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Chemical and sensory analyses of juice, cider and vinegar produced from different apple cultivars

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Cover illustration: Apple cider of ten different cultivars just before decanting. From left: Aroma, Baldwin, Belle de Boskoop, Bramley, Cortland, Gravensteiner, Ingrid-Marie, Jonathan, Rubinola and Spartan.

Abstract

The interest for locally produced food is increasing due to consumer concern about the environment, distrust of industrial foods and a demand for high quality products. Apple is the predominant fruit crop in Sweden, and by processing apples into cider and vinegar, these products could significantly contribute to the development of the market of local foods.

In this study different yeast types and different bacterial cultures were evaluated for their suitability in cider and vinegar production from cloudy apple juice. Ten apple cultivars (Aroma, Baldwin, Belle de Boskoop, Bramley, Cortland, Gravensteiner, Ingrid-Marie, Jonathan, Rubinola and Spartan) were also evaluated for their suitability for production of juice, cider and vinegar. Chemical analyses including total soluble solids, titratable acidity and total phenols were performed on the products along with sensorial evaluation by taste panels.

The yeast strains were shown to have an effect on fermentation rate and the resulting content of total phenols in ciders fermented from cloudy apple juice. Dry commercial starter strains gave a higher appreciated cider compared to cider that was spontaneously fermented, and the ale yeast Safale S-04 was concluded to be the most suited for fermentation of cloudy apple juice.

For vinegar production, the bacterial culture had an effect on TSS, but not on any other chemical or taste characteristics. Clear differences in acceptability were found between the cultures; the culture from Alles um den Essig, intended for the submerged method, seemed to better be suited to the used production system compared to cultures developed for the surface method.

The cloudy apple juice from the different cultivars varied significantly in chemical composition, with TSS in the range of 9.6–15.1%, TA 0.41–1.24% and total phenols 123.9–850.0 mg GAE/L. The comparatively sweet juices of Jonathan and Spartan obtained the highest acceptance whereas juices with lower TSS/TS ratios were less acceptable by the taste panel. During fermentation into cider, the TSS decreased differentially in the cultivars, whereas the differences in TA and total phenols were unaffected. Ciders that were perceived to be comparatively sweet were accepted to a higher degree. The fermentation enhanced the taste differences between the cultivars, and Jonathan and Spartan, were also most accepted as ciders. For vinegar, the differences in traits decreased, and of all the chemical parameters only content of total phenols separated the cultivars. There was however a tendency of lower acceptance of vinegar from Belle de Boskoop, Gravensteiner and Jonathan.

It was concluded that several aspects influence the quality of cloudy apple juice, cider and vinegar, including cultivar, ripeness and choice of microorganisms for fermentation. For juices and ciders, sweeter products were preferred to a large extent, and the TSS/TA ratio appears to be a good predictor of consumer acceptance. In this study, an apple cultivar with a juice of good taste generally also produced a good cider, whereas the cultivar was of less importance for vinegar production.

Sammanfattning

Under senare år har intresset för lokalproducerad mat ökat, av marknads-, miljö- och kvalitetsskäl. I Sverige domineras fruktproduktionen av äpple, och genom vidareförädling av dessa till cider och vinäger kan nya produkter introduceras och bredda utbudet av lokal mat.

I den här studien jämfördes olika jästsorters och bakteriekulturers lämplighet för framställning av cider och vinäger. Tio olika äppelsorter (Aroma, Baldwin, Belle de Boskoop, Bramley, Cortland, Gravensteiner, Ingrid-Marie, Jonathan, Rubinola and Spartan) pressades till must som sedan fermenterades till cider och vinäger med de tidigare utvalda mikroorganismerna. Kemiska analyser av socker- och syrainnehåll samt totala fenoler genomfördes på musten, cidern och vinägern, medan de sensoriska egenskaperna utvärderades av en smakpanel.

Resultaten visade att typen av jäst påverkade jäshastigheten och innehållet av fenoler i cidern. Kommersiella torrjäster gav en cider som i högre utsträckning gillades av smakpanelen jämfört med vildjäst cider. Ale-jästen Safale S-04 befanns vara lämpligast för fermentering av äppelmust.

Vid produktionen av vinäger hade valet av bakteriekultur effekt på innehållet av löslig torrsubstans (TSS), men inte på några andra analyserade kemiska eller sensoriska parametrar. Det var emellertid stora skillnader i gillande av de olika vinägerprodukterna. Bakteriekulturen från Alles um den Essig, bedömdes vara mest lämpad för det fermenteringssystem som användes.

Det var stor variation hos äppelmust tillverkad från de olika äppelsorterna i samtliga analyserade egenskaper; TSS (9,6–15,1%), TA (0,41–1,24%) och totalhalten fenoler (123,9–850,0 mg GAE/L). Det var en lägre acceptans för must med låg kvot mellan TSS och TA, och de jämförelsevis söta mustarna av äppelsorterna Jonathan och Spartan gillades mest av smakpanelen.

Vid fermentering till cider minskade TSS i varierande grad hos de olika sorterna medan skillnaderna i TA och totalhalten fenoler bestod. En cider med högre sötma mottogs bättre av smakpanelen och även som cider hade Jonathan och Spartan högst acceptans.

I vinägern framställd av de olika äppelsorterna, var skillnaderna i både de kemiska och smakmässiga egenskaperna små och endast innehållet av fenoler varierade signifikant. Ingen signifikant skillnad fanns mellan sorterna beträffande acceptans, men det var tendens för lägre gillande av vinäger som framställts av Belle de Boskoop, Gravensteiner och Jonathan.

Slutsatserna var att kvaliteten i must, cider och vinäger påverkas av många olika faktorer, så som äppelsort och mognadsgrad samt val av jäst och bakteriekultur. En must eller cider med mer sötma uppskattades i högre utsträckning, och kvoten mellan socker och syra skulle kunna användas för att förutsäga konsumentens gillande. Slutligen visades att en äppelsort som hade en välsmakande must ofta även gav en god cider, men att äppelsorten hade mindre betydelse vid vinägerframställning.

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1 Introduction

The consumer demand for locally produced and regional foods is increasing, since these products are perceived as having better quality and taste, and are considered to be friendlier to the environment (Jordbruksverket, 2010). There is also an increased interest from the consumer to know the producer behind the product. In addition, by consuming local foods the agriculture and the job market in the neighbourhood can be supported.

Fruits and vegetables produced in Sweden are said to have extraordinary quality, because of the cold climate and long summer days (Landsbygdsdepartementet, 2010). These factors make them suitable as raw material for further processing. Apple is the major fruit crop in Sweden and the produce is mainly aimed for the fresh market. In processing industry, apples are often used for production of sauce and juice, but can also be processed into other products, such as cider and cider vinegar. Cider and vinegar could be new products suitable for small scale production and valuable supplements to the today rather small number of local foods.

Cider is a common beverage in England, France, Germany and some other countries with a long tradition, whereas the Swedish true cider production is limited but increasing since the middle of 1990's (Sveriges Bryggerier, 2010). Due to the focus on production for the fresh market, the apple cultivars grown in Sweden are often dessert fruits that lack some of those quality attributes that characterise typical cider apple cultivars that have long been bred and selected for in traditional cider countries.

Apple cider vinegar is a product with increasing interest in the recent years, because of proposed health benefits brought about by a diet containing vinegar, besides its wide culinary usability. Vinegar is sometimes the result of failed cider fermentation or poor storage conditions for cider, but the modern commercial production is a highly refined process.

Cider and vinegar are both produced by fermentation. During alcoholic fermentation, yeasts utilise sugar in apple juice to produce ethanol, an anaerobic process that results in cider. The production of vinegar involves an additional aerobic fermentation step, where acetic acid bacteria convert ethanol in cider into acetic acid. Except for the cultivars used, the sensorial characteristics of cider and vinegar are highly dependent on the microorganisms used in the fermentation processes along with the selected processing techniques (Downing, 1989; Lea, 1989).

This report consists of a literature study that will give an overview of processing methods for apple juice, cider and vinegar, and an experimental part where different yeasts, bacteria and apple cultivars are evaluated for juice, cider and vinegar production.

1.1 The stepwise process of juice, cider and vinegar production

The production of cider and vinegar from apples are fermentation processes, with three different products; the initial juice, the intermediary cider and the final vinegar (Figure 1). Several different treatments can be applied along the production line to modify and influence the processing of juice, cider and vinegar. Which treatments that are used depend on the requirements on the product and its desired quality.

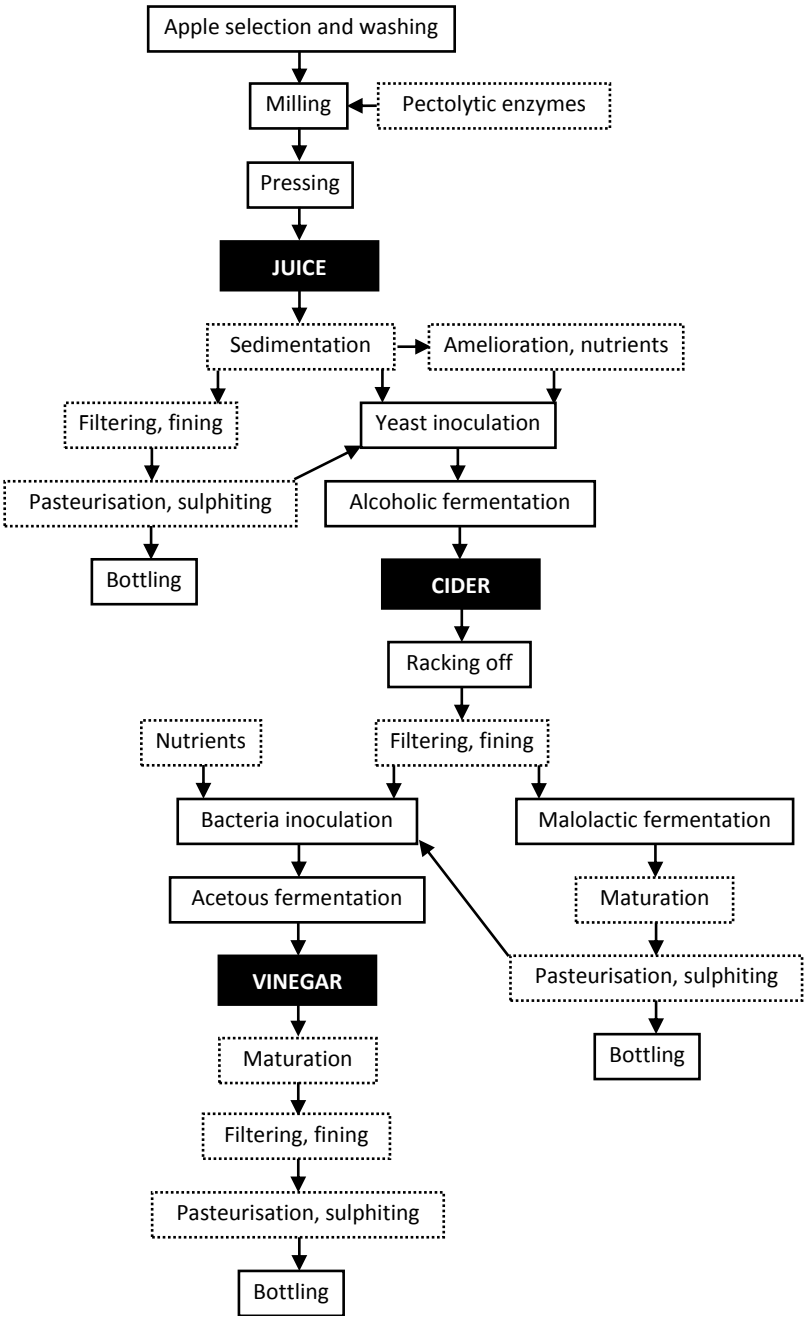

Figure 1. Process flow chart of juice, cider and vinegar from apples. Boxes with dotted lines are optional steps depending on desired production efficiency and quality characteristics. Chart modified from Joshi & Sharma (2009).

1.2 Apple juice

Apple juice is a well-known and appreciated beverage all over the world, with the dark golden and clear types being the most common (Bump, 1989). Cloudy apple juice, also called natural, is more similar to the juice immediately obtained after juice extraction and contains small insoluble particles, such as plant cell fragments, pectins and proteins that make it more or less turbid (Kilara & Van Buren, 1989). In production of cloudy apple juice, the largest particles are allowed to settle and the juice can be decanted once, but no further treatments are made to change the opacity (Bates et al., 2001). Both clear and cloudy apple juice can be used for production of cider and vinegar, but often only a rough filtration of the juice is made before fermentation, and if needed and desired, clarification treatments are applied either to the finished cider or vinegar (Lea, 1989).

1.2.1 Apple juice extraction

The basic principle of apple juice extraction is to disintegrate the fruits into smaller pieces and then extract the juice out from this pomace. Before extraction, the selected apples are washed in cold water and all damaged fruits are discarded. The apples can be crushed, grinded or milled with different methods, where the most ancient is to place the apples in a container and mash them mechanically with a pole (Proulx & Nichols, 2003). In modern production, the pomace is obtained by a hammer mill or a grating mill, where the former gives variable particle sizes whereas the latter gives uniform pieces (Bump, 1989). By changing the screens for the mills, the particle size giving the most efficient extraction can be chosen (Bates et al., 2001).

To extract the juice from the mash, three main types of presses are used in commercial production of apple juice; the hydraulic press, the screw press and the belt press (Bump, 1989). In the basic type of hydraulic press, the rack and frame press, the pomace is enclosed in cloths, and by use of a frame the pomace is formed to a cheese (Bates et al., 2001¸ Bump, 1989). Several cheeses are placed on each other before application of a constant vertical force by a horizontal plate. In more advanced hydraulic presses no cloth is used and the pomace is loaded to a stainless steel chamber to which the force is applied. Unlike the hydraulic press, the screw press is operated continuously and has a high working capacity (Bates et al., 2001). The large screw is enclosed in a cylinder screen of stainless steel (Bump, 1989). When operating, the mash is pressed radially against the screen, and as the juice is being extracted the press cake is moved through the cylinder and released in the end. The belt press is also used for continuous operation, where the pomace is transported on a permeable belt and passes over rotating rolls that presses the juice out of the pomace (Bates et al., 2001).

Two additional types of presses are the bladder press and the basket press. These are mainly used in small and medium scale production (Bates et al., 2001; Proulx & Nichols, 2003). The bladder press extracts in a batch operation, and is composed of a rubber bladder enclosed by a mesh

stainless steel cylinder (Bates et al., 2001). The pomace is put between the bladder and the cylinder, and by filling the bladder with water or air, it expands and presses the pomace against the cylinder screen walls and juice can trickle through the mesh. The basket press is one of the most ancient types of presses. It is made of a wooden basket where the pomace, enclosed in a cloth, is placed and a screw applied from above on a vertical plate compresses the pomace, and juice is drained off at the bottom (Proulx & Nichols, 2003).

The raw juice from all presses is cloudy due to its content of small apple pieces, and also sensitive to browning because of oxidation of phenolic compounds (Bump, 1989). Decreased browning can be achieved by addition of ascorbic acid during the mashing process, or by excluding as much air as possible during processing, for example by adding a preventive layer of nitrogen gas (Bates et al., 2001). The yield at juice extraction is dependent on several factors, such as cultivar, maturity and equipment (Bump, 1989). Pectolytic enzymes can be added when milling the apples. Their ability to enhance the breakdown of cellular structures and release the juice, can help to improve the yield considerably (Joshi & Sharma, 2009).

1.3 Cider

There are several different cider types, and traditional cider countries as Great Britain and France have their own specialities (Morgan & Richards, 1993). The French cider is often produced in a more natural way without additives and other modern treatments, compared to the English cider production. Because of the different production methods, the French cider has more fruitiness whereas the English has a higher alcohol content.

In North America and Australia, the word ‘cider’ refers to the raw pressed unfermented apple juice, while ‘hard cider’ is more or less synonymous with the alcoholic beverages from Western Europe (Downing, 1989). The Swedish regulations for what kind of beverages, that can be marketed as ‘cider’, differ from those in some other European countries. In Sweden, ‘cider’ is defined as a fermented juice of apple or pear, with a minimum of 15% of fruit juice (SLV, 2009). The juice can be blended with water, sugars, unfermented juice, aroma substances and approved additives, but the alcoholic content must be derived from the fermentation process. An additional Swedish classification, made by a Swedish brewer organisation, is into strong and weak cider with above or below 2.25% (v/v) alcohol (Sveriges Bryggerier, 2010). From now on, in this thesis, the word ‘cider’ is used for the traditional European type of beverages made by fermentation of 100% fruit juice.

