Characterization of the Coffee Mucilage Fermentation Process Using Chemical Indicators: A Field Study in Nicaragua

SUSAN C. JACKELS AND CHARLES F. JACKELS

ABSTRACT: The recent “crisis” brought about by the collapse of the worldwide commodity coffee market has caused severe economic conditions for coffee producers in developing countries, including those of Central America. As a result, many coffee producers desire to improve the quality and consistency of their product to enter the specialty market. With the ultimate aim of assisting coffee producers in their quality control efforts, this study was designed to determine the feasibility of simple chemical measurements of the fermentation process on remote farms and to assess the potential of these measurements for assisting the producers in control and optimization efforts. Temperature, pH, and the concentrations of glucose, ethanol, and lactic acid were measured throughout the course of 7 coffee mucilage fermentation batches on 4 farms. In each batch, a pattern was observed in which the pH was initially in the range 5.5 to 5.7 and decreased sharply to about 4.6 as fermentation neared completion. Glucose concentration was seen to drop throughout the course of most batches, whereas either ethanol or lactic acid increased sharply near completion. The pH profile may prove useful in predicting the time of fermentation completion and in preventing over-fermentation of coffee mucilage.

Keywords: coffee, fermentation, field study, Nicaragua, pH

Introduction

By 2001, the collapsing world coffee market yielded inflation-adjusted prices only 25% of the 1960 level (Oxfam Intl. 2003). According to Brown (2004), this collapse, which had been caused by global oversupply from increased production, falling coffee demand, and an abundance of low-quality coffee, has given rise to serious social, environmental, and health problems in the producing nations. Among suggested remedies to this crisis are promotion of agricultural diversification, global promotion of coffee consumption, and support of coffee producers to secure specialty market premiums (Scholer 2004).

As of 1999-2000, the 30000 Nicaraguan coffee producers, with an average farm size of 3.2 ha, accounted for 26.5% of the country’s total exports (Varangis and others 2003). In Nicaragua, shade-grown, ripe coffee cherries (Coffee arabica) are picked daily and processed by the “wet process” (Castelein and Verachtert 1981), in which the outer skin and soft flesh are removed mechanically, and the remaining viscous, slippery mucilaginous layer is loosened from the parchment coffee by natural fermentation without added water (“dry fermentation”) and then removed by washing with water. The washed parchment coffee is then ready for drying and storage.

Traditionally, producers determine that mucilage fermentation is “complete” by manual inspection of the fermenting mass. Before completion, the intact mucilage layer is slippery, and the parchment coffee readily slides over itself. But at completion, the coffee is no longer slippery, and the mucilage layer is loosened and can be completely washed off. “Over-fermentation” (beyond that necessary to loosen the mucilage) is generally considered detrimental to coffee quality (Gibson and Butty 1975; Castelein and Verachtert 1981; Lo-pez and others 1989; Puerta-Quintero 1999, 2001), and its control is usually a component of quality and consistency improvement programs that are aimed at gaining access to specialty markets.

Contrary to earlier suggestions (Castelein and Verachtert 1981), recent studies of the fermentation process (Avallone and others 2001a, 2001b, 2002) are interpreted to indicate that the pectin-rich mucilage is degraded neither by endogenous pectolytic enzymes nor by pectolytic bacteria. Rather, it is suggested (Avallone and others 2001a) that physicochemical changes of the carbohydrate matrix, in concert with limited pectolysis, may be occurring. During the course of a single fermentation batch, several strains of aerobic bacteria, lactic-acid bacteria, and yeasts increased in number (Avallone and others 2001b). The microflora consumed simple sugars from the mucilage and produced significant amounts of acetic and lactic acids, resulting in lowered pH. Only low levels of ethanol (produced by yeasts) and other organic acids were detected, and the yeast population grew to significance only after 10 to 15 h of fermentation. Because it has been suggested that yeasts (Avallone and others 2001b) and the ethanol generated by them (Castelein and Verachtert 1981) may play some role in the degradation of coffee aroma and flavor due to over-fermentation, a control strategy could also include measures aimed at affecting the balance between bacteria and yeasts.

With the ultimate aim of assisting coffee producers in their quality control efforts, this study was designed to determine the feasibility of simple chemical measurements of the fermentation process on remote farms and to assess the potential of these measurements for assisting the producers in control and optimization efforts. We present the results of these measurements and discuss their implications toward an understanding and optimization of the fermentation process.

