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Influence of Fruit Variety, Harvest Technique, Quality Sorting, and Storage on the Native Microflora of Unpasteurized Apple Cider

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ABSTRACT

Apple variety, harvest, quality sorting, and storage practices were assessed to determine their impact on the microflora of unpasteurized cider. Seven apple varieties were harvested from the tree or the ground. The apples were used fresh or were stored at 0 to 4°C for ≤5 months and were pressed with or without quality selection. Cider yield, pH, Brix value, and titratable acidity were measured. Apples, postpressing apple pomace, and cider samples were analyzed for aerobic bacteria, yeasts, and molds. Aerobic bacterial plate counts (APCs) of ciders from fresh ground-picked apples (4.89 log CFU/ml) were higher than those of ciders made from fresh, tree-picked apples (3.45 log CFU/ml). Quality sorting further reduced the average APC to 2.88 log CFU/ml. Differences among all three treatment groups were significant ($P < 0.0001$). Apple and pomace microbial concentrations revealed harvest and postharvest treatment-dependent differences similar to those found in cider. There were significant differences in APC among apple varieties ($P = 0.0001$). Lower counts were associated with varieties exhibiting higher Brix values and higher titratable acidity. Differences in APC for stored and fresh apples used for cider production were not significant ($P > 0.05$). Yeast and mold counts revealed relationships similar to those for APCs. The relationship between initial microbial load found on incoming fruit and final cider microbial population was curvilinear, with the weakest correlations for the lowest apple microflora concentrations. The lack of linearity suggests that processing equipment contributed to cider contamination. Tree-picked quality fruit should be used for unpasteurized cider production, and careful manufacturing practices at cider plants can impact both safety and quality of the final product.

Before 1990, few disease outbreaks caused by bacterial pathogens were associated with consumption of unpasteurized juice. The pH of apple cider, orange juice, and other acidic juices was considered sufficiently low to kill or inhibit growth of pathogens. Since 1991, however, a number of disease outbreaks have been attributed to the consumption of unpasteurized juices *(2, 4–6, 12, 24).* Among these outbreaks, disease caused by *Escherichia coli* O157:H7 is of particular concern because of its low infectious dose and frequent association with childhood kidney failure caused by hemolytic uremic syndrome *(3, 13).* In exploring the physiological basis for the survival of these bacteria in acidic juices, several laboratories have found extremely acid-tolerant strains of *E. coli* O157:H7 *(1, 10, 15–19).* Storage at low temperature, even in highly acidic environments, prolonged the viability of contaminating bacteria.

In response to these outbreaks, the U.S. Food and Drug Administration (FDA) issued a regulation requiring the implementation of hazard analysis critical control point (HACCP) programs by juice manufacturers *(26).* The HACCP plan must identify the potential hazards in the process and the critical control points used to control the hazards. The regulation also requires that juice manufacturers implement a process capable of reducing pertinent pathogens by 100,000-fold (5 log units).

As the first step toward the establishment of an HACCP plan, the potential hazards related to the production of apple cider must first be clearly understood. Although contaminated fruit could be responsible for the outbreaks, it is unclear whether the outbreaks were the consequence of contaminated fruit or of hazards introduced into the cider-processing system. Without clear knowledge of sources of contamination, points of hazard introduction into production or the processing chain, or other risk factors, the establishment of an HACCP plan is difficult.

Unfortunately, the sporadic nature of the outbreaks and the low likelihood of finding human pathogens on fruit contribute to the difficulty in establishing conclusive cause and effect evidence *(20).* Therefore, other means of estimating the likelihood and levels of microbial hazards are required to establish the impact of various practices on food safety. Although aerobic plate counts are not relevant for public health per se, the overall concentrations of microbes, particularly when higher than normal or when increasing over time and production for a particular food, can reflect the

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overall exposure of foods to environmental microbiological sources and the effectiveness of sanitation practices, which are directly related to public health *(9, 11, 21, 22).* The relationship of aerobic bacteria on or in apples and in cider may also reflect sanitary practices in orchards, during harvesting, or during transport to the processing facility.

To further understand factors that may contribute to outbreaks, the FDA performed a multistate field study in 1997 on unpasteurized apple cider *(25).* Although this study provided a general assessment of sanitation effectiveness in small cider plants, it did not assess concentrations of native microflora occurring on fruit or systematically examine the factors that might lead to contamination of the fruit, the processing plant, or the (cider) product.

