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A Preliminary Study of Yeast Strain Influence on Chemical and Sensory Characteristics of Apple Cider

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Abstract: During the fermentation of apple juice, yeast metabolism creates complex biosynthetic pathways which produce a range of compounds responsible for the organoleptic qualities of cider. In this study, basic cider quality parameters were measured to investigate the influence of six yeast strains on cider made from three apple varieties ('Pink Lady', 'Sturmer', and 'Bulmer's Norman'). Measurement of pH, titratable acidity, and total phenolic content revealed that yeast can influence cider attributes, albeit variety and season dependent. Descriptive sensory analysis using a trained sensory panel was conducted on cider made from 'Pink Lady' apples and the same six yeast strains. The sensory panel significantly differentiated the yeast strains on the attributes of 'fresh apple', 'earthy' and 'pear'. Identifying the variety specific influence of individual yeast strains on chemical and sensory characteristics of apple cider will provide cider makers with an enhanced understanding when choosing yeast strains.

Keywords: cider; apple; variety; fermentation; phenolic; sensory analysis; yeast

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1. Introduction

The idea that microorganisms could influence the flavour profiles of beverages was first presented by Louis Pasteur in the 1860s [1]. Yeast activity is a critical factor in the production of cider, primarily for the purpose of producing alcohol [2–4]. Alcoholic fermentation is the result of yeast converting sugars into ethanol [5,6]. During the fermentation process, yeast metabolises the sugars available in the substrate, which leads to the production of volatile (e.g., alcohols, esters, and fatty acids) and nonvolatile compounds (e.g., malic acid), which influence both the final aroma and flavour of the cider [6–10]. The development of flavour in ciders is similar to wine, where, through a long history of fermentation research, studies have identified which flavour influencing compounds are: (a) grape-derived, (b) grape-derived then altered by yeast, and (c) the result of the primary metabolism of yeast [11]. The interaction between compounds found naturally in the fruit juice and those developed by the yeast create the sensory experience for the consumer [11].

The most common yeast species used for wine and cider fermentation is *Saccharomyces cerevisiae* [4,11,12]. The industrial popularity of *S. cerevisiae* comes from its durability under extreme fermentation conditions, as it can tolerate high sugar content, low pH, and high ethanol [11,13]. The ability to tolerate these environments and produce consistent aroma and taste, as well as for complete fermentation, aids quality control and gives *S. cerevisiae* a competitive edge over other yeasts [4,11,12].

In the process of converting sugars to ethanol and carbon dioxide, yeast produces a variety of metabolites, which can have organoleptic qualities [6]. Yeast metabolites include esters, higher alcohols, carbonyl compounds, volatile acids, volatile phenol, and sulfur compounds [11]. The production of these compounds is influenced by the yeast strain, as well as fermentation conditions and nutrient availability [6,14]. In previous studies, different yeast strains have been shown to produce a varying intensity of flavour characteristics such as 'apple or pear' (in this case correlating with ethyl esters and terpenes) [15], 'tropical fruity aroma' (correlating with high production of 3-methylbutyl acetate), 'nail polish' (correlating with 2-methylbutan-1-ol, 3-methylbutan-1-ol, and 2-methyl-1-propanol) [12], and 'sulfur' (proposed to be driven by hydrogen sulfide) [16]. The spike in different characteristics is driven by the yeast as it creates different biosynthetic pathways during metabolism and, thus, forms different compounds [6,11,14]. The relationship between varietal characteristics of the fruit and the yeast's ability to form these compounds creates the sensory complexity of fermented beverages such as wine and cider, compared to the sweet, relatively simplistic fruit juices they originated from [11]. The ability for yeast strains to influence flavour in fermented beverages highlights the importance of yeast selection, especially for those fermenting on a commercial scale [17].

In apple cider, the chemical properties that relate to flavour are influenced by the physical cider making process [18], yeast strain [19], variety, and ripeness of the apples being fermented [20]. Phenolic content is commonly used as a quality indicator of apple cider, as phenolic compounds are responsible for colour, mouthfeel, bitterness, and astringency, factors that relate directly to sensorial perception [21–24]. Consequently, it is expected that changes to total phenolic content (TPC) are likely to be reflected in the sensory experience [21]. Due to the large number of yeast strains and apple varieties to choose from for cider fermentation, it is important that cider producers and yeast manufacturers understand the influence of yeast strain on key cider characteristics and consumer perception in the context of a range of apple varieties [12,25].

This study investigated the influence of yeast on a range of common cider quality attributes. We hypothesized that ciders produced from the same apple variety fermented with different yeasts will be differentiated through base cider chemistry parameters and sensory analyses. To address this hypothesis, we analysed TPC, titratable acidity, pH, and total soluble solids in ciders produced from three apple varieties and six commercial yeast strains, as well as a wild ferment. Of the three apple varieties, cider made from 'Pink Lady' apples was used for sensory analysis, using descriptive analysis to test if consumers could differentiate the sensory properties of ciders fermented with different yeast strains.

