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3 March 2004

RSS 308029658

AN ASSESSMENT OF CHEMICAL MARKERS FOR THE ESTABLISHMENT OF JUICE CONTENT IN CIDERS

FSA PROJECT Q01057 A

EXECUTIVE SUMMARY

- A literature review was undertaken to examine those components of apple juice, especially phenolics, which might provide quantitative markers of juice content in ciders.
- The juice content of UK ciders was estimated from published data as typically 30 – 50%.
- Potassium as a primary marker and sorbitol as a secondary marker were identified as the most suitable components for quantitative assessment of juice content. Phenolics were considered to be of no value due to their variable levels and losses during processing.
- Based on published data for potassium in apple juice, it was concluded that juice content in ciders could be estimated with a confidence interval ± 25% of the true value, taking a mean value of 1000 mg/l potassium to represent pure juice.
- Experimental fermentations and dilutions were carried out using juices, juice concentrates and glucose syrup, followed by potassium and sorbitol analyses to validate the proposed approach. The values determined were within the expected confidence interval.
- Commercial ciders and perries were analysed for potassium and sorbitol levels, and gave data for juice content which was consistent with the range expected from the published literature.
- It is concluded that analysis of potassium and sorbitol is an appropriate method for determining the juice content of ciders.

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PART 1

INTRODUCTION

This report forms a part of FSA project Q 01057 "Identification of Markers in Ciders and Fruit Drinks". The Agency's interest in cider arises from a perception that most consumers assume that cider is made primarily from apple juice rather than from other forms of fermentable sugars, and therefore as a matter of public interest it would be valuable to have a way of determining this. The Agency consequently called for a review of methodologies that might be appropriate for assessing the juice content of ciders (and perries), with a particular focus on the potential use of polyphenols for this purpose. This report (Q 01057A) provides such a review and also some experimental work in support of the proposed methodology. A second separate report (Q 01057B) deals with the fruit juice content of unfermented fruit drinks.

PART 2

LITERATURE REVIEW

2.1 Historical Background

The need for a way of identifying the juice content of fermented cider is not new. In 1909, a Government Laboratory analysis of a 'Champagne Cider' showed that it consisted primarily of sweetened water acidified with tartaric acid and only slightly flavoured with cider, and in that year the Board of Agriculture initiated two successful prosecutions for "selling under the name of cider an article devoid of apple juice" (Marsh 1983).

To support such prosecutions, a chemical test for the presence of apple juice was required. The Director of the National Fruit and Cider Institute (BTP Barker) and the Bristol Public Analyst (E Russell) reviewed the data available to them at the time on cider composition in terms of sugar-free extract, acid, alcohol, ash and 'tannin' (Barker and Russell 1909). They also devised a quick colorimetric test for 'tannin' based on the treatment of an ethyl acetate extract of cider with lime water, which gave a claimed detection limit of 1 part per thousand of apple juice in cider. Such a test, as the authors knew, could only pick out ciders which were grossly fraudulent and contained no apple juice at all. It could not define the actual juice content of a cider.

There were a number of legitimate reasons why a fermented cider might be watered to some extent prior to sale. Chief amongst these were the high alcohol content (sometimes exceeding 8%) which might result from the fermentation of juice in a good year when the starting gravities were high, though a more typical figure was 6%. A 'small cider' for drinking in quantity by farm labourers engaged in thirsty work without the risk of over-intoxication might also be required at a much lower alcohol level of say 3.5%. In those circumstances, the watering of ciders was considered desirable and acceptable. But the addition of water to cider otherwise was regarded by Herbert Durham (the then Chief Chemist of HP Bulmer) as "a fraud, especially if there is attempted concealment by adding sugar also". Durham (1908) proposed minimum alcohol and sugar-free extract levels which he felt to be essential to support a legal definition of cider.

In 1909 Barker lamented that there was in the UK no such legal definition of cider. In 1931 a short-lived 'National Mark' scheme for cider was introduced by the Ministry of Agriculture but it had been discontinued by the late 1930's (Beech and Carr 1977). There is still no legal definition today, although the NACM (National Association of Cidermakers) Code of Practice in its various forms from 1970 onwards has been adopted by HM Customs and Excise as Notice 162, which appears to have been accepted as a *de facto* definition by Trading Standards (a relevant extract is included as an Appendix to this report). The NACM Code of Practice has always permitted the addition of sugar or syrup and water "before or after fermentation". It also allows the addition of up to 25% pear juice to cider and up to 25% apple juice to perry (Beech and Carr 1977, NACM 1998).

2.2 Present Day Practice

The current situation in the UK therefore permits a cider to be made with the unlimited addition of fermentable syrup before fermentation to a high alcohol level, followed by 'breaking back' with water prior to retail sale (Beech 1993, Jarvis 1993). The final juice equivalent may therefore be substantially lower than 100%. Currently there is no requirement for this information to be disclosed by cidermakers. It is probable that the practice began on a significant scale during the 1960's when post-war sugar rationing had ceased and when glucose syrups (hydrolysed corn starch) became widely and cheaply available as brewing adjuncts. As brewery practices became more widely adopted throughout the UK cider industry during the 1970's, this form of 'chaptalisation' appears to have become the norm.

The product made in this way is very different from a traditional 'pure juice' cider, and effectively has redefined the meaning of the word 'cider' in the UK marketplace over the last 30 years. The mainstream industry can point to a considerable increase in sales volume over that period, and can argue that they have responded to consumer demand by producing a lighter style of cider to compete successfully with lager amongst their target consumers in pubs and other outlets. The fact that it contains far less apple juice than hitherto is seen as a benefit, not a drawback, since it makes the product less 'heavy' on the palate and easier to consume.

Manufacturers do not reveal the juice content of their ciders and they are under no obligation to do so. Two pieces of published information do enable an estimate to be made, however. A discussion of cider chaptalisation by Jarvis (1993), the then Technical Director of HP Bulmer, suggests that sugar syrups are added to fresh or reconstituted juice of 12º Brix (potential alcohol 6 %) to give a juice of 24º Brix (potential alcohol 12%) prior to fermentation. The dilution back to an alcohol of 6 % would therefore give 50% apple juice equivalent, and a further dilution to a product of 3.5% alcohol would give 30% juice equivalent.

