# Characteristics and fermentability of cider apple cultivars grown in Sweden

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## Abstract

In countries with a long apple cider-making tradition, such as United Kingdom or France, cider is traditionally made from a blend of special cider apple cultivars, that for example, by its content of phenolic compounds give the cider a desired bitter taste. There is a growing interest among cider producers in cultivating cider apple cultivars in Sweden, in order to produce a wider range of ciders than is possible using the table apple cultivars that are typically grown. The aim of this study is therefore to investigate how British and French cider apple cultivars perform when grown in Sweden, based on chemical traits of both the apple and cider. A further objective was to increase the understanding of apple juice fermentability and how it is affected by different juice traits by monitoring the progress of fermentation. The results showed, for example, that the included cultivars differed in fermentability, possibly influenced by nitrogen, and indicated good fruit quality, based on the investigated parameters, in French and British cider cultivars when grown in Sweden. Classification of most cultivars were, in this study, consistent with traditional classification of the cultivars according to Long Ashton Research Station's classification system.

*Keywords:* Apple, Malus domestica, juice, cider, fermentation, fermentability, traits, characteristics, YAN, FAN, phenolic content, soluble solids, acidity

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# Abbreviations

| | |
|---|---|
| DAP | Diammonium phosphate |
| FAN | Free amino nitrogen |
| LARS | Long Ashton Research Station |
| SG | Specific gravity |
| SSC | Souluble solids content |
| TA | Titratable acidity |
| TPH | Total phenolic content |
| YAN | Yeast assimilable nitrogen |

# 1. Introduction

In countries with a long apple cider making-history cider is traditionally produced using a blend of special cider apple cultivars, with different characteristics of its juice and cider compared to the table apple cultivars usually grown in Sweden. The traits affects the fermentability of the juice and the quality of the finished cider. Some of the cider cultivars used in traditional cider blends have a higher content of phenolic compounds that gives the cider its characteristic bitter taste. Along with the growing interest for production of craft apple cider in Sweden, there is an interest of introducing more bitter cultivars, for example British or French cider apple cultivars, to enable production of a wider range of types of ciders.

Since there is a lack of tradition of growing special cider apple cultivars in Sweden there is also a lack of knowledge on how the cultivars perform in the Swedish climate. Apple juice and cider traits can show annual differences within the same cultivar (Bourvellec et al. 2015) and it is therefore likely that the traits are dependent on the regional climate. Against this background a trial of British and French cider apple cultivars was established at SLU's former research station Balsgård in 2014 and later expanded in 2020. The traits of the juice from some of the cultivars as well as susceptibility to blue mould, caused by *Penicillium expansum*, have previously been evaluated (Spoor et al. 2019). This study aimed to continue the evaluation of these cultivars.

An aspect that has not been covered in the former studies from Balsgård, and for which data also appears to be lacking internationally, is the concentration of yeast assimilable nitrogen, *YAN*, in apple juice. YAN is a crucial parameter in cider production since it affects the fermentability and the quality of the final product. For example, intentionally having low levels of YAN are important for the production of naturally sparkling cider with residual sweetness, a traditional practice in French cider making (Nogueira et al. 2008). However, it may also cause quality issues, and nitrogenous supplements are often recommended and used in cider production.

With this background, the traits of apple juice and cider from cider apple cultivars grown in Sweden will be investigated, with the content of YAN in the juice and fermentability as novelties.

## 1.1 Background

### 1.1.1 Cider apple cultivars

The term *cider apple cultivar* is commonly used to refer to cultivars used for cider production, however, there is no strict definition of what qualifies as a cider apple cultivar. It has been suggested that there may be a genetic differentiation between cider apples and table fruit, with cider apples showing greater ancestry from *Malus sylvestris*; however, the results have been inconsistent (Migicovsky et al. 2016). One definition is that cider cultivars are cultivars "selected and cultivated primary for cider making use" (Copas et al. 2002). Traditionally, according to Soomro et al. (2022) the cultivars seen as cider apples are generally smaller and more tannic than table fruit while table fruit is suitable for fresh consumption and generally the taste

is perceived as sweeter. Lea (2015) states that the “true” cider apple cultivars, except for the taste, differentiates itself by its fibrous structures that enables easier pressing than table fruit. Even if cider apples is a diverse group, in groupwise comparison of French cider apple cultivars, they were proven to be more phenolic than table fruit (Soomro et al. 2022). However, considering that cider is usually made from a blend of cultivars with different traits makes generalizations harder (Soomro et al. 2022).

There are several characterization systems for cider apple cultivars. One of the most commonly used characterization systems is the British classification system (Table 1) developed at Long Ashton Research Station (LARS) that classifies cultivars, based on content of malic acid and tannins, as sharp, sweet, bittersweet and bittersharp (Karl et al. 2023). To make a balanced cider a blend of different types of cultivars is typically used.

*Table 1: The Brittish classification system from LARS based on concentrations of tannins and malic acid (Karl et al. 2023)*

| Classification | Tannins (g $L^{-1}$) | Malic acid (g $L^{-1}$) |
|---|---|---|
| Sharp | <2 | >4.5 |
| Sweet | <2 | <4.5 |
| Bittersweet | >2 | <4.5 |
| Bittersharp | >2 | >4.5 |

Many of the traditional cider cultivars have occurred as chance seedlings, or been found as wild seedlings and have a more or less long history of cultivation in the region of its origin. For example ‘Dabinett’ and ‘Yarlington Mill’, two popular British cultivars, were found as chance seedlings in Somerset (Jolicoeur 2022). However, some cider cultivars are the result of more recent breeding programs. For example, in 2007 a series of cultivars commonly referred to as *The Girls* was released from LARS (Harper et al. 2020). The background of the development of the cultivars was the need for the cider production industry of early maturing cultivars, namely the industry faced processing problems with a large part of cider cultivars matured in October (Harper et al. 2020). A breeding programme was therefore initiated in the 1980’s (Copas et al. 2002). Except for early maturing the aim was to develop cultivars with regular cropping, good sized and bittersweet fruits with an easily managed tree shape (Harper et al. 2020). The parents in the breeding programme resulting in The Girls series were ‘Dabinett’ or ‘Michelin’ as female parents and ‘James Grieves’ or ‘Worcester Pearmain’ as male parents (Harper et al. 2020). An example of a French cultivar released from a breeding programme is ‘Judeline’, released in the 1980’s from the breeding programme of INRA Fruit Tree Station at Angers for processing and table fruit (Boré 1994). ‘Judeline’ is a sharp cultivar with high juice yield, used both for cider and juice production.

### 1.1.2 Balsgård trial

To determine whether English and French cider cultivars are suitable for commercial orchards in Sweden an observation trial was set up at Balsgård in 2014 (Spoor et al. 2019). The traits of the juice from some of the cultivars as well as susceptibility to blue mould, caused by *Penicillium expansum*, was evaluated by (Spoor et al. 2019). The cultivars included in the study were found to ripen

somewhat later compared to when grown in their countries of origin, but they generally demonstrated the ability to achieve adequate fruit quality under Swedish climatic conditions. Most cultivars also corresponded to the same categories in the LARS classification system as they traditionally do. Overall, cider cultivars had higher content of *souluble solids* (SSC) and *total phenolic content* (TPH) but lower *titratable acidity* (TA) compared to the table cultivars included in the study. A concerning discovery was the unexpectedly high susceptibility to blue mould of the cider cultivars. However, the possibility that this result was influenced by the shared genetic background of the cultivars should be considered, as all those tested for susceptibility to blue mould belonged to the The Girls series of Long Ashton cultivars and shared 'James Grieve' as a common parent. The Balsgård trial was expanded in 2020 with additional cider apple cultivars some of which were included in the present study.

### 1.1.3 Cider production, market and styles

The term cider covers a range of beverages in variating in accordance to national legislation, local regulations and traditions. One attempt to define cider would be "a beverage made from fermented apple juice" even though regulations of alcohol content, minimum juice content etc. varies between countries. Jolicoeur (2022) captures the complexity in the cider market:

> In one extreme of this range, we find small producers who grow their own apples, juice them, and make cider from the natural fermentation of that juice with no other addition. On the other extreme, we find large multinational industrial facilities that produce alcopop-type cider from water, sugar, glucose syrup, imported apple juice concentrate, and chemical flavouring and additives, but often, no fresh apples at all. (Jolicoeur 2022:3)

Jolicoeur (2022) further argues that it is problematic that the two, in his opinion, different products that he calls "real cider" and "alcopop-type cider" can be marketed using the same epithet. He argues that this development has led to a desire from small-scale cider makers to differentiate their product from the industrial ciders using the following terms: *Farm- Traditional-, Real-* or *Craft Cider*. The terms covers different aspects of cider making, shortly: Farm Cider refers to cider produced at the same farm as the apples are grown; Traditional Cider follows the regional traditions mainly in choice of cultivars, style and production methods; Real Cider cover cider fermented from freshly made juice with minimal additives and Craft Cider may include all of the aspects above or simply be seen as the opposite of industrial cider. Svenska Ciderfrämjandet defines craft cider as a beverage composed of at least 95% apples or pears, produced without the addition of water or sugar, except when required for bottle fermentation, with all alcohol derived exclusively from the natural fermentation process (Svenska Ciderfrämjandet u.å.).

There are many different methods and traditions used for cider production: Different countries and regions traditionally follow processes with more or less additives (Al Daccache et al. 2020). However some fundamental elements of the cider making process are: harvesting, washing & storing, mashing, settling, fermenting, aging and bottling.

*Production methods, traditions and regulations of some important cider producing countries*

In Europe, the main cider producing countries are Great Britain, France, Spain and Germany (Coton et al. 2016). The following section provides a brief overview of production methods, traditions and regulations in some of the important cider producing countries.

French cider differentiates from cider from others by its low acidity together with high residual sugar and tannins (Jolicoeur 2022) and by using a process with less additives than British cider (Al Daccache et al. 2020). According to French regulations cider has to be made from 100% juice (Jolicoeur 2022), however some of the juice may be obtained from concentrate (Légifrance see Rosend et al. 2020). There is a general sensory difference between French industrial cider, that is described as having fruity and cooked odours, and French small scale produced cider, that is rather described with terms associated to astringency and bitterness (Le Quéré et al. 2006). The French production methods includes several practices that aims to induce a slow fermentation to favour a fruity aroma (Nogueira et al. 2008).

British cider, generally containing less residual sugar and usually have a higher alcoholic content than French cider (Al Daccache et al. 2020). According to British legislation, cider must be produced through the fermentation of apple or pear juice and may not exceed an alcohol content of 8.5% (Jolicoeur 2022). Addition of certain permitted substances is allowed. The juice content must be at least 35% both in the pre fermentation mixture and in the final cider. Some examples of permitted substances are water, sugars, carbon dioxide, certain preservatives and acids. A few large companies dominates the British cider market with industrial ciders, however, according to Lea (2015), hundreds of craft cider producers within the country creates a diversity in the cider market. According to Lea (2015), a common practice for the Brittish large industrial producers is to use low content of juice and sometimes concentrate from table fruit imported from e.g. China, as well as fermenting to high alcohol content using glucose syrup and then diluting the beverages with water – a process called chaptalization. This, he argues, is commercially meaningful but does not create quality cider such as craft cider produced from mainly raw apple juice. The consumer organization Campaign for Real Ale, CAMRA, use the term “real cider” to separate cider made fully from apples from the ciders made using concentrate or chaptalized juices (CAMRA u.å.).

In the United States cider, commonly referred to as “hard cider”, once was a widely consumed beverage that to a large extent disappeared after the Prohibition. The hard cider has been rediscovered and cider production and consumption has largely increased during the past 20 years, especially within the younger generations (Miles et al. 2020). Miles et al. (2020) suggest that the increasing demand could motivate and enable apple producers to focus on production of apples for cider production, e.g in a shift to more cultivation of cider apple cultivars.

However, American consumers have thus far been satisfied with ciders primarily made from table fruit cultivars. Additionally, there is a need to forecast the demand, as the long term investments is needed to change the production. United States Association of Cider Makers (u.å.) makes a difference between *modern cider*, premilary made from table fruit, and *heritage cider*, premilary made from cider cultivars, generelly with higher tannins than modern cider. An interest in growing more apples for cider production among the american growers has been showed in interviews by Ostrom et al. (2022), however some obstacles are the lack of knowledge in regional suitability of cider cultivars and pest management as well as uncertinty of the producers willingness to pay for the apples.

The use of some amount of concentrate in cider is allowed in all of Europe and is used due to economical advantages. This practice results in chemically different starting material for fermentation compared to the fermentation of pure apple juice (Rosend et al. 2020). The effects of the finished cider from usage of concentrate is not well studied, however Rosend et al. (2020) reported that fermentation of concentrate overall can be concidered efficent and can result in equal kinetics and quality of the finished cider as fermentation of fresh apple juice. However the fermentation might need additional nutritional supplements to compensate losses from the concentration process to be successful Rosend et al. (2020).

*Production methods, traditions and regulations of Sweden*

The regulations from the Swedish National Food Agency's states that cider is a fermented beverage made from juice of apple or pear with all alcohol derived exclusively from the natural fermentation process. (LIVSFS 2005:11). However, the regulations allow addition of non-fermented juice of apple or pear, sugar or water, and the content of fruit juice only needs to be 15% of the finished product. Some larger Swedish producers of the type of cider Jolicoeur (2022) would refer to as “alcopop-type cider” have created and branded their cider style as *Swedish cider*, Sweden's Brewers' Association reports that 75% of the industry cider produced in Sweden is exported (Sveriges Bryggerier u.å.). There are ongoing efforts to differentiate large scale cider production from craft cider production and a proposal for a EU definition of cider is presently being discussed.

### 1.1.4 Important juice and cider traits

A combination of several traits of the finished cider gives the cider its taste. The perceived taste depends of complex chemical and sensorial interactions. Determining e.g. the perceived dryness of a cider from its chemical composition can be challenging (Picchi et al. 2023). An example of a factors affecting the perceived taste is ethanol content. High ethanol content can increase the perceived bitterness at the same time as it decreases the astringency, and high sugar content can decrease the perceived bitterness (Lea & Arnold 1978).

However, the chemical composition is not only important for cider taste. A proper level of acidity is e.g. important to avoid microbial spoilage, and the sugar content determines the potential alcohol content of the finished cider. The biochemical composition of the juice and cider depends of several factors with cultivar as one of the major factor, but maturity, season, climate, and management practices are also important (Al Daccache et al. 2020) VanderWeide et al. (2022)

reports that chemical traits of cultivars show annual variation and some cultivars may alternate between cider apple classes between seasons. Cultivars with high phenolic and/or malic acid content show greater variation between seasons than others and is suggested suitable for production of vintage quality ciders (VanderWeide et al. 2022). Considering phenolic content, the cultivar seems to have greater influence than season (Anastasiadi et al. 2017; Marks et al. 2007 see VanderWeide et al. 2022).

*Fermentable sugars*

The fermentable sugars provide substrate for the yeast. They are therefore crucial for cider production, and will determine the potential ethanol content (Karl et al. 2023). Residual sugar, either leftover from the fermentation or added after completed fermentation, add sweetness to the finished cider (Karl et al. 2023).

The fermentable sugars in apple are glucose and fructose, the main monosaccharides and sucrose, the main disaccharide (Al Daccache et al. 2020). However sucrose needs to be broken down into glucose and fructose to become fermentable. In apple juice fructose accounts for up to 70% of the total fermentable sugar content (Wang et al. 2004). The total sugar content is commonly quantified as SSC, reported as Brix° in fresh juice or *specific gravity* (SG) along with alcoholic fermentation. It is also possible to analyze specific sugars through HPLC or enzymatic tests were enzymes transforms the sugars to NADPH of which the amount can be measured trough absorbance. Previous data from the Balsgård trial reported SSC levels ranging from 9.8 to 16.0 Brix° with a mean value of 13.7 Brix° in juices, with higher levels of juices from cider cultivars than of table fruit cultivars (Spoor et al. 2019). Plotkowski and Cline (2021) reported SSC levels ranging from 10.6 and 18.3 Brix° from a trial with 28 cider cultivars.

*Acidity and total acidity*

Acidity and total acidity are crucial in cidermaking for two reasons: To achieve the perceived taste and to prevent microbial spoilage (Lea & Drilleau, 2003 see Karl et al. 2023). A pH of maximum 3.8 of the juice is usually recommended to reduce the risk for microbial spoilage during fermentation (Karl et al. 2023). Malic acid is the main organic acid in apples (Al Daccache et al. 2020). As fruit ripens during storage, the level of organic acids typically decreases because ethylene triggers their metabolism for respiration (Defilippi et al. 2004).

Total acidity in apple juice is commonly for ease quantified as TA and acidity as pH. TA content is usually reported as malic acid equivalents. Previous data from the Balsgård trial show TA of 8.1 on average in table fruit and 5.6 g malic acid equivalents $L^{-1}$ on average in cider cultivars. Plotkowski and Cline (2021) reported TA values ranging from 3.1 to 19.1 g malic acid equivalents $L^{-1}$ and pH ranging from 2.88 to 4.76.

*Phenolic content*

Polyphenols, that have been associated with health benefits of consumption of apples e.g. due to antioxidant properties, may in cider primarily contribute to taste, color and perceived texture (Karl et al. 2023). Typically polyphenols add bitterness and astringency to the cider.

Polyphenols is a complex group of substances, synthesized by plants and commonly conjugated to organic acids or sugars (Crozier et al. 2009). The basic structure of polyphenols consist of at least one aromatic ring with one or more hydroxyl group attached (Crozier et al. 2009). The polyphenols occurring in plant tissues are commonly classified into two groups: flavonoids and the non-flavonoids (Crozier et al. 2009), the basic structure of some of the subclasses are found in Figure 1. Flavonoids consist of two aromatic rings connected by a three carbon bridge and include important classes such as flavan-3-ols and anthocyanidins (Feng et al. 2021), e.g. polymers of flavan-3-ols can contribute with desired bitter taste and astringent mouthfeel and high levels of anthocyanidins in the flesh can be utilized to make rosé cider (Karl et al. 2023). Principal non-flavonoids in apples include phenolic acids, such as gallic acid (Feng et al. 2021). Polyphenolic composition and concentrations varies between tissues and cultivars of apples (Feng et al. 2021; Spoor et al. 2019). As previously stated, the bitter cider cultivars generally contains higher levels of polyphenols than other apple cultivars.

