

Communication

# A Preliminary Evaluation to Establish Bath Pasteurization Guidelines for Hard Cider

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**Abstract:** Though in-package water bath heat pasteurization for hard cider production is commonly employed to improve product safety and stability, there is a considerable lack of research-based guidelines to inform industry practices. In this study, fermented cider was bottled and inoculated with high populations of *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* yeast. The bottles were then subjected to water bath pasteurization 60 °C at varying lengths of time. For both yeast species, populations were reduced to undetectable levels after just 1 min of processing time. Though validation of each individual process is recommended, cider producers may be able to sufficiently reduce the risks of spoilage organisms with minimal water bath pasteurization, especially when combined with other methods to reduce the presence of spoilage organisms.

**Keywords:** *Saccharomyces*; *Zygosaccharomyces*; alcoholic fermentation; spoilage prevention; apple

## 1. Introduction

Cider, also known as “hard cider” in North America, is the beverage produced from the alcoholic fermentation of apple juice. Though cider production practices may vary, it is fairly common for the finished product to be pasteurized in its final package to preserve product quality and product safety. Cidermaking is in practice similar to winemaking, but cider typically contains much lower ethanol levels (around 5–7%) compared to wine (11–15%). It is therefore more prone to microbial spoilage.

Refermentation in the package and subsequent bottle explosions have been a concern in beverage industries for decades, and refermentation from *Zygosaccharomyces* spp. in beverages have been observed to cause bodily harm, specifically to the eye [1]. *Zygosaccharomyces* spp., particularly *Zygosaccharomyces rouxii*, have also been isolated from grape juice concentrate, and organisms from this genus are known to cause spoilage in wine [2].

The pasteurization process is typically conducted using a water bath pasteurizer and measured in terms of pasteurization units (PUs) which are defined as the minutes a product is held at 60 °C [3]. PUs can also be calculated by the following equation:

$$PU = t \times 1.393^{(T-60)}$$

where  $t$  = time in minutes and  $T$  = temperature in degrees Celsius [3].

Currently, there is no cider industry standard regarding a sufficient number of PUs. Comparatively, in the beer industry, 15 PUs have been cited as the industry standard [3]. Research has found that while 5 PUs has been noted to be theoretically sufficient to destroy microorganisms of concern in beer, many operations even employ up to 30 PUs [3–6]. In comparison, some cidermakers have reported using 10–25 PUs, while some sources cite as high as 60 PUs for sufficient reductions in

microorganisms [7]. Using an insufficient amount of PUs may result in insufficient microorganism destruction and subsequent spoilage and refermentation, while using excessive PUs may result in economic losses through energy expenditures and additional labor costs associated with monitoring the batch process.

Though microbial testing is necessary to validate any unique process, the aim of this research is to begin the establishment of general bath pasteurization guidelines to assist cidemakers in achieving a microbially stable product.

## 2. Materials and Methods

### 2.1. Cider Fermentation

In four cleaned and sanitized 5-gallon glass carboys, commercial juice concentrate (FruitSmart<sup>®</sup>, Grandview, WA, USA) was rehydrated achieve a soluble solids content (SSC) of 13 °Brix to obtain a potential alcohol content of 7.0% alcohol by volume (ABV) post-fermentation. SSC was measured using a Palm Abbe Digital Refractometer (MISCO, Cleveland, OH, USA). Before inoculation, juice pH was measured using a Starter 5000 OHAUSE pH meter (OHAUSE<sup>®</sup> Corporation, Parsippany, NJ, USA). Titratable acidity (TA) was reported in malic acid equivalents and measured by titrating against a 0.2 N NaOH solution to a pH endpoint of 8.27. Free and total SO<sub>2</sub> were measured using the Ripper Titration [7]. The juice was inoculated with 0.25 g/L *Saccharomyces cerevisiae* var. *bayanus* DV10 (DV10) yeast (Lallemand, Montreal, QC, Canada) and supplemented with the standard addition of 0.37 g/L Fermaid<sup>®</sup> K yeast nutrient (Lallemand, Montreal, Canada). Fermentation was considered complete once the cider had 0.5% or less residual sugar, measured with AimTab<sup>™</sup> Reducing Substances tablets (Germaine Laboratories, San Antonio, TX, USA). The cider was then racked and bottled into sanitized 12 oz beer bottles and stored at 36 °C until further analysis. Post-fermentation measurements for pH and TA were also taken at this time.