Materials and Methods

Portable laboratory measurements were made at each producer’s beneficio, the facility on the farm where coffee cherries are...
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pulped and the parchment coffee is fermented and washed. Because in some cases the benefits are remote and unelectrified, instrumentation must be battery-powered, and it must be possible to carry the equipment for a distance of 1 to 2 km. The laboratory was transported in a closed plastic container approximately 60 × 80 × 80 cm that could be carried on trails. Equipment included the following: battery-powered reflectometer (Model BQflex 2TM; Merck, Darmstadt, Germany); quantitative strip tests for pH, glucose, lactic acid, and ethanol (ReflectoquantTM strips; Merck); battery-powered thermometer (TraceableTM Flip-Stick; Control Co., Friendswood, Tex., U.S.A.); and wrist watch with a second-hand for timing. The laboratory also included volumetric plasticware for dilutions and measurements and utensils for sampling and transporting parchment coffee. Purified bottled water was obtained in Matagalpa, Nicaragua and carried to each site.

**Sampling**

Samples were collected from the masses of fermenting, pulped cherries placed by the producers in their fermentation tanks. Generally, the producers did not add water to these tanks, which were allowed to drain during fermentation. At sites A, B, and C, samples of the fermenting mass were collected from locations approximately 15 cm beneath its surface and 30 cm from the edge of the fermentation tanks, which were wooden, and approximately 70 cm deep, 70 cm wide, and 1 to 2 m long. At site D, which had much larger, tile-lined, concrete tanks (1.2 × 1 × 3 m), fermenting coffee was collected across the width of the tank from 3 points approximately 60 cm from 1 end and 15 to 30 cm deep, and these 3 portions were mixed in a bowl before proceeding with the analysis. In all cases, the sample used for analysis had a volume of 50 mL and contained approximately 110 to 120 beans. Any pulp or dried unpulped fruit found in the sample was excluded before proceeding with the analysis. The sample was mixed thoroughly with 50 mL purified water, and the tests were performed on the resulting aqueous suspension without further preparation.

**Measurements**

Reflectoquant test strips were used according to product instructions to make the following measurements for each sample: pH (range 4.0 to 9.0), glucose (1 to 100 mg/L), lactic acid (1.0 to 60.0 mg/L), and ethanol (20 to 200 mg/L). Dilutions were necessary in some cases to bring the concentration into the measurable range. Tests were performed in the following sequence immediately after sampling: pH, lactic acid, alcohol, and glucose. Glucose was tested last, as experience indicated that a delay of about 30 min between the addition of water and the measurement was required to allow its concentration to stabilize. The ambient temperatures at the beneficio and in the fermenting mass were also recorded.

In general, at each sampling time the data were collected for a single sample. However, in the case D-1, 3 separate samples were taken from the mixture collected as described previously and tested at each time, providing an indication of the data's precision. The standard deviation and coefficient of variance (CV) were calculated for each triplicate data point and then averaged over the duration of run D-1 for each of the species being measured. The average standard deviation of pH for run D-1 is 0.09, and the average %CVs for the other species are as follows: glucose (6.9%), lactic acid (12.2%), and ethanol (9.5%). These values can serve as an indication of the precision limitations due to both the analytical procedure and the heterogeneity of the fermenting mass.

At the time of measurement, qualitative observations were also made of the fermenting coffee, including the degree to which the mucilage layer remained adherent and slimy. The time at which the coffee “washed clean” of its mucilage in the sampling water was noted as the time when it appeared to the authors to be sufficiently fermented. This was not necessarily the time at which the producer would judge the fermentation to be “complete.”

**Fermentation pH**

As an illustration of the fermentation pH measurements, the results from batch D-1 are presented in Figure 1. Using the traditional method, the producer judged fermentation to be complete after approximately 20 h. The pH was relatively constant, in the range 5.5 to 5.7, during the first 15 h of fermentation and then dropped sharply, reaching 4.7 shortly after 18 h and 4.4 at 20 h. Because the final data point (at t = 20 h) was collected after the producer determined fermentation to be complete, the actual time of completion t4.6 was bracketed by these last 2 data points.