In association to cider or wine, tannins may be a more frequently used term than polyphenols. Tannins are a subgroup of phenolics that is a subgroup of polyphenols. Traditionally analysis of tannin content has been made by titration with potassium permanganate (Spoor et al. 2019). However, a useful contemporary method is Folin-Ciocalteu chromatography, measuring the total phenolic content, that can be performed in micro plates as a high throughput method (Spoor et al. 2019). The Folin-Ciocalteu method generates results that are reported as gallic acid equivalents. The results from the two methods have been shown to give almost identical levels in apple juice and cider and the results of TPH can therefore be compared to historical data of tannin content (Spoor et al. 2019). Measurement of individual phenolic compounds is possible by HPLC.

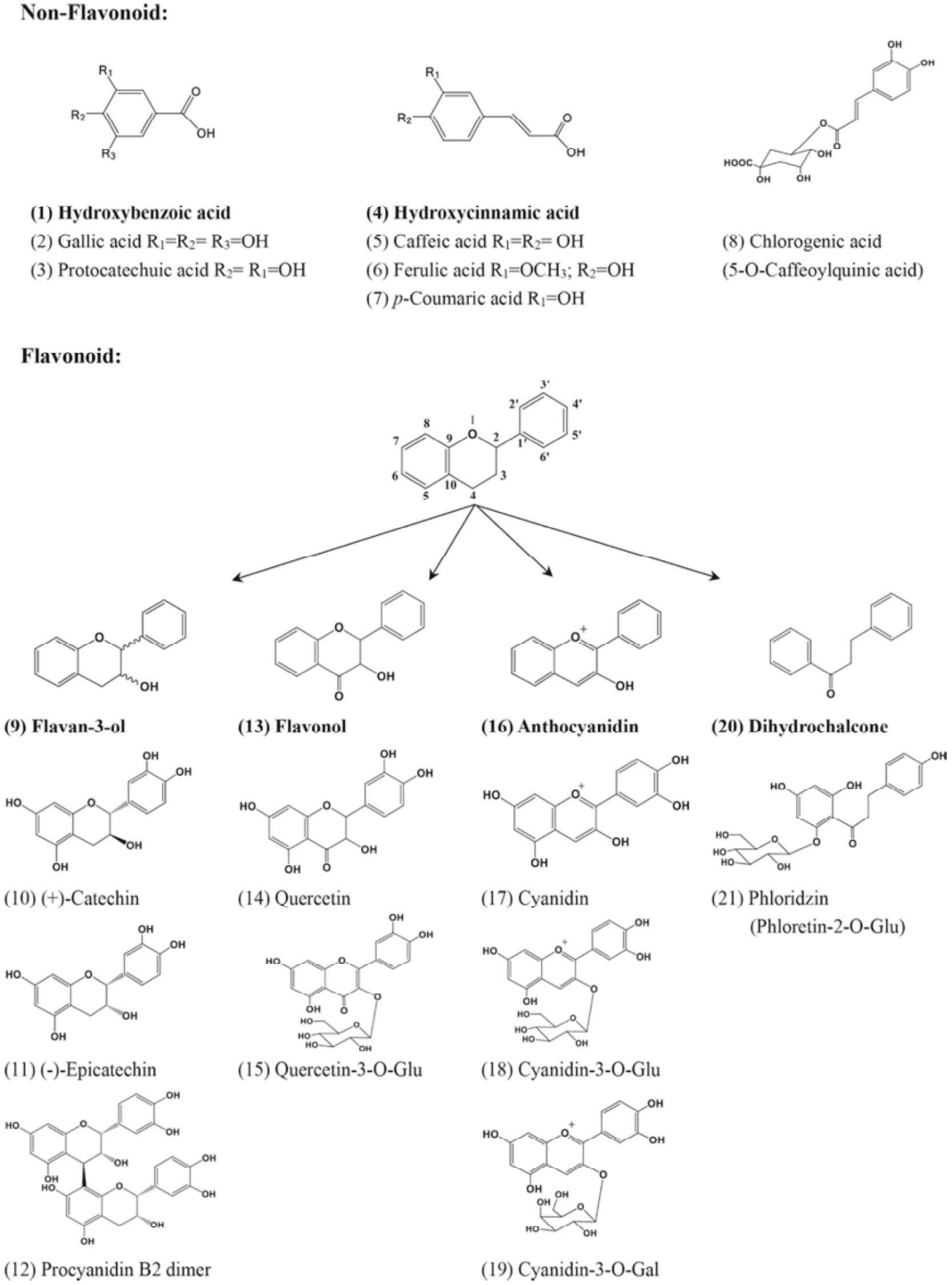


*Figure 1: Chemical structure of some of the polyphenols found in apple (Feng et al. 2021).*

*Content of yeast assimilable nitrogen*

YAN is essential for yeast growth and metabolism during fermentation. YAN consist of two components: Ammonium ions and *free amino nitrogen* (FAN). YAN is therefore estimated by separete mesurements of its components. In average FAN comprised 85% of YAN in a study including 12 cultivars (Boudreau IV et al. 2018).

There are indicatians of a correlation between FAN and YAN, and it is suggested that it may be possible to estimate YAN correctly only measuring FAN (Boudreau IV et al. 2018).

YAN is well studied in grape juice and wine but yet there is limited information of YAN concentrations in apple juice (Boudreau IV et al. 2018). In wine deficit tends to lead to slow or incomplete fermentations (Bisson, 1999; Blateyron & Sablayrolles, 2001; Ingledew & Kunkee, 1985 see Boudreau IV et al. 2018). Deficiency is also associated with defects in the cider from production of hydrogen sulfide ($H_2S$). Reported levels of YAN in apple juice are e.g. 20 to 138 mg N $L^{-1}$ (Valois et al. 2006), 9 and 249 mg N $L^{-1}$ with a mean value of 59 (Boudreau IV et al. 2018) and 60 and 256 mg N $L^{-1}$ (Plotkowski & Cline 2021). There are no well-established target concentrations of YAN for apple juice or guidelines for nitrogen fertilization, one of the efficient means for increasing the YAN content, for cultivation of apples for cider production (Karl et al. 2023). In comparison concentrations of 200–350 mg $L^{-1}$ is recommended for successful fermentation in wine (Karl et al. 2023). Karl et al. (2023) concludes that concerning recent findings apple juice often seems to be deficent in YAN for fermentation using wine standards, however, Boudreau IV et al. (2018) suggest that, since apple juice has lower soluble solid content (SSC) than grape juice, the requirements of YAN for cider production could be lower. Target concentrations for cider is suggested to depend of a number of factors such as fermentation conditions, yeast strains, raw material and aimed cider type (Karl et al. 2023). Concentrations of YAN in cider apples have shown significant cultivar and seasonal variation (Boudreau IV et al. 2018).

Adding *diammonium phosphate* (DAP) is a common practice to rise the YAN levels (Cline & Plotkowski 2021). Song et al. (2020) reports that in general lower YAN concentrations led to higher hydrogen sulfide ($H_2S$) concentrations, however DAP supplements did not always give the desired outcome: Moderate DAP supplements to juices with low YAN content was associated to increased $H_2S$ production. Song et al. suggest that further research of the mechanisms of $H_2S$ production is needed given its importance for cider producers.

*Juice yield*

Juice yield depends primarily of cultivar, maturity and design of the press (Wilczyński et al. 2019). Wilczyński et al. (2019) reported juice yields in the range of 61.9 to 71.6 % in a study comparing the efficacy of a screw press and a basket press using three table apple cultivars. The screw press over all showed higher efficiency, this was suggested to be to the wider openings of the cell membranes caused by the method. (Lea 2015) suggests that a good juice yield is around 75 %, even though the juice yield depends on the method and scale of processing. Except for being dependent of cultivar and processing method, the juice yield is also affected by storage duration (Lahaye et al. 2023). The juice yield can be improved by heat treatment or by enzymes, however heat treatment does not only affect the juice yield but also the traits of the juice such as increasing the content TPH and SSC content (Gerard & Roberts 2004) and usually affects aroma. Gerard & Roberts (2004) suggested that heating the mash to 60 °C was the optimal treatement to increase juice yield and quality.

### 1.1.5 Fermentation

Alcoholic fermentation is an anaerobic metabolic process where sugars are transformed into ethanol and carbon dioxide by microorganisms. The process is affected by several parameters such as yeast and the qualities of the apple juice (Al Daccache et al. 2020). There can be many different yeast species present during fermentation, but the yeast genus *Saccharomyces* seems to be dominating in spontaneous fermentation (Al Daccache et al. 2020). *Saccharomyces cerevisiae* may be considered as the most important microbe for human civilization due to its role in alcoholic fermentation. Except for fermenting beverages, *Saccharomyces cerevisiae* also functions as baking yeast as well as it is used for biofuel production (Money, 2018 see Walker & Walker 2018). Other than yeasts bacteria can be present during cider fermentation, for example malolactic fermentation sometimes takes place (Al Daccache et al. 2020).

Yeast fermentation convert glucose to ethanol and carbon dioxide in two steps, a way for the microorganism to release energy in anaerobe conditions (Walker & Walker 2018): In the first step, called glycolysis, glucose is converted to pyruvate together with the release of 2 ATP. In the second step is pyruvate first converted to acetaldehyde and carbon dioxide and then the acetaldehyde is reduced to ethanol. In total 12 enzymes are involved in catalyzing the reactions. The reaction can be concluded as $C_6H_{12}O_6 \rightarrow 2C_2H_5OH+2CO_2$, but is described more in detail as follows (Walker & Walker 2018):

Conversion of glucose to pyruvate:
$C_6H_{12}O_6 \rightarrow 2C_3H_4O_3+2ATP+2NADH$
Conversion of pyruvate to acetaldehyde and carbon dioxide:
$2C_3H_4O_3 \rightarrow 2C_2H_4O+2CO_2$
Reduction of acetaldehyde to ethanol:
$2C_2H_4O+2NADH+2H^+ \rightarrow 2C_2H_5OH+2NAD^+$

In order to convert glucose to ethanol and carbon dioxide the yeast needs proper nutrients such as fermentable carbohydrates, nitrogen, vitamins and minerals (Walker & Walker 2018). In cider fermentation fermentable carbohydrates mainly are glucose and fructose, even though fructose needs to be converted to fermentable forms first. However, *Saccharomyces* appears to be glucophillic and a high ratio of fructose to glucose content can cause problems with unwanted residual sweetness in wine production (Berthels et al. 2004). Fructose utilization also appears to be more sensitive to high ethanol content which also may be a problem in wine production (Berthels et al. 2004). The difference in efficiency of fructose and glucose utilization may change during the fermentation process, which may depend on differences in sensitivity to ethanol stress, between *Saccharomyces* strains and by nitrogen content. Namely fructose utilization showed to be stimulated by nitrogen supplementation to a larger extent than glucose utilization. In terms of minerals magnesium and zinc are considered particularly important due to its important roles in enzymatic functions (Walker & Walker 2018). Contradictory, even though alcoholic fermentation is an anaerobic process, oxygen is essential for efficient fermentation: Proper oxygenation before onset of the fermentation makes the yeast cells more fermentatively active and stress tolerant (Walker & Walker 2018). Vitamins are involved in many of the yeast metabolic pathways including

processes affecting the taste, and certain levels are therefore needed for efficient fermentation and sufficient quality of the product (Evers et al. 2021). E.g. deficiency in pantothenic acid can lead to accumulation of $H_2S$ (Hosono et al., 1972 see (Evers et al. 2021) that is, as earlier mentioned, sought to be avoided in production of alcoholic beverages.

The progress of fermentation is, as explained above, affected by many factors, i.e. it has been known for a long time that YAN is a limiting factor in fermentation of apple juice as well as that the maturity of the fruit and cultivar affects the fermentation rate (Barker 1908). It is however a common traditional practice to use biomass reducing techniques to reduce the fermentation rate, an example of that the urge is not always to give the yeast optimal conditions for fast growth. The two main methods to do this are keeving and biomass reduction by settling, or more contemporary filtration or centrifuging, followed by racking (Nogueira et al. 2008). Keeving is a process of clarification done before the start of fermentation while the measurements can be done once or repeatedly during the fermentation process.

The fermentation changes the chemical content of the juice or later cider, e.g. concentrations of polyphenols and organic acids (Ye et al. 2014). Malic acid may increase during fermentation and lactic acid can be synthesized.

## 1.2 Aim & research questions

The overall aim of this study was to generate knowledge relevant to Swedish cider producers regarding characteristics of juice and cider derived from British and French cider cultivars grown in the Swedish climate by investigating some essential traits of the juice before fermentation and the cider after fermentation. The aim was also to increase the knowledge of fermentability of apple juice and how it is affected by different traits, with a special focus of YAN. The research questions was: “How do British and French cider apple cultivars perform, in terms of essential juice and cider traits important for cider production, when grown in the Swedish climate?” and “How is the fermentability affected by YAN and other traits of the juice?” The hypothesizes were, given the earlier results from Balsgård, that the characteristics of the juice and cider will somewhat be affected by the Swedish climate but not to the extent that the cultivars are reclassified into new categories compared to traditional categorization and that that the YAN content in the apple juices will be a limiting factor for the fermentation.

# 2. Material & methods

18 apple cultivars were evaluated in biological triplicates, thus the study comprised in total 54 juice and 54 cider samples. After reduction of the natural microflora in the juice, and adding a selected yeast strain, the aim was to let the fermentation carry out until it stopped spontaneously. Juice traits, fermentation progress and cider traits were evaluated.

## 2.1 Cultivars, harvest and fruit quality

18 apple cultivars were selected to be included in the trial (Table 2). 15 of those were cider cultivars and one a table cultivar all trees grown in the trial at SLU Balsgård research station, Fjälkestad, Kristianstad in eastern Scania. Two cultivars were obtained from a commercial orchard, Solnäs, Fjelie, in southwestern Scania. The distance between the sites were approximately 100 km. The cider cultivars included originated from either France or UK.

The trees grown at Balsgård were grafted on different rootstocks, planted either in 2014 or in 2020, and the trees were trained and pruned with a central leader (Table 3). In some cases trees of the same cultivar within the Balsgård trial was grafted on different rootstocks (Table 3) and the management was equal to organic management. There is no information on rootstocks or year of planting of the cultivars from Solnäs, but the trees were managed using conventional management practices.

*Table 2: Origin and type of the apple cultivars included in the trial. *'Amorina' is a red coloured mutant of 'Aroma' that is not yet commercially available.*

| Cultivar | Origin | Type of apple cultivar |
|---|---|---|
| 'Amorina'* | Balsgård | Table |
| 'Angela' | LARS, UK | Cider, bittersharp |
| 'Aroma' | Balsgård | Table |
| 'Black Dabinett' | Somerset, UK | Cider, bittersweet |
| 'Dabinett' | Somerset, UK | Cider, bittersweet |
| 'Debbie' | LARS, UK | Cider, sharp |
| 'Fiona' | LARS, UK | Cider, sharp |
| 'Frequin Rouge' | Britany, France | Cider, bittersweet |
| 'Harry Master´s Jersey' | Somerset, UK | Cider, bittersweet |
| 'Jane' | LARS, UK | Cider, bittersweet |
| 'Judeline' | INRA, France | Cider, sharp |
| 'Lizzy' | LARS, UK | Cider, bittersweet |
| 'Muscadet de Dieppe' | Normandy, France | Cider, bittersweet |
| 'Prince William' | LARS, UK | Cider, bittersweet |
| 'Santana' | WUR, The Netherlands | Table |
| 'Three Counties' | LARS, UK | Cider, bittersweet |
| 'Tremlett's Bitter' | Devon, UK | Cider, bittersweet |
| 'Yarlington Mill' | Somerset, UK | Cider, bittersweet |

*Table 3: Data of cultivation conditions and maturity of the fruit used in the trial. Maturity is reported as Streif index, a lower value indicates more mature fruit than a higher value. The mean value of the Streif index from six apples per cultivar were calculated.*

| Cultivar | Place for cultivation | Year for planting | Rootstock | Harvest date | Streif index mean value (standard deviation) |
|---|---|---|---|---|---|
| 'Amorina' | Balsgård | 2020 | M111 | Oct 3 | 0.06 (±0,019) |
| 'Angela' | Balsgård | 2014 | MM106/111 | Oct 10 | 0.05 (±0,007) |
| 'Aroma' | Solnäs | Unknown | Unknown | Obtained Oct 3 | 0.03 (±0,005) |
| 'Black Dabinett' | Balsgård | 2020 | A2/M9/M7 | Nov 11 | 0.06 (±0,013) |
| 'Dabinett' | Balsgård | 2020 | M111 | Oct 22 | 0.05 (±0,006) |
| 'Debbie' | Balsgård | 2014 | MM106 | Oct 3 | 0.05 (±0,012) |
| 'Fiona' | Balsgård | 2014 | MM106/111 | Oct 3 | 0.07 (±0,010) |
| 'Frequin Rouge' | Balsgård | 2014 | MM106/111 | Oct 10 | 0.10 (±0,036) |
| 'Harry Master´s Jersey' | Balsgård | 2014 | MM106/111 | Oct 22 | 0.06 (±0,007) |
| 'Jane' | Balsgård | 2014 | MM106/111 | Oct 10 | 0.05 (±0,014) |
| 'Judeline' | Balsgård | 2014 | MM106/111 | Oct 10 | 0.04 (±0,004) |
| 'Lizzy' | Balsgård | 2014 | MM106/111 | Oct 10 | 0.04 (±0,009) |
| 'Muscadet de Dieppe' | Balsgård | 2014 | MM106/111 | Sep 23 | 0.04 (±0,005) |
| 'Prince William' | Solnäs | Unknown | Unknown | Obtained Oct 3 | 0.04 (±0,004) |
| 'Santana' | Balsgård | 2014 | MM106/111 | Oct 10 | 0.03 (±0,004) |
| 'Three Counties' | Balsgård | 2020 | THC/M7 | Oct 10 | 0.03 (±0,006) |
| 'Tremlett's Bitter' | Balsgård | 2014 | MM106/111 | Oct 10 | 0.03 (±0,001) |
| 'Yarlington Mill' | Balsgård | 2020 | M111 | Oct 10 | 0.05 (±0,010) |

The fruits were harvested during the period September to November in 2024 (Table 3). The choice of time for harvest was based on previous experience of fruit maturity in the trial and repeatingly picking of fruits and estimating maturity using Starch index. To assess starch degradation a slice from the center of each apple were dipped in iodine solution and then left to react for two minutes. The slices were then compared to an index standard chart for starch degradation, using a scale of 1–10 (Tahir 2014). The aim was to harvest the fruit as mature as possible, without being overripe, corresponding to Starch index 8–10. The fruit was harvested from the trees to avoid bruising of the fruit. However, since some cultivars have a tendency to drop its fruit before reaching full maturity it was not always possible to wait until starch index 8–10, therefore e.g. 'Muscadet de Dieppe' were harvested already at Starch index 6. After harvest the fruit was kept in cold storage for a few weeks. The maturity was then evaluated again just before processing (Table 3).