Ministry of Agriculture statistics for 1999 permit the data shown in Table 2.1 to be calculated. The total amount of cider apples, non-cider apples and imported concentrate used by the industry in 1999 (from their own figures) amounted to 183 M litres of juice at 11.2º Brix single strength juice equivalent (SG 1.045 and potential alcohol 5.6%). The total claimed production for 1999 (NACM figures) was 600M litres of cider. Assuming a mean alcohol level of 5.6% for all ciders, this gives a mean juice content of 183/600 = 31%. From these data it would appear that the overall juice content of ciders produced in the UK is about one-third of the product volume.

Table 2.1

Apple and Cider Production Figures for 1999

	Tonnes	**Yield**	**Million L**	**% of Production**
Cider Apples	78900	0.78	61.5	10.3%
non Cider Apples	44000	0.78	34.3	5.7%
Imported concentrate (assumed 70° Brix to 11.2°)	14600	5.98	87.3	14.5%
SUBTOTAL (apples and AJC)			183.1	30.5%
TOTAL PRODUCTION (NACM figures)			600	100.0%

Apple and concentrate figures are taken from MAFF Statistics issued 18th July 2000. Cider production figures are taken from 'Cider 2000' (NACM). Juice yield is assumed at 780 litres per tonne. Concentrate is assumed to be at 70° Brix. 11.2° Brix is taken to be SG 1.045 with a potential alcohol of 5.6% v/v. No allowance has been made for imported fresh apples used forUK cider production (if any).

2.3 Apple and Cider Components as Juice Content Markers

Any chemical assessment of juice content should ideally be based on an analytical parameter which is stable, easy to detect, expensive to purchase, unique to the fruit in question, varies naturally between only very narrow limits and is not subject to losses during processing. It goes almost without saying that there is no such ideal material! Nevertheless a range of possibilities present themselves and will be most conveniently examined in the following classes:

- Isotopic composition of ethanol
- Sugar-free extract
- Non-fermentable carbohydrates
- Organic acids
- Nitrogenous components
- Mineral components
- Polyphenols
- Other materials

It should be mentioned in passing that the concept of RSK values for cider (similar to those used for pure fruit juices) has been suggested and explored in Germany (Scholten 1992), but the proposed list would be impracticably restrictive for cider made under the permissions of C&E 162.

2.3.1 Isotopic composition of ethanol

In many other fermented beverages, such as grape wine or distilled spirits, isotopic analysis of ethanol can indicate the botanical source of the original sugar. For instance the ratio of ^{12}C to ^{13}C or the ratio of deuterium to hydrogen in the terminal methyl group of ethanol can indicate whether a source is non-agricultural, derived from cane or corn, or derived from beet, whey or apple. However, in most such products, the permissible fermentable sugars are very limited and therefore any deviations constitute adulteration. In the case of cider, all sources of fermentable sugar are allowable and the quantitative detection of apple within such a wide envelope becomes impracticable. The use of beet and inulin syrups, with identical carbon ratios to that of apple and

very similar D/H values, makes it impossible to measure apple content in ciders with any degree of confidence by isotopic analysis of the sugar-derived ethanol.

2.3.2 Sugar-Free Dry Extract

The 'sugar-free dry extract' of fermented beverages has been used for many years as an indicator of minimum juice content, and was suggested by Durham as a criterion for ciders in 1908. It is generally estimated by the difference in density between that of the original cider and that of its distillate under standard conditions - this gives a figure which can be expressed as 'total dry extract'. The sugar content is analysed and then subtracted to leave the value for sugar-free dry extract (SFDE). The sugars were traditionally estimated as Fehling's reducing sugars, but are nowadays normally estimated by HPLC.

Principal contributors to the SFDE include polyols and unfermentable sugars, non-volatile organic acids, inorganic ash, and polyphenols. Barker (1909) in reviewing the literature to that date, remarked that the SFDE of authentic ciders showed a minimum of around 16 g/litre and that “it would be a valuable constant, provided that standard methods for the determination of total solids and sugars were invariably adopted”. He noted that Allen (1902) found a range of SFDE in commercial English ciders from 5.5 up to 40 g/l but that “there is no means of distinguishing how many of these ciders were really genuine”. Burroughs (1984) in work commissioned by MAFF in 1980, determined the SFDE in 25 authentic UK apple juices to be 19, 22 and 14 g/l in Bramley, Cox and Golden Delicious juices respectively. The mean value was 19.2 g/l with a CV of 21%.

The SFDE continued to be a useful and widely used measure for minimum juice content in ciders until very recent years. Thus Possmann (1992) could report that the minimum SFDE values for ciders accepted in Germany, France and the UK were 18, 16 and 13 g/l respectively. The value of 13 g/l had been adopted by the NACM in 1970 and was incorporated into the Code of Practice from that time (Beech and Carr 1977).

However, the SFDE has a serious drawback as a measure of juice content if fermentable sugar syrups are added during cidermaking. Such syrups are prepared by incomplete hydrolysis of glucose or fructose polymers (starch and inulin respectively) and therefore inevitably contain significant quantities of unfermentable oligosaccharides (i.e. maltose and higher, although maltose may be fermentable by certain yeasts). These remain unchanged after fermentation and therefore raise the apparent SFDE level. For instance, a '95% dextrose equivalent' (95DE) syrup sold as a fermentation adjunct could contain 50 g/kg of unfermentable oligosaccharides (e.g. Cargill 'Clearsweet' Datasheet). If added in the proportions given in the previous example (i.e. to a 12º Brix juice to give a 24º Brix juice which was diluted back after fermentation), it could add 3 g/l to the SFDE, which is around half the SFDE contributed by the original apple juice. Lower DE syrups contain even more oligosaccharides. In that situation the SFDE becomes worthless as a measure of juice content, and only remains of value if the oligosaccharides can be removed or otherwise accounted for by analysis to provide an 'oligosaccharide free' SFDE value. Although a number of possibilities could be entertained for this (e.g. by total enzymic hydrolysis of glucose or fructose oligomers to monosaccharides before estimation of SFDE), this would require extensive validation on a range of fermented juice / syrup mixtures which is beyond the scope of the current exercise.

SFDE is no longer included in the NACM Code of Practice (1998) nor in C&E Notice 162. Although the concept of a SFDE remains valuable in assessing juice content, it is not now usable in its original simple form. Rather, it is necessary to analyse and investigate the individual components within it rather than relying on the total value.

