

1 **Title: Effects of the Co-inoculation of Commercially Available Yeast and Bacteria**
2 **on the Organoleptic Properties of Unaged Pot Distilled Rum**

3
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6 **Degree Program: MSc Brewing and Distilling**

7
8 **Abstract**

9 This project investigated the effects of co-inoculation of a commercially available yeast and
10 three strains of lactic acid bacteria on the organoleptic properties of unaged, pot distilled rum in
11 a commercial setting. Four production trials were performed in triplicate, with respective
12 distillate portions aggregated: control (Lalvin EC-1118™), trial 1 (Lalvin EC-1118™ + DistilaBact®
13 LP), trial 2 (Lalvin EC-1118™ + EnoFerm Alpha™), and trial 3 (Lalvin EC-1118™ + SafSour LP
14 652™). For each triplicate, a production-style spirit run was performed from low wines (85%),
15 heads (10%), and tails (5%): control (WDC), trial 1 (WDC 1), trial 2 (WDC 2), trial 3 (WDC 3).
16 Fermentation performance was largely unaffected by the inclusion of lactic acid bacteria, with
17 little difference between final levels of trial pH, specific gravity, and alcohol concentration (v/v),
18 versus the control, except trial 2, which had a significantly higher pH. Gas chromatography
19 showed that all trials had similar concentrations of higher alcohols and esters to their respective
20 controls, except for isobutanol and active-amyl and isoamyl alcohols, which were distinctly
21 different for trials 2, 3, WDC 2, and WDC 3. A trained sensory panel found that each trial was
22 distinctly different than their respective control with trial 3 having a greater overall relative
23 score than the control (1.11 vs 0.43) and WDC 3 scoring higher than WDC (0.54 vs 0.40). The
24 results suggest that co-inoculation fermentations using lactic acid bacteria can produce rums
25 with improved organoleptic characteristics, without yield loss or significant additional process
26 complexity.

27
28 **Introduction**

29 Rum is a globally produced distilled spirit, with deep historical ties to the Caribbean, and
30 defined by CARICOM as:

- 31
32 1. 'a spirit obtained exclusively by alcoholic fermentation and distillation of sugar cane
33 molasses, sugar cane syrups, sugar cane juices or cane sugar produced during the
34 processing of sugar cane.'
35 2. 'a spirit drink distilled at an alcohol content of less than 96.0% alcohol by volume at
36 20°C.'
37 3. 'a spirit drink produced in such a way that the product has the organoleptic
38 characteristics derived from the natural volatile elements contained in the above raw
39 materials or formed during the fermentation or distillation process of the named raw
40 materials; and which includes mixtures solely of the above distillate.'(CROSQ, 2008).

41
42 Many factors play a role in the organoleptic properties of the resulting distillate including base
43 materials and their treatment, fermentation and distillation conditions, and the maturation

44 program carried out by the distillery. This paper will primarily focus on molasses-based rum
45 production, although sugarcane juice-based rums and cachaça will also be discussed. The
46 starting point for all quality rums and cachaças begins with understanding the microbial ecology
47 of the fermentation, primarily the yeast and bacteria driving it, since their diligent efforts are
48 responsible for many of the organoleptic compounds found in rum: aldehydes, ketones, fatty
49 acids, fatty acid esters, a variety of alcohols and more (Greg, 1895c; Allan, 1906; Lehtonen and
50 Soumalainen, 1977).

51

52 **The microbiology of rum fermentation and its role in product quality**

53 The microbiology of rum fermentation and the conditions through which many of the above-
54 mentioned organoleptic compounds are created have been studied since the 1890s (Greg,
55 1895a; Greg, 1895b; Greg, 1895c; Greg, 1895d; Pairault, 1903; Allan, 1906; Ashby, 1907; Ashby,
56 1911). From this foundation, numerous studies have furthered our understanding of the
57 microflora present during the production of molasses/sugarcane juice-based rums and primarily
58 found that numerous species of *Schizosaccharomyces*, *Saccharomyces*, *Bacillus*, *Clostridium*,
59 *Propionibacterium*, *Lactobacillus*, *Leuconostoc*, and *Torulopsis* were present (Allan, 1905; Ashby,
60 1911; Hall et al., 1935; Shehata, 1960; Parfait and Sabin, 1975; Ganou-parfait et al., 1987;
61 Fahrasmane et al., 1988; Ganou-Parfait et al., 1989; Fahrasmane and Ganou-Parfait, 1998; Fleet
62 and Green, 2010). However, even with 130+ years of research, our understanding of many of
63 the microbiological processes taking place at various production stages, remains very limited
64 particularly when considering the resources available to beer, wine, and whisky producers
65 (Green, 2015).

66

67 Recent developments by companies such as Lallemand and Fermentis to create useable bacteria
68 products have justified the need for contextualizing historic and contemporary rum production
69 techniques, specifically how the deliberate use of selected yeast and bacterial strains can lead
70 to beneficial improvements in the organoleptic qualities of the resulting distillate, and to
71 provide small-scale producers a proper foundation from which to approach the development of
72 organoleptically complex rums.

73

74 **Background**

75 **Rum production 1890 – 1950s**

76 In the first decades of the 20th century as the microbiology of rum fermentation became better
77 understood, the research community was divided, with one group (Pairault, 1903) concluding
78 that pure fermentations using selected yeasts in an environment low in bacteria presence would
79 lead to improved rum production and efficiency, and the other group (Allan 1905; Ashby 1909)
80 concluding that bacteria play a major role in the organoleptic qualities of heavy rums
81 (Fahrasmane and Ganou-Parfait, 1998). At the time, a variety of different rum styles were
82 produced throughout Jamaica via spontaneous fermentation, with most estates offering several
83 different marques, each being distinguished by their ester content and organoleptic
84 characteristics (Allan, 1905; Cousins, 1907; Ashby, 1911). Before going further, it is necessary to
85 define these historic rum styles and their fermentation/production components, which can
86 respectively be found in **Table 1** and **Table 2**.

87 **Table 1. Early 20th century Jamaican rum styles and descriptions.**

Reference	Rum Style	Description
Cousins (1907)	Common clean	A light rum, pot distilled, with ester content 90 – 300 g/hLAA, with a principal aroma of ethyl acetate, with variation between estates due to trace amounts of other higher acid esters, traces of caprylic alcohol, and other aromatic higher alcohols.
Cousins (1907)	UK Home trade	Pot distilled rum with an ester content of 300 – 500 g/hLAA, produced from slow fermentation, characterized by a heavy residual body, mainly esters of higher molecular weight acids which originate from the large presence of bacteria in the fermentation.
Cousins (1906)	Tea rum	Pot distilled medium bodied rum with an ester content of 400 – 700 g/hLAA, primarily to enrich afternoon tea.
Cousins (1906)	Flavored/German	Produced from highly acidic fermentations, typically 15 – 21 days in length, utilizing fission yeasts, and double retort stills, with an ester content 700-1600 g/hLAA. Primarily used for rum blending.

88

89 **Table 2. Early 20th century Jamaican rum fermentation components and definitions.**

Reference	Item	Description
Ashby (1911)	Skimmings	The solid-liquid slurry skimmed from the surface of the sugarcane juice clarifiers, typically with a brix range of 10-20 brix.
Ashby (1911)	Dunder	Spent rum wash leftover in the still after distillation is finished. Rich in acids and typically with a brix range of 10-25 brix.
Ashby (1911)	Acid (cane vinegar)	Soured skimmings or cane juice, rich in acetic acid.
Ashby (1911)	Muck (flavor)	A liquid/sludge, rich in butyric and other higher acids, produced via a slow, controlled, putrefactive fermentation process of the liquid and solid portions of dunder, wash bottoms (dead yeast), spent stillage (low or high wines), and cane trash.

90

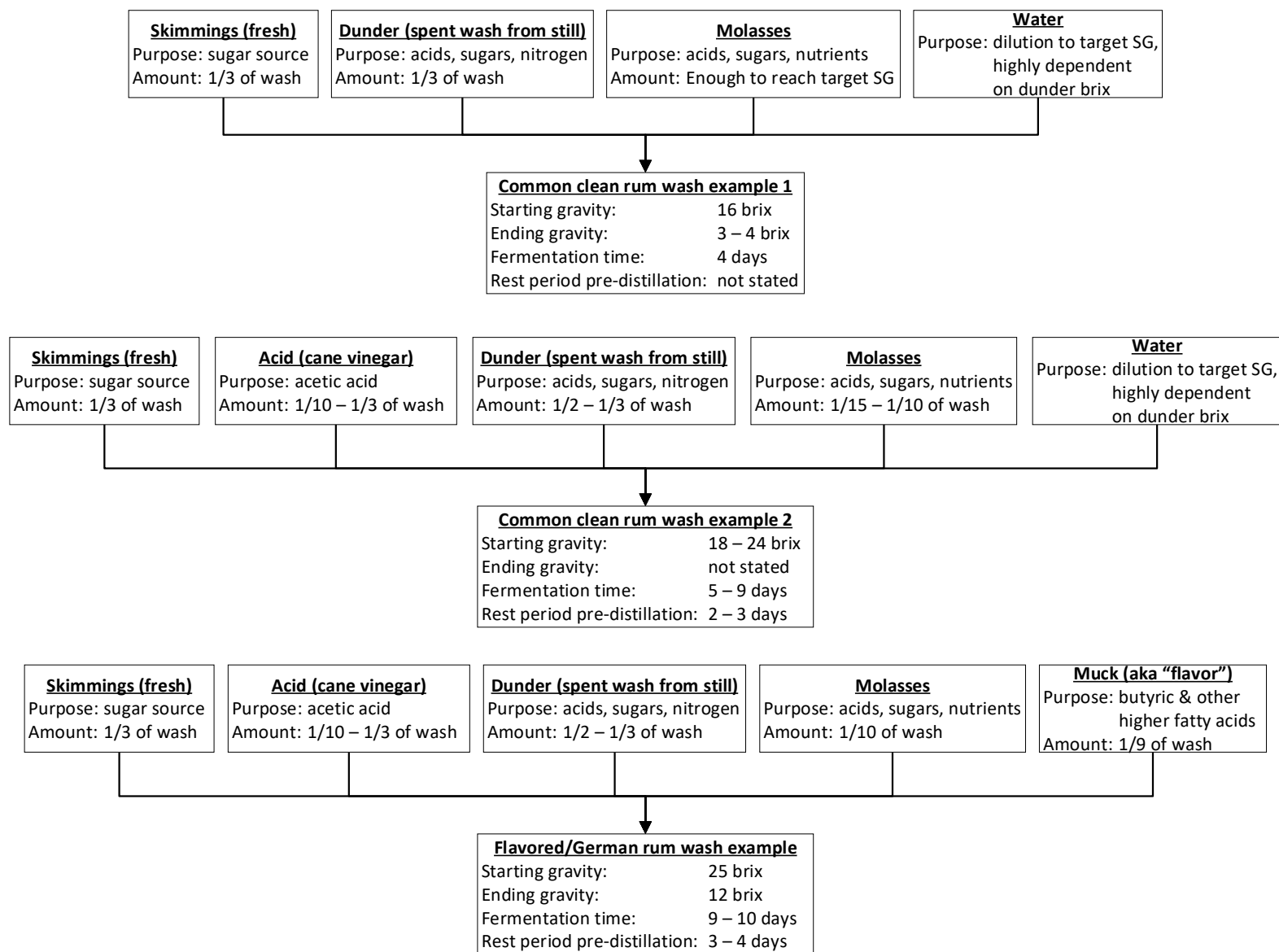
91 During his work in Jamaica, Ashby (1911) reported on three styles of rum production capable of
 92 yielding two versions of common clean rum (CCR1 and CCR2, with ester contents of 100 g/hLAA
 93 and 1,000 g/hLAA) and one version of the heavier bodied “flavored” rum (ester content up to
 94 1,600 g/hLAA). Process diagrams for each style can be found in **Figure 1**. Two species of yeasts
 95 were commonly found in the fermentations (1) *Saccharomyces* spp. was most prevalent in the
 96 faster fermenting CCR1 and (2) as wash acidity and bacteria presence increased in both CCR2
 97 and the flavored rum, *Schizosaccharomyces* spp. became dominant and fermentation time
 98 significantly increased (Allan, 1905; Ashby, 1911). Ashby (1907) reported that acetic, propionic,
 99 butyric, caprylic, capric, and lauric acids were present in rum fermentations and upon

100 distillation, their esters were found in rum at the following concentrations: ethyl acetate (98%),
101 (2%) combination of butyric, caprylic, capric, lauric and other higher alcohol esters, which were
102 found to provide body and flavor characteristics.

