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Chemical and Chromatic Effects of Commercial Wine Yeast Strains (*Saccharomyces* spp.) on 'Dolgo' Crabapple Rosé Cider

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Abstract

Hard apple cider produced locally in North Dakota is receiving increased commercial interest, but no research has been conducted on locally adapted apple cultivars and specific yeast strains (*Saccharomyces* spp.) to produce fermented cider products. 'Dolgo', a red fleshed crabapple with persistent production history in North Dakota was fermented using 15 different commercial yeast strains to produce rosé cider. To gain knowledge concerning the role of *Saccharomyces* spp. yeast strains in the fermentation process and fermented products, yeast strain performances were compared through multiple dimensions. Ethanol, glycerol, residual sugar, sorbitol content and acid properties were analysed to compare the impact of yeast strains on main chemical components of apple cider. Chromatic properties were determined to evaluate the yeast strain influence on final cider color. Yeast strains impacted rosé cider color as monitored by lightness (L*), redness (a*), hue, and color density. Antioxidant activity was assessed; however, there were no significant differences among yeast strains. The amount of malic acid and acetic acid varied significantly among yeast strains, as well as the final ethanol content. This research provided a basis for further research into rosé apple cider production and to continue refining microorganism pairing for fermented products in cool climates.

Keywords – cider color – deacidification – fermentation – hard cider – low-alcohol products

Introduction

Hard apple cider is a traditional beverage with regional increases in consumption (Kline & Cole 2017, Farris et al. 2019, Yenerall et al. 2022). Apple cider quality depends on intrinsic (color, aroma, and flavor) and extrinsic (origin and branding) characteristics (Mora et al. 2020). The crabapple 'Dolgo' *(Malus baccata*) was brought to South Dakota, USA from Russia by Professor Niels Ebbesen Hansen and was selected and introduced to the market in 1917. Due to its cold-hardiness, early production, and capacity for heavy crop load, 'Dolgo' was successfully adopted in extreme climates as a pollinizer with tolerance of biotic and abiotic stresses (Chatfield & Draper 1995, Matsumoto et al. 2008).

While the tart flavor of crabapple makes it popular for canning and making jellies, limited work has addressed the use of 'Dolgo' crabapple in cider production. Previous research has shown that 'Dolgo' was more sensitive to drought stress when compared to five other crabapple cultivars (Li et al. 2017). As an ornamental crabapple, 'Dolgo' received some interests due to its flower color and

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structure, and disease tolerance (Smith & Treaster 1985, Hagan et al. 1993, 2000, Gul et al. 2019). 'Dolgo' was included in the research on the comparative transcriptome analysis among ornamental apple species (Gul et al. 2019). Similarly, pollen was examined and compared with other apple cultivars via electron microscopy (Zhang et al. 2019). 'Dolgo' fruit was also examined to understand the volatile profiles of *Malus* spp. (Sugimoto et al. 2015). Regionally, 'Dolgo' fruit has been increasingly used by commercial cideries, but there is no research addressing the cider quality characteristics.

Cider quality depends on apple quality and selection, such as cultivars, ripening stage, storage conditions and growing conditions (Perestrelo et al. 2019). Beyond this, yeast strains and fermentation conditions are important factors influencing the development of sensory properties in ciders (Laaksonen et al. 2017, Tarko et al. 2018, Rosend et al. 2019). The mechanisms via which yeast strains influence alcohol beverages, beyond their secondary metabolites, fermentation rate, and ideal fermentation conditions include their interactions with polyphenols such as through adsorbing and removing anthocyanin and tannin (Mazauric & Salmon 2006, Monagus et al. 2007). Furthermore, the fermented products such as mannoproteins, could influence the taste, aroma, and color (Escot et al. 2001). Yeast also influences the polysaccharide profiles via expression of pectinolytic activity. Yeast-derived polygalacturonase expression is related to genetic regulation and modification, which can be influenced by environmental conditions. Therefore, the pectinolytic activity is variable during fermentation depending on fermentation conditions, yeast strain, and must chemistry (Belda et al. 2016). Commercially available yeast strains can improve fermentation ease and consistently attain required chemical parameters in a repeatable fashion (Tarko et al. 2018). With the elevated levels of alcohols and esters driven by certain yeast strains, fermented beverages may have different final aroma profiles (McKay et al. 2011). The influences of yeast strains include the formation of aroma compounds in both quantity and nature for specific compounds (Witener et al. 2014).

Several isolated yeast strains and commercial wine yeast strains were evaluated in Asturian apple juice and resulted in differentiations of ethyl acetate, acetaldehyde and isobutanol in the final cider product (Madrera et al. 2015). Yeast strains also affected chemical composition, aromatic profiles and sensorial properties of ice ciders obtained by cryo-extraction (Bedriñana et al. 2020). Non-*Saccharomyces* yeast strains, such as *Williopsis* spp., have also been investigated for their capacity to modulate cider flavors (Aung et al. 2015). Yeast strains were also reported to influence the amino acid composition of sparking ciders (Valles et al. 2005). It has been noted that the existence of amino acids could cause large differences among yeast in the cider production of fusel alcohols and ethyl esters (Santos et al. 2015). Meanwhile, it has been reported that nitrogen concentrations influence yeast gene expression and hydrogen sulfide production in cider fermentation (Song et al. 2020). Lorenini et al (2019) observed that yeasts used in single or mixed apple juice fermentations for cidermaking, may amplify the volatile compounds. To efficiently control volatile compound formation, one should adjust the inoculation time of the yeast strains when making cider (Xu et al. 2006).