2.3.3 Non-Fermentable Carbohydrates

The major carbohydrates in apple juice are fructose, glucose and sucrose which are fully fermentable by all normal yeasts used in cider-making and therefore have no value as juice content markers. Maltose and low glucose oligomers are variably fermentable depending on yeast strain, but arise only from the use of glucose syrups or possibly from the breakdown of apple starch (in early season fruit) by added amylases. However, it was reported by Whiting (1960) that xylose (an unfermentable pentose) is a normal constituent of apple juice and could be present in fermented ciders at levels up to 0.05%. This observation was confirmed with a range of xylose concentrations from 0.01% to 0.11% (CV 56%) in 77 Canadian apple juices prepared from 11 different cultivars (Fuleki *et al* 1994).

The major non-fermentable carbohydrate of apples (and pears) is the sugar alcohol sorbitol. As a primary metabolite derived from the photosynthetic pathway (and as the transport sugar in *Rosaceous* fruits in general), sorbitol has often been suggested as an authenticity marker in apples, pears, peaches, apricots etc. It has always been regarded as one of the major individual contributors to the SFDE of ciders, together with glycerol which is synthesised in variable quantities by yeast during alcoholic fermentation. Direct analysis of sorbitol is now possible by HPLC and enzymatic techniques. Sorbitol values reported in 93 US apple juices by Mattick and Moyer (1983) lie from 0.16 - 1.2 % (mean 0.52) with a CV of 41%. The Canadian study on 77 samples gave a mean of only 0.29% but with a similar CV of 34%, with levels rising slightly on storage for most cultivars except Golden Delicious (Fuleki *et al* 1994). A US study (Elkins / NFPA 1995) of 87 apple juice concentrate samples sourced worldwide over a three-year period gave a mean sorbitol level of 0.39% with a CV of 26% (data normalised to 11.5 Brix). Data for 59 Chinese apple juices at 11.5 Brix (Hammond *et al* unpublished) showed a mean of 0.64% with a CV of 35%. The mean of most data is quoted between 0.3 and 0.5% by Lee and Wrolstad (1988), and the RSK values for sorbitol in apple juice lie between 0.2 and 0.7%. It is generally accepted that sorbitol levels in pears are much higher than those in apples - the RSK range lies between 0.9 and 2.5%. For this reason, sorbitol and its ratio to the content of total sugars has often been suggested as a marker to detect the addition of pear juice to apple (Lee and Wrolstad

1988). Unfortunately this would not of itself be of any value for a fermented beverage such as cider.

Pectin breakdown products (free galacturonic acid and its oligomers) are likely to be too variable with regard to fruit maturity and processing for use as juice content markers. Lea and Jarvis (2000) recorded levels of free galacturonic acid between 380 and 2000 mg/l in nine commercial UK ciders. With the widespread use of depectinised juice concentrates and pectolytic enzymes during cidermaking, pectin and its breakdown products are not likely to be helpful parameters.

The direct analysis of the oligosaccharides added from the fermentation syrups themselves would also be of little value, since their patterns will not be consistent and they may range from hydrolysed starch and inulin sources to cane or beet sucrose. The levels of measurable oligosaccharides could vary from zero to significant amounts over a wide range of chemistries and yet still say nothing about the juice content of the cider.

2.3.4 Organic Acids

The chief organic acid of apples is L-malic acid, at levels which vary from 0.2 up to 2%. Malic acid is not generally affected by yeast fermentation although some yeasts can synthesise it and others can metabolise it. However, L-malic can be converted to variable amounts of D and L-lactic acids with decarboxylation during the bacterial malo-lactic fermentation which may take place during cider maturation. Hence the absence of malic acid is not an indication that a cider is not authentic. In addition, C & E Notice 162 permits the use of all food grade acidulants (malic, lactic, citric, tartaric) to cider *quantum satis.* Both L and DL malic could be added. Organic acid analysis would therefore seem to be a non-starter as regards juice content.

However, it is not generally appreciated that the second major organic acid of apples is quinic acid whose levels may even exceed that of malic in the early stages of fruit development (Hulme 1958). Quinic is a relatively unusual material and not readily available from most other fruit juices, cranberry being the notable exception. In apples and pears it may be especially associated

with the peel. Fuleki and co-workers (1995) found quinic acid levels from 10 - 750 mg/l (CV 53%) in their 77 juice samples from 11 different apple cultivars, with some indication that its levels dropped on storage. Quinic acid in two sources of single strength pear juice was quoted by Pilando and Wrolstad (1992) at 340 and 730 mg/l, within the range established for apple. Work by various authors quoted by Lee and Wrolstad (1988) indicates ranges in apple between 28 - 2000 mg/l depending on source. The ratio of quinic to the total acid varied from 4 to 23%. It is possible that some of this variation depends on the analytical techniques used, since quinic acid has a weak UV chromophore and is not easy to quantify in small amounts even by modern analytical techniques. Quinic acid in cider may also be subject to loss by bacterial metabolism during the malo-lactic fermentation (Beech and Carr 1977). For these reasons it is unlikely to be a robust marker of juice content in ciders.

2.3.5 Nitrogenous Components

The total nitrogenous composition of apple juice is much lower than in many other fruits (e.g. grapes). There is little soluble protein or peptide material and > 90% of it is in the form of aspartic acid or asparagine (Burroughs 1984, Lea 1995b). For this reason, most cider fermentations are reinforced with the addition of ammonium salts and thiamine in order to stimulate yeast growth. The primary amino acids are metabolised by the yeast and hence disappear at the end of fermentation. Hence the measurement of nitrogen content or of specific amino acids would seem to offer little of any value for the assessment of juice content.

However, not all amino acids are metabolised by yeast during fermentation and Burroughs (1957, 1960) identified the presence of 4-hydroxy methyl proline (4HMP) and amino cyclopropane carboxylic acid (ACC), which persisted after fermentation in both apples and pears. ACC was later identified as a critical intermediate in the synthesis of ethylene as a plant-ripening hormone, and for this reason is presumably present in small amounts in all climacteric fruits. The compound 4HMP appears to be of restricted distribution and does not seem to have been investigated to any significant extent although its use as an authenticity marker for pear juice has been suggested (Rossetti 1977). Clearly its analysis could be useful in this regard

although very few figures are available. The only quantitative data known to us is that of Roozen and Janssen (1982) who reported it at 35 mg/l in apple juice and 60 mg/l in an orange/peach nectar though it was not present at all in orange juice.

Proline itself is characteristic of pears by comparison with apples and is one of the parameters used by the RSK system to assess adulteration of apple juice with pear. It is normally regarded as being non-assimilable by fermenting yeasts and might therefore have some value as a marker of pear in cider - however, it does not appear in the paper chromatogram of perry shown by Burroughs (1957) and this point may need some clarification.