To get a more complete view of maturity of the fruit at the time for processing Streif index was used. Streif index combined firmness (kg/cm2), SCC (°Brix) and starch content (Starch index). Firmness was measured using a penetrometer (Fruit Penetrometer 41050, STEP Systems GmbH, Germany) of two points of the opposite peeled sides of the apple. SCC was measured in duplicates, using juice from a slice from the middle of the apple with a refractometer (HI96801, Hanna Instruments, USA). Streif index was calculated using the following formula (Tahir 2014):

Streif index = Mean value of kg/cm2 / (Mean value of °Brix x Starch index)

A lower value indicates more mature fruit than a higher value. The mean value of the Streif index from six apples per cultivar were calculated.

## 2.2 Processing

### 2.2.1 Pressing of the fruit

Every cultivar was processed in triplicates using 2000 g of apples. The fruits were washed, crushed using an electrical fruit shredder (Fruit Mill 600kg/h 230V 50Hz, Inderst GmbH, Italy) and pressed using a hydro press (Bladder Press frutty 6lt, Inderst GmbH, Italy) (Figure 2). The juice samples were frozen in bag in boxes and 50 mL test tubes for later fermentation and analyzes.

*Figure 2: Processing and fermentation of the fruit. To the left: Pressing of the crushed fruit using a hydro press (Bladder Press frutty 6lt, Inderst GmbH, Italy). To the right: The bottles of juice, placed in a heating cabinet, to ferment at the start of the trial.*

### 2.2.2 Fermentation

300 mL of the thawed samples were, after measuring SG and pH, and if necessary adjusting pH, put in 330 mL glass bottles together with 750 µL of potassium disulfide (Vinoferm) 40 g $L^{-1}$ solution, i.e. the concentration in the prepared samples of potassium disulfide were 100 mg $L^{-1}$, according to instructions from the manufacturer. The boundary to adjust pH was set to pH <3.8 and all samples with higher pH than 3.8 was adjusted to 3.8 using 1 M malic acid solution. The bottles were shaken and then covered with a clean paper tissue and placed in room temperature for 24 hours. The Lalvin EC-1118 (Lallemand), strain of *Sacharomyces bayanus*, yest were rehydrated in sulfur treated apple juice, according to instructions from the manufacturer. The yeast was rehydrated for 20 minutes as the juice were kept between 35–37 °C while being stirred. 750 µL of the

yeast solution (100 g $L^{-1}$) were pippeted into each bottle, thus the yeast concentration in the bottles were 0.25 g $L^{-1}$. The bottles were shaken, covered with an air lock and placed in a heating cabinet keeping the temperature at 22°C (Figure 2).

The aim was to ferment the juice until it spontaneously stopped. This was considered to be achieved when no signs of continued fermentation were observed based on weight loss of the bottles. Not all samples had completed fermentation when the trial was terminated after 48 days.

Samples of cider were transferred to 50 mL test tubes, with addition of 100 µL of potassium disulfide (Vinoferm) 40 g $L^{-1}$ solution to each tube, to prevent further fermentation, and the test tubes were frozen or kept in fridge for later analyzes (Figure 3).


*Figure 3: Some of the ciders at termination of the trial after 48 days. From the left biological triplicates of 'Angela', 'Santana' and 'Yarlington Mill'.*

## 2.3 Analyses of traits

### 2.3.1 Chemical analyses

All chemical analyses were performed on thawed samples.

*Sugar content*

SSC was measured in juice samples in triplicates using a refractometer (RFM 80 Digital Refractometer, Xylem Water Solutions, UK). SG was measured using a density meter (EasyDens, Anton Paar ConsumerTec GmbH, Austria) in both juice and cider samples. Since the density meter proved to be very consistent it was decided to not measure SG in the juice samples in triplicates, the reason for this was to save time during the preparation of samples, so that the fermentation should be initiated for all samples in as short timespan as possible. SG in the cider was however measured in triplicates.

Content of glucose and fructose was measured in juice using Megazyme's enzymatic kit *D-Fructose/D-Glucose Assay Kit* on microplate following the instructions from the manufacturer for the sequential assay procedure for microplate (*D-Fructose/D-Glucose Assay Kit* u.å.): 200 µL of distilled water and 10 µL of either distilled water (for blank), standard or sample (diluted in distilled

water 1:99) were added to each well of the microplates together with 10 µL of Buffer (pH 7.6) and sodium azide (0.02% w/v) solution and 10 µL of a solution containing NADP+, ATP and PVP. The microplate was shaken using the micro plate readers shake function for two minutes. The absorbance $A_1$ was read after additionally 3 minutes at 340 nm. The first reaction was then started by adding 2 µL of hexokinase plus glucose-6-phosphate dehydrogenase suspension to each well. The microplate was shaken for two minutes and the absorbance $A_2$ was read after 5 minutes. The second reaction was then started by adding 2 µL of phosphoglucose isomerase suspension to each well. The microplate was shaken for two minutes and the absorbance $A_3$ was read after 10 minutes. The glucose content was calculated from the difference between $A_1$ and $A_2$ and the fructose content from the difference between $A_2$ and $A_3$ using the absorbance of the standard.

*Acidity and total acidity*

Acidity was analyzed by measuring pH and total acidity was analyzed as TA in juice samples. pH was measured using a handheld pH meter (VWR® PH 20 pH Meter, Avantor, Inc., USA) in juice samples, while adjusting pH in the juice. TA was measured in triplicates using a titrator (TitroLine *easy,* SI Analytics, Germany). Before the titration the test tubes with samples were shaken a few times by hand, 5 mL of sample were then diluted into 25 mL of distilled water in a beaker and placed with a magnetically stirrer in the titrator. 0,1 N sodium hydroxide solution was used as titrant. The pH electrode was rinsed between the measurements with distilled water. The result of the titration was calculated as malic acid equivalents using the following formula:

$$\text{TA (g malic acid equivalents } L^{-1}) = 67 \times \text{mL consumed NaOH solution} \times 0{,}1\ N/5$$

*Total phenolic content*

TPH was measured in juice and cider samples in triplicates using the Folin-Ciocâlteu microplate method described by Bobo-García et al. (2015). The analysis of juice and cider samples was performed at different times. Therefore new solutions as well as a standard curve was prepared for the analysis of cider samples.

1 mL of each sample of apple juice was pipetted into an Eppendorf tube and centrifuged for five minutes at 14 000 rpm. Initially samples of juice were diluted to a concentration of 1:4 by pipetting 200 µL of the sample and 800 µL of water into Eppendorf tubes. Later it was noticed that some of the samples had higher absorbance than acceptable. Samples were therefore diluted to 1:9 with 100 µL of the sample and 900µL of water before analysis.

Sodium carbonate solution was prepared by adding 8.5 g of sodium carbonate (Na2CO3) to 100 mL of water. Folin-Ciocâlteu regens was diluted 1:4 by mixing 5 mL of Folin-Ciocâlteu regent with 20 mL of water. A stock solution with concentration of 400 mg $L^{-1}$ of gallic acid was prepared by adding 40 mg of gallic acid to 100 mL of water. Double distilled water was used to prepare all of the solutions and also to dilute the samples. The 400 mg $L^{-1}$ gallic acid solution was diluted in a dilution series of 400, 200, 100, 50 and 25 mg $L^{-1}$ to be used as standard on the microplates and to make a standard curve (Appendix 1).

20 µL of sample (diluted 1:4 or 1:9), standard (400 mg $L^{-1}$ gallic acid solution) or destilled water (blank) and 100 µL of the diluted Folin-Ciocâlteu regent were added to each well of a 96-well microplate. The microplate was put in the spectrophotometer and shaken for one minute and was then left in the spectrophotometer for additionaly four minutes. 75 µL of sodium carbonate solution was added to each well and the plate was then once again shaken for one minute. The microplate was then placed in a dark cabinet in room temperature for two hours. The absorbance was then measured at 750 nm. The obtained value for absorption was reduced with the value obtained for absorption of the control. The result was calculated to g gallic acid equivalents (GAE) $L^{-1}$ using the function generated by the standard curve (Appendix 1). The result was multiplicated 5 or 10 times to compensate for the dilution of the samples.

*Yeast assimilable nitrogen*

Content of YAN was measured in juice by adding the measurements of ammonia to the measurements of FAN. FAN was also measured in cider.

Ammonia was measured using Megazyme's enzymatic kit *Ammonia Assay Kit (Rapid)* on microplate following the instructions from the manufacturer (*Ammonia Assay Kit (Rapid)* u.å.): 200 µL of distilled water and 10 µL of either distilled water (for blank), standard or sample were pipetted to each well of the microplates together with 30 µL of a solution containing buffer (pH 8.0) and 2-oxoglutarate and sodium azide (0.02% w/v) and 20 µL of NADPH solution. The microplate was shaken using the micro plate readers shake function for two minutes. The absorbance $A_1$ was read after additionally 2 minutes at 340 nm. The reaction was then started by adding 2 µL of glutamate dehydrogenase suspension. The microplate was shaken for two minutes and the absorbance $A_2$ was then read after 5 minutes. The ammonia content was calculated from the difference between $A_1$ and $A_2$ using the absorbance of the standard.

FAN was measured using Megazyme's enzymatic kit *Primary Amino Nitrogen Assay Kit (PANOPA)* on microplate following the instructions from the manufacturer (*Primary Amino Nitrogen Assay Kit (PANOPA)* u.å.): 300 µL of N-acetyl-L-cysteine solution and 5 µL of either distilled water (for blank), standard or sample were added to each well of the microplates. The microplate was shaken using the micro plate readers shake function for two minutes. The absorbance $A_1$ was read after additionally 2 minutes at 340 nm. The reaction was then started by adding 10 µL of a solution containing Ortho-phthaldialdehyde in 12 mL of ethanol (96% v/v). The microplate was shaken for two minutes and the absorbance $A_2$ was then read after 15 minutes. The FAN content was calculated from the difference between $A_1$ and $A_2$ using the absorbance of the standard.

*Ethanol content*

Potential ethanol content from SG and Brix° values of the juice as well as estimated ethanol content of ciders based on loss of SG during fermentation was calculated using the following formulas (Jolicoeur 2013):

*Brix ° values were estimated as SG:*
$SG \approx 261.3/(261.3 - °Bx)$
*The sugar content (g $L^{-1}$) was estimated from SG:*

$S_{avg}$ = 2130 * (SG - 1)
*The potential ethanol content is estimated as:*
Apavg = 0.06 * Savg

Ethanol content of cider was measured using two methods: By a combination of the density meter and a refractometer (SmartRef Digital Refractometer: EasyDens, Anton Paar ConsumerTec GmbH, Austria), a relatively recent, rapid, and cost-effective method as well as by using Megazyme's enzymatic kit *Ethanol Assay Kit* (*Ethanol Assay Kit* u.å.). The enzymatic analyse was performed on microplate following the instructions from the manufacturer: 200 µL of distilled water and 10 µL of either distilled water (for blank), standard or sample (degassed and diluted 1:499) were added to each well of the microplates together with 20 µL solution containing buffer (pH 9.0) and sodium azide (0.02% w/v), 20 µL NAD+ solution and 5 µL aldehyde dehydrogenase solution. The microplate was shaken using the micro plate readers shake function for two minutes. The absorbance $A_1$ was read at 340 nm after additionally 2 minutes. The reaction was then started by adding 2 µL of alcohol dehydrogenase suspension. The microplate was shaken for two minutes and the absorbance $A_2$ was then read after 5 minutes. The ethanol content was calculated from the difference between $A_1$ and $A_2$ using the absorbance of the standard. The ethanol content was converted from g $L^{-1}$ to % (v/v), using the conversion factor 0.1266 (*Ethanol Assay Kit* u.å.).

### 2.3.2 Other analyses

*Progress of fermentation*
The progress of the fermentation was monitored by weighing the bottles using a precision balance (KERN 572-39, KERN & Sohn GmbH, Germany) to follow the weight loss from carbon dioxide production. The bottles were weighed every 24 hours from the addition of yeast for 10 days, after that they were weighed in intervals of at first 2 days, later 3 days and finally 6 days. The percentual weight loss of every sample was calculated from the initial weight of the bottle.

*Juice yield*
The percentual juice yield was measured in triplicates and calculated from the weight (Digital Scale IMF) of the batch of fruit before crushing and the weight of the finished juice. The fruit shredder was scraped out between the batches, to make sure no pulp was left, to enable accurate measurements of juice yield.

*Classification of cultivars from LARS classification system*
Cider cultivars are, as previously mentioned, commonly classified by their chemical content. The cultivars in this study were classified using Long Ashton Research Station's classification system that from the content of malic acid and tannins classifies cultivars as sharp, sweet, bittersweet and bittersharp (Karl et al. 2023). Threshold value to classify as bitter is >2 g tannins $L^{-1}$ and for sharp >4.5 g malic acid $L^{-1}$, values lower than that is classified as sweet. The classification is here done based on TPH content (GAE g $L^{-1}$) and TA g malic acid equivalents $L^{-1}$ as is done by Spoor et al. (2019).

## 2.4 Statistical analyses

Data were analyzed by one-way ANOVA and Tukey's HSD multiple comparisons test to reveal differences between the cultivars. Significant differences were defined as $P < 0.05$. Data are reported as mean ± standard deviation of the mean for $n = 3$ replicates for juice and cider traits. Associations between different parameters was determined using Pearson correlation tests. Data was then analysed sample by sample including all cultivars. Statistical tests were carried out using Minitab statistical software (version 19.2020.1.0).

# 3. Results

Mean values are presented for each of the investigated traits, and significant differences are shown. The results of the of the traits, where it was considered to be relevant, are other than by cultivar reported merged together for the following groups of cultivars: *French- LARS-, other British-* and *table cultivars*. Correlations between the different traits are there after presented.

## 3.1 Sugar content

Sugar content is reported as SSC (Brix°) of juice, SG of juice and cider (Table 4 & 5) as well as content of glucose (g $L^{-1}$) and fructose (g $L^{-1}$) of juice (Table 6 & 7). For every trait there was significant differences between cultivars.

The group Other British cultivars had the highest mean values and the table cultivars the lowest both for SSC and SG of the juice (Table 5). The cultivar with the highest mean value was 'Tremlett's Bitter' with 17.70 and the lowest 'Aroma' with 11.90 Brix°. The cultivar with the highest mean value was 'Tremlett's Bitter' with 1.070 and the lowest 'Aroma' and 'Santana' with 1.048 SG.

'Tremlett's Bitter' had the highest mean value of SG in cider as well, whereas 'Three Counties' had the lowest in cider: 0.997 (Table 4). The mean value of SG in cider for all cultivars was 1.004, equal to an average drop of 0.044 SG during fermentation.

*Table 4: Mean values and standard deviation of SSC (Brix°) of juice and SG of juice and cider. Means that do not share a letter are significantly different.*

| Cultivar | N | Mean value Brix°, juice | | | | Mean value SG, juice | | | | Mean value SG, cider | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 'Amorina' | 3 | 13.83 | ± | 0.15 | hij | 1.056 | ± | 0.001 | efg | 1.005 | ± | 0.008 | cdef |
| 'Angela' | 2 | 13.95 | ± | 0.21 | ghij | 1.055 | ± | 0.001 | efg | 1.000 | ± | 0.002 | def |
| 'Aroma' | 3 | 11.90 | ± | 0.10 | m | 1.048 | ± | 0.001 | k | 0.998 | ± | 0.000 | f |
| 'Black Dabinett' | 3 | 15.30 | ± | 0.35 | de | 1.060 | ± | 0.002 | cd | 1.001 | ± | 0.001 | def |
| 'Dabinett' | 3 | 16.53 | ± | 0.21 | b | 1.065 | ± | 0.001 | b | 1.004 | ± | 0.003 | cdef |
| 'Debbie' | 3 | 12.50 | ± | 0.20 | lm | 1.051 | ± | 0.001 | ij | 1.000 | ± | 0.001 | def |
| 'Fiona' | 3 | 12.67 | ± | 0.15 | kl | 1.051 | ± | 0.001 | ijk | 0.999 | ± | 0.001 | ef |
| 'Frequin Rouge' | 3 | 14.70 | ± | 0.27 | efg | 1.058 | ± | 0.001 | de | 1.000 | ± | 0.001 | def |
| 'Harry Master´s Jersey' | 3 | 16.10 | ± | 0.20 | bc | 1.065 | ± | 0.001 | b | 1.014 | ± | 0.002 | bc |
| 'Jane' | 3 | 15.73 | ± | 0.29 | cd | 1.062 | ± | 0.001 | bc | 1.009 | ± | 0.011 | bcde |
| 'Judeline' | 3 | 13.33 | ± | 0.25 | ijk | 1.053 | ± | 0.000 | ghi | 1.018 | ± | 0.001 | b |
| 'Lizzy' | 3 | 14.47 | ± | 0.12 | fgh | 1.058 | ± | 0.001 | de | 0.999 | ± | 0.000 | ef |
| 'Muscadet de Dieppe' | 3 | 13.30 | ± | 0.27 | jk | 1.052 | ± | 0.001 | hi | 0.998 | ± | 0.000 | f |
| 'Prince William' | 3 | 14.40 | ± | 0.20 | ghij | 1.057 | ± | 0.001 | def | 0.998 | ± | 0.001 | f |
| 'Santana' | 3 | 11.97 | ± | 0.21 | m | 1.048 | ± | 0.001 | jk | 0.998 | ± | 0.001 | ef |
| 'Three Counties' | 3 | 15.13 | ± | 0.29 | def | 1.059 | ± | 0.002 | d | 0.997 | ± | 0.000 | f |
| 'Tremlett's Bitter' | 2 | 17.70 | ± | 0.14 | a | 1.070 | ± | 0.000 | a | 1.033 | ± | 0.000 | a |
| 'Yarlington Mill' | 3 | 14.00 | ± | 0.10 | hij | 1.054 | ± | 0.001 | fgh | 1.010 | ± | 0.002 | bcd |

*Table 5: Mean values of SSC (Brix°) of juice and SG of juice and cider based on categories.*

| Category | Mean value Brix°, juice | | | Mean value SG, juice | | | Mean value SG, cider | | |
|---|---|---|---|---|---|---|---|---|---|
| French cultivars | 13.78 | ± | 0.80 | 1.054 | ± | 0.003 | 1.005 | ± | 0.011 |
| LARS cultivars | 14.12 | ± | 1.20 | 1.056 | ± | 0.004 | 1.000 | ± | 0.004 |
| Other Brittish cultivars | 15.93 | ± | 1.38 | 1.063 | ± | 0.006 | 1.012 | ± | 0.013 |
| Table cultivars | 12.57 | ± | 1.09 | 1.051 | ± | 0.005 | 1.000 | ± | 0.004 |

'Muscadet de Dieppe' had the highest mean value of glucose, 27.4 g $L^{-1}$, and 'Angela' the lowest, 8.7 g $L^{-1}$. 'Tremlett's Bitter' had the highest mean value of

fructose, 99.0 g $L^{-1}$and 'Santana' the lowest 45.6 g $L^{-1}$ (Table 6). The mean values of glucose were on average highest by the French and lowest in the table cultivars (Table 7). For Fructose other British cultivars were highest and LARS cultivars the lowest.