The application of different yeast strains has been widely conducted in wine research and the wine industry (Synos et al. 2015). But little information is known on the effects of different yeast strains on apple cider. Two examples of broadly used commercial wine yeast strains that are also utilized for apple cider fermentation are Lalvin 71B-1122 and Lalvin EC-1118. Building on the commercial yeast strains used for wine fermentation, for this research, we chose 15 white wine yeast strains including 71B-1122 and Lalvin EC-1118 to investigate their effects on apple cider fermentation. The objective of this study was to examine the 15 commercial yeast strains (*Saccharomyces* spp.) on 'Dolgo' apple cider fermentation and to develop an understanding of their use in rosé apple cider production.

Materials & Methods

Orchard

Own-rooted 'Dolgo' crabapples were field planted at the North Dakota State University

Horticulture Research Farm, near Absaraka, ND (46.987, -97.355) in 1988 and 1989. Soil for the orchard is a mixture of Antler (Fine-loamy, mixed, superactive, frigid Aeric Calciaquolls)-Wyard (Fine-loamy, mixed, superactive, frigid Typic Endoaquolls) loams and Warsing sandy loam (Fine-loamy, mixed, superactive, frigid Oxyaquic Hapludolls), with 0 to 2 percent slope (Soil Survey Staff, 2022). Three field replicates of approximately 30 kg of fruit were hand-harvested from 'Dolgo' apple trees on Sept. 03, 2020. Fruit was held in a refrigerated walk-in cooler at 2 °C overnight until processing the following day.

Cider making

On Sept. 04, 2020, individual field replicates of 'Dolgo' apples were processed using a motorized apple shredder (WE208, MoreWine Pro, Pittsburg, CA, USA) and pressed using a 20 L stainless steel bladder press (Speidel, Ofterdingen, DE). Musts were manually transferred to individual 23 L carboys and dosed with sulfur dioxide (SO_2) at a rate of 40 mg/L using potassium metabisulfite. Musts were transported to Fargo, ND, USA, and experimental lots of 1 L were homogeneously partitioned into individual 1.5 L capacity, clear, glass bottles for fermentation.

Fermentations were initiated 24 hr after pressing. Dehydrated commercial yeast strains were inoculated at a rate of 0.264 g/L, following rehydration. One day after inoculation, fermenting musts received 0.264g/L of a yeast nutrient supplement (Fermaid ® K, Lallemand Inc., Montreal, Québec, CAN). Fifteen *Saccharomyces* spp. yeast strains were inoculated into apple must individually. The strains included 58W3 (YSEO, France), 71B (Lalvin, CA, US), Assmanhausen (Enoferm AMH, Germany), BA11 (Lalvin, Portugal), CY3079 (Lalvin, CA, US), D47(Lalvin, CA, US), EC1118 (Lalvin, CA, US), K1-V1116 (Lalvin, CA, US), QA23 (Lalvin, CA, US), R2 (Lalvin, CA, US), Rhone4600 (Lalvin, CA, US), Sensy (Lalvin, CA, US), T306 (Lallemand, CA, US), VL3 (Laffort, CA, US), and W15 (Lalvin, CA, US).

Fermentations were conducted at the temperature of 19 °C; primary fermentation lasted approximately three weeks. After fermentations were completed, ciders were transferred to 750 mL bottles where they were held for 60 days at room temperature until bottling to allow any final fermentation to complete. Finished ciders were transferred into sanitized, argon purged, amber bottles and sealed with oxygen absorbing bottle caps until further analysis. Analytical samples were collected three months after bottling and frozen until final analysis.

Analysis of chemical and chromatic properties of ciders

Soluble solid content (SSC) was recorded using a Pal-1 digital refractometer (Atago Co., Tokyo, JPN). Titratable acidity and pH were monitored using an Orion Star A111 pH meter (Thermo Fisher Scientific, Waltham, MA, USA). Total acidity was measured using an ATAGO pocket Brix-Acid meter (Atago, Co., Tokyo, JPN). Pre-fermentation measurements also included measurements with kits specific to malic acid, sucrose, fructose, glucose, sorbitol, ammonia, and primary amino nitrogen (Megazyme Ltd., Wicklow, IRL).

Final samples were submitted to the Midwest Grape and Wine Industry Institute at Iowa State University for laboratory analysis of malic acid, acetic acid, glucose, fructose, ethanol, and glycerol content via high performance liquid chromatography (HPLC) according to their standard practices for wine analysis (Cuzmar et al. 2018). Briefly, the samples were filtered through 0.45µm nylon syringe filters in preparation for analysis. Agilent 1200 series HPLC (Agilent Technologies, Inc., Santa Clara, CA, USA) were equipped with two HPX-87H columns (Biorad labs, Richmond, CA, USA) in series protected with a cation H guard column. The operation parameters were 35 min runs, flow rate of 0.5000 mL/min at temperature of 65 °C. The samples were eluted with 0.045N sulfuric acid with 6% acetonitrile. The acids were detected and quantified by a diode array detector and residual sugars and ethanol were quantified by a refractive index detector and similar to the method described by Castellari et al. (2000).