A basic classification of cider is into sweet, dry, sparkling, champagne or carbonated (Downing, 1989). Dry cider is fermented until the sugar is totally consumed, and has an alcohol concentration of 6–7%, depending on initial sugar content. Sweet cider can be produced by two methods, where the first is to just add sugar to the dry cider until a pleasant flavour is reached (Proulx & Nichols, 2003).

Sweet cider can also be produced by interrupting the fermentation before all sugars in the apple juice have been converted into ethanol. This gives a beverage with lower alcoholic content (Downing, 1989; Proulx & Nichols, 2003). Dry and sweet ciders are still types, while sparkling- and champagne-ciders contain bubbles. The cider is made sparkling by addition of some extra sugar just before the dry cider is bottled and closed with a cap. When this added sugar is fermented, carbon dioxide is produced and retained in the cider (Proulx & Nichols, 2003). This requires a yeast strain that has enough vitality at higher ethanol concentrations, and that the cider is not pasteurised or sulphited after the first fermentation. Carbonated cider is made sparkling by commercial carbon dioxide, and both dry and sweet ciders can be used for this purpose (Downing, 1989).

1.3.1 Cider apples

Choice of apple cultivars for the production of cider influences the final product to a very high degree. In countries with old cider traditions, special apple cider cultivars are grown, with a higher acid and tannin content compared to dessert apple cultivars (Downing, 1989). However, it is also common to make use of any kind of apples, that for different reasons cannot be sold on the fresh market (Joshi & Sharma, 2009).

Tannins, the common name for procyanidins, are a group of polyphenols that bring bitterness and astringency to the cider (Lea & Drilleau, 2003). These are important for a high organoleptic quality. Astringency is sensed over the whole tongue as a drying and wrinkling experience, and is mainly due to polymeric procyanidins. Bitterness is caused by lower molecular weight procyanidins. This taste is sensed mostly at the sides and at the back of the tongue, and can be perceived as unpleasant. English apples are classified into four different groups depending on the acid and tannin content (Downing, 1989). A sweet or bittersweet apple has an acidity below 0.45 g malic acid/100 ml, whereas sharp and bittersharp apples have a content above this level. The tannin content is below (sweet and sharp apples) or above 0.2 g/100 ml (bittersweets and bittersharp apples). Bittersweet apples are the most important source of specific bitterness and astringency that characterise cider.

Only a few cider apple cultivars have sufficiently complex characteristics of sweetness, acidity, aroma and tannins to be successfully used in single-variety ciders (Proulx & Nichols, 2003). It is more common to make a blend based on a juice of high sweetness that will result in efficient alcoholic fermentation. To facilitate the development of a pleasant flavour and aroma, juice from acidic, aromatic and astringent apples are then added to the sweet base after pressing.

1.3.2 Yeast

Alcoholic fermentation is a process where the sugar in a substrate is converted into ethanol. The main micro-organisms responsible for the fermentations are yeasts belonging to the class Saccaromycetes in the fungal phylum Ascomycota (Rainieri & Zambonelli, 2009). Yeast has a high

tolerance to acidity, which facilitates survival and growth in fruit juices that have pH values below the tolerance level for several other microorganisms (Jay et al., 2005). Compared to other fungi, yeasts differ by their growth as single cells instead of a mycelium. Reproduction is vegetative by fission or budding, a characteristic shared with many other fungi. Growth is possible in both aerobic and anaerobic conditions, where the former results in high growth rate, and the latter (oxygen limiting) results in a slow biomass production with energy being stored in the produced ethanol (Rainieri & Zambonelli, 2009).

The substrate for yeast metabolism is mainly monosaccharides, like glucose, fructose and mannose that are metabolised into two molecules of pyruvate in the glycolysis, also called Embden-Meyerhof-Parnas pathway (Bai et al., 2008). The produced pyruvate is further reduced to ethanol and carbon dioxide by the enzymes pyruvate decarboxylate and alcohol dehydrogenase. The overall chemical reaction is thus:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 + 55\ Kcal$$ (Lea, 1989)

Theoretically, the yield of ethanol is about 65% (v/w) of the initial glucose content, but the actual conversion efficiency is reduced to about 60% due to loss of glucose for production of minor compounds and growth (Bai et al., 2008; Raineri & Zambonelli, 2009).

The fermentation is affected by several environmental factors. Non-optimal conditions can stress the yeast and reduce the productivity (Bai et al., 2008). The concentration of both substrate and product affects the yeast. A high sugar content (>25% w/v) results in osmotic stress whereas a high ethanol content (>15% v/v) reduces cell vitality and causes damage to enzymes and membranes, resulting in a reduced fermentation rate. High temperatures and low pH values are additional stressors, and in the presence of more than one unfavourable factor they have a synergistically detrimental effect.

1.3.3 Yeasts in cider production

The traditional way of producing cider is to utilize the naturally occurring microflora present on the skin of the fruit or the equipment. If a newly pressed apple juice if left for some days under favourable conditions, the fermentation takes place spontaneously. In apple cider fermentation, the wild yeasts mainly belong to the species *Saccharomyces*, *Kloeckera*, *Candida* and *Pichia* (Joshi & Sharma, 2009). The problem with natural fermentation is that the desirable yeasts have to be present at sufficiently high concentrations. If the amount is too low, there is a risk for development of unwanted microorganisms that can produce off-flavours or even make the cider hazardous to consume. A fermentation based on spontaneous fermentation is therefore unpredictable. Well defined yeast cultures, based on a pure strain, are commonly used today thus facilitating a

standardised production. The yeast strains used for commercial cultures, are selected on their rate of fermentation without production of undesired compounds, tolerance to high alcohol, sugar and sulphite concentrations, ability to sediment and low level of mutation (Downing, 1989). For cider production, the strains commonly used today belong to the species *Saccharomyces cerevisiae* or *S. bayanus* (Lea, 1989).

The choice of yeast strain as starting culture can have a high impact on the flavour profile of fermented beverages (Downing, 1989; Joshi et al., 2002; Nurgel et al., 2002). During fermentation of apple juice, the rate and content of ethanol, sugars, tannins, esters, methanol and volatile acids are some of the quality characteristics that can be affected by the specific yeast strain (Joshi et al., 2002). One disadvantage of using a pure starting strain is that a natural mixture of different strains sometimes can result in a more complex organoleptic quality, because of their production of different secondary metabolites (Nurgel et al., 2002).

1.3.4 Pretreatments

Before fermentation, the apple juice can be treated in different ways to improve the process and the product, depending on the desired quality. Amelioration, where the juice composition of acids, sugars and nutrients is adjusted, can enhance the fermentation process, the development and flavour and the final alcohol concentration (Downing, 1989). The sugar concentration basically determines the alcohol percentage in the finished cider. An undesirably low concentration can be raised by the addition of sugar, to a recommended level of 10–11% (w/w) which gives a cider with 5–6% (v/v) alcohol (Lea, 1989). Acidity should be in the pH range of 3.2–3.8, and can be corrected by addition of water, citric or malic acid, or another juice. To provide more efficient fermentation conditions for the starting yeast, different nutrients, especially nitrogen, can be added to the juice (Proulx & Nichols, 2003).

The natural microflora that compete for substrate or produce undesirable metabolites, can be inactivated by different methods. The most commonly used inactivation method in cider production is the addition of sulphite (Downing, 1989). When sulphite is dissolved in the juice, it produces gaseous SO_2 which is harmful to many microorganisms by causing functional cell damage (Jay et al., 2005). The starter yeast strain cannot be added until most of the SO_2 has been consumed for killing the microorganisms, which can take up to two days (Downing, 1989). The natural microflora can also be reduced by pasteurisation, but heat treatment can have negative effects on the flavour (Choi & Nielsen, 2005).

1.3.5 Alcoholic fermentation methods

Small-scale cider production for home consumption can be made using glass demijohns or more modern polyethylene barrels. Traditionally, fermentation of larger volumes has been performed in

wooden barrels. Present-day commercial production is commonly carried out with vertical stainless steel tanks with volumes in the range of 2000–9000 litres (Downing, 1989; Singh & Sooch, 2009).

The fermentation process is initiated in the presence of oxygen, and starts some hours after inoculation with the yeast (Singh & Sooch, 2009). The first phase is characterised by an intensive bubbling and foaming on the surface of the juice. Once this activity has ceased, a fermentation lock is put on the barrels. This facilitates anaerobic conditions, through carbon dioxide production of the yeast, which is necessary for alcoholic fermentation. The fermentation rate depends on several factors, including starter yeast strain, density of yeast cells, temperature and sugar concentration (Downing, 1989). The optimum temperature depends on the strain, but is often in the range of 12–18°C (Singh & Sooch, 2009). A lower temperature results in slower fermentation, which is said to develop a cider of better quality (Downing, 1989). It is therefore a balance between the need for decreasing production time and the need for sufficient development of flavour and aroma. A juice with a higher sugar content takes longer time to ferment into dryness, but eventually produces a higher alcoholic content when all the sugars have been metabolised (Lea, 1989). In addition, the rate of fermentation is affected by different pretreatments of the juice, where sulphiting reduces and heating increases the rate (Downing, 1989; Nurgel et al., 2002).

When the main fermentation is finished after 1–3 weeks, the yeast is settled at the bottom of the container and can easily be separated from the cider by racking it off into a new barrel, leaving the sediment behind (Lea, 1989). In industrial production, this biomass is removed faster and more efficient by centrifugation, but the investments required for the equipment are not feasible in a small-scale production (Joshi & Sharma, 2009). After racking off or centrifugation, the cider is left for maturation, sometimes for several months, until the desired product is achieved (Downing, 1989).

In a cider made of unsulphited and unheated juice, malolactic fermentation by naturally present lactic acid bacteria can take place during storage, which can improve the flavour and aroma (Lea & Drilleau, 2003; Rainieri & Zambonelli, 2009). Lactic acid bacteria can also be added as a starting culture, together or after the addition of a yeast starting culture, and allow malolactic fermentation also in cider from pretreated juice (Singh & Sooch, 2009). Malolactic fermentation is the result of the activity of lactic acid bacteria, that convert malic acid into lactic acid and forms CO_2, which can be an undesirable side-effect in bottled products (Lea & Drilleau, 2003).

1.3.6 New fermentation techniques

In industrial production of cider, the main issues is to decrease the fermentation time and reduce labour costs to make the production more profitable, but still retain the high quality (Verbelen et al., 2006). Two different bioreactors for alcoholic fermentation have been developed, the homogenous and the heterogeneous system (Singh & Sooch, 2009).

The homogenous system is similar to the traditional methods, with cells suspended in the liquid, but with the difference that the yeast cells are reused in the next batch of fermentation. Yeast cells are retained in the bioreactor either by filtration or centrifugation and have the advantage of being accustomed to the conditions. Problems associated with the homogenous bioreactor system are that centrifugation can stress the cells and reduce viability, and it is also difficult to avoid clogging of the system at filtration (Sing & Sooch, 2009).

In the heterogeneous bioreactor, there are two different phases with the yeast cells in a solid phase, immobilised from the liquid phase. This system increases the yeast cell density and viability through an increased protection from unfavourable conditions, and makes it easy to reuse the yeast (Bai et al., 2009). Immobilisation is achieved by entrapping the yeast cells in a supporting material, as a gel or a membrane, or by aggregation of the yeast cells, a process that occurs spontaneously by flocculation or can be induced by cross-linking agents (Sing & Sooch, 2009). The fermentation rate can be enhanced in the bioreactor by increasing the contact time between the two phases. This is achieved by agitation, either mechanical or by air flow, or with columns packed with the immobilised cells allowing liquid to flow through (Singh & Sooch, 2009). The immobilisation system is promising in decreasing the conversion rate of sugars into ethanol, especially if applied to continuous production systems (Verbelen et al., 2006). In a continuous system, the medium in the bioreactor is kept at a steady state, by a constant inflow of substrate parallel to a constant outflow of finished product.

Implementation of the new techniques in commercial production is still limited and more research is required (Bai et al., 2009; Verbelen et al., 2006). Problems associated with immobilisation are an overly high growth of the yeast cells resulting in stressful conditions due to limited space, nutrient deficiency and accumulation of toxic substances (Bai et al., 2009). In addition, the continuous methods are difficult to keep at the required balance of in- and outflow that is needed for a functional fermentation process.

1.4 Cider vinegar

Vinegar is described as a sour and sharp liquid used as condiment and for preservation of food. It is produced by double fermentation of a carbohydrate-containing solution with agricultural origin (FAO/WHO, 2000). Over the world there are several different types of vinegar based on widely varying raw materials, such as grapes, rice, apples, different berries, grains, whey and honey (Solieri & Giudici, 2009). In 2005, balsamic vinegar, made of grapes, had the largest world market share with about one third, while the cider vinegar share was 7% (The Vinegar Institute, 2006).

In Europe, there are regional standards for different vinegars, but a threshold value for wine vinegar is a minimum of 6% acetic acid (w/v), obtained by acetous fermentation of wine (European Commission, 2010). In vinegars produced from other alcoholic bases than wine, the acetic acid

concentration threshold minimum is 5% (w/v) (Solieri & Giudici, 2009). In North America, the vinegar must have an acetic acid content of at least 4% (w/v). The maximum ethanol content in vinegars has been set to a maximum 0.5% and 1% (v/v) for wine vinegar and other vinegars, respectively, by the Codex Alimentarius commission (FAO/WHO, 2000).

Other ingredients that are permitted in the vinegar, apart for the raw material of agricultural origin, are fruit juices, sugars, honey, whey, plant parts or extracts adding flavour, and salts. Additionally, some food additives are permitted and include specified antioxidants, colours, flavour and flavour enhancers, stabilisers and processing aids, as bacterial nutrients, and also agents for clarification (FAO/WHO, 2000).

1.4.1 Acetic acid bacteria

The acetous fermentation of ethanol into acetic acid is performed by acetic acid bacteria belonging to the family Acetobacteriaceae and the genera *Acetobacter* and *Gluconobacter* (Lea, 1989). The total chemical reaction that takes place is as follows:

$CH_3CH_2OH + O2 \rightarrow CH_3COOH + H_2O + 493$ kJ (García-García et al., 2009)

There are two steps in the oxidation of ethanol to acetic acid, driven by the enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) (Raspor & Goranovič, 2008). The first step is oxidation to acetaldehyde by ADH, which is further oxidised to acetic acid by ALDH. The reaction is exothermic, thus increasing the temperature in the medium. The acetic acid can be further oxidised to carbon dioxide in the tricarboxylic cycle. This is an unwanted process in vinegar production but it can occur when the ethanol concentration is limited (Lea, 1989). The process, called overoxidation, is only made by bacteria belonging to *Acetobacter,* because two of the key enzymes required for oxidation are non-functional in species of *Gluconobacter*.

Acetic acid bacteria are gram negative, strictly aerobic and differ from other bacteria by their survival at low pH values (down to pH 3–4), even if their optimum is pH 5.5–6.3 (Raspor & Goranovič, 2008). The rate of acetic acid production is dependent on temperature, availability of oxygen, concentration of substrate (ethanol) and concentration of product (acetic acid) (García-García et al., 2009). Acetic acid bacteria can grow in temperatures of 8–35°C, but with an optimum in vinegar production at 31°C (Tesfaye et al., 2002). Because the bacteria are obligate aerobs**,** even a short interruption in oxygen availability can result in death (García-García et al., 2009). The ethanol content affects the bacteria both in the beginning and at the end of fermentation. If the initial ethanol concentration is too high, bacterial vitality can decrease due to the antimicrobial effect of ethanol. If, instead, initial concentration is too low, down to 0.1–0.2%, the risk for overoxidation increases (Lea, 1989). When the acetic acid concentration is increasing during fermentation, the pH decreases, and

will reduce the bacterial activity and set a limit for the concentration of acetic acid that is possible to produce (Raspor & Goranovič, 2008).

1.4.2 Bacteria in vinegar production

As in the production of cider, vinegar making can rely on a spontaneous fermentation, a method used traditionally but only in a small scale (Solieri & Giudici, 2009). The acetic acid bacteria are present in the environment and in the raw material, but they cannot grow during alcoholic fermentation because of the anaerobic conditions. When the alcoholic liquid is exposed to oxygen, the acetic acid bacteria are able to start to grow on the surface. The method is sensitive to spoilage and is rarely used in commercial production. The most common method is instead to make use of bacterial cultures from earlier production batches as a seed culture (Gullo & Giudici, 2008). During the acetous fermentation, the layer of bacteria that is produced on the surface can easily be collected and transferred to another batch. These cultures consist of a mixture of several different, often unspecified, acetic acid bacteria (Raspor & Goranovič, 2008). A third method, but with limited implementation in commercial production, is the use of defined starting cultures with high density of cells (Solieri & Giudici, 2009). The selection of starter cultures must take several aspects into concern, such as tolerance to high levels of acetic acid and low pH, preference for ethanol as substrate, no production of off flavours or cellulose, and no overoxidation (Gullo & Giudici, 2008). Only a few starter cultures are available, because of difficulties in culturing and preserving the bacteria outside the fermentation barrel, and the higher costs for production compared with using seed cultures from previous production batches (Solieri & Giudici, 2009). The advantage of the starting culture is that it facilitates a more controlled process that is easier to predict and gives a standardized product (Gullo & Giudici, 2008).

