

Project Title: Novel Use of a Sustainable, Perennial Sorghum Hybrid for Tennessee Whiskey

Introduction: Globally, Tennessee Whiskey is the best-selling American whiskey holding a Protected Geographical Indication (PGI) for its unique production method. In addition to required manufacturing and bottling in Tennessee, all Tennessee whiskey undergoes the “Lincoln County process”, or charcoal mellowing, where the spirits are slow dripped through sugar maple charcoal before aging, creating a unique and smooth flavor. By utilizing locally grown sorghum in combination with the famed Lincoln County process and both traditional and rapid aging methods, this research will create a new and distinct Tennessean product while utilizing an environmentally conscious cereal grain.

Background: Cultivated sorghum discovered at Nabta Playa archaeological site in Egypt dating to 8000 B.C. indicates domesticate sorghum has existed for centuries.¹ Soil erosion of traditional annual cereal grains is a large environmental concern for growers who are now considering perennial grains, like sorghum. A recently developed perennial sorghum hybrid has shown subsequent growth yields perform similarly to the first year². If adopted by farmers, this hybrid could reduce soil erosion and increase fertility in areas where it is incorporated³. While cereals are predominantly grown for animal feed or biofuel, an increasing amount is funneled into alcoholic beverage production. From 2014 to 2019, bourbon and Tennessee whiskey beverages have grown 37.3%, with over 26 million 9-liter cases sold throughout the U.S. in 2019 alone⁴ which indicates a potential to substitute annual grains with perennial sorghum within the industry. The new sorghum hybrid must demonstrate qualities that are interchangeable with existing cereal grains in production yields, processes, and products, while imparting similar or new desirable flavor characteristics to Tennessee whiskey.

Previous results were presented at the TN Academy of Science Annual Conference and MTSU Research and Creative Activity Open House in Fall 2022.

Purpose: The purpose of this research is to determine if a significant analytical difference exists between novel perennial sorghum hybrid grains aged by traditional charcoal-mellowed barrels and cubed barrel staves using literature-reviewed, key sorghum and oak standards.

Materials and Methods: Sorghum whiskey will be produced using two different mash bills (70% sorghum, 20% corn, 10% malted barley and 70% corn, 20% sorghum, 10% malted barley) hydrolyzing in 100 C water for 1 hour. The mash will be cooled to 60 C to encourage liquefaction by exogenous glycolytic enzymes. Mash will then be cooled below 30 C and inoculated with *Saccharomyces cerevisiae*. The fermentation process will be monitored daily and stopped when the fermentable sugars are depleted. After confirmation of sugar depletion by specific gravity hydrometer, the fermentation will be distilled using a Grainfather 500 until purified. Distillate will then be transferred to an 800ml barrel for aging. Stainless steel containers pressure fitted with a charred oak barrel head will be used to mimic commercial aging. Rapid maturation will be achieved using (1) high surface area to volume ratio barrels and (2) cubed barrel staves to maximize extraction and interaction between the wood and distillates. Samples will be removed from the barrels at regular intervals and then analyzed using high performance liquid chromatography and gas chromatography. Anthocyanins and phenolic compounds found in each will be quantified using standard curves prepared by using literature-reviewed, key sorghum and oak wood standards.

Description of Duties: The student will prepare and manage all fermentation, distillation, and sample analysis processes under the weekly supervision and direction of Dr. O'Brien.