2.3.6 Mineral Components

The major inorganic components of apple juice are potassium, magnesium, calcium and phosphorous. Of these, the latter three have no value as juice markers. Phosphate is frequently added to cider as a yeast nutrient, while the magnesium and calcium levels may be significantly distorted by the addition of water to apple juice concentrate prior to fermentation or by 'breaking back' afterwards.

The native potassium level (*ca* 1000 mg/l) will be a fair reflection of fruit content and is unlikely to be distorted greatly by the addition of water to concentrate or product. The RSK range for potassium is 900 - 1500 mg/l although Mattick and Moyer (1983) quote a wider range from 685 - 1510 mg/l with a CV of 18% in their survey of 93 US apple juices. The US study (Elkins / NFPA 1995) of 87 apple juice concentrate samples sourced worldwide over a three-year period gave a mean potassium level of 1009 mg/l with a CV of 9% (data normalised to 11.5 Brix). Burroughs (1984) found a mean value of 1020 mg/l with a CV of 17% in 30 authentic single strength UK apple juices. There was no potassium loss on bentonite treatment and sheet filtration. Data for 59 Chinese apple juices at 11.5 Brix (Hammond *et al* unpublished) showed a mean of 969 mg/l with a CV of 14%. Inclusion of data from Chinese sources is important since this now represents one of the largest contributors to world trade in apple juice concentrate, and is utilised by

cidermakers. There is far less data available for potassium in pear juice but the RSK range is given as 1050 to 1600 mg/l.

Potassium may be elevated by the addition of permitted additives in their potassium salt form e.g. sorbate and metabisulphite. It may also be elevated by the permitted use of 'cidrasse' which is the non-volatile residue from Calvados or cider brandy production and which could contain high levels of minerals. Potassium may be reduced or increased by ion-exchange treatment of the juice or concentrate or the resulting cider, but it is not clear whether this would be covered by the scope of C & E 162 since ion-exchange treatment is only permitted if it does not alter the characteristics of the cider. Potassium carbonate addition to ciders to ameliorate acidity is listed as a permitted additive in the NACM Code of Practice (1998), but not specifically in C & E 162 - this procedure could significantly increase potassium levels.

A mineral sometimes associated with apples is boron (*ca* 4 - 31 mg/kg dry weight according to Hulme (1958)). Barker and Russell (1909) suggested that it might be of value as an authenticity marker (although in their case because boric acid was frequently added at that time as an illegal preservative!). Most modern data is from the standpoint of fruit nutrition, since boron is a required trace element for healthy apple tree growth. Boron levels reported by Burroughs (1984) in 30 UK apple juices showed a mean of 2.5 mg/l with CV of 31%. Pilano and Wrolstad (1992) give figures of 13-18 mg/kg in pear concentrate (*ca* 2.5 mg/l at single strength). Of course small amounts of boron could easily be added to distort the levels if it were known to be in use as an authenticity marker.

Any discussion of minerals such as potassium or boron makes the assumption that the isotopic composition is of natural abundance and that the uptake profile into the fruit is similar for all origins. With modern ICP-MS analysis this assumption could conceivably be tested for the major isotopes of potassium (mass 39, 93% abundance; mass 41, 7% abundance) and of boron (mass 11, 80% abundance; mass 10, 20% abundance). A major difference in isotope uptake by the plant could conceivably lead to a way of distinguishing between the 'natural' element absorbed via the plant and one which has been added from mineral sources. There appears to be

no literature data on the isotopic fractionation of these minerals during uptake into apple fruit. In the case of potassium there seem to be significant experimental difficulties in determining isotope ratios by ICP-MS due to the formation of argon hydride ions of similar m/z values, although these can be overcome using specialised instrumentation (Becker and Dietze 1998)

2.3.7 Other organic components

There are few organic components known to be almost restricted to apples and two of those (4HMP and phloridzin) are covered elsewhere in this review. An unusual alcohol so far reported only from apples and pears is 1,3 octanediol, whose occurrence in a glycosidically bound form has been reported at *ca* 100 mg/l (Berger *et al* 1988, Beuerle and Schwab 1997), and which is known to react with acetaldehyde during fermentation to produce the volatile component 2-methyl 4-pentyl-1,3-dioxan (Dietrich *et al* 1997). No study of its native occurrence in juices or ciders has been carried out so far as we know. The presence of arbutin (a hydroquinone glycoside) is sometimes taken to be characteristic of pear. Spanos and Wrolstad (1990) reported levels in various pear juices of 6 - 16 mg/l, but in a recent German survey (Schieber *et al* 2001) values not exceeding 1 mg/l were found. In our experience the reliable detection of arbutin from pear juice added to apple is problematic due to chromatographic interferences and a very short retention time.

2.3.8 Polyphenols

The polyphenols of apples are frequently regarded as characteristic, and indeed the high 'tannin' of traditional bittersweet cider apples from the UK and NorthWestern France is a distinguishing feature of those fruits. However, the proportion of such fruit used in modern cider blends is low and so by itself the total amount of 'tannin' is not a criterion for juice content. Moreover, the variation between cultivars can be as much as tenfold or more. This was noted even in 1909 by Barker and Russell who tabulated the 'tannin' content of authentic single variety ciders made at the Long Ashton Research Station and observed a range of 0.04 to 0.37%. While total 'tannin' may not be a very useful measure, the distribution of individual phenolics within this may be of

more value. In this review we were specifically commissioned to look at phenolics in more detail and the discussion below reflects that request.

As with many fruits, the phenolics of apple may initially be divided into two major sub-groups, the hydroxy-cinnamates and the flavonoids. Each of these is then divided into further sub-groups as described below. By and large the same sub-classes occur in pears also.

2.3.8.1 Hydroxy-cinnamates

The main representatives of this class are

- Chlorogenic acid (5-caffeoyl quinic acid)
- *p*-coumaroyl quinic acid

Both these compounds are conjugates of caffeic and p-coumaric acid with quinic acid. Small amounts of the parent phenols are sometimes found - free quinic acid has been described earlier. The hydroxy-cinnamates are distributed throughout the apple, principally in the flesh. Chlorogenic and free caffeic acid are found in pear juice, while p-coumaroyl quinic may only be present at lower levels than found in apple.