Multiple spectral properties were evaluated to estimate apple cider composition using metrics developed for wine from Iland et al. (2004) by using a 1 mm path length quartz cell measured in a UV-Vis spectrophotometer (Genesys^{Tm}10S UV-Vis Spectrophotometer, ThermoFisher Scientific,

Waltham, MA, USA). Values were adjusted to a path length of 10 mm. CIELab color coordinates, including lightness (L*), chroma (C*), hue (h*), red-green (a*), and yellow-blue (b*) were calculated with MSCV® software.

Phenolic and antioxidant property assays

Three assays were used to monitor functional food characteristics (Table 1). The ABTS assay was used to measure inhibition of free radicals in samples using the Antioxidant Assay kit (CS0790, Sigma-Aldrich, St. Louis, MO, USA). The results were converted into equivalent Trolox amount (mM). To directly evaluate the antioxidant capacity, ferric reducing antioxidant power (FRAP) assays were used with a kit (MAK369, Sigma-Aldrich, St. Louis, MO, USA). The results were converted into ferrous equivalent (nmol/L). The phenolic compounds assay kit (MAK365, Sigma-Aldrich, St. Louis, USA) provided a method for measuring the total amount of phenolic compounds in 'Dolgo' samples. Catechin equivalents (CEs) were used to represent the phenolic compounds.

Statistical analysis

Data were analysed and graphed using R software version 4.0.5 (R Core Team 2022). When differences were significant ($P \leq 0.05$), means were separated using Tukey's honestly significant difference test (HSD). Comparison of individual cider's composite color were conducted by calculating the relative ΔE value between two samples, where ΔE was calculated as below:

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

These samples were then plotted in Google Slides (Google, Mountain View, CA, USA) to allow for visible evaluation of contrasting colors simultaneously with intersecting ΔE values. Any ΔE value below 1.0 indicated the difference between two colors should not be perceptible to the human eye, while any ΔE value between 1.0 and 2.0 indicated the difference may be detectable via intense observation, and ΔE values above 3.0 indicated a rapidly perceptible variation to the human eye due to substantial differences.

Results

The initial 'Dolgo' must had a soluble solid content of 12.93±0.32 Brix. Fructose content was about 47.28 g/L, which was the main fermentable sugar (Table 1). The must environment was highly acidic with a pH of 2.77 driven by almost 26 g/L of malic acid (Table 2). Meanwhile, initial must had a bright pink to red color prior to fermentation with a color density of 0.77 and total phenolics measured at 45.76 AU across reps (Table 3).

Table 1 Initial soluble solid, sugar, and sorbitol characteristics of 'Dolgo' crabapple musts.

Soluble solid content (Brix)		Glucose (g/L)		Fructose (g/L)		Sucrose (g/L)		Sorbitol (g/L)	
12.93	±0.32	20.79	±1.60	47.28	±2.69	33.95	±4.21	26.40	±4.20

Table 2 Initial acid characteristics of 'Dolgo' crabapple musts.

pH		Total acidity (g/L)		Malic acid (g/L)	
2.77	±0.04	31.87	±0.34	25.67	±0.52

Table 3 Initial spectrophotometric characteristics of 'Dolgo' crabapple musts.

Total phenolics (AU)		Total red pigments (AU)		Degree Red Pigment %		Color Density (AU)		Color
45.76	±1.41	1.08	±0.83	26.95	±5.28	0.77	±0.24	

In the fermented ciders, yeast strains depleted most of glucose and fructose, although several ciders still had some glucose and fructose residues in the products (Table 4). Yeast strain VL3 was the only cider with notably different glucose content. Fructose was generally fermented to completion by most yeast strains; however, musts with yeast strains VL3 (5.35), R2 (3.55), and Assmanhausen (1.21) all exceeded 1 g/L of fructose in final ciders.

The ethanol content ranged from 4.59 ± 0.12% to 5.24 ± 0.10% by volume (Table 4). Ciders from yeast strains Assmanhausen and K1-V116 had the significantly ($p<0.05$) higher ethanol content, both above 5.20%. The average ethanol content for all ciders was 5%. Yeast strain VL3 resulted in the lowest overall ethanol content, in line with the significantly ($p<0.05$) higher content of residual glucose and fructose in the cider. The mean glycerol content for ferments was 4.97 g/L. The glycerol content varied from 4.49 ± 0.13g/L (D47) to 5.46 ± 0.31g/L (QA23). No significant ($p>0.05$) differences were observed in sorbitol content following yeast treatments.

Table 4 Ethanol, glycerol, residual sugar, and sorbitol content of 'Dolgo' crabapple rosé ciders fermented by 15 commercial *Sacharomyces* spp. yeast strains.

