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Determination of the Validation Frequency for Commercial UV Juice Processing Units

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ABSTRACT

The CiderSure 3500 is one of the most commonly used UV juice processing units in the United States for the nonthermal processing of apple cider and fulfills the 5-log performance standard established by the federal juice HACCP regulation. However, the appropriate validation frequency of this machine's quartz tubes is currently unknown by juice processors and regulatory agencies. Presently, an annual validation is recommended by the manufacturer. Historical validation data from 1998 to 2013 of commercially used quartz tubes underwent comprehensive statistical analysis. A total of 400 tubes were validated one time, and 212 of those units were revalidated at least once over the evaluated time frame. Validations were performed at 14 $mJ \cdot cm^{-2}$ UV dose and under turbulent flow conditions. Every validation showed a greater than 5-log reduction of *Escherichia coli* ATCC 25922, a nonpathogenic surrogate for pathogenic *E. coli* O157:H7, in each of three replicates. For initial validations, a mixed-effect model with log reduction of *E. coli* as response was constructed (400 tubes analyzed in triplicate). The model showed that the year of analysis and the initial inoculum level significantly affected the log reduction of *E. coli* ($P < 0.0001$), which on average was 7.0 ± 0.7. A quadratic relationship between the year of analysis and the response was found. Likewise, for revalidations (212 tubes analyzed in triplicate), the constructed random coefficient model showed that the year of analysis, quadratic effect of year of analysis, and initial inoculum level significantly affected the log reduction of *E. coli* ($P < 0.0001$). For this model, the major source of variance was explained by the year of analysis. The models describe the UV reactor's performance over time and suggest that a validation frequency of every 3 years would be conservatively adequate during the first 8 years of quartz tube use. After that, due to the reported quadratic trend, yearly validation would be recommended.

In 2000, the U.S. Food and Drug Administration recognized UV light treatment as an alternative for thermal pasteurization of juices and beverages. The requirements state in 21 Code of Federal Regulations 179.39 that UV radiation may be safely used for the processing of juice products when the treatment is provided by low-pressure mercury lamps emitting 90% of the emission at a wavelength of 253.7 nm and the juice undergoes turbulent flow through tubes, with a minimum Reynolds number of 2,200 *(25)*. Since then, and due to an increased consumer demand for more fresh products with enhanced nutritional properties *(2, 23),* the applications of this nonthermal technology have increased, with the advantage that UV light is characterized by low energy requirements and reduced initial investment in comparison with thermal pasteurization *(3, 15, 18)*. Furthermore, with the intention of preventing potentially negative effects, due the application of traditional heat treatments, on the organoleptic properties of cider, and given that unpasteurized and contaminated apple cider has caused several foodborne outbreaks *(6, 21),* many small- and medium-sized cider producers have acquired a commercial UV juice processing unit to safely treat this beverage. The CiderSure 3500 (FPE Inc., Rochester, NY) is one of the most commonly used commercial UV processing machines in the United States for the nonthermal processing of apple cider. This machine has been proven effective to ensure more than 5-log reductions of *Escherichia coli* O157:H7 and *Cryptosporidium parvum* in cider *(1, 13),* microorganisms that represent the pertinent pathogens likely to occur in this juice. Therefore, this technology fulfills the 5-log performance standard established in the federal juice HACCP regulation *(24)*.

Since 1998, the application of UV light treatments to juices has been actively researched, and in a 16-year period, a large number of individual quartz tubes used on this UV apparatus have been validated. In 2000, 70 of these tubes were subjected to a comprehensive statistical analysis, and the variability within and between tubes, plus the distributions of the mean log reductions of *E. coli* ATCC 25922 and the between-replicate variability were determined *(9)*. However, until now, the appropriate frequency of revalidation of these tubes is still unknown by juice processors and regulatory agencies. Presently, an annual validation is recommended, due to the lack of knowledge regarding the tube variation over time in the commercial-use setting. Thus, it is important for the UV-treated cider industry and regulatory agencies to accurately identify the adequate