Although several investigators have determined the concentrations of naturally occurring microflora in commercial ciders produced under various conditions *(7, 8, 23),* there has not been a systematic evaluation of the relationships among apple quality, apple variety, harvesting technique, and concentrations of native microflora in the cider. In studies conducted on apple cider manufactured in Connecticut, the low *E. coli* concentrations were similar to those found in the FDA survey, but no information on indigenous microflora concentrations was provided *(8).*

Senkel et al. *(23)* provided background concentrations of microflora found on apples used for production by several Maryland cider processors. Populations on incoming apples, including ground-harvested apples, varied from a 5.59 log CFU/g to <3.40 log CFU/g. For apples used by processors who excluded ground-harvested apples, the highest incoming aerobic population was 4.76 log CFU/g. Despite the wide difference in initial microbial populations in these apples, the authors noted no increase in final aerobic population in cider made from ground-harvested apples.

In a more recent study on apple cider production in Iowa, Cummins et al. *(7)* reported concentrations of aerobic microflora on incoming apples similar to those in the Maryland study. Other factors, such as apple variety, the extent of culling, the effect of culling on microfloral concentrations in cider, and treatment of the incoming fruit were not investigated.

To better assess the sources influencing the safety of unpasteurized cider, in this study we used relatively controlled commercial production conditions to compare the effects of fruit variety, harvest technique, quality sorting, and storage on the native microflora on apples and in apple cider.

MATERIALS AND METHODS

Harvest and storage. Apples of seven varieties, McIntosh, Gala, Golden Delicious, Red Delicious, Red Rome, Fuji, and Granny Smith, were procured from local sources in the Placerville, Calif., area. All apples were manually harvested. Tree-harvested fruit was either processed immediately or placed into cold storage at 0 to 4°C for up to 5 months. Ground-harvested fruit was processed separately. No ground-harvested fruit was stored. To determine the effect of apple quality on cider production, some lots of tree-harvested fruit were sorted and culled prior to processing. Sorting and culling was performed by experienced workers, according to U.S. Department of Agriculture standards for U.S. Cider Grade apples. U.S. Cider Grade apples are free from decay, worm holes, and internal breakdown; culls are apples that fail to meet U.S. Cider Grade requirements. Apples considered U.S. Cider Grade and above were used for selected batches of cider. Ground-harvested fruit was not culled prior to being processed into cider.

Apple cider production. Cider was produced in a small commercial facility as described previously *(14).* The facility has a peak production capacity of 757 liters (200 gal) of cider per hour using a five-person crew. During the studies, each production run utilized approximately 85 kg (188 lb) of fruit. Apples were weighed prior to each production run, and cider and pomace were weighed after production. Yields were calculated as the percentage of juice extracted from apples (wt/wt). None of the cider produced during the study was used for human consumption.

At the processing plant, apples were loaded directly onto an elevator without washing or other treatment and then macerated using a hammer mill containing a 1.27-cm (0.5-in.) sizing screen. The wet pomace (not yet pressed for juice extraction) was collected in a stainless steel hopper. In some cases, the wet pomace was mixed for 10 min before collection for analysis by circulating it with a pump. Wet pomace was collected from tree-picked apple trials only. The pomace was then transferred to a vertical rack-and-cloth cider press. The cider was extracted at a maximum pressure of 121 kg/cm^2 (1,720 psi). Expressed cider was collected in a covered stainless steel holding tank and then pumped to a larger stainless steel bottling tank. Cider held in the holding and bottling tanks was stirred before samples were collected for microbiological and chemical analyses. After pressing, dry pomace was thoroughly mixed before samples were collected for analysis.

Preliminary data indicated that an aerobic plate count standard deviation of approximately 1 log unit could be expected on individual apples in control groups. Computer simulations were performed to determine how many composites of six apples were necessary to obtain 70% power of detecting a 1-log difference in treatment effect. Four composites of six apples were found to provide sufficient power. The microbial concentrations from each composite were log transformed, and the four values were averaged.

Microbiological profiles. Incoming apples were sampled for microbiological analysis as described by Keller et al. *(14)* by collecting four groups of six randomly selected apples. Each composite was pureed in a 4-liter blender using an equal mass of 0.1% peptone (wt/wt) (Difco, Becton Dickinson, Sparks, Md.). Serial dilutions were made using 0.1% peptone. Pour plates were prepared by transferring 1 ml of appropriate dilutions to petri dishes and adding approximately 20 ml of previously tempered (45°C) plate count agar (Difco, Becton Dickinson) and swirling to mix thoroughly. For aerobic plate counts (APCs), petri dishes were aerobically incubated for 48 h at 32°C. The temperature of the apple puree was monitored after blending to ensure there was no increase (+2°C) over ambient temperature during blending. For some runs, four samples of wet pomace (approximately 50 ml in volume) were withdrawn at the pomace hopper prior to cider extraction. Wet pomace samples and apple samples were processed in the same way. Four samples (approximately 50 ml in volume) of dry pomace were withdrawn from press cloths after pressing. Dry pomace samples were diluted 10-fold by weight, then processed in the same ways as the apples. Triplicate cider samples (25 ml each) were removed from the collection tank after stirring. Pomace and cider samples were plated as described in Keller et