Yeast Strain	Ethanol (%, v/v)	Glycerol (g/L)	Glucose (g/L)	Fructose (g/L)	Sorbitol (g/L)
58W3	5.08 ±0.09 abc[1]	5.04 ±0.14 abcde	0.17 ±0.13 b	0.00 ±0.12 c	28.72 ±1.32 ns
71B	5.04 ±0.08 abc	5.31 ±0.29 abc	0.17 ±0.13 b	0.00 ±0.12 c	30.47 ±1.03
Assmanhausen	5.23 ±0.17 a	4.74 ±0.29 cde	0.69 ±0.39 b	1.21 ±1.11 bc	26.27 ±0.67
BA11	5.08 ±0.04 abc	5.40 ±0.17 a	0.16 ±0.13 b	0.00 ±0.12 c	28.55 ±0.70
CY3079	4.96 ±0.06 abc	5.02 ±0.12 abcde	0.64 ±0.23 b	0.94 ±0.94 c	26.97 ±1.71
D47	5.18 ±0.06 ab	4.49 ±0.13 e	0.18 ±0.12 b	0.00 ±0.12 c	26.88 ±0.75
EC1118	5.13 ±0.05 ab	4.69 ±0.29 cde	0.17 ±0.13 b	0.00 ±0.12 c	29.34 ±1.48
K1-V1116	5.24 ±0.10 a	4.70 ±0.21 cde	0.26 ±0.05 b	0.00 ±0.12 c	26.88 ±0.35
QA23	4.99 ±0.13 abc	5.46 ±0.31 a	0.18 ±0.13 b	0.00 ±0.12 c	33.10 ±2.21
R2	4.70 ±0.09 bc	4.77 ±0.20 bcde	1.36 ±0.46 b	3.55 ±0.36 ab	29.42 ±1.25
Rhone4600	5.04 ±0.10 abc	5.38 ±0.24 ab	0.17 ±0.13 b	0.00 ±0.12 c	29.25 ±1.00
Sensy	5.11 ±0.06 abc	5.12 ±0.11 abcd	0.17 ±0.13 b	0.00 ±0.12 c	26.53 ±1.46
T306	5.06 ±0.05 abc	4.78 ±0.13 bcde	0.41 ±0.08 b	0.00 ±0.12 c	29.77 ±2.78
VL3	4.59 ±0.12 c	4.59 ±0.07 de	4.92 ±1.53 a	5.35 ±0.88 a	29.51 ±0.51
W15	4.88 ±0.14 abc	5.03 ±0.18 abcde	0.17 ±0.12	0.00 ±0.12 c	29.25 ±1.14
F-test	3.2500	2.2160	7.2488	11.1114	1.6203
p-value	0.0038	0.0356	<0.0001	<0.0001	0.1346

[1] Values and standard error in a column followed by different letters indicate means are significantly different at $P<0.05$ with means separated by Tukey's HSD; ns = not significant.

Although pH values started around 2.77, they finished in the range of 3.02 to 3.16 (Table 5). Final cider total acidity range was from 20 g/L to 30 g/L. Yeast strain Assmanhausen resulted in the lowest overall total acidity of 20.0 g/L, while yeast strains T306 and CY3079 resulted in the highest

total acidities. Malic acid amount was between 20.8 to 27 g/L in the ciders. Yeast strains Assmanhausen and K1-V1116 resulted in ciders with the lowest malic acid content. They were the only yeasts that resulted in ciders with malic acid levels below 21.50 g/L. Ciders fermented with 71B, CY3079, QA23, R2, Rhone4600, T306, and W15 had the highest acid content. Acetic acid was generated at the content of 0.17 g/L, on average, but the values varied from 0.11 ± 0.02 g/L (BA11) to 0.27 ± 0.02 g/L (QA23) in the ciders.

Table 5 Acid properties of 'Dolgo' crabapple rosé ciders fermented by 15 commercial *Sacharomyces* spp. yeast strains.

Yeast Strain	pH		Total acidity (g/L)		Malic acid (g/L)		Acetic acid (g/L)	
58W3	3.10	±0.01 ns	26.2	±1.0 ab	24.98	±0.70 ab	0.16	±0.01 bc
71B	3.04	±0.01	27.9	±3.0 ab	26.98	±0.33 a	0.21	±0.02 ab
Assmanhausen	3.21	±0.13	20.0	±3.8 b	20.80	±3.18 c	0.16	±0.01 bc
BA11	3.03	±0.03	27.5	±0.7 ab	24.45	±0.76 abc	0.11	±0.02 c
CY3079	3.02	±0.03	29.5	±0.6 a	26.07	±0.61 a	0.17	±0.01 bc
D47	3.09	±0.03	24.9	±2.1 ab	24.16	±1.76 abc	0.14	±0.02 bc
EC1118	3.14	±0.05	24.2	±0.8 ab	21.75	±1.62 bc	0.16	±0.02 bc
K1-V1116	3.16	±0.05	21.1	±2.1 ab	21.04	±1.67 c	0.15	±0.01 bc
QA23	3.02	±0.03	28.7	±0.7 ab	26.89	±0.59 a	0.27	±0.02 a
R2	3.03	±0.04	29.2	±0.2 ab	26.19	±0.51 a	0.15	±0.00 bc
Rhone4600	3.03	±0.03	28.8	±1.8 ab	26.34	±1.03 a	0.21	±0.01 ab
Sensy	3.09	±0.02	27.5	±1.2 ab	25.21	±0.69 ab	0.19	±0.01 ab
T306	3.04	±0.03	30.0	±0.9 a	25.87	±0.61 a	0.14	±0.01 bc
VL3	3.05	±0.01	29.1	±0.9 ab	25.45	±0.48 ab	0.17	±0.00 bc
W15	3.02	±0.03	27.7	±1.2 ab	25.76	±0.44 a	0.18	±0.04 bc
F-test	1.4773		2.9765		2.4984		6.5156	
p-value	0.1840		0.0068		0.0190		<.0001	

[1] Values and standard error in a column followed by different letters indicate means are significantly different at P<0.05 with means separated by Tukey's HSD; ns= not significant.

