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Abstract: 'King of the North' ('KON'), as a cold-hardy grape, has many advantages, such as tolerance to a wide range of soil conditions and harsh winter climate. Due to the adapting demand of North Dakota's fruit and wine industry, optimized quality of wine from regionally productive grapevines is required. In this study, yeast strain, one of the primary fermentation tactics, was tested on 'KON' grapes. Five different commercial yeast strains, including 71B, EC1118, Maurivin B, Rhône 4600, and W15, were added to initiate fermentation. The analysis of grape must characteristics and the fermentation dynamic changes indicated a high correlation between color and acid metrics. Yeast strains have influenced the color dynamic changes and fermentation process. The panelist sensory evaluations confirmed that yeast strains contributed differently to the perceived aromas and flavors within 'KON' wines. Rose, apple, grape, and apricot aromas were distinguished in 'KON' wines. The lemon taste was the dominant flavor detected in 'KON' wines. However, wines were also varied based on the extent of the aroma or taste observed. Therefore, exploring the use of different yeast strains for fermentation provides information for further application to cold-hardy grape cultivars and other high-acid fruit, aiding winemakers in using North American grapes with diverse fruit chemistry.

Keywords: 'King of the North'; wine; yeast

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1. Introduction

Wine fermentation is a process by which yeast obtains energy by converting sugar into alcohol and carbon dioxide [1,2]. The fermentation process is traditionally carried out with *Saccharomyces cerevisiae* [3]. In addition to using *Vitis vinifera* varieties for winemaking, cold-hardy grapes, the interspecific hybrids of *Vitis vinifera* with *Vitis labrusca* and *Vitis riparia* are increasing in prevalence [4]. One of the varieties, 'King of the North', was identified as a highly hardy interspecific hybrid containing *Vitis riparia* and *Vitis labrusca*, which can be made into various food products, such as wine, juice, or jelly [5]. Although it is highly disease-resistant, vigorous vine with reasonable productivity, its application was limited because of its high titratable acidity. Its rich, aromatic, grapey quality positively impacts its red wines; therefore, optimized grape wines are considered necessary for this cold-hardy grape [6].

Many strategies can be used to improve grape quality, directly influencing wine quality. Different trellis and training systems have been tested to enhance the quality of grapes in North Dakota [7,8]. Breeding and selection are another effective method for improving cold-hardy grape qualities [9,10]. Field management, such as foliar applications of nutrients, has promoted grapevine performance in cold conditions [11,12]. Environmental variables and harvest dates impact the quality of the grapes and wine [13]. Lastly, storage and postharvest treatment can be applied for several cold-hardy grapes to alter chemistry [12].

In addition to improving grape quality through agricultural management and postharvest treatments, multiple fermentation strategies can be applied to wine fermentation. Pre-fermentative strategies, such as cryogenic treatments, have impacted the aroma compounds in 'Sauvignon Blanc' and 'Chenin Blanc' wine [14]. Fermentation conditions, such as temperature, were reported to influence cold-hardy grape wine chemical characteristics and sensory profiles [14–16]. Maceration affects wine quality, especially the phenolic compounds—steam extraction and maceration duration impact Frontenac wine color and phenolics [17]. Fermentation with grape skins, seeds, and rachis could improve wine quality by enhancing tannins and anthocyanins [18]. Whole-cluster fermentation has improved the phenolics in cold-hardy hybrid 'Marquette' and 'Frontenac' wines [19]. Microbes, including yeasts and bacteria, can also be used to modify the wine fermentation process and products [20,21]. Malolactic fermentation is a strategy commonly used to decrease the acidity in cold-hardy grape wines [22].

Among many fermentative strategies, yeast strains are often used as a simple and direct method to improve cold-hardy grape wine fermentation. Wine yeast extracts and metabolizes compounds from the grapes, which likely influences a wine's flavor, stability, and metabolites. Fermentation conditions affect different yeast strains' activities, including temperature, sugar concentration, pH, and ethanol, directly influencing the wine's qualities [23]. Different yeast strain inoculation can often affect the variations of phenolic compounds, organic acids, and volatile compounds in wines [24]. An example includes the gene-modified yeast strain, I-328 ΔCAR1_MLF, which results in reduced ethyl carbamate and malic acid levels in wine [25]. Outside of fungal organisms, winemakers utilize bacteria, such as *Oenococcus oeni*, to convert malic acid into lactic acids; this technique alters the wine pH, acid content, chemistry, and aroma [26]. However, due to the impacts on aroma, sensory characteristics, and targeted wine style, winemakers may avoid using *O. oenoi* [27].

In this study, reducing the high acidity via biological means was the core challenge assessed when fermenting cold-hardy grapes. Different yeast strains have different metabolic outcomes, which synthesize different compounds and cause changes in the fermentation process [28]. *Saccharomyces* yeast and non-*Saccharomyces* yeast species are applied in winemaking to solve various challenges in fermentation parameters. The yeast *Lachancea thermotolerans* can degrade small amounts of L-malic acid, but it is most widely used for acidification via the conversion of hexose sugars into L-lactic acid [29,30]. Some strains of *S. cerevisiae* are reported to degrade malic acid by up to 40% [31]. Meanwhile, different yeast strain usage contributes to variable alcohol profiles and volatile and non-volatile products in wine [32]. Currently, *S. cerevisiae* and other *Saccharomyces* spp. remain the dominant yeasts of choice in winemaking, with non-*Saccharomyces* yeasts increasingly under exploration as an added tool for winemaking practices.

To support local winemaking practices, we tested five commercial yeast strains' performance in fermenting 'King of the North' ('KON') must to assess their potential in cold-hardy grape winemaking. EC1118, one of the most used yeast strains, is often treated as a yeast control treatment [33]. 71B was introduced to metabolize malic acids, produce high esters, and alter the aromatic profiles for wines based on the Lallemand website. Since cold-hardy grapes contain high levels of malic acids, 71B might be a good option to improve the taste of cold-hardy grape wines. Akin to 71B, Maurivin B was chosen because it is reported to consume up to 56% malic acid during fermentation from the product introduction (https://www.abbiotek.com/). Rhône 4600 has been mentioned to enhance the one cold-hardy grape cultivar, 'Frontenac's, fruity characteristics [34]. W15 has enhanced the cool-climate 'Riesling' wine esters compared to EC1118 [35]. Based on the advantages of each yeast, EC1118, 71B, Maurivin B, Rhône 4600, and W15 were chosen to assess their effects on 'KON' wines. In addition to the fundamental physiochemical analysis, this research also included a formal investigation of sensory evaluation on 'KON' red wines.

This study evaluated five representative yeast strain for their effects on 'KON' wine fundamental characteristics, sugar and color dynamic changes, and sensorial qualities. The experimental results will help to understand the relationship between yeast strains and cold-hardy grape fermentation. Future studies will consider modern technology to support the results.