### 2.2. Microbial Inoculation

The stored bottled cider was brought to room temperature then inoculated with one of two yeast strains in each part of the study. In Part I, DV10 was chosen, as it is a commonly used yeast strain in the cider industry as well as a relatively strong commercial yeast strain. DV10 yeast was added at rates to achieve populations of 10<sup>5</sup> cells/mL as has been done in previous studies [8,9]. This population density represents a case in which yeast counts would be much higher than in most finished ciders and provides a scenario in which processing steps to reduce yeast activity is of utmost importance for product stability. Once cultures were added, bottles were immediately sealed with a sanitized crown cap and subjected to a pasteurization treatment.

In Part II, *Z. rouxii* derived from NCYC 381 KWIK-STIK<sup>™</sup> Plus (Microbiologics, St. Cloud, MN, USA) was cultured according to manufacturer instructions. The *Z. rouxii* culture was then used to inoculate room temperature Difco<sup>™</sup> Potato Dextrose Agar (PDA) (BD Biosciences, Sparks, MD, USA) plates. The inoculated plates were then placed in a 25 °C incubator and grown for eight days. This culture was then used to inoculate additional PDA plates which were again placed in a 25 °C incubator and grown for eight days. The cells were then washed off the plates using sterile peptone buffer and sterile swabs and transferred into a sterile peptone buffer solution. This solution was then used to inoculate sample cider bottles to be pasteurized. Sucrose was also added at a level of 3% (30 g/L) to each 355 mL bottle before inoculation with *Z. rouxii* to simulate a back-sweetened cider.

### 2.3. Pasteurization

The bottles were immediately sealed with a sanitized crown cap and subjected to a pasteurization treatment of 0, 1, or 5 PUs. Cider was pasteurized in a 60 °C Precision<sup>™</sup> Reciprocal Shaking Bath (Thermo Scientific, Waltham, MA, USA) water bath to achieve treatments of 0.03, 1, 2.5, 5, 13, and 23 PU. The control treatment, 0 PU, was not pasteurized. The internal temperature of a bottle was recorded

using a temperature probe placed into a reference bottle. PUs were calculated based on in internal time and temperature of the reference bottle using the PU equation. Once each treatment reached its number of PUs in the water bath, the bottles were cooled using ice water baths. Internal bottle temperature dropped to 30 °C in 10 min and were then removed from the cooling baths for enumeration.

#### 2.4. Enumeration

After the pasteurization treatment, ciders were immediately sampled for microbial analysis. Wallerstein Laboratory (WL) Nutrient (MilliporeSigma, Burlington, MA, USA) agar was used to spread plate 100 µL of full strength and dilutions of  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  of the cider sample. The plates were incubated at 30 °C and counted after 2, 3, and 8 days.

#### 2.5. Statistical Analysis and Replicates

The mean and standard deviation for each treatment were calculated from the experimental replicates using GraphPad Prism Version 8.2.1 software (GraphPad Software, San Diego, CA 92108, USA). For each experiment, each of the four carboys represented a fermentation replicate. Three bottles were taken from each fermentation replicate and labeled B1, B2, and B3. Each bottle was then inoculated with either *S. cerevisiae* DV10 in Part I or *Z. rouxii* in Part II, subjected to a pasteurization treatment, and then sampled for microbial analysis. Plates were inoculated in duplicate.

### 3. Results and Discussion

Table 1 details the chemistry of the apple must, the chemistry of the fermented cider, and the inoculated organisms for each part of the treatment. SO<sub>2</sub> was not added before or after fermentation but was measured at both of these stages to account for additions during juice concentrate processing and yeast metabolic processes.

**Table 1.** Chemistries of juice and cider prior to pasteurization treatments.

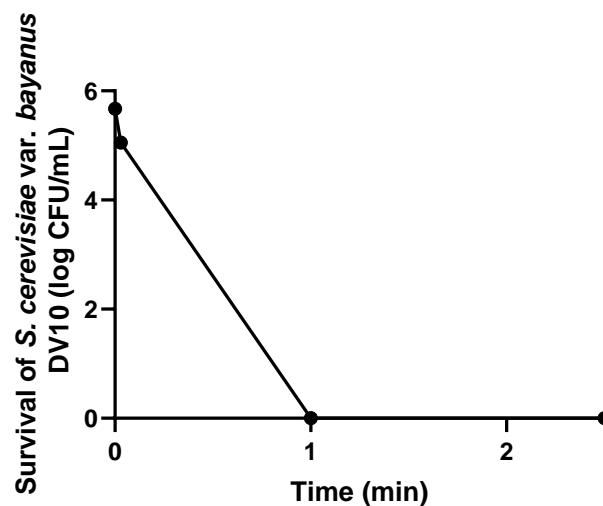
Parameter	Part I	Part II
Chemistries of juice prior to fermentation.		
SSC (°Brix)	13.0 ± 1.0	13.0 ± 1.0
Titrateable Acidity (g/L)	4.0 ± 0.0	3.9 ± 0.1
pH	3.5 ± 0.0	3.5 ± 0.0
Free SO <sub>2</sub> (mg/L)	13.5 ± 0.6	8.3 ± 1.7
Total SO <sub>2</sub> (mg/L)	28.0 ± 2.6	14.8 ± 1.3
Chemistries of fermented cider prior to pasteurization treatments.		
Titrateable Acidity (g/L)	5.6 ± 0.1	5.8 ± 0.0
pH	3.6 ± 0.0	3.6 ± 0.0
Free SO <sub>2</sub> (mg/L)	8.3 ± 1.3	12.3 ± 0.5
Total SO <sub>2</sub> (mg/L)	21.0 ± 1.4	33.8 ± 3.0