TABLE 1. *Influence of harvest method, culling, and storage on total aerobic bacterial plate counts (APCs) on apples, in dry apple pomace (cider expressed), and in apple cider*

Harvest and postharvest treatment	Sample type	Mean APC (log CFU/g or CFU/ml)							Pooled SD[a]
		Fuji	Gala	Golden Delicious	Granny Smith	McIntosh	Red Delicious	Red Rome	
Fresh, tree harvested, culled	Apples	2.21	2.06	1.60	1.47	1.40	2.97	1.72	0.59
	Pomace	3.87	2.39	2.99	2.80	2.49	4.01	2.28	0.73
	Cider	3.40	2.61	1.90	2.75	2.27	3.14	2.23	0.64
Fresh, tree harvested, unculled	Apples	2.33	2.21	2.11	2.54	2.32	1.74	1.74	0.53
	Pomace	4.00	2.41	3.82	3.46	3.30	3.88	4.58	0.81
	Cider	3.93	2.65	3.57	3.32	2.47	3.77	3.85	0.53
Fresh, ground harvested, unculled	Apples	5.81	5.63	4.53	5.01	4.46	4.86	4.24	0.51
	Pomace	6.00	6.23	5.48	5.77	4.94	5.90	4.90	0.30
	Cider	5.32	5.43	4.94	5.11	4.19	5.03	4.67	0.14
Stored, tree harvested, culled	Apples	2.72	1.69	2.12	1.77	1.96	3.30	2.28	0.45
	Pomace	3.43	3.56	2.91	2.99	3.94	5.24	3.58	0.73
	Cider	2.89	2.67	2.36	2.49	3.28	4.33	3.50	0.41
Stored, tree harvested, unculled	Apples	3.45	3.26	2.71	2.29	1.64	2.53	3.18	0.46
	Pomace	4.42	4.29	3.95	3.04	3.82	3.98	3.94	0.62
	Cider	3.59	3.72	3.51	2.91	3.57	3.27	3.80	0.45

[a] Pooled across variety within treatment group.

al. *(14).* For yeast and mold populations, appropriate apple puree, pomace, or cider samples were diluted, and 1-ml aliquots were plated onto Yeast and Mold Petrifilm (3M, Minneapolis, Minn.). Petrifilm plates were incubated aerobically at 25°C for 5 days.

Chemical analysis. Brix values, pH, and titratable acidity were determined for all cider samples. A standard combination pH electrode (Fisher Scientific, Pittsburgh, Pa.) was used for pH measurements. Brix was determined using a standard handheld refractometer (Fisher Scientific). Titratable acidity was determined by titration of 10 ml of cider to a pH end point of 8.2 using 0.1 N NaOH (Fisher Scientific). Titratable acidity was calculated as the percentage of malic acid (%TA).

Cleaning and sanitation. Cleaning and sanitizing were conducted before the first trial each day, after each trial, and at the end of the production day. Cleaning and sanitizing procedures for the equipment and facility were described previously *(14).* The procedure consisted of an initial potable water rinse, followed by spray washing with FoamClor (Zep Company, Atlanta, Ga.) at 45°C, a second potable water rinse, then a treatment with Zepamine (a quaternary amine disinfectant; Zep Company) at pH 7.0 to 7.7. Zepamine was left in place for 5 min and then rinsed off with potable water before continuing production. At the end of each production day, Zepamine was left in contact with the equipment overnight. Excess pomace on press cloths was removed with potable water. The cloths were then washed in a commercial washer with commercial chlorine bleach and dried in a commercial drier. Press cloths were stored in a dry closed plastic container between uses.

Statistical analysis. All statistical analyses and computer simulations were performed using PC SAS (SAS Systems, Cary, N.C.) version 8.2. Data were grouped according to harvest and postharvest conditions into one of five groups: tree-picked culled, tree picked, ground harvested, stored tree-picked culled, and stored tree picked. The five harvest and postharvest conditions were treated as a one-way structure in the analyses. Variables contributing to the observed variance in cider pH, Brix, %TA, and yield were analyzed using the SAS MIVQUE variance components procedure. Paired comparisons between means in analyses of variance (ANOVAs) were made using the Ryan-Einot-Gabriel-Welsch multiple comparisons procedure at the $\alpha = 0.05$ level. Paired comparisons in analyses of covariance (ANCOVAs) were made using least significant difference *t* tests. Residuals from all models were found to be normally distributed for parametric analyses using the Wilk-Shapiro test.