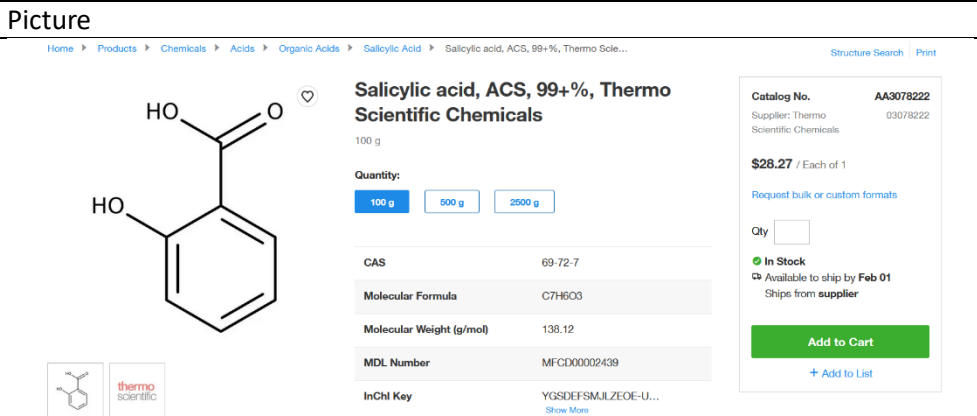
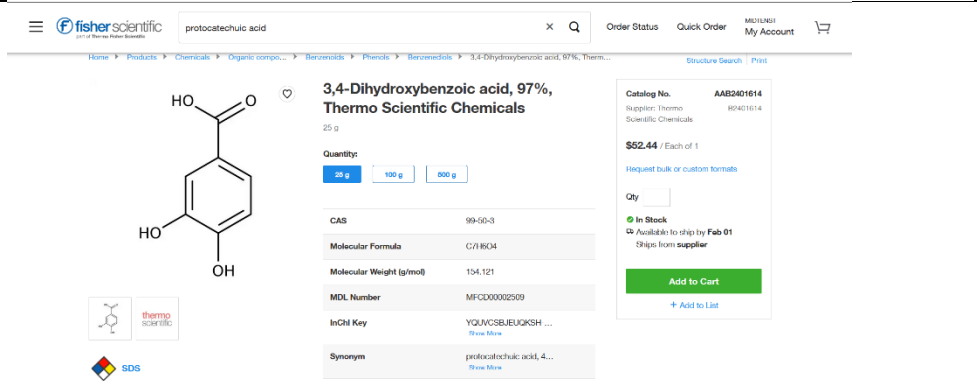
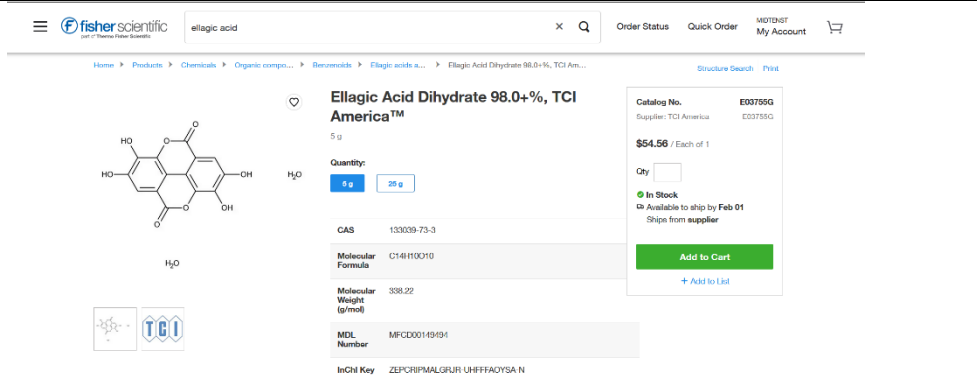
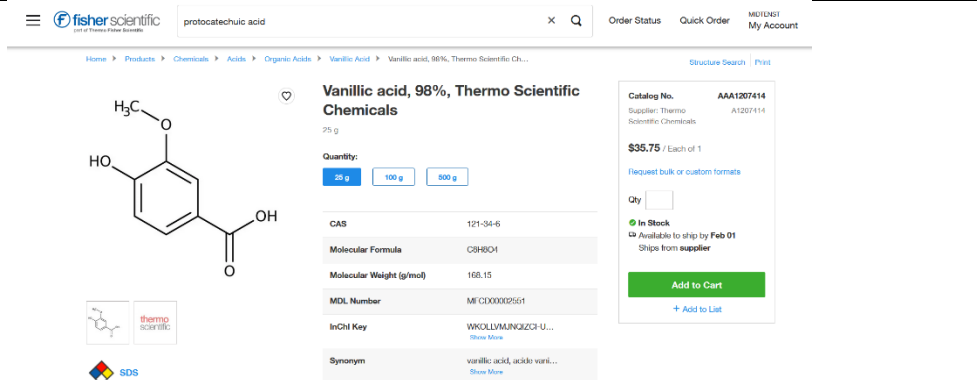
Role of Mentors: Using Dr. O'Brien's expertise, the methods in the manufacture and subsequent chemical analysis will be refined during weekly meetings. Dr. O'Brien will also provide instruction and guidance for statistical analysis, interpretation of results, and writing and presentation of results.