103
104 As seen in **Figure 1**, the reason for the significant increase in ester content is due to the
105 complexity of the fermentation. The addition of acid and the increase in dunder further
106 acidified the wash, which when combined with the longer fermentation time and pre-distillation
107 rest time, allowed the necessary bacterial reactions to take place, and thus seeded the wash
108 with copious amounts of acids and ester precursors (Allan, 1906; Ashby, 1911). Flavored rum
109 took this further with the addition of muck, which has its own incredibly complex production
110 method, alongside acid and dunder, to dramatically acidify the wash and lead to the production
111 of incredibly aromatic rums with high ester contents.

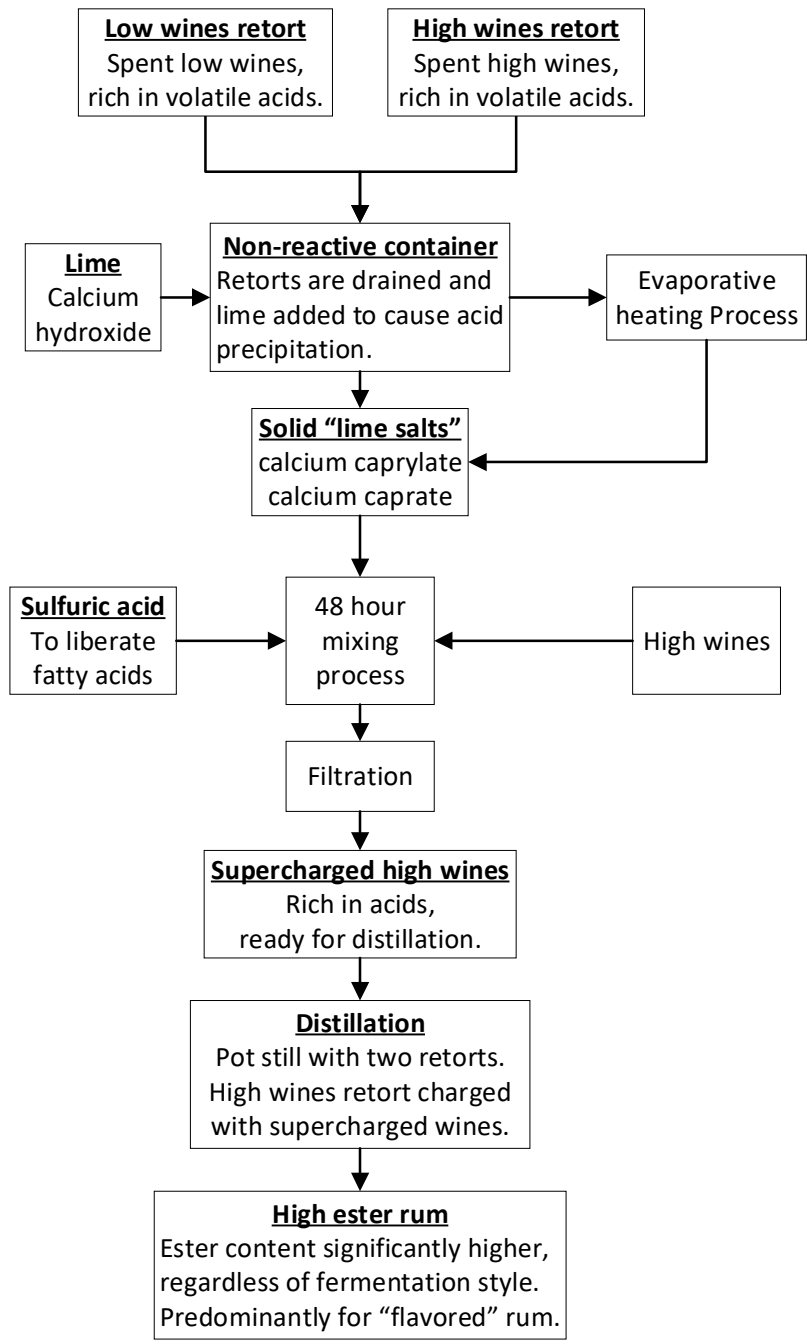
112
113 Allan (1905) thought the flavored rum fermentation techniques were overly complicated and a
114 crude attempt to foster specific strains of bacteria for acid development. Since most of the
115 esters found in rum are formed via direct esterification, it can therefore be said that the greater
116 the rum wash acidity, the greater the ester content in the resulting rum, however, this does not
117 mean that all acids present in the wash will undergo esterification (Cousins, 1906; McFarlane,
118 1946). These acids are produced via sugar metabolism and hence why if a distiller wants to
119 produce a “high ester” rum, the alcohol yield from that fermentation will be significantly
120 decreased, and the bottle will justifiably command a significantly higher price (McFarlane,
121 1946). Additionally, many of the organic acids found in rum, either in their free state or as esters
122 have high boiling points and are typically collected near the end of distillation and are therefore
123 concentrated in the spent wines in the retorts (McFarlane, 1946).

124
125 Cousins developed a high ester rum production process to alleviate the efficiency losses of
126 traditional flavored rum production and to deal with the high import tariffs of the German
127 market (Pietrek and Smith, 2022). Scale implementation of his process allowed any estate to
128 produce common clean variants or high ester rum without undertaking the long and
129 complicated traditional fermentation-based approach for producing “high flavored” rum, or be
130 forced to work with highly acidic dunder and the detrimental effects it would have on their
131 standard rum production process (Cousins, 1906). The “Cousins Process” can be seen as a
132 process diagram in **Figure 2**. This enabled distilleries to produce rums with ester contents up to
133 4,000-6,000 g/hLAA, designed for blending and were primarily used in the German market to
134 create authentic blended Jamaican rum products to combat the rise of rum verschnitt (Cousins,
135 1906; Pietrek and Smith, 2022). Thirty years later, these products had significantly affected the
136 demand for traditional Jamaican rum, and the significant backlash across the Jamaican rum
137 industry led to the establishment of a maximum ester content of 1,600 g/hLAA (Jamaica, 1935;
138 Pietrek, 2022).



139
140
141

Figure 1. Fermentation process for three styles of Jamaican rum, two versions of common clean, and one version of flavored/German rum. This process diagram is based on descriptions by Ashby (1911).



142
 143 **Figure 2. The legendary “Cousins Process” for producing very high ester rums used for blending. This**
 144 **process diagram is based on the description by Cousins (1906).**

145
 146 Some Caribbean distilleries followed the protocols of Pairault (1903), and used pure culture
 147 yeast strains, which lead to the production of more neutral rums, with lower levels of acids and
 148 esters, and higher amounts of higher alcohols, but most distilleries went back to using wild
 149 fermentations so as to produce more richly flavored rums regardless of production efficiency
 150 losses (McFarlane, 1946; Fährsmane and Ganou-Parfait, 1998). In Puerto Rico throughout the
 151 1940s, extensive investigations were conducted by Arroyo to determine commercially viable
 152 means to use cultured strains of yeast and bacteria in a controlled fermentation environment to

153 produce heavy rums (Arroyo, 1945a; Arroyo, 1945b). He determined that strains from the
154 propionic acid or butyric acid bacteria groups are ideal candidates for producing heavy rums,
155 and developed a list of specifications for selecting them (Arroyo, 1945b). In 1945, Arroyo
156 patented his production process wherein *Schizosaccharomyces pombé Lindner* was used
157 alongside *Clostridium saccharobutyricum* or *Propionibacterium technicum* to produce heavy
158 rum from treated molasses, and this can be seen in **Figure 3** (Arroyo, 1945a; Arroyo, 1945b).

159
160 As of 1946, although fermentation control was found to be the most important factor in rum
161 production, and higher pH and/or constant pH fermentations were just starting to be
162 considered, no Jamaican rum distilleries seriously attempted to correlate wash composition
163 with final distillate qualities or to explore the fermentation conditions where these compounds
164 are easily produced (McFarlane, 1946). However, the Jamaican rum style classification did
165 change to its current version and can be seen in **Table 3**. Surprisingly, by the 1950s, “production
166 targets in organoleptic properties seemed not to have been taken into account in choosing the
167 correct moment for modification of the fermentation stage (Fahrasmane and Ganou-Parfait,
168 1998).” Ultimately, economics caused many distilleries to standardize their production methods
169 and therefore use selected pure yeast cultures in fermentation (Fahrasmane and Ganou-Parfait,
170 1998).