Each of the 7 fermentation batches exhibited a pH profile similar to that of Figure 1, and these data were examined to ascertain whether there was a common pH value that could be generally used as an indication of completion. Columns 2 through 5 of Table 1 show the sampling times and pH values that bracket completion for each fermentation batch. With the exception of batch B-1, the value pH = 4.6 falls within this interval and is assigned tentatively as a marker for completion of the process. In batch B-1, the value pH = 4.6 was achieved approximately 30 min after the producer had indicated fermentation was complete. Although the value pH = 4.7 would have been acceptable for B-1 and most of the other batches, it would have been somewhat early for run D-1 (under-fermentation). Because the consequences of under-fermentation can include small amounts of mucilage remaining on the dried parchment coffee, which can in turn lead to both clumping and growth of microorganisms, the value pH = 4.6 was selected as a choice that would err, if at all, slightly in the direction of over-fermentation. For each batch, t4.6, the time at which pH = 4.6, was determined by a linear interpolation of the data in Table 1. These interpolated values t4.6 appear in the last column of the table.

**Schedule**

During January and February 2004, a total of 7 fermentation batches were studied at 4 Matagalpa region coffee farms (designated as sites A-D). Sites A-C have between 2 and 15 ha of cultivated coffee at altitudes ranging from 700 to 930 m. Site D is a large farm of 210 ha at approximately 1200 m. In each case, the fermentation process was initiated by the producer when pulping had been completed, usually in the late afternoon. Sampling and testing were then begun immediately and continued at 3-h to 4-h intervals throughout the night, without any modification of the normal procedures of each farm. The next day, the fermentation process was halted and washing begun at the discretion of the producer, who made this judgment using traditional guidelines (discussed subsequently). A final set of measurements was made immediately after the producer had decided that fermentation was complete.

Producers determine when fermentation is “complete” by inserting a long straight object such as a tool handle or stick into the fermenting mass and subsequently removing it. Before completion of fermentation, the intact mucilage layer is very slippery, and the coffee beans readily slide past each other to fill in the hole as the tool is removed. At completion, however, the mucilage layer is no longer intact, and the beans are not slippery. They experience sufficient friction against each other to maintain the hole formed by the inserted tool for 1 min or longer. The fermentation process is then judged to be complete; washing is started as soon as feasible and completely removes the mucilage layer. (Fermentation does not actually halt at this point, but if uninterrupted, continues on to eventually degrade the quality of the parchment coffee.)

**Results and Discussion**
Although the producer-determined fermentation time of the batches varied from less than 10 to nearly 24 h, the pH decrease at completion generally occurred during the preceding 3-h to 4-h period. To facilitate comparison of these fermentation processes, a shifted time coordinate was defined as $t - t_{4.6}$: the total elapsed time in the fermentation tank minus the elapsed time at which pH = 4.6. By definition, this time coordinate is universally zero when pH = 4.6, negative prior to it, and positive subsequently. The pH profiles of the 7 batches are presented in Figure 2, where this shift of abscissa permits them to share a common "completion point." It is observed that, with this adjustment, the 7 curves are nearly overlaid near completion and are quite similar overall.

At the beginning of the fermentation process, the pH is at 5.5 or above and remains in this range until about 3 to 4 h before completion, when it begins to decrease linearly until at least 2 h after completion. In general, the curves in Figure 2 represent the entire fermentation process; processes that proceeded more rapidly begin at later (less negative) times on this scale. The single exception is batch D-2. For practical reasons, D-2 data collection could only begin after fermentation had already proceeded for approximately 20 h. At that point, the pH began its decrease as fermentation neared completion. Batch D-2 had been started at approximately $t - t_{4.6} = -24$ h and is, in fact, the longest fermentation process studied, even though there are no early data to present for the D-2 curve.

Examination of the data shows that pH can serve as a reliable gauge of the progress of fermentation, and the predictive ability of the pH measurements could be very useful on the farm. Regardless of how much time a particular fermentation may require or at what pH it may start, a pH value dropping below 5 indicates that fermentation will be complete within the next 2 h. Similarly, a pH of about 4.0 could be used to determine that the fermentation process had become complete approximately 2 to 3 h earlier. A fermentation that is much too extended would clearly yield pH values of 4.0 or below.

The producers often operate on a daily cycle in which the fermentation batch is started in late afternoon and is 1st checked for completeness when the producer begins work at dawn. Although this schedule is strongly recommended by the daily rhythm of farm life, it means that often the fermentation batch is not checked before approximately 11 to 12 h of fermentation. To avoid over-fermentation, it would be useful for the producer to know at this point (from a pH measurement) whether the batch has been ready for several hours and is perhaps becoming over-fermented. If, on the other hand, fermentation is incomplete at dawn, a pH measurement would be useful in planning the day's work by predicting when the washing of the coffee would begin. Thus, the ability to quantify fermentation progress would assist in both developing a more consistent product and increasing the efficiency of the farm operation.