2.3.8.2 Flavonoids

All flavonoids are characterised by a C15 structure in which two aromatic rings (the A and B) are connected by a three-carbon chain which is often cyclised through an adjacent hydroxyl group to form a heterocyclic C ring. Substituents on the A and B rings may be varied, the oxidation state of the C ring may differ, and the C ring often bears a sugar unit. This multitude of possible permutations means that more than 6000 flavonoids are currently known in nature, with widely differing chemistries.

Those found in apples fall into the following groups:

- **Anthocyanins** (bright red pigments) found only in the skin of red or flushed apples or pears. The anthocyanin pattern of apple is rather simple compared to that of grapes (which may contain 10 - 20 different anthocyanins) and consists largely of cyanidin 3-galactoside.

- **Flavonol glycosides** (very pale yellow) which are found in all apple skins, but not in the flesh. There are six significant flavonol glycosides from apple, based on the quercetin aglycone with a variety of substituted sugars:
    - quercetin 3-glucoside (isoquercitrin)
    - quercetin 3-arabinoside (avicularin)
    - quercetin 3-xyloside (reynoutrin)
    - quercetin 3-galactoside (hyperin)
    - quercetin 3-rhamnoside (quercitrin)
    - quercetin 3-rhamnoglucoside (rutin)

  Note that the quercetin aglycone (which is water insoluble) does not occur in the free state and that other flavonols such as kaempferol, myricetin and iso-rhamnetin derivatives are practically absent from apples. The quercetin glycosides, although moderately water soluble, do not normally appear in apple juice since they are cell-bound in the skin. They may be liberated by alcoholic, enzymic or hot-water extraction which disrupts the cells.

  In pears the situation is somewhat contrasting, and iso-rhamnetin glycosides appear to be present in pear *flesh* or juice at levels exceeding those of the quercetin derivatives (Spanos and Wrolstad 1990). They are therefore regarded to be characteristic of pear juice and have been suggested on various occasions as authenticity markers for the presence of pear in apple (e.g. Schieber *et al* 2001 and references therein)

- **Dihydrochalcones** (colourless) which are found to a small extent in apple flesh, but are principally associated with skin, seeds and root tissue. The principal dihydrochalcones are phloretin 2'-glucoside (commonly known as phloridzin) and similar amounts of phloretin 2'-xylosylglucoside. The parent aglycone phloretin, and the hydroxylated form 3-hydroxy phloridzin, are also found in much lesser amounts. Phloretin derivatives are of very limited distribution in plants and are restricted to apples amongst common fruits. They are not found at all in pears.

- **Catechins** (colourless), which are also known as flav**a**n-3-ols or flav**a**noids (note the small but significant vowel change!). The principal catechin in apples and pears is (-)-epicatechin, with only minor amounts of (+)-catechin.
- **Procyanidins**, which may be regarded essentially as catechin (flavanol) oligomers or polymers. The major procyanidin dimer of apples and pears is an epicatechin 4-6 linked dimer with the trivial name of procyanidin B2. Smaller amounts of the 4-8 linked dimer (B5) also occur, as does the mixed epicatechin-catechin 4-6 dimer known as B1. Higher oligomers, predominantly epicatechin based, have been isolated from apples in the free state up to DP7 (the heptamer), but the degree of structural certainty falls as the molecular weight rises. Recent work in France has shown that very high molecular weight polymers (DP30) may be extracted from certain apple cultivars by use of organic solvents, but these are not soluble in water.

Procyanidin chemistry is complex and difficult - it is almost impossible to isolate and to purify any but the dimers, they cannot be crystallised, and none are commercially available as pure reference standards. They are also extremely prone to oxidation and eventual polymerisation to ill-defined and coloured forms.

Procyanidins are especially astringent and hence they have been selected for at a much lower level in modern dessert apples than in bittersweet cider apples. Because of their astringency and their protein-binding properties, procyanidins are also known as and function as 'tannins'. However, not all 'tannins' are procyanidins and conversely the term 'tannin' has sometimes been mis-applied to cover all plant polyphenols. Such loose terminology is not helpful and is best avoided. Measurement of total ‘tannin’ by the permanganate titration or the Folin colorimetric reagent includes all polyphenols unless specific steps are taken to selectively fractionate certain groups before assay.

2.3.8.3 The Function of Polyphenols in Fruits

For completeness, it is worth recording that the primary functions of polyphenols in fruits are now believed to be as follows:

- The bright anthocyanin skin colours of ripe fruits attract the attention of animals such as rodents and birds which eat them and thereby disperse the seeds.
- Polyphenols in fruit skin and plant leaves (especially anthocyanins, flavonol glycosides and hydroxy-cinnamates) provide screening against UV-radiation and help to protect plant cells against environmental damage. Hence greater exposure to sunlight generally results in higher levels of skin flavonoids.

Many polyphenols have weak anti-microbial properties in their own right. The active polyphenol / polyphenoloxidase system (see below) also synthesises further phenolic polymers with anti-microbial action in response to physical injury of fruits or leaves, thereby protecting the plant against invasion by pathogens.

As 'secondary metabolites', and by comparison with the 'primary metabolites' such as sugars and acids, the synthesis of plant polyphenols is subject to wide variations depending upon environmental and selection pressures. In cultivated fruits such as apples, many biosynthetic pathways have been deliberately manipulated by plant breeders, for instance to enhance skin colour or to suppress astringency. Hence the levels of polyphenols in apples can vary widely (up to tenfold) depending upon cultivar, nutrient supply, and weather patterns.

2.3.8.4 Effects of Apple Processing

Apples, unlike citrus fruits, contain a very active polyphenoloxidase (PPO) system. In the living fruit the PPO system is compartmentally separated from the polyphenols and the interactions between them are under strict biosynthetic control. When the fruit is milled prior to juicing, however, this control is disrupted and in the presence of air very rapid oxidative changes take place, which result in the familiar golden-straw colour of apple juice. In essence the chlorogenic acid is first oxidised to give chlorogenoquinone, which then acts as a redox shuttle resulting in the preferential oxidation of the procyanidins, catechins and dihydrochalcones. The

chlorogenoquinone is consequently reduced back to chlorogenic acid and becomes available to be oxidised once more. This cycle continues until the PPO activity is lost, for instance by pasteurisation or by product-induced inactivation, or until all the substrates are oxidised. It is the ill-defined products of this oxidative polymerisation which generate the colour and which result in an increase in molecular weight. These materials are always present in apple juice and cider (though not in fresh apples) as the so-called 'derived polyphenols', and cannot be chemically characterised except in the most general terms.