RESULTS AND DISCUSSION

Impact of harvest practices, storage, and culling on concentrations of aerobic bacteria, yeasts, and molds. A key objective of this investigation was to test the hypothesis that cider from ground-harvested apples is more contaminated with microorganisms than is cider from tree-harvested fruit. Composite data for aerobic bacterial populations in cider, pomace, and apples for the five treatment groups by variety are given in Table 1. Microbial populations on apples, in pomace, and in cider were higher for all varieties when apples were harvested off the ground. To further compare the effect of harvest practice, quality sorting, and storage, data from all apple varieties were pooled for a one-way ANOVA (Fig. 1). Treatment group means were compared using the Ryan-Einot-Gabriel-Welsch multiple range test. The overall trends for apples, dry pomace, and cider were similar: elevated aerobic bacterial concentrations were found for ground-harvested apples. In the final cider, the average APC for all pooled varieties in the ground-harvested group was 4.89 log CFU/ml compared with 3.45 log CFU/ml for the fresh tree-picked unculled group and 2.88 log CFU/ml for the fresh tree-picked culled group. Pairwise differences between these three groups were significant ($P < 0.001$).

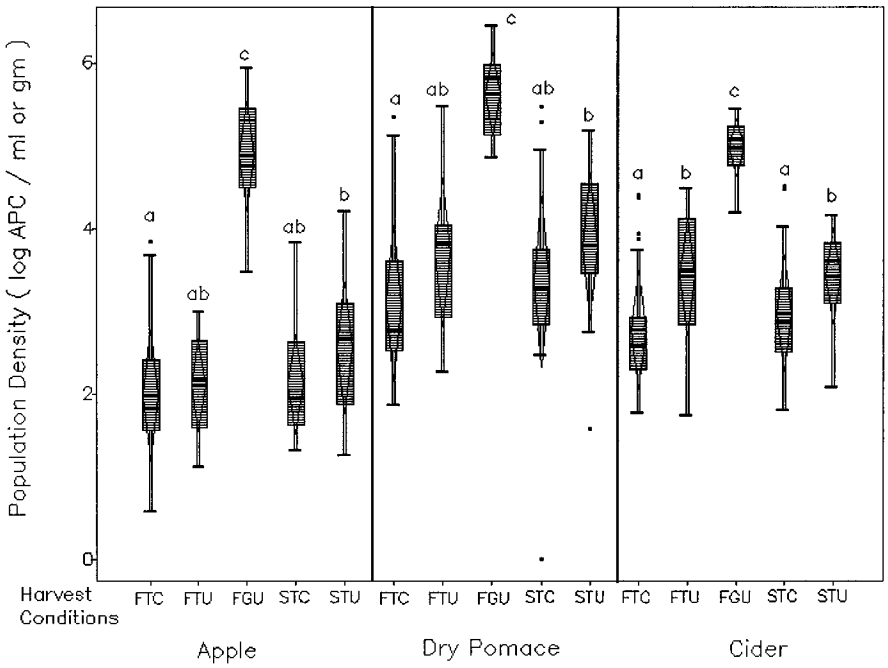

FIGURE 1. *Differences in Tukey box plots of APCs of pooled apple varieties based on harvest conditions, storage, and culling. Means sharing a letter were not significantly different at the $\alpha = 0.05$ level. FTC, fresh, tree harvested, culled; FTU, fresh, tree harvested, unculled; FGU, fresh, ground harvested, unculled; STC, stored, tree harvested, culled; STU, stored, tree harvested, unculled.*

Culling did not produce a significant effect on apple and pomace APCs but did result in a small but significant difference ($P < 0.05$) on cider APCs (Fig. 1). This result supports the hypothesis that visibly damaged fruit is more likely to be contaminated than is undamaged fruit. However, storage did not have a significant effect ($P > 0.05$) on APCs for culled or unculled fresh fruit. The storage time utilized here may have not been sufficient to produce larger changes in aerobic bacterial populations, or the types of microorganisms present may have changed while the total numbers remained the same.

Yeast and mold populations were also examined for all varieties and treatment groups, and results were similar to those for aerobic bacterial populations except that mean differences between treatment groups were smaller (Fig. 2).

Impact of harvest practices, storage, and culling on cider yield. Cider yield for all varieties and treatment conditions for each variety are shown in Table 2. Cider yield was expected to vary based on the variety and the condition of the apples. There were significant effects of treatment, variety, and treatment × variety on yield ($P < 0.0001$). Among varieties tested, Gala and Red Rome produced the lowest yields with means of 54 and 57%, respectively. These means were significantly different from each other ($P < 0.05$) and from the remaining varieties, whose mean yields ranged from 61 to 64%. Averaging across varieties, ground-harvested apples had the lowest mean yield at 57%. This mean was significantly different ($P < 0.0004$) from that for tree-harvested apples, with a mean yield of 61%. Culling did not have a significant effect on cider yield ($P > 0.05$).