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frequency for examining the performance of these quartz tubes. The availability of data corresponding to a large quantity of validations and revalidations represents an exceptional opportunity to determine this frequency, based on a comprehensive statistical approach. The purpose of this study was to statistically analyze historical validation and revalidation data for the quartz tubes from commercial UV processing units from 1998 to 2013, with the aim to describe the reactor's performance and its variability over time. We hope that our findings and recommendation of a validation frequency will ultimately help the juice industry and regulatory agencies establish the most appropriate frequency of revalidation for the CiderSure 3500 UV processing unit.

MATERIALS AND METHODS

Microbiological analysis. Locally purchased (Geneva, NY) nonpasteurized apple cider, which did not contain any preservatives or other additives, was inoculated with *E. coli* ATCC 25922, a clinical isolate from the American Type Culture Collection obtained from the Food Microbiology Laboratory at the New York State Agriculture Experiment Station (Geneva), and a nonpathogenic surrogate that has shown similar UV sensitivity to *E. coli* O157:H7 *(20).* A single isolated colony of *E. coli* grown on Trypticase soy agar (TSA; Difco, BD, Sparks, MD) was transferred to 10 ml of Trypticase soy broth (TSB; Difco, BD) and incubated for 5 $\pm$ 1 h at 35 $\pm$ 2°C. The inoculum was then transferred into 400 ml of TSB and incubated for 20 $\pm$ 2 h at 35 $\pm$ 2°C to stationary phase in an Innova 2300 rotary platform shaker (New Brunswick Scientific Co., Edison, NJ) at 250 rpm. Prior to the validation of the quartz tubes, approximately 1.8 liters of cider was inoculated with a 20-ml aliquot of the *E. coli* inoculum. An initial concentration of 6 to 7 log CFU·ml^{-1} of the surrogate was targeted. Inoculated ciders were aseptically sampled before and after UV processing and analyzed immediately. For UV-treated samples, 1 ml of cider and two serial dilutions in sterile 0.1% peptone water were aseptically plated (in duplicate) in petri dishes, to which approximately 20 ml of sterilized TSA was pour plated and mixed thoroughly. For untreated samples, six serial dilutions in sterile 0.1% peptone water were required. Petri dishes were incubated for 20 $\pm$ 2 h at 35 $\pm$ 2°C before enumeration, and the level of microbial reduction was calculated as $\log(N/N_0)$ where N refers to the after treatment *E. coli* count, and N_0 to the initial count, both in CFU per milliliter. Each quartz tube was evaluated in triplicate.

UV juice processing unit. Apple cider was run through a commercial CiderSure 3500 UV juice processing unit (FPE Inc.) at a wavelength of 254 nm, guaranteeing a turbulent flow regime and a constant UV dose of 14 mJ·cm^{-2}. The UV processing unit is composed of stainless steel housing and an inner quartz tube. The apple cider is pumped between the outer steel housing and inner quartz tube using a positive displacement pump, and the product is exposed to eight germicidal low-pressure mercury lamps placed concentrically within the interior of the quartz–stainless steel cylinder *(1).* Two UV light sensors, located at the bottom and top of the outer cylinder, measure the UV light transmittance through the cider every 50 ms. Based on the product transmittance measurements, this machine has been programmed to automatically adjust the pump flow rate ensuring a constant UV dose exposure throughout the UV process *(1, 20).* This apparatus has been provided with an automatic system designed to shut down the process if a UV light sensor fails or if the sensor indicates that a minimum of 14 mJ·cm^{-2} UV dose, which represents the critical limit on the application of this treatment, has not been met.