2. Materials and Methods

2.1. Grapes

Mature own-rooted 'KON' grapevines (*Vitis* spp.) were obtained from a commercial vineyard located in Fertile, MN, USA (47°33′52.3″ N 96°11′00.7″ W). 'KON' is a hardy grape with tart, often tight, clusters. This variety is adapted to North Dakota conditions, which encompass the USDA's hardiness zone 4a and below [7]. In late spring, the vines were spur-pruned and cordon-trained within a vertical shoot positioning system with cordon wires at 0.4 to 0.6 m above the soil. Grape vines were planted 2.4 m within rows and 3.0 m between rows in an east–west orientation within a Chapett fine sandy loam (mixed, superactive, frigid Alfic Argiudolls) with a slope of 6 to 12 percent [30]. Three field replicates of 45 kg were manually harvested on 30 September 2020, transported in plastic harvest bins to Fargo, ND, and stored in a refrigerated walk-in cooler at 4 °C for 16 h.

2.2. Winemaking

On 1 October 2020, the fruit was transported to the North Dakota State University Horticulture Research Farm located near Absaraka, ND, where individual field replicates were automatically crushed and destemmed (Fratelli Baesso Crusher Destemmer, Curtarolo, Italy) to remove stems. Must was obtained using a 20 L stainless steel bladder press (Speidel, Ofterdingen, Germany). More than 40 L of must was manually transferred to two 23 L carboys and dosed with sulfur dioxide (SO_2) at a rate of 40 mg/L to prevent spoilage, along with a specific inactivated yeast-derived nutrient at a rate of 0.35 g/L (BoosterBlanc®, Lallemand Inc., Montréal, QC, Canada). Afterwards, musts were transported to Fargo, ND, and homogenized before being portioned into individual fermenters (Little Big Mouth Bubbler® Northern Brewer) fitted with three-piece airlocks. Each fermenter held 2.2 L of must, and each treatment included three replicates (fermenters). One day later, yeast strains were reactivated by dissolving them in 35–37 °C water; rehydration was followed by inoculation of musts at a rate of 0.264 g/L. The selected *Saccharomyces* yeast strains for fermentation comparison included 71B, EC1118, Maurivin B, Rhône 4600, and W15 (Table 1).

Table 1. Yeast strain information for strains examined in this study.

Yeast	Yeast Species	Company	Location of Origin
71B	*Saccharomyces cerevisiae*	Scott Lab (Petaluma, CA, USA)	Narbonne, FRA
EC1118	*Saccharomyces cerevisiae* (*ex-bayanus*)	Scott Lab (Petaluma, CA, USA)	Champagne, FRA
Maurivin B	*Saccharomyces cerevisiae*	Lallemand Inc. (Montreal, QC, Canada)	Burgundy region of France
Rhône 4600	*Saccharomyces cerevisiae*	Scott Lab (Petaluma, CA, USA)	Côtes du Rhône, FRA
W15	*Saccharomyces cerevisiae*	Lallemand Inc., (Montreal, QC, Canada)	Wädenswil, CHE

Two days after the yeast inoculation, fermenting musts received 0.264 g/L of a yeast nutrient supplement (Fermaid ® K, Lallemand Inc., Montreal, QC, Canada). Fermentation for this study occurred at 17 °C. Daily fermentation monitoring involved measuring density using a DMA 35 digital density meter (Anton Paar, Graz, Austria). Concurrently, a daily 10 mL must sample was collected and frozen at −20 °C to analyze the must's acid and chromatic properties. As the wines neared the completion of primary fermentation, they were racked into argon-purged, clear 1.89 L glass carboys and held at 17 °C until bottling.

2.3. Analysis of Chemical and Chromatic Properties of Must and Wine

Soluble solid content (SSC) was recorded using a Pal-1 digital refractometer (Atago Co., Tokyo, Japan). Titratable acidity and pH were monitored using an Orion Star A111 pH meter (Thermo Fisher Scientific, Waltham, MA, USA). Titratable acidity was measured via manual titration of a 5 mL juice sample to an endpoint pH of 8.2. Malic acid was measured using an enzymatic method using an L-malic acid assay kit (Megazyme, Wicklow, Ireland). Meanwhile, the sugar content in the must was measured by a sucrose/D-fructose/D-glucose assay kit (Megazyme, Wicklow, Ireland). The primary-amino nitrogen assay kits PANOPA and K-LARGE L-arginine/Urea/Ammonia assay kits (Megazyme, Wicklow, Ireland) measured the YAN, ammonia, and PAN contents in pre-fermentation musts.

Spectral properties of musts and fermentation products were evaluated to understand wine characteristics. The color density was calculated by A420 nm + A520 nm, the color intensity was calculated by A420 nm + A520 nm + A620 nm, and the color hue (h) was calculated as A420 nm/A520 nm [36]. These color metrics were calculated based on absorbance at each wavelength was evaluated using a 1 mm path length quartz cell measured in a UV-Vis spectrophotometer (Genesys$^{\text{TM}}$ 10S UV-Vis Spectrophotometer, ThermoFisher Scientific, Waltham, MA, USA); values were adjusted to a path length of 10 mm. The estimated total phenolic content and total red pigment were monitored in an acidified wine in 10 mm pathlength polymethyl methacrylate UV-cuvette cells (UV-Cuvette semi-micro, BrandTech ® Scientific, Inc., Essex, CT, USA) [37]. The total phenolic content was recorded as A280 nm, and the total red pigment was calculated as A520 for these acidified samples. The CIELab color coordinates were calculated with MSCV® software (https://www.unirioja.es/color/descargas.shtml) to obtain values for lightness (L*), chroma (C*), hue (h*), red-green (a*), and yellow-blue (b*) based on measurements collected from undiluted samples in a 1 mm path length quartz cells. The final wines were assessed for glycerol content, ethanol content, and acids (malic acid, tartaric acid, lactic acid, and acetic acid) using high-performance liquid chromatography as previously described [38,39].

2.4. Descriptive Sensory Analysis

The study was reviewed and approved by North Dakota State University IRB Federal Wide Assurance with the Department of Health and Human Services (FWA00002439). A sensory panelist aged 25 to 60 (eight females and five males) was trained in the descriptive evaluation of wines. All panelists work at North Dakota State University (NDSU) or were members of the North Dakota Grape and Wine Association (NDGWA) and have broad expertise in the sensory evaluation of wine beverages. The evaluation of 'KON' wine samples was carried out at temperatures (21–22 °C) during three different sessions, and each session included five samples. Each sample had three replicates for evaluation, and all samples were distributed randomly. Approximately 30 mL of wine was served into standard red wine glasses (Dailyware^{TM}, Hudsonville, MI, USA), together with the appropriate questionnaire. Unsalted crackers and water were provided to the panelist to cleanse the palate between samples. The wine samples were assessed with descriptive sensory analysis. The description was included in the visual, olfactory, and gustative phases. The 'KON' aroma and taste descriptives were based on the UC Davis wine aroma wheel with slight modifications, which described most red and white wines, regardless of the grape variety [40].

A complete list of color, aroma, flavor terms, and intensity levels was applied for wine sensory analysis. The scale for each descriptor ranged from the lowest (0) to the highest (5).