Impact of apple variety. Significant differences were observed in total aerobic bacterial populations among apple varieties that were not explained by harvest method, cull-

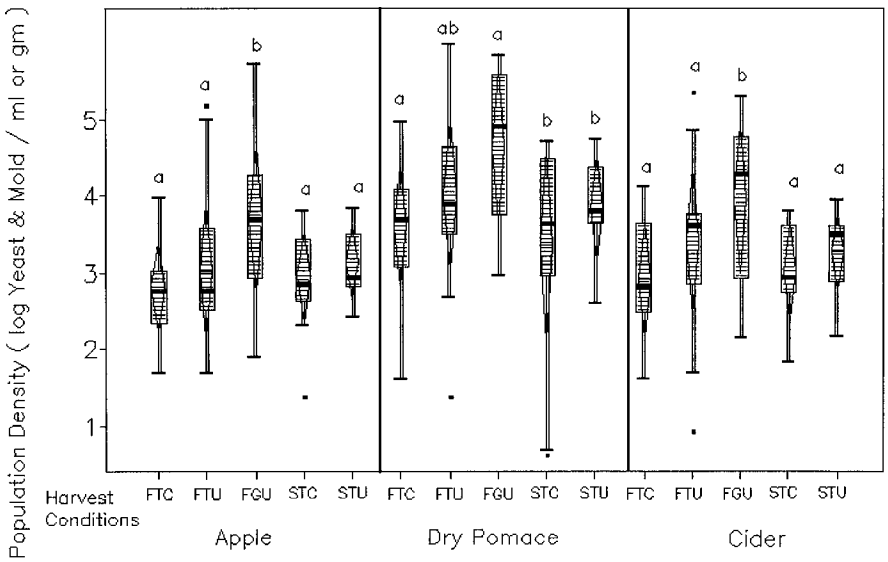

FIGURE 2. *Differences in Tukey box plots of yeast and mold populations in pooled apple varieties based on harvest conditions, storage, and culling. Means sharing a letter were not significantly different at the $\alpha = 0.05$ level. FTC, fresh, tree harvested, culled; FTU, fresh, tree harvested, unculled; FGU, fresh, ground harvested, unculled; STC, stored, tree harvested, culled; STU, stored, tree harvested, unculled.*

TABLE 2. *Cider yield based on fruit variety and treatment group*

Treatment group[a]	Mean cider yield (%)							
	Fuji	Gala	Golden Delicious	Granny Smith	McIntosh	Red Delicious	Red Rome	Pooled SD[b]
FTC	57.3	52.7	62.6	60.2	63.0	60.1	56.5	0.03
FTU	59.4	50.1	63.4	59.6	63.2	63.5	53.3	0.02
FGU	54.6	56.4	59.7	56.1	58.3	58.8	55.9	0.02
STC	63.6	54.2	59.3	65.8	60.2	59.5	57.9	0.02
STU	65.1	56.8	61.0	64.4	60.9	59.1	59.0	0.01

[a] FTC, fresh, tree harvested, culled; FTU, fresh, tree harvested, unculled; FGU, fresh, ground harvested, unculled; STC, stored, tree harvested, culled; STU, stored, tree harvested, unculled.
[b] Pooled across variety within treatment group.

ing, and storage. Consequently, cider pH, Brix, and %TA were examined for all varieties in each treatment group (Table 3). All three variables had highly significant overall ANOVA *P*-values for the effects of apple variety ($P < 0.0001$), treatment group ($P < 0.0003$), and variety × treatment group ($P < 0.0001$) interactions.

The strong association between apple variety and treatment condition within these three physicochemical characteristics suggests an interaction of pH, Brix, and %TA on the microbial populations found on apples and in cider. This relationship is shown in Table 4. Here, pooled data from all tree-harvested varieties are arranged according to mean values. In general, apple varieties with lower %TA, higher pH, and higher Brix had higher aerobic bacterial populations both on incoming fruit and in the finished cider. Of the apple varieties tested, Fuji and Red Delicious tended to have highest bacterial concentrations, lowest %TA, highest pH, and highest Brix. At the opposite end of the ranges were Granny Smith and McIntosh, with the lowest bacterial concentrations, highest %TA, lowest pH, and lowest Brix.

To further explore the relationship between intrinsic apple factors and cider microbiology, cider APCs were modeled using an ANCOVA. Brix, pH, %TA, and yield were utilized as covariates, and the five treatment groups and the apple varieties were utilized as categorical factors. Examination of cider APC data, using ANCOVA models, indicated that differences in cider APCs between the culled or unculled fresh and the culled or unculled stored categories were not significant ($P = 0.67$ and 0.58 for culled and unculled, respectively). Consequently, data from fresh and stored apples were pooled for subsequent analyses. The final model for cider APCs (4 df, $r^2 = 0.67$) included three treatments (tree-harvested culled, tree-harvested unculled, and ground harvested) with Brix and %TA as covariates. The coefficients for Brix and %TA were 0.127 (SE = 0.31, $P = 0.0001$) and −1.015 (SE = 0.364, $P = 0.0061$), respectively. There were no significant interactions ($P > 0.05$) among any of the model factors. These analyses reaffirm the relationship between the intrinsic physicochemical parameters of each apple variety and cider APC.