*Table 6: Mean values and standard deviation of glucose (g $L^{-1}$) and fructose (g $L^{-1}$) of juice. Means that do not share a letter are significantly different.*

| | Glucose, juice | | | | | Fructose, juice | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **N** | **Mean value (g $L^{-1}$)** | | | | **N** | **Mean value (g $L^{-1}$)** | | | |
| 'Amorina' | 3 | 12.9 | ± | 1.6 | efgh | 3 | 84.8 | ± | 12.9 | abc |
| 'Angela' | 2 | 8.7 | ± | 0.2 | h | 2 | 56.3 | ± | 7.9 | de |
| 'Aroma' | 2 | 16.4 | ± | 0.3 | cde | 2 | 69.0 | ± | 0.9 | cde |
| 'Black Dabinett' | 3 | 27.0 | ± | 2.1 | a | 3 | 75.2 | ± | 4.2 | bcd |
| 'Dabinett' | 3 | 18.5 | ± | 1.8 | bcd | 3 | 84.8 | ± | 7.8 | abc |
| 'Debbie' | 3 | 11.4 | ± | 1.0 | fgh | 3 | 55.0 | ± | 2.8 | de |
| 'Fiona' | 3 | 10.0 | ± | 0.8 | gh | 3 | 55.4 | ± | 8.2 | de |
| 'Frequin Rouge' | 3 | 19.2 | ± | 1.9 | bc | 3 | 73.6 | ± | 2.8 | bcd |
| 'Harry Master´s Jersey' | 2 | 19.3 | ± | 1.8 | bc | 2 | 91.6 | ± | 4.3 | abc |
| 'Jane' | 3 | 15.9 | ± | 1.3 | cde | 2 | 70.4 | ± | 17.0 | bcd |
| 'Judeline' | 2 | 15.4 | ± | 0.6 | cdef | 2 | 70.8 | ± | 0.1 | bcd |
| 'Lizzy' | 3 | 13.1 | ± | 0.5 | efgh | 3 | 59.9 | ± | 3.4 | de |
| 'Muscadet de Dieppe' | 3 | 27.4 | ± | 1.3 | a | 3 | 63.0 | ± | 3.6 | de |
| 'Prince William' | 3 | 13.2 | ± | 1.0 | efgh | 3 | 60.2 | ± | 4.7 | de |
| 'Santana' | 3 | 10.3 | ± | 0.2 | gh | 3 | 45.6 | ± | 1.8 | e |
| 'Three Counties' | 3 | 21.5 | ± | 2.0 | b | 3 | 93.2 | ± | 8.0 | ab |
| 'Tremlett's Bitter' | 2 | 14.1 | ± | 0.1 | defg | 2 | 99.0 | ± | 0.8 | a |
| 'Yarlington Mill' | 2 | 12.0 | ± | 0.3 | efgh | 2 | 52.7 | ± | 8.8 | de |

*Table 7: Mean values of glucose (g $L^{-1}$) and fructose (g $L^{-1}$) of juice based on categories.*

| | Glucose, juice | | | Fructose, juice | | |
|---|---|---|---|---|---|---|
| **Category** | **Mean value g** $L^{-1}$ | | | **Mean value g** $L^{-1}$ | | |
| French cultivars | 20.67 | ± | 6.13 | 69.13 | ± | 5.49 |
| LARS cultivars | 13.40 | ± | 4.27 | 64.34 | ± | 13.77 |
| Other British cultivars | 18.18 | ± | 5.79 | 80.66 | ± | 17.92 |
| Table cultivars | 13.20 | ± | 3.06 | 66.47 | ± | 19.72 |

## 3.2 Acidity and total acidity

Acidity reported as pH and total acidity as TA (g malic acid equivalents $L^{-1}$) of juice is presented for cultivars in Table 8 and as merged mean values of the groups in Table 9. Significant differences between cultivars could be seen in both parameters. The table cultivars stood out by having lower pH and higher TA than the other groups and other British cultivars by having higher pH and lower TA. Both the French and the LARS cultivars showed a high variation within the groups for both parameters. 'Three Counties' had the highest mean value of pH, 4.20 and 'Debbie' the lowest with 2.90, Debbie had the highest mean value of TA, 10.42 and 'Three Counties' the lowest with 1.58 g malic acid equivalents $L^{-1}$.

*Table 8: Mean values and standard deviation of pH and TA (g malic acid equivalents $L^{-1}$) of juice. Means that do not share a letter are significantly different.*

| Cultivar | N | Mean value pH, juice | | | | Mean value TA, juice | | | |
|---|---|---|---|---|---|---|---|---|---|
| 'Amorina' | 3 | 3.21 | ± | 0.21 | e | 7.18 | ± | 0.42 | b |
| 'Angela' | 3 | 3.16 | ± | 0.03 | e | 7.35 | ± | 0.51 | b |
| 'Aroma' | 3 | 3.13 | ± | 0.03 | ef | 7.20 | ± | 0.13 | b |
| 'Black Dabinett' | 3 | 3.85 | ± | 0.08 | cd | 3.22 | ± | 0.15 | de |
| 'Dabinett' | 3 | 4.19 | ± | 0.08 | a | 2.11 | ± | 0.10 | ef |
| 'Debbie' | 3 | 2.90 | ± | 0.06 | f | 10.42 | ± | 1.19 | a |
| 'Fiona' | 3 | 3.19 | ± | 0.11 | e | 7.19 | ± | 0.40 | b |
| 'Frequin Rouge' | 3 | 3.87 | ± | 0.02 | bcd | 3.56 | ± | 0.17 | d |
| 'Harry Master´s Jersey' | 3 | 4.09 | ± | 0.05 | ab | 2.36 | ± | 0.04 | def |
| 'Jane' | 3 | 4.08 | ± | 0.03 | abc | 1.83 | ± | 0.07 | f |
| 'Judeline' | 3 | 3.13 | ± | 0.04 | ef | 6.49 | ± | 0.55 | bc |
| 'Lizzy' | 3 | 3.96 | ± | 0.04 | abcd | 2.12 | ± | 0.08 | ef |
| 'Muscadet de Dieppe' | 3 | 3.90 | ± | 0.08 | bcd | 2.74 | ± | 0.21 | def |
| 'Prince William' | 3 | 4.19 | ± | 0.02 | a | 1.82 | ± | 0.18 | f |
| 'Santana' | 3 | 3.12 | ± | 0.01 | ef | 7.40 | ± | 0.20 | b |
| 'Three Counties' | 3 | 4.20 | ± | 0.05 | a | 1.58 | ± | 0.09 | f |
| 'Tremlett's Bitter' | 2 | 3.78 | ± | 0.03 | d | 5.32 | ± | 0.01 | c |
| 'Yarlington Mill' | 3 | 3.77 | ± | 0.13 | d | 3.57 | ± | 0.90 | d |

*Table 9: Mean values and standard deviation of pH and TA (g malic acid equivalents $L^{-1}$) of juice based on categories.*

| Category | Mean value pH, juice | | | Mean value TA, juice | | |
|---|---|---|---|---|---|---|
| French cultivars | 3.63 | ± | 0.44 | 4.26 | ± | 1.97 |
| LARS cultivars | 3.67 | ± | 0.56 | 4.62 | ± | 3.62 |
| Other British cultivars | 3.94 | ± | 0.19 | 3.32 | ± | 1.27 |
| Table cultivars | 3.15 | ± | 0.05 | 7.26 | ± | 0.12 |

## 3.3 Total phenolic content

Data of phenolic compounds content is presented in terms of TPH (g GAE $L^{-1}$) of juice and ciders of based on cultivars (Table 10) and categories (Table 11). Significant differences between cultivars could be seen in both parameters.

'Tremblett's Bitter' had the highest mean value both in juice and cider, 4.47 and 4.74 respectively, Santana had the lowest mean value in juice of 0.21 and Santana together with Aroma in cider with 0.21 respectively. The results for 'Amorina' were inconsistent and the data was therefore excluded.

Mean values for the groups were slightly higher in the juices than the ciders for all but the table cultivars, however it should be noted that the mean value for juice is based on only 'Aroma' and 'Santana' since data for 'Amorina' is missing. There was a large variation between the groups with the group other British had approximately ten times higher TPH content than the table cultivars.

*Table 10: Mean values and standard deviation of TPH (GAE g $L^{-1}$) of juice and cider. Means that do not share a letter are significantly different.*

| | TPH, juice | | | | TPH, cider | | | |
|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **N** | **Mean value, GAE g $L^{-1}$** | | | **N** | **Mean value, GAE g $L^{-1}$** | | |
| 'Amorina' | 3 | - | ± | - | - | 3 | 0.49 | ± | 0.12 | gh |
| 'Angela' | 3 | 0.99 | ± | 0.06 | fgh | 3 | 0.98 | ± | 0.05 | ef |
| 'Aroma' | 3 | 0.25 | ± | 0.05 | gh | 3 | 0.21 | ± | 0.03 | h |
| 'Black Dabinett' | 3 | 3.14 | ± | 0.40 | b | 3 | 2.63 | ± | 0.20 | b |
| 'Dabinett' | 3 | 2.42 | ± | 0.18 | bcd | 3 | 2.36 | ± | 0.17 | bc |
| 'Debbie' | 3 | 0.51 | ± | 0.10 | fgh | 3 | 0.43 | ± | 0.02 | gh |
| 'Fiona' | 3 | 1.12 | ± | 0.03 | fgh | 3 | 1.04 | ± | 0.04 | ef |
| 'Frequin Rouge' | 3 | 2.48 | ± | 0.08 | bc | 3 | 2.18 | ± | 0.23 | c |
| 'Harry Master´s Jersey' | 3 | 2.19 | ± | 0.24 | bcde | 3 | 2.25 | ± | 0.18 | bc |
| 'Jane' | 3 | 2.38 | ± | 0.03 | bcd | 3 | 2.19 | ± | 0.14 | c |
| 'Judeline' | 2 | 0.76 | ± | 0.01 | fgh | 3 | 0.66 | ± | 0.09 | fg |
| 'Lizzy' | 3 | 0.88 | ± | 0.07 | fgh | 3 | 0.98 | ± | 0.07 | ef |
| 'Muscadet de Dieppe' | 3 | 1.36 | ± | 0.08 | def | 3 | 1.20 | ± | 0.05 | de |
| 'Prince William' | 3 | 0.82 | ± | 0.05 | fgh | 3 | 0.67 | ± | 0.12 | fg |
| 'Santana' | 3 | 0.21 | ± | 0.04 | h | 3 | 0.21 | ± | 0.03 | h |
| 'Three Counties' | 3 | 1.30 | ± | 0.13 | efg | 3 | 1.59 | ± | 0.13 | d |
| 'Tremlett's Bitter' | 2 | 4.47 | ± | 0.16 | a | 2 | 4.74 | ± | 0.12 | a |
| 'Yarlington Mill' | 3 | 2.88 | ± | 0.24 | b | 3 | 2.54 | ± | 0.19 | bc |

*Table 11: Mean values of juice and cider of TPH based on categories. *'Amorina' is excluded.*

| | TPH, juice | | | TPH, cider | | |
|---|---|---|---|---|---|---|
| **Category** | **Mean value, GAE g $L^{-1}$** | | | **Mean value, GAE g $L^{-1}$** | | |
| French cultivars | 1.53 | ± | 0.87 | 1.35 | ± | 0.77 |
| LARS cultivars | 1.14 | ± | 0.60 | 1.13 | ± | 0.59 |
| Other Brittish cultivars | 3.02 | ± | 0.89 | 2.90 | ± | 1.04 |
| Table cultivars | 0.23* | ± | 0.03 | 0.30 | ± | 0.16 |

## 3.4 Yeast assimilable nitrogen reported as FAN

Data of YAN is presented in terms of FAN (mg $L^{-1}$) of juice and cider (Table 12). Ammonia content was analysed in juice samples, however the method showed extremely large variation and the results was considered not reliable and therefore excluded from the study. Even in the FAN data there were some inconsistencies and due to this some data had to be excluded from the analysis. For this reason there are only data from two samples for several cultivars included in the analysis. When there where only reliable data for one of the replicates it was also excluded from the analysis.

Analysis of variance showed significant differences between cultivars for FAN in juice, (p 0.000) but not in cider (p 0.053). However Tukey's HSD multiple comparisons test revealed significant difference between the ciders of 'Yarlington Mill', 39.2, and 'Debbie', 8.1 mg $L^{-1}$. In juice 'Santana' and 'Muscadet de Dieppe' had the highest mean content of FAN, 50.9 and 42.1 mg $L^{-1}$. Santana had significantly higher value than all of the others but 'Muscadet de Dieppe' and 'Muscadet de Dieppe' had significantly higher value than all but 'Santana', 'Yarlington Mill' and 'Aroma'

*Table 12: Mean values and standard deviation of FAN (mg $L^{-1}$) of juice and cider. Means that do not share a letter are significantly different. Some data has been excluded from the analysis because of inconsistent results of the technical triplicates. Therefore there are only data from two samples for some cultivars. When there was only data for one replicate the cultivar was excluded from the analysis.*

| Cultivar | N | Mean, FAN juice, mg $L^{-1}$ | | | | N | Mean, FAN cider, mg $L^{-1}$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 'Amorina' | 3 | 15.6 | ± | 3.0 | c | 3 | 10.3 | ± | 3.0 | ab |
| 'Angela' | 2 | 16.0 | ± | 6.0 | c | 3 | 25.0 | ± | 19.2 | ab |
| 'Aroma' | 2 | 22.0 | ± | 3.9 | bc | 2 | 17.8 | ± | 2.5 | ab |
| 'Black Dabinett' | 2 | 15.7 | ± | 4.8 | c | 2 | 6.2 | ± | 0.2 | ab |
| 'Dabinett' | - | - | ± | - | - | - | - | ± | - | - |
| 'Debbie' | 3 | 12.0 | ± | 5.0 | c | 3 | 8.1 | ± | 3.1 | b |
| 'Fiona' | 2 | 18.8 | ± | 2.2 | c | - | - | ± | - | - |
| 'Frequin Rouge' | 2 | 14.4 | ± | 7.6 | c | 2 | 14.8 | ± | 12.5 | ab |
| 'Harry Master´s Jersey' | 3 | 9.6 | ± | 3.9 | c | - | - | ± | - | - |
| 'Jane' | - | - | ± | - | c | - | - | ± | - | - |
| 'Judeline' | - | - | ± | - | c | 2 | 7.7 | ± | 3.3 | ab |
| 'Lizzy' | 3 | 8.8 | ± | 4.6 | c | 2 | 17.9 | ± | 3.1 | ab |
| 'Muscadet de Dieppe' | 3 | 42.1 | ± | 5.8 | ab | 2 | 22.3 | ± | 0.8 | ab |
| 'Prince William' | 3 | 16.1 | ± | 8.8 | c | 2 | 11.7 | ± | 1.6 | ab |
| 'Santana' | 2 | 50.9 | ± | 4.0 | a | 2 | 42.7 | ± | 0.9 | ab |
| 'Three Counties' | 2 | 20.1 | ± | 14.7 | c | - | - | ± | - | ab |
| 'Tremlett's Bitter' | - | - | ± | - | c | 2 | 18.8 | ± | 0.0 | ab |
| 'Yarlington Mill' | 2 | 20.4 | ± | 3.5 | bc | 2 | 54.7 | ± | 39.2 | a |

## 3.5 Ethanol content

The potential ethanol content as % was estimated both from SG and SSC of the juice (Table 13). The estimations made from SSC are overall slightly higher than the ones based on SG.