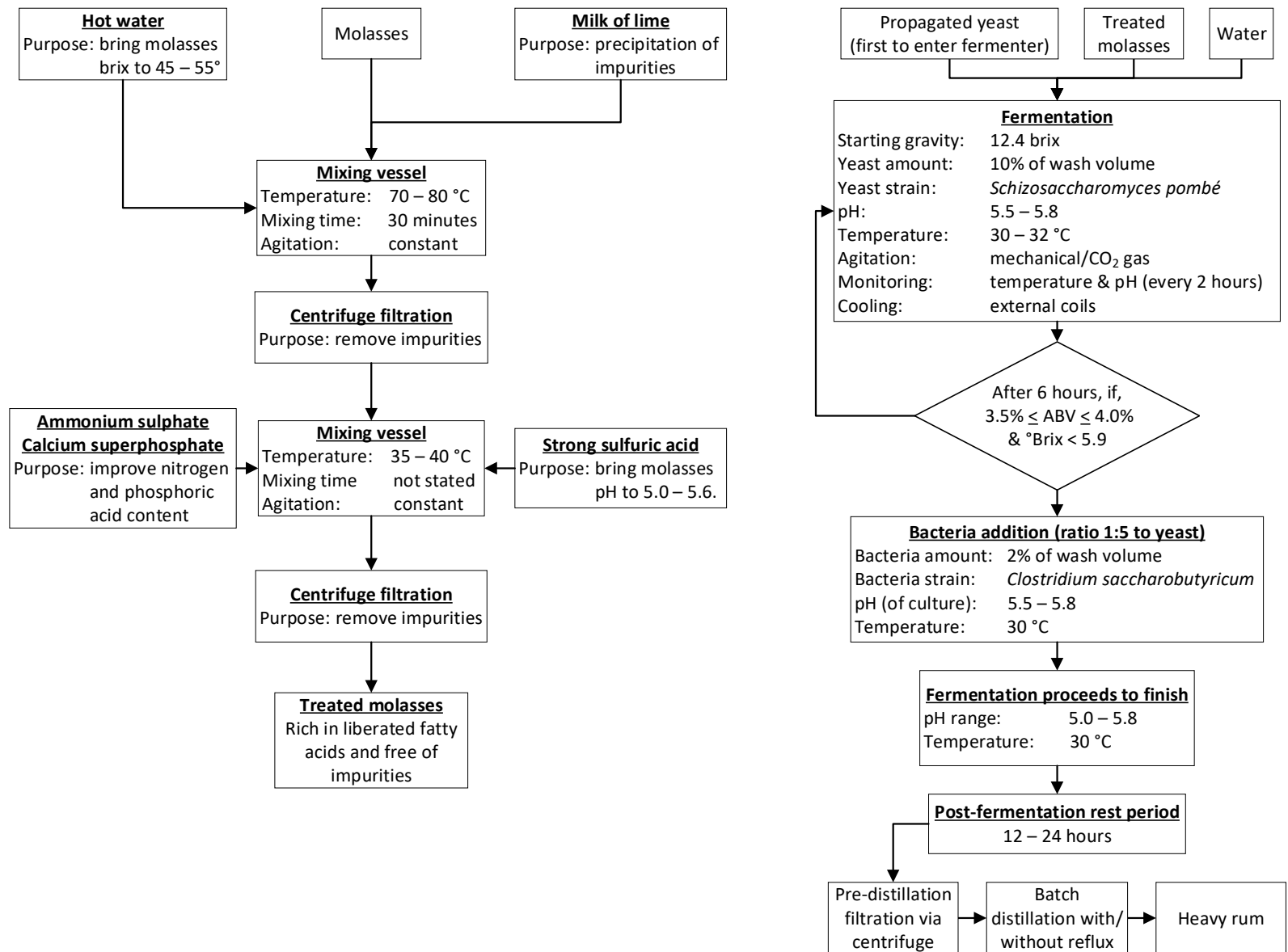
171

172 **Rum production and research 1960s – present**

173 As process standardization and the use of pure yeast cultures in fermentation became the
174 norm, most distilleries shifted away from wild fermentation in preference of controlled
175 fermentations, with the faster fermenting *Saccharomyces* spp. finding significantly more use
176 than the slower fermenting *Schizosaccharomyces* spp. yeasts (Fahrasmane et al., 1988). Just in
177 time, as consumer tastes were changing from heavier pot distilled rums towards lighter
178 continuously distilled rums, causing many distilleries to stop producing pot distilled rums
179 (l'Anson, 1971; Burglass, 2011). Although production styles were changing, researchers
180 continued to investigate the microbiology of rum and cachaça production.

181

182 In Brazil, Shehata (1960) found *Saccharomyces*, *Candida*, *Pichia*, and *Torulopsis* species were
183 prevalent on sugarcane plants and in fresh juice, however, only *Saccharomyces*, *Candida*, and
184 *Schizosaccharomyces* species were isolated from fermenting sugarcane juice. In the French West
185 Indies, Parfait et al. (1972), investigated the effects of *S. cerevisiae* and several non-
186 *Saccharomyces* strains on ester production from molasses-based and synthetic mediums and
187 found that *S. pombé* produced the greatest content of esters, and the non-*Saccharomyces*
188 strains, *Hansenula anomala* and *Candida krusei* produced the lowest. In Martinique, where
189 molasses-based Grand Arôme rum is produced, *S. pombé* and *Clostridium acetobutylicum* are
190 often found during fermentation and lead to heavy rums, rich in esters (>500 g/hLAA), volatile
191 acids (propionic, isobutyric, and butyric), and other organoleptic characteristics (Ganou-Parfait
192 and Parfait, 1980; Fahrasmane et al., 1983; Fahrasmane and Ganou-Parfait, 1997). Fahrasmane
193 et al. (1986), found that *S. pombé* appeared to have specific nutritional requirements which
194 could only be found in molasses-based fermentation media and required lengthy fermentations.



195
196 **Figure 3. Process diagrams showing Arroyo's molasses treatment and heavy rum production methods. These were created based on**
197 **description by Arroyo (1945b).**

198 **Table 3. Classifications of Jamaican rum styles circa 1940s, which has since become standardized.**

Reference	Rum style	Ester content
McFarlane (1946)	Common clean	80 – 150 g/hLAA
McFarlane (1946)	Plummer	150 – 200 g/hLAA
McFarlane (1946)	Wedderburn	200 – 300 g/hLAA
McFarlane (1946)	Flavored	700 – 1,600 g/hLAA

199
 200 From 1970-2010, much work was performed to characterize the yeasts and bacteria found in
 201 rum fermentation media and to understand how fermentation control affected their
 202 contributions to final distillate organoleptic quality. The extensive work performed by Lehtonen
 203 and Soumalainen (1977) has provided the most comprehensive analysis of rum organoleptic
 204 compounds (~200), the factors affecting their production, and the biochemical pathways leading
 205 to their formation (Green, 2015). Green (2015) has also compiled a comprehensive list. Ganou-
 206 Parfait et al. (1989), has provided a comprehensive list of 50 different bacteria species that are
 207 active during sugarcane fermentation and in which media (sugarcane juice, sugarcane syrup, or
 208 molasses) they are typically found.

209
 210 Fahrasmane and Ganou-Parfait (1998) further investigated the role of bacteria in rum
 211 fermentation and their major work, showing bacteria origins, location in the production cycle,
 212 optimum temperature and pH, fermentation features, and positive and/or negative effects on
 213 fermentation and distillate quality should be the starting point for any distiller seeking to
 214 incorporate bacteria into their fermentation program(s). The microaerophilic *Lactobacillus*
 215 species and *Propionibacterium* species were found to be the most significant bacteria in rum
 216 fermentation, as the acids they produce can be esterified, and positively contribute to the
 217 organoleptic characteristics of the resulting rum (Fahrasmane and Ganou-Parfait, 1997;
 218 Fahrasmane and Ganou-Parfait, 1998).

219
 220 For aromatic rum production, Lehtonen and Soumalainen (1977) recommended a fermentation
 221 temperature up to 30 °C and a pH range of 5.5-5.8, and a pH of 5.0 or greater for mixed
 222 fermentations using yeast and bacteria. Fleet and Green (2010) found several species of
 223 *Clostridium*, *Bacillus*, *Zymomonas*, lactic acid bacteria, and propionic bacteria and found that
 224 higher pH fermentation (i.e., > 5.5) offered the best chance for their contribution. However, very
 225 few studies have investigated how the combined use of selected yeast and bacteria affects the
 226 organoleptic qualities of rum.

227
 228 Nemoto (1975) built on Arroyo's 1945 work and investigated two methods for producing heavy
 229 rums from molasses-based media (1) the symbiotic fermentation of *S. pombé* and *Clostridium*
 230 *butyricum* and (2) adding a highly acidic wash (acidified by butyric acid bacteria) to a standard
 231 rum wash fermentation prior to distillation. Symbiotic fermentation was not successful above
 232 sugar concentrations greater than 14% glucose and heavy rum could only be produced in (2)
 233 when the pH was lowered to 2.0, thus freeing the butyric acid into solution to esterify and
 234 increase the ester content of the resulting rum (Nemoto, 1975).

235

236 From 2006-2010, Green (2015) systematically investigated the microbial ecology of the
237 molasses-based rum production process at the Bundaberg Distilling Company, Bundaberg,
238 Australia, including the contributions of bacteria during fermentation, the microbial ecology of
239 dunder (fresh and aged) and its effect on fermentation and rum organoleptic qualities. She
240 conducted a series of controlled molasses-based fermentation experiments (7.5% dunder, pH
241 5.5, 30° Brix) using *S. cerevisiae* and the three isolated lactic acid bacteria, *Lactobacillus*
242 *fermentum*, *Lactobacillus plantarum*, and *Lactobacillus* spp., in both single and mixed conditions
243 (Green, 2015). The control (*S. cerevisiae*) fermented at a nearly constant pH (5.2-5.3), whereas
244 the mixed fermentations experienced a significant pH drop (5.2 to 4.2-4.7) (Green, 2015).
245 Additionally, each mixed fermentation produced significantly more ethanol than the control
246 (6.0-7.9% vs 5.1% ABV), with the *S. cerevisiae* & *L. fermentum* trial producing significantly
247 greater concentrations of organoleptic compounds in the fermentation and the resulting
248 distillate (Green, 2015).

249
250 Hill et al. (2017), characterized the microbiology of dunder at a Scottish distillery, and assessed
251 its effect on fermentation and organoleptic characteristics when added to a controlled 96-hour
252 molasses-based fermentation. Five strains of *Lactobacillus* were isolated and identified from the
253 dunder (Hill et al., 2017). Additionally they found that the amount of dunder added (5% of
254 volume) was not sufficient to cause significant changes in the fermentation profile and thought
255 that by increasing the amount added or extending the fermentation time would've allowed
256 additional acid production or for the "symbiotic fermentation" described by Arroyo when he
257 worked with *S. pombe* and *C. saccharobutyricum* to produce heavy rums (Arroyo, 1945b; Hill et
258 al., 2017). However, there were significant increases in the amounts of organoleptic compounds
259 in the resulting distillate.

260
261 In Brazil, Duarte et al. (2011), investigated the effects of co-inoculation of *S. cerevisiae* and *L.*
262 *fermentum* on the quality of cachaça and found that co-inoculation yielded cachaça with higher
263 concentrations of acetaldehyde, ethyl acetate, and 2,3-butanedione, while cachaça produced
264 solely by the yeast had higher concentrations of ethyl lactate, propionic acid, butyric acid, and
265 1-pentanol. Finally, there is growing interest in using non-*Saccharomyces* yeast or a mixed
266 inoculation with *S. cerevisiae* for cachaça production (Duarte et al., 2013; Amorim et al., 2016).

267
268 The above-mentioned studies have shown that bacteria, particularly lactic acid bacteria, play a
269 positive role in the organoleptic properties of cachaça, rum, and whisky production, and thanks
270 to recent technological developments by Lallemend and Fermentis, these strains can be easily
271 used by producers to enhance the organoleptic characteristics of their spirits. This study is the
272 first to explore their use in commercial rum production.