2.5. Statistical Analysis

Data were assessed and graphed using R software version 4.0.5. Principle component analysis and other plots were constructed in 'tidyverse' packages [18,19]. Color data for samples were processed with the colorspace package [20]. Date visualization was created into figures using the ggplot2 package v0.9.0 [21].

3. Results and Discussions

3.1. General Parameters of 'KON' Musts

The initial characteristics of the analyzed 'KON' must indicate an average SSC (°Brix) value of 17.53 (Table 2). The harvested 'KON' grapes had more fructose (~90.78 g L^{-1}) than glucose (88.28 g L^{-1}). Compared to the standard red wine grape cultivar's target SSC (approximately 22–26°Brix), the 'KON' SSC for this study was lower [41]. The SSC observed for the 'KON' must was close to, but slightly lower than, prior observations in the region, which is approximately 20°Brix and above. The ideal target SSC of 'KON' fruit is yet unknown, as is the same for many other cold-hardy grapevines [42].

The 'KON' must pH was lower than common grape cultivars (2.9~4.0) [43]. Titratable acidity was used to describe the acidity based on titration with a strong base (NaOH) to a specified endpoint pH of 8.20 [44]. It is also a measure of the amount of acid people may detect. Titratable acidity in typical wines was approximately 4–8 g/L [45], whereas 'KON' exhibited a high acidity of 22.7 g/L, indicating that the 'KON' must was very sour.

The malic acid content in 'KON' was high, approximately 13.44 g/L, which is quite common for cold-hardy grapes [46]. The high level of malic acid poses a challenge for cold-hardy grape applications, particularly in winemaking. Thus, the primary solution involves reducing the malic acids in 'KON' musts.

Table 2. Initial characteristics of 'King of the North' musts.

Sugar content		
SSC (°Brix)	Glucose (g/L)	Fructose (g/L)
17.53 ± 0.47	88.28 ± 3.10	90.78 ± 1.87
Acid attributes		
pH	Titratable acidity (g/L)	Malic acid (g/L)
2.58 ± 0.01	22.7 ± 0.05	13.44 ± 0.57
Total phenolics and total red pigments		
Total phenolics (AU)	Total red pigments (AU)	
12.56 ± 1.05	5.15 ± 1.11	
Nitrogen content		
YAN (mg/L)	Ammonia (mg/L)	PAN (mg/L)
196.05 ± 15.99	7.71 ± 0.69	188.34 ± 15.44

YAN—Yeast assimilable nitrogen; PAN—Primary amino nitrogen. Values are listed as mean ± standard error of replicates.

The spectrophotometric properties of the 'KON' musts showed that its total phenolics were approximately 12.56 AU, and anthocyanins averaged 1/3–1/2 of the total phenolics. Anthocyanins are the main class of phenolic compounds and are fundamentally responsible for the color of red grapes and wines [47]. Yeast assimilable nitrogen (YAN) in 'KON' must was 196.05 mg L^{-1}, and ammonia contributed only a small portion—only approximately 7.71 mg L^{-1}. Ammonia is the primary form of assimilable nitrogen for yeast throughout the fermentation process. The primary amino nitrogen content was 188.34 mg L^{-1} in the must. Commonly, at least 140 mg L^{-1} is required for fermentation [48]. Therefore, the total amount of YAN in the fermenter was sufficient for 'KON' wine fermentation.

'KON', while highly vigorous and cold-hardy, often has overwhelmingly high levels of acidity. Malolactic fermentation is usually considered the first option to reduce acidity but can detract from certain wine styles due to aroma and flavor notes such as a buttery taste [26]. Meanwhile, malolactic fermentation may produce undesirable compounds (such as ethyl carbamate and biogenic amines) and increase acetic acid content, adversely affecting wine quality [22]. Yeast strain decisions remain one key option to reduce acidity in wines, contributing a direct strategy for winemakers to ferment this cold-hardy grape.

3.2. Effect of Yeast Strains on Color Metrics and Acid Metrics

The overall effect of yeast strain on color metrics and acid metrics was assessed for fermentation samples (Figure 1A). The correlation matrix indicated that the day of fermentation was positively correlated with the pH and lightness (L) of wine, whereas it was negatively associated with SSC. Meanwhile, malic acid contents might moderately correlate with tartaric acid in ferments with R around 0.7. pH was negatively correlated ($R = 0.6$) with the density and intensity of the wine color. A significantly positive correlation was observed between color density, intensity, color space c, and color space a. A high negative correlation was observed between color density and lightness of ferments. The value c in CIE lab indicates the chroma (positive values = brighter; negative values = duller). Therefore, this indicated that along with fermentation, the color density of the must was lower, accompanied by brighter and lighter colors.