The rapid pH decrease observed near fermentation completion (Figure 2) suggests that it would be useful to systematically study the completion of fermentation as a function of pH and determine the highest pH value that corresponds to the mucilage layer being ready to be washed from the parchment coffee. If use of pH measurements resulted in slightly shorter fermentation times, compared with those obtained traditionally, this information could be used to optimize the process and eliminate over-fermentation. Having identified pH as a generally useful indicator of fermentation progress, we now present data collected for other chemical species.

### Lactic acid

Lactic acid is generated as a by-product of the growth of certain bacteria during fermentation. In combination with other acids that can also be generated during this process, lactic acid is responsible for the decrease in pH as fermentation progresses. Figure 3 presents the total lactate (acid plus anion) concentration for each batch studied. In all cases, the lactate concentration starts near zero and increases during the fermentation process. Near completion ($t - t_{4.6} = 0$), the batches fall into 2 groups: batches D-1, D-2, and D-3 show

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**Table 1—pH data taken during the time course of fermentation**

<table>
<thead>
<tr>
<th>Fermentation batch</th>
<th>Data before completion</th>
<th>Data after completion</th>
<th>Total time to completion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t$ (h)</td>
<td>pH</td>
<td>$t$ (h)</td>
</tr>
<tr>
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<td>11.5</td>
</tr>
<tr>
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<tr>
<td>C-1</td>
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<td>5.0</td>
<td>15.5</td>
</tr>
<tr>
<td>D-1</td>
<td>18.4</td>
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</tr>
<tr>
<td>D-2</td>
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<td>5.1</td>
<td>24.0</td>
</tr>
<tr>
<td>D-3</td>
<td>15.8</td>
<td>5.4</td>
<td>19.1</td>
</tr>
</tbody>
</table>

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2Last data collected before determination by the producer that fermentation was complete.
3Collected as soon as possible after determination by the producer that fermentation was complete.
4Elapsed time from initiation of fermentation until value of pH = 4.6 was achieved.
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Exponentially or super-linearly increasing lactate concentrations, whereas the other 4 exhibit more nearly linear increases. Because in all cases pH (a logarithmic quantity) is decreasing linearly near completion (Figure 2), the hydrogen ion and therefore the acid concentration must be generally increasing exponentially. The data represented in Figure 3 suggest that the observed pH changes at site D may be driven primarily by the changes in lactic acid concentration. For example, the concentration of approximately 150 mg/L of lactic acid (pKₐ = 3.86 [Lide 2004], molecular weight = 90.08) at completion would yield pH = 3.4, neglecting all other interactions. At the other sites, increases in other acids must be responsible. Acetic acid, which was not measured here, is a likely possibility in those cases (Avallone and others 2001b). Controlled studies are planned to determine which of the variable conditions may be responsible for the dominance of lactic acid production during fermentation.

Ethanol

Ethanol can be produced by yeasts, which thrive at the lower pH values achieved in the latter stages of fermentation (Avallone and others 2001b). Because the ethanol would be expected to be largely removed by the washing, it is not clear if it serves only as an indicator of yeast activity (and perhaps over-fermentation) or if it is actively involved in the chemical transformations leading to the presence of the undesirable volatile esters associated with over-fermentation (Bade-Wegner and others 1997). Figure 4 presents the measured ethanol concentrations for this study, which generally begin to increase during the last 5 h of fermentation. The batches labeled A-1, B-1, and C-1 have ethanol concentrations at completion that are above 100 mg/L, having begun to increase exponentially or at least super-linearly 1 to 2 h prior to it. Although the other batches exhibit ethanol concentrations that increase more gradually (and linearly) before completion, it is noted that batch A-2 exhibits a very sharp ethanol concentration increase shortly thereafter. The sharply increasing rates of ethanol concentration in some cases may signal the point at which yeasts are beginning to flourish. To the degree that yeast fermentation is underrun-able and other acids must be responsible. Acetic acid, which was not measured here, is a likely possibility in those cases (Avallone and others 2001b). Controlled studies are planned to determine which of the variable conditions may be responsible for the dominance of lactic acid production during fermentation.