2. Materials and Methods

2.1. Cider Making

Over two growing seasons, apples of four varieties ('Pink Lady' (dessert), 'Sturmer' (culinary), 'Bulmer's Norman' (2018 only) (cider), and 'Frequin Rouge' (2019 only) (cider)) were collected from the Huon Valley, southern Tasmania, Australia (43.0295° S, 147.0580° E). Apple varieties were chosen to represent the varieties that are readily available and preferred by local cider makers, as well as providing a range of TPC and organoleptic attributes [21]. 'Dessert' varieties have typically been bred to provide a balance between crunch and sweetness for eating, whilst also having disease resistance. 'Cider' varieties are usually derived from a European background, have a higher phenolic content than dessert and are used primarily for the purpose of cider making [21,26]. 'Culinary' (or heritage) varieties are often regarded as dual-purpose apples, as they can be eaten fresh, used in cooking, or for juice or cider. 'Sturmer' can be consumed as a dessert variety, added to juice or cider, or be used in cooking.

All apples were harvested aligning with commercial harvest in both seasons. It was not possible to source sufficient 'Bulmer's Norman' apples in 2019 due to climatic conditions affecting the orchard, so 'Frequin Rouge' was chosen as a replacement cider variety. Apples were milled and crushed according to the method detailed by Way et al. [21]. Following milling, three $\times$ 50 mL juice samples for each apple variety were taken and frozen for juice laboratory analysis. Fermentation (in triplicate) was completed and monitored as in Way et al. [21], with the same protocol followed for all yeast strain treatments, with the only exception being that the ciders were fermented in 1 L glass bottles (4 replicates $\times$ each variety $\times$ strain). Three commercial yeast strains, Lalvin ICV

OKAY (Lallemand, Edwardstown, SA, Australia), Lalvin EC1118 (Lallemand, Edwardstown, SA, Australia), and IOC Be Fruits (Danstar Fermentation A.G., Australia), were used as treatments in the 2018 experiment (Table S1). The trial was repeated in 2019 with an additional three yeast varieties including: Maurivin Elegance (AB Biotek, North Ryde, NSW, Australia), Maurivin UOA Maxithiol (AB Biotek, North Ryde, NSW, Australia), and Mauribrew Weiss (AB Biotek, North Ryde, NSW, Australia) (Table S1). A 'wild' treatment was used in both 2018 and 2019 experiments, where the juice was treated the same as it was for the commercial yeast addition, but instead of adding 1 mL of a rehydrated commercial yeast, 1 mL of pure apple juice of the same variety was added to each wild ferment.

2.2. Chemical Analysis

The ciders were centrifuged for 10 min at 4000 rpm and brought to room temperature of 20 °C. pH was measured using a handheld WP-81 pH-Cond-Salinity Meter (TPS, Brisbane, Australia), and titratable acidity (TA) was measured using an automatic titrator (Mettler Toledo g20 Compact Titrator, Greifensee, Switzerland) where results were expressed as g/L of malic acid. The total soluble solids (TSS) of apple juice were measured using a handheld digital refractometer (A. Kruss Optronic, Hamburg, Germany) with results measured in °Brix. The TPC of both juice and base cider was quantified using the Somers method [21], as validated for use in cider by Way et al. [21] by diluting the samples 1:50 in 1M HCl and using a spectrophotometer (SPECTROstar Nano, BMG LABTECH, Windsor, NSW, Australia). The spectrophotometer results, as expressed in absorbance units, were multiplied by the dilution factor (×50); therefore, results are represented as total phenolic index (TPI).

2.3. Descriptive Sensory Analysis

The purpose of the sensory analysis was to quantify potential differences in the flavour profiles of ciders made from different yeast strains by a trained panel of consumers. Descriptive analysis was selected as the most suitable approach due to the volume of cider available as well as the intention of the study. An alternative approach, such as the discriminate method 'triangle test', may have differentiated ciders from one another, but cannot provide insight into how they differed and what was driving this difference, as descriptive analysis can [27]. Descriptive analysis allows a tailored approach, where descriptors can be derived from the attributes of the ciders being analysed [28]. To ensure that differences in flavour profiles were attributed to yeast strain, rather than apple variety, only ciders made from one apple variety were used in the sensory analysis. Ciders made from the dessert variety 'Pink Lady' were selected for the assessment given the high proportion of dessert apples that are used in cider making (due to their availability) compared to true cider varieties. Pink Lady is representative of dessert varieties in general and was readily available at the time of harvest. Each cider was fermented with a different yeast: Lalvin ICV OKAY (Lallemand), Lalvin EC1118 (Lallemand), IOC Be Fruits (Danstar Fermentation A.G.), Maurivin Elegance (AB Biotek), Maurivin UOA Maxithiol (AB Biotek), Mauribrew Weiss (AB Biotek), and a 'wild' ferment.