Results from the pasteurization treatments for each experiment can be found in Table 2. The data from 2 days of incubation was used, as no changes were observed with 3 and 8 days of incubation. These results demonstrate that in cases of high yeast populations, as little as 1 PU was sufficient in reducing DV10 populations from  $4.6 \times 10^5$  CFU/mL to undetectable levels, and in the case of *Z. rouxii*, one PU was sufficient for reducing  $4.8 \times 10^3$  CFU/mL to undetectable levels.

**Table 2.** Effect of various applications of pasteurization units (PUs) on yeast populations for water bath pasteurized bottled ciders.

Organism	PUs Applied	Incubation Time (Days)	Mean (CFU/mL)	Standard Deviation (CFU/mL)
<i>S. cerevisiae</i>	0	2	$4.6 \times 10^5$	$1.7 \times 10^4$
	0.03	2	$1.1 \times 10^5$	$1.4 \times 10^4$
	1	2	<1	0
	2.5	2	<1	0
	5	2	<1	0
	13	2	<1	0
<i>Z. rouxii</i>	23	2	<1	0
	0	2	$4.8 \times 10^3$	$2.5 \times 10^3$
	1	2	<1	0
	5	2	<1	0

The Decimal Reduction Time at 60 °C ( $D_{60\text{ °C}}$ ) for DV10 in cider was calculated to be 0.05 min using the 0 and 0.03 PU data points. The log reduction of this organism can be found in Figure 1.

**Figure 1.** Deactivation of *Saccharomyces cerevisiae* var. *bayanus* DV10 in bottled cider during water bath pasteurization at 60 °C. Decimal Reduction Time at 60 °C ( $D_{60\text{ °C}}$ ) for this organism in cider was calculated to be 0.05 min.

The results indicate that in these ciders, at little as 1 PU was sufficient in reducing populations of *S. cerevisiae* DV10 and *Z. rouxii* in the experimental ciders. Related studies on wine pasteurization have also found that relatively low levels of pasteurization are suitable for reducing yeast populations. In a study by Splittstoesser et al. (1975), most yeast species had D-values at 49 °C of 0.6 min or below in grape wine containing 12.1% ethanol. When calculated in terms of PUs, this pasteurization treatment is equivalent to 0.016 PUs. Other organisms in this study, however, had higher levels of heat resistance. For example, one strain of *S. cerevisiae* had a D-value at 49 °C of 1.6 min, and the bacterial species *Lactobacillus fructivorans* WBM had a D-value at 60 °C of 1.7 min [10].

The chemistry of a particular cider may also contribute to the pasteurization procedure followed. Previous studies have indicated that ethanol is an important contributor to heat processes [10,11] but factors such as pH, TA, sulfur dioxide concentrations, and others may also cause variations in pasteurization processes. Practices during commercial cider production, such as filtration or addition of chemical preservatives, might further reduce yeast populations. However, though  $\text{SO}_2$  is very effective against bacterial spoilage organisms, commercial yeast strains in particular tend to have a greater tolerance against the preservative.

#### 4. Conclusions

The results of this work indicate that as little as one pasteurization unit is sufficient to reduce even high populations of spoilage yeast, though further research will be necessary to evaluate the possible effects of pH, ethanol concentration, and added preservatives on cider pasteurization recommendations. The efficacy of heat pasteurization on other microorganisms, including additional *S. cerevisiae* strains and *Zygosaccharomyces* species should be evaluated. Relatively heat resistant bacterial species, such as *Lactobacillus* species, should also be evaluated. Though there is yet to be enough research to establish a pasteurization standard for the cider industry, it is likely that recommendations of 50–60 PUs are much too high and result in energy and labor over-expenditures. Regardless, it is important for each individual process to be validated with microbial testing to ensure sufficient heat processing and resulting reduction of microbial viability.

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