TABLE 3. *Physical characteristics (means) of cider based on fruit variety and treatment group*

Variable	Treatment group[a]	Fuji	Gala	Golden Delicious	Granny Smith	McIntosh	Red Delicious	Red Rome	Pooled SD[b]
pH	FTC	3.91	3.86	3.64	3.42	3.45	4.10	3.63	0.02
	FTU	3.85	3.86	3.62	3.44	3.45	4.10	3.55	0.05
	FGU	3.88	4.04	3.66	3.54	3.56	4.18	3.73	0.03
	STC	3.81	4.02	3.83	3.42	3.48	3.97	3.46	0.01
	STU	3.97	3.90	3.81	3.49	3.48	3.97	3.55	0.04
Brix	FTC	18.0	14.1	12.9	12.5	11.6	12.5	12.9	0.8
	FTU	16.3	14.6	12.5	13.1	11.8	12.7	14.5	0.8
	FGU	17.8	14.6	13.4	13.5	12.2	14.4	12.8	0.5
	STC	16.6	15.2	13.5	12.1	13.1	14.0	14.4	0.3
	STU	15.1	14.4	12.7	14.4	11.3	14.4	12.4	0.8
%TA	FTC	0.47	0.30	0.37	0.55	0.61	0.23	0.44	0.03
	FTU	0.47	0.29	0.38	0.67	0.62	0.19	0.52	0.02
	FGU	0.42	0.21	0.38	0.57	0.56	0.21	0.36	0.01
	STC	0.33	0.26	0.31	0.52	0.48	0.19	0.41	0.04
	STU	0.31	0.24	0.29	0.56	0.46	0.16	0.41	0.04

[a] FTC, fresh, tree harvested, culled; FTU, fresh, tree harvested, unculled; FGU, fresh, ground harvested, unculled; STC, stored, tree harvested, culled; STU, stored, tree harvested, unculled.
[b] Pooled across variety within treatment group.

TABLE 4. *Effect of variety and physical parameters on aerobic bacterial populations on apples and in cider*

	Mean values for each apple variety[a]			
	≥2.5 log CFU/g	2.5–2.3 log CFU/g		≤2.3 log CFU/g
APC, apples	Fuji A	Red Delicious AB Gala AB		Red Rome B Golden Delicious B Granny Smith B McIntosh B
	≥3.3 log CFU/ml	3.3–2.8 log CFU/ml		≤2.8 log CFU/ml
APC, cider	Red Delicious A Fuji A	Red Rome AB Golden Delicious AB Gala AB		Granny Smith B McIntosh B
	≤0.25	0.24–0.32	0.33–0.45	≥0.46
%TA	Red Delicious A	Golden Delicious B Gala B	Red Rome C Fuji C	Granny Smith D McIntosh D
	≥4.00	3.99–3.80	3.79–3.51	≤3.50
pH	Red Delicious A	Fuji B Gala B	Golden Delicious C	Red Rome D Granny Smith D McIntosh D
	≥15.0	14.9–13.9	13.8–12.8	≤12.7
Brix	Fuji A	Gala B	Red Rome C Red Delicious C Golden Delicious C	Granny Smith CD McIntosh D

[a] Values are means for tree-harvested apples in all treatment groups. Different letters within each parameter group indicate significant differences ($P < 0.05$).

Use of this model allows the adjustment of means of cider APC for the influence of %TA and Brix. Calculated adjusted means for the three groups, tree-picked culled, tree-picked unculled, and ground-harvested apples, were 2.88 log CFU/ml (SE = 0.078, $P < 0.0001$), 3.45 log CFU/ml (SE = 0.078, $P = 0.0001$), and 4.89 log CFU/ml (SE = 0.127, $P = 0.0001$), respectively. These group means, when adjusted for the influence of Brix and %TA, still displayed a 2-log difference between cider from tree-picked culled and that from ground-harvest apples and a 0.5-log difference due to culling.

The association of higher microbial populations with higher Brix might seem counterintuitive, because large increases in Brix decrease water activity, which suppresses microbial growth potential. However, over the limited range of Brix examined here, there would be little or no change in water activity. Consequently, these small increases in Brix would merely increase available fermentable carbohydrate. The methods applied here did not allow us to identify changes in dominant microbial species found in cider, only changes in total populations. Increases in Brix could result in increases in yeast populations, which are likely to be included in total APCs.

Effect of processing on cider microbiology. Analysis of pooled cider data revealed that aerobic microbial populations were correlated (all at $P < 0.0001$) with dry pomace APC ($r^2 = 0.77$), wet pomace APC ($r^2 = 0.81$), and apple composite APC ($r^2 = 0.59$). However, the correlation between apple composite APC and cider APC did not seem as high as might be expected if microbial populations in cider were derived primarily from those found on the original apples. Poor correlation may indicate some contamination with microorganisms during processing. Examination of total mean APC of apples compared with that in cider (Table 4) indicates that cider APC is typically higher than that of incoming apples.