Several different species of acetic acid bacteria have been isolated from commercial production of vinegar, but the most common is *Acetobacter aceti* (Raspor & Goranovič, 2008). Each species includes several different strains with their own fermentation characteristics that affect the quality of the end product (Lea, 1989).

1.4.3 The Orleans process

The traditional way of producing vinegar at a larger scale, is the surface method in wooden barrels, the history of which goes back to France in the 14th century (Mazza and Murooka, 2009). This method, called the Orleans process after the city of invention, is said to produce the highest quality vinegar (Raspor & Goranovič, 2008). The Orleans process relies on the natural acetic acid bacteria present in the raw material, or makes use of a seed culture from a previous production batch (Mazza & Murooka, 2009). The bacteria, usually belonging to the species *Acetobacter xylium*, grow on the liquid-air interface of the medium because of their oxygen requirement. Due to the capacity of this

species to produce cellulose, a thick mat of a gelatinous substance containing bacterial cells and cellulose will develop over time on the top of the liquid (Lea, 1989). Oxygen moves into the mat and is used for the oxidation of ethanol into acetic acid. This produces a concentration gradient within the barrel, with a continuous diffusion of finished vinegar down-wards and a diffusion of ethanol towards the mat (Raspor & Goranovič, 2008). The acetification process takes a long time compared to more recent methods, with a production rate of about 1% acetic acid per week (Lea, 1989).

The fermentation rate depends on the oxygen availability. In order to increase the surface area between air and liquid, the barrels are only filled to about two thirds of their volume (Raspor & Goranovič, 2008). Oxygenation also takes place through diffusion of air through the wood into the liquid. Production by the Orleans method can be performed as a semi-continuous process, because once the vinegar has developed to a desired quality, about half to three-fourth of the volume of the finished vinegar can be removed from the bottom while the same volume of non-acetified alcoholic substrate is simultaneously added from the top (Mazza & Murooka, 2009). The bacteria in the vinegar that is left in the barrel is used as inoculate for the next production volume, and each circle can take up to three months.

The product from the Orleans process is high quality vinegar since the slow production process promotes the development of flavour and aroma (Lea, 1989; Raspor & Goranovič, 2008). Additionally, this way of processing provides a constant availability of finished vinegar. The drawback is the long time required, resulting in high costs per volume produced even though the investment in equipment and the running costs are low (Lea, 1989).

1.4.4 The Generator process

Development of new processes for vinegar fermentation have been driven by an increased demand for shorter production time, and are based on enhancing the surface area exposed to oxygen (Mazza & Murooka, 2009). During the 19th century, several methods with a similar, basic generator design were developed, as the Quick, German, Luxemburgian and Schutzenlaub process (Lea, 1989; Raspor & Goranovič, 2008; Tesfaye et al., 2002). This fermentation is most commonly performed in tanks made of wood or steel, with a volume of 50 000–60 000 litres. The surface area where bacteria are exposed to oxygen, is increased by a packing material in the tank, on which the bacteria are immobilised (Raspor & Goranovič, 2008). The mostly used packing is made of beech wood shavings, over which the liquid is sprayed and then allowed to drip through to the bottom of the reactor. Air is blown in from the lower part to maintain high oxygen availability. The partly finished vinegar, that accumulates at the bottom of the tank, is re-circulated to the top again, until the desired concentration of acetic acid is obtained. Once the vinegar is finished, 90% is removed from the

bottom of the tank and replaced with the same volume of alcoholic substrate. The process is performed at 27–30°C and a cooling coil in the tank prevents overheating.

The increased surface exposed to bacteria and the active inflow of oxygen in the generator process, results in an efficient production where time is highly reduce compared to the traditional method, and facilitates a conversion rate of 1% acetic acid per 24 hours (Lea, 1989). The reactor can be driven continuously for one year, and then the packing material has to be renewed, which is relatively costly. There are also several other disadvantages associated with the process, as high risk of clogging when cellulose-producing bacteria grow in the generator, accumulation of dead bacteria and infection with vinegar eels (Tesfaye et al., 2002). Another disadvantage is a relatively high loss of ethanol by evaporation, which makes it difficult to produce vinegar with high acetic acid concentration (Raspor & Goranovič, 2008).

1.4.5 The Submerged process

The most commonly method used nowadays for commercial vinegar production is the submerged-culture fermentor, where the bacteria is suspended in the medium, in contrast to the traditional and the generator process (Tesfaye et al., 2002). The first bioreactor of the submerged type was Fring´s acetator in the early 1950´s, and it was followed by other patented methods as the Cavitator, bubble column fermentor and Effigas turbine vinegator (Mazza & Murooka, 2009; Tesfaye et al., 2002).

The fermentor is normally made by stainless steel with several different volumes, but most commonly in the range 10 000–40 000 litres (Tesfaye et al., 2002). The basic principle is that the bacteria are free in the substrate, and air is forced into the medium by a stirrer at the bottom of the tank where an air inlet is positioned (Mazza & Murooka, 2009). A mechanical agitation gives a fine bubbling in the system. New substrate can be let in at the bottom, while finished vinegar can be pumped out at the surface level. At the top of the fermentor there is an air outflow. To enable a carefully controlled production, the fermentor is equipped with thermometer, cooling coils and sometimes an alcograph, and a system to control and remove the build-up of foam (Lea, 1989; Tesfaye et al., 2002). The system is very sensitive, and since the bacteria are dispersed in the medium, even a short interruption of the air inflow and stirring can result in cell death (Lea, 1989).

The submerged process can be used for production of vinegar in either a discontinuous, semi-continuous or continuous system (García-García et al., 2009; Tesfaye et al., 2002). In the discontinuous system, vinegar is produced in batches where a volume of substrate is loaded and inoculated with bacteria, and after the acetification the volume is completely unloaded from the fermentor (Tesfaye et al., 2002). The semi-continuous system is the most commonly used, and it requires a start-up period when the fermentor is loaded and inoculated (García-García et al., 2009). When the acetification has proceeded to the desired level, about 40–50% of the volume is unloaded,

while the vinegar left behind is used as inoculum for the next cycle. The advantage of this system is a natural selection of the best adapted bacteria over time, and a shortened bacterial lag time for growth, which gives a more efficient production. The continuous system is based on a constant composition of the medium at a state where the bacteria are in the exponential growth phase and therefore have their highest growth rate (Tesfaye et al., 2002). This system also requires a start-up period, but is then maintained by a constant volume of in- and outflow of substrate and product.

Irrespective of which of the described systems is used, the submerged method is highly efficient and can produce a conversion of 8–9% acetic acid in 24–48 hours (Tesfaye et al., 2002). A drawback is that this fast conversion results in a limited production of esters and other volatiles that contribute to flavour and aroma, and therefore a lower organoleptic quality compared with slower processing methods (Raspor & Goranovič, 2008).

1.4.6 Maturation

Maturation of the vinegar is required for development of a pleasant aroma and a high quality product. In the old days, vinegar could be stored for up to 1–2 years in wooden barrels, whereas today vinegar is stored, at the most, for 1–2 months in barrels or in stainless steel tanks before bottling (Lea, 1989).

1.5 General treatments of juice, cider and vinegar

1.5.1 Clarification and fining

Clarification of apple juice, cider and vinegar is undertaken to improve the appearance and stability of the product (García-García, 2009). Turbidity in the product is the result of larger particles as plant debris, yeast and bacterial cells, and smaller material as carbohydrates, polyphenols and proteins (Kilara & Van Buren, 1989). The cells and plant particles are removed by sedimentation followed by decanting, and this is the only clarification procedure in production of natural, cloudy products (Bates et al., 2001). Further clarification to remove smaller particles can be achieved through filtration, but in small-scale production without proper equipment, this can be associated with a high risk of contamination or unwanted exposure to air (Proulx & Nichols, 2003). Clarity can also be improved by pectolytic enzymes, which decrease the viscosity by breaking down the polymeric carbohydrates pectins and cause flocculation of dissolved material (Kilara & Van Buren, 1989). The flocculated particles are then removed by filtration.

An alternative or complement to the clarification, that is used both in small and larger scale, is fining (Lea, 1989). This treatment improves the clarity even more, and decreases the risk of developing turbidity during storage (Kilara & Van Buren, 1989). Turbidity after packaging is due to smaller particles that are difficult to remove by sedimentation and filtration (Kilara & Van Buren,

1989). Fining is achieved by addition of substances that bind the unwanted particles, often gelatine or bentonite, and make them flocculate and settle at the bottom (Joshi & Sharma, 2009). The clear liquid is then decanted to another tank after approximately one week. The drawback with fining is that different polyphenols, sometimes desired for contributing to aroma, flavour and health benefits, are also removed by the treatments (Lea & Drilleau, 2003). Clarification and fining treatments of apple juice should be avoided if the juice is aimed for further processing to cider, since pectolytic enzymes as well as addition of bentonite can reduce the fermentation rate (Duenas et al., 1997).

1.5.2 Preservation

Irrespective of whether the product is apple juice, cider or vinegar, it has a restricted shelf life and stability, even if stored cold. Furthermore, a raw product can contain potentially hazardous microorganisms that can be present without spoiling it. Various methods can be used to eliminate unwanted microorganisms, including pasteurisation, sterile filtration and different additives (Lea, 1989).

Pasteurisation is applied as a last step before bottling, and different combinations of temperature and time are used depending on product and equipment. A lower heating temperature requires a longer treatment time to obtain the same reduction of microorganisms (Jay et al., 2005). A drawback with pasteurisation is that the heat can decrease the organoleptic quality by affecting colour and flavour (Choi & Nielsen, 2005).

Cold sterile filtration through a membrane with pore size less than 0.2 µm, is an alternative to pasteurisation (Lea, 1989). This method can only be applied to clear products, otherwise the fine membranes that are used will be clogged.

Sulphiting is an effective method for inactivation of microorganisms, and it is used to limit the natural microflora in cider fermentation. This method should not be used when the cider is further processed into vinegar, since possibly occurring residue of sulphite after the alcoholic fermentation inhibits the acetic acid bacteria (Lea, 1989). Sulphiting has also been shown to decrease the rate of alcoholic fermentation (Duenas et al., 1997). However, both the finished cider and vinegar can be sulphited to inhibit development of haziness and unwanted growth of residual microorganisms during storage.

1.6 Health aspects

1.6.1 Polyphenols

Polyphenols are secondary plant metabolites that influence the flavour, aroma, colour and clarity of processed apple products (Lea, 1989; Spanos & Wrolstad, 1992). In addition, phenolic compounds in apples can prevent different chronic disorders such as cancer and cardiovascular disease (Dai and

Mumper, 2010; Weichselbaum et al., 2010). Polyphenols present in apples are classified into flavanols, hydroxycinnamic acids, dihydrochalcones, flavonols and anthocyanins (Tsao et al., 2005).

The content of polyphenols varies between different apple cultivars, but is also affected by growth conditions, maturity and processing (Spanos & Wrolstad, 1992). In juice from cider apples, the content of flavanols is higher compared to juice from dessert apples, resulting in a higher bitterness and astringency. The sensory attributes are ascribed to procyanidins, a group of flavanols (Kahle et al., 2005). The main procyanidins are epicatechin and procyanidin B2, and the content of these compounds is correlated with high antioxidant activity in the apple juice (Tsao et al., 2005).

During processing of apples, several steps are involved which can have significant effects on the content of polyphenols. At milling and pressing, oxidation by polyphenol oxidases usually takes place and decreases the content of phenolic compounds in the juice (Spanos & Wrolstad, 1992). The oxidation can be reduced by inhibition of enzyme activity, either by heating or by addition of ascorbic acid or sulphur dioxide (Bates et al., 2001). The polyphenol content, especially of procyanidins, is affected by the length of time and level of oxygenation when the juice and the pomace are in contact (Lea & Drilleau, 2003; Renard et al., 2010). When the procyanidins are oxidized, they become irreversibly retained in the solid particles and are then removed with the pomace at pressing or sedimentation. Additionally, the concentration is also dependent on clarification and fining treatments with gelatine or bentonite, because the polyphenols are entrapped in flocculation material and removed by the process. A cloudy juice therefore contains higher amounts of phenolic compounds compared to a clarified juice, making it an important source of natural antioxidants (Kahle et al., 2005; Oszmianski et al., 2007).

1.6.2 Vinegar benefits

Apart from the apple-derived polyphenols that are present in vinegar, additional health benefits have also been proposed. Vinegar has been used for thousands of years, both in food preparation and in some cultures to treat wounds and infections (Mazza & Murooka, 2009). The antimicrobial property of vinegar in food preservation, due to the low pH, is well established but scientific research about medicinal properties is still scarce (Johnston & Gass, 2006). It has however been shown that vinegar can decrease the glycemic index in a meal (Leeman et al., 2005). The glycemic index is a measure of how the blood sugar is affected by the food. In both healthy people and in diabetes patients, an antiglycemic effect can provide health benefits. A positive correlation has also been demonstrated between vinegar consumption and an increased satiety after having a meal, and this effect can be used for dietary recommendations to treat obesity (Östman et al., 2005). The biochemical mechanism behind the antiglycemic and satiety effects are not fully understood, but could involve a delayed gastric emptying or effects on enzymes in the metabolism of sugar (Johnston & Gass, 2006).

1.7 Aim

The aim of this thesis was to characterise cloudy apple juice extracted from different cultivars and to ferment the juice into cider and further into vinegar to determine the suitability of cultivars for the different products. Before evaluation of cultivars, some yeast strains and bacterial cultures were evaluated for their applicability in apple cider and apple cider vinegar production. In addition to the laboratory work, a literature study of common processing techniques was performed.

2 Material and methods

2.1 Evaluation of yeast strains

Two different cloudy apple juices were provided by a commercial producer (Öspab) and used for evaluation of yeast strains for ethanol fermentation. Both of the apple juices were blends pressed from unknown proportions of the cultivars Aroma, Elise and Katja (juice A), and one of them also contained juice from the cultivars Cox Orange and Kim (juice B). The juice was kept for 3 days at 8°C and then stored in a freezer at -18°C until use. After thawing, 950 ml juice was poured into 1000 ml glass bottles. The different yeasts used for fermentation were the commercial dry starting strains Lalvin EC-1118 and Safale S-04. The Lalvin EC-1118 yeast is a strain of *Saccharomyces bayanus*, selected in the Champagne region in France (Lallemand, 2006). Safale S-04 is an English ale yeast strain of *Saccharomyces cerevisiae* (Fermentis, 2009). Additional to the dry starters, the naturally present wild yeast in the juice was utilized as a third treatment. Each combination of juice and yeast was tested in five replications.

Before inoculation of the dry starter yeast, the juice was slightly heated in a convection oven and kept at 50°C for 20 minutes to reduce the natural microflora. The juice for wild yeast fermentation was not heated. The dry starter cultures were prepared according to manufacturers' instructions by dehydration in boiled juice followed by stirring until all yeast was dissolved. The highest recommended dose for normal fermentation was used, thus 40 g/hL and 80 g/hL of Lalvin EC-1118 and Safale S-04 respectively.

The juices were placed at 20°C and the bottlenecks were covered with a clean tissue to prevent contamination from the air. In the treatment with dry starter yeasts, the first foaming stage of fermentation started after half a day, and in the treatment with the wild yeast not until after one and a half day. When the foaming fermentation had ceased, the inside of the necks was cleaned and a rubber cap and fermentation lock was applied. This was done after 2.5 days for Safale-04, after 3.5 days for Lalvin EC-1118 and after 4 days for the wild yeast.

The fermentation was followed by regular measurements of total soluble solids (TSS). After 10 days, at a TSS of approximately 4%, the cider was racked off to clean bottles, leaving the sediment behind. The bottles were enclosed with metal caps and kept at 8°C for 22 days before evaluation of taste and analysis of chemical characteristics. Based on the outcome of the taste test, Safale S-04 was chosen for the following experiments.