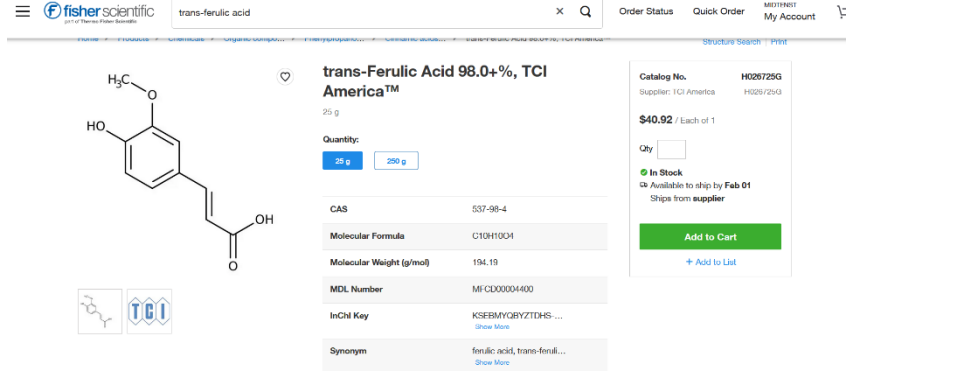
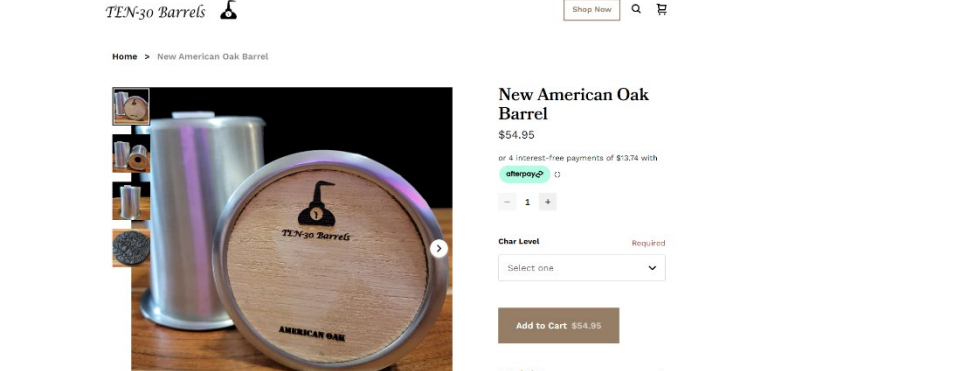
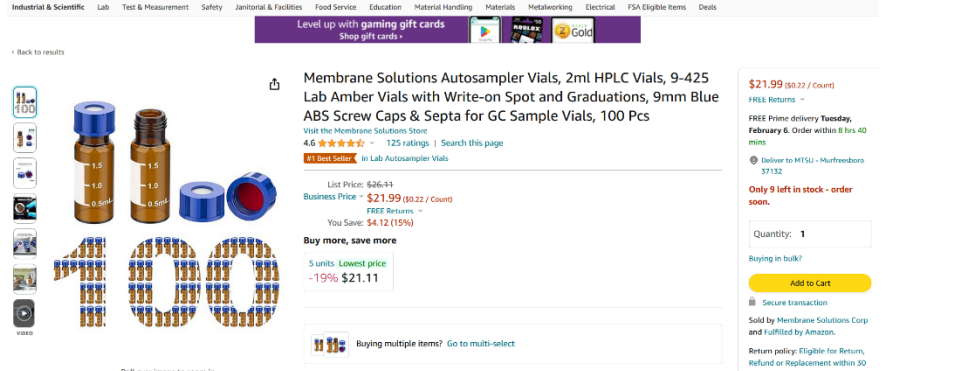
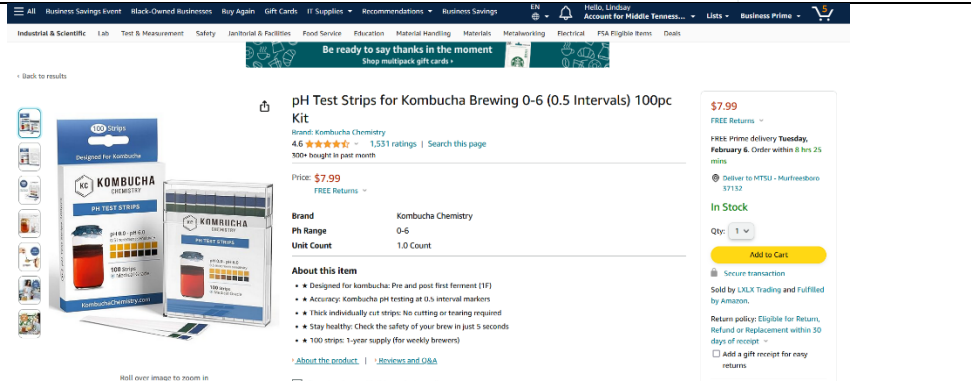
Timeline:

Date	Approximate Hours	Activity
Early February	15	Mill, Cook, and Mash grains. Inoculate with yeast. Monitor specific gravity and pH daily.
Late February	20	Distill the two mashes, sample and analyze the new make spirits, and barrel for maturation.
March	10	Weekly sampling of rapid aged and traditionally aged spirits as they age for chemical analysis and comparison.
April	20	Further sampling and comparison of rapid aged spirits to traditionally aged spirits.
Early May	10	Analyze and interpret final data; finalize and turn in report

References

- 1 Dahlberg, J. A.; Wasylikowa, K. Image and Statistical Analyses of Early Sorghum Remains (8000 B.P.) from the Nabta Playa Archaeological Site in the Western Desert, Southern Egypt. *Vegetation History and Archaeobotany* 1996, 5 (4), 293–299.
- 2 Cox, S.; Nabukalu, P.; Paterson, A.; Kong, W.; Nakasagga, S. Development of Perennial Grain Sorghum. *Sustainability* 2018, 10 (2), 172. <https://doi.org/10.3390/su10010172>.
- 3 Glover, J. D.; Reganold, J. P.; Bell, L. W.; Borevitz, J.; Brummer, E. C.; Buckler, E. S.; Cox, C. M.; Cox, T. S.; Crews, T. E.; Culman, S. W.; DeHaan, L. R.; Eriksson, D.; Gill, B. S.; Holland, J.; Hu, F.; Hulke, B. S.; Ibrahim, A. M. H.; Jackson, W.; Jones, S. S.; Murray, S. C. Increased Food and Ecosystem Security via Perennial Grains. *Science* 2010, 328 (5986), 1638–1639. <https://doi.org/10.1126/science.1188761>.
- 4 *On America's Whiskey Trail*. <https://www.distilledspirits.org/wp-content/uploads/2020/04/American-Whiskey-2019.pdf>