Sensory Training

Sensory training was conducted at the Tasmanian Institute of Agriculture in June 2020. Ten individuals (aged 25 to 65, six male, four female) were selected based on their availability and desire to participate. The panel was trained over five × 1 h sessions before participating in three formal descriptive analysis sessions. During the sessions, the panel was trained to evaluate and rate intensity of selected attributes. In the first session, the panel was asked to assess four × 30 mL cider samples for odour, taste, and mouthfeel and record three to five attributes they detected in the sample either from a list of potential attributes as in Qin et al. [29], or create their own. Training sessions 2 and 3 involved providing the panel with reference standards according to the attributes chosen in session 1. Thirteen attributes were chosen: nine aroma, and four taste/mouthfeel (Table 1). The odour attributes chosen were: 'floral', 'fresh apple', 'cooked apple', 'pear', 'citrus', 'tropical fruit', 'earthy', 'yeasty', and 'chemical', and for taste, they were: 'astringent', 'sweet', 'sour', and 'alcoholic'. In the second training session, thirteen glasses were labelled with letters and the participants were asked to match each glass with one of the panel-generated attributes provided to them in a list. In the third session, two sets of the reference standards were presented; one set had weaker reference standards than the previous session, and the other set was stronger. The panel were asked to rate the intensity of each attribute from 0 to 9 on a 9 cm unstructured scale, 0 being 'absent' and 9 being 'high intensity'. In the fourth and fifth training sessions, in preparation for formal analysis, two cider samples that were not included in the formal analysis were presented to each panellist to rate the intensity of each of the thirteen attributes in the provided list. Freshly opened bottles of cider, which had been refrigerated for 10 months, were used for all training sessions.

Table 1. Sensory attributes chosen by sensory panel and corresponding reference standards' compositions.

Attribute	Reference Standard [a]
Odour	
Floral	120 mL elderflower juice
Fresh apple	1 chopped fresh apple, 50 mL apple juice (Spreyton Fresh)
Cooked apple	1 chopped fresh apple in boiling water for 5 min
Pear	1 chopped fresh pear, 50 mL pear juice (Bickford's)
Citrus	50 mL juice and half of one chopped fresh grapefruit and one half of one chopped lemon
Tropical fruit	1 cup of fresh chopped pineapple, $\frac{1}{2}$ cup of chopped melon
Earthy	1 chopped large potato, 4 chopped cup mushrooms
Yeasty	1.5 g of commercial yeast (Lalvin EC1118 (Lallemand))
Chemical	10 µL ethyl acetate
Taste	
Sweet	15 g white sugar (CSR)
Sour	50 mL apple cider vinegar (Cornwell's)
Astringent	20 mL commercial winemaking tannin (Enartis)
Alcoholic	10 mL of 95% ethanol

[a] In 500 mL of 50:50 filtered water: base cider (made from 'Pink Lady' apples, fermented by EC1118 cider) to have 30 mL poured into an ISO/INAO clear glass for each panellist.

Formal sensory analysis occurred over three sessions where each panel member was presented with seven × 30 mL ciders per session, rating the intensity for all 13 attributes. The cider samples (30 mL) were served in 215 mL covered ISO/INAO clear glasses in an open-plan room, with all glasses presented at once. Panel members were placed at individual tables over 1.5 m apart and performed individual sensory analysis. Each session, a panel member analysed seven ciders made from 'Pink Lady' apples, as described above, each fermented by a different yeast strain. Each panellist was presented with a different randomised order of glasses. For each glass of cider, the panel rated the intensity of each attribute from 0 to 9 on an unstructured 9 cm line scale. Social science ethics approval for the collection of tasting data was obtained from the University of Tasmania's Social Sciences Human Research Ethics Committee (Ref No: H0018534).

2.4. Statistical Analysis

Chemistry results were analysed using SPSS (IBM SPSS Statistics Version 24) with a multivariate general linear model approach to determine differences between yeasts. Yeast strain and variety were considered as the main effects. A two-way analysis of variance in SPSS was used to determine significant differences between varieties for each of the analytical methods at $p \leq 0.05$. A Tukey's post hoc test was used to determine differences between groups of samples. JMP (ver. 14, SAS Institute, Cary, NC, USA) was used to determine difference between sensory analysis results using a two-way ANOVA with panellist and treatment as the main factors.

3. Results and Discussion

3.1. Base Cider Parameters

The basic physiochemical properties of both juice and cider samples including titratable acidity, pH, and total phenolic content are presented in Table 2. Apple variety had the greatest impact on cider chemical attributes across both seasons consistent with other studies reporting similar findings. Rosend et al. [17] investigated the effect of variety, maturity, and yeast strains on the volatile composition of apple cider and found that variety was the most influential attribute and any yeast effect was variety-specific. During a study comparing the effect of two commercial strains of *S. bayanus*, Bandić et al. [30] also found variety strongly affected chemical parameters (which included alcohol, sugar-free extract, reducing sugars, and titratable and volatile acidity). Another study found results to be variety-dependent when examining the effects of maceration and press fraction [31]. The TSS (°Brix) of the juice in 2018 was 11 for 'Pink Lady', 10.6 for 'Sturmer', and 10.8 for 'Bulmers Norman', while in 2019, the TSS was measured as 14 for 'Pink Lady', 14.2 for 'Sturmer', and 15.5 for 'Frequin Rouge'. In both seasons, no significant differences were observed between varieties for TSS.

Table 2. Mean TA (malic acid mg/mL), pH, and TPC (TPI) of initial juice (N = 1) samples and ciders for each variety as well as ciders fermented using different yeast strains (average of all three apple varieties) in 2018 and 2019. Cider results represent the average of all yeast strains combined. (* represents $p \leq 0.05$, ** is $p < 0.01$, and *** $p < 0.001$. Different letters denote significant differences between means at $p \leq 0.05$. No significant difference is denoted by "ns").