2.2 Evaluation of bacterial cultures

The cider used as raw material for acetous fermentation was made of the same two apple juices as in the yeast evaluation. These were fermented into cider in larger batches in 15 L glass barrels. Juice A

was fermented in duplicate, whereas juice B in triplicate. The juice was heated in a large sauce pan to 50°C and kept at this temperature for 20 minutes. The juice was transferred to 8°C to allow a quick cooling to ambient temperature before inoculation. The yeast strain Safale S-04 was prepared as described above and inoculated in a concentration of 80 g/hL. The juice was fermented at 20°C for three weeks. The finished cider was and distributed to 1000 ml glass bottles, with 500 ml in each.

To enhance the rate of the acetous fermentation, a small scale lab system was set up with the aim to mimic the submerged method. The aeration was increased by use of air pumps for aquariums. From each pump, with a total capacity of 150 L/h, the air was distributed to ten bottles with PVC airlines. To the end of each airline, an air stone for aquariums was connected to produce a dispersion of the air into small bubbles (Figure 2). The aeration was approximately 0.5 vvm (volume air*volume liquid^{-1}*minute^{-1}).

Figure 2. Lab scale fermentor system with enhanced aeration by use of air stones and air pumps for aquariums, giving a fine dispersed bubbling.

Three different bacterial cultures were evaluated; two cultures intended for use in surface culture systems (Bockmeyer and Rheinhessen-Nahe), and one culture developed for submerged systems (Alles um den Essig). The mother cultures were pre-cultured into larger volumes before inoculation, in the same system as the fermentation was performed. For the cultures from Bockmeyer and Rheinhessen-Nahe, culturing was made by adding 1:10 of mother culture to ciders from the yeast evaluation, whereas Alles um den Essig was added in 1:20 according to manufacturers' recommendations. The bottles were placed at 28–30°C and in the dark for one week.

The prepared mother cultures were inoculated to the batch ciders in a ratio of 1:10 and each combination of cider and bacterial culture was made in five replications. The fermentation was made in the same conditions as above, for 9 days, and followed by measurement of titratable acidity (TA).

The vinegar was roughly filtered in a tea strainer to remove the cellulose build-up and then pasteurised in the bottle in a water bath at 67°C. Chemical properties were analysed and the vinegar was evaluated in a taste test. The bacterial culture which produced the most highly appreciated vinegar, Alles um den Essig, was used for further experiments.

2.3 Evaluation of apple cultivars for cider and vinegar production

2.3.1 Apple cultivars

Ten different apple cultivars were compared for their suitability for making apple cider and vinegar. Three of the cultivars (Aroma, Ingrid-Marie and Rubinola) were provided by a commercial orchard (Öspab), while the other (Baldwin, Belle de Boskoop, Bramley, Cortland, Gravensteiner, Jonathan and Spartan) were grown at Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Science, Balsgård. All fruits very picked in late October-early November and stored in a cold room until juice extraction.

The selection of cultivars was made with the aim to include cultivars of interest for commercial growers in Sweden and/or known to be suitable for juice and cider production. The cultivars Aroma, Belle de Boskoop, Ingrid-Marie and Gravensteiner are cultivars that have been, or are much grown in orchards in Sweden (Näslund & Sandberg, 2010). The North American cultivars Cortland, Jonathan and Spartan are grown in Sweden in a very limited amount, whereas Baldwin and Bramley are rarely grown. Rubinola is a relatively new cultivar but is increasing in popularity and could be interesting because of a very restricted browning of the fruit flesh (Näslund & Sandberg, 2010). All chosen cultivars, except Bramley and Rubinola, are described as both dessert- and cooking fruits (Morgan & Richards, 1993; Näslund & Sandberg, 2010). Bramley is mainly a cooking apple whereas Rubinola mostly is used as a dessert apple. The cultivars Baldwin, Bramley, Cortland, Gravensteiner and Jonathan have been used for cider production in England and North America, but then often in blends with other cultivars (Downing, 1989; Morgan & Richards, 1993; Proulx & Nichols, 2003).

2.3.2 Juice extraction

The apples of the different cultivars were washed and shredded in a small-scale commercial system with a centrifugal grating mill (Voran Machinery). The mash was packed in a 20 L bladder press (Pillan Enotechnica) and extraction was operated until a pressure of maximum 3 bars. The juice was roughly strained and stored at 8°C in a plastic bucket for some hours and then frozen at -18°C until preparation for fermentation. Samples were taken for chemical and sensory analysis and frozen separately.

2.3.3 Alcoholic fermentation

The juice of the cultivars was thawed over night at room temperature, and 950 ml was distributed into 1000 ml glass bottles. For each cultivar the juice was fermented in five replicates. The juice was heated to 50°C in a convection oven for 20 minutes and cooled to ambient temperature before inoculation. The yeast strain, Safale S-04, was dehydrated in boiled apple juice according to manufacturer's instructions and inoculated at a concentration of 80 g/hL. The bottles were placed at 20°C and a tissue cloth was put in the neck during the first days of the foaming stage of fermentation. After 3.5 days the bottles were closed with a rubber cap and a fermentation lock was mounted.

After 30 days the cider was racked off to new bottles and stored for 3 days at 8°C before sensorial evaluation. Samples were taken and stored frozen at -18°C until chemical analyses.

2.3.4 Acetous fermentation

After racking off, 500 ml of each cider replicate of the different cultivars (except one replicate that was used for sensorial evaluation of the cultivar cider), was poured into clean bottles and inoculated in 1:10 with the bacterial culture form Alles um den Essig, prepared as described in the test of bacterial cultures. The bottles were placed dark in 28°C, and mounted with aeration system as described previously. The fermentation was run over 16 days, and then the products were roughly filtered using a tea strainer to remove the produced slime. The vinegars were pasteurised at 67°C in a water bath before chemical analysis and sensory evaluation. For sensory evaluation, vinegar replicates with as equal titratable acidity as possible were selected.

2.4 Chemical analyses

2.4.1 Total soluble solids

Total soluble solids (TSS) in the juice, cider and vinegar were measured as % Brix with a portable digital refractometer (Atago). The measurements were made in triplicate for each sample.

2.4.2 Titratable acidity

The content of acid in the apple juice, cider and vinegar was analysed by titration with an automatic titration equipment (Radiometer Copenhagen). Juice and cider samples were analysed in a volume of 5 ml diluted in 15 ml of distilled water, whereas the ratio used for vinegar was 2:18. Each sample was measured in triplicate. The titration was made with 0.1 M NaOH until pH end point 8.4 and the acidity was calculated as concentration of malic acid in juice and cider and as acetic acid in vinegar.

2.4.3 Total phenols

Analysis of total phenols was made spectrophotometrically with Folin-Ciocalteau's method. The phenols of the juice, cider and vinegar samples were analysed in triplicate. Phenol extraction was

made of 2 ml sample and 8 ml 50% ethanol with addition of 50 mM H_3PO_4 in 15 ml centrifuge tubes. The extraction was made overnight on a vibrating plate at 200 rpm and 8°C, and then centrifuged 10 minutes at 4500 rpm. Of the extract, 100 µl sample or 50 µl sample and 50 µl of 5% ethanol was used for analysis, depending on phenol content, to give an absorbance within the standard curve. The sample was mixed with 200 µl Folin-Ciocalteau's reagent, 2 ml 15% Na_2CO_3 and 1 ml distilled water in a cuvette according to the procedure described by Gao et al. (2000). The mixture was left for 2 hours before measurement of absorbance at 765 nm in a UV-VIS scanning spectrophotometer (Shimadzu). Each cuvette was read two times and the absorbance was compared to a standard curve of gallic acid, and the content in the sample was calculated as mg gallic acid equivalents (GAE)/L.

2.4.4 Specific gravity

The specific gravity was measured with a non-professional hydrometer for a quick and easy estimation of the produced alcohol in the cider. The reading was made three times per sample. The reading was compared to a conversion table to alcohol, provided by Proulx & Nichols (2003).

2.5 Sensory evaluation

The taste of the juice, cider and vinegar was evaluated as a descriptive analysis of flavour attributes and as an acceptance test (Appendix I). The untrained taste panel consisted of staff at the Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Science, at Balsgård. The panellist was asked about the intensity of the flavour attributes sweetness, acidity and astringency and marked their perceived sense on a 100 mm line ranging from 'very weak' to 'very strong'. In the acceptance test the panellist rated the sample on a 100 mm line ranging from 'dislike very much' to 'like very much'. The samples were served at an ambient temperature in clear plastic glasses. Potable water was available for rinsing the mouth between testing the samples.

2.6 Statistical analysis

The collected data were treated in the statistical software Minitab 16. Analysis of variance (ANOVA; One way and General Liner Model) and a multiple pairwise comparison of means (Tukey´s test) was used to determine significant differences ($p \leq 0.05$). Correlation analysis (Pearson´s correlation coefficient) was made between the perceived taste and the chemical composition.

Because of the small number of participants in the sensory evaluations, all data were not normally distributed and therefore non-parametric statistical methods could have resulted in more reliable results.

3 Results

3.1 Evaluation of yeast strains

The chemical composition of the two apple juices used for cider production during yeast evaluation was relatively similar (Table 1). The 5-cultivar juice (B) had a slightly higher TSS/TA ratio and was perceived as a little more sweet than the 3-cultivar juice (A) (personal observation). No difference in appearance of the two juices was seen when visually assessed.

Table 1. Chemical properties of apple juices utilized for evaluation of yeast strains. Juice A contained Aroma, Elise and Katja and juice B contained Aroma, Cox Orange, Elise, Katja and Kim.

Juice	TSS (%)	TA (%)[1]	TSS/TA	Total phenols (mg GAE/L)
A	9.6	0.61	15.8	320.3
B	10.4	0.56	18.8	356.4

[1] w/v malic acid

Figure 3. Apple ciders fermented for 10 days and stored 21 days in 8°C, each with different combination of yeast strains and apple juices. Juice A was made of Aroma, Elise and Katja and juice B was made of Aroma, Cox Orange, Elise, Katja and Kim.

At time of racking off the cider to new bottles, cider made from juice B was generally slightly clearer than cider made from juice A, as observed by the naked eye (Figure 2). The cider fermented with Lalvin EC-1118 was almost totally clear, whereas the Safale S-04 cider was intermediate and the wild cider very opaque. After storage the opacity had decreased in the Safale S-04 and wild yeast ciders, but was still not totally clear. In addition to the change in opacity, all combinations of juice blend and yeast strain had a lighter yellow colour than before fermentation.

The chemical composition of the ciders, that was calculated as an average of the five replicates within each combination of juice and yeast, the TSS and the specific gravity were highest in the ciders fermented with Safale S-04, which indicates that the cider was not totally fermented into dryness (Table 2 and Appendix II). Evidence of still ongoing fermentation in the cider was also provided by the slight sparkling from produced CO_2 when opening the bottle.

The TA increased slightly during fermentation, but the difference between treatments was an effect of the initial fruit juice and not the yeast strain. Because of the effect of yeast strain on TSS, but not on TA, the TSS/TA ratio, which is important for the perceived taste, was higher in the cider fermented with Safale S-04 (Figure 4). The composition of total phenols decreased during fermentation and there were differences between the two dry starter cultures compared to the wild yeast, where the latter decreased most.

Table 2. Chemical composition of apple ciders fermented with different yeast strains (Lalvin EC-1118, L; Safale S-04, S, and Wild yeast, W) with two different cloudy juices (A made of Aroma, Elise and Katja; B made of Aroma, Cox Orange, Elise, Katja and Kim). Results are means of five replicates and different letters within a column and between lines indicate significant differences between the combination of yeast and juice (p≤0.05).

Yeast	Juice	TSS (%)	TA (%)[1]	Total phenols (mg GAE/L)	Alcohol (%)[2]
Lalvin EC-1118	A	4.0 bc	0.79 ab	291.1 bc	4.9 b
Lalvin EC-1118	B	4.1 b	0.76 bc	316.7 a	5.3 a
Safale S-04	A	4.8 a	0.80 a	294.3 b	4.1 c
Safale S-04	B	4.7 a	0.75 c	321.7 a	4.5 c
Wild	A	3.8 c	0.75 bc	219.0 d	4.8 bc
Wild	B	4.1 b	0.76 abc	273.0 c	5.2 a
Lalvin EC-1118	A/B	4.1 b	0.77	303.9 a	5.1 a
Safale S-04	A/B	4.8 a	0.77	308.0 a	4.3 b
Wild	A/B	4.0 b	0.76	246.0 b	5.0 a
L/S/W	A	4.2	0.78 a	268.1 b	4.6 b
L/S/W	B	4.3	0.76 b	304.8 a	5.0 a

[1] w/v malic acid
[2] tabulated v/v estimated from the change in specific gravity (see Appendix II)

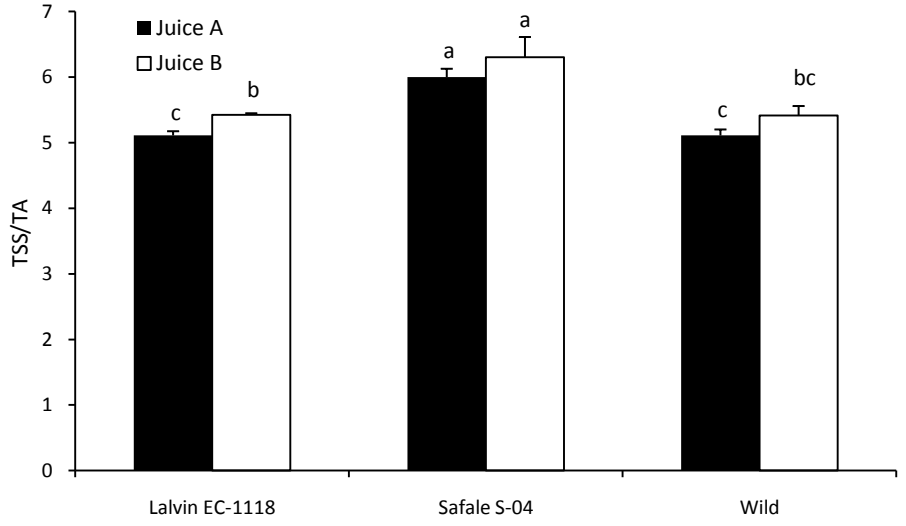

Figure 4. Ratio between total soluble solids (TSS) and titratable acidity (TA) in apple ciders fermented with three different yeasts (Lalvin EC-1118, Safale S-04 and wild yeast) from two different cloudy apple juices (A made of Aroma, Elise and Katja; B made of Aroma, Cox Orange, Elise, Katja and Kim). Bars show mean of five replicates and error bars indicate standard deviation. Different letters indicate significant differences between the combinations of juice and yeast (p≤0.05).

In a comparison of all combinations of yeast strain and juice, the ciders fermented with Safale S-04 were perceived as significantly sweeter by the taste panel than ciders fermented with wild yeast and one of the Lalvin EC-1118 ciders (Table 3). Ciders made from juice A was perceived as more sour than ciders made of juice B, but there was no effect of the yeast strains. Astringency varied between some of the treatments, but no clear-cut effects of either yeast or juice could be determined. Cider made with Safale S-04 was accepted to a higher degree than cider made with the wild yeast (Figure 5). There was a positive correlation between acceptance and perceived sweetness, TSS and TTS/TA, whereas a negative correlation was present with acceptance and titratable acidity (Table 4). The perceived sweetness did also correlate with the TSS in the cider.

Table 3. Sensory evaluation of apple ciders fermented with different yeast strains (Lalvin EC-1118, L; Safale S-04, S, and Wild yeast; W) with two different cloudy juices (A made of Aroma, Elise and Katja; B made of Aroma, Cox Orange, Elise, Katja and Kim). The attributes were scored on a 100 mm line ranging from 'very weak' (0) to very strong (100). Results are means of the response from ten panellists, and different letters within the same column indicates significant differences between the combination of yeast and juice (p≤0.05).

Yeast	Juice	Sweetness	Acidity	Astringency
Lalvin EC-1118	A	20.7 b	58.5	59.0 a
Lalvin EC-1118	B	24.6 ab	46.9	37.6 ab
Safale S-04	A	35.9 a	45.5	33.8 b
Safale S-04	B	36.2 a	37.1	38.5 ab
Wild	A	17.1 b	56.5	43.7 ab
Wild	B	21.9 b	41.6	37.2 ab
Lalvin EC-1118	A/B	22.7 b	52.7	48.3
Safale S-04	A/B	36.0 a	41.3	36.1
Wild	A/B	19.5 b	49.0	40.4
L/S/W	A	24.6	53.5 a	45.5
L/S/W	B	27.5	41.8 b	37.8

Table 4. Correlation (Pearson's correlation coefficient) between sensorial and chemical attributes of apple ciders in the evaluation of yeast strains.