273 274 **Project Aims**

275 Over the summer of 2022, as part of an American Distilling Institute grant, the author
276 investigated the effects of co-inoculation of commercially available yeast (Lalvin EC-1118™) and
277 three bacteria (DistilaBact® LP, EnoFerm Alpha™, SafSour LP 652™) on the organoleptic
278 properties of unaged, pot distilled rum, produced from Louisiana blackstrap molasses and raw
279 cane sugar. The bacteria were expected to increase the quantity of specific acids during

280 fermentation, which the yeast would metabolize into esters and ester precursors. All work was
 281 performed around the summer production schedule at Windon Distilling Company, the home of
 282 LYON RUM, in Saint Michaels, Maryland, USA. The distillery is representative of small producers
 283 and lacks the modern laboratory equipment which allows for in-depth analysis of fermentations
 284 (i.e., plating and culturing, cell counts, microscopy, etc.) or distillates (i.e., GC-MS).
 285

286 **Materials & Methods**

287 **Materials**

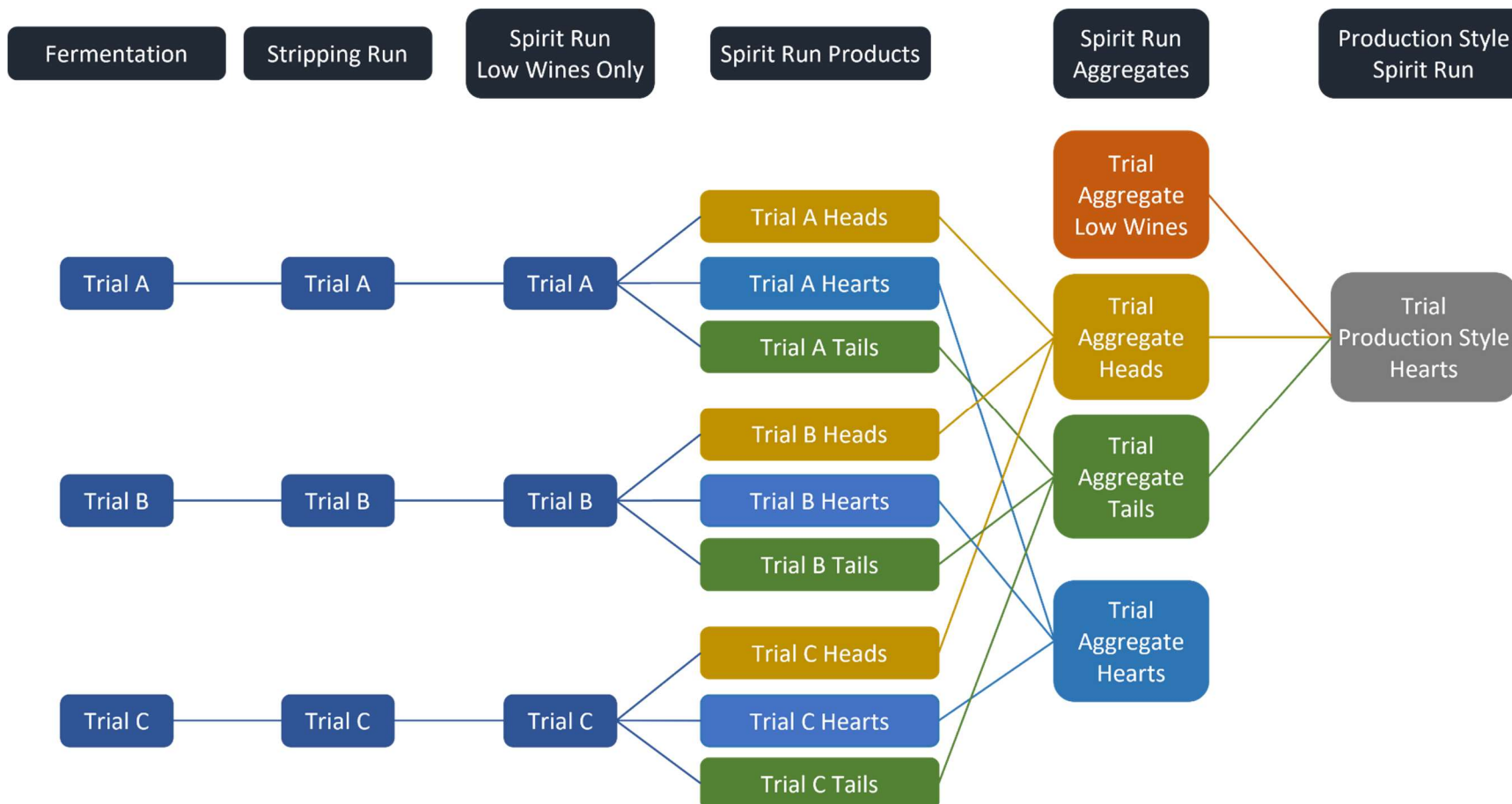
288 The blackstrap molasses and raw cane sugar are both non-GMO products of the Lula-Westfield
 289 Sugar Factory in Paincourtville, Louisiana, USA. The yeast and bacteria products are listed in
 290 **Table 4** and a complete list of equipment in **Table A** of the appendix.

291 **Table 4: Yeast and bacteria products used during this project.**
 292

Product	Strain	Supplier	Description
Lalvin EC-1118™	<i>S. cerevisiae bayanus</i>	Lallemand	Popular yeast in the American distilling scene, noted for its fermentation performance, neutral sensory contribution, and ability to showcase raw ingredients.
DistilaBact® LP	<i>L. plantarum</i>	Lallemand	Lactic acid bacteria product for use in the distilling industry, capable of producing sour mash related organoleptic properties, such as lactic (creamy), citrus, and tropical fruit notes.
EnoFerm Alpha™	<i>Oenococcus oeni</i>	Lallemand	Malolactic fermentation bacteria product typically used in the wine industry to add roundness, mouthfeel, red fruit, pear, and tropical fruit notes to wines.
SafSour LP 652™	<i>L. plantarum</i>	Fermentis	Kettle-souring bacteria used in the brewing industry to add citrus, tropical, and other fruity notes to various beer styles.

293
 294 **Methods**
 295 Each research trial was performed in triplicate. The experimental design is shown in **Figure 4**,
 296 with each trial having an A, B, and C segment. All fermentation vessels and related equipment
 297 were cleaned and sanitized before use. The yeast and bacteria were both rehydrated and added
 298 to the fermentation according to manufacturer directions, and the trial compositions and pitch
 299 rates are stated in **Table 5**.

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301
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303
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Figure 4. Project experimental design showing the composition of trial and production-style spirit runs. Each A, B, and C segment represents a complete trial triplicate. All triplicate low wines, heads, hearts, and tails were aggregated, as would be more typical in industry. This allowed a “production style” spirit run to be performed.

307 **Table 5. Trial compositions and component pitch rates. Each trial was performed in triplicate.**

Trial	Composition	Pitch rate (g/L)
Control	Lalvin EC-1118™	0.26
1	Lalvin EC-1118™ + DistilaBact® LP	0.26 0.1
2	Lalvin EC-1118™ + EnoFerm Alpha™	0.26 0.01
3	Lalvin EC-1118™ + SafSour LP 652™	0.26 0.1

308
309 Fermentation performance was tracked with a standard glass fermentation hydrometer and an
310 Oakton pH meter (calibrated weekly in pH 7 and pH 4 solutions, and properly stored between
311 uses). For stripping runs, a standard glass distillation hydrometer was used to track starting and
312 ending alcohol concentration (Percent alcohol by volume; %ABV). During fermentation, daily
313 measurements were taken for specific gravity, pH, and temperature, including a visual/sensory
314 check of activity. Fermentation was complete when there was no change in specific gravity
315 within a 24-hour period and no fermentation activity was visually present.

316
317 **Fermentation**

318 Each fermentation was 76 L with a composition of 10.89 kg blackstrap molasses, 9.07 kg raw
319 cane sugar, and 62.78 kg filtered municipal water. The target fermentation temperature was 30
320 °C, to aid in ester development. The molasses and sugar were weighed into the fermentation
321 vessel and then heated water (31-34 °C) was added. Then, each fermentation was thoroughly
322 mixed using a commercial immersion blender before yeast and bacteria additions. The setup for
323 weighing ingredients and heating the water is shown in **Figure 5**. Trial 2 had different
324 temperature requirements than the others and the water for this trial was heated to 29 °C, to
325 ensure a pitch temperature below 30 °C, and that the fermentation temperature would drop
326 below 27 °C when the fermentation was ~10% ABV. All fermentations took place in lidded 208 L
327 stainless steel drums and were completed after 144-163 hours, with an average wash strength
328 of 10.84% ABV.

329



330
331 **Figure 5. Equipment setup for weighing fermentation ingredients and heating the water.**

332 **Distillation**

333 Stripping runs were performed in 100 L pot stills, heated by an internal electric element (**Figure**
334 **6**). Once heated, the stills operated at 11 amps (out of 20) for an average of 8.4 hours. Low
335 wines were collected into glass carboys and had initial and final alcohol concentrations of 66%
336 ABV and 16% ABV, respectively, with an average yield of 15.14 L at 42% ABV per run.

337
338 Spirit runs were performed on an 11.36L (US 3 gallon) still, heated by an electric hot plate
339 (**Figure 7**). Once heated, the still operated at a heat setting of 4.5 (out of 5.0). Two sets of spirit
340 runs were performed, and the distillates for each set had the same cut points and collected
341 volumes. Additionally, the still, onion head, lyne arm, and worm tub condenser were rinsed
342 multiple times with hot water and dried between uses. The first set was distilled entirely from
343 low wines and will be referred to as **control**, **trial 1**, **trial 2**, and **trial 3**. For each triplicate, the
344 heads, hearts, tails, and remaining low wines were blended to create respective aggregates. The
345 second set was a “production style” spirit run using a ratio of low wines (85%), heads (10%), and
346 tails (5%), 11.36 L in total, and will be referred to as control (**WDC**), trial 1 (**WDC 1**), trial 2 (**WDC**
347 **2**), trial 3 (**WDC 3**). The hearts were then slowly proofed to 45% ABV using carbon filtered
348 municipal water, as is standard practice at Windon Distilling Company. The choice of 45% ABV
349 was not arbitrary and served as a point of comparison to our standard white rum, which is also
350 produced from a champagne-style yeast and bottled at 45% ABV.

351



352
353 **Figure 6. The 100 L stills that were used for stripping runs, showing controllers and the carboys used**
354 **for low wines collection.**

355



356
357 **Figure 7. The 11.36 L (3 US gallon) alembic pot still and hot plate used for all spirit runs.**
358

359 **Data analysis**

360 For each trial, samples were taken from the proofed aggregate hearts and brought to Brewing
361 and Distilling Analytical Services (BDAS Testing, <https://bdastesting.com>) in Lexington, Kentucky,
362 USA, for gas chromatography and sensory panel analysis. The services are detailed in **Table 6**.
363 After testing the data was analyzed using Microsoft Excel.

364
365 **Table 6: BDAS testing services utilized for this project.**

Testing service	Description
<u>CP09: Distilled Spirits Comprehensive Chemical Profile</u>	Alcohol % By Volume and Weight, Acidity (Volatile and Total), Haze, Higher Alcohols and Esters via GC, pH, and Residual Extracts/Total Solids.
<u>CP11: Taste panel evaluation</u>	Single evaluations and multi-time/date evaluations offered. A full 36 attribute descriptive sensory profile with radar (spider) charts, bar charts, and sensory summary.