Because of the active PPO system, levels of free polyphenols remaining in an apple or pear juice are very dependent on the oxidation history at the point of milling and pressing. In general, oxidation will sharply diminish the free polyphenols observed (Lea 1995 a, b). Use of diffuser extraction, or addition of enzymes to the mash, can also markedly increase phenolic levels (up to 5 fold) especially of epicatechin, phloridzin and flavonol glycosides. Storage of apple juice as concentrate for 9 months can lead to marked losses in chlorogenic acid (36%), phloridzin (50%) and a total loss in procyanidins (Spanos, Wrolstad and Heatherbell 1990).

Fermentation has little effect on most apple polyphenols since they are not metabolised by yeast, and the reducing conditions which prevail during fermentation tend to inhibit rather than to promote their further degradation. However, the subsequent use of protein-based fining agents (e.g. gelatin) or filter media may reduce the levels of procyanidins and 'derived polyphenols' quite markedly. Processing through carbon or adsorbent resins (e.g. in the manufacture of 'white cider') is likely to remove many of the phenolics almost entirely (Ritter and Dietrich 1996). The use of non-volatile cider residues such as ‘cidrasse’ may be presumed to increase the level of total phenolics in the cider, though many of them will be in the ‘derived’ or oxidised form.

2.3.8.5 Analysis of apple polyphenols

Polyphenols have strong UV chromophores and are readily observed by modern HPLC techniques. In a typical trace of an apple juice or a cider, the following components are usually discernible and quantifiable - chlorogenic and p-coumaroyl quinic acid, phloridzin and phloretin xyloglucoside, and possibly some quercetin glucosides (if they have been released from the skin

during processing). If the juice is not too far oxidised during processing, epicatechin and procyanidin dimer B2 may be identifiable as well. Higher procyanidins and the 'derived polyphenols' are not normally evident, even if present in significant quantities, since they are not homogeneous chemical entities and they become 'smeared-out' during the elution of the chromatogram.

Published levels of individual polyphenols found in UK commercial fermented ciders are very scarce. Table 2.2 shows data published on two commercial ciders nearly twenty years apart from which it is evident that the range may be very wide.

Table 2.2
Polyphenol levels in commercial ciders

Component (mg/l)	**Lea 1984 'Woodpecker'**	**DuPont 2002 'Scrumpy Jack'**
Chlorogenic Acid	256	9.5
p-coumaroyl quinic acid	na	3.9
Epicatechin	52	2.4
Phloridzin	22	3.1
Phloretin xyloglucoside	na	1.5
Procyanidin Dimer B2	18	na
Procyanidin Oligomers	10	na
Procyanidin Polymers	64	na
Total Quercetin glycosides	na	0.37

na – not analysed

Data collated by Herrmann (1998 b) on phenolics in dessert apple juices prepared in Germany, Holland and USA gives ranges shown in Table 2.3. It has been contrasted with figures from Lea (1995 a) on juice obtained from 'Dabinett', a typical bittersweet cider cultivar.

Table 2.3

Range of individual polyphenols found in apple juices

Component	**Maximum**	**Minimum**	**'Dabinett'**
Values in mg/l	*USA, Holland and Germany*		*UK*
Chlorogenic acid	178	6	690
Epicatechin	30	0	300
Procyanidin B2	59	0	300
Phloridzin	45	2	200
Quercetin galactoside	14	0	NA

The differences in the first two columns reflect both cultivar and processing variations across a range of normal dessert apple cultivars. The final column simply reflects the very high levels of phenolics associated with UK bittersweet fruit. Given that all samples are by definition 'apple', and that all could be used in UK cidermaking, the chances of using polyphenolic components as quantitative markers of apple content seem slight indeed.

2.4 Conclusions

The brief for this review included a specific request to examine the possibility of using the polyphenols from apples and pears as quantitative markers of juice content in ciders. Due to the wide range of concentrations encountered across apple cultivars, and the effect of processing in reducing them, it seems fairly unlikely that they could realistically play such a role. On the other hand, the specific presence of markers such as phloridzin and iso-rhamnetin for apple and pear respectively might be of value in determining whether admixture with either alternate fruit had taken place, although it would be difficult to determine the exact level. Such information could be of value for instance if sorbitol were considered one of a set of parameters for determining juice content, since the sorbitol ranges are substantially different for both apple and pear. The phloridzin / iso-rhamnetin ratio might be used to help interpret the sorbitol value.

Of all the other parameters reviewed above, the three most promising are as follows:

- Oligosaccharide-free sugar-free dry extract (OFSFDE)
- Potassium
- Sorbitol

The drawback to the OFSFDE is that there is no openly published or agreed protocol for digestion and removal of the wide range of glucose and fructose oligosaccharides which might occur in modern fermentable syrups. Furthermore, there is no modern published database of SFDE values in authentic apple juices, and it would be necessary to fall back in the first instance on an assumed value, such as the previous NACM standard of 13 g/l or the Burroughs (1984) mean value of 19 g/l (CV 21%). The OFSFDE would also need laboratory validation of the oligosaccharide removal procedure before it could be brought into use, and a publicly accessible database would need to be constructed. Its restricted applicability to UK-fermented ciders would always tend to limit the amount of analytical information which would be available for the database.

The advantage of potassium and sorbitol values is that they are single measurements of defined chemical entities, using relatively robust analytical methodologies. They are measured and will continue to be measured by food analysts worldwide, not just by those concerned with the UK cider industry. The data cited in this review draws on several major studies of apple juices from all over the world and there is every expectation that further data will be published. Tables 2.4 and 2.5 below summarise the information for potassium and sorbitol levels which was cited in the review.

Table 2.4

Summary data for Potassium in Single-Strength-Equivalent Apple Juice

Study	Juice Source Country	Mean (mg/l)	CV %	Sample Numbers
Mattick 1983	USA	1073	18	93
Burroughs 1984	UK	1020	17	36
NFPA 1995	Argentina, Austria, Chile, France, Germany, Hungary, Mexico, New Zealand, Poland, Spain, USA,	1009	9	87
Hammond 2003	China	969	14	59
Collated		**1001**	**12.5**	**201**

Table 2.5

Summary data for Sorbitol in Single-Strength-Equivalent Apple Juice

Study	Juice Source Country	Mean (mg/l)	CV %	Sample Numbers
Mattick 1983	USA	5200	41	93
Fuleki 1994	Canada	2900	34	36
NFPA 1995	Argentina, Austria, Chile, France, Germany, Hungary, Mexico, New Zealand, Poland, Spain, USA,	3900	26	87
Hammond 2003	China	6400	35	59
Collated		**4200**	**45.6**	**242**

The collated values have been recalculated from the raw data (where accessible). In the case of the Mattick study only a reduced data set of mean values for 16 cultivars is available in the published literature.