Validations and revalidations. Throughout 16 consecutive years (from 1998 to 2013), a total of 400 quartz tubes were validated at least one time, and 212 of those same tubes have been revalidated at least once. All quartz tubes were brought to the Food Microbiology Laboratory at the New York State Agricultural Experiment Station (Geneva, NY) and subjected to the standard validation procedure designed by this laboratory and described previously. During the evaluated time frame, all quartz tubes were validated and revalidated by the same analyst, using the same UV juice processing unit, which included a pump, UV light sensors, software, and eight germicidal UV lamps. Over the years, regular maintenance has been provided to the UV unit, which includes repairs to the pump and replacement of the UV light sensors and lamps.

Statistical analyses. The mixed-effect and random coefficient models were constructed and analyzed using JMP, version 11 (SAS Institute Inc., Cary, NC). Effects were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Initial tube validations. All initial validations showed greater than 5-log reductions of *E. coli* ATCC 25922 in each of the three replicates: if a tube failed to achieve the minimum 5-log pathogen reduction during the first validation, that tube was not sold to the cider industry, and therefore it was not considered for further statistical analysis. Prior to this study, it was found that the risk of obtaining a less than 100,000-fold reduction of *E. coli,* by using these UV light processing tubes for treating apple cider, occurs less than 0.2% of the time *(9).*

For the analysis of the initial validations, a mixed-effect model with log reduction of *E. coli* as response was constructed. As stated by Wang et al. *(26),* a mixed-effect model can specify a realistic model for the correlation existing between repeated measurements, which in this case, were represented by the logarithmic reduction of *E. coli* due to the UV light exposure, measured in the same tube on three occasions. The results associated with 400 tubes, analyzed in triplicate, and evaluated between 1998 and 2013 gave a total of 1,200 observations. The variables of initial *E. coli* count, year of analysis (with year 0 corresponding to 1998), and a quadratic effect of year of analysis were added to the model as continuous fixed effects, while the tube identification was included as a random effect.

An average of 7.0 $\pm$ 0.7-log reductions of *E. coli* was obtained, and as observed in Figure 1, the histogram of log reductions shows a skew to the left, with a minimum value of 5.01-log reduction, the boundary at which the data set was artificially truncated when the tubes that obtained a lower than 5-log reduction were not considered for further analysis. Additionally, the upper limit was found at 8.29-log reduction. Note that this value may not reflect the maximum effectiveness of the apparatus. Instead, it suggests that the efficacy of these UV processing quartz tubes could be limited, in part, by the initial and targeted *E. coli* concentration in the test cider. Likewise, using a subset of this data (70 tubes), Duffy et al. *(9)* previously reported high and low log reduction tails when @RISK and Analytica simulations were used to model the data's distribution,

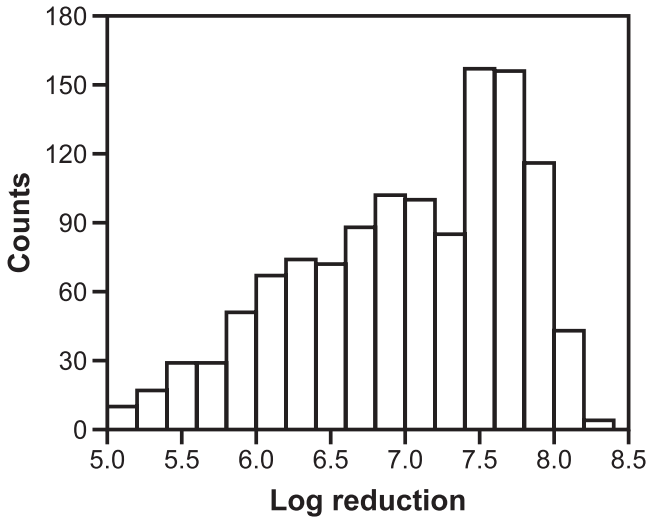

FIGURE 1. *Histogram of log reductions of* E. coli *ATCC 25922 in apple cider subjected to UV treatment at 14 mJ·cm^{-2} UV dose by using a commercial UV juice processing unit, corresponding to validation trials (*n *= 1,200).*

indicating that those tails are probably an accurate reflection of the UV processing unit's performance rather than an artifact of simulation.