Chromatic properties of 'Dolgo' final products indicated there were variations among the lightness, red color, and hue from apple cider products based on fermentation with different yeast strains (Table 6). The overall color for the apple ciders varied from light yellow to pinkish yellow color. Lightness (L*), red color (a*) and hue (H*) had significant differences (p<0.001) among the final products. Cider from yeast strain EC1118 was the lightest with the greatest L* value (94.90), whereas cider from yeast strain VL3 was the darkest (L* = 91.43). This trend followed in the red (a*) values where cider from yeast strain EC1118 had the second lowest value (0.92), and cider from yeast strain VL3 had the greatest a* value (5.63). The red (a*) values averaged 2.90 across all ciders, while only cider from yeast strains EC1118, 71B (1.90), and Sensy (0.86) were below 2.00. The extent of cider yellowness (b*) did not vary among ferments, nor did the chroma (C*). Hue (H*) varied between 75.75 and 87.95 for cider from yeast strains VL3 and Sensy, respectively, with a mean of 82.10.

Table 6 Chromatic properties of 'Dolgo' crabapple rosé ciders fermented by 15 commercial *Sacharomyces* spp. yeast strains.

Yeast strain	Lightness L*		Red a*		Yellow b*		Chroma C*		Hue H*	
58W3	93.27	±0.28 abc	3.13	±0.18 abcd	20.26	±0.82 ns	20.50	±0.82 ns	81.14	±0.18 abcd
71B	93.17	±0.83 abc	1.90	±1.08 cd	21.18	±2.51	21.26	±2.55	84.65	±1.36 ab

Table 6 Continued.

Yeast strain	Lightness L*		Red a*		Yellow b*		Chroma C*		Hue H*	
Assmanhausen	94.03	±0.51 ab	2.24	±1.89 bcd	19.36	±0.85	19.58	±0.88	83.06	±3.14 abc
BA11	92.30	±0.45 bc	3.39	±0.86 abcd	21.21	±1.07	21.50	±1.14	80.85	±0.98 abcd
CY3079	91.90	±0.26 bc	3.38	±0.30 abcd	23.14	±0.27	23.39	±0.25	81.66	±0.55 abcd
D47	93.37	±0.67 abc	2.79	±0.67 bcd	19.36	±1.39	19.58	±1.42	81.88	±0.64 abcd
EC1118	94.90	±0.29 a	0.92	±1.02 d	17.10	±1.54	17.14	±1.58	87.04	±2.23 ab
K1-V1116	93.57	±0.35 abc	2.88	±0.69 bcd	18.90	±0.68	19.15	±0.73	81.30	±1.11 abcd
QA23	92.90	±0.60 abc	3.04	±0.81 bcd	21.15	±2.17	21.39	±2.17	81.63	±1.23 abcd
R2	92.30	±0.22 bc	4.75	±0.77 ab	21.35	±1.05	21.90	±1.05	77.35	±1.25 cd
Rhone4600	93.60	±0.57 abc	2.30	±0.22 bcd	20.11	±2.56	20.24	±2.55	83.21	±0.51 abc
Sensy	92.37	±0.13 bc	0.86	±0.44 d	23.60	±1.02	23.62	±0.96	87.95	±1.09 a
T306	91.60	±0.13 bc	2.75	±0.46 bcd	23.77	±1.09	23.94	±1.07	83.39	±0.74 abc
VL3	91.43	±0.36 c	5.63	±0.69 a	22.23	±0.71	22.96	±0.64	75.75	±1.00 d
W15	92.57	±0.48 abc	3.56	±0.76 abc	21.67	±1.26	21.96	±1.29	80.59	±0.91 bcd
F-test	4.0774		6.3684		1.5645		1.6154		5.1914	
p-value	0.0008		<.0001		0.1522		0.1361		0.0001	

[1] Values and standard error in a column followed by different letters indicate means are significantly different at P<0.05 with means separated by Tukey's HSD; ns = not significant.

Comparison of individual cider's composite color by calculating the relative ΔE value between samples from two yeast strains indicated that the largest ΔE value (60.5) occurred between VL3 and EC1118, (Fig. 1). In total, 11 ΔE values were below 1.0, and thus had similar color and unlikely discernible based on color by the human eye. These ciders included 58W3-D47 (0.9), 58W3-QA23 (0.9), 58W3- Rhone4600 (0.8), Assmanhausen-D47 (0.7), Assmanhausen-Rhone4600 (0.8), BA11-QA23 (0.5), BA11- W15 (0.3), CY3079-T306 (0.9), D47-K1 V1116 (0.3), D47-Rhone 4600 (0.9), and QA23-W15 (0.6). Ciders from yeast strains EC1118 and Sensy had the most unique color with every cider color comparison from the other yeast strains resulting in ΔE values above 3.0.