	Acceptance	Sweetness	Acidity	Astringency
Sweetness	0.256 (p=0.048)			
Acidity	- 0.132 (p=0.314)			
Astringency	- 0.069 (p=0.599)			
TSS/TA	0.422 (p=0.001)			
TSS	0.487 (p=0.000)	0.403 (p=0.001)		
TA	0.392 (p=0.002)		0.024 (p=0.858)	
Total phenols	0.497 (p=0.000)			- 0.055 (p=0.675)

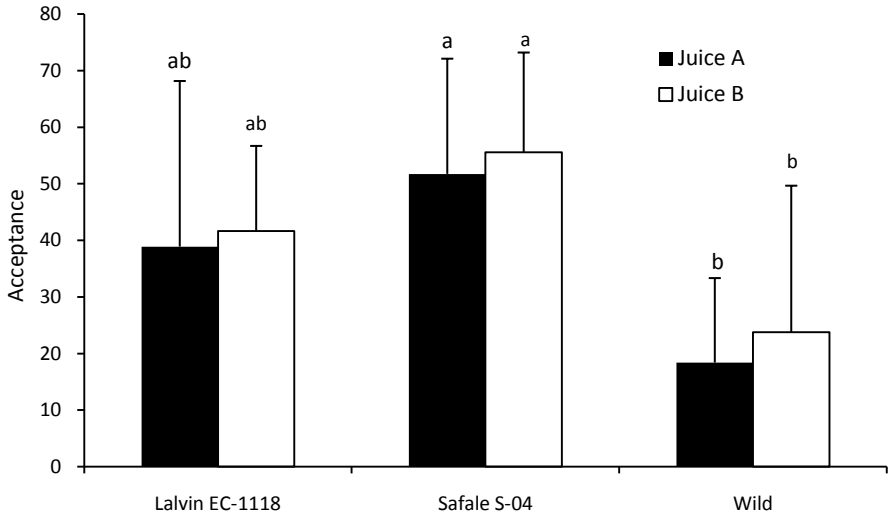

Figure 5. Acceptance of apple ciders fermented with three different yeasts (Lalvin EC-1118, Safale S-04 and wild yeast) from two different cloudy apple juices (A made of Aroma, Elise and Katja; B made of Aroma, Cox Orange, Elise, Katja and Kim). The acceptance was scored on a 100 mm line, ranging from 'dislike very much' (0) to 'like very much' (100). Bars show means of five replicates and error bars indicate the standard deviation. Different letters indicate significant differences between the combinations of juice and yeast (p≤0.05)

The panellists were invited to provide their own comments about the ciders. On the whole, these comments reported that the ciders fermented with Lalvin EC-1118 had a nice appearance and fragrance, and the Safale S-04 was perceived as a little bit sparkling. Several panellists could sense an unpleasant taint in the cider from the wild yeast fermentation, described as resemblant to chemicals, medicine, acetone or nail polish.

When choosing the yeast strain for further experiments, the most important factor was the overall acceptance. Comparing the treatments irrespective of juices there was no difference between the two dry starters, but Safale S-04 was chosen because both juices fermented with this strain were significantly better than the spontaneously fermented cider. In addition, Safale S-04 was easier to rehydrate and prepare for inoculation than Lalvin EC-1118.

3.2 Evaluation of bacterial cultures

The chemical composition of the ciders produced by batch fermentation, to be used in the bacterial evaluation for production of vinegar, was approximately the same as in the ciders obtained in the yeast test (Table 5). The TSS and specific gravity was lower than in the yeast test for ciders made of Safale S-04, because the fermentation proceeded for one week extra, to dryness of the cider.

Table 5. Chemical composition of cider made in 15 L batches with the dry starter yeast Safale S-04 and from two different cloudy apple juices (A was made of Aroma, Elise and Katja; B was made of Aroma, Cox Orange, Elise, Katja and Kim.

Cider	TSS (%)	TA (%)[1]	TSS/TA	Total phenols (mg GAE/L)	Alcohol (%)[2]
A	4.1	0.75	5.5	274.4	5.05
B	4.2	0.73	5.8	319.9	5.15

[1] w/v malic acid

[2] tabulated v/v estimated from the change in specific gravity (see Appendix II)

During acetification of the ciders, the acidity increased when ethanol was converted into acetic acid, but no significant differences were observed for the different combinations of cider and bacterial culture (Figure 6). It is worth noting that it was difficult to achieve a homogenous air flow in all the bottles. The flow to each bottle was regulated by screws on two 5-way valves, and these screws were tricky to fine-tune. In addition, the air stones showed varying resistance to flow. These factors resulted in a large variation of the acetification in each of the five replicates within each treatment.

Regarding the other chemical parameters, the TSS decreased during fermentation and was also differentially affected by the bacterial cultures, where Bockmeyer decreased most (Table 6). As in the cider, the content of phenols was higher in vinegar from juice B, but no effect was noted from the use of different bacterial cultures. The content of total phenols increased slightly during fermentation, but this difference between the cider and the vinegar can be the result of the concentration of the liquid itself. It was observed that the volume in the bottles decreased during the acetification, due to evaporation enhanced by the high temperature in the production chamber and the aeration.

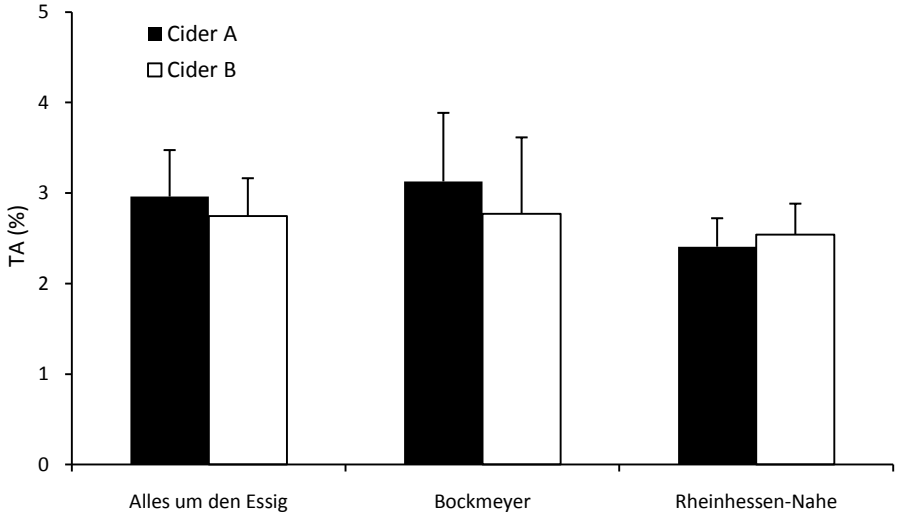

Figure 6. Titratable acidity (w/v acetic acid) in apple vinegars fermented with three different bacterial cultures (Alles um den Essig, Bockmeyer, Rheinhessen-Nahe) and from two different ciders (A made of Aroma, Elise and Katja; B made of Aroma, Cox Orange, Elise, Katja and Kim). Bars show mean of five replicates and error bars indicate standard deviation. Different letters indicate significant differences between the combinations of cider and culture ($p \leq 0.05$).

Table 6. Chemical composition of cider vinegars fermented with three different bacterial cultures (Alles um den Essig, A; Bockmeyer, B and Rheinhessen-Nahe, R) and from two different ciders (A made of Aroma, Elise and Katja; B made of Aroma, Cox Orange, Elise, Katja and Kim). Results are means of five replicates and different letters within a column and between lines indicate significant differences between the combination of yeast and juice (p≤0.05).

Bacterial culture	Cider	TSS (%)	TSS/TA	Total phenols (mg GAE/L)
Alles um den Essig	A	3.5 c	1.2	310.8 ab
Alles um den Essig	B	3.6 abc	1.3	333.7 ab
Bockmeyer	A	3.8 a	1.3	314.9 ab
Bockmeyer	B	3.7 ab	1.5	330.5 ab
Rheinhessen-Nahe	A	3.4 bc	1.4	287.9 b
Rheinhessen-Nahe	B	3.4 c	1.4	348.2 a
Alles um den Essig	A/B	3.5 b	1.3	322.3
Bockmeyer	A/B	3.7 a	1.4	322.7
Rheinhessen-Nahe	A/B	3.4 b	1.4	318.0
A/B/R	A	3.5	1.3	304.5 b
A/B/R	B	3.5	1.4	337.5 a

Table 7. Sensory evaluation of apple ciders fermented with three different bacterial cultures (Alles um den Essig, A; Bockmeyer, B and Rheinhessen-Nahe, N) and from two different apple ciders (A made of Aroma, Elise and Katja; B made of Aroma, Cox Orange, Elise, Katja and Kim). The attributes were scored on a 100 mm line ranging from 'very weak' (0) to very strong (100). Results are means of the response from seven panellists and different letters within the same column and between lines indicates significant differences between the combination of yeast and juice (p≤0.05).

Bacterial culture	Cider	Sweetness	Acidity	Astringency
Alles um den Essig	A	29.2	59.1	26.1
Alles um den Essig	B	30.4	64.1	26.9
Bockmeyer	A	27.1	60.6	23.8
Bockmeyer	B	21.6	67.1	33.9
Rheinhessen-Nahe	A	24.9	66.0	29.6
Rheinhessen-Nahe	B	27.6	59.3	26.6
Alles um den Essig	A/B	29.8	61.6	26.5
Bockmeyer	A/B	24.3	63.9	28.8
Rheinhessen-Nahe	A/B	26.3	62.6	28.1
A/B/R	A	27.1	61.9	26.5
A/B/R	B	26.5	63.5	29.1

The sensorial evaluation of the vinegars showed no differences in sweetness, acidity and astringency between the different cultures or ciders used (Table 7). In addition, there was no correlation between any of the taste characteristics and the chemical properties (Table 8). Thus, the perceived acidity was positively correlated with acceptance. When comparing the acceptance of all different vinegars, the bacterial culture from Alles um den Essig was significantly higher accepted than the two other cultures (Figure 7). The vinegar from Alles um den Essig was also described as having a fresh and clear taste by the panellists. The vinegar made by the culture from Bockmeyer was described as having an unpleasant taint and a sweaty smell whereas the Rheinhessen-Nahe was too mild and insipid. The culture from Alles um den Essig was thus used for further experiments.

Table 8. Correlation (Pearson's correlation coefficient) between sensorial and chemical attributes of apple cider vinegars in evaluation of bacterial cultures.

	Acceptance	Sweetness	Acidity	Astringency
Sweetness	0.077 (p=0.631)			
Acidity	0.353 (p=0.024)			
Astringency	- 0.097 (p=0.548)			
TSS/TA	- 0.038 (p=0.813)			
TSS	- 0.171 (p=0.285)	- 0.000 (p=0.998)		
TA	- 0.094 (p=0.557)		- 0.006 (p=0.968)	
Total phenols	- 0.224 (p=0.159)			- 0.024 (p=0.880)

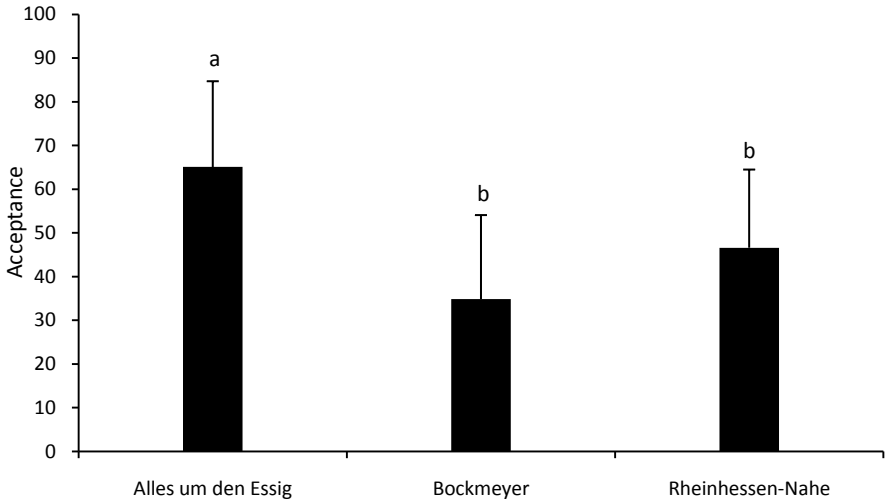

Figure 7. Acceptance of apple cider vinegars fermented with three different bacterial cultures (Alles um den Essig, Bockmeyer, Rheinhessen-Nahe) and from two different ciders (A made of Aroma, Elise and Katja, B made of Aroma, Cox Orange, Elise, Katja and Kim). The acceptance was scored on a 100 mm line, ranging from 'dislike very much' (0) to 'like very much' (100). Bars show means of the response from seven panellists over both ciders, and error bars indicate ± standard deviation. Different letters indicate significant differences between the combinations of cider and bacterial culture (p≤0.05).

3.3 Evaluation of apple cultivars

3.3.1 Juice characterisation

The juice yield of each cultivar was calculated from the weight of the mash loaded into the bladder press and the final juice weight. Several of the cultivars had a yield below 50% (Table 9). Apparently, the bladder press was inappropriate for those cultivars giving a pomace with a pulpy structure, since the mesh size of the metal basket was too large. The highest yield was obtained from Ingrid-Marie, where no mash was pressed through the pores. By contrast, for cultivars with comparatively low yield, like Jonathan and Rubinola, the amount of mash pressed through the pores was large.

Figure 8. Cloudy apple juices of ten different cultivars extracted with a bladder press.

Many of the juices were perceived as having a high viscosity, because some pulpy pomace was blended with juice at extraction. In addition, some of the juices became highly oxidised during the extraction (Figure 8).

The chemical composition of the apples varied with cultivar (Table 9). The cultivar Belle de Boskoop had a relatively high TSS compared to the others, and also the acidity was higher. TSS is often used as rough measurement of sugar content, but it is also influenced by amount of organic acids. The cultivars differed considerably (from 10 to 30) in TSS/TA, a ratio that is important for the perceived taste (Figure 9).

The total phenolic content showed a wide range, where the content in Belle de Boskoop was sevenfold the content in Aroma. It is notable that the two juices that were visually observed as brightest, Belle de Boskoop and Rubinola, also had highest initial content of phenols. By contrast, the darkest colour was observed in Aroma and Ingrid-Marie, both of which had low levels of phenols.

Table 9. Chemical composition of cloudy apple juices from ten different cultivars extracted with a bladder press.

Cultivar	Yield (%)	TSS (%)	TA (%)[1]	Total phenols (mg GAE/L)
Aroma	54.7	11.3	0.70	123.9
Baldwin	53.2	13.7	0.73	398.7
Belle de Boskoop	47.9	15.1	1.24	850.0
Bramley	40.5	10.2	1.02	464.0
Cortland	43.6	9.6	0.38	166.5
Gravensteiner	59.5	10.5	0.50	264.7
Ingrid-Marie	65.1	11.1	0.76	193.3
Jonathan	39.2	13.6	0.59	406.2
Rubinola	43.5	13.3	0.64	690.6
Spartan	47.2	12.2	0.41	324.5

[1] w/v malic acid

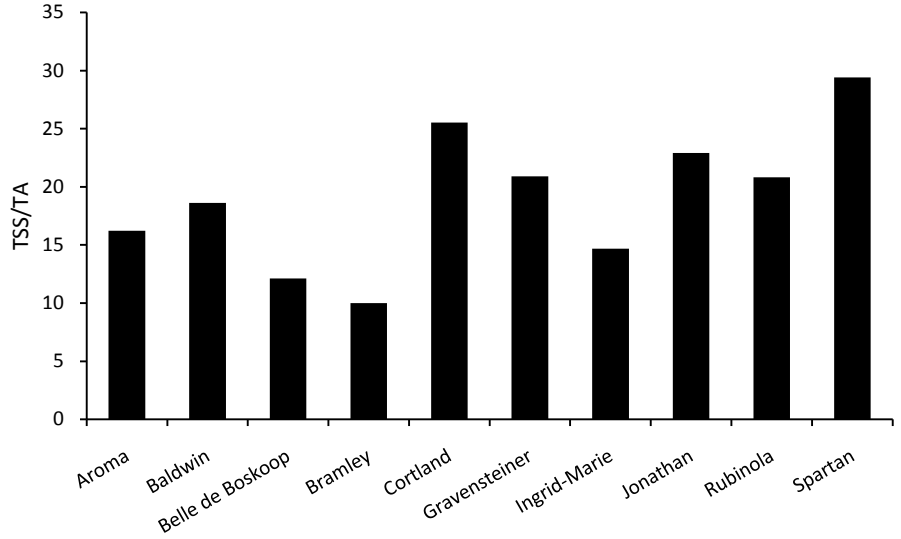

Figure 9. Ratio between total soluble solids (TSS) and titratable acidity (TA) in cloudy apple juices made from ten different cultivars.