Regression analysis of the APC of cider on that of dry pomace and on that of wet pomace revealed linear relationships, with slopes of 0.701 (SE = 0.034, $r^2 = 0.57$) and 0.832 (SE = 0.077, $r^2 = 0.90$) and intercepts of 0.761 (SE = 0.138) and 0.437 (SE = 0.025), respectively (Fig. 3). The regression of cider APC on apple APC was not linear but could be fitted with a cubic equation of cider APC $= 3.62 - 1.19x + 0.53x^2 - 0.05x^3$ using a weighted regression ($r^2 = 0.72$, Fig. 4), with weight inversely proportional to cider variance. The lack of a linear relationship between aerobic bacterial populations on apples and those in cider, as found for pomace and cider, is noteworthy. If microbial populations found in cider arose primarily from the incoming apples, a linear relationship with a slope close to one and an intercept close to zero would be expected. Such a linear relationship does exist for pomace, albeit without a zero intercept, but not for incoming apples. The lack of a zero intercept and the nonlinear relationship between incoming apples and cider suggests that contamination occurs during cider processing and makes the predic-

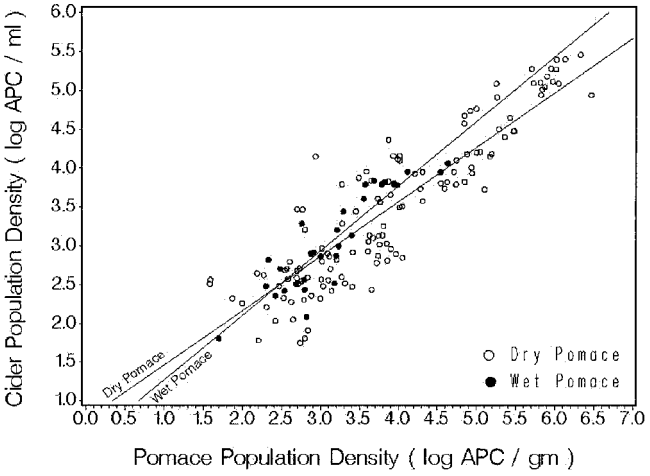

FIGURE 3. *Relationships between aerobic microbial populations found in dry or wet pomace and the final aerobic populations found in the cider.*

tion of cider quality based on the quality of incoming apples difficult.

The primary aim of this research was to determine the effects of apple variety, various harvest practices, quality sorting, and storage on apple cider microbial concentrations and to determine how microbial concentrations on apples relate to those in cider. The use ground-harvested apples increases microbial concentrations in finished apple cider and results in reduced cider yield. Although not as dramatic in scale, culling of low-quality fruit reduced microbial concentrations in cider. There were also significant differences in total microbial populations based on the variety of apple used. This effect was associated with differences in concentrations of acid and sugars typical for each variety. Apple cider microbiological quality was also suggestively linked to processing plant sanitation. The results of this study and that of Keller et al. *(14)* indicate that common sanitation measures may be inadequate to prevent cross-contamination of cider from food-processing equipment and from the plant environment. The results of this study support the need for control measures to reduce or inactivate microbes after the cider has been expressed.

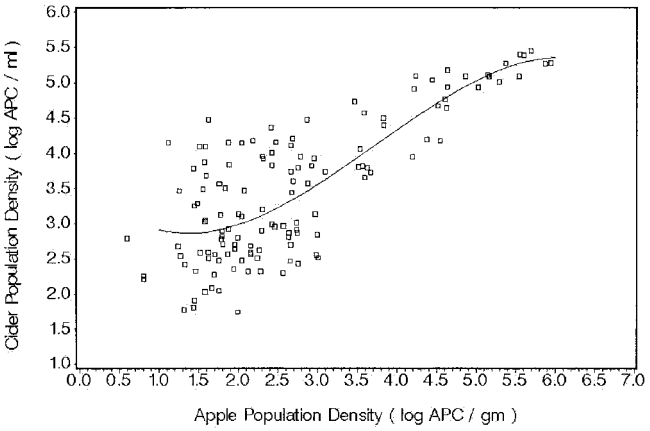

FIGURE 4. *Relationship between aerobic microbial populations found on apples and the final aerobic populations found in the cider.*