Cider color hue averaged 2.69 AU (Fig. 2A). The color hue varied based on yeast strain used ($p < 0.0001$). The greatest color hue value was for Sensy fermented ciders (2.74 AU) and the lowest was for VL3 fermented ciders (2.25 AU). Color density was affected by yeast strains ($p = 0.0106$) (Fig. 2B). The ciders with the greatest color density resulted from VL3 and T306, both averaging 5.23 AU. The cider with the lowest color density was EC1118 (3.44 AU).

Total phenolics was the only metric of phenolics or antioxidants that was altered by yeast strain (Table 8). Total phenolic compounds as measured via catechin equivalent, ABTS, and FRAP did not differ among fermented cider samples. Trolox equivalent ABTS assay measured the free radicals of apple ciders with 'Dolgo' ciders averaging 7.48 mg Trolox antioxidant capability. The FRAP assay for 'Dolgo' ciders which tested substances, such as polyphenols, flavonoids, vitamins, enzymes, and other compounds with antioxidant capabilities, averaged 48.02 mM Ferrous equivalents. In this research, yeast strains did not cause significant differences among the 'Dolgo' apple ciders for antioxidant or phenolic capacity except through total phenolics as measured by AU at 280nm.

	58W3	71B	Ass-man	BA11	CY 3079	D47	EC-1118	K1-V1116	QA23	R2	Rhone 4600	Sensy	T306	VL3	W15
58W3	0.0	2.4	2.2	1.9	10.2	0.9	17.5	2.0	0.9	4.8	0.8	17.1	15.3	13.5	2.7
71B	2.4	0.0	4.2	3.0	7.6	4.1	20.6	6.3	1.4	8.9	1.5	7.6	9.9	18.0	3.4
Assman	2.2	4.2	0.0	7.7	20.1	0.7	7.6	0.8	5.1	13.3	0.8	22.6	25.6	26.5	9.2
BA11	1.9	3.0	7.7	0.0	3.9	4.9	29.8	7.2	0.5	1.9	4.1	12.1	7.5	6.8	0.3
CY3079	10.2	7.6	20.1	3.9	0.0	16.8	51.5	21.0	5.1	5.2	13.2	6.8	0.9	6.1	2.6
D47	0.9	4.1	0.7	4.9	16.8	0.0	10.9	0.3	3.5	8.9	0.9	22.7	22.6	20.1	6.6
EC1118	17.5	20.6	7.6	29.8	51.5	10.9	0.0	8.9	24.9	39.5	12.7	48.7	58.7	60.5	33.3
K1-V1116	2.0	6.3	0.8	7.2	21.0	0.3	8.9	0.0	5.5	11.1	1.8	27.6	27.6	23.2	9.1
QA23	0.9	1.4	5.1	0.5	5.1	0.5	5.1	3.5	0.0	3.3	2.1	11.0	8.6	10.0	0.6
R2	4.8	8.9	13.3	1.9	5.2	8.9	39.5	11.1	3.3	0.0	9.2	20.2	10.3	2.3	1.6
Rhone 4600	0.8	1.5	0.8	4.1	13.2	0.9	12.7	1.8	2.1	9.2	0.0	15.8	17.6	20.3	5.1
Sensy	17.1	7.6	22.6	12.1	6.8	22.7	48.7	27.6	11.0	20.2	15.8	0.0	4.2	25.5	11.1
T306	15.3	9.9	25.6	7.5	0.9	22.6	58.7	27.6	8.6	10.3	17.6	4.2	0.0	10.7	6.0
VL3	13.5	18.0	26.5	6.8	6.1	20.1	60.5	23.2	10.0	2.3	20.3	25.5	10.7	0.0	5.9
W15	2.7	3.4	9.2	0.3	2.6	6.6	33.3	9.1	0.6	1.6	5.1	11.1	6.0	5.9	0.0

Fig. 1 – Mean colors of 'Dolgo' crabapple rosé ciders fermented by 15 commercial *Sacharomyces* spp. yeast strains and the calculated ΔE values for relative difference of color between any pair of yeast strains.