Fermentation temperature

Because microbial growth releases heat and the rate of fermentation due to either enzymatic or microorganismic mechanisms should be favored by temperature increases, rapidly increasing fermentation temperature could be an indication of very rapid or "run-away" fermentation. Figure 5 exhibits the temperature of the fermentation mass for the 7 runs reported here. Under a wide variety of conditions, the fermentation temperature is maintained within the range 21 °C to 23 °C before completion. Only in cases A-1 and B-1 did the temperature begin to increase rapidly after (and even before) completion. Even though the degree of tank insulation and the size of the batches varied considerably, it was observed that the temperatures did not vary greatly. The night air temperatures varied considerably among the 7 batches, with the overnight low temperatures spanning the range 17 °C to 21 °C. In general, the fermentation temperatures were maintained at 1 °C to 4 °C above that of the ambient air. Of all the runs, only batch A-1 displayed a significant increase in T_mass - T_air, with the temperature of the fermentation mass increasing to 6 °C or more above that of the air. Although increasing temperature might serve in a particular case as an indication of a "run-away" excessive fermentation, temperature profiles do not generally change in a way that would make them useful diagnostically for fermentation progress.

It was noted that the cherries in batch A-1 were not as well sorted as in the other cases, and that the pulper had not been as well adjusted as otherwise seen. The result of these differences was that

**Figure 3—Total lactate concentration (mg/L) during coffee mucilage fermentation**

**Figure 4—Ethanol concentration (mg/L) during coffee mucilage fermentation**
the fermentation mass contained more over-ripe dark red cherries ("seccas") and more pieces of skin and shreds of fruit than did the other batches. These differences are suggestive but have not been proven to have influenced the nature of the fermentation in a way that would account for the temperature behavior.

Overall fermentation process

From the field data collected in this study, it is clear that the coffee mucilage fermentation process on remote farms is amenable to on-site study. The overall results obtained from the 7 fermentation batches are consistent among themselves and with previous results obtained in the laboratory using more sophisticated analytical instrumentation (Avallone and others 2001b). The pH decreases as fermentation proceeds and microbial action builds up concentrations of acids. The glucose concentration decreases as it is consumed by microorganisms during fermentation. Finally, the concentrations of lactic acid and ethanol are seen in some cases to increase sharply as fermentation progresses toward completion.

The robustness of specific conclusions drawn here is strengthened by the considerable variation among the processes studied. The 7 batches ranged in size from approximately 60 to 2500 L. The fermentation tanks were large tile-angled concrete tanks at site D, but were smaller and made of wood at the other 3 sites. At site D, the cherries were moved through a large, heavily mechanized beneficio in a very large stream of water both before and after pulping, whereas at the other 3 sites the amount of water used in transporting the cherries varied from very little to a modest flow. All sites used the same traditional means of determining completion of fermentation, and all of them used prodigious amounts of water to wash the parchment clean after fermentation and before sun-drying commenced.

Both lactic acid (Figure 3) and ethanol (Figure 4) arise as products of the fermentation. These concentrations remain low during the earliest stages of fermentation and then increase near completion. Because in the presence of glucose, lactic acid is generated prodigiously by lactic acid–producing bacteria and ethanol by yeasts, an exponential growth in either substance would be an indication that the corresponding microorganism is flourishing. In Figure 3 it is seen that only in batches D-1, D-2, and D-3 is the lactic acid concentration growing rapidly at completion, suggesting that lactic acid bacterial growth may be dominant at those sites. Similarly, in Figure 4 it is seen that, in batches A-1, B-1, and C-1, ethanol concentration is growing rapidly at completion. These differences suggest that the fermentation batches may differ in the nature of the dominant process (yeasts or lactic acid bacteria) at completion. More work is needed to test this hypothesis and to identify the variables that are involved.

Conclusions

The most important conclusion reached in this study is that chemical measurements in the field are feasible, as demonstrated on the coffee farms near Matagalpa. Data were obtained that can serve the producers as diagnostic and predictive tools as well as supporting on-site controlled studies of fermentation. The results obtained are also consistent with the framework provided by previous laboratory biochemical studies. Systematic measurement of pH alone can predict when fermentation will be complete and can be an indicator of when over-fermentation may be occurring: the value of pH = 4.6 was observed to be an indication of completion in the batches studied. None of the other quantities measured here exhibited profiles possessing such general predictive ability. Future studies are planned to correlate coffee quality as determined by cupping laboratories with control of fermentation and the predominance of lactic acid or lack thereof.

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