		TA		pH		TPC	
		2018	**2019**	**2018**	**2019**	**2018**	**2019**
Juice	Pink Lady	4.36	4.67	3.77	3.58	4.50	4.00
	Sturmer	6.76	6.90	3.65	3.45	9.00	9.50
	Bulmer's Norman	2.06		3.65		45.0	
	Frequin Rouge		2.24		4.06		71.0
Cider	Pink Lady	6.26	5.28	3.66 b	3.72	7.96 a	7.63 a
	Sturmer	7.67	7.11	3.59 a	3.54	12.45 b	14.79 b
	Bulmer's Norman	6.09		3.56 a		54.62 c	
	Frequin Rouge		4.33		3.88		128 c
	Significance			***		***	***
Yeast Strain	Wild	6.29	5.66	3.62 ab	3.72	23.48	49.96
	OKAY	6.55	5.47	3.63 b	3.72	26.03	49.24
	EC1118	6.53	5.78	3.60 ab	3.70	25.14	48.85
	BE Fruits	7.31	5.71	3.57 a	3.69	25.40	48.70
	Weiss		5.38		3.79		50.95
	Elegance		5.55		3.69		53.51
	UOA Maxithiol		5.58		3.73		52.18
	Significance			**		ns	ns
	Interaction (cider variety × yeast strain)	**	**	ns	***	ns	ns

3.1.1. Titratable Acidity (TA)

For TA (malic acid content), interactions were found between yeast strain and apple variety in both seasons. In 2018, a significant ($p < 0.01$) interaction was found between yeast strains (Lalvin ICV 'OKAY', Lalvin 'EC1118', IOC 'Be Fruits', and the wild ferment) and variety, which was heavily influenced by the TA results for cider made from 'Sturmer' apples (Figure 1). The 'Sturmer' apple cider had significantly greater TA compared to the other two varieties when fermented using the three commercial yeasts 'EC1118', 'OKAY', and BE 'Fruits' (Figure 1). When the same 'Sturmer' juice went through wild fermentation, the malic acid content was not significantly different to ciders produced from the other apple varieties. Differences in TA results between the wild ferment and the commercial yeasts, at least for this variety, are likely caused by the inability of the wild yeast to ferment comparably to the commercial yeasts. In contrast, the following season, the wild ferment produced a mid-range TA cider (Table 2). This finding demonstrates the unpredictable nature of using wild ferments, producing inconsistent flavours in comparison to *Saccharomyces* species, and indicates seasonal variation in results [7]. The changes for wild ferments between the two seasons also explain the possibility that the microorganisms responsible for the wild ferments in season one could be different to those in the second season, again alluding to the unpredictability of an uncontrolled wild ferment. 'Sturmer' ciders fermented using 'Weiss' and wild yeasts in 2019 had significantly higher TA results than those ciders made using 'Pink Lady' and 'Frequin Rouge' apples (Figure 2). This interaction is opposite to what was observed in 2018 (Figure 1), suggesting that apple variety and seasonality are strong drivers of variation in TA results during the fermentation process. The interaction between the yeast and variety is likely to be variety-driven; for example, if using Weiss yeast, cider made using 'Sturmer' apple juice may have more malic acid content than cider made using dessert or cider varieties. This understanding of how varieties may interact with different yeast strains to produce a range of organoleptic qualities could have a powerful and real impact on the decision making for cider makers [31].

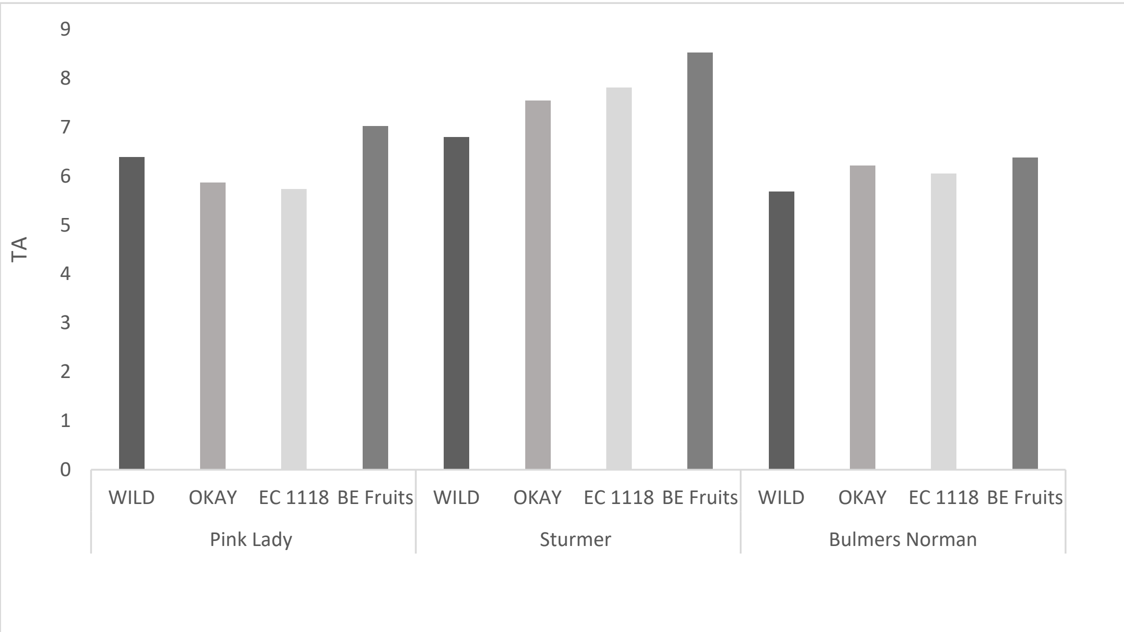

Figure 1. Titratable Acidity (TA) of cider made with each yeast strain for all three apple varieties in 2018.