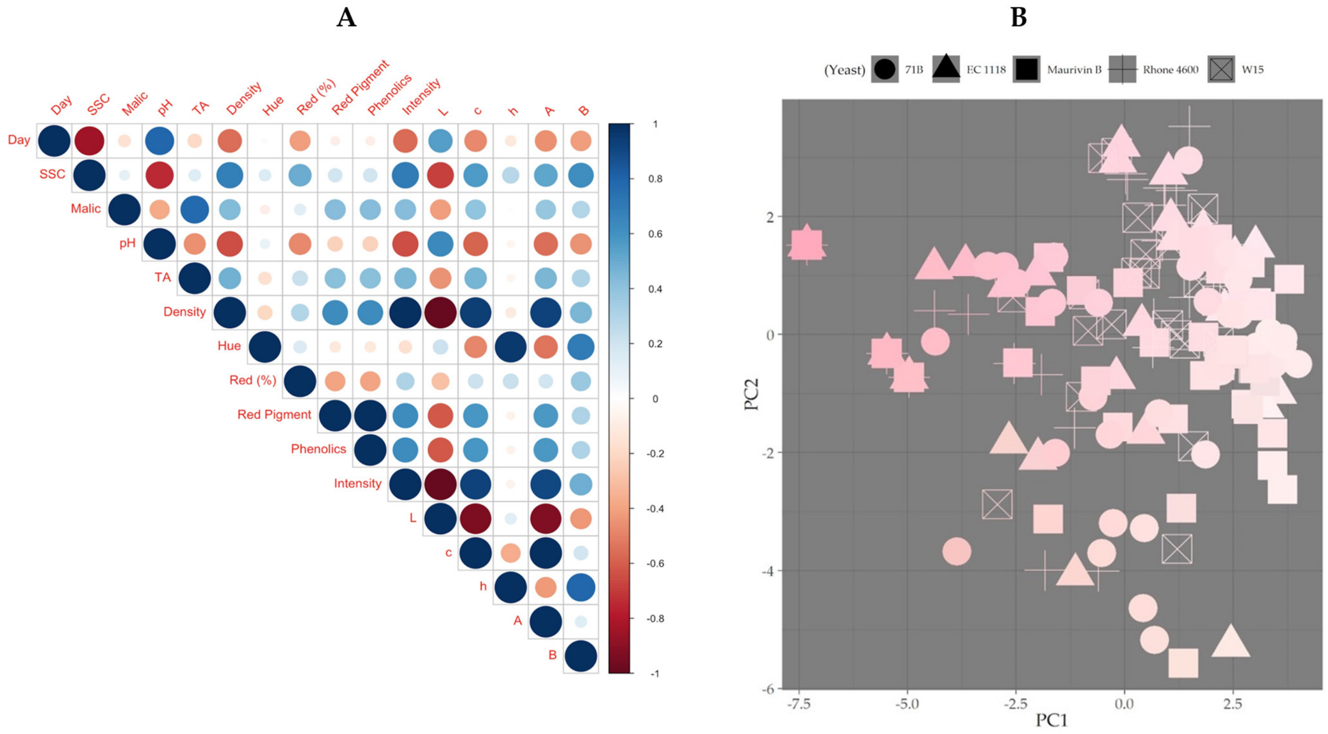

Figure 1. Fermentation Metric Correlation. (**A**) The overall correlation matrix of KON wine is influenced by color and acidity metrics. Day refers to the number of days after inoculation; SSC represents soluble solid content; Malic indicates malic acid content in grams; TA denotes tartaric acid content (10 µL in 490 µL H_2O); Density refers to color density. Hue signifies the color hue; Red (%) indicates the level of red pigments; Phenolics represent the total phenolic content; Intensity denotes color intensity; L, A, and B refer to the CIELAB color space parameters; c denotes CIELAB color space c; and h represents CIELAB color space h. (**B**) PCA of yeast strain contribution across multidomain data with individual samples. Different colors correspond to distinct wine hues for each sample, while various shapes represent different yeast strains, as indicated at the top of the figure. PC1 exemplifies color and lightness, while PC2 illustrates the ratio of wine visual evaluation (420 nm/520 nm) correlated with each sample.

Red pigment directly indicates the anthocyanin content of the grape wines [49]. The color metrics of 'KON' also revealed that red pigments were highly correlated with the amounts of phenolics and moderately correlated with color intensity, chroma, a (red-green parameter), and L. In summary of the physicochemical metrics, along with the progress of fermentation, the sugar dropped gradually, but the pH increased. The color metrics indicated the must got lighter with less red color and intensity.

The direct color-indicated in results from PCA also showed that, along with the positive shift direction of PC1, samples with greater lightness exhibited less color (Figure 1B). The visual evaluation of wine color nuance at 420/520 nm generally revealed a negative association (PC2). Throughout the fermentation process, the color gradually decreased in all fermentation treatments. W15 retained the greatest red coloration among the treatments, while Maurivin B failed to retain red coloration.

3.3. Fermentation Lightness Changes with Different Yeast Strains

Generally, the lightness of wine increased with the fermentation process (Figure 2). Initially, yeasts 71B and EC1118 were the most active strains on the first day; afterward, other strains caught up. During the middle stages (3–5 days), 71B, EC1118, and Maurivin B exhibited similar trends, with the clarity of the wines decreasing before increasing again, possibly due to the heightened yeast growth causing lower clarity. Meanwhile, the wine with W15 and Rhône 4600 gradually exhibited increased lightness. After fermentation (DAI 15), the results indicated that the wines made with 71B and Maurivin B displayed the most clarity (L* around 92.5) compared to the others. Wines made with EC1118 and Rhône 4600 still had L* values exceeding 90, whereas W15 had less than 90 lightness, indicating that the wine with W15 was less bright than the other wines.

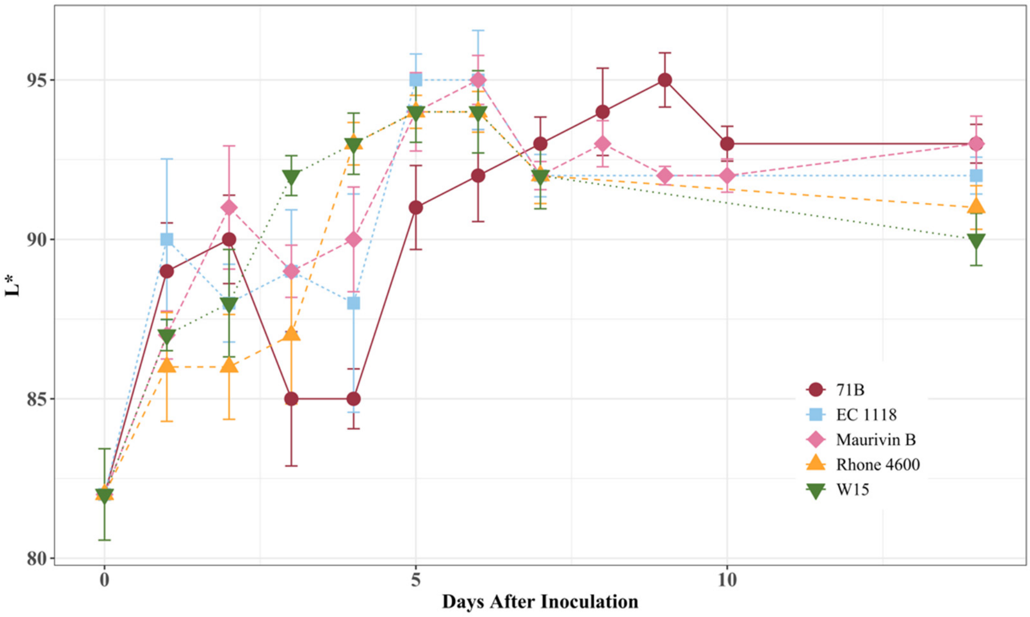

Figure 2. Perceptual lightness (L*) dynamic change of King of the North Wine with five yeast treatments. Bars indicate the standard error of the mean for individual treatments and three replicates in each treatment. The yeast strains include 71B, EC1118, Maurivin B, Rhône 4600, and W15.

In conclusion, 71B and Maurivin B may have resulted in increased clarity of 'KON' wines. Wine with EC1118 had slightly lower lightness after fermentation compared to 71B and Maurivin B wines. The Rhône 4600 and W15 yeasts were generally slow starters, resulting in fewer bright wines.

Yeast diversity and its effects can be optimized, resulting in better organoleptic characteristics, such as color and aroma. Yeast can impact wine color through different mechanisms, including, but not limited to, the release of metabolites that stabilize wine color and increase the content of stable pigments, enzymatic activities, and the adsorption of phenolic compounds by yeast cells. Therefore, yeast selection can be an essential strategy for improving the color of cold-hardy grape wines.

3.4. Sugar Transformation Differences with Different Yeast Strains

Sugar consumption indicated that 71B did not start fermentation until day 6 but began effectively digesting sugar between days 6 and 10 (Figure 3). Similarly, the fermentation with EC1118 also had an incubation stage, during which the sugar consumption did not start until day 4. The Rhône 4600 and W15 yeasts were the fastest strains to use sugar in juice because, with both, SSC decreased quickly to near zero by around day 6. Maurivin B's performance in sugar consumption was distinguished with a low decrease from day 2 to day 15. All the yeast strains could finish fermentation successfully with 'KON' musts.