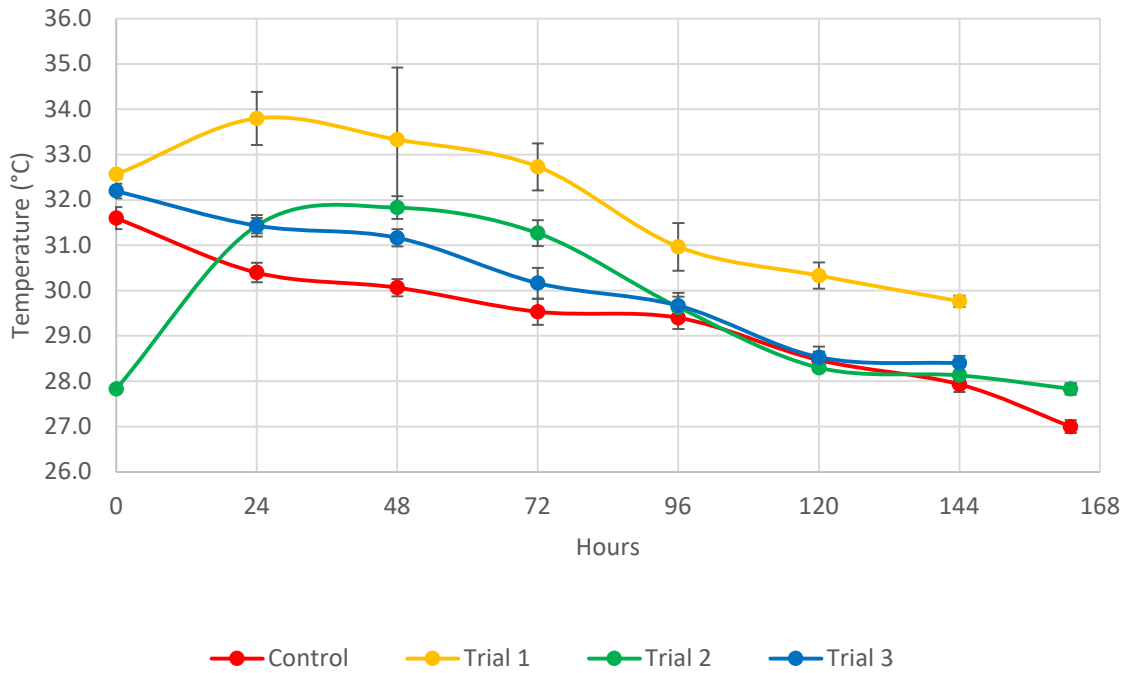
366
367 **Results**

368 **Fermentation results**

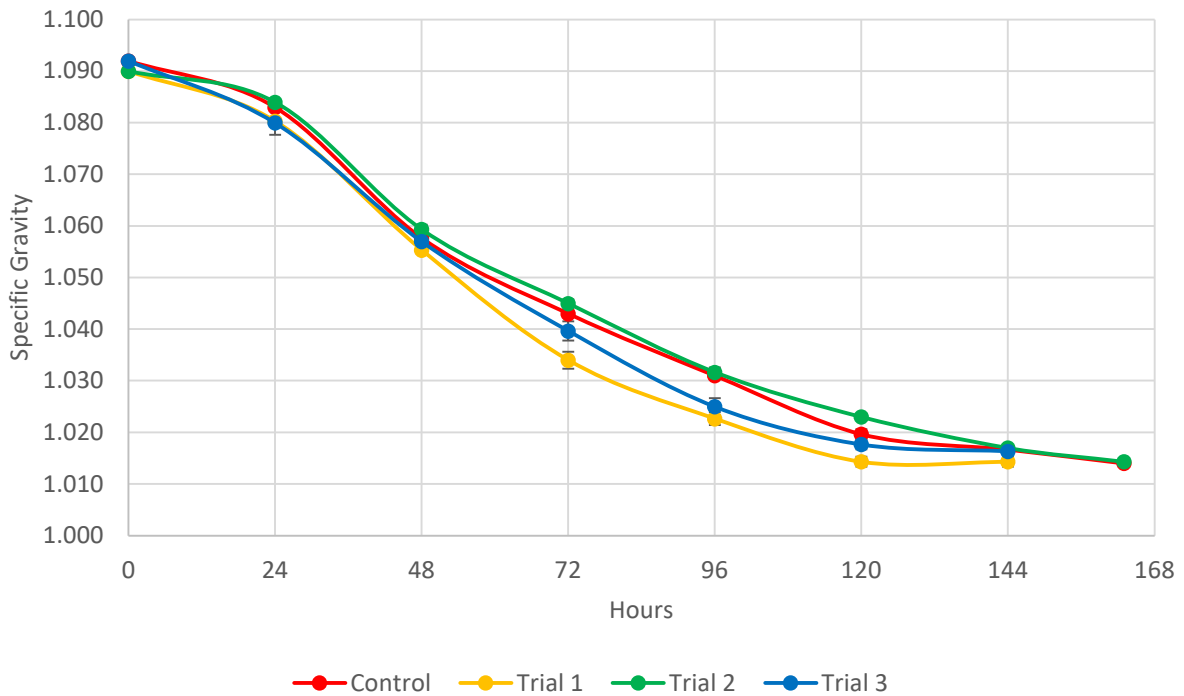
369 Fermentation performance was largely unaffected, with little difference between final levels of
370 trial pH, specific gravity, and %ABV, versus the control, except for trial 2, which had significantly
371 higher pH (**Table 7** and **Figures 8-11**). Fermentation length varied between 144-163 hours and
372 was found to be significantly different for trial 1 and trial 3 compared to control (144 hours vs
373 163 hours). No differences were found in fermentation length for trial 2 (163 hours).
374

Table 7. Trial fermentation data showing triplicate averages and standard deviations for each factor.

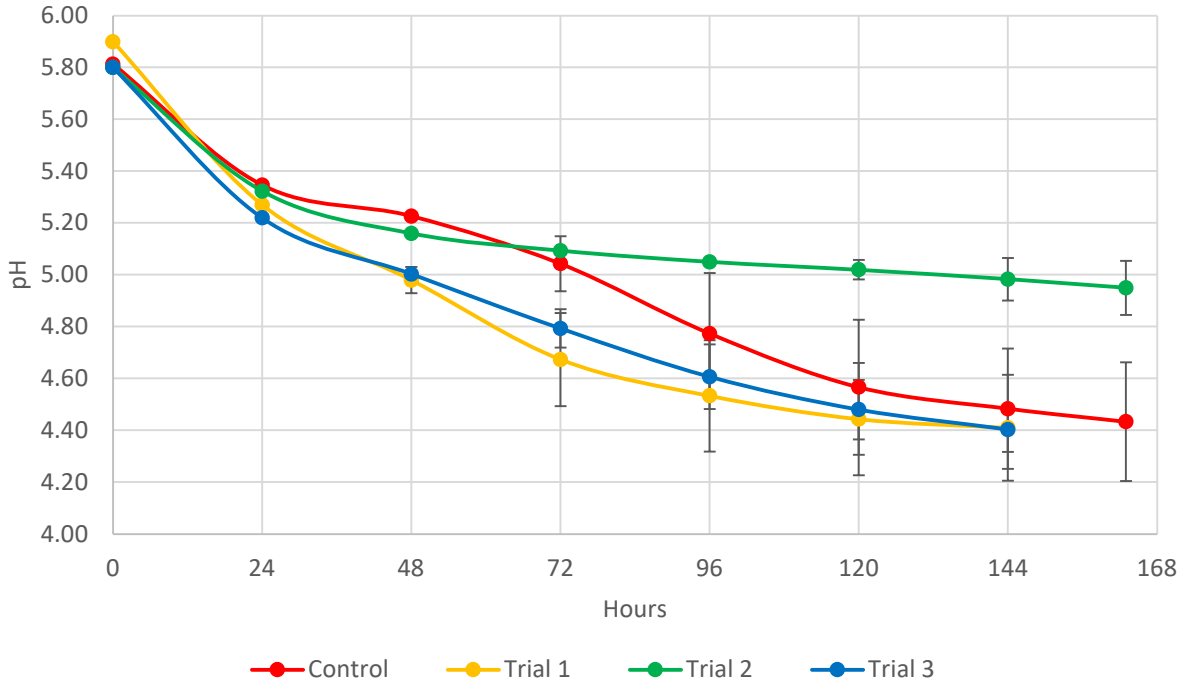
Trial	Hours	Temperature (°C)	Specific Gravity	pH	%ABV
Control	0	31.6 ± 0.2	1.092 ± 0.000	5.81 ± 0.01	00.00 ± 0.00
	24	30.4 ± 0.2	1.083 ± 0.000	5.35 ± 0.01	01.37 ± 0.00
	48	30.1 ± 0.2	1.058 ± 0.001	5.23 ± 0.01	05.09 ± 0.14
	72	29.5 ± 0.3	1.043 ± 0.000	5.04 ± 0.11	07.17 ± 0.00
	96	29.4 ± 0.2	1.031 ± 0.000	4.77 ± 0.23	08.82 ± 0.00
	120	28.5 ± 0.2	1.020 ± 0.001	4.57 ± 0.26	10.35 ± 0.13
	144	27.9 ± 0.2	1.017 ± 0.000	4.48 ± 0.23	10.74 ± 0.06
	163	27.0 ± 0.1	1.014 ± 0.000	4.43 ± 0.23	11.10 ± 0.00
Trial 1	0	32.6 ± 0.1	1.090 ± 0.000	5.90 ± 0.00	00.00 ± 0.00
	24	33.8 ± 0.6	1.080 ± 0.003	5.27 ± 0.00	01.46 ± 0.39
	48	33.3 ± 1.6	1.055 ± 0.000	4.98 ± 0.05	05.12 ± 0.07
	72	32.7 ± 0.5	1.034 ± 0.002	4.67 ± 0.18	08.11 ± 0.21
	96	31.0 ± 0.5	1.023 ± 0.001	4.53 ± 0.21	09.63 ± 0.17
	120	30.3 ± 0.3	1.014 ± 0.001	4.44 ± 0.22	10.74 ± 0.12
	144	29.8 ± 0.1	1.014 ± 0.001	4.41 ± 0.20	10.74 ± 0.12
Trial 2	0	27.8 ± 0.0	1.090 ± 0.000	5.80 ± 0.00	00.00 ± 0.00
	24	31.4 ± 0.2	1.084 ± 0.000	5.32 ± 0.00	00.91 ± 0.00
	48	31.8 ± 0.2	1.059 ± 0.001	5.16 ± 0.00	04.55 ± 0.14
	72	31.3 ± 0.3	1.045 ± 0.001	5.09 ± 0.00	06.58 ± 0.11
	96	29.6 ± 0.2	1.032 ± 0.001	5.05 ± 0.01	08.42 ± 0.13
	120	28.3 ± 0.1	1.023 ± 0.000	5.02 ± 0.04	09.59 ± 0.00
	144	28.1 ± 0.0	1.017 ± 0.000	4.98 ± 0.08	10.38 ± 0.00
	163	27.8 ± 0.1	1.014 ± 0.000	4.95 ± 0.10	10.74 ± 0.06
Trial 3	0	32.2 ± 0.2	1.092 ± 0.000	5.80 ± 0.00	00.00 ± 0.00
	24	31.4 ± 0.2	1.080 ± 0.000	5.22 ± 0.01	01.82 ± 0.00
	48	31.2 ± 0.2	1.057 ± 0.001	5.00 ± 0.00	05.19 ± 0.11
	72	30.2 ± 0.3	1.040 ± 0.002	4.79 ± 0.07	07.63 ± 0.26
	96	29.7 ± 0.3	1.025 ± 0.002	4.61 ± 0.12	09.63 ± 0.22
	120	28.5 ± 0.2	1.018 ± 0.001	4.48 ± 0.12	10.61 ± 0.12
	144	28.4 ± 0.2	1.016 ± 0.001	4.40 ± 0.09	10.79 ± 0.12



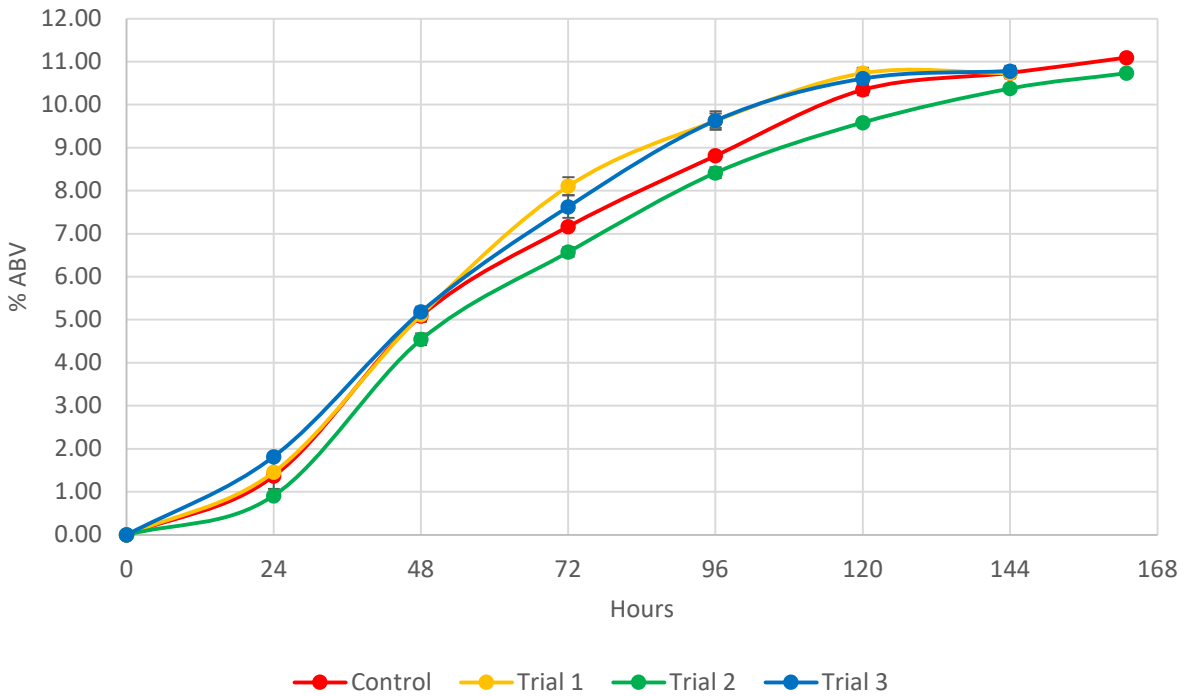
377
 378 **Figure 8. Trial fermentation temperature. Each data point and associated error bar represents the trial**
 379 **triplicate average and standard deviation.**
 380