It is evident from the summary data that potassium is the best single parameter for measurement of cider juice content. Its distribution is relatively tight across the data sets available to us. With

an overall CV of 12.5% and a mean of 1000 mg/l, any estimate of apple juice content based on potassium analysis would have an uncertainty of ± 25% at the 95% confidence level. The situation with sorbitol is less helpful, since its CV is much wider (46%). However, sorbitol could act as a useful secondary marker of juice content. Hence, to gain a current understanding of juice content in UK ciders, the use of potassium and sorbitol as joint markers seems to be most appropriate.

For obvious reasons, multiple authenticity markers should be independent variables, which are not correlated with one another. To confirm that potassium and sorbitol in apple juices are essentially independent, a linear regression between potassium and sorbitol levels was performed on the NFPA and the Chinese data sets described above (in which each individually identifiable sample was analysed for both analytes). The r^2 value was 0.01, showing that there is no correlation between them. Hence the potassium and sorbitol levels in apple juices may be regarded as essentially independent, which confirms their suitability as joint authenticity markers.

Despite the independence of the potassium and sorbitol levels in any single sample, it is worth noting that on a *mean* basis over the 400 data points cited, the ratio of sorbitol to potassium was 4.2. This mean ratio should be conserved for any sufficiently large sample set, and would be essentially independent of any dilution of the juices analysed. Hence a sorbitol / potassium regression line for a set of juices or ciders prepared at various dilutions should tend to a slope of 4.2 .

PART 3

EXPERIMENTAL

3.1 Outline of the experimental programme

The purpose of this project was to examine the methodologies most appropriate for assessing the juice content of ciders, paying particular attention to the role of secondary metabolites such as polyphenols. The recommendation from the literature review is that the measurement of potassium and sorbitol is likely to provide the most robust current data, and that polyphenols were not likely to be helpful. The experimental programme described here was designed to test that hypothesis, and falls into the following parts:

- Preparation and analysis of authentic small scale apple juices and fermented ciders, to confirm that potassium and sorbitol are not materially affected by yeast fermentation
- Analysis to demonstrate that the markers are absent from fermentable glucose syrup
- Analysis of 'cidrasse' for potassium, iron and copper levels
- Small scale fermentation of high alcohol juice concentrate ciders with added glucose syrup to demonstrate that potassium and sorbitol levels are appropriate markers of juice content
- Analysis of a range of commercial ciders for polyphenol levels
- Analysis of a range of commercial ciders for potassium and sorbitol levels, and an assessment of their value as an indication of juice content.

3.2 Materials

3.2.1 Small scale apple juices and ciders

Authentic apple juices were milled and pressed from fully ripe fruit in 2001 from the cultivars shown in Table 3.1. Small scale ciders were prepared on the 5 litre scale by direct addition of dried fermenting yeast (Uvaferm BC) at 400 mg/l, to sulphited juices after standing overnight.

Macer8 pectolytic enzyme was also added at 200 mg/l to each 5 litre batch. All samples were fermented to dryness at ambient temperature under airlocks. They were racked in March 2002 into tightly closed containers and were finally bottled in crown-capped glass bottles with the addition of 50 mg/l sulphur dioxide. All apples were grown near Wallingford, Oxfordshire, except Michelin which was grown near Martock, Somerset.

Table 3.1

Small Scale Cider Fermentations

Cultivar	Date	SG	pH	SO_2 (mg/l)
Harry Masters	12 Nov	1.052	4.2	150
Michelin	12 Nov	1.048	3.9	150
Kingston Black	12 Nov	1.050	3.6	100
Stoke Red	12 Nov	1.046	3.6	100
Dabinett	25 Nov	1.052	4.2	150
Yarlington Mill	2 Dec	1.052	4.0	150

3.2.2 Small scale 'high gravity' fermentations

Authentic apple and pear juice 70 Brix concentrates of Austrian origin (Ybbstaler) were supplied ‘suitable for cider making’ as a gift from David Berryman Associates. Fermentable glucose syrup (Cerestar grade 02779) was kindly obtained by Aspall Cyder, Suffolk.

Fermentations were made up to a nominal 22° Brix ‘double strength’ according to the following table, where the mass of concentrate or syrup is shown. Each blend was made up to a volume of 900 ml and the Brix was measured. The musts were sulphited overnight at 100 mg/l SO_2 and 0.5g of Difco Yeast Nutrient base was added, before pitching dried yeast EC1118 at 1000 mg/l. Fermentations were racked after completion and samples were serially diluted with water to 50%

and 25% by volume, prior to analysis of potassium and sorbitol (see Section 3.3).

Table 3.2

Composition of 'high gravity' fermentations (mass in grams per 900 ml)

Sample Code	PJC 20.827	AJC 12.720	Glucose G02779	pH	Measured Brix	Juice Content v/v at 11.2 Brix
301		146	155	3.45	22.5	48%
302		290		3.48	21.0	97%
303	78	224		3.51	20.0	100%
304	152		151	3.57	22.9	49%
305	294			3.61	21.6	101%
306	226	76		3.58	22.1	102%

Following commercial practice as described in the review, a high gravity fermentation gives a high alcohol 'base' which would be diluted for retail sale. For the sake of simplicity, therefore, the nominal juice content after such dilution (i.e. at 11.2 Brix) has been calculated as follows:

Mass of conc x 70/11.2 = mass of juice used.
Mass of juice x 11.2/measured Brix = mass of juice at single-strength (SS) equivalent
Mass at SS/1.045 = volume of SS juice
Volume of SS juice / 900 = percentage of SS juice in sample by volume

3.2.3 Supply of 'cidrasse'

A sample of 'cidrasse', the non-volatile residue from the distillation of cider, was kindly supplied by the Somerset Cider Brandy Company, Martock, Somerset. It was analysed as received for potassium, iron and copper.

3.2.4 Supply of draught ciders and perries for analytical purposes

By kind permission of the organisers, samples of ciders and perries were obtained from the cider and perry bar at the CAMRA festival held at Kings Meadow, Reading, in May 2002. All these were dispensed on draught from unpressurised barrels or plastic containers. Samples were held in Sterilin pots and were kept refrigerated until analysed during the following month.