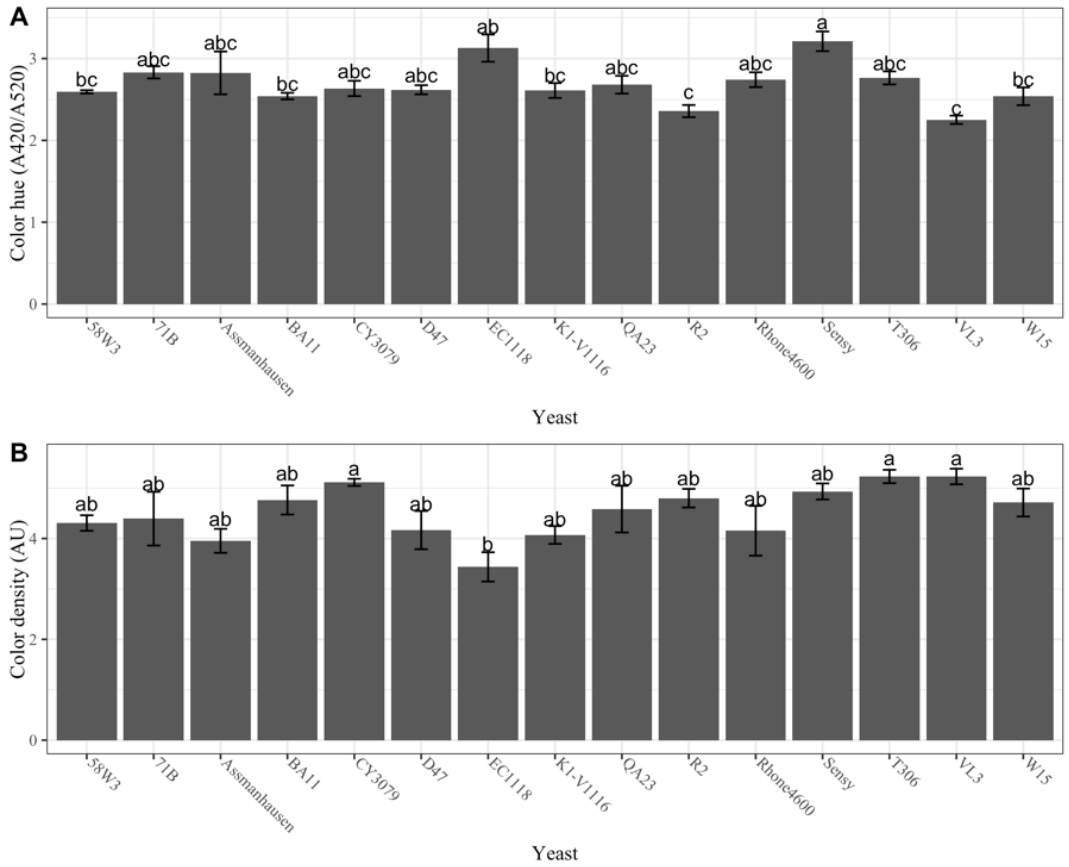

Fig. 2 – (A) Color hue and (B) color density of 'Dolgo' crabapple rosé ciders fermented by 15 commercial *Sacharomyces* spp. yeast strains; error bars = standard error of the mean for each cider; bars with different letters are significantly different based on Tukey's HSD test.

Table 8 Phenolic and antioxidant properties of 'Dolgo' crabapple rosé ciders fermented by 15 commercial *Sacharomyces* spp. yeast strains.

Yeast strain	Total phenolics (AU)		Phenolic compounds (mM catechin equivalents)		ABTS (µM TE/g FM)		FRAP (µM TE/g FM)	
58W3	38.69	±1.05 abc	1.09	±0.15 ns	6.29	±1.83 ns	48.08	±2.64 ns
71B	31.55	±3.94 abc	1.07	±0.10	9.23	±2.51	45.76	±3.04
Assmanhausen	36.91	±3.20 abc	0.93	±0.10	7.48	±1.78	48.90	±1.52
BA11	34.48	±3.14 abc	1.23	±0.09	7.65	±0.80	44.08	±1.98
CY3079	38.79	±0.33 abc	1.15	±0.04	7.34	±1.04	48.39	±2.52
D47	38.59	±4.00 abc	0.97	±0.10	7.38	±1.06	46.07	±4.34
EC1118	27.98	±1.70 c	1.09	±0.07	9.21	±1.97	51.34	±4.38
K1-V1116	34.04	±1.05 abc	0.94	±0.03	6.96	±1.71	51.01	±5.53
QA23	34.48	±4.24 abc	1.11	±0.07	9.74	±1.74	46.41	±5.00
R2	37.54	±2.32 abc	1.09	±0.09	8.03	±0.86	48.22	±1.24
Rhone4600	29.03	±3.36 bc	1.01	±0.03	7.74	±0.84	45.58	±0.90
Sensy	44.31	±4.20 ab	1.12	±0.15	7.38	±1.90	51.57	±2.77
T306	44.99	±2.42 a	0.99	±0.05	7.83	±1.91	47.16	±2.19
VL3	41.01	±1.38 abc	1.14	±0.09	5.10	±0.87	49.26	±2.40
W15	37.55	±2.36 abc	1.05	±0.09	4.88	±0.59	48.80	±6.84
F-test	2.7498		0.8270		0.7860		0.3776	
p-value	0.0110		0.6364		0.6758		0.9707	

[1] Values and standard error in a column followed by different letters indicate means are significantly different at $p<0.05$ with means separated by Tukey's HSD; n s = not significant.

Discussion

From the initial characteristics of 'Dolgo' musts, the soluble solid content (13 °Brix) was relatively low when compared with 16 apple cultivars from eight states (Lee & Mattick 1989). This low level of sugars may have been the result of an early harvest. Sucrose, glucose, and fructose from 'Dolgo' were also in range of most cultivars in the US. The pH of 'Dolgo' was 2.77, indicating fruit was acidic; the pH was lower than average for apple cultivars (Lee 2012). Malic acid in 'Dolgo' was high. Therefore, without additional malolactic fermentation, the acid in the final cider product was still highly acidic.

