganisms do not contaminate the seed coating.

First, the cacao fruits were washed and cut. The seeds were then separated from the shell. After which the seed coating was removed by gently rubbing the seeds wrapped in a piece of cloth. The removed seed coating was placed in a clean basin and



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Table 1

Materials used in the Study

Materials	Description
1. Cacao seed coating	The substance that was fermented to produce the vinegar.
2. Sugar	This was used to add to the sweetness of the seed coating for a better fermentation.
3. Yeast	This contains enzymes that causes the breaking-up of glucose and fructose.
4. Phenolphthalein	This is an indicator that was used to find the percentage acetic acid.
5. pH Meter	This was used to find the pH of the vinegar produced.

Table 2

Equipment used in the Study

Equipment	Descriptions
1. Knife	This was used to cut the cacao fruits.
2. Strainer	This was used to separate the liquid from solid particles.
3. Measuring cup	This was used to measure the seed coating and other materials for an exact proportion.
4. 2 pieces of cloth	Clean previously boiled cloths used to extract the seed coating from the cacao seeds and was also used cover these extracts during fermentation.
5. Teaspoon	This was used to measure the yeast.
6. 2 Clean basins	This held the seed coating seeds and the juice produced by the seed coating.
7. 2 sterilized bottles	This contained the vinegar produced.
8. Graduated cylinder	This was used to measure the volume of the vinegar produced.
9. Digital balance	This was used to get the mass of the vinegar produced.
10. Erlenmeyer flask	This was used to hold the vinegar for titration.
11. Burette with stand	This was used during titration.

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measured by using a measuring cup. To every cup of seed coating we added 3 cups of water to make diluted juice from the seed coating. This mixture was soaked overnight. After soaking, we separated the solid particles from the juice by using the strainer. We then placed the juice in the other clean basin and measure it again using the measuring cup. To every 36 cups of diluted juice we added 8 cups of sugar. This new mixture was pasteurized at 60 degrees Centigrade for 20 minutes or at 73 degrees Centigrade for 15 minutes. After pasteurizing we let the mixture cool and then measured it again using the measuring cup. We added 2 teaspoons of yeast for every 36 cups of the mixture. After adding the yeast we covered the mixture with one of the previously boiled clean pieces of cloth for 20 minutes. The mixture was then distributed among five sterile bottles with tight corners.

Fermentation

This second part consisted of the Fermentation process of the seed coating and post-treatment of the substance produced.

We allowed the prepared mixture to ferment. While the mixture is fermenting, we made observations and took note of the number of days it took to finish the fermentation of the mixture

into vinegar. You can determine that the fermentation process is complete if the mixture smells like and tastes like vinegar. After the fermentation process, we filtered the vinegar and pasteurized it to kill the microorganisms (if ever there are any) before placing the vinegar produced in the sterilized bottles.

Testing

Finding the chemical properties of the vinegar produced such as pH, percentage acetic acid, specific gravity and density fall under Testing.

In finding the pH of the vinegar produced, we used the pH Meter. We tested five replicates for a more precise data. We got the mean of the pH of the five replicates and that will serve as the overall pH of the product.

In finding the percentage acetic acid we diluted 10 milliliters sample of the vinegar produced with recently boiled and cooled water until it appeared only slightly colored and we titrated it with 0.5 N alkali, using phenolphthalein as indicator. One mL 0.5 alkali = 0.04 g percentage acetic acid (HOAC).

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In determining the density of the vinegar produced we measured 10 g of the vinegar produced and measure the volume using a graduated cylinder. We determined the density using the formula:

$$\text{Density} = \text{mass per unit volume.}$$

These tests were also employed in determining the chemical properties of the two other vinegar.

Comparison

To test if the product has no significant difference from two other commercial vinegar in terms of pH, percentage acid, specific gravity, and density, we used the One-way Analysis of Variance (ANOVA) and the Duncan's Multiple Range Test, both set at 0.05 alpha, as statistical tools. This fell under Comparison.