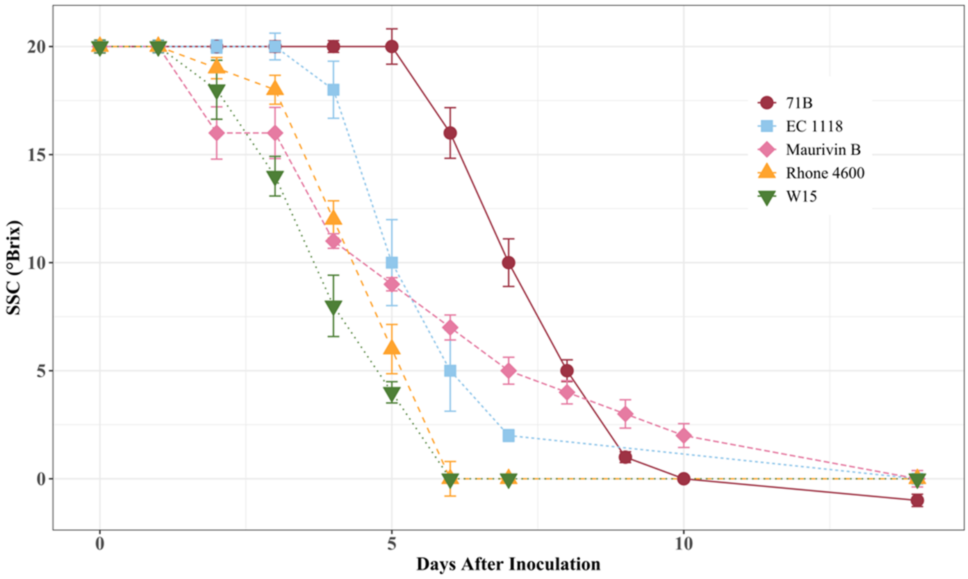

Figure 3. Sugar dynamic changes during fermentation with five different yeast strains. The *y*-axis is SSC (soluble solid content). Bars indicate the standard error of the mean for individual treatments. Three biological replicates are included in each treatment.

Yeast strains have different fermentation kinetics due to their differential fermentation performance and ethanol tolerance [32,50]. All the commercial strains selected for this study displayed similar slopes during sugar consumption, although strain 71B initially had a slow start.

Yeast fermentation behavior largely depends on the available nitrogen in grape musts. A nitrogen supplement was added to the musts to meet the yeast's requirements (Table 2). Additionally, studies have shown that yeast strains vary in their ability to adapt to different nitrogen compositions [22]. Therefore, further research should assess specific yeast nitrogen profiles.

Fermentation operations, such as the mode of inoculation in the preparation stage, could also influence yeast fermentation dynamics and wine compositions [51]. This study used yeast rehydration and inoculation; further studies should also consider preculturing yeast strains before inoculation. Must composition, such as fatty acids, may also influence the yeast performance [52]. A comprehensive investigation is needed to understand the yeast strain differences. The yeast characteristics, including fermentation initiation speed, resistance to high ethanol contents, requirements for growth factors, and development of organoleptic profiles, shall all be considered for further studies. Co-inoculation with yeast and bacteria, yeast and yeast, or sequential inoculation, also could influence yeast behavior [24]. In this study, bacteria/yeast co-inoculation was not introduced since it might introduce more complexities to the results. However, there is good potential for this to be employed with different kinds of fermentations. Wine fermentation kinetics as physical analysis are necessary yet insufficient tools for a complete understanding of the dynamics of wine fermentation [25]. Therefore, the optimization of cold-hardy grape wine requires more research. Combined research technologies and computer engineering may provide more promising detection and evaluation strategies for wine fermentation.

3.5. Wine Chemistry

The final wine pH ranged from 3.06 (W15) to 3.15 (71B) (Table 3). Malic acid content was lowest for 71B and Maurivin B, with 9.83 and 8.93 g/L malic acid in the final wines, respectively. The remaining yeast produced wines with malic acid content at or above 12 g/L. Tartaric acid was between 1.12 and 1.51 g/L; 71B fermented wines had the lowest overall tartaric acid content. The titratable acidity of wines followed the observations of the malic and acid content, with 71B (11.2 g/L) and Maurivin B (11.7 g/L), both yielding wines with lower levels of titratable acidity. Lactic acid was below 0.10 g/L for all yeast treatments. Acetic acid content was greatest for Maurivin B fermented wines (0.33 g/L) and lowest for EC1118 (0.05 g/L) and Rhône 4600 (0.07 g/L) wines.

Table 3. Acid properties of 'King of the North' grape wines fermented using five yeast strains.

Yeast	Malic Acid (g/L)	Tartaric Acid (g/L)	Lactic Acid (g/L)	Acetic Acid (g/L)	pH	Titratable Acidity (g/L) [1]
71B	9.83 ± 0.39 ^{b2}	1.12 ± 0.20 ^{b}	0.05 ± 0.01 ^{ns}	0.15 ± 0.01 ^{b}	3.15 ± 0.02 ^{a}	11.2 ± 0.2 ^{c}
EC1118	13.27 ± 0.48 ^{a}	1.21 ± 0.06 ^{ab}	0.05 ± 0.01	0.05 ± 0.01 ^{c}	3.11 ± 0.05 ^{ab}	13.0 ± 0.2 ^{ab}
Maurivin B	8.93 ± 0.39 ^{b}	1.33 ± 0.19 ^{ab}	0.09 ± 0.04	0.33 ± 0.02 ^{a}	3.08 ± 0.03 ^{b}	11.7 ± 1.0 ^{bc}
Rhône 4600	13.03 ± 0.39 ^{a}	1.50 ± 0.13 ^{a}	0.06 ± 0.01	0.07 ± 0.01 ^{c}	3.05 ± 0.01 ^{b}	12.9 ± 0.3 ^{ab}
W15	12.81 ± 0.39 ^{a}	1.51 ± 0.17 ^{a}	0.05 ± 0.01	0.14 ± 0.01 ^{b}	3.06 ± 0.02 ^{b}	13.5 ± 0.1 ^{a}

[1] Titratable acidity expressed as tartaric acid equivalents. [2] Values and standard error in a column followed by different letters indicate mean values are significantly different based on means separation via Tukey's HSD; ns = not significant.

Final wines varied in their glycerol content (Table 4). Glycerol content was greatest for 71B (6.31 g/L), followed by W15 (6.15 g/L), while they were lowest for EC1118 (5.39 g/L). Ethanol percentages were between 8.01 (71B) and 8.20 (EC1118 and Maurivin B).

Table 4. Glycerol and ethanol content of 'King of the North' grape wines fermented using five yeast strains.

Yeast	Glycerol (g/L)	Ethanol (% *v*/*v*)
71B	6.31 ± 0.12 ^{a1}	8.01 ± 0.11 ^{ns}
EC1118	5.39 ± 0.14 ^{b}	8.20 ± 0.10
Maurivin B	5.81 ± 0.15 ^{ab}	8.20 ± 0.12
Rhône 4600	5.94 ± 0.15 ^{ab}	8.15 ± 0.11
W15	6.15 ± 0.29 ^{a}	8.12 ± 0.11

[1] Values and standard error in a column that are followed by different letters indicate mean values are significantly different based on means separation via Tukey's HSD; ns = not significant.