The results from the taste test showed that juice from the cultivars Jonathan, Rubinola and Spartan were perceived as the sweetest while Belle de Boskoop and Bramley produced the least sweet juice. Juice from Belle de Boskoop and Cortland was perceived as the most and the least acidic, respectively (Table 10). The astringency did not distinguish the cultivar juices to any larger extent, and the only significant difference was observed between Bramley on one hand and Cortland, Rubinola and Spartan on the other.

When comparing the chemical composition of the juices and the results from the taste test, there was no correlation between measured TSS and perceived sweetness (Table 11). By contrast, the panellists were better able to perceive the real acid content, shown by the correlation with the measured TA. There was also a positive correlation between acceptance and sweetness but a negative correlation between acceptance and acidity as well as acceptance and astringency.

Table 10. Sensory evaluation of cloudy apple juices of ten different cultivars. The attributes were scored on a 100 mm line ranging from 'very weak' (0) to very strong (100). Results are means of the response from eleven panellists and different letters within the same column indicates significant differences between the cultivars ($p \leq 0.05$).

Cultivar	Sweetness	Acidity	Astringency
Aroma	56.3 abc	54.1 abc	36.5 abc
Baldwin	60.2 abc	49.0 abcd	35.7 abc
Belle de Boskoop	38.5 c	72.3 a	47.1 ab
Bramley	36.5 c	68.3 ab	50.3 a
Cortland	62.7 ab	17.8 e	23.4 bc
Gravensteiner	55.0 abc	56.8 abc	36.8 abc
Ingrid-Marie	48.1 bc	55.6 abc	39.6 abc
Jonathan	73.6 a	43.1 bcde	33.0 abc
Rubinola	77.5 a	36.6 cde	22.4 bc
Spartan	78.0 a	25.0 de	18.7 c

Table 11. Correlation (Pearson's correlation coefficient) between sensorial and chemical attributes of cloudy apple juices made of ten different cultivars.

	Acceptance	Sweetness	Acidity	Astringency
Sweetness	0.575 (p=0.000)			
Acidity	- 0.336 (p=0.000)			
Astringency	- 0.233 (p=0.016)			
TSS/TA	0.227 (p=0.018)			
TSS	0.144 (p=0.238)	0.096 (p=0.320)		
TA	- 0.216 (p=0.025)		0.562 (p=0.000)	
Total phenols	- 0.069 (p=0.479)			0.104 (p=0.282)

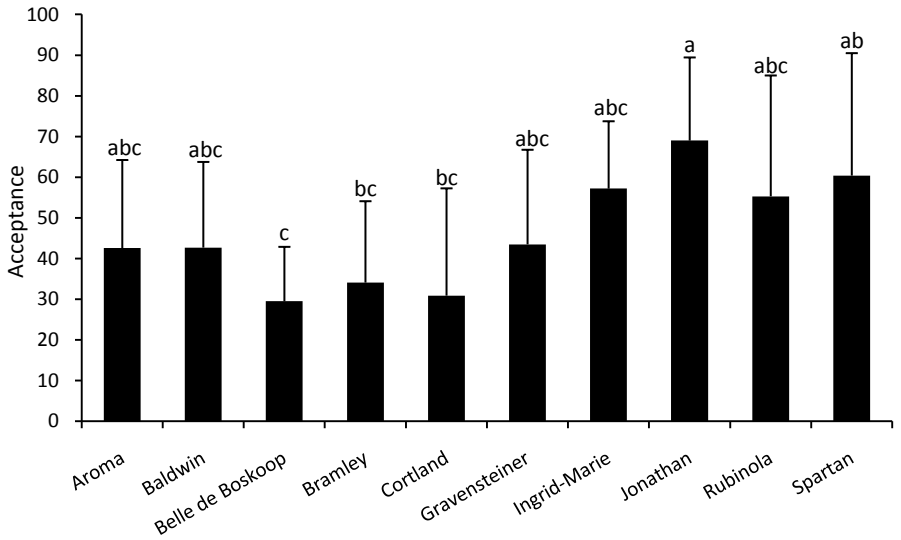

Figure 10. Acceptance by a taste panel of cloudy apple juices of ten different cultivars. The acceptance was scored on a 100 mm line ranging from 'dislike very much' (0) to 'like very much' (100). Results are means of the response from eleven panellists and error bars indicate standard deviation. Different letters indicates significant differences between the cultivars (p≤0.05).

Juice of Jonathan had a significantly higher acceptance than juice of Belle de Boskoop, Bramley and Cortland (Figure 10). Cortland was perceived as less accepted compared to juices with similar TSS/TA ratio, but this could be due to the watery and weak taste and a taint of overripeness, characters mentioned by the panellists. According to the comments, the juice of Rubinola was different both in appearance and taste. The colour was more similar to orange juice and the flavour was not recognized as typical for apple, which made it less acceptable to some people in the panel but interesting to others.

3.3.2 Cider characterisation

A colour change to a lighter appearance was observed in several of the juices after heating to 50°C when preparing them for the fermentation (mild pasteurisation). During fermentation into cider, the degree of clarification varied between the cultivars. None of five replicates in any of the tested cultivars produced cider of the same clarity as in the ciders in the yeast evaluation (Figure 11).

Figure 11. Ciders of ten different cultivars fermented with the dry yeast strain Safale S-04.

Significant differences between the cultivars were found for all analysed chemical parameters (Table 12). During fermentation, the TSS decreased in all cultivars, but to a varying degree. Cortland, with lowest TSS in the juice, also had the lowest TSS after fermentation, whereas Belle de Boskoop with highest TSS in the juice, did not have the highest content in the cider. The titratable acidity was more or less non-affected by the fermentation, whereas the content of phenols was differentially affected. For some of the cultivars the content was stable (Aroma, Gravensteiner, Jonathan), but it decreased in others (Baldwin, Belle de Boskoop, Bramley, Cortland, Ingrid-Marie, Rubinola, Spartan). All ciders except Jonathan and Spartan were more or less fermented into dryness. Because of initial differences in specific gravity (Appendix II), the hydrometer-based estimates of alcohol content were very variable with more than twice the content of estimated alcohol in Belle de Boskoop compared to Spartan (Table 12). Jonathan and Spartan were the cultivars that also had the highest TSS/TA ratio, because of the residual sugar that was present (Figure 12).

Table 12. Chemical composition of apple ciders made from cloudy apple juice of ten different cultivars. Results are means of five replicates, and different letters within a column indicate significant differences between the ciders (p≤0.05).

Cultivar	TSS (%)	TA (%)[1]	Total phenols (mg GAE/L)	Alcohol (%)[2]
Aroma	4.1 e	0.71 cde	124.3 f	5.5 e
Baldwin	5.2 c	0.72 cd	288.7 d	6.6 c
Belle de Boskoop	6.1 b	1.11 a	731.8 a	7.7 a
Bramley	4.1 e	1.00 b	395.5 c	4.5 g
Cortland	3.7 f	0.52 g	129.0 f	5.2 f
Gravensteiner	4.0 e	0.58 f	272.6 de	5.2 f
Ingrid-Marie	4.1 e	0.73 c	159.2 f	5.8 d
Jonathan	7.5 a	0.66 e	381.9 c	5.1 f
Rubinola	4.8 d	0.67 de	505.8 b	7.0 b
Spartan	7.3 a	0.52 g	249.7 e	3.8 h

[1] w/v malic acid
[2] tabulated v/v estimated from the change in specific gravity

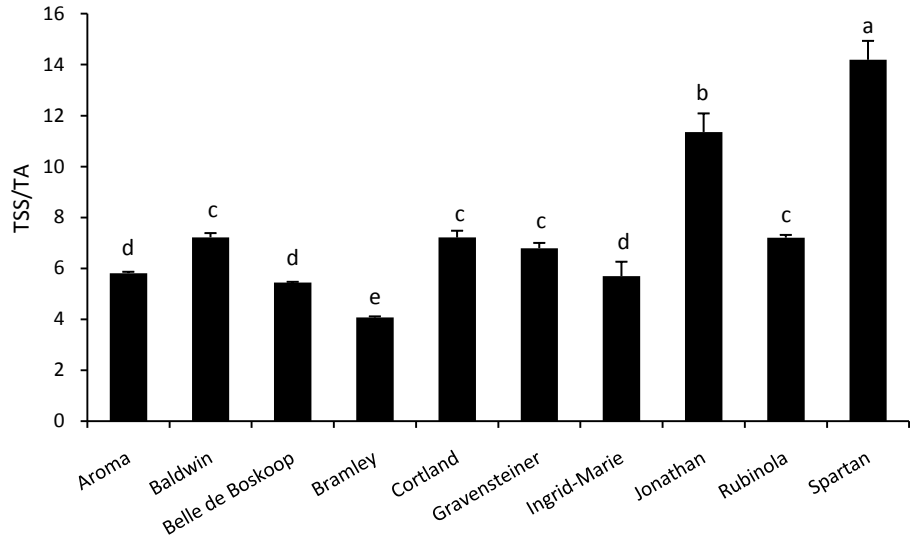

Figure 12. Ratio between total soluble solids (TSS) and titratable acidity (TA) in ciders made from cloudy apple juices of ten cultivars. Bars show mean of five replicates and error bars indicate standard deviation. Different letters indicate significant differences between the cultivars (p≤0.05).

Table 13. Sensory evaluation of apple ciders of ten different cultivars. The attributes were scored on a 100 mm line ranging from 'very weak' (0) to very strong (100). Results are means of the response from seven panellists and different letters within the same column indicates significant differences between the combination of yeast and juice (p≤0.05).

Cultivar	Sweetness	Acidity	Astringency
Aroma	28.3 c	53.1 abc	29.9 ab
Baldwin	33.8 c	44.4 abcd	30.4 ab
Belle de Boskoop	24.1 c	70.9 a	54.1 a
Bramley	25.1 c	71.9 a	44.4 ab
Cortland	37.3 c	37.0 bcd	30.4 ab
Gravensteiner	42.5 bc	39.4 bcd	34.0 ab
Ingrid-Marie	32.5 c	56.6 ab	43.6 ab
Jonathan	69.9 ab	24.6 cd	30.4 ab
Rubinola	25.4 c	62.8 ab	30.4 ab
Spartan	74.3 a	20.8 d	22.9 b

In the taste test, cider of Spartan and Jonathan were perceived as the sweetest (Table 13). The ranking of the cultivars according to acidity was similar to the corresponding ranking for acidity in juice, but Cortland was rated higher. Regarding astringency, Belle de Boskoop was rated higher than Spartan, just as for the juices. The ciders of Jonathan and Spartan had the highest acceptance, whereas Aroma, Baldwin, Belle the Boskoop and Bramley were less accepted (Figure 13). A general comment about most of the ciders in the sensorial evaluation was that appearance and consistency of the ciders more resembled cloudy apple juice than what is normally recognised as for cider, because of the low degree of clarification. The least accepted cider, of Bramley, was considered brown, sour and insipid, whereas Jonathan, one of the favourites, was described as having a rich and sweet flavour with much character of apple.

The panellists were good in sensing the real content of acids and sugars, showed by the strong correlation between the tasted acidity and sweetness with the analysed TA and TSS values, respectively (Table 14). In contrast to the juice test, astringency was positively correlated to the content of phenols.

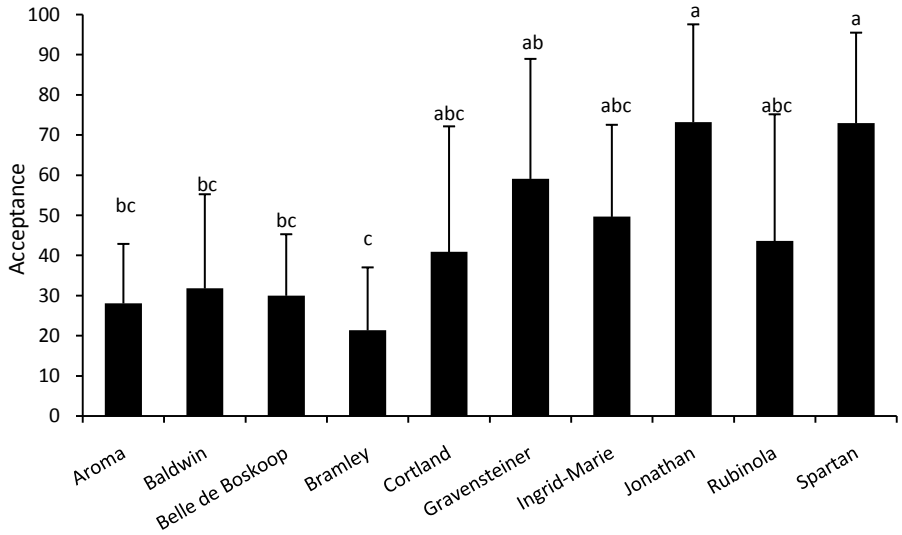

Figure 13. Acceptance of apple ciders of ten different cultivars. The acceptance was scored on a 100 mm line ranging from 'dislike very much' (0) to 'like very much' (100). Results are means of the response from seven panellists and error bars indicate standard deviation. Different letters indicates significant differences between the cultivars (p≤0.05).

Table 14. Correlation (Pearson's correlation coefficient) between sensorial and chemical attributes of apple ciders made of ten different cultivars.

	Acceptance	Sweetness	Acidity	Astringency
Sweetness	0.418 (p=0.000)			
Acidity	- 0.360 (p=0.002)			
Astringency	- 0.170 (p=0.160)			
TSS/TA	0.512 (p=0.000)			
TSS	0.377 (p=0.001)	0.528 (p=0.000)		
TA	- 0.376 (p=0.001)		0.526 (p=0.000)	
Total phenols	- 0.088 (p=0.467)			0.347 (p=0.003)

3.3.3 Vinegar characterisation

The visually observed changes in appearance during acetification were minor except for the vinegar made from Cortland which clarified during the process (Figure 14). Sediment appeared in some of the vinegars when the aeration was removed.

The chemical properties of the inoculation culture made of Alles um den Essig is shown in Table 15. The composition of the vinegars of the different cultivars was not corrected for the addition of the starter culture, since the added amount was small and identical for all cultivars.

Figure 14. Cider vinegars of ten different apple cultivars fermented with a bacterial culture from Alles um den Essig.

Table 15. Chemical composition of the bacterial culture used as vinegar inoculate (1:10) to ciders of ten different cultivars, prepared from a vinegar starter culture from Alles um den Essig.

Bacterial culture	TSS (%)	TA (%)[1]	Total phenols (mg GAE/L)
Alles um den Essig	3.3	2.38	308.3

[1] w/v acetic acid

During the acetification, the volume in the bottles decreased as a result of evaporation of the water. This process was enhanced by the high temperature and the additional aeration by the air pump. It was also observed that and the decrease was varying between the four replicates within each cultivar. The chemical composition was analysed in the final products, but data was also recalculated on the initial volume in each replicate, before calculation of mean values within each cultivar, to obtain a more reliable comparison between the cultivars.

The TSS value in the vinegars decreased compared to the TSS value in the ciders, both in compensated and in non-volume compensated data, but the differences were larger after compensation (Table 16). The largest change in TSS values was observed in those cultivars that had the highest cider TSS values, namely Jonathan and Spartan, with a decrease of 3.2 TSS% and 3.4 TSS%, respectively. For content of phenols, the cultivars were ranked in more or less the same order whether based on original phenol data or on volume-compensated data. After volume compensation, three cultivars (Aroma, Cortland and Ingrid-Marie) showed a higher phenol content in the vinegar, whereas the others remained the same or showed a slight decrease.

The acidity increased during fermentation, as expected when ethanol was converted to acetic acid by the bacterial culture. The individual replicates showed large variation in TA before volume correction, with the highest TA, 4.20%, in one replicate of Belle de Boskoop, and the lowest in one replicate of Baldwin, with 1.95%. Within-cultivar, variation was substantial, and no significant differences in acidity of the vinegars were therefore found between cultivars when based on original data. After volume compensation, Belle de Boskoop had significantly higher acidity than Baldwin and Gravensteiner, whereas the other cultivars were intermediate (Figure 15).

Table 16. Chemical composition of apple cider vinegars made of ten different cultivars with or without compensation for the volume loss during the acetification. Results are means of four replicates and different letters within a column indicate significant differences between the cultivars (p≤0.05).