381
 382 **Figure 9. Trial fermentation performance averages for specific gravity. Each data point and associated**
 383 **error bar represents the trial triplicate average and standard deviation.**
 384



385
 386 **Figure 10. Trial fermentation performance averages for pH. Each data point and associated error bar**
 387 **represents the trial triplicate average and standard deviation.**
 388



389
 390 **Figure 11. Trial fermentation performance averages for alcohol concentration (v/v). Each data point**
 391 **and associated error bar represents the trial triplicate average and standard deviation.**
 392

393 **Gas chromatography and sensory panel results**

394 All trials had similar concentrations of higher alcohols and esters to their respective controls,
395 except for isobutanol and active amyl and iso-amyl alcohols, which were distinctly different for
396 trials 1, 2, WDC 1, and WDC 2. Additionally, the total fusel oil content, which is the sum of n-
397 Propanol, Isobutanol, 1-Butanol, and active amyl and iso-amyl alcohols, was distinctly different
398 in trials 1-3, WDC 1 and WDC 2 (**Table 8** and **Figure 12**).

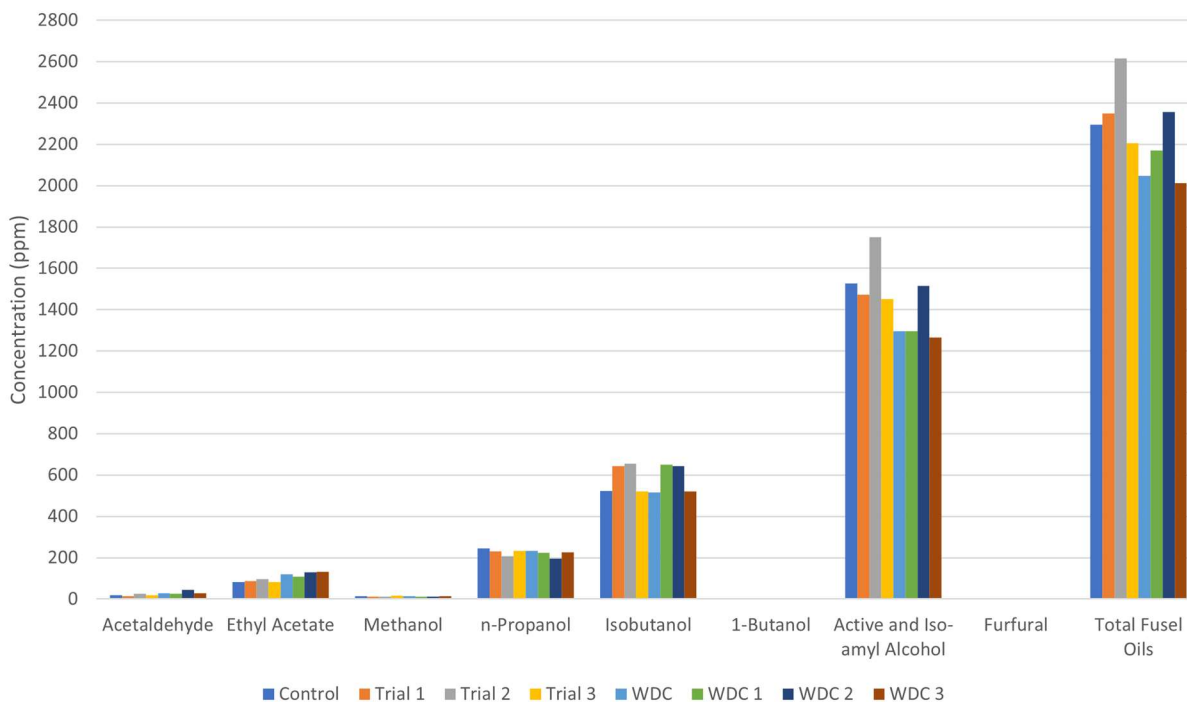
399
400 A trained sensory panel evaluated all distillates using a 36-point attribute ballot (**Table 9**). Each
401 trial was found to be distinctly different than their respective control with trial 2 having a
402 greater overall score than the control (1.11 vs 0.43) and WDC 3 scoring higher than WDC (0.54
403 vs 0.40) (**Figure 13** and **Figure 14**, respectively). BDAS testing states on their sensory panel
404 results that, "A consistently well produced spirit beverage with little to fault it and one
405 appropriate to intended style, class, or type, and at its best, fresh release, would potentially
406 earn a score of between 1.0- 2.0 and a zero score represents a sample with little to fault but
407 nothing to note.". The investigator self-assessed each distillate set and found them to be
408 distinctly different than their respective controls, with trial 1 preferable to the control and WDC
409 1 preferable to WDC (**Table 10**).

410

411 **Table 8. Gas chromatography results for each trial showing the concentrations of compounds detected.**
 412 **Ester and higher alcohol concentrations were determined in accordance with the SSD:TM:200 testing**
 413 **method. Total fusel oils represent the sum of n-Propanol, Isobutanol, 1-Butanol, and Active amyl and**
 414 **iso-amyl alcohols. Compound threshold values from Hill (2023): acetaldehyde (8.1 ppm), ethyl acetate**
 415 **(16.8 ppm), n-propanol (7.8 ppm), isobutanol (6.5 ppm), 1-butanol (5 ppm), isoamyl alcohol (5-10**
 416 **ppm), furfural (30 – 230 ppm).**

	Control	Trial 1	Trial 2	Trial 3	WDC	WDC 1	WDC 2	WDC 3
Acetaldehyde (ppm)	17.85	13.14	25.10	17.94	29.24	25.68	45.42	27.27
Ethyl acetate (ppm)	83.44	85.89	96.69	81.91	119.57	108.71	129.67	131.87
Methanol (ppm)	13.75	12.81	12.74	15.22	13.29	12.26	11.01	14.29
n-Propanol (ppm)	245.37	231.32	206.66	232.35	233.52	224.74	196.33	225.86
Isobutanol (ppm)	521.91	644.08	655.27	519.73	516.41	649.12	642.31	520.65
1-Butanol (ppm)	1.14	0.91	0.56	0.91	1.10	1.08	0.69	1.02
Active amyl and Iso-amyl alcohols (ppm)	1527.34	1472.95	1751.77	1452.06	1295.7	1295.47	1516.14	1265.66
Furfural (ppm)	2.93	2.78	1.23	1.55	1.59	1.72	0.94	0.8
Total fusel oils (ppm)	2295.77	2349.27	2614.26	2205.06	2046.73	2170.42	2355.47	2013.19

417



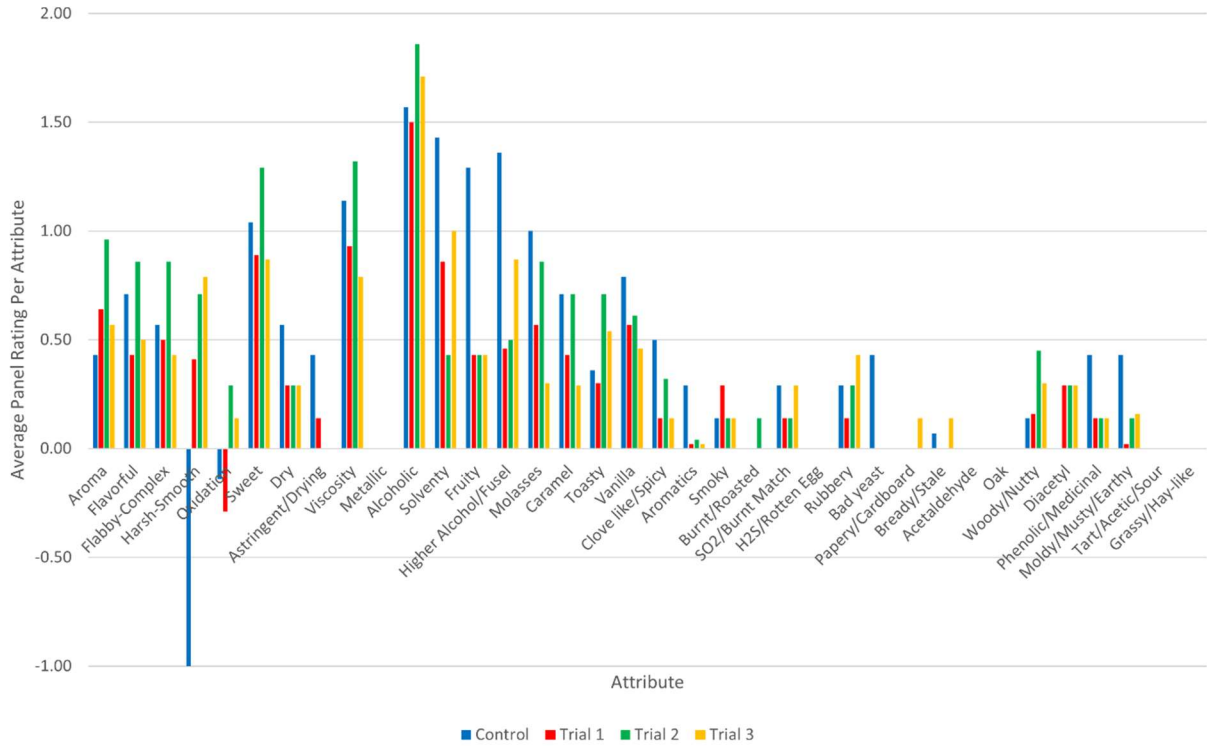
418

419 **Figure 12. Gas chromatography results for each trial showing compound concentrations. Note: total**
 420 **fusel oils represent the sum of n-Propanol, Isobutanol, 1-Butanol, and Active amyl and iso-amyl**
 421 **alcohols.**