3.2.5 Supply of bottled and canned ciders and perries for analytical purposes

Samples of bottled and canned ciders and perries were purchased from supermarkets in the Reading area during 2002 and 2003. They were stored at room temperature until opened shortly before analysis.

3.3 Analytical procedures

3.3.1 Analysis of potassium

Potassium was assayed using standard procedures (RSSL method B15 – see appendix) by nitric acid dissolution, followed by atomic absorption spectroscopy. Data was expressed as mg/l.

3.3.2 Analysis of sorbitol

Sorbitol was assayed by ion-chromatography HPLC using a Dionex instrument , a CarboPac PA-100 column running in buffered ammonium acetate, and a pulsed amperometric detector (RSSL method B17 – see appendix). Data was expressed as mg/l.

3.3.3 Analysis of polyphenols

Apple polyphenols were analysed using reverse-phase gradient HPLC with diode-array detection, by an RSSL in-house method. Chlorogenic acid and phloridzin were quantified with respect to commercial external standards. *p*-coumaryl quinic acid and phloretin xyloglucoside were quantified as chlorogenic and phloridzin equivalents. These components can be satisfactorily determined in ciders by direct injection of the samples without pre-concentration or dilution.

3.4 Results and Discussion

3.4.1 Analysis of potassium and sorbitol in authentic juices and their ciders

The data obtained from the fermentation trials is shown as follows (Table 3.3):

Table 3.3

Marker levels in authentic juices and ciders

Cultivar	Potassium mg/l		Sorbitol mg/l	
	Juice	**Cider**	**Juice**	**Cider**
Harry Masters	1160	1180	5720	5240
Michelin	980	1030	4450	4270
Kingston Black	1270	1320	5390	5060
Stoke Red	1160	1130	3200	3150
Dabinett	980	1000	4650	4420
Yarlington Mill	1170	1210	7590	7360
Mean	**1120**	**1145**	**5170**	**4920**

Statistical analysis (paired t-test) showed no significant differences in the potassium level between juice and cider. A slight bias was seen in the sorbitol data indicating a possible loss of *ca* 5% during fermentation, but this is within the limits of measurement accuracy and is unlikely to be significant in the context of the present exercise.

3.4.2 Analysis of glucose syrup for potassium and sorbitol

The potassium level found in the glucose syrup was 7 mg/kg. The sorbitol was below the detection limit of 50 mg/kg. These data indicate, as expected, that glucose syrup would not contribute any significant amounts of juice content markers.

3.4.3 Analysis of cidrasse

The following figures were obtained for single strength cidrasse, expressed in mg/l

Potassium	Iron	Copper
1310	1.5	12

The potassium level is slightly higher than expected from a single strength juice or cider, which is not unreasonable given that cidrasse is the residue from distillation and so some concentration of non-volatile material would be expected. The copper level is notable and arises from the copper construction of the still. Although there is no way of knowing from a single analysis how typical this figure might be of all such samples, it does indicate a potential limit on the use of cidrasse (or its concentrate) to enhance the apparent juice content of a cider, since the copper level in cider must not exceed 2 mg/kg.

3.4.4 Analysis of 'high gravity' fermentations

The data obtained from analysis of the 'high gravity' fermentations is given in Table 3.4. Data was obtained on the original fermentations and again after serial dilution with Reading tap water to 50% and 25% (shown by the x1 and x2 suffixes respectively). For the sake of comparison with normal retail product, the measured potassium and sorbitol values have also been recalculated to a notional base value of 11.2 Brix before fermentation. The predicted juice content has been calculated on the basis of equating 1000 mg/l potassium to 100% juice (for both apple and pear), and is shown for comparison with the actual juice content as given in Table 3.2.

The predicted juice content is slightly higher than the actual content as may be seen from the last two columns. This is in effect due to the fact that the concentrates used for this work (which were arbitrarily taken from trade and not specially selected by us beforehand) have potassium levels higher than the mean database value of 1000 mg/l (standardised to 11 Brix). The data also show, as expected, that the potassium and sorbitol levels are not affected by dilution with tap water and that these parameters remain effectively linear on serial dilution.

Table 3.4

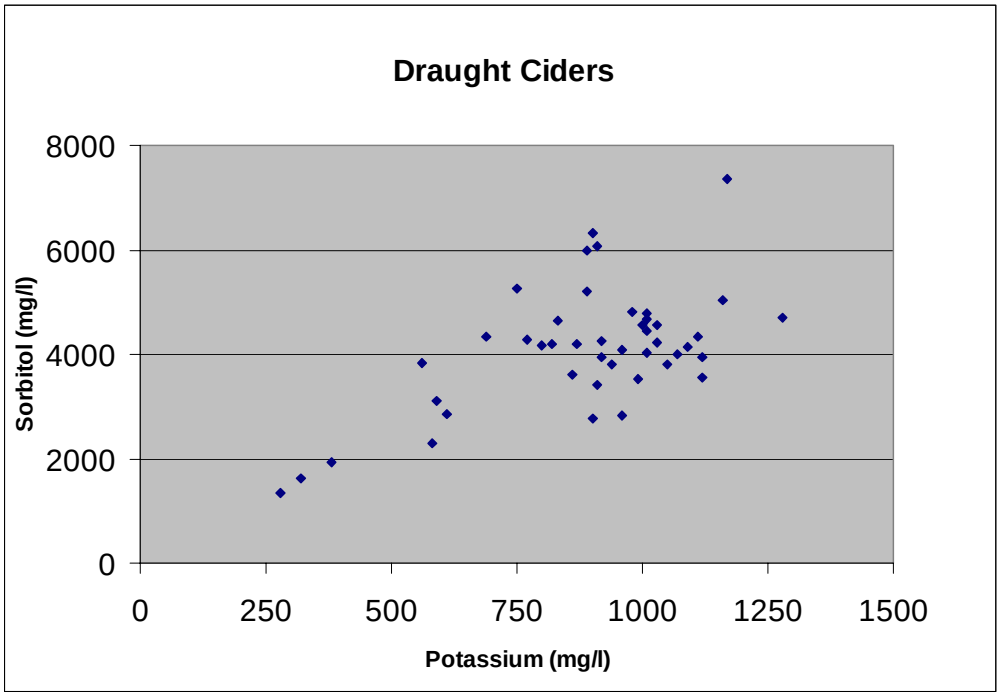
Analytical Data and Juice Content of 'High Gravity' Fermentations