It is clear that season to season variability can influence the flavour outcomes of the ciders produced. In contrast to 2018, the TA results for 2019 ciders showed no significant difference between the ciders fermented by different yeast strains (Table 1). Previous studies have reported comparable results. For example, Leguerinel et al. [19] used 13 strains of *S. uvarum* fermented with two different apple varieties and showed that the amount of malic acid was mainly dependent on the variety and TA was not significantly affected by yeast strain. Lachowicz et al. [32] showed similar findings using a red flesh apple variety to make cider with both *S. bayanus* and *S. cerevisiae*, where malic acid content was unaffected by the different yeast strains. Whilst the yeast used in this trial had a negligible influence on TA and no correlations were found between TA and sensory results (see below), perhaps trialling a yeast such as *S. pombe*, which has been found to reduce malic acid [33], may have had greater influence on sensory experience. It has been shown that reducing organic acids, particularly malic acid, leads to an increase in pH, which potentially could soften harsh attributes such as sourness and astringency [34,35].

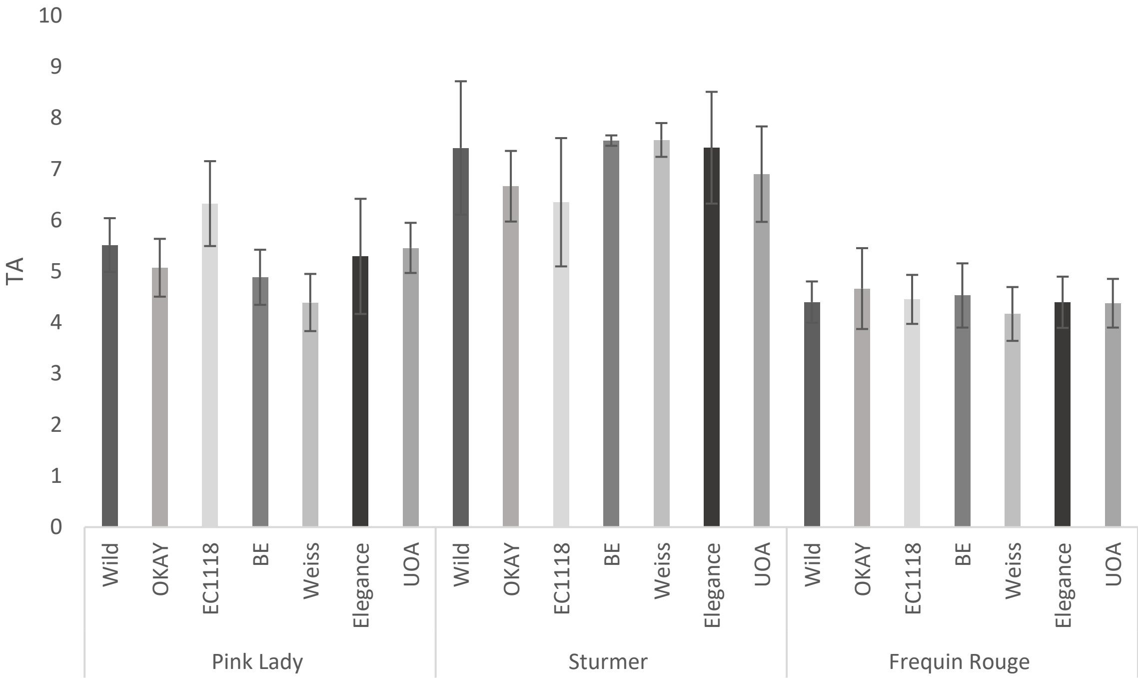

Figure 2. Titratable Acidity (TA) of cider made by each yeast strain for all three apple varieties in 2019.

3.1.2. pH

In 2018, a significant ($p < 0.001$) difference was found in the pH of ciders made with different yeast strains as a main effect, whilst in 2019, a significant ($p < 0.001$) interaction was found between apple variety and yeast strain (Table 2). In 2018, ciders fermented using the yeast 'BE Fruits' were significantly more acidic than those fermented by 'OKAY'. Whilst significant ($p < 0.001$), the difference in 2018 between pH measurements of ciders made from different yeasts was 0.06. This difference is unlikely to influence a preference for a particular yeast as pH is often adjusted during cider production if it is >3.8 to prevent microbiological spoilage [26]. In 2019, there was no significant difference between the yeast 'BE Fruits' and 'OKAY' for any variety. Instead, in 2019, the yeast 'Weiss' was the main driver of an interaction between yeast strains and apple variety. 'Weiss' yeast produced ciders with significantly greater pH than other yeast strains for both 'Pink Lady' and 'Frequin Rouge' ciders. However, regardless of the yeast strain used, there was no significant difference for the pH results for all ciders made using 'Sturmer' apples in 2019.