*Table 13: Mean values and standard deviation of estimated potential ethanol content (%) of cider.*

| Cultivar | N | Potential ethanol content (%) estimated from SG of juice | | | N | Potential ethanol content (%) estimated from Brix° of juice | | |
|---|---|---|---|---|---|---|---|---|
| 'Amorina' | 3 | 7.1 | ± | 0.1 | 3 | 7.1 | ± | 0.1 |
| 'Angela' | 3 | 7.1 | ± | 0.1 | 2 | 7.2 | ± | 0.1 |
| 'Aroma' | 3 | 6.1 | ± | 0.1 | 3 | 6.1 | ± | 0.1 |
| 'Black Dabinett' | 3 | 7.7 | ± | 0.2 | 3 | 7.9 | ± | 0.2 |
| 'Dabinett' | 3 | 8.3 | ± | 0.1 | 3 | 8.6 | ± | 0.1 |
| 'Debbie' | 3 | 6.5 | ± | 0.1 | 3 | 6.4 | ± | 0.1 |
| 'Fiona' | 3 | 6.5 | ± | 0.1 | 3 | 6.5 | ± | 0.1 |
| 'Frequin Rouge' | 3 | 7.4 | ± | 0.1 | 3 | 7.6 | ± | 0.1 |
| 'Harry Master´s Jersey' | 3 | 8.3 | ± | 0.1 | 3 | 8.4 | ± | 0.1 |
| 'Jane' | 3 | 8.0 | ± | 0.2 | 3 | 8.2 | ± | 0.2 |
| 'Judeline' | 3 | 6.8 | ± | 0.0 | 3 | 6.9 | ± | 0.1 |
| 'Lizzy' | 3 | 7.4 | ± | 0.1 | 3 | 7.5 | ± | 0.1 |
| 'Muscadet de Dieppe' | 3 | 6.6 | ± | 0.2 | 3 | 6.8 | ± | 0.2 |
| 'Prince William' | 3 | 7.3 | ± | 0.1 | 3 | 7.4 | ± | 0.1 |
| 'Santana' | 3 | 6.1 | ± | 0.2 | 3 | 6.1 | ± | 0.2 |
| 'Three Counties' | 3 | 7.5 | ± | 0.3 | 3 | 7.8 | ± | 0.1 |
| 'Tremlett's Bitter' | 2 | 8.9 | ± | 0.0 | 2 | 9.3 | ± | 0.1 |
| 'Yarlington Mill' | 3 | 6.9 | ± | 0.1 | 3 | 7.3 | ± | 0.1 |

The enzymatic method gave unlikely and inconsistent results and the results was therefore excluded from the study. Ethanol content (%) estimated from the decrease of SG during fermentation as well as measured by the Anton Paar devices are reported in Table 14. Mean values obtained by the Anton Paar devices were slightly higher than what was calculated from decrease in SG.

*Table 14: Mean values and standard deviation of ethanol content of cider (%) and estimations of ethanol content calculated from decrease of SG. Means that do not share a letter are significantly different.*

| | Ethanol content of cider, % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **N** | **Calculated from decrease of SG** | | | | **N** | **Anton Paar method** | | | |
| 'Amorina' | 3 | 6.5 | ± | 1.1 | bcd | 3 | 6.9 | ± | 1.2 | abcd |
| 'Angela' | 3 | 7.0 | ± | 0.2 | abcd | 3 | 7.3 | ± | 0.1 | abc |
| 'Aroma' | 3 | 6.4 | ± | 0.1 | cd | 3 | 6.6 | ± | 0.2 | bcd |
| 'Black Dabinett' | 3 | 7.5 | ± | 0.2 | abc | 3 | 8.1 | ± | 0.3 | ab |
| 'Dabinett' | 3 | 7.9 | ± | 0.5 | ab | 3 | 8.2 | ± | 0.6 | a |
| 'Debbie' | 3 | 6.5 | ± | 0.2 | cd | 3 | 6.7 | ± | 0.2 | bcd |
| 'Fiona' | 3 | 6.6 | ± | 0.1 | abcd | 3 | 7.0 | ± | 0.1 | abcd |
| 'Frequin Rouge' | 3 | 7.4 | ± | 0.1 | abc | 3 | 7.9 | ± | 0.2 | abc |
| 'Harry Master´s Jersey' | 3 | 6.6 | ± | 0.4 | abcd | 3 | 6.9 | ± | 0.3 | abcd |
| 'Jane' | 3 | 6.9 | ± | 1.3 | abcd | 3 | 7.5 | ± | 1.4 | abc |
| 'Judeline' | 3 | 4.5 | ± | 0.2 | e | 3 | 4.6 | ± | 0.2 | e |
| 'Lizzy' | 3 | 7.6 | ± | 0.1 | abc | 3 | 7.9 | ± | 0.1 | abc |
| 'Muscadet de Dieppe' | 3 | 6.9 | ± | 0.1 | abcd | 3 | 7.4 | ± | 0.2 | abc |
| 'Prince William' | 3 | 7.6 | ± | 0.1 | abc | 3 | 7.8 | ± | 0.2 | abc |
| 'Santana' | 3 | 6.4 | ± | 0.1 | cd | 3 | 6.6 | ± | 0.1 | bcd |
| 'Three Counties' | 3 | 7.9 | ± | 0.3 | a | 3 | 8.4 | ± | 0.3 | a |
| 'Tremlett's Bitter' | 2 | 4.7 | ± | 0.0 | e | 2 | 5.5 | ± | 0.0 | de |
| 'Yarlington Mill' | 3 | 5.7 | ± | 0.3 | de | 3 | 6.4 | ± | 0.3 | cd |

## 3.6 Juice yield

Significant differences between cultivars could be seen in juice yield (%) between cultivars (Table 15). 'Santana' had the highest mean value, 69.3% and 'Prince William' the lowest, 32.0%, i.e. markedly lower than the next lowest, 'Lizzy' with 49.0%. The groups with highest mean values were the French and the table cultivars, LARS cultivars showed large variation (Table 16).

*Table 15: Mean values of juice yield (%). Means that do not share a letter are significantly different.*

| **Cultivar** | **N** | **Mean value, juice yield (%)** | | | |
|---|---|---|---|---|---|
| 'Amorina' | 3 | 63.3 | ± | 2.9 | bcd |
| 'Angela' | 3 | 58.3 | ± | 1.5 | def |
| 'Aroma' | 3 | 57.0 | ± | 1.7 | ef |
| 'Black Dabinett' | 3 | 53.7 | ± | 0.6 | fg |
| 'Dabinett' | 3 | 53.0 | ± | 2.0 | fg |
| 'Debbie' | 3 | 65.3 | ± | 1.2 | abc |
| 'Fiona' | 3 | 67.3 | ± | 2.1 | ab |
| 'Frequin Rouge' | 3 | 63.0 | ± | 2.0 | bcd |
| 'Harry Master´s Jersey' | 3 | 53.3 | ± | 1.2 | fg |
| 'Jane' | 3 | 58.3 | ± | 1.2 | def |
| 'Judeline' | 3 | 66.3 | ± | 3.8 | abc |
| 'Lizzy' | 3 | 49.0 | ± | 2.0 | g |
| 'Muscadet de Dieppe' | 3 | 64.7 | ± | 0.6 | abc |
| 'Prince William' | 3 | 32.0 | ± | 1.0 | h |
| 'Santana' | 3 | 69.3 | ± | 0.6 | a |
| 'Three Counties' | 3 | 54.7 | ± | 1.5 | ef |
| 'Tremlett's Bitter' | 2 | 60.5 | ± | 2.1 | cde |
| 'Yarlington Mill' | 3 | 56.7 | ± | 1.2 | ef |

*Table 16: Table 16: Mean values of of juice yield (%) based on categories.*

| **Category** | **Mean value, juice yield (%)** | | |
|---|---|---|---|
| French cultivars | 65 | ± | 2 |
| LARS cultivars | 55 | ± | 12 |
| Other Brittish cultivars | 55 | ± | 3 |
| Table cultivars | 63 | ± | 6 |

## 3.7 Fermentability

Fermentability, i.e. the rate and degree of fermentation, is presented first by mean values of percentual weight loss, from a selection of timepoints, in Table 17 & 18 and as the mean fermentation rate for all cultivars is visualised in Figure 4-7. The cultivars are shown in four figures by categories to increase readability.

Significant differences between cultivars could be seen in weight loss (%) between cultivars in all of the selected timepoints: 3, 5, 10, 20 and 48 days after addition of yeast (i.e. start of fermentation). Mean values of weight loss for all cultivars were as follows: Day 3 0.86 %, day 5 1.51 %, day 10 2.49 %, day 20 3.86 % and day 48 5.51 %. At day 3 'Santana', 'Muscadet de Dieppe' and 'Aroma', in descending order with significant difference in between, stood out with a higher degree of weight loss (Table 17). The pattern of the three of them having a greater weight loss than the other persisted until day 10 (Table 18). In day 20 there were no significant differences among them, what stands out is instead 'Judeline', 'Tremlett's Bitter' and 'Yarlington Mill' with significantly lower weight loss than most of the others. This remains at the final day of the trial, day 48, for 'Judeline' and 'Tremlett's Bitter'. The highest mean weight loss at the end of the trial was 6.48% ('Three Counties') and the lowest 3.74% ('Judeline').

*Table 17: Mean values of weight loss three respectively five days after addition of yeast. Means that do not share a letter are significantly different.*Mean values are based on two instead of three samples, one bottle is missing.*

| Cultivar | Mean day 3 | | | | Mean day 5 | | | |
|---|---|---|---|---|---|---|---|---|
| 'Amorina' | 0.42% | ± | 0.13% | def | 0.86% | ± | 0.26% | de |
| 'Angela' | 0.42% | ± | 0.05% | def | 0.84% | ± | 0.09% | de |
| 'Aroma' | 1.89% | ± | 0.37% | c | 3.31% | ± | 0.61% | b |
| 'Black Dabinett' | 0.90% | ± | 0.17% | d | 1.65% | ± | 0.29% | c |
| 'Dabinett' | 0.50% | ± | 0.06% | def | 0.99% | ± | 0.12% | cde |
| 'Debbie' | 0.18%* | ± | 0.08% | ef | 0.60%* | ± | 0.09% | de |
| 'Fiona' | 035% | ± | 0.08% | ef | 0.85% | ± | 0.13% | de |
| 'Frequin Rouge' | 0.69% | ± | 0.14% | de | 1.25% | ± | 0.23% | cd |
| 'Harry Master´s Jersey' | 0.40% | ± | 0.03% | def | 0.78% | ± | 0.06% | de |
| 'Jane' | 0.45% | ± | 0.11% | def | 0.86% | ± | 0.21% | de |
| 'Judeline' | 0.17% | ± | 0.02% | f | 0.42% | ± | 0.01% | e |
| 'Lizzy' | 0.67% | ± | 0.07% | def | 1.25% | ± | 0.13% | cd |
| 'Muscadet de Dieppe' | 2.49% | ± | 0.22% | b | 4.32% | ± | 0.35% | a |
| 'Prince William' | 0.72% | ± | 0.10% | de | 1.29% | ± | 0.18% | cd |
| 'Santana' | 3.47% | ± | 0.38% | a | 4.80% | ± | 0.05% | a |
| 'Three Counties' | 0.63% | ± | 0.22% | def | 1.26% | ± | 0.42% | cd |
| 'Tremlett's Bitter' | 0.28%* | ± | 0.01% | ef | 0.53%* | ± | 0.01% | de |
| 'Yarlington Mill' | 0.37% | ± | 0.06% | ef | 0.69% | ± | 0.09% | de |

*Table 18: Mean values of weight loss ten, twenty respectively forty eight days (i.e. the final day of the trial) after addition of yeast. Means that do not share a letter are significantly different.*Mean values are based on two instead of three samples.*

| Cultivar | Mean day 10 | | | | Mean day 20 | | | | Mean day 48 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 'Amorina' | 1.72% | ± | 0.48% | cdefg | 3.16% | ± | 0.83% | def | 5.37% | ± | 0.85% | abcd |
| 'Angela' | 1.78% | ± | 0.13% | cdefg | 3.34% | ± | 0.24% | cdef | 5.81% | ± | 0.12% | abcd |
| 'Aroma' | 4.85% | ± | 0.09% | a | 5.03% | ± | 0.04% | ab | 5.36% | ± | 0.12% | abcd |
| 'Black Dabinett' | 3.35% | ± | 0.43% | b | 5.40% | ± | 0.41% | a | 6.43% | ± | 0.58% | ab |
| 'Dabinett' | 2.02% | ± | 0.23% | cdef | 3.95%* | ± | 0.13% | abcde | 6.23% | ± | 0.40% | abc |
| 'Debbie' | 1.48%* | ± | 0.13% | defg | 2.94%* | ± | 0.17% | def | 5.24%* | ± | 0.12% | abcde |
| 'Fiona' | 1.87% | ± | 0.29% | cdefg | 3.56% | ± | 0.47% | bcde | 5.46% | ± | 0.12% | abcd |
| 'Frequin Rouge' | 2.36% | ± | 0.43% | cde | 4.23% | ± | 0.70% | abcd | 5.98% | ± | 0.10% | abc |
| 'Harry Master´s Jersey' | 1.60% | ± | 0.11% | defg | 3.00% | ± | 0.23% | def | 5.26% | ± | 0.30% | cd |
| 'Jane' | 1.72% | ± | 0.42% | cdefg | 3.19% | ± | 0.75% | def | 5.54% | ± | 1.00% | abcd |
| 'Judeline' | 0.97% | ± | 0.03% | g | 1.93% | ± | 0.07% | f | 3.74% | ± | 0.10% | f |
| 'Lizzy' | 2.44% | ± | 0.24% | bcd | 4.40% | ± | 0.40% | abcd | 6.07% | ± | 0.01% | abc |
| 'Muscadet de Dieppe' | 5.34% | ± | 0.05% | a | 5.47% | ± | 0.04% | a | 5.73% | ± | 0.04% | abcd |
| 'Prince William' | 2.42% | ± | 0.29% | bcd | 4.39% | ± | 0.48% | abcd | 5.99% | ± | 0.17% | abc |
| 'Santana' | 4.92% | ± | 0.09% | a | 5.03% | ± | 0.09% | ab | 5.30% | ± | 0.10% | bcd |
| 'Three Counties' | 2.65% | ± | 0.78% | bc | 4.76% | ± | 1.08% | abc | 6.48% | ± | 0.11% | a |
| 'Tremlett's Bitter' | 1.11%* | ± | 0.01% | fg | 2.13%* | ± | 0.01% | ef | 3.83%* | ± | 0.03% | ef |
| 'Yarlington Mill' | 1.42% | ± | 0.16% | efg | 2.66% | ± | 0.25% | ef | 4.73% | ± | 0.21% | def |

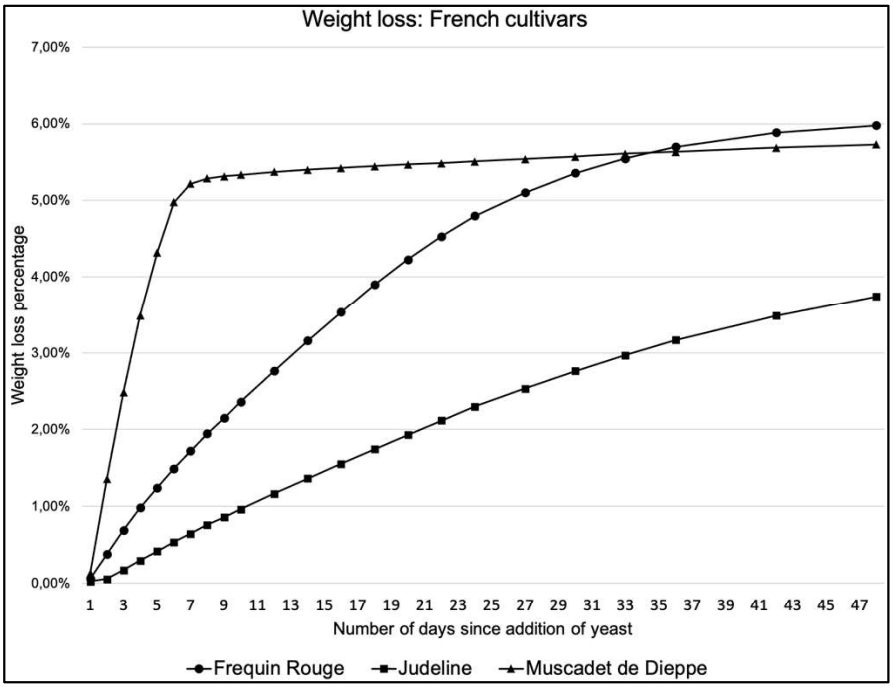


*Figure 4: Mean values of weight loss percentage of the different cultivars from day 1 to 48 after addition of yeast of the three French cultivars included in the study.*

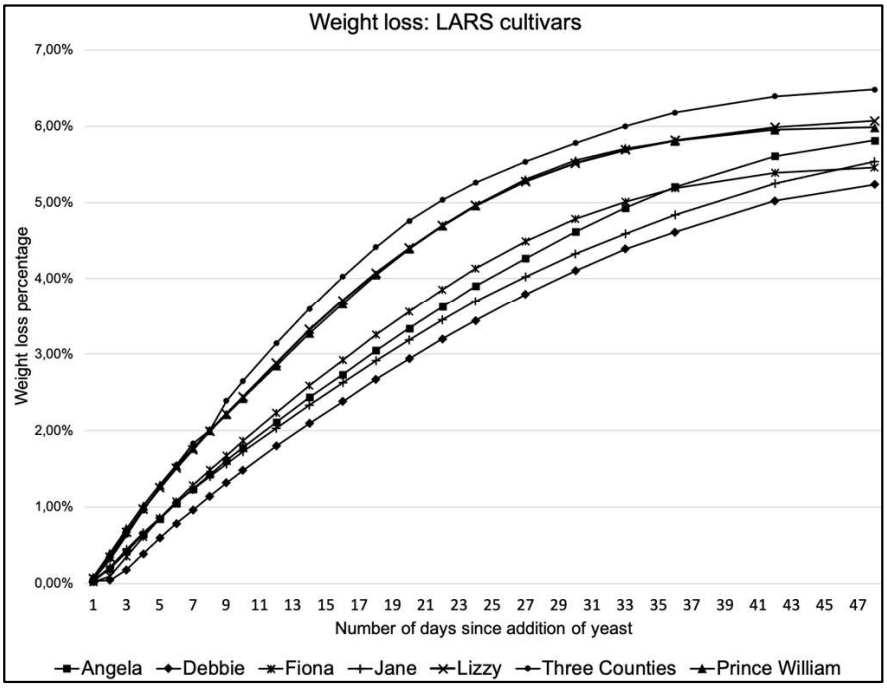


*Figure 5: Mean values of weight loss percentage of the different cultivars from day 1 to 48 after addition of yeast of the seven cultivars lanced from LARS that was included in the study.*