Color density and intensity did not differ among yeast treatments (Table 5). Color density ranged from 0.56 to 0.73, and color intensity ranged from 0.57 to 0.77. Color hue was greatest for 71B wines (1.87) and below 1.50 for all other wines. Total phenolics ranged from 10.65 (EC1118) to 11.62 (71B), with no differences among yeast.

Table 5. Color density, color intensity, color hue, and total phenolics in 'King of the North' grape wines fermented using five yeast strains.

Yeast	Color Density	Color Intensity	Color Hue	Total Phenolics (AU)
71B	0.56 ± 0.03 ^{ns1}	0.57 ± 0.03 ^{ns}	1.87 ± 0.43 ^{a}	11.62 ± 1.37 ^{ns}
EC1118	0.73 ± 0.06	0.77 ± 0.08	1.41 ± 0.20 ^{b}	10.65 ± 1.57
Maurivin B	0.73 ± 0.04	0.77 ± 0.04	1.49 ± 0.20 ^{b}	10.68 ± 1.06
Rhône 4600	0.68 ± 0.03	0.70 ± 0.04	1.32 ± 0.13 ^{b}	11.52 ± 1.43
W15	0.66 ± 0.12	0.67 ± 0.13	1.46 ± 0.17 ^{b}	11.02 ± 1.11

[1] Values and standard error in a column that are followed by different letters indicate mean values are significantly different based on means separation via Tukey's HSD; ns = not significant.

CIELAB chromatic characteristics of ‘KON’ wines were not altered by yeast strain (Table 6). Wine values for L* ranged from 95.57 (Maurivin B) to 97.77 (71B). The a* values were between 2.13 (71B) and 3.50 (EC1118 and Rhône 4600). The b* values fell between 3.73 (Rhône 4600) and 4.42 (Maurivin B), while chroma values were between 4.83 (71B) and 5.52 (Maurivin B). Wine hue angles were as low as 46.92 (Rhône 4600) and as high as 63.74 (71B), with no differences observed between yeast treatments.

Table 6. Chromatic properties of ‘King of the North’ grape wines fermented using five yeast strains.

Yeast	Lightness (L*)	Red (a*)	Yellow (b*)	Chroma (C*)	Hue (h°)
71B	97.77 ± 0.29 ^{ns}	2.13 ± 0.57 ^{ns}	4.26 ± 0.15 ^{ns}	4.83 ± 0.16 ^{ns}	63.74 ± 6.69 ^{ns}
EC1118	96.43 ± 0.49	3.50 ± 0.56	4.07 ± 0.21	5.41 ± 0.42	49.76 ± 4.44
Maurivin B	95.57 ± 0.17	3.19 ± 0.75	4.42 ± 0.17	5.52 ± 0.46	55.17 ± 6.25
Rhône 4600	96.73 ± 0.18	3.50 ± 0.42	3.73 ± 0.30	5.14 ± 0.33	46.92 ± 4.21
W15	97.03 ± 0.67	3.44 ± 0.77	4.07 ± 0.51	5.37 ± 0.82	50.42 ± 4.56

^{ns} means not significant.

3.6. Descriptive Sensory Analysis of ‘KON’ Wine with Different Yeast Strains

The panelists described the ‘KON’ wine colors primarily as ‘Pink’ and ‘Salmon’. The descriptive sensory analysis focused mainly on aroma and taste. The aroma evaluation showed that the aroma intensity was rated between 2 and 3 among the five yeast-fermented samples (Figure 4). Overall, fruity and floral aromas were the predominant characteristics of the fermented ‘KON’ wines. W15 received a nearly 3 rating for its fruity aroma, followed by Rhône 4600 (2.5) and Maurivin B (2.2). EC1118 and W15 had lower fruity ratings, ranging from 1 to 1.5. The floral aroma of the five yeast-fermented wines was rated between 1 and 1.5. The wine from EC1118 had the highest floral aroma score at 1.5, while Maurivin B had the lowest floral aroma rating of 1. Spice was sensed mainly in Rhône 4600, and the aroma was not strong at a rate of 0.75. Spice was barely sensed in Maurivin B wines. Vegetal and yeast aromas were only identified in some ‘KON’ wines.

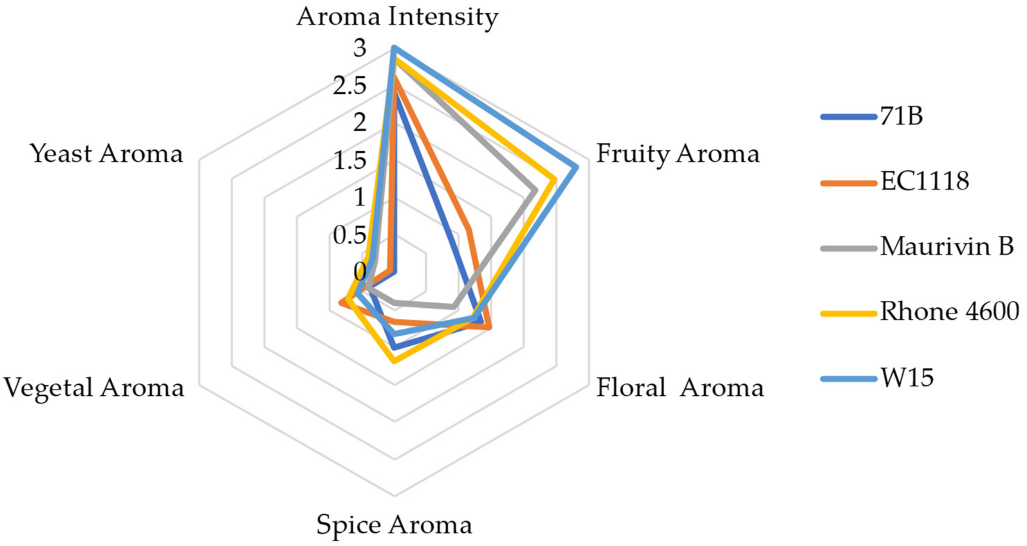

Figure 4. ‘KON’ wine aroma evaluation. Five different yeasts (71B, EC1118, Maurivin B, Rhône Rhône W15) were evaluated through aroma intensity, fruity aroma, floral aroma, spice aroma, vegetal aroma and yeast aroma. The levels for evaluation were described from lowest (0) to highest (5). Bars indicate the standard error of the mean for individual treatments, n = 3.

The wine taste evaluation (Figure 5) showed that the acidity taste was strong in all of the samples, with a high rate of 3. Fruity tastes were sensed in all wines, ranging from 2.5 to 2.75. Both tannin and floral tastes were rated as 1 to 1.5, mostly in the wines. Rhône

4600 had the highest floral and tannin tastes, followed by Maurivin B, W15, and 71B. Although W15 had the highest acidity, its sweetness was also the highest. However, the sweetness was rated lower than 1. Spice was mainly sensed in Rhône 4600, with the rate slightly lower than 1. The vegetal taste was the least perceived flavor detected across all the wines.