The influence that the yeast strain 'Weiss' had on the pH for both 'Pink Lady' and 'Frequin Rouge' ciders in 2019 may be due to this yeast being intended for brewing beer (Figure 3) (Table S1). It is unclear why there was not a similar effect for 'Sturmer'. Perhaps this is because the pH of 'Sturmer' was generally lower across ciders made using all yeasts compared to the other two varieties (Figure 3). This may indicate that regardless of yeast strain, the pH of cider made from the variety 'Sturmer' will be unaffected. This is a similar finding to a study using a red-flesh apple that found yeast strain did not significantly affect the pH value of cider [32]. The more complex results occurred for 'Frequin Rouge' ciders fermented using 'Elegance' and 'UOA' were different to wild, but for 'Pink Lady', wild and 'UOA' were different to 'BE Fruits'. These findings show how yeast selection can alter base cider properties whilst being variety-specific.

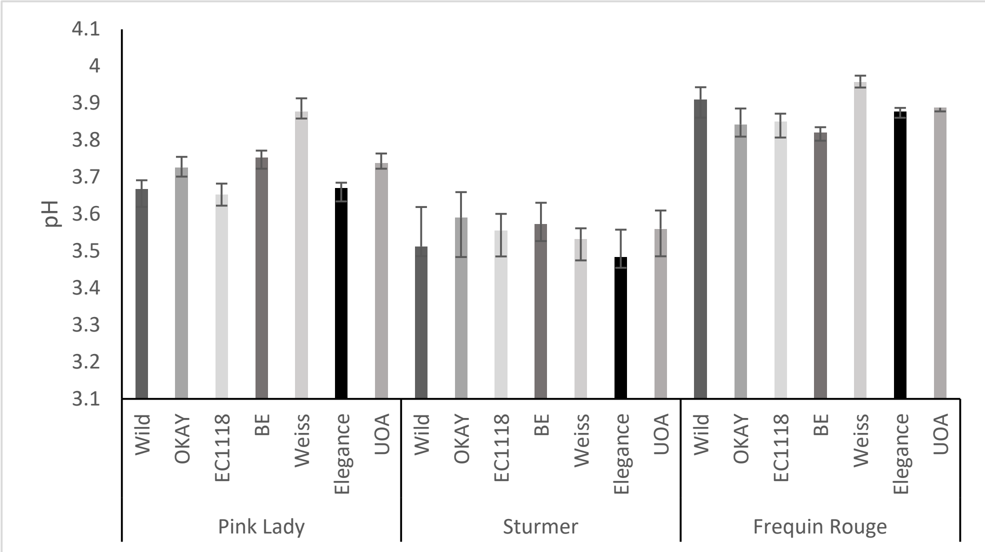

Figure 3. pH of cider made by each yeast strain in 2019, for all three apple varieties.

In 2019, for both juice and cider, 'Frequin Rouge' had the highest pH, followed by 'Pink Lady' and then 'Sturmer'; this trend was evident for all yeast strains. The difference was driven by varietal interactions with certain strains. For example, cider made with the variety 'Pink Lady' had a significantly greater pH when fermented using the 'Weiss' strain compared to when fermented using the 'Elegance' strain, but this interaction was not seen for other apple varieties. This may indicate to cider makers that yeast strain will not significantly alter the original range of the juice pH when looking at single varieties.

For yeast, the focus is on how the pH influences the fermentation conditions, rather than how the yeast influences the pH [11,36]. Although not statistically comparable, the pH results of the ciders fermented with different yeast strains had less variation in pH than between individual apple varieties across the two seasons. For example, 'Pink Lady' cider pH was 3.66 in 2018, then 3.72 in 2019 (Table 2). This is not uncommon as pH has been found to be influenced by season [37] and vary by variety [23,38]. Sadras et al. [37] found there was a complex, variety-specific interaction between seasonal effects, such as temperature and pH in juice.

3.1.3. Total Phenolic Content (TPC)

TPC is a measure of phenolic compounds which are secondary plant metabolites that influence sensorial properties such as bitterness astringency, mouthfeel, and also colour [23–25,39–41]. As a result, phenolic content is considered a key indicator of the quality of apple cider [21,42]. When ciders were analysed for TPC, it was found that yeast strain had no significant effect on the produced cider. Instead, the phenolic content of the cider was strongly influenced by apple variety (Table 2), particularly observed in the difference between dessert and cider varieties [20,25,31,34,41]. The limited influence of yeast strain on TPC is consistent with findings by Laaksonen et al. [25], who showed that yeast strain had less impact on the phenolic composition of apple ciders than the cider making technique, apple variety, or ripening stage. Yeast has been found to have an effect on phenolic content when using four non-*Saccharomyces* yeasts as both monocultures and mixed fermentations [12]. In this case, some of the non-*Saccharomyces* monocultures were able to produce significantly different phenolic contents in specific mixed-yeast fermentations [12].