	Final product				Corrected composition		
Cultivar	TSS (%)	TSS/TA	Total phenols (mg GAE/L)	Volume loss (%)	TSS (%)	TSS/TA	Total phenols (mg GAE/L)
Aroma	3.3 b	1.1 c	220.6 fg	24.3	2.5 c	1.1 c	167.3 g
Baldwin	3.8 b	1.4 bc	388.6 cd	31.3	2.6 c	1.4 bc	266.9 de
Belle de Boskoop	4.9 a	1.4 bc	812.5 a	19.3	3.9 a	1.4 bc	649.4 a
Bramley	3.7 b	1.2 c	456.7 c	29.1	2.6 c	1.2 c	322.2 c
Cortland	2.9 b	1.0 c	194.6 g	20.5	2.3 c	1.0 c	154.9 g
Gravensteiner	3.1 b	1.1 c	321.5 def	29.3	2.1 c	1.1 c	255.2 ef
Ingrid-Marie	3.6 b	1.1 c	243.3 efg	23.0	2.7 c	1.1 c	178.45 fg
Jonathan	5.1 a	1.8 a	336.9 de	15.5	4.3 a	1.8 a	285.0 cd
Rubinola	3.8 b	1.2 c	578.3 b	20.7	3.0 bc	1.2 c	457.8 b
Spartan	5.3 a	1.7 ab	244.7 efg	27.3	3.9 ab	1.7 ab	177.3 fg

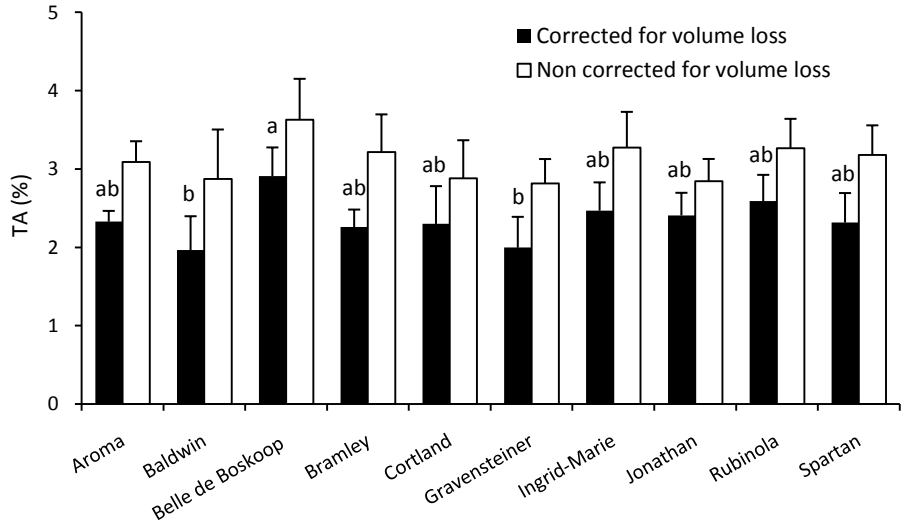

Figure 15. Titratable acidity (acetic acid w/v) in apple cider vinegars of ten different cultivars. Bars show mean of four replicates and error bars represent standard deviation. Different letters within the same bar colour indicates significant differences between the cultivars (p≤0.05).

In the sensory evaluation of the vinegars, the panellists could not sense any differences in sweetness or acidity of the vinegars, and only a few differences in astringency were perceived (Table 17). This was further concluded from correlation analysis, where none of the sensory parameters were correlated to their chemical counterparts (Table 18). Concerning the acceptance, no significant differences were found (Figure 16) due to the small data set and large variation between the panellists. However, a tendency was found for lower acceptance of the vinegars made of Belle de Boskoop, Gravensteiner and Jonathan. It was noted in the comments from the panellists that some of the vinegars had a flavour of ethyl acetate, especially in the less preferred Belle de Boskoop and Jonathan. Other comments repeated by several panellists, were a taste of honey in the vinegar made of Cortland, and a nice clear and bright colour in Ingrid-Marie vinegar.

Table 17. Sensory evaluation of apple cider vinegars of ten different cultivars. The attributes were scored on a 100 mm line ranging from 'very weak' (0) to very strong (100). Results are means of the response from seven panellists. Different letters within the same column indicates significant differences between the combination of yeast and juice (p≤0.05).

Cultivar	Sweetness	Acidity	Astringency
Aroma	28.0	60.8	31.8 ab
Baldwin	22.6	48.9	21.9 ab
Belle de Boskoop	26.9	71.1	16.9 ab
Bramley	27.9	69.3	29.0 ab
Cortland	27.9	66.9	31.0 ab
Gravensteiner	19.0	58.9	24.4 ab
Ingrid-Marie	30.0	60.6	13.0 b
Jonathan	33.2	59.3	34.6 a
Rubinola	22.1	57.1	17.4 ab
Spartan	35.9	58.2	37.4 a

Table 18. Correlation (Pearson's correlation coefficient) between sensorial and chemical attributes of apple cider vinegars made of ten different cultivars.

	Acceptance	Sweetness	Acidity	Astringency
Sweetness	0.146 (p=0.226)			
Acidity	- 0.091 (p=0.452)			
Astringency	0.200 (p=0.096)			
TSS/TA	- 0.231 (p=0.054)			
TSS	- 0.224 (p=0.062)	0.158 (p=0.191)		
TA	0.073 (p=0.550)		- 0.079 (p=0.518)	
Total phenols	- 0.187 (p=0.120)			- 0.193 (p=0.109)

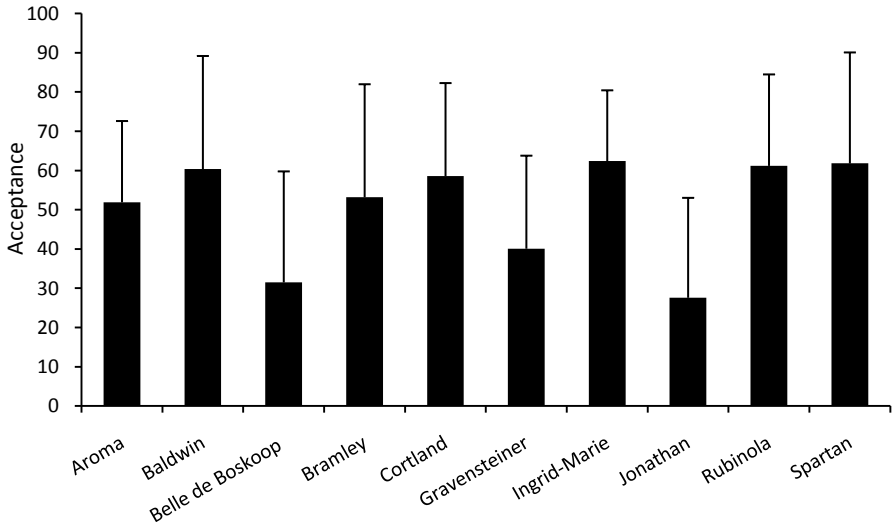

Figure 16. Acceptance of apple cider vinegars of ten different cultivars. The acceptance was scored on a 100 mm line ranging from 'dislike very much' (0) to 'like very much' (100). Results are means of the response from seven panellists and error bars indicate standard deviation.

4 Discussion

4.1 Evaluation of yeast strains

It is well known that yeast strains influence the production and the quality of fermented beverages, and that individual strains therefore may be suited to different uses (Joshi et al., 2002). In this study, different yeasts were evaluated to find a suitable culture for fermentation of cloudy apple juice into cider, in a small-scale production process.

Large changes in appearance were observed during fermentation of the two juice blends. The turbidity decreased during fermentation when plant debris and yeast cells settled, as reflected by the accumulation of lees at the bottom of the bottles. The main clarification is however the result of enzymatic breakdown of pectins, originating from the middle lamella of the plant cell wall (Blanco et al., 1999). Yeasts of the species *Saccharomyces* are able to produce pectolytic enzymes, mainly polygalacturonases. The capacity to produce and secrete these enzymes varies between different strains, which results in different degrees of clarification depending on the utilised yeast strain (Blanco et al., 1997). In the cider that was fermented spontaneously in this study, several different species and strains of yeasts may have competed, resulting in less clarification compared to the tested dry starter strains. An additional factor that can influence the clarification process is the flocculation of yeast cells towards the end of the fermentation process (Soares, 2010). As with pectinase production, the ability to flocculate is dependent on the specific yeast strain, and may have contributed to the differences in loss of turbidity that was observed in this study.

In addition to the clarification, the ciders had a lighter colour than the juices. A change to brighter appearance during the fermentation has been reported previously (Lea, 1989; Lea & Drilleau, 2003). The change in colour is caused by chemical modifications of the phenols. Yeasts are very reductive and the chromophore is therefore removed from some pigments during the fermentation, thus reducing the colour intensity (Lea & Drilleau, 2003). Another reason for colour change is reactions between proanthocyanidins and secondary metabolites from the yeasts, like acetaldehyde, which results in formation of new colourless or yellow pigments (Fulcrand et al., 2006). The phenolic content of the cider is not only important for the colour, but also for the flavour and proposed health benefits (Spanos & Wrolstad, 1992). A yeast strain that affects the phenol content as little as possible is therefore desired. All strains that were evaluated in this study reduced the content of total phenols, but the losses were largest when the juice was fermented with wild yeast strains. This observation is in contradiction to what has been found by Satora et al. (2009), where apple wines fermented with wild microflora resulted in higher content of phenolics compared to fermentation with dry starter yeasts. A reduction of the phenols may occur, since the yeast cells have the capacity to adsorb phenols to their cell membranes. The content of phenols is then reduced

when the cells settle into the lees, which are discarded (Nogueira et al., 2008). Yeasts of different strains can, however, have varying adsorbance capacity and different effect on the content of phenols during fermentation.

The yeast strains affected the fermentation rate, as indicated by the different end value of TSS and specific gravity of the cider when different strains were used. The Safale S-04 yeast had a slower conversion efficiency of sugar to ethanol compared to Lalvin EC-1118 and the wild yeast. Safale S-04 did not reach dryness (specific gravity close to 1.000) within the study period, as the Lalvin EC-1118 and the wild yeast did. The fermentation rate is dependent on the temperature (Bai et al, 2008), and the chosen fermentation temperature may have been less optimal for the Safale S-04 yeast cells than for the Lalvin EC-1118 and the wild yeast. A rapid fermentation is often desired in order to achieve a fast and thus efficient cider production, but a slower fermentation could result in better organoleptic quality of the cider (Downing, 1989).

Ciders fermented with wild yeast were significantly less appreciated than ciders fermented with the commercial starter cultures. This confirms the unpredictability and the limited control that are associated with the use of wild yeasts (Downing, 1989). Starter cultures are developed and selected because of their known capacity to produce a product with a desired characteristic. The benefits of using a defined starter culture were also shown in this study, since the ciders produced by these strains had better taste, higher content of phenols and a nicer appearance.

4.2 Evaluation of bacterial cultures

Each strain of acetic acid bacteria has its own fermentation characteristic, affecting the quality of the produced vinegar (Lea, 1989). Commercial starter cultures consist of many different strains, and thus the differences between the starters can be large. Bacterial starter cultures were evaluated to find a culture that could be suitable for cider vinegar production in the fermentation system that was designed in this study.

None of the bacterial cultures produced an acidity of 5%, and can therefore not strictly be marketed as vinegars. The estimated alcohol content in the ciders was above 5%, a concentration that should facilitate an acidity higher than the 2.5–3% that was obtained. However, the acetification was stopped before all ethanol had been converted into acetic acid. The reason to stop before completion was that the sensorial characteristics of the different treatments varied sufficiently. Thus it was already possible to select the best bacterial culture for further experiments.

The different bacterial cultures did not differ to any large extent in their effect on the chemical characteristics of the vinegars. The significant differences that thus were obtained should be interpreted with caution. No compensation was made for initial chemical differences of the mother cultures, and also not for the volume losses during acetification. These aspects were however

considered as less important, because the sensory evaluation was in focus when a suitable culture was chosen.

As expected from the small differences in chemical parameters, the panellist in the sensory evaluation of the vinegars had problems to separate any of the vinegars based on their sweetness, acidity and astringency. The panellists nevertheless showed a high preference for the culture from Alles um den Essig. The fermentation system in this study was constructed to mimic the submerged method, and the culture from Alles um den Essig had been developed for this type of system. In contrast, the cultures from Bockmeyer and the Rheinhessen-Nahe had been selected for surface systems. The turbulence in the fermentation system that was caused by the aeration could have been a stressor for these strains that had been selected for a stationary liquid. Under unsuitable conditions the bacteria can be expected to produce unpleasant secondary metabolites, giving off flavour to the vinegar and thereby less acceptance for the product.

4.3 Evaluation of apple cultivars

4.3.1 Juice characterisation

Cloudy apple juice is often produced from fruits that cannot be sold on the fresh market. Often the apple juice is produced from a mix of different cultivars, but single cultivar juices are an option, that may be even higher appreciated by consumers. Important aspects for the acceptance of cloudy apple juice by the consumers are a pleasant aroma and a good balance between sweetness and acidity. Additional aspects that the consumers consider when judging the quality are the colour and the consistency (Jaros et al., 2009).

Many of the cultivars that were pressed to cloudy apple juice had low yield and the juices were viscous and had a high degree of browning. These parameters are partially results of the processing technique. The yield could have been higher and the consistency improved by use of a better combination of mashing and pressing equipment, as well as different pectolytic enzymes. A traditional grating mill that provides larger pieces could have resulted in a decreased amount of particles when the bladder press was used for extraction. The grinding method that presently was used produced a pomace that probably had been better suited for a belt press. The smaller mesh size of the belt press could have facilitated smaller pieces of apple fruit flesh to be separated from the juice. Another aspect is that the juices were not left for sedimentation followed by decanting. This is common practise in commercial production of cloudy apple juices (Bates et al., 2001), and could have decreased the content of particles in the juice.

Extraction with a bladder press may be considered unsuitable, since the surface area of the pomace that is exposed to air during extraction is large by this method. This resulted in a high degree of oxidation for some of the juices. The browning of juice is the result of oxidation of phenols by

polyphenol oxidase (PPO) in presence of oxygen. It has been shown that the activity of PPO differs between cultivars (Joshi et al., 2007). The oxidation can be decreased by addition of pure ascorbic acid during the mashing of the apples (Bates et al., 2001). A problem with the use of ascorbic acid is the increasing public hesitation to additives, especially since a cloudy apple juice is often considered to be as natural as possible. However, in Sweden, ascorbic acid is a permitted additive also in organic products. The additive is considered as natural, being present in products with vegetable origin and therefore safe (KRAV, 2010).

If the juices are pasteurised, the high degree of browning may be a smaller problem. A change to a brighter colour was observed when the juices were heated during preparation for fermentation. This has also been observed by Choi & Nielsen (2005) that analysed the colour of cloudy apple juice by the Hunter Lab-system. A pasteurised juice obtained an increase in L*-value, that indicates more brightness, and a decrease in the a-value from positive to negative, which means a shift from red to green colouration. The change in colour has been explained as a result of fast degradation of anthocyanins during heating. Thus, even if pasteurisation sometimes is concerned as unfavourable to the flavour and aroma, it can raise the organoleptic quality by providing a better appearance to the juice.

The chemical composition differed between the cultivars, but was in general comparable with values reported in literature for cloudy apple juices produced by single dessert cultivars (Jaros et al., 2009; Wilson et al., 2003). Notable was the high acidity in Belle de Boskoop and Bramley of 1.24% and 1.02% respectively, that are in line with the acidity of 0.97% that has been reported in a crab apple cultivar (Wilson et al., 2003).

The cultivars differed in total phenols, and this can depend on an initial variation between apple cultivars, but also a varying degree of oxidation during extraction, that can result in loss of some phenols. Generally, the phenol content is lower in dessert cultivars compared to cider cultivars, but the values listed in literature are variable. Kahle et al. (2005) have reported a phenol content of 154–178 mg/L in dessert apples and up to 970 mg/L in cider apples. Thus several of the cultivars in the present study should then belong to the cider apple group. On the other hand, Jaros et al. (2009) found phenol contents of 585–655 mg/L in dessert apples, and Nogueira et al. (2008) found up to 2200 mg/ in cider apples.

In the acceptance test, the juice of Jonathan apples was most appreciated. This may be explained by the fact that this cultivar is one of the most commonly used for commercial apple juice production. The panellists could unconsciously have recognized it as a standard apple juice taste and therefore liked it most. Poll (1981) evaluated different cultivars for juice production, including Aroma, Belle de Boskoop, Bramley, Ingrid-Marie and Spartan. The overall aroma and taste were in that study equally good for Aroma, Belle de Boskoop, Ingrid-Marie and Spartan, whereas Bramley

was scored lower. This observation is in alignment with the acceptance of the cultivars in the present study, except for Belle de Boskoop, that was less liked. Both in this study and in Poll (1981), the juices from Belle de Boskoop and Bramley were described as sour, and had relatively low TSS/TA ratios.