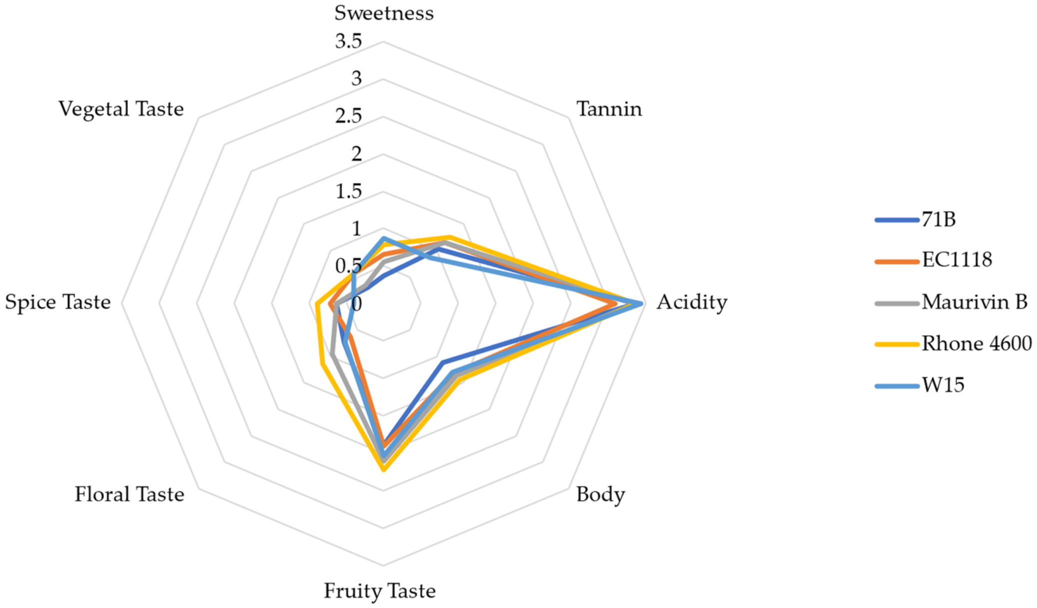

Figure 5. 'KON' wine taste evaluation. Five different yeasts (71B, EC1118, Maurivin B, Rhône Rhône W15) were evaluated through different characteristics of taste, including sweetness, tannin, acidity, body, fruity taste, floral taste, spice taste and vegetal taste. The levels for evaluation were set from lowest 0 to highest 5. Bars indicate the standard error of the mean for individual treatments, n = 3.

The 'KON' wine-specific aromas indicated that most panelists detected intense floral and fruity scents (Figure 6). Among the floral aromas, rose was the most prominent, followed by jasmine. Regarding fruity scents, the grape, apple, apricot, pear, plum, and raspberry were the most frequently identified, with intensity levels exceeding 20. Additionally, the panelists recognized aromas such as banana, blackcurrant, honeydew, lemon, pear, pineapple, prune, raisin, and watermelon, which had intensity levels below 20. Regarding spice aromas, some 'KON' wines exhibited scents of cinnamon, cloves, black pepper, and red pepper. For vegetal aromas, some panelists noted hints of asparagus, bell pepper, grass, hay, leaves, and mushrooms. Aroma intensities varied among the different wines. For example, the rose aroma was particularly noted in W15, as its aroma stack bar height was the highest. In contrast, the 71B and Maurivin B wines exhibited lower levels of rose aroma. Furthermore, aroma specificity was observed among the five wines; red pepper was a distinctive scent in the W15 wine samples but not in the others.

The 'KON' wine flavors indicated that the most dominant taste was lemon, suggesting that 'KON' wine exhibited high-acidity characteristics (Figure 7). Based on the intensity of the lemon flavor, 71B may have more capability to reduce the acids in 'KON'. The primary fruity flavors identified included rose, apple, cranberry, grape, pineapple, plum, raspberry, and watermelon. Like the aromas, rose remained the strongest among the floral flavors (Figure 7). Lavender was distinctive in 71B and EC1118 'KON' wines, while a lily flavor was not noted in W15 wines.

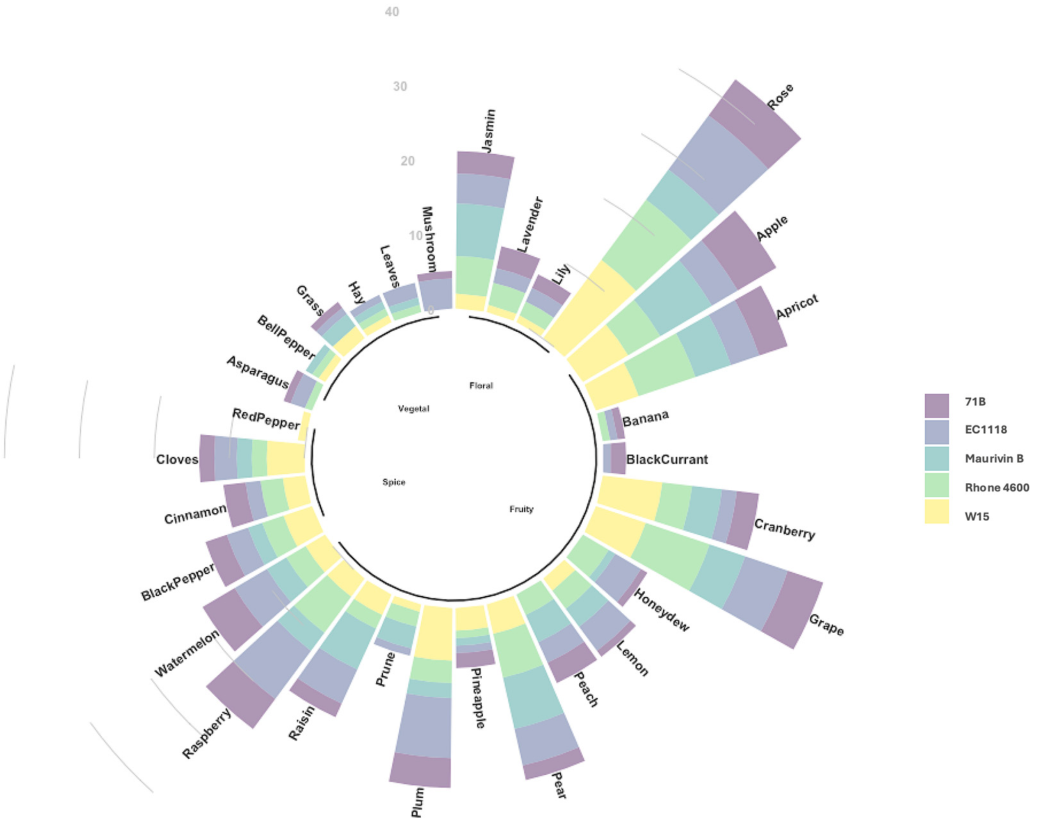

Figure 6. Specific aroma evaluation of 'KON' wines. Five different yeasts (71B, EC1118, Maurivin B, Rhône Rhône W15) effects on aroma characteristics were evaluated through a circular plot. The five yeasts were listed with the corresponding colors within the legend. The top of each bar indicates the specific aroma. The stacked bar height indicates the corresponding intensity levels of each fermented yeast. The bar-plot line values (from 0 to 40) specified the height of aroma intensity levels. Inside the circular plots, four categories of aromas (fruity, floral, spice and vegetal) are labeled.