The constructed mixed-effect model showed that the effects of initial *E. coli* count and year of analysis significantly affected the log reduction of *E. coli* ($P < 0.0001$; Table 1), and the relationship between the response variable and the year of analysis was found to be quadratic, with an increasing and then diminishing effect over time. Moreover, the model showed that the variance between the quartz tubes was considerably higher, representing 70% of the total variance, in comparison with the variance observed within tubes (Table 2).

Tube revalidations. With the objective of establishing the appropriate frequency of validation of the quartz tubes, revalidation data from 1998 to 2013 underwent a comprehensive statistical analysis. The results of 212 UV processing quartz tubes (each analyzed in triplicate) and revalidated at least once (more than two validations were conducted) over the selected time frame (a total of 1,740 observations) were used to construct a random coefficient model with random intercept, with log reduction of *E. coli* as response. According to Cudeck and Harring *(5),* in the context of repeated measure studies, the random coefficient models are based on the idea that the process of change is defined for each unit of study; in this case, each quartz tube is also related to the population mean trajectory over time. The variables of initial *E. coli* count, year of analysis (with year 0 corresponding to 1998), and a quadratic effect of year of analysis, were considered as the fixed effects, while the tube identification, and the year of analysis (as a nominal variable and nested within tube identification), were added as the random effects.

TABLE 1. *Parameters estimates of the fixed effects included in the mixed-effect model used to analyze the initial validations of the quartz tubes with log reduction of* E. coli *ATCC 25922 as response*

Term	Estimate	Standard error	$P > \|t\|$
Intercept	0.6	0.6	0.3542
Initial *E. coli* count	0.74	0.08	<0.0001
Year of analysis	0.22	0.03	<0.0001
(Year of analysis) × (year of analysis)	−0.011	0.002	<0.0001

TABLE 2. *Variance components of the random effects included in the mixed-effect model used to analyze the initial validations of the quartz tubes with log reduction of* E. coli *ATCC 25922 as response*

Random effect	Variance component	Percentage
Tube identification	0.30	70
Residual	0.13	30
Total	0.43	100

The parameters estimates of the constructed model are given in Table 3. As observed, the initial *E. coli* count and year of analysis significantly affected the log reduction of *E. coli* ($P < 0.0001$). Once again, a quadratic behavior of year of analysis with an increasing and then diminishing effect on the log reduction of *E. coli* over time was found. Considering the overall mean initial count of 7.57 log of *E. coli,* it was found that the maximum predicted value of *E. coli* reduction as a response, which corresponds to 8.00 log, was observed at 8.86 years after the first validation. After this time, the bacterial reduction levels begin to decrease. The residual unexplained variance within tubes was 29%, whereas the variance between tubes caused only 5% of the total variance. The rest, and most, of the variance was explained by the random effect of year of analysis (Table 4). The higher level of variance caused by the year of analysis is likely due to the expected and widely reported differences of the physicochemical characteristics of the apple ciders used over the 16-year period. Although a slight reduction of *E. coli* in the juices (without subjecting the beverage to the UV radiation) is possible, the effect was not deemed substantial. The chemical and nutritional composition of apple cider has been determined in several studies for different apple varieties, and significant differences between apple cultivars have been consistently reported *(1, 4, 10, 12, 14, 17, 19).* Also, variables, such as the growing season and storage conditions, represent some of the parameters that influence the physicochemical properties of fruits and

TABLE 3. *Parameters estimates of the fixed effects included in the random coefficient model used to analyze the revalidations of the quartz tubes with log reduction of* E. coli *ATCC 25922 as response*

Term	Estimate	Standard error	$P > \|t\|$
Intercept	0.5	0.5	0.3113
Initial *E. coli* count	0.74	0.07	<0.0001
Year of analysis	0.23	0.03	<0.0001
(Year of analysis) × (year of analysis)	−0.013	0.002	<0.0001