The results in Table 2 show ciders fermented in 2018 had approximately half the TPC when compared to those fermented and grown in 2019, regardless of the yeast strain used. The juice and cider TPC for both 'Pink Lady' and 'Sturmer' remained unchanged across the seasons, so it is most likely the change in cider varieties, not seasonality, that increased the overall average TPC in the 2019 trial (Table 2). A greater range of TPC has been found to exist between cider varieties alone than between cider and dessert varieties [31]. This observation is repeated in this study, where the TPC results reported for 'Bulmer's Noman' cider in 2018 were 54.62, while the results for 'Frequin Rouge' in 2019 were 127.65 (Table 2).

3.2. Sensory Analysis

The results from the sensory descriptive analysis from 'Pink Lady' cider made in 2019 showed that yeast selection can have a significant ($p < 0.05$) effect on cider sensory attributes. Of the 13 attributes selected by the trained panel, 'fresh apple', 'earthy', and 'pear' were found to significantly differ between samples (Figure 1). The yeast strains varied in their influence across the three attributes. For example, 'wild' yeast was the only treatment where the attribute 'pear' was distinguished with statistical significance, while the attributes 'fresh apple' and 'earthy' were identified with statistical significance with the yeast strains: 'EC1118', 'OKAY', and 'UOA' (Figure 4). There was no trend between the three significant attributes, as they were found to differentiate between the yeast strains independently of each other. These results suggest that yeast strains affect the sensory properties of cider, albeit in a manner that is specific to the yeast strain. A potential reason the attribute 'pear' may have been differentiated in the wild fermented cider from the other yeasts is due to incomplete fermentation compared to the ciders using commercial yeast strains. The commercial strain, *S. cerevisiae*, is a characteristically strong and fast fermenter, able to tolerate sulfur and alcohol better than other strains [26]. Ciders that do not ferment through to 'completion' or 'dryness' contain a proportion of unfermented sugars that completed fermentations do not. These incomplete fermentations can result in more 'fruity', 'fresh', and 'sweet' flavours in the cider [43]. Conversely, ciders that have fermented through to dryness are known to be less 'sweet' and 'fruit' and more 'dry' or 'astringent'.

The panel successfully differentiated the ciders according to yeast strains for the attribute 'fresh apple'. 'Be Fruits', 'Weiss', and 'UOA MaxiThiol' are all expected to promote 'fruity esters'. When separated by significant ($p < 0.05$) differences for sensory results, 'Be Fruits' and 'Weiss' ranked high for 'fresh apple' and are denoted with an 'a', while 'UOA MaxiThiol' ranked the poorest and is denoted 'c', which suggests vast differences between the two 'a' attributes (see Figure 4). While 'UOA MaxiThiol' was expected to promote 'fruity esters', it is likely such esters are associated with more tropical-fruit attributes rather than apple. Different yeast strains produce different concentrations and types of esters, and 'fresh apple' aroma compounds are often associated with acetates [44]. Lorenzini et al. [7] found acetic acid content to vary between yeast strains and Riekstina-Dolge et al. [44] found acetic acids to be characteristic by-product compounds of yeasts.

The panel determined that ciders fermented by 'UOA Maxithiol' and 'OKAY' had significantly more intense 'earthy' aromas when compared to ciders fermented by 'Weiss', 'Elegance', and wild yeast. The attribute 'earthy' has been used in wine sensory studies [45–48] as well as those relating sensory aspects to yeast strain [36]. Gürbüz et al. [49] suggested high 'earthy' values relate to either more oak contact time or greater skin contact time in California Cabernet, neither of which can be directly translated to a cider context. Darriet et al. [50] found through gas chromatography–mass spectrometry that the compound geosmin (trans-1,10-dimethyl-trans-9-decalol) is responsible for the 'earthy' aroma, and suggested it is derived from microorganisms that develop on the grape. One commercial yeast strain may not always create a greater quality cider than another, but the strain chosen may help to enhance certain aspects or characteristics that are desired by the cider maker or consumer.