Regarding spice flavors, black pepper was detected across all wines, though cinnamon, cloves, and red pepper were perceived in some varieties. The intensity of vegetal flavors mirrored that of spice aromas, being lower than the floral and fruity flavors. A grass flavor was noted in each type of fermented wine, while that of mushroom, leaves, hay, bell pepper, and asparagus were only faintly perceived by some panelists.

A wide range of compounds significantly influences the sensory properties of wine, and different yeast strains typically result in wines with varied sensory characteristics, even when using the same grape cultivar. In this study, 'KON' wines exhibited fruity and floral aromas and tastes, with each yeast strain leading to variable intensity of these aromas and flavors (Figures 4 and 5). Additionally, each strain caused variations in the characteristics of specific aromas and flavors (Figures 6 and 7). Aside from yeast strains, no other factors were introduced. However, fermentation conditions were included in some publications to compare the effects of yeast strains. Previously, low-temperature fermentation correlated with high ester levels and low terpene concentrations in Merlot wines, and the concentrations of alcohols and fatty acids were dependent on the yeast strain [26]. Wines are also affected by the nutrients available during fermentation [27]. Although the primary wine aroma and flavor compounds originate from fermentation, yeast can modulate these compounds. Co-inoculation of yeast with yeast or bacteria, whether sequential or simultaneous, can influence the wine's aroma and flavor compounds [3]. The chromatographic technology and electronic sensory analysis (e.g., electronic nose (E-nose) and electronic tongue (E-tongue)) provide helpful methods for the study of flavor and aroma in wines [53]. Future research on 'KON' wine profiles can be completed using these techniques.

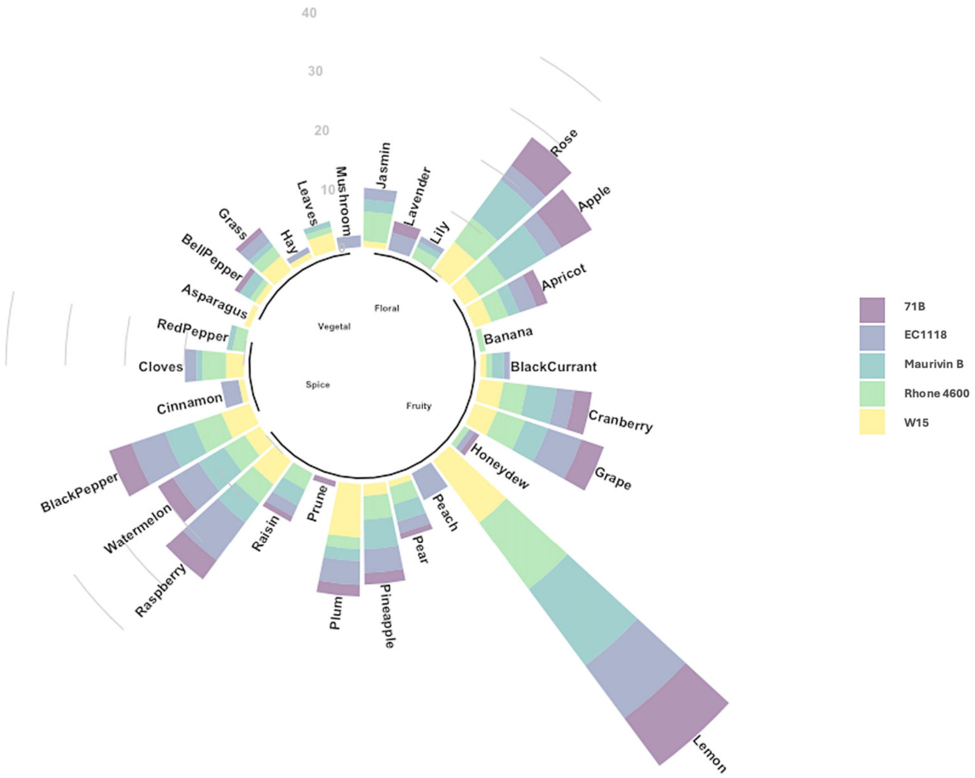

Figure 7. Specific flavor evaluation of 'KON' wines. Five different yeasts (71B, EC1118, Maurivin B, Rhône 4600, and W15) effects on flavor/taste characteristics were evaluated through a circular plot. The five yeasts were listed with the corresponding colors as legends. The top of each bar indicates the specific flavor. The stacked bar height indicates the corresponding intensity levels of each fermented yeast. The bar plot line values (from 0 to 40) specified the height of flavor intensity levels. Inside the circular plots, four flavor categories (Fruity, Floral, Spice, and Vegetal) are labeled based on the variety of tastes.

In addition to yeasts, the microflora used in grape winemaking must also consider the influence of lactic acid bacteria [54]. Malolactic fermentation is another method to reduce the high acidity and may alter the overwhelming lemon taste in 'KON' wines. The organoleptic profile from microbiota development is based on grape materials and the microorganisms used in wine production.

4. Conclusions

'King of the North' is a cold-hardy grape cultivar with a high survival rate during harsh winters in North Dakota. In response to new and adapting demands of the fruit and wine industry, especially in North Dakota, improved processing techniques for regionally developed grapevine cultivars must be developed; yeast strain decisions are among the most immediately accessible and applicable tools for regional winemakers to refine and define their products.

This is the first study to evaluate yeast's impact on 'KON' wine from cold-hardy grapes. Cold-hardy grapes often have high acidity, limiting their application. With the expanded wine production and market in local areas of North Dakota, developing wine research using local cultivars is crucial. Yeast was used in this research as the main factor influencing wine sensory values.

'King of the North' grape musts were inoculated with five commercial yeast strains. The impact was assessed through both changes in fermentation dynamics and sensory evaluation. The five yeast strains varied in their ability to transform sugars and color characteristics during fermentation. All the fermented musts showed reduced red pigments, although the extent of color reduction differed. These five yeast strains also affected the specific aroma and flavor profiles, yet all the wines exhibited intense floral and fruity aromas and flavors. Different strains led to varying acidity reduction; however, the high acidity was retained, and lemon taste remained the predominant flavor in 'KON' wines.

This evaluation will help grape growers and winemakers predict the sensory characteristics of fermentation tactics and provide usage possibilities for newly released grapevine genotypes in North Dakota. More methods and strategies must be developed to improve the quality and produce pleasant, cold-hardy wines.

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Data Availability Statement: Data are available upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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