TABLE 4. *Variance components of the random effects included in the random coefficient model used to analyze the revalidations of the quartz tubes with log reduction of* E. coli *ATCC 25922 as response*

Random effect	Variance component	Percentage
Tube identification	0.02	5
Year of analysis	0.28	66
Residual	0.12	29
Total	0.42	100

therefore ciders. Worth noting, it has been also demonstrated that these differences between the physicochemical properties of apple ciders may affect the survival of *E. coli* during the application of UV light treatments, due to potential differentiated antimicrobial advantages and disadvantages provided by the juice *(1)*. Interestingly, it has been reported that apple cider produced from stored apples shows less inhibition of *E. coli* O157:H7 than that made with freshly harvested apples *(7, 8, 22)*. This may explain, in part, the observed variability among the revalidations performed in different years, and therefore using different ciders.

Nonetheless, considering exclusively the revalidation data, a greater than 5-log reduction of *E. coli* ATCC 25922 was observed for all the tubes tested and on each of the three corresponding replicates. This result suggests that although the differences among ciders are important, and probably explain most of the variance of the resulting model, the inclusion of new apple varieties to produce cider, and the normal differences between the physicochemical properties of the fruits, did not compromise the performance of the tubes during the studied time frame. Therefore, it is the authors' opinion that this source and magnitude of variability does not justify performing yearly revalidations, as it is currently recommended by the manufacturer.

Different elements may explain the quadratic trend found with the random coefficient model. A loss of the UV sensitivity of sensors, lamp degradation, and darkening of the quartz caused by cider contact over the years—besides other changes in the quartz tubes that have not been completely understood—could be the cause of the observed results. For instance, if UV light sensors are not recalibrated, they may lose their UV transmittance sensitivity over time, thus slowing the flow rate of cider through the reactor and causing extended times of UV light exposure that, ultimately, translates into higher microbial reductions. A reduction of the germicidal capacity of the UV lamps is also expected over time. The aging of lamps influences the emitted UV energy intensity and is primarily caused by two factors: solarization of the lamp wall material and blackening due to deposits of sputtered oxides from the electrodes *(16)*. The content within the lamps is continuously exposed to changes in pressure and temperature, thus the electrodes inside decay and deposit material on the interior quartz, reducing the lamps output. Moreover, the efficacy of the lamp is directly related to the saturated mercury pressure inside the lamp, and many parts on the UV lamps, such as the glass bulb, quartz bulb, electrode emitter, and metal parts consume mercury during the lamp's life and reduce its efficacy *(11)*. Note that these effects are expected to be controlled, to some extent, by the UV light sensors and their effect on the apparatus's pump, which, in turn, affects the flow of cider through the quartz tubes.

The constructed models reported in this study describe the quartz tubes' performance and their variability over time. As indicated by Duffy et al. *(9)*, quantifying the variability of a food process could be extremely useful to formulate better predictive models and also to separate the uncertainty from variables as much as possible. This study aims to establish an appropriate frequency of revalidation for the quartz tubes of a commercial UV processing unit. Based on the constructed random coefficient model, we recommend revalidating the quartz tubes every 3 years during the first 8 years of use. After that, and due to the reported quadratic trend that predicts the *E. coli* reduction to decrease after 8.86 years, a yearly validation is recommended instead. We also suggest that any changes in the UV juice processing unit that may negatively compromise the performance of the reactor and, ultimately, the safety of the cider, such as changes in the computer and software, or replacement of the pump, UV light sensors, and mercury lamps should be followed by the application of the validation procedure of the quartz tubes, regardless of the time and results associated with the last validation performed. Likewise, the use of this UV machine for treating different juices or beverages and changes in the formulation of already validated liquid products must be subjected to the validation protocol before launching the beverage to market. Furthermore, it is important to clarify that the frequency of validation suggested in this study does not substitute for regular verification procedures, which include periodical maintenance of UV light sensors, pump, and computer and the standard cleaning and sanitizing protocols of the UV unit, recommended by the manufacturer.

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