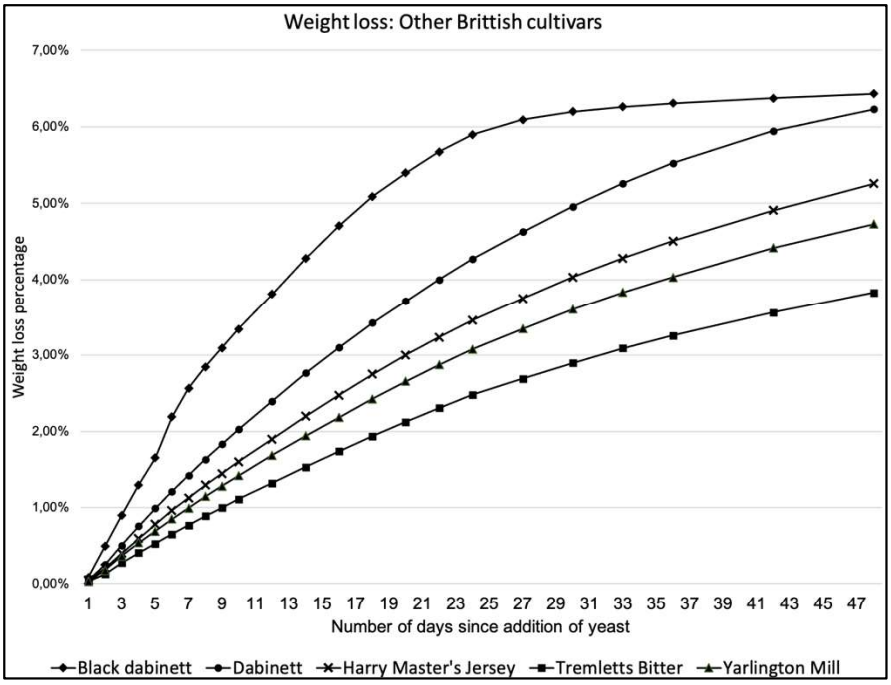


*Figure 6: Mean values of weight loss percentage of the different cultivars from day 1 to 48 after addition of yeast of the other British cultivars included in the study.*

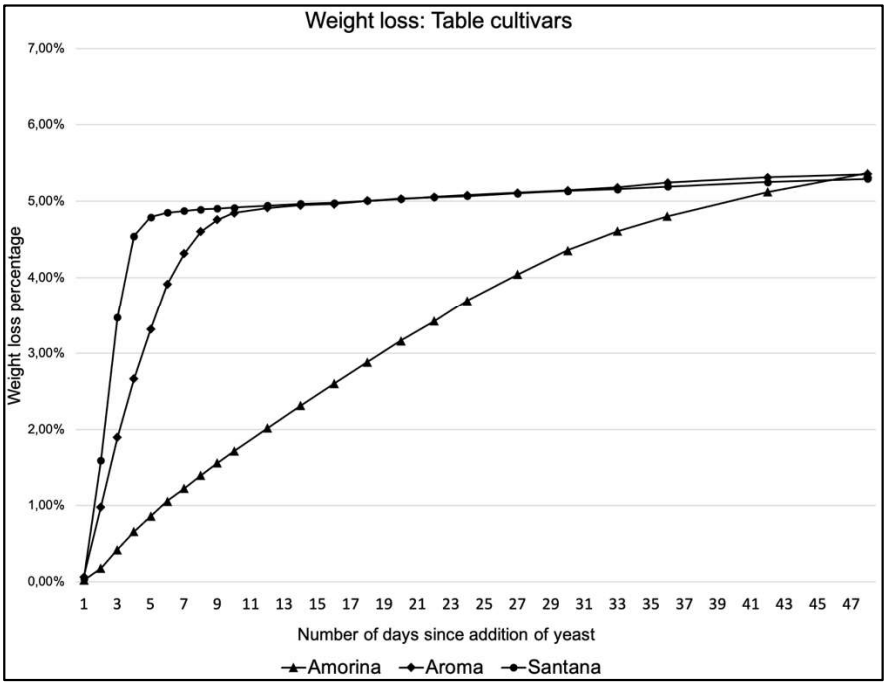


*Figure 7: Mean values of weight loss percentage of the different cultivars from day 1 to 48 after addition of yeast of the three table cultivars included in the study..*

### 3.7.1 Classification by LARS system

The results are presented and compared to the traditional classifications in Table 19. Eleven out of 15 of the cultivars fell in to the same classes as traditionally. The ones that differed was 'Angela', 'Prince William', 'Three Counties' and 'Tremlett's Bitter'. 'Angela' that was in this study classified as sharp instead of bittersharp was far below the limit to be classified as bitter.

*Table 19: Classification of the cultivars, based on mean values of titratable acidity and total phenolic compound content using LARS classification system. The results from this study is compared to the traditional classification of the cider cultivars. *The three table cultivars are here classified as if they were cider cultivars, for comparison. 'Amorina' was not classified due to the lack of data of TPH in juice, but based on the data of TPH in 'Amorina' cider it would most likely be classified as sharp.*

| Cultivar | Mean value TA, juice | Mean value, TPH juice | Classification in this study | Traditional classification |
|---|---|---|---|---|
| 'Amorina' | 7.18 | - | - * | Table |
| 'Angela' | 7.35 | 0.98 | Sharp | Cider, bittersharp |
| 'Aroma' | 7.20 | 0.21 | Sharp * | Table |
| 'Black Dabinett' | 3.22 | 2.63 | Bittersweet | Cider, bittersweet |
| 'Dabinett' | 2.11 | 2.36 | Bittersweet | Cider, bittersweet |
| 'Debbie' | 10.42 | 0.43 | Sharp | Cider, sharp |
| 'Fiona' | 7.19 | 1.04 | Sharp | Cider, sharp |
| 'Frequin Rouge' | 3.56 | 2.18 | Bittersweet | Cider, bittersweet |
| 'Harry Master´s Jersey' | 2.36 | 2.25 | Bittersweet | Cider, bittersweet |
| 'Jane' | 1.83 | 2.19 | Bittersweet | Cider, bittersweet |
| 'Judeline' | 6.49 | 0.66 | Sharp | Cider, sharp |
| 'Lizzy' | 2.12 | 0.98 | Bittersweet | Cider, bittersweet |
| 'Muscadet de Dieppe' | 2.74 | 1.20 | Bittersweet | Cider, bittersweet |
| 'Prince William' | 1.82 | 0.67 | Sweet | Cider, bittersweet |
| 'Santana' | 7.40 | 0.21 | Sharp * | Table |
| 'Three Counties' | 1.58 | 1.59 | Sweet | Cider, bittersweet |
| 'Tremlett's Bitter' | 5.32 | 4.74 | Bittersharp | Cider, bittersweet |
| 'Yarlington Mill' | 3.57 | 2.54 | Bittersweet | Cider, bittersweet |

## 3.8 Associations between traits as well as results obtained from between different analytical methods

The significant correlations between the different juice and cider traits are reported in tables in this sections, all analysed correlations including the non-significant are reported in Appendix 2.

### 3.8.1 Correlations between juice and cider traits and fermentability

The strongest correlations between the different juice traits were a strong negative correlation between TA and pH of the juice (n 53, r -0.959, p 0.000) and a strong positive between SSC and SG of the juice (n 52, r 0.989, p 0.000). Among the other stronger correlations between juice traits were the ones between TPH and SSC (n 48, r 0.792, p 0.000) or SG (n 49, r 0.753, p 0.000) as well as between SSC and pH (n 52, r 0.748, p 0.000) indicating that cultivars had a tendency to either have high phenolic and sugar content and low acidity or low phenolic and sugar content and high acidity.

*Table 20: Significant correlations between juice traits.*

| Juice traits | | N | R-value | P-value |
|---|---|---|---|---|
| TPH juice | TA juice | 49 | -0.445 | 0.001 |
| TPH juice | SSC juice | 48 | 0.792 | 0.000 |
| TPH juice | pH juice | 49 | 0.512 | 0.000 |
| TPH juice | SG juice | 49 | 0.753 | 0.000 |
| TPH juice | Glucose juice | 44 | 0.384 | 0.010 |
| TPH juice | Fructose juice | 43 | 0.597 | 0.000 |
| TA juice | SSC juice | 52 | -0.660 | 0.000 |
| TA juice | pH juice | 53 | -0.959 | 0.000 |
| TA juice | SG juice | 53 | -0.612 | 0.000 |
| TA juice | Glucose juice | 48 | -0.551 | 0.000 |
| TA juice | Fructose juice | 47 | -0.357 | 0.014 |
| SSC juice | pH juice | 52 | 0.748 | 0.000 |
| SSC juice | SG juice | 52 | 0.989 | 0.000 |
| SSC juice | FAN juice | 36 | -0.437 | 0.008 |
| SSC juice | Glucose juice | 47 | 0.341 | 0.019 |
| SSC juice | Fructose juice | 46 | 0.708 | 0.000 |
| pH juice | SG juice | 53 | 0.704 | 0.000 |
| pH juice | Glucose juice | 48 | 0.531 | 0.000 |
| pH juice | Fructose juice | 47 | 0.421 | 0.003 |
| SG juice | FAN juice | 37 | -0.480 | 0.003 |
| SG juice | Glucose juice | 48 | 0.284 | 0.050 |
| SG juice | Fructose juice | 47 | 0.695 | 0.000 |
| Glucose juice | Fructose juice | 47 | 0.440 | 0.002 |

The strongest correlation between a juice trait and a cider trait was between TPH of juice and cider (n 49, r 0.979, p 0.000) (Table 21), evident given the small change in phenolic content during fermentation previously showed. The second strongest correlations were between TPH of cider and SSC (n 52, r 0.834, p 0.000) or SG (n 53, r 0.796, p 0.000) of juice, pointing out the same tendency as previously mentioned to either have high phenolic and sugar content and low acidity or the opposite.

*Table 21: Significant correlations between juice and cider traits.*

| Juice trait | Cider trait | N | R-value | P-value |
|---|---|---|---|---|
| TPH juice | SG cider | 49 | 0.609 | 0.000 |
| SSC juice | SG cider | 52 | 0.487 | 0.000 |
| SG juice | SG cider | 53 | 0.493 | 0.000 |
| Fructose juice | SG cider | 47 | 0.433 | 0.002 |
| TPH juice | TPH cider | 49 | 0.979 | 0.000 |
| TA juice | TPH cider | 53 | -0.483 | 0.000 |
| SSC juice | TPH cider | 52 | 0.834 | 0.000 |
| pH juice | TPH cider | 53 | 0.564 | 0.000 |
| SG juice | TPH cider | 53 | 0.796 | 0.000 |
| Glucose juice | TPH cider | 48 | 0.357 | 0.013 |
| Fructose juice | TPH cider | 47 | 0.548 | 0.000 |
| TA juice | Ethanol cider % | 53 | -0.510 | 0.000 |
| SSC juice | Ethanol cider % | 52 | 0.280 | 0.044 |
| pH juice | Ethanol cider % | 53 | 0.533 | 0.000 |
| SG juice | Ethanol cider % | 53 | 0.272 | 0.049 |
| Glucose juice | Ethanol cider % | 48 | 0.378 | 0.008 |

The juice trait strongest correlated to weight loss was FAN (Table 22). Out of the analysed timepoints there was a significant positive correlation day 3, 5, 10 and 20 but not day 48. The correlation was as strongest day 3 (n  36, r 0.885, p 0.000) (Figure 8) and then declined (Figures of the correlation day 5-48 are found in Appendix 3). In the beginning of the trial there were several correlations indicating a negative correlation between the different parameters showing sugar content and weight loss. However, later in the trial there was a positive correlation between glucose and weight loss that was as strongest day 20 (n 46, r 0.494, p 0.000).

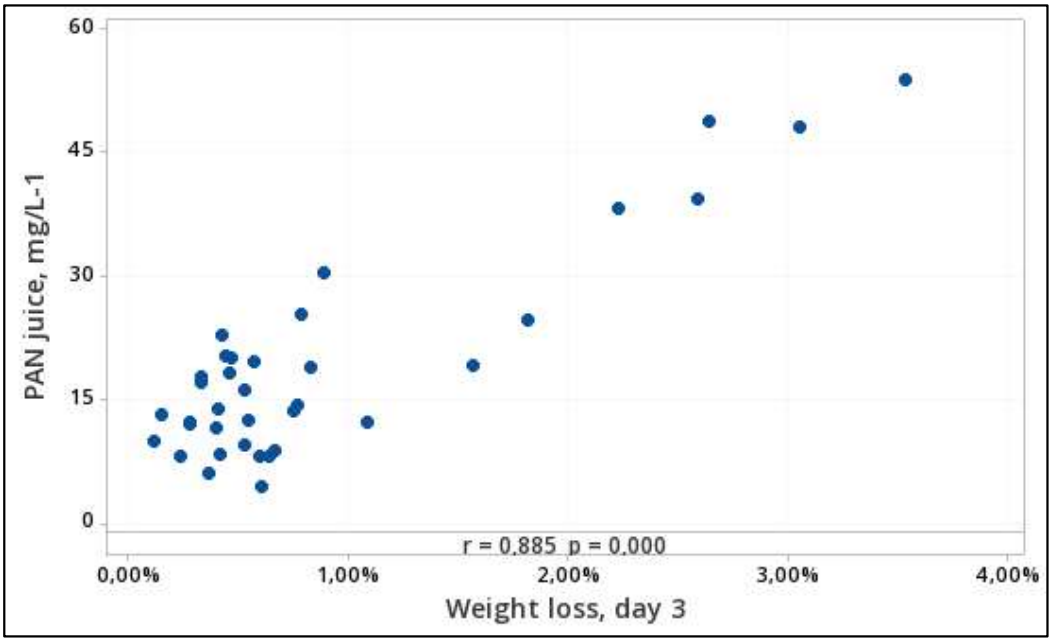


*Figure 8: Correlation between FAN content of juice and weight loss percentage day 3.*

*Table 22: Significant correlations between fermentability and juice traits.*

| | Juice trait | N | R-value | P-value |
|---|---|---|---|---|
| Weight loss day 3 | TPH juice | 48 | -0.414 | 0.003 |
| | SSC juice | 51 | -0.492 | 0.000 |
| | SG juice | 52 | -0.499 | 0.000 |
| | FAN juice | 36 | 0.885 | 0.000 |
| | Fructose juice | 46 | -0.373 | 0.011 |
| Weight loss day 5 | TPH juice | 48 | -0.424 | 0.003 |
| | SSC juice | 51 | -0.500 | 0.000 |
| | SG juice | 52 | -0.509 | 0.000 |
| | FAN juice | 36 | 0.876 | 0.000 |
| | Fructose juice | 46 | -0.350 | 0.017 |
| Weight loss day 10 | TPH juice | 48 | -0.405 | 0.004 |
| | SSC juice | 51 | -0.453 | 0.001 |
| | SG juice | 52 | -0.466 | 0.000 |
| | FAN juice | 36 | 0.784 | 0.000 |
| | Glucose juice | 47 | 0.398 | 0.006 |
| Weight loss day 20 | FAN juice | 36 | 0.565 | 0.000 |
| | Glucose juice | 46 | 0.494 | 0.000 |
| Weight loss day 48 | TA juice | 52 | -0.407 | 0.003 |
| | pH juice | 52 | 0.407 | 0.003 |
| | Glucose juice | 47 | 0.367 | 0.011 |

### 3.8.2 Correlation between results obtained from different analytical methods

Some traits was measured using two or several analytical methods. Correlations between the results of all of this cases were analysed and is reported in Table 7 and two examples can be seen in Figure 9 & 10. The results were strongly correlated in all cases.

*Table 23: Correlations between results of the same parameter obtained from different analytical methods. All parameters that were measured by more than one method are included in the table and show strong significant correlations.*

| Parameter | | N | R-value | P-value |
|---|---|---|---|---|
| **Sugar content of the juice** | | | | |
| SG juice | SSC juice | 52 | 0.989 | 0.000 |
| **Fermentation rate** | | | | |
| Weight loss day 48 | Decrease SG | 52 | 0.974 | 0.000 |
| **Potential alcohol content** | | | | |
| SSC Pot alc (%) 1 | SG Pot alc (%) | 52 | 0.989 | 0.000 |
| **Ethanol content of cider** | | | | |
| Ethanol cider % (Anton Paar) | Weight loss day 48 | 52 | 0.959 | 0.000 |
| Ethanol cider % (Anton Paar) | Calculated ethanol cider % | 53 | 0.977 | 0.000 |

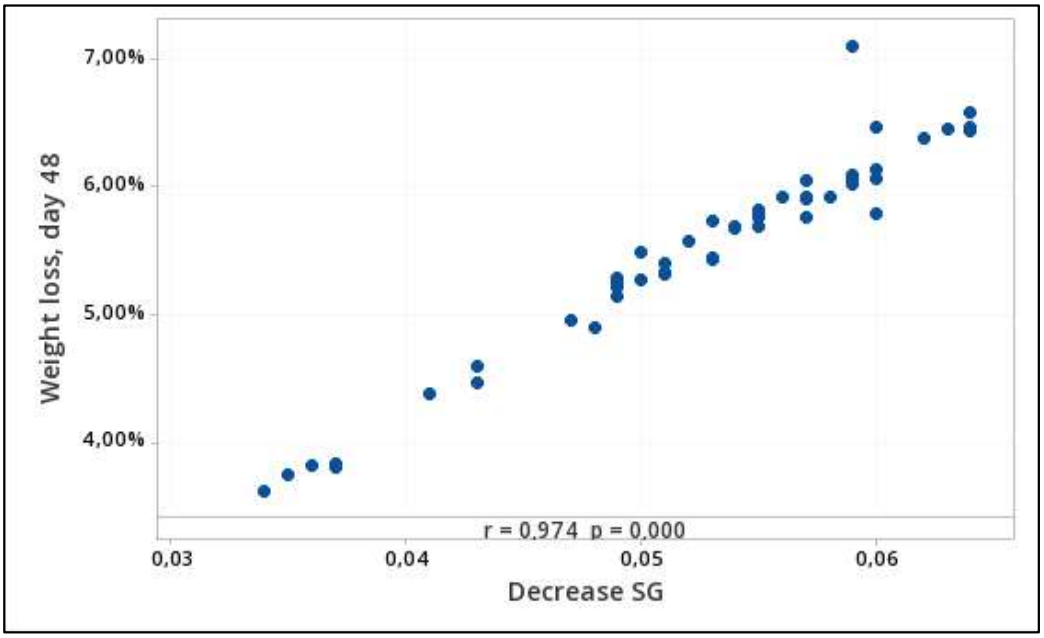


*Figure 9: Correlation between decrease of SG during fermentation and weight loss percentage day 48. There was strong positive correlation.*

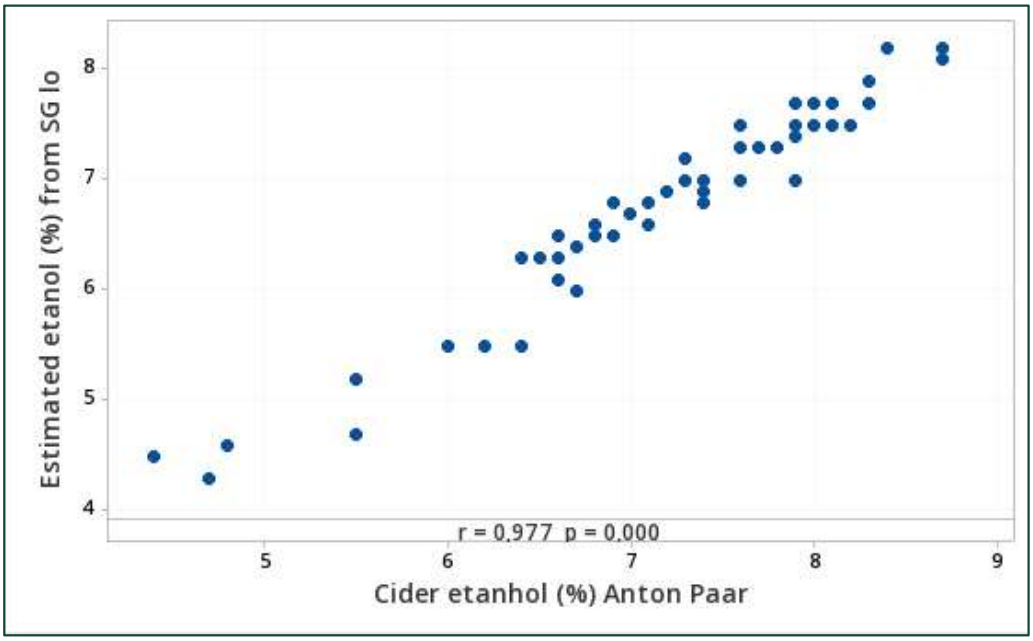


*Figure 10: There was a strong correlation between ethanol content measured using the Anton Paar method and the ethanol content estimated from loss of SG during fermentation.*

# 4. Discussion

The overall aim of this study was to generate knowledge relevant to Swedish cider producers regarding characteristics of juice and cider derived from British and French cider cultivars grown in the Swedish climate by investigating some essential traits of the juice before fermentation and the cider after fermentation. The aim was also to increase the knowledge of fermentability of apple juice and how it is affected by different traits, with a special focus of YAN. In the study important quality traits of the juice were analyzed before fermentation and of the cider after fermentation, fermentability of juice was also investigated. The study was based on two research questions: "How do British and French cider apple cultivars perform, in terms of essential juice and cider traits important for cider production, when grown in the Swedish climate?" and "How is the fermentability affected by YAN and other traits of the juice?".