One-way Analysis of Variance

The One-way ANOVA, set at 0.05 alpha, determined if there is significant difference in the pH, percentage acetic acid, specific gravity, and density of the product and two other commercial vinegar.

Statistical Data Analysis

This study had employed the mean and standard deviation as descriptive statistical tools. The One-way Analysis of Variance (ANOVA) and the Duncan's Multiple Range Test was employed as inferential statistical tools.

Mean

The mean was used to determine the average pH, percentage acetic acid, specific gravity, and density of the product from 5 preparations.

Standard Deviation

The standard deviation was used to determine the homogeneity of the data from their means.

One-Way Analysis of Variance

The One-Way ANOVA, set at 0.05 alpha, determined if there is significant difference in the pH, percentage acetic acid, specific gravity, and density of the product and two other commercial vinegar.

Scheffe Test

The Scheffe Test, set at 0.05 alpha, will be used as post-hoc multiple comparison tests.

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Chapter 4

Results

The study aimed to produce quality vinegar from the seed coating of ripe cacao. Specifically, it determined whether or not quality vinegar could be produced from the seed coating of ripe cacao. It also determined the chemical properties of the vinegar produced in terms of pH, percentage acetic acid and density. It was hypothesized that there exists no significant difference as to pH, percentage acetic acid and density among the product and two other commercial vinegar.

Feasibility of producing quality vinegar from the seed coating of ripe cacao. It was proven that cacao seed-coating can be used to produce vinegar but the quality of the resulting product was not comparable to commercial ones because there was a significant difference in the pH, percentage acetic acid and density between the product and the two commercial ones.

Length of fermentation. The fermentation of the vinegar was completed after one month.

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pH of the product and two commercial vinegar. The result of the study showed that the average pH of the product was 2.522. The average pH of the cane vinegar was 2.408, while that of the palm vinegar was 2.454.

Table 3 shows the data.

Percentage acetic acid of the product and two commercial vinegar. The result of the study showed that the average percentage acetic acid of the product was 4.304%. The average percentage acetic acid of the cane vinegar was 6.512%, while that of the palm vinegar was 6.976%.

Table 3 shows the data.

Density of the product and two commercial vinegar. The results of the study showed that the average density of the product was 0.871 g/mL. The average density of the cane vinegar was 0.8786 g/mL, while that of the palm vinegar was 0.8744 g/mL.

Table 3 shows the data.

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Table 3
pH, Percentage Acetic Acid and Density of the Product and two commercial vinegar.

Vinegar	Mean		
	pH	Percentage Acetic Acid(%)	Density(g/mL)
Product	2.522	4.304	.8710
Cane	2.408	6.512	.8786
Palm	4.454	6.976	.8744

Significant difference in the pH of the product and two commercial vinegar. The One-way ANOVA showed that there was a significant difference in the pH of the product and the two commercial vinegar, as reflected by the $F(12) = .002, p < .05$.

Table 4 shows the data.

Furthermore, the Scheffe test showed that there was a significant difference in the pH of the product and the cane vinegar as reflected by the significance of $.002, p < .05$. It also showed a significant difference in the pH of the product and the palm vinegar as reflected by the significance of $.044, p < .05$.

Table 5 shows the data.

Significant difference in the percentage acetic acid of the product and two commercial vinegar. The One-way ANOVA showed that there was a significant difference in the percentage acetic acid of the product and the two commercial vinegar, as reflected by the $F(12) = .000, p < .05$.

Table 4 shows the data.

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Table 4 One-way ANOVA showing the differences in the pH, percentage acetic acid and density of the product and two commercial vinegar.

Category	Source of Variations	Sum of Squares	df	Mean Square	F	Sig.
PH	Between Groups	3.289E-02	2	1.645E-02	7.877	.002
	Within Groups	1.688E-02	12	1.407E-03		
	Total	4.977E-02	14			
Percent HOAc	Between Groups	20.384	2	10.192	53.409	.000
	Within Groups	2.290	12	.191		
	Total	22.673	14			
Density	Between Groups	1.499E-04	2	7.247E-05	11.692	.007
	Within Groups	1.104E-04	12	9.200E-06		
	Total	2.553E-04	14			

Table 5 Test for the multiple comparison of the One-way ANOVA in Table 4.