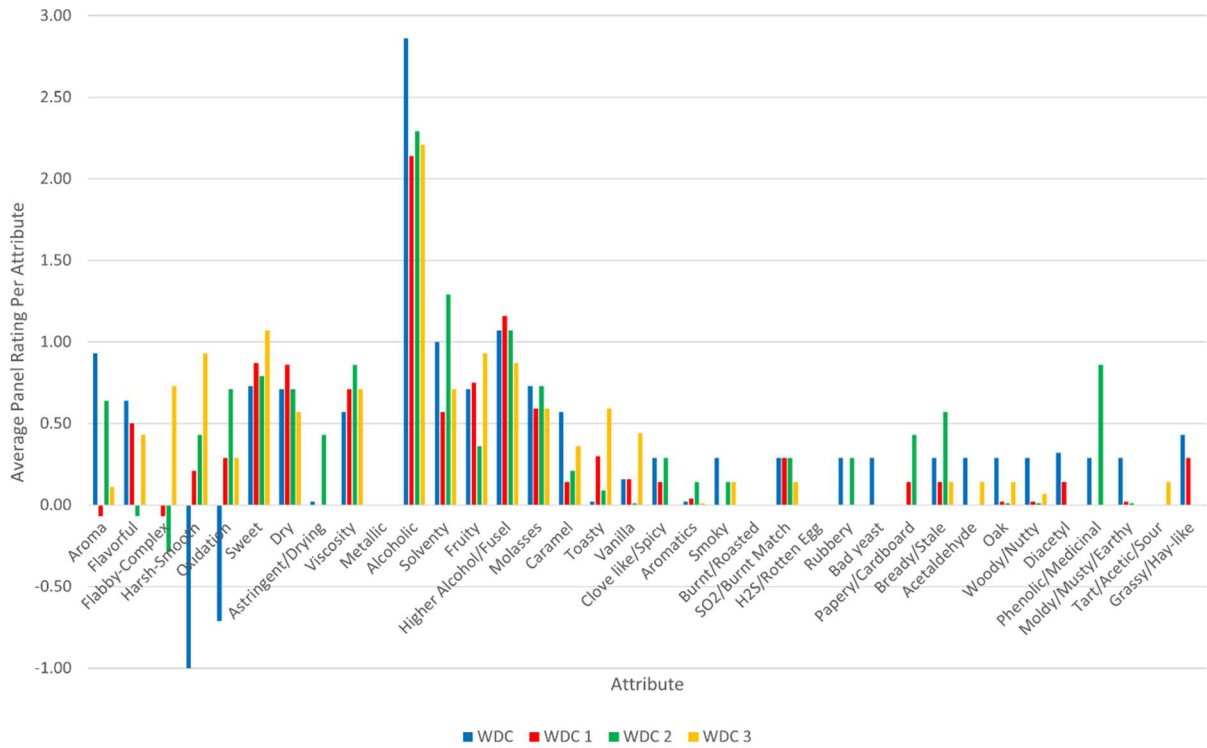
422 **Table 9. Sensory panel evaluation results from BDAS Testing in Lexington, Kentucky, USA. All values**
 423 **represent the average panel scores for each parameter. Bold values indicate (1) trial values greater**
 424 **than the control or (2) control values greater than trials.**

Sample ID/Parameter	Control	Trial 1	Trial 2	Trial 3	WDC	WDC 1	WDC 2	WDC 3
Overall Score	0.43	0.68	1.11	0.40	0.40	0.29	-0.21	0.54
Aroma	0.43	0.64	0.96	0.57	0.93	-0.07	0.64	0.11
Flavorful	0.71	0.43	0.86	0.50	0.64	0.50	-0.07	0.43
Flabby-Complex	0.57	0.50	0.86	0.43	0.00	-0.07	-0.29	0.73
Harsh-Smooth	-1.00	0.41	0.71	0.79	-1.00	0.21	0.43	0.93
Oxidation	-0.14	-0.29	0.29	0.14	-0.71	0.29	0.71	0.29
Sweet	1.04	0.89	1.29	0.87	0.73	0.87	0.79	1.07
Dry	0.57	0.29	0.29	0.29	0.71	0.86	0.71	0.57
Astringent/Drying	0.43	0.14	0.00	0.00	0.02	0.00	0.43	0.00
Viscosity	1.14	0.93	1.32	0.79	0.57	0.71	0.86	0.71
Metallic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alcoholic	1.57	1.50	1.86	1.71	2.86	2.14	2.29	2.21
Solventy	1.43	0.86	0.43	1.00	1.00	0.57	1.29	0.71
Fruity	1.29	0.43	0.43	0.43	0.71	0.75	0.36	0.93
Higher Alcohol/Fusel	1.36	0.46	0.50	0.87	1.07	1.16	1.07	0.87
Molasses	1.00	0.57	0.86	0.30	0.73	0.59	0.73	0.59
Caramel	0.71	0.43	0.71	0.29	0.57	0.14	0.21	0.36
Toasty	0.36	0.30	0.71	0.54	0.02	0.30	0.09	0.59
Vanilla	0.79	0.57	0.61	0.46	0.16	0.16	0.01	0.44
Clove like/Spicy	0.50	0.14	0.32	0.14	0.29	0.14	0.29	0.00
Aromatics	0.29	0.02	0.04	0.02	0.02	0.04	0.14	0.01
Smoky	0.14	0.29	0.14	0.14	0.29	0.00	0.14	0.14
Burnt/Roasted	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00
SO2/Burnt Match	0.29	0.14	0.14	0.29	0.29	0.29	0.29	0.14
H2S/Rotten Egg	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rubbery	0.29	0.14	0.29	0.43	0.29	0.00	0.29	0.00
Bad yeast	0.43	0.00	0.00	0.00	0.29	0.00	0.00	0.00
Papery/Cardboard	0.00	0.00	0.00	0.14	0.00	0.14	0.43	0.00
Bready/Stale	0.07	0.00	0.00	0.14	0.29	0.14	0.57	0.14
Acetaldehyde	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.14
Oak	0.00	0.00	0.00	0.00	0.29	0.02	0.01	0.14
Woody/Nutty	0.14	0.16	0.45	0.30	0.29	0.02	0.01	0.07
Diacetyl	0.00	0.29	0.29	0.29	0.32	0.14	0.00	0.00
Phenolic/Medicinal	0.43	0.14	0.14	0.14	0.29	0.00	0.86	0.00
Moldy/Musty/Earthy	0.43	0.02	0.14	0.16	0.29	0.02	0.01	0.00
Tart/Acetic/Sour	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14
Grassy/Hay-like	0.00	0.00	0.00	0.00	0.43	0.29	0.00	0.00

425



426
 427 **Figure 13. Sensory panel results showing the average attribute score for the low-wines-only distillates.**
 428 **The overall scores were – control 0.43, trial 1 0.68, trial 2 1.11, and trial 3 0.40.**
 429



430
 431 **Figure 14. Sensory panel results showing the average attribute score for the production-style**
 432 **distillates. The overall scores were – WDC 0.40, WDC 1 0.29, WDC 2 -0.21, WDC 3 0.54.**

433 **Table 10: Self-assessment sensory notes for low-wines-only and production-style distillate samples.**

Trial	Self-assessment notes	Trial	Self-assessment notes
Control	<u>Nose</u> Neutral, but with hints of molasses and grass, and very slight notes of phenols	WDC	<u>Nose</u> Creamy, grassy, with hints of custard cream and vegetal notes, and alcohol presence.
	<u>Palate</u> Creamy profile and mouthfeel, with hints of grassy/herbaceous notes and a very, very faint phenol presence.		<u>Palate</u> Very creamy and grassy! Coconut crème, grass, with alcohol presence and a shorter finish.
Trial 1	<u>Nose</u> Citrus and tropical fruit notes on top of a grassy and coconut crème mid note and a gentle floral and molasses base note.	WDC 1	<u>Nose</u> Incredible nose! Very good balance of creamy, grassy, citrus, and tropical notes.
	<u>Palate</u> More complex than Lalvin EC-1118™, with tropical fruit and citrus, grass, coconut crème. No phenolic notes. Long, delightful finish.		<u>Palate</u> Very much like the nose. Great balance of creamy, grassy, citrus, and tropical notes. Long creamy, grassy, tropical finish! This would continue to develop in barrel and be a perfect candidate for a cognac cask.
Trial 2	<u>Nose</u> Creamy, floral, red apple and peel notes, gentle molasses aromas, faint grass, and alcohol vapors.	WDC 2	<u>Nose</u> Very different than the above two WDC samples. More apple/red apple, pear, grass, and spices. There is an unpleasant phenol off-aroma that comes through.
	<u>Palate</u> Molasses/coconut crème, grassy, floral with red fruit notes, apples, and a long, layered finish.		<u>Palate</u> Somewhat flatter, with a not too pleasant phenolic note. Alcohol presence isn't balanced. Finish is long but not complex. Burns.
Trial 3	<u>Nose</u> Grassy, with coconut crème, alcohol, faint molasses, and hints of tropical and citrus fruits.	WDC 3	<u>Nose</u> Bigger and bolder than the other SafSour sample, with significantly more citrus and tropical creamy notes, mild alcohol presence with a grassy, coconut crème complex.
	<u>Palate</u> Grassy with coconut crème notes, citrus, and tropical fruits, bright and very creamy, hints of almonds and slightly floral.		<u>Palate</u> Less well combined than the nose. Mostly alcohol, with hints of citrus, tropical flavors, coconut crème and grass. Barrel aging would improve.
Ranking	Trial 1, trial 3, trial 2, control	Ranking	WDC 1, WDC, WDC 3, WDC 2

435 **Discussion**

436 **Fermentation**

437 Except for trial 2 pH, all trials had similar fermentation performance to the control, including
438 yield (control 11.10 %ABV vs trials 10.74-10.79 %ABV). This is noteworthy since the deliberate
439 use of bacteria in fermentation can be detrimental to overall performance and yield however,
440 these modern bacteria products have mostly demonstrated a beneficial ability to work with this
441 selected yeast strain to produce rum with unique characteristics. During visual inspections, each
442 co-inoculation fermentation had a better fermenting appearance than the control and this may
443 be due to the added bacteria strains dominating other strains present in the fermentation
444 media. Both the DistilaBact® LP and SafSour LP 652™ were developed specifically for the
445 distilling industry and functioned as expected. However, EnoFerm Alpha™ was developed
446 specifically for the wine industry, and its lower pitch temperature, along with other temperature
447 conditions, may have affected the yeast performance during fermentation leading to off-note
448 development.

449
450 Although yields weren't dramatically affected, off-notes were detected by the sensory panel,
451 and there are many potential factors for this. Lack of temperature control (**Figure 8**) may have
452 played a role in the presence of several off-notes found by the sensory panel, particularly for
453 trial 2, where fermentation temperatures were above 27 °C when the fermentation was ~10%
454 ABV. It was surprising to see how EnoFerm Alpha™ affected the fermentation pH. It was
455 expected that the pH would have dropped similarly to the other bacteria trials, however, it
456 stalled at just under pH 5.0. The fermentation composition could have played a role in buffering
457 the effects of the bacteria. Had time allowed, it may have been best to wait a day between
458 fermentation being confirmed as "complete" to allow the bacteria more time to work, as was
459 suggested by others (Allan, 1905; Ashby, 1911; Pietrek and Smith, 2022).

460

461 **Distillation**

462 This project took place around the summer production schedule of LYON RUM which meant
463 that all distillations were performed on weekends and two stripping runs needed to happen on
464 Saturdays. Since each run required at least 8.4 hours, lack of distilling time is why the low wines
465 were not collected down to 5% ABV as is more typical at the distillery. Therefore, the cut points
466 for both stripping and spirit runs likely played a role in the concentrations of compounds found
467 in the distillates. Many of the acid-based esters come over late in the distillation and one reason
468 the effects of the selected bacteria were not more pronounced is likely due to the cut points.
469 McFarlane (1946) found this to be true and the primary reason for the success of the Cousins
470 Process as these acids are typically concentrated in the retorts. The addition of heads and tails
471 also played a role in the final distillate organoleptic qualities, and for WDC 2, may have added
472 compounds that later resulted in the significant phenolic off-notes present in the distillate. The
473 decision to use in-house pot stills for distillation instead of a column still or a pot still with
474 several plates, played a major role in final distillate quality, since the greater reflux of the latter
475 still types could make it more difficult to distinguish the beneficial organoleptic effects of the
476 bacteria. Additionally, the worm tub condenser (**Figure 7**) may have added perceived fault notes
477 as these types of condensers are known to maintain sulfur notes and produce heavier spirits
478 that benefit from longer maturation.