### 4.1.1 Juice traits and classification of cultivars

*Sugar content*

'Tremletts Bitter', one of the bittersweet British cultivars, had significantly higher SSC, 17.70 and 'Santana' and 'Aroma', table cultivars, had significantly lower values, 11.97 and 11.90 Brix°. This was reflected by the categories where the table cultivars had lower mean values than the cider cultivars. To have a bittersweet cider cultivar in the top and table fruit cultivars in the bottom, as well as how the categories were ranked was expected, given the earlier results from Balsgård and the characters of the cultivars. Brix° values of some cultivars could be compared to Spoor et al. (2019), earlier Balsgård results and from Plotkowski and Cline (2021) that investigated the juice traits of some of the cultivars grown in Ontario, Canada, in 2017 and 2018. For the overlapping cultivars of the study, nothing deviating was found in the results of this study, especially considering the large annual variation reported by Spoor et al. Interestingly, 'Amorina', a mutant of 'Aroma' had a significantly higher mean value than 'Aroma', 13.83 Brix°. The two of them were however grown at different locations with different cultivation practices and harvested at different times so it is uncertain to say whether it is a general difference between the two of them or a coincidence. As expected the content of fructose accounted for the large part of the fermentable sugar content with mean values of glucose and fructose of juice for all cultivars was 16.09 and 69.60 g $L^{-1}$, respectively. Additional measurement of saccharose would have been favorable giving a more complete picture of the fermentable sugar content.

As expected there was a strong positive correlation between Brix and SG content of the juice (N 52, r 0.989, p 0.000), suggesting that the SG data of the juice is reliable even though not performed in triplicates and that the Anton Paar density meter performed adequately.

*Acidity and total acidity*

As it concerns acidity and total acidity the table cultivars as a group had notably low pH and high TA compared to the cider cultivars. However there were exceptions from the overall trend, especially the LARS cultivars but also the French showed a large variation within the groups. In both parameters the two LARS cultivars 'Debbie' and 'Three Counties' was the most and least acidic of all cultivars. It is wort to note that the LARS cultivars are derived from crosses between table and cider cultivars and that the French cultivars include 'Judeline' that is originally bred as a table cultivar but that had come to be used as a sharp cider cultivar, a large variation is therefore expected. As expected a range of cider cultivars had pH values above 3,8 and needed to be lowered to enable safe fermentation. In TA Plotkowski and Cline (2021) result are lower in all cultivars, however they conclude that their data of TA is all over lower than historical data. Their results are as well lower in three of four of the overlapping cultivars in pH, suggesting that some cultivars may be more acidic in the Swedish climate than the Canadian. This could however, of course, be due to chance and would benefit from further investigation. The results for 'Dabinett', 'Angela' and 'Jane' seems to, considering yearly variation, be inline with earlier data from Balsgård: Dabinett 0.14% (the result from this study is 0.21%), Angela 0.94% and 0.95% (results from two years, can be compared to the results from this study that is 0.73%) and Jane 0.21% and 0.24% (results from two years, can be compared to the results from this study that is 0.18%) (Spoor et al. 2019)."

Unfortunately pH was not measured in triplicates which, the variation within the cultivars was however quite small indicating accurate results. Data of acidity in the cider would have been able to add an interesting factor considering any possible malolactic fermentation that could have taken place.

*Total phenolic content*

As expected some traditional bittersweet cider cultivars was placed in the top of TPH content, with 'Tremlett's Bitter' having the highest mean value of 4.47 g GAE $L^{-1}$, and the table fruit cultivars 'Santana' and 'Aroma' in the bottom. The trend is reflected by the mean values of the groups with the group Other British having approximately ten times higher TPH content than the table cultivars. This was as expected due to that the categories contains only cultivars traditionally classified as bittersweet.

TPH mean value of all cultivars of this study is 1.6 g GAE $L^{-1}$, which can be compared with the mean value of not specified 442 apple genotypes reported by Karl et al. (2023): 1.3 g GAE $L^{-1}$, indicating that the values, considering the considerable part of bitter cultivars in this study are in a reasonable range. Spoor et al. (2019) does however overall report higher values, in eight out of eleven overlapping cultivars, if this depends on methodological differences or yearly variation is uncertain to say.

Unfortunately no data for 'Amorina' for juice could be presented, however looking at the TPH content of cider it had a surprisingly high mean value compared to 'Aroma'. One explanation to this could be the red skin of 'Amorina' compared to the greener skin of 'Aroma'. Differences in nutrient supply is another potential influential factor, 'Aroma' was grown commercially and 'Amorina' at Balsgård,

given that it has been suggested that reduced nutrient supply may increase phenolic content (Lea & Arnold 1978).

*Classification by LARS system*

To give an overwiev of the cider cultivars performance in the Swedish climate the data obtained in this study was used to classify the cultivars using the LARS classification system (Table 1). The results should be interpreted as examples of how the cultivars may perform in Sweden. As previously mentioned, traits of apple juice show annual variation and is dependant of the microclimate and it cannot be seen as a guarantee of fruit quality, however the results may hopefully contribute with knowledge of potential fruit quality when grown in Sweden. With that said: Most of the cultivars classified as traditionally, however, interestingly some cultivars were in this study reclassified due to low TPH values. With one expection, 'Tremlett's Bitter' that is in this study classified as bittersharp instead of bittersweet, the other three reclassified cultivars follows the same trend: TPH values of 'Angela' juice differed considerably from Spoor et al. (2019) that presented a TPH content of 2.43 GAE g $L^{-1}$. 'Prince William' shows a similar result: It was in this study classified as sweet instead of as traditionally bittersweet while Spoor et al. (2019) reports a mean value over the threshold, 2.18 GAE g $L^{-1}$. 'Three Counties' follows the same trend and is in this study classified as sweet instead of bittersweet but is however closer to the threshold value, Spoor et al. (2019) reports a higher value of 3.27 that is well above the threshold. Concerning the other results from Spoor et al. (2019) they conclude that most of the cultivars then included fell into the same categories as traditionally, however the three out of 16 that differed did that due to too low TPH content to reach the threshold value to be classified as bitter. Two of those cultivars were though included in this study as well, 'Lizzy' and 'Yarlington Mill', and did not show deviating values in this study. It is however worth noting, even if it does not concern the same cultivars and if the main part of the cultivars is not reclassified, that if the results are merged, in total six out of seven cases of reclassification this is done due to low TPH values.

Another aspect of the characteristics of the cultivars worth noting is that there was a tendency of the cultivars having either high phenolic and sugar content and low acidity, as the bittersweet cultivars, or low phenolic and sugar content and high acidity, as the table cultivars. This addresses the need of complementing the table cultivars currently grown with bittersweet cultivars to be able to produce ciders more similar to the British and French.

*Juice yield*

The average juice yield of the categories was higher of the table cultivars and the French cultivars, however the LARS cultivars did show large variation even in this trait. Overall the results were expected, except for the very low juice yield of 'Prince William'. They are a little bit lower than what is usually suggested to be acceptable but this is likely to be due to the small baches for pressing of 2000 g. Interestingly significant differences could be seen between cultivars and even if the juice yield probably would be higher when pressing in a larger scale the proportions between the juice yield of different cultivars can hopefully provide some knowledge.

It should be kept in mind that the maturity stage influences the traits of the juice (Tahir 2014). Determining the exact optimal time for harvest is a complex and time consuming procedure and reflects the same issue as the growers faces. It is likely that harvesting at another time point would have given other results. However, the aim has been to harvest as close to the optimal stage as possible for cider production, and the results are thereby relevant for cider producers even though they may vary depending on maturity stage. In one case the maturity most certainly had major influence of the results: Namely 'Prince William' with Streif Index of 0.03 that was overripe and transformed to purée when pressed and therefore gave a very low juice yield of 32%. If 'Prince William' were harvested earlier this would probably not have been the case. Another thing worth highlighting is that several other cultivars had as low Streif Index as 'Prince William' but had significantly higher juice yield, indicating the difference between cultivars in fruit texture and optimal maturity stage for harvest.

The results of this study should be interpreted with caution when compared to other studies using different methodologies. Concerning juice yield the results are dependent on production system and the results from this study may not be applicable in a larger production facility or when using other methods. However, the differences between the cultivars in juice yield can hopefully provide some insight about the characteristics of the cultivars. Given the high juice yield of 'Santana' this may be, in this aspect a suitable cultivar to be used in a blend as a sharp cider cultivar.

### 4.1.2 YAN, fermentability and ethanol content

*YAN*

The hypothesis was that the YAN content of the juice would be a limiting factor for fermentation of the juice. The content was therefore examined in both juice and ciders. Unfortunately, due to inconsistent results from the ammonia measurements, no data for ammonia was presented. One source of error is thought to be, since the nitrogen analyses are the only ones performed on microplate with undiluted samples, difficulties with pipetting as small volumes as 10 µL of slightly viscous samples. Another hypothetical source of error was the colour of the samples, it is possible that some of them had to strong colour and should have been treated according to the manufactures instructions for "strongly coloured samples" (*Ammonia Assay Kit (Rapid)* u.å.). Ideally, had time and resources permitted, greater effort would have been devoted to investigating why the method failed and how it could be improved.

However FAN, that is expected to account for the large part of YAN (Boudreau IV et al. 2018) values is presented for both juice and cider. Some data had to be excluded from the FAN analysis as well. Despite this a few significant differences between cultivars could be seen, supporting continues evaluations of the YAN content of apple juice.

Due to the high standard deviations for the cultivars it did not seem meaningful to compare them merged into categories. FAN values of the juice were in a range of 8.8 and 50.9 mg $L^{-1}$ and in cider 0.2 and 39.2. Assuming that the FAN values of

the juice represent the largest part of YAN the overall low values was expected, considering earlier reports and the management practices, except for for the cultivars obtained from a commercial grower, with little or none fertilization. Namely, nitrogen fertilization can increase YAN content of the juice (Karl et al. 2023.), Lea & Arnold (1978) report a considerable decrease of 50% of nitrogen content between fruit fertilized and not fertilized 'Dabinett' trees.

*Fermentability*

Results of fermentability of the different juices was reported trough several traits: SG values from before and after fermentation to capture the consumption of sugar by the yeast, ethanol content to check the final product of fermentation and as weight loss of the samples, a way to monitor the fermentation process, in terms of $CO_2$ production, without disrupting or contaminating the samples.

First of all, the fermentation rate, i.e. the weight loss percentage, varied a lot between the samples but was overall slower than expected. The plan was to let all samples ferment until they stopped spontaneously, this was however not possible within the time frame of the study given the fermentation rate. A few cultivars stood out by a rapid fermentation already from the first day of the trial, namely 'Santana', 'Muscadet de Dieppe' and 'Aroma', even 'Black Dabinett' fermented quite fast. The significant differences between 'Aroma' and 'Amorina' until day 20 was unexpected and indicates that cultivation practices, maturity or location influenced the results. Unlike the large difference within the Table and French cultivars that showed large variability, the LARS cultivars showed surprisingly similar progress (Figure 5).

The correlation between weight loss of bottles and content of FAN in apple juice supports the hypothesis that yeast assimilable nitrogen is a limiting factor for yeast growth and fermentation. Of course the lack of data of ammonia complicate conclusions. However, given that the hypothesis was true there should have been a positive correlation between YAN content and weight loss. A such correlation, of declining strength, between FAN and weight loss could be seen in the beginning of the experiment, suggesting that higher FAN content led to faster start of fermentation. However, those analyses were affected by that a few cultivars, 'Santana' and 'Muscadet de Dieppe' had much higher FAN content and also fermented much faster than most of the others, i.e. a few samples had great impact of the results. However, the fact remains, there was a correlation, and given the fact that nitrogen is an important source of nutrition for the yeast it is suggested that FAN content of the juice seems to have had an impact on the fermentation rate. Given that most cultivars seems to have low YAN values compared to e.g. recommended values for wine of 200-350 mg $L^{-1}$ (Karl et al. 2023), this could have been a contributing factor to the slow fermentation rate.

It seems to be consensus that apple juice, concerning YAN, compared to standards for wine, is deficient. However the consequences of this can be seen in different perspectives, e.g. Valois et al. (2006) concludes:

> Based on these contributing factors and the YAN values that we observed, it may be advisable to supplement juice from low N varieties with available N sources for consistent fermentation, unless the cider-maker intends to make a bottle conditioned

> naturally effervescent cider with 4 to 5% alcohol and 1-2% residual sugar content. (Valois et al. 2006:14)

Considering that many craft cider makers intentionally aims to do exactly this, the low YAN levels in apple juice are not necessarily a problem. Rather, they represent an important aspect of the cider-making process that producers needs to be aware of, and one that would benefit from further research. Since YAN content seems to be dependent of cultivation practices, YAN content within the same cultivars grown in different cultivation systems is suggested as a topic for further research.

Considering the glucophillic character of *Saccharomyces* (Berthels et al. 2004) it is also worth noticing that 'Muscadet de Dieppe' and 'Black Dabinett', the two cultivars with highest mean values of glucose content, significantly different from the others, also were cultivars with considerably high fermentation rate in the beginning of the trial. However the lower results of 'Santana' and 'Aroma', the other fastest fermenting cultivars is contradictive and the overall association between glucose content of juice and fermentation rate measured as weight loss of the samples was weak. However, it is possible that part of the explanation to why some cultivars fermented so much faster lies in a combination of YAN and glucose content. One aspect that was not covered in this study is vitamins, that may beside sugar content, YAN etc. play a great role in the fermentability of the cider. This would hence be an interesting perspective to add if repeating the study. There was, a positive correlation between final weight loss, day 48, and pH (and a corresponding negative correlation for TA) (n 52, r 0.407, p 0.003). The correlation is not strong, but significant, and could potentiallt indicate an inhibitory effect on yeast growth of high acidity.

### 4.1.3 Cider traits

TPH was measured in both juice and cider, mean values of all cultivars were 1.61 in juice and 1.46 g gallic acid equivalents $L^{-1}$ in cider, indicating a general small decrease during fermentation. One explanation of this decrease is likely to be the sedimentation that took place in most of the samples during fermentation. Concerning residual sweetness, a desirable trait for many cider producers, the highest SG values of cider after 48 days of fermentation are worth noting: 'Tremletts Bitter' with SG of 1.033 that is equal to residual sugars of 70.3 g $L^{-1}$, 'Judeline', 1.018, equal to 38.3 g $L^{-1}$, and 'Harry Master's Jersey', 1.014 equal to 29.8 g $L^{-1}$.

To evaluate the methods used correlations were analysed: The decrease in SG was strongly and positively correlated to weight loss of the bottles at the end of the trial (N 52, r 0.974, p 0.000). This suggests that the sugar have been consumed and that the samples lost weight from $CO_2$ to the same degree, indicating that alcoholic fermentation took place as supposed and that the two methods worked satisfactory. Something went wrong with the enzymatic analysis. As with the ammonia analysis, had time and resources permitted, greater effort would have been devoted to investigating why the method failed and how it could be improved.

The estimations of potential alcohol content made from SG and SSC of the juice gave similar results that was strongly correlated, however the estimations based on SSC were slightly higher. The hypothesis is that SSC measurements slightly may

have been overestimated the sugar content due to larger interference of other soluble solids. Another explanation lays in that SSC values have to be estimated as SG, it is possible that this formula do not gave accurate results in this case. However, the difference between the methods was small. The calculated ethanol content, from decrease in SG, was strongly correlated to the measured values, obtained by the Anton Paar devices (n 53, r 0.977, p 0.000) supporting the accuracy of the Anton Paar method. The Anton Paar devices can, based on this study, be recommended as a fast and cost-efficient method of measuring alcohol content in cider. Interestingly, it was noted that the measured values overall was a little bit higher than the calculated, the difference was not big, but seemed to increase in samples with higher TPH content. This suggest that one or both of the methods potentially needs to be slightly adjusted to be suitable for bittersweet apples.