Category	Vinegar Type	Vinegar Type	Mean Difference	Significance
PH	Product	Cane	.1140*	.002
	Product	Palm	6.800E-02*	.044
	Cane	Palm	-4.60E-02	.195
Percent HOAc	Product	Cane	-2.2080*	.000
	Product	Palm	-2.6720*	.000
	Cane	Palm	-.4640	.282
Density	Product	Cane	-7.6E-03*	.007
	Product	Palm	-3.4E-03	.248
	Cane	Palm	4.20E-03	.133

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Furthermore, the Scheffe test showed that there was a significant difference in the percentage acetic acid of the product and the cane vinegar as reflected by the significance of .000, $p < .05$. It also showed a significant difference in the percentage acetic acid of the product and the palm vinegar as reflected by the significance of .000, $p < .05$.

Table 5 shows the data.

Significant difference in the density of the product and two commercial vinegar. The One-way ANOVA showed a significant difference in the density of the product and two commercial vinegar, as reflected by the $F(12) = .007$, $p < .05$.

Table 4 shows the data.

Furthermore the Scheffe test showed that there was a significant difference in the density of the product and the cane vinegar as reflected by the significance of .007, $p < .05$. But the Scheffe showed that there was no significant difference in the density of the product and the palm vinegar as reflected by the significance of .248, $p < .05$.

Table 5 shows the data.

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Chapter 5

Findings, Conclusions and Recommendations

The study aimed to produce quality vinegar from the seed-coating of ripe cacao. Specifically, it sought answers to the following questions:

1. Can quality vinegar be produced from the seed-coating of ripe cacao?
2. What are the chemical properties of the product in terms of (a) pH, (b) percentage acetic acid and (c) density?
3. How long will the fermentation process last?
4. Is there a significant difference among the vinegar produced from cacao and two other commercial vinegar?

Based on these questions, it was hypothesized that there is no significant difference as to (a) pH, (b) percentage acetic acid and (c) density among the vinegar produced from cacao seed-coating and the two other commercial vinegar.

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Findings

Based on the data of the study, the following are the findings:

1. Quality vinegar cannot be produced from the seed coating of cacao because there was a significant difference in the pH, percentage acetic acid and density between the product and two commercial vinegar.

2. The product has a mean pH of 2.522, has a mean percentage acetic acid of 4.304% and a density of 0.871 g/mL.

3. The fermentation process lasted one month.

4.a. There was a significant difference in the pH of the product and the cane vinegar, which shows that the product is inferior to the cane vinegar in terms of pH.

4.b. There was a significant difference in the pH of the product and the palm vinegar, which shows that the product is inferior to the palm vinegar in terms of pH.

4.c. There was a significant difference in the percentage acetic acid of the product and the cane vinegar, which shows that the product is inferior to the cane vinegar in terms of percentage acetic acid.

4.d. There was a significant difference in the percentage acetic acid of the product and the palm vinegar, which shows that

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the product is inferior to the palm vinegar in terms of percentage acetic acid.

4.e. There was a significant difference in the density of the product and the cane vinegar, which shows that the product is inferior to the cane vinegar in terms of density.

4.f. There was no significant difference in the density of the product and the palm vinegar, which shows that the product is comparable to the palm vinegar in terms of density.

Conclusion

After the study was conducted and the results gathered and analyzed, we conclude that the vinegar produced was inferior to commercial ones because there was a significant difference in the pH, percentage acetic acid and density between the product and two commercial vinegar showing inferiority in the vinegar produced.

Recommendations

After conducting the study, the researchers recommend that the time for fermentation be lengthened. They also recommend that clay pots be used to contain the vinegar during fermentation instead of glass bottles.

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