To be able to judge the quality of an apple juice, it is important to know which chemical parameters that are relevant. In the present study the acceptance was positively correlated with the TSS value and negatively correlated with the TA value. However, the strongest correlation was obtained between acceptance and the TSS/TA ratio. In Jaros et al. (2009) and Poll (1981) it has also been concluded that the TSS/TA ratio is the most important parameter for predicting consumer preference of cloudy apple juice, and an optimal ratio of 15–18 was found. In the present study the less accepted Belle de Boskoop and Bramley juices had TSS/TA ratios clearly lower than 15, but juices with ratios up to 29 were equally well accepted. Jaros et al. (2009) yet also reflected on that there is a segment of consumers that generally prefer sweeter juices with higher TSS/TA ratio.

Compared to the TSS/TA ratios reported for Aroma, Belle de Boskoop, Bramley, Ingrid-Marie and Spartan by Poll (1981), the ratios were higher in the present study. This may indicate that some of the fruits had passed the optimal stage for pressing. The chemical composition of apples changes with ripening. The sugar content increases and the acid content decreases over time (Bates et al., 2001), resulting in increasing TSS/TA ratio. These changes affect the taste of the produced juice. The studied cultivars were all harvested and pressed at the same time without considering their optimal ripening stage. Both the results of the chemical analyses and the acceptance ratings of the cultivars must therefore be taken with caution. The results could have been different if the juice had been extracted at another time of the year. To provide the most reliable evaluation, each cultivar should be harvested and pressed at its own optimal stage, and the juice thereafter stored frozen until analyses.

4.3.2 Cider characterisation

Apple cider of the traditional English or French type is a relatively uncommon beverage in Sweden. Cider in Sweden has for a long time been a sweet and sparkling soft drink with relatively low content of juice, sometimes even without alcohol. Quality parameters that characterise traditional ciders are rather different, with stronger colour intensity, varying degree of clarity, less sweetness and higher astringency and higher alcohol content.

Ciders produced from the different cultivars in this study were extremely turbid. Both the appearance and consistency resembled more a juice than a cider. This may be due to the juices that were utilised for the cider production, since they had a high degree of solid particles. The content of pectins in the cell walls was probably higher than the yeast pectinases could degrade. The clearest cider was produced from Ingrid-Marie, the cultivar that also had highest yield and least amount of

pulpy mash dissolved in the juice after pressing. To improve the viscosity and turbidity, the finished ciders can be filtered. It is however better to change the practises during juice extraction, and thereby avoid too high amounts of solid particles in the cloudy juice that shall be fermented.

In addition to the clarity and colour, the phenol content is important since the phenolic procyanidins provide astringency and bitterness to the ciders. These are preferable characteristics in traditional ciders (Kahle et al., 2005). During fermentation the phenol content changed differently between the cultivars in this study, where it was either stable or decreased. As mentioned for the yeast evaluation (4.1), a decrease in phenols can take place during fermentation due to adsorbance to the yeast cell wall. The phenols can also bind to proteins, precipitate and be removed with the lees (Nogueira el al., 2008). In addition, a decrease can take place initially during fermentation, as a result of oxidation by PPO. Krapfenbauer et al. (2006) demonstrated that pasteurisation at 80°C is required to totally inactivate PPO. The mild heating of the juices at 50°C in the present study may not have been high enough to inactivate the enzyme. The PPO could then continue to oxidise the phenols during fermentation, before anaerobic condition was established. Both the content of proteins and the activity of PPO depend on the cultivar (Spanos & Wrolstad, 1992), thus resulting in different effect on the phenol content in the different cultivar ciders during fermentation.

The importance of the phenols for the astringency of the ciders was confirmed from the taste test, where the perceived astringency and the actual value of total phenols were strongly correlated. In the apple juice there was no significant correlation between astringency and total phenols, probably due to the higher sweetness of the juice, masking the differences.

In absolute values, the phenolic content of the ciders was lower than what has been reported for traditional ciders. Picinelli et al. (2000) analysed the composition in Asturian ciders, and reported a content of 0.8–1.3 g/L, which is higher than for all of the cultivars analysed in this study. The lower phenol content could have an effect on the taste of the ciders in this study, making them less astringent than ciders made of true cider cultivars.

The alcohol content of the ciders was varying, and some ciders had lower content than the 6–7% that according to Downing (1989), is common in dry English ciders. In Picinelli et al. (2000) the alcohol content was 5.3–5.6% in ciders made from blends of different cultivars. These values are in better agreement with the estimated alcohol content of the ciders in the present study. The alcohol content that theoretically can be achieved in a cider is determined by the sugar content of the juice. The final alcohol content also depends on the fermentation efficiency of the yeasts and it was observed that the ciders of Jonathan and Spartan apple juice had low alcohol content compared to what would have been possible from their sugar content (estimated as TSS). In addition to sugars, the yeast cells also require other nutrients, like nitrogen, minerals and vitamins, and the content of

nutrients always varies between cultivars. If the yeast cells lack nutrients the viability can decrease, and the fermentation process may be reduced or interrupted (Rainieri & Zambonelli, 2009).

As for the juices, the acceptance of the ciders was strongly correlated with the TSS/TA ratio. The ratio could thus be used as a predictor for consumer acceptability. The sensory panel had a preference for a high ratio, that indicates a sweeter cider with less sour taste. This could be because ciders on the Swedish market (which the panellists probable recognize) are often sweet, soda-resembling beverages. An option to satisfy consumer preferences could be to stop the alcoholic fermentation before dryness and in that way keep some residual sugar and sweetness in the cider. In this way the alcohol content is also decreased. The acceptance rating of the cultivar ciders was relatively stable compared to the rating of the juices, with the sweetest, Jonathan and Spartan, as favourites. It can be concluded that a well tasting juice most probably also will result in a cider with high acceptability.

4.3.3 Vinegar characterisation

No detailed studies of the effect of different apple cultivars on the characteristics of apple cider vinegar have been found in literature. Vinegar has however been used for long time as condiment and for preservation (Joshi & Sharma, 2009). For quality, safety and stability issues it is therefore important that the utilised apple cultivar can provide a proper acid content, which is determined by the initial sugar content of the fruit.

None of the fermented ciders from the different cultivars reached an acid level of 5%, and could thus not strictly be called vinegars. The alcohol content in most of the cultivar ciders was however high enough to reach a sufficient acidity, but time and also the fermentation method (discussed in 4.4) were probably not optimal. However, the cultivars have potential for vinegar production, if the process is improved. Apple cider vinegar has proposed health benefits and is already used by many people as a daily shot, but then often diluted in water due to the very sour taste. The lower acidity of the vinegars from the different apple cultivars could be seen as an advantage, since these vinegars are easier to drink undiluted. Of course, the health benefits of the vinegars produced with lower acetic acid content may not be the same as tested vinegars, and must be evaluated in scientific studies before any claims are made.

The phenolic content is also important for the proposed health benefits of the vinegar. During fermentation from cider into vinegar the total phenols decreased for some of the cultivars, however, the rating between the different cultivars was almost the same. It may be concluded that the content of phenols in the raw material is the major determiner for the final content in the vinegar. The initial differences before the double fermentation are thus more or less preserved during the production. Andlauer et al. (2000) analysed the change in phenols during acetification in different types of

vinegars, and obtained a 40% decrease in cider vinegar. This is a much higher decrease than in the vinegars in the present study.

Even if the content of acid in the vinegars was low in this study, it was high enough to mask other taste characteristics. This resulted in difficulties for the taste panel to recognize any differences between the vinegars from the different cultivars. Some of the vinegars had a smell and taste of ethyl acetate, that provides an unpleasant chemical fruitiness to the vinegar. Ethyl acetate is a reaction product of ethanol and acetic acid, and can be formed in vinegars during storage, if there is any residual alcohol (Joshi & Sharma, 2009). Before sensory evaluation, the vinegars were stored for three days, a time that could have been long enough for the ethyl acetate formation. The vinegars that were perceived as having a hint of ethyl acetate were especially those of Belle de Boskoop and Jonathan. Belle de Boskoop contained highest amount of alcohol in the cider, and it could be expected that more ethanol was remaining compared to the other cultivars after the acetous fermentation. This also results in a higher risk for formation of ethyl acetate. Jonathan, on the other hand, had intermediate alcohol content in the cider, but the cider was not fermented into dryness. Some additional alcohol could have been produced by the yeasts even after the start of acetous fermentation, and contributed to formation of ethyl acetate. Belle the Boskoop and Jonathan had the lowest values for acceptance. It cannot be excluded that the ethyl acetate was the main reason for the low acceptability instead of unfavourable cultivar characteristics.

There was a change in acceptance for some of the cultivars when fermenting into vinegar compared to the ciders, and this shows that different cultivars may be suited for different applications. Jonathan, that produced juice and cider with the highest acceptability, showed a tendency to be least accepted as vinegar. On the other hand, Cortland was not liked as juice but among the best accepted vinegars, whereas Ingrid-Marie was well accepted as juice and vinegar, but not as cider.

4.4 Acetous fermentation system

The conversion of ethanol to acid by acetic acid bacteria is a process that requires large volumes of oxygen (García-García, 2009). As mentioned previously, within the 16 days of acetous fermentation, none of the vinegars obtained the acidity that theoretically was possible, as calculated from the content of alcohol in the cider. In this study a small-scale fermentor system was designed, with increased aeration by air pumps and air stones intended for aquariums, in an attempt to achieve an efficient and quick fermentation process. The system was based on the submerged method, where the bacteria constantly are submerged in the liquid, and oxygen is provided through fine air bubbles. In a commercial submerged fermentor the yield of acetic acid can be up to 95–98% of the initial ethanol content, and the conversion can take place within one or a few days (García-García et al.,

2009, Raspor & Goranovič, 2008). This efficiency was far from reached in the present study, and several things should be considered in an attempt to improve the method.

Firstly, each pump provided air to ten bottles, and it was difficult to finely tune the aeration for each individual bottle. The variation in aeration between the bottles caused differences in acetic acid production, and may explain the large variation between the replicates within each cultivar. In addition, the flow of air should have been higher. This could have been achieved by stronger pumps.

Secondly, during the acetous fermentation the air stones successively became covered by a mucus layer. This layer clogged the stones and decreased the permeability, and thereby the supply of oxygen over time. Bacteria belonging to the species *Gluconacetobacter xylinus* have the ability to produce cellulose, which is the major part of the slime (Joshi & Sharma, 2009).

Thirdly, the bottles were covered with rubber caps during fermentation, but these proved not to be completely airtight. Because of the high temperature and the aeration, a considerable large volume of the initial liquid evaporated during the weeks of fermentation. This resulted in a concentration of sugar, acid and phenols in the final products. Unfortunately, the content of ethanol was not possible to measure per se, and it can therefore not be excluded that some ethanol also evaporated. This resulted in lower content of acidity than theoretically possible.

Fourthly, the number of bacteria in the inoculation culture was not determined. A low number could be one cause to the slow fermentation rate. The submerged culture from Alles um den Essig was diluted and prepared as the cultures for surface systems. This may also have resulted in too low concentration of bacteria. An additional factor to the suboptimal acidity level of the final vinegar could have been the initial acidity of the substrate. Grewal et al. (1988) used a laboratory scale fermentor to produce vinegar from apples, and found that the optimal initial acidity, after bacteria inoculation, was 1.5%. In the present study, the bacterial culture used for inoculation had an acidity of 2.38% (w/v), thus only contributing with about 0.2% acidity to the ciders.

Traditional vinegar production takes several months by the surface method (Joshi & Sharma, 2009). Despite the suboptimal conditions, this study has however shown that a faster vinegar production is possible to achieve, and in a relatively easy way with inexpensive equipment.

5 Conclusions

The results presented in this pilot study show that several factors influence the chemical and sensorial characteristics of cloudy apple juice, cider and vinegar produced in a small scale. The processing techniques obviously affected the consistency, colour and clarity of the products. The choice and control of microorganisms used for fermentation was of importance to obtain a product with proper appearance and without off taste. The apple cultivars that were compared showed high variability in chemical composition, which influenced the sensorial characteristics of the juice, cider and vinegar. It is well known that the ripeness of the apple affects the chemical composition, and it can be recommended to use fruits of different cultivars at their respective optimal ripeness stage, to perform a more fair comparison. It was possible to produce products of acceptable quality from the Swedish-grown dessert apples, but it was also shown that only a few cultivars had sufficient flavour and balance between sweetness and sourness to be used alone. Based on the data presented in this report, further product developments can be performed e.g. by making blends of cultivars with different properties to get the desired fragrance, flavour and aroma.

A further important step would be to evaluate the market opportunities for locally produced cider and vinegar. Until now, many of the Swedish consumers are not familiar with the traditional ciders and vinegars, and large efforts are probably needed to market these rather novel beverages. An introduction could require an adaption of the product to the local taste preferences that was shown in the sensory analyses, which means a sweeter and less astringent cider compared to the common traditional types. For this purpose, the Swedish-grown cultivars that were evaluated have been shown to be suitable.

It was difficult to develop a rapid and efficient vinegar production process in small scale. However, the low acid vinegars that were produced could have a future as health promoting drinks, if the beneficial effects on the health that has been shown for true apple cider vinegars also can be established for the developed low acid vinegars.

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Appendix I – Sensory evaluation form

Smaktest äppelmust/cider/vinäger/*Taste test apple juice/cider/vinegar*

Provnummer/*Sample number* _____

Smakegenskaper/*Flavour characteristics*
Markera på linjen för att ange intensiteten av olika smakkomponenter.
Draw a mark on the line to indicate the intensity of different flavour attributes.

Sötma/*Sweetness*

Mycket svag — Mycket stark
Very weak — *Very strong*

Syrlighet/*Acidity*

Mycket svag — Mycket stark
Very weak — *Very strong*

Strävhet/*Astringency*

Mycket svag — Mycket stark
Very weak — *Very strong*

Acceptans/*Acceptance*
Betygsätt provet generellt med en markering på linjen.
Rate the acceptance of the sample by marking on the line.

Ogillar mycket — Gillar mycket
Dislike very much — *Like very much*

Övriga kommentarer (doft. färg. bismak etc)/*Other comments (fragrance. colour. taint etc)*

Appendix II – Specific gravity

Table II-1. Specific gravity of apple juices and ciders in yeast evaluation. Results in ciders are means of five replicates and different letters within the column and between lines indicate significant differences between the combination of yeast and juice (p≤0.05)

Yeast	Juice	Juice specific gravity	Cider specific gravity
Lalvin EC-1118	A		1.000 b
Lalvin EC-1118	B		1.000 b
Safale S-04	A		1.006 a
Safale S-04	B		1.005 a
Wild	A		1.001 b
Wild	B		1.001 b
Lalvin EC-1118	A/B		1.000 b
Safale S-04	A/B		1.006 a
Wild	A/B		1.001 b
L/S/W	A	1.040	1.002
L/S/W	B	1.043	1.002

Table II-2. Specific gravity of apple juices, ciders and vinegars in bacterial evaluation. Results in vinegars are means of five replicates and different letters within the column and between lines indicate significant differences between the combination of bacteria and juice (p≤0.05).

Bacteria	Juice	Juice specific gravity	Cider specific gravity	Vinegar specific gravity
Alles um den Essig	A			1.010
Alles um den Essig	B			1.009
Bockmeyer	A			1.010
Bockmeyer	B			1.008
Rheinhessen-Nahe	A			1.009
Rheinhessen-Nahe	B			1.008
Alles um den Essig	A/B			1.010
Bockmeyer	A/B			1.009
Rheinhessen-Nahe	A/B			1.008
A/B/R	A	1.040	0.999	1.009
A/B/R	B	1.043	1.001	1.009

Table II-3. Specific gravity of apple juice, cider (fermented with Safale S-04 dry starter yeast) and vinegar (Alles um den Essig bacterial culture) in cultivar evaluation. Values are mean for five and four replicates for cider and vinegar respectively. Different letter within column of cider or vinegar indicates significant differences between the cultivars (p≤0.05).

Cultivar	Juice specific gravity	Cider specific gravity	Vinegar specific gravity
Aroma	1.044	1.000 de	1.013 bc
Baldwin	1.054	1.002 bc	1.016 abc
Belle de Boskoop	1.062	1.002 b	1.017 ab
Bramley	1.040	1.002 b	1.015 abc
Cortland	1.041	0.999 de	1.011 c
Gravensteiner	1.043	1.001 cd	1.012 bc
Ingrid-Marie	1.046	1.000 de	1.013 bc
Jonathan	1.056	1.015 a	1.017 ab
Rubinola	1.053	0.998 e	1.013 bc
Spartan	1.047	1.016 a	1.019 a