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REFERENCES

1. Benjamin, M. M., and A. R.-À. Datta. 1995. Acid tolerance of enterohemorrhagic *Escherichia coli. Appl. Environ. Microbiol.* 61: 1669–1672.
2. Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, T. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA* 269:217–222.
3. Buchanan, R. L., and M. E. Doyle. 1997. Foodborne disease significance of *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli. Food Technol.* 51:69–76.
4. Centers for Disease Control and Prevention. 1996. Outbreaks of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice—October, 1996. *Morb. Mortal. Wkly. Rep.* 45:975.
5. Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider—Connecticut and New York, October 1996. *Morb. Mortal. Wkly. Rep.* 46:4–8.
6. Centers for Disease Control and Prevention. 1999. Outbreak of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice—United States and Canada, June 1999. *Morb. Mortal. Wkly. Rep.* 48:582–585.
7. Cummins, A., C. Reitmeier, L. Wilson, and B. Glatz. 2002. A survey of apple cider production practices and microbial loads in cider in the state of Iowa. *Dairy Food Environ. Sanit.* 22:745–751.
8. Dingman, D. W. 1999. Prevalence of *Escherichia coli* in apple cider manufactured in Connecticut. *J. Food Prot.* 62:567–573.
9. Edberg, S. C., E. W. Rice, R. J. Karlin, and M. J. Allen. 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. *J. Appl. Microbiol.* 88:106S–116S.
10. Garren, D. M., M. A. Harrison, and S. M. Russell. 1998. Acid tolerance and acid shock reponse of *Escherichia coli* O157:H7 and non-O157:H7 isolates provide cross protection to sodium lactate and sodium chloride. *J. Food Prot.* 61:158–161.
11. Griffith, C. J., R. A. Cooper, J. Gilmore, C. Davies, and M. Lewis. 2000. An evaluation of hospital cleaning regimes and standards. *J. Hosp. Infect.* 45:19–28.
12. Health Canada. 1999. An outbreak of *Escherichia coli* O157:H7 infection associated with unpasteurized non-commercial custom-pressed apple cider—Ontario 1998. *Can. Commun. Dis. Rep.* 25: 113–120.
13. Hilborn, E. D., P. A. Mshar, T. R. Fiorentino, Z. F. Dembek, T. J. Barrett, R. T. Howard, and M. L. Cartter. 2000. An outbreak of *Escherichia coli* O157:H7 infections and haemolytic uraemic syndrome associated with consumption of unpasteurized apple cider. *Epidemiol. Infect.* 124:31–36.
14. Keller, S. E., R. I. Merker, K. T. Taylor, H. L. Tan, C. D. Melvin, S. J. Chirtel, and A. J. Miller. 2002. Efficacy of sanitation and cleaning methods in a small apple cider mill. *J. Food Prot.* 65:911–917.
15. Leyer, G. J., L. Wang, and E. A. Johnson. 1996. Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. *Appl. Environ. Microbiol.* 61:3752–3755.
16. Lin, J., I. S. Lee, J. Frey, J. L. Slonczewski, and J. W. Foster. 1995. Comparative analysis of extreme acid survival in *Salmonella typhimurium, Shigella flexneri,* and *Escherichia coli. J. Bacteriol.* 177: 4097–4104.
17. Lin, J., M. P. Smith, K. C. Chapin, H. S. Baik, G. N. Bennett, and J. W. Foster. 1996. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli. Appl. Environ. Microbiol.* 62:3094–3100.
18. McKellar, R. C., and K. P. Knight. 1999. Growth and survival of

various strains of enterohemorrhagic *Escherichia coli* in hydrochloric and acetic acid. *J. Food Prot.* 62:1466–1469.
19. Miller, L. G., and C. W. Kaspar. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. *J. Food Prot.* 57:460–464.
20. Riordan, D. C. R., G. M. Sapers, T. R. Hankinson, M. Magee, A. M. Mattrazzo, and B. A. Annous. 2001. A study of U.S. orchards to identify potential sources of *Escherichia coli* O157:H7. *J. Food Prot.* 64:1320–1327.
21. Rothe, A. 2000. Durchfuhrung und dokumentation der reinigung und desinfektion in lebesmittelbetrieben. *Fleischwirtsch* 80:114–117.
22. Russell, A. D., W. B. Hugo, and G. A. Ayliffe (ed.). 1982. Principles and practice of disinfection, preservation and sterilization. Blackwell Scientific Publications, Boston.
23. Senkel, I. A. J., R. A. Henderson, B. Jobitado, and J. Meng. 1999. Use of hazard analysis critical control point and alternative treatments in the production of apple cider. *J. Food Prot.* 62:778–785.
24. Steele, B. T., N. Murphy, G. S. Arbus, and C. P. Rance. 1982. An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple cider. *J. Pediatr.* 101:963–965.
25. U.S. Food and Drug Administration. 1999. Report of 1997 inspections of fresh, unpasteurized apple cider manufacturers. Available at: http://vm.cfsan.fda.gov/~dms/ciderrpt.html. Accessed 13 January 2004.
26. U.S. Food and Drug Administration. 2001. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice. Final rule. 21 CFR Part 120. *Fed. Regist.* 66:6137–6202.