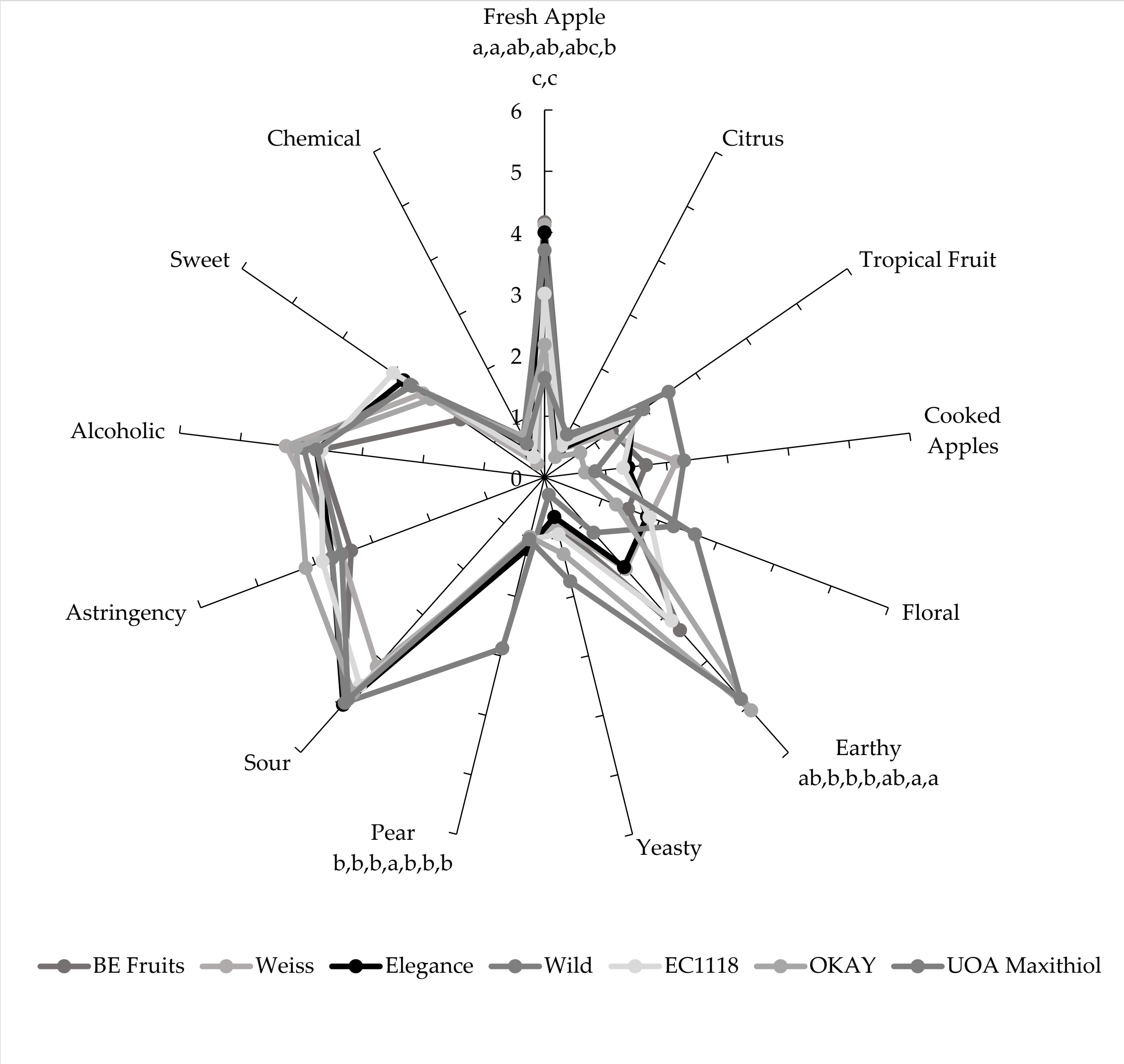

Figure 4. Sensory profiles of 'Pink Lady' ciders made using 7 different yeast strains shown for 'BE Fruits', 'Weiss', 'Elegance', Wild, 'EC1118', 'OKAY'. And 'UOA Maxithiol' yeasts, respectively). Lletters under the attributes 'fresh apple', 'pear', and 'earthy' indicate a statistically significant difference at $p < 0.001$.

The results suggest that although the panel were able to detect differences between the ciders made with different yeasts, the chemical analyses were possibly not testing the chemical compounds responsible for the differences perceived. For example, TPC is strongly linked to astringency [25,26,51], yet this study found no relationship between the sensory property 'astringency' or TPC relating to the different yeast strains used. Riekstina-Dolge et al. [24] showed that variation in cider sensory properties was dependent on apple physiochemical composition in a study looking at the TPC and sensory evaluation of different apple varieties for cider. However, the correlations made between sensory results and physiochemical properties in that study were reliant on the composition of phenolic compounds rather than the total phenolic content [41]. As there was no overlap between the basic physiochemical cider properties measured, and the attributes were found to significantly differ between yeast strains, it is likely the difference in sensory perception was driven by properties not measured during this study, such as volatile compounds [12,29,36,52,53].

4. Conclusions

Improving the understanding of the impact of individual yeast strains on base cider characteristics when using different apple varieties may lead to cider makers choosing specific yeast strains depending on the apple varieties available or preferred. The yeast strain can clearly influence the flavour profile of cider; however, its influence on the flavour profile will differ and depend on the properties being analysed, as shown in this study. We showed that base cider attributes are strongly influenced by variety, and less so yeast strains, yet also are subject to seasonal influences. There was limited overlap between the findings of the base cider characteristics measured analytically and the attributes observed by the panel in this study. Further research focusing on the link between other base cider characteristics not studied here (such as volatiles or specific phenolic compounds) and sensory properties may still find relationships existing between yeast strains and apple varieties, as occurred within the treatments used in this study.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8090455/s1, Table S1: Condensed description and technical information of the commercial yeast strains used.

Author Contributions: Conceptualization and methodology of this research were carried out by M.L.W., J.E.J. and N.D.S.; formal analysis, investigation, and data curations by M.L.W. and R.L.; writing—original draft preparation by M.L.W.; writing—review and editing was a joint effort between M.L.W., J.E.J., R.L., R.G.D. and N.D.S. The research was supervised by J.E.J. and N.D.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Not applicable.

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