### 4.1.4 Limitations

The experimental design was not optimal in all aspects and limits the generalizability of the results. The trial was performed with a variation of rootstocks which can have had an influence of the results, since the rootstock has been showed to have an impact on the biochemical content in the fruit (Kviklys et al. 2014). Another factor to consider is the maturity of the fruit, which impacts the chemical content of the fruit (Tahir 2014). The method for measuring fruit maturity gave inconsistent results and it is therefore not certain to say that all cultivars were harvested at the optimal time. It is however reasonable to assume that 'Frequin Rouge' were less mature than the others and that all of the others were in a range of Streif index of somewhere around 0.03-0.07, i.e. all of the batches used in the study seemed to be very mature compared to what is suitable for storage, e.g. Streif index 0.18-0.22 is recommended for fruit for fresh consumption to be stored in ULO storage (Tahir 2014). Even if maturity stage possibly had an impact of some factors it did probably not affect the TPH content, namely tannin concentration is relatively stable during fruit development even if TPH can increases during storage, possibly due to water losses (Ewing et al. 2019). It has however been shown that not only juice yield but also juice traits such as SSC, TPH and acidity can be affected by the press construction (Wilczyński et al. 2019). The differences is suggested to i.e. be due to more intensive mixing and grinding of the raw material by some methods than others (Wilczyński et al. 2019). This addresses the complexity in comparing the results with other data, but also the need of not only choosing sufficient cultivars for production of cider – but also sufficient press constructions.

Another obstacle, that was already discussed, was the lack of data of ammonia that would have provided a more complete picture of the YAN content and its effect of fermentability. The enzymatic methods for analysis of ammonium, but also for ethanol, needs to be further developed for apple juice.

Given what was mentioned above, there are some obstacles with making general conclusions based on the study. Regardless, seen as preliminary investigation it may hopefully provide some useful information of the potential characteristics of juice and ciders from cider apples grown in Sweden.

## 4.2 Conclusions

- Classification, based on the results of this study, of most cider apple cultivars tested were consistent according to the LARS classification system and compared to published information, even though some cultivars showed lower TPH content, indicating a good quality of French and British cider apple cultivars is possible to achieve when they are grown in Sweden.
- There was a tendency of the cultivars having either high phenolic and sugar content and low acidity or, as table cultivars that is currently grown in Sweden, low phenolic and sugar content and high acidity. This addresses the need of complementing the table cultivars with bittersweet cultivars to be able to obtain cider qualities more similar to the British and French.
- The apple cultivars included in the study differed in fermentability, possibly affected by nitrogen.
- The content of free amino nitrogen was overall low, with can be seen as an advantage if the aim is to produce naturally effervescent, low alcohol cider with residual sugar.
- The enzymatic methods for analysis of ammonium and ethanol needs to be further developed for apple juice.

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# Popular science summary

I some countries, with long history of cider making, special cider apple cultivars of apples is used for cider production. Those cultivars usually have higher content of bitter tasting compounds than fruit used for fresh consumption. It is possible to make cider out of the apples typically used for fresh consumption, that is currently grown in Sweden, as well, but it will not get the bitter taste that is characteristic for traditional apple cider from for example France of UK. There is therefore an interest, from cider producers in Sweden, to start cultivating cider apple cultivars in Sweden. To do this successfully there is a need for knowledge in how the cultivars perform in the Swedish climate. Therefore levels of sugars, acidity and bitter tasting compounds was measured in juice made from apples of cider apple cultivars that was grown as a test in Sweden. The measured levels of this traits did not show anything deviating compared to earlier results, taking into account that there normally is a large annual variation within the same cultivar.

In cider making apple juice is fermented, an process where yeast converts sugars to carbon dioxide and alcohol. To do this the yeast needs a sufficient supply of nutrients, for example of nitrogen. The content of nitrogen and its effect of how fast the juices fermented was therefore also investigated in the study. Interestingly there were quite large differences in how fast the ciders made from different cultivars fermented. Due to some methodological problems to measure the nitrogen, there unfortunately were some obstacles with making definite conclusions based on the nitrogen content. However, the results from this study indicated that the nitrogen content positively affected the rate of the fermentation process. The overall nitrogen levels in the juices were comparatively low. With that said, a low nitrogen content and a slow rate of fermentation can be a good thing, it is actually commonly utilized in French cider making to enable the aromas to fully develop.

No recommendations of which cultivar to grow or predictions of exactly how it will behave in Sweden can be given from this study, but hopefully it can provide some insight in the possibilities in cider making to apple growers and cider producers in Sweden.

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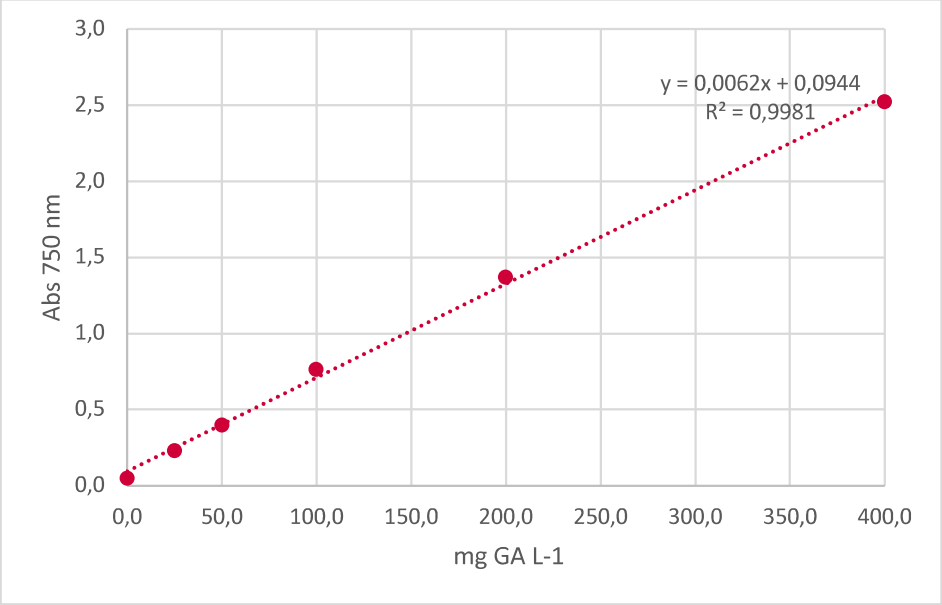
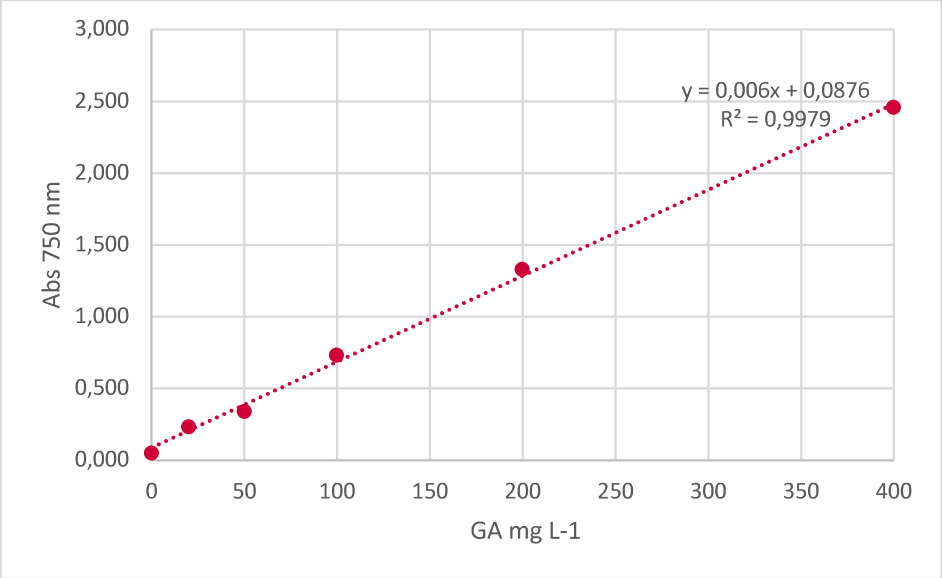
# Appendix 1: Standard curves for TPH analyses




*The standard curve for micro plate assays of TPH, used for juice samples, showing absorbance of different concentrations of gallic acid at 750 nm.*



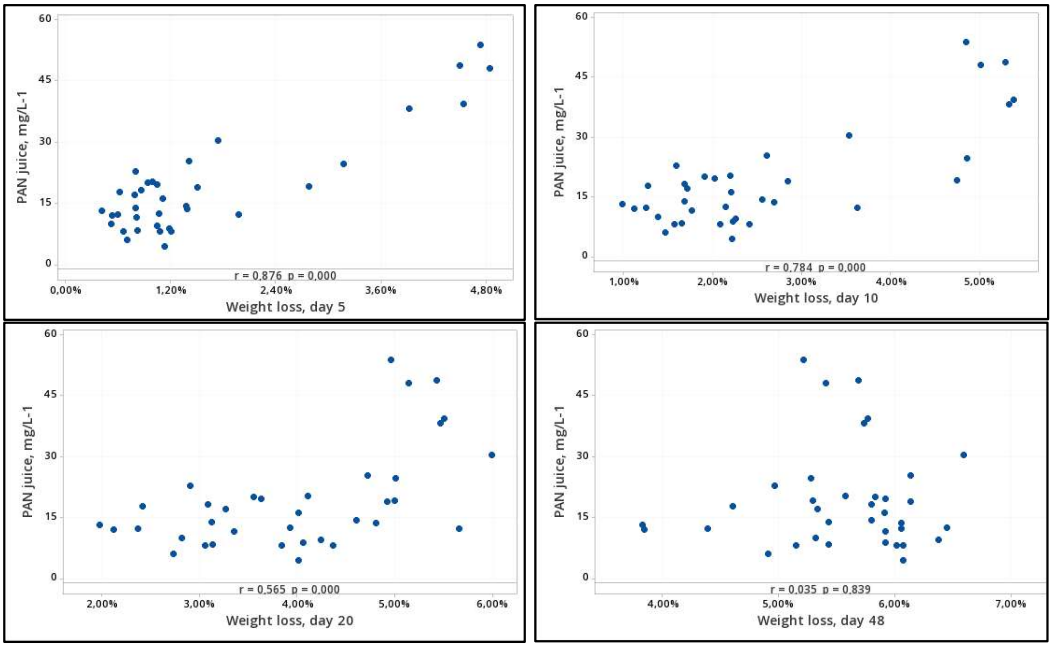

*The standard curve for micro plate assays of TPH, used for cider samples, showing absorbance of different concentrations of gallic acid at 750 nm.*

# Appendix 2: Correlations between different parameters

| Correlations juice trait - weight loss | | N | Correlation | P-Value |
|---|---|---|---|---|
| TPH juice | Day 3 | 48 | -0,414 | 0,003 |
| TA juice | Day 3 | 52 | 0,118 | 0,407 |
| SSC juice | Day 3 | 51 | -0,492 | 0,000 |
| pH juice | Day 3 | 52 | -0,169 | 0,231 |
| SG juice | Day 3 | 52 | -0,499 | 0,000 |
| FAN juice | Day 3 | 36 | 0,885 | 0,000 |
| Glucose juice | Day 3 | 47 | 0,163 | 0,275 |
| Fructose juice | Day 3 | 46 | -0,373 | 0,011 |
| TPH juice | Day 5 | 48 | -0,424 | 0,003 |
| TA juice | Day 5 | 52 | 0,096 | 0,499 |
| SSC juice | Day 5 | 51 | -0,500 | 0,000 |
| pH juice | Day 5 | 52 | -0,149 | 0,291 |
| SG juice | Day 5 | 52 | -0,509 | 0,000 |
| FAN juice | Day 5 | 36 | 0,876 | 0,000 |
| Glucose juice | Day 5 | 47 | 0,245 | 0,097 |
| Fructose juice | Day 5 | 46 | -0,350 | 0,017 |
| TPH juice | Day 10 | 48 | -0,405 | 0,004 |
| TA juice | Day 10 | 52 | 0,006 | 0,965 |
| SSC juice | Day 10 | 51 | -0,453 | 0,001 |
| pH juice | Day 10 | 52 | -0,059 | 0,678 |
| SG juice | Day 10 | 52 | -0,466 | 0,000 |
| FAN juice | Day 10 | 36 | 0,784 | 0,000 |
| Glucose juice | Day 10 | 47 | 0,398 | 0,006 |
| Fructose juice | Day 10 | 46 | -0,275 | 0,065 |
| TPH juice | Day 20 | 47 | -0,306 | 0,036 |
| TA juice | Day 20 | 51 | -0,230 | 0,104 |
| SSC juice | Day 20 | 50 | -0,229 | 0,109 |
| pH juice | Day 20 | 51 | 0,198 | 0,163 |
| SG juice | Day 20 | 51 | -0,241 | 0,088 |
| FAN juice | Day 20 | 36 | 0,565 | 0,000 |
| Glucose juice | Day 20 | 46 | 0,494 | 0,000 |
| Fructose juice | Day 20 | 45 | -0,151 | 0,322 |
| TPH juice | Day 48 | 48 | -0,115 | 0,437 |
| TA juice | Day 48 | 52 | -0,407 | 0,003 |
| SSC juice | Day 48 | 51 | 0,130 | 0,365 |
| pH juice | Day 48 | 52 | 0,407 | 0,003 |
| SG juice | Day 48 | 52 | 0,117 | 0,407 |
| FAN juice | Day 48 | 36 | 0,035 | 0,839 |
| Glucose juice | Day 48 | 47 | 0,367 | 0,011 |
| Fructose juice | Day 48 | 46 | 0,017 | 0,913 |
| Correlations juice trait – juice trait | | N | Correlation | P-Value |
| TA juice | TPH juice | 49 | -0,445 | 0,001 |
| SSC juice | TPH juice | 48 | 0,792 | 0,000 |
| pH juice | TPH juice | 49 | 0,512 | 0,000 |
| SG juice | TPH juice | 49 | 0,753 | 0,000 |
| FAN juice | TPH juice | 34 | -0,252 | 0,150 |
| Glucose juice | TPH juice | 44 | 0,384 | 0,010 |
| Fructose juice | TPH juice | 43 | 0,597 | 0,000 |
| SSC juice | TA juice | 52 | -0,660 | 0,000 |
| pH juice | TA juice | 53 | -0,959 | 0,000 |
| SG juice | TA juice | 53 | -0,612 | 0,000 |
| FAN juice | TA juice | 37 | 0,056 | 0,744 |
| Glucose juice | TA juice | 48 | -0,551 | 0,000 |
| Fructose juice | TA juice | 47 | -0,357 | 0,014 |
| pH juice | SSC juice | 52 | 0,748 | 0,000 |
| SG juice | SSC juice | 52 | 0,989 | 0,000 |
| FAN juice | SSC juice | 36 | -0,437 | 0,008 |
| Glucose juice | SSC juice | 47 | 0,341 | 0,019 |
| Fructose juice | SSC juice | 46 | 0,708 | 0,000 |
| SG juice | pH juice | 53 | 0,704 | 0,000 |
| FAN juice | pH juice | 37 | -0,121 | 0,474 |
| Glucose juice | pH juice | 48 | 0,531 | 0,000 |
| Fructose juice | pH juice | 47 | 0,421 | 0,003 |
| FAN juice | SG juice | 37 | -0,480 | 0,003 |
| Glucose juice | SG juice | 48 | 0,284 | 0,050 |
| Fructose juice | SG juice | 47 | 0,695 | 0,000 |
| Glucose juice | FAN juice | 34 | 0,237 | 0,177 |
| Fructose juice | FAN juice | 33 | -0,324 | 0,065 |

| Correlations juice traits – cider traits | | N | Correlation | P-Value |
|---|---|---|---|---|
| TPH juice | SG cider | 49 | 0,609 | 0,000 |
| TA juice | SG cider | 53 | -0,012 | 0,929 |
| SSC juice | SG cider | 52 | 0,487 | 0,000 |
| pH juice | SG cider | 53 | 0,058 | 0,680 |
| SG juice | SG cider | 53 | 0,493 | 0,000 |
| FAN juice | SG cider | 37 | -0,302 | 0,069 |
| Glucose juice | SG cider | 48 | -0,083 | 0,573 |
| Fructose juice | SG cider | 47 | 0,433 | 0,002 |
| TPH juice | TPH cider | 49 | 0,979 | 0,000 |
| TA juice | TPH cider | 53 | -0,483 | 0,000 |
| SSC juice | TPH cider | 52 | 0,834 | 0,000 |
| pH juice | TPH cider | 53 | 0,564 | 0,000 |
| SG juice | TPH cider | 53 | 0,796 | 0,000 |
| FAN juice | TPH cider | 37 | -0,232 | 0,168 |
| Glucose juice | TPH cider | 48 | 0,357 | 0,013 |
| Fructose juice | TPH cider | 47 | 0,548 | 0,000 |
| TPH juice | FAN cider | 27 | 0,085 | 0,674 |
| TA juice | FAN cider | 31 | -0,128 | 0,494 |
| SSC juice | FAN cider | 30 | -0,101 | 0,597 |
| pH juice | FAN cider | 31 | 0,073 | 0,698 |
| SG juice | FAN cider | 31 | -0,154 | 0,409 |
| FAN juice | FAN cider | 23 | 0,245 | 0,259 |
| Glucose juice | FAN cider | 27 | -0,234 | 0,240 |
| Fructose juice | FAN cider | 27 | -0,272 | 0,170 |
| TPH juice | Ethanol cider % | 49 | 0,057 | 0,697 |
| TA juice | Ethanol cider % | 53 | -0,510 | 0,000 |
| SSC juice | Ethanol cider % | 52 | 0,280 | 0,044 |
| pH juice | Ethanol cider % | 53 | 0,533 | 0,000 |
| SG juice | Ethanol cider % | 53 | 0,272 | 0,049 |
| FAN juice | Ethanol cider % | 37 | -0,033 | 0,848 |
| Glucose juice | Ethanol cider % | 48 | 0,378 | 0,008 |
| Fructose juice | Ethanol cider % | 47 | 0,094 | 0,530 |

# Appendix 3: Correlations between FAN content of juice and fermentability




*Correlations between FAN content of juice and weight loss percentage day 5, 10, 20 and 48. Significant positive correlations day 5 and 10 and a weak positive correlation day 20. No correlation day 48.*

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