479 **Sensory impact**

480 In general, all distillates exceeded respective compound threshold values except for 1-butanol
481 and furfural (**Table 8**). Compared to their respective control, each bacteria had distinct effects
482 on distillate organoleptic characteristics – isobutanol and active amyl and iso-amyl alcohols
483 concentrations for trial 1, trial 2, WDC 1, WDC 2, and total fusel oil concentration for trial 2,
484 WDC 1, and WDC 2 – and echoes the findings of Duarte et al. (2011) and Green (2015) that co-
485 inoculation of *S. cerevisiae* and *L. fermentum* or *L. plantarum* in sugarcane-based fermentation
486 media can produce distillates with enhanced organoleptic characteristics and compound
487 concentrations.

488
489 Sensory panel results (**Table 9, Figure 13**) show that the trials had lower average scores than the
490 control, specifically: harshness, dryness, astringent/drying, stale, phenolic, and musty/earthy.
491 However, the control had greater average scores for solventy, fruity, higher alcohol/fusel
492 (surprising), molasses, vanilla, clove-like/spicy, aromatic. For all other attributes, at least one of
493 the trials had the same or greater average score than the control. For the production-style
494 distillates (**Table 9, Figure 14**), WDC was found to have more fault notes than the trials,
495 specifically: harshness, oxidation, bad yeast, smokiness, and moldy/musty/earthiness.
496 Additionally, WDC had greater scores for aroma, flavorful, alcoholic, caramel, acetaldehyde, oak
497 (surprising since it's not matured), woody/nutty, diacetyl, and grassy/hay like. For all other
498 attributes, at least one of the trials had the same or greater average score than the control.

499
500 It was quite surprising that the overall scores for the production-style distillates were lower than
501 the low-wines-only distillates. This was most significant for WDC 2 compared to trial 2 (-0.21 vs
502 1.11), with the former containing noticeable phenolic off-notes. This suggests the amounts of
503 heads and tails negatively affected distillate quality. For both distillate sets, the sensory panel
504 preferred at least one trial to their respective control (trial 1, trial 2, and WDC 3 each scoring
505 higher). Overall impressions suggest that each bacteria adds significant roundness and
506 fruity/sweet notes to the distillates. During self-assessment, this presented as tropical/citrus
507 notes, with enhanced creaminess on the profile. Low wines and heads, hearts, and tails cut
508 points likely played a role in the organoleptic qualities of each distillate. Process refinement
509 would improve these qualities and reduce the presence of off-notes, as would maturation and
510 the beneficial effects of oak and air contact.

511 512 **Conclusions and future work**

513 This project has shown that co-inoculation fermentations are capable of increasing product
514 organoleptic characteristics, without significant process complexity or yield loss. These results
515 are from one-shot trials with a champagne-style yeast, *S. cerevisiae bayanus*, noted for its
516 neutral effects on the resulting distillate. With continued process refinement, and focusing on a
517 single bacteria product, the presence of off-notes would be significantly reduced.

518
519 Future work should focus on (1) the effects of cut points, and heads and tails additions on final
520 distillate quality; (2) determining the ideal spirit run cut points for each bacterial product to best
521 showcase their effects on final distillate quality. The latter case could be accomplished by first
522 determining cut points for the control and then, repeating these exactly for the first trial, with

523 subsequent trials modifying the heads and tails cuts. Then, if desired, “production style”
524 distillate trials would be similarly performed, with trials varying the amounts of heads and tails
525 added, to further determine their effects on final distillate organoleptic quality. Changing the
526 yeast strain or using multiple yeasts in concert with these novel bacterial products, could yield
527 remarkably complex rums. Maturation also plays a significant role in spirit quality and can
528 improve the harsh/unpleasant characteristics of new make distillates through the numerous
529 reactions taking place within the barrel over time and thus transform it into excellent aged
530 spirit. Therefore, if time and budget allow, performing the above future work as part of a
531 longitudinal study, would show the effects of maturation on the resulting distillates over time,
532 and yield commercially actionable data on the utility of these bacteria products.

533
534 It is imperative that distilleries and suppliers continue to openly communicate, discuss, develop,
535 and trial novel bacteria products, or yeast-bacteria combination products. It’s truly an exciting
536 time to be a distiller! In the near future, suppliers could offer “starters” which would be
537 combinations of yeast and bacteria, tailored to provide specific profiles from a particular raw
538 ingredient base and/or beverage category. And when that day comes, the industry will have
539 come full circle to considerations made by Greg (1895d), Pairault (1903); Allan (1905), Ashby
540 (1909), and countless others over 100 years ago. Except this time, we will be able to select
541 specific strains of bacteria and yeast that can work together to create rums with specific profiles
542 brought about through fermentation control, understanding of fermentation microbiology,
543 timing for bacteria addition, and the effects that distillation cut points have on the
544 concentration of compounds found in the rum. Clearly there is much work to be done in this
545 area and this research topic is wide open for those researchers intrepid enough to make their
546 mark.

547

548 **Acknowledgements**

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555 80+ hour weeks required to perform project work around the summer production schedule.
556 Lastly, thank you to my family for always supporting my decisions, even when they initially seem
557 crazy or unrealistic. Often, many ideas appear crazy or foolhardy up until the point where those
558 ideas change the world.

559

560 **References**

561

562 ALLAN, C. 1905. Report on the Manufacture of Jamaica Rum. *In: COUSINS, H. H. (ed.) Report on*
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Appendix

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Table A. Complete list of equipment used during this project, including their associated costs.

Item	Model / Type	Brand	Website	Quantity	Unit Cost	Total
RV water filter	KDF/Carbon filter	Camco	https://www.amazon.com/Camco-TastePURE-Flexible-Protector-40043/dp/B0006IX87S/ref=sr_1_5?crd=150PB8AWQIMJC&keywords=camco+water+filter&qid=1681663726&sprefix=camco+%2Caps%2C143&sr=8-5	1	\$ 16.66	\$ 16.66
Stainless steel drum with lid	55-gallon	Bubba's Barrels	https://www.bubbasbarrels.com/55-gallon-open-head-drum-20-gauge	3	\$ 523.00	\$ 1,569.00
Stainless steel stock pot with lid	80 Qt	Vigor	https://www.webstaurantstore.com/vigor-80-qt-heavy-duty-stainless-steel-aluminum-clad-stock-pot-with-cover/4735SPOT80.html	2	\$ 189.93	\$ 379.86
Induction cooktop, portable	208 – 240 V, 3500 W	Avantco	https://www.webstaurantstore.com/avantco-ic3500-countertop-induction-range-cooker-208-240v-3500w/177IC3500.html	2	\$ 208.62	\$ 417.24
Table stove	120 V, 900 W	Oster	https://www.amazon.com/Oster-CKSTSB100-B-2NP-Adjustable-Temperature-Control/dp/B0082JMCB6	1	\$ 60.00	\$ 60.00
Immersion blender	1.25 HP, 14" shaft	Avamix	https://www.webstaurantstore.com/avamix-ibhd14-14-heavy-duty-variable-speed-immersion-blender-1-1-4-hp/928IBHD14.html	1	\$ 349.99	\$ 349.99
Platform scale	650 lbs x 0.25 lbs	Global Industrial	https://www.globalindustrial.com/p/industrial-bench-floor-scale-660-lb-x-0-25-lb	1	\$ 300.95	\$ 300.95
Digital scale	500g x 0.01g	Brifit	https://www.amazon.com/Upgraded-Digital-Kitchen-Back-Lit-Included/dp/B08DXWFZLZ?th=1	1	\$ 11.99	\$ 11.99
Fermentation hydrometer	Specific gravity	Northern Brewer	https://www.northernbrewer.com/products/beer-and-wine-triple-scale-hydrometer	1	\$ 7.99	\$ 7.99
Alcohol hydrometer	%ABV & Proof	Brewer's Supply Group	https://www.amazon.com/Proof-Tralle-Hydrometer-200/dp/B01C7MRFYW/ref=sr_1_3?crd=19A7X4EDXSCE&keywords=bsg+hydrometer&qid=1681662064&sprefix=bsg+hydrometer%2Caps%2C162&sr=8-3	1	\$ 14.95	\$ 14.95
Thermometer	CDT300	Comark	https://www.amazon.com/Comark-Instruments-PDT300-Waterproof-Thermometer/dp/B001U59MDA/ref=sr_1_8?crd=1UPF69FHBZF56&keywords=comark+cdt+300&qid=1681660992&s=home-garden&sprefix=comark+cdt+300%2Cgarden%2C110&sr=1-8	1	\$ 27.75	\$ 27.75
Electronic alcohol meter	Snap 41	Anton-Paar	https://www.anton-paar.com/corp-en/products/details/snap/	1	\$1,803.00	\$ 1,803.00
pH Meter with calibration & storage liquids	pHTester® 50	Oakton	https://www.coleparmer.com/i/oakton-phtestr-50-waterproof-pocket-ph-tester-premium-50-series/3563415	1	\$ 240.07	\$ 240.07
Graduated cylinder	1,000 mL	Pyrex	https://www.coleparmer.com/i/pyrex-3025-1l-cylinder-brand-3025-graduated-1000-ml/3454627	1	\$ 112.50	\$ 112.50
Graduated cylinder	100 mL	Pyrex	https://www.coleparmer.com/i/pyrex-3025-100-brand-graduated-cylinder-100-ml/3454604	1	\$ 43.00	\$ 43.00
Glass jar	32 fl oz	Mason	https://www.amazon.com/Ball-Mouth-Quart-Mason-Bands/dp/B07MZ8ZKSR/ref=sr_1_4?crd=2C2D46TKVGG3D&keywords=mason+jar&qid=1681663850&sprefix=mason+jar%2Caps%2C1067&sr=8-4	4	\$ 11.95	\$ 47.80
Glass jar	12 fl oz	Mason	https://www.amazon.com/Ball-Regular-Mouth-Mason-2-Pack/dp/B07MZCXCV4/ref=sr_1_11?crd=2C2D46TKVGG3D&keywords=mason+jar&qid=1681663850&sprefix=mason+jar%2Caps%2C1067&sr=8-11	2	\$ 8.50	\$ 17.00
Glass jar	112 fl oz	IKEA	https://www.ikea.com/us/en/p/ikea-365-jar-with-lid-glass-plastic-s1927767/	8	\$ 9.99	\$ 79.92
Pot still	26-gallon	Hillbilly Stills	https://www.hillbillystills.com/store/26-Gallon-Boiler-p322064814	3	\$1,400.00	\$ 4,200.00
Pot still	3-gallon	Al-Ambiq	https://www.copper-alembic.com/en/traditional-riveted-alembic-stills/10-l-traditional-riveted-alembic-still	1	\$ 172.69	\$ 172.69
Glass carboy	5-gallon	North Mountain Supply	https://www.amazon.com/gp/product/B09B4FMMMPH/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&psc=1	8	\$ 53.65	\$ 429.20
Glass carboy	3-gallon	Geo Sports Bottles	https://www.amazon.com/gp/product/B074Q3511Y/ref=ppx_od_dt_b_asin_title_s00?ie=UTF8&psc=1	8	\$ 49.99	\$ 399.92
					Total	\$10,701.48

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