Three yeasts (Assmanhausen, EC1118, and K1-V1116) resulted in malic acid below 22 g/L. Of these strains, EC1118, and K1-V1116 are typically vigorous fermenting yeasts with short lag phases, while Assmanhausen has a long lag phase and slower fermentation rate. The manufacturer of Assmanhausen notes that indigenous microbiota may flourish during the extended lag phase, thus, it is plausible that additional microbiota may have played a role in deacidification of the Assmanhausen ferments, despite sulfur additions.

To estimate yeast fermentation nutrition requirements, yeast assimilable nitrogen (YAN) and ammonia were evaluated pre-ferment. YAN measurements averaged 41.2 mg/L and ammonia took small amount of it (2.78 mg/L) (data not shown). The primary amino nitrogen content was approximately 38.40 mg/L in 'Dolgo' cider musts. For sufficient yeast nutrition, 350 mg ammonia

and amino acid per liter are required to force sluggish fermentation (Varela et al. 2004). As a result, extra nitrogen supplement was added to assist yeast fermentation in our research methods. Despite this, there were indications that some yeast strains, such as VL3, may not have completed fermentation based on lower ethanol and higher sugar concentrations in final ciders. Yeasts that struggled to complete fermentation may have higher nitrogen requirements than what was provided or may not be adapted to the extremely low pH environment of the 'Dolgo' ciders.

Beyond working with specifically available commercial yeast strains, alternative research efforts may assist in understanding the effect of fermentation on cider composition. Ecological interactions among yeast strains have been shown as a dominant factor in determining the outcome of the fermentation and fermentation dynamics, and as such, they are a point of further interest for apple cider fermentation (Bagheri et al. 2020). Technology assisted fermentation and processing of ciders may also enhance yeast performance, such as ultrasound processing (Al Daccachea et al. 2020).

Some research has focused on cider yeast screening based on molecular identification, tolerance to alcohol, production of volatile acidity and hydrogen sulphide, and volatile composition (Suárez Valles et al. 2008). Along with this, analysis of the volatile composition of ciders associated with aromas would also benefit specific yeast strain selection (Villière et al. 2012 and Pello-Palma et al. 2017).

Genetic identification and modification make yeast strain isolation and commercial application potentially a faster process for new strain development than traditional methods. Spontaneous fermentation of cider also receives interests for yeast interaction screening (Suárez Valles et al. 2007). The imprinting of global diversity and domestication of wine and cider yeast could provide valuable ecological data and information for strain selection (Almeida et al. 2014). Indigenous isolated strains are also an important method to ferment apple cider (Daccache et al. 2020). Beyond *Saccharomyces*, enzymatic activity of non-*Saccharomyces* yeast strains may also play a role in cider yeast fermentation activity (Bedriñana et al. 2012).

While yeast strain affected color parameters, the color of final ciders was substantially reduced compared to the fresh cider must. Polyphenolic profiles and pH are two factors that affect red color retention in red-fleshed apple juices (Fevrier et al. 2017). Enzymatically driven oxidation can lead to anthocyanin degradation in the musts; thus, yeast strains and fermentation techniques that limit enzymatic oxidation while mitigating polyphenolic profiles and pH may be a collected means to preserve color in rosé ciders. Argon atmosphere storage conditions to prevent oxidation have also been explored as a suitable means to preserve color in red-flesh apple juice (Malec et al. 2014). Malec et al. also found that argon controlled-atmosphere preserved color stability while sulfur, ascorbic acid, and copper had substantially smaller impacts on color stability.

Conclusion

These results indicated that fermented 'Dolgo' crabapple rosé ciders' chemical and chromatics properties are altered by yeast strain choice. The strains examined in this work focused on commercially available *Saccharomyces* strains; however, there remains a need for further characterization of cider-specific strains, beer-specific strains, and local indigenous strains of *Saccharomyces* to completely profile the impact of yeast on rosé ciders. Alongside profiling additional yeast strains, a more detailed screening of fermentation dynamics may help to identify what role the yeast play in final fermentation outcomes. Finally, in the future, sensory evaluation might be valuable for commercial cideries.

Rosé ciders offer an opportunity for ciders to standout with their distinct appearance and unique anthocyanin profiles (Shoji et al. 1999, 2002, Sugrue and Dando 2018). Rosé ciders are also receiving increased interest for potential processed products (van Nocker & Gottschalk 2017). Methods to increase color retention in rosé ciders must focus on each step of the cider production path. Breeders must make efforts to identify and select new apple cultivars with greater extractable color and more stable pigments (Sekido et al. 2010). They must do this selection without sacrificing favorable fruit volatile profiles, yield characteristics, and disease resistance. Processors must identify optimal means

of color preservation from harvest to shredding ant then pressing of cider musts. Finally, fermentation efforts must continue to focus on identifying ideal fermentation conditions (temperature, treatments, and *Saccharomyces* and non-*Saccharomyces* strain utilization) to preserve color of rosé ciders.

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