Item	Cost	Picture
Salicylic Acid Standard	28.27	 <p>Home > Products > Chemicals > Acids > Organic Acids > Salicylic Acid > Salicylic acid, ACS, 99+%, Thermo Scientific Chemicals</p> <p>Salicylic acid, ACS, 99+%, Thermo Scientific Chemicals</p> <p>100 g</p> <p>Quantity: <input type="button" value="100 g"/> <input type="button" value="500 g"/> <input type="button" value="2500 g"/></p> <p>CAS: 69-72-7</p> <p>Molecular Formula: C7H6O3</p> <p>Molecular Weight (g/mol): 138.12</p> <p>MDL Number: MFCD00002439</p> <p>InChI Key: YGSDIEFSMLZEOE-U...</p> <p>Catalog No. AA3078222</p> <p>Supplier: Thermo Scientific Chemicals</p> <p>\$28.27 / Each of 1</p> <p><input type="button" value="Add to Cart"/></p> <p><input type="button" value="+ Add to List"/></p>
Protocatechuic Acid Standard	52.44	 <p>Home > Products > Chemicals > Organic compo... > Benzenoids > Phenols > Benzenoids > 3,4-Dihydroxybenzoic acid, 97%, Thermo Scientific Chemicals</p> <p>3,4-Dihydroxybenzoic acid, 97%, Thermo Scientific Chemicals</p> <p>25 g</p> <p>Quantity: <input type="button" value="25 g"/> <input type="button" value="100 g"/> <input type="button" value="500 g"/></p> <p>CAS: 99-50-3</p> <p>Molecular Formula: C7H6O4</p> <p>Molecular Weight (g/mol): 154.121</p> <p>MDL Number: MFCD00002509</p> <p>InChI Key: YQUVCSBJEUKSH ...</p> <p>Synonym: protocatechuic acid, 4...</p> <p>Catalog No. AAB2401614</p> <p>Supplier: Thermo Scientific Chemicals</p> <p>\$52.44 / Each of 1</p> <p><input type="button" value="Add to Cart"/></p> <p><input type="button" value="+ Add to List"/></p>
Ellagic Acid Standard	54.56	 <p>Home > Products > Chemicals > Organic compo... > Benzenoids > Ellagic acids a... > Ellagic Acid Dihydrate 98.0+%, TCI America</p> <p>Ellagic Acid Dihydrate 98.0+%, TCI America™</p> <p>5 g</p> <p>Quantity: <input type="button" value="5 g"/> <input type="button" value="25 g"/></p> <p>CAS: 133039-73-3</p> <p>Molecular Formula: C14H10O10</p> <p>Molecular Weight (g/mol): 338.22</p> <p>MDL Number: MFCD00149494</p> <p>InChI Key: ZEPCRIPMALGRUR UHFFFAOYSA N</p> <p>Catalog No. E03755G</p> <p>Supplier: TCI America</p> <p>\$54.56 / Each of 1</p> <p><input type="button" value="Add to Cart"/></p> <p><input type="button" value="+ Add to List"/></p>
Vanillic acid Standard	35.75	 <p>Home > Products > Chemicals > Acids > Organic Acids > Vanillic Acid > Vanillic acid, 98%, Thermo Scientific Chemicals</p> <p>Vanillic acid, 98%, Thermo Scientific Chemicals</p> <p>25 g</p> <p>Quantity: <input type="button" value="25 g"/> <input type="button" value="100 g"/> <input type="button" value="500 g"/></p> <p>CAS: 121-34-6</p> <p>Molecular Formula: C8H8O4</p> <p>Molecular Weight (g/mol): 168.15</p> <p>MDL Number: MFCD00002561</p> <p>InChI Key: WKOLLUMINQZCF-U...</p> <p>Synonym: vanillic acid, acids vani...</p> <p>Catalog No. AAA1207414</p> <p>Supplier: Thermo Scientific Chemicals</p> <p>\$35.75 / Each of 1</p> <p><input type="button" value="Add to Cart"/></p> <p><input type="button" value="+ Add to List"/></p>

<p>Trans-ferulic acid standard</p>	<p>40.92</p>	
<p>Barrel</p>	<p>54.95</p>	
<p>HPLC Vials</p>	<p>21.99</p>	
<p>pH strips</p>	<p>7.99</p>	
<p>Total</p>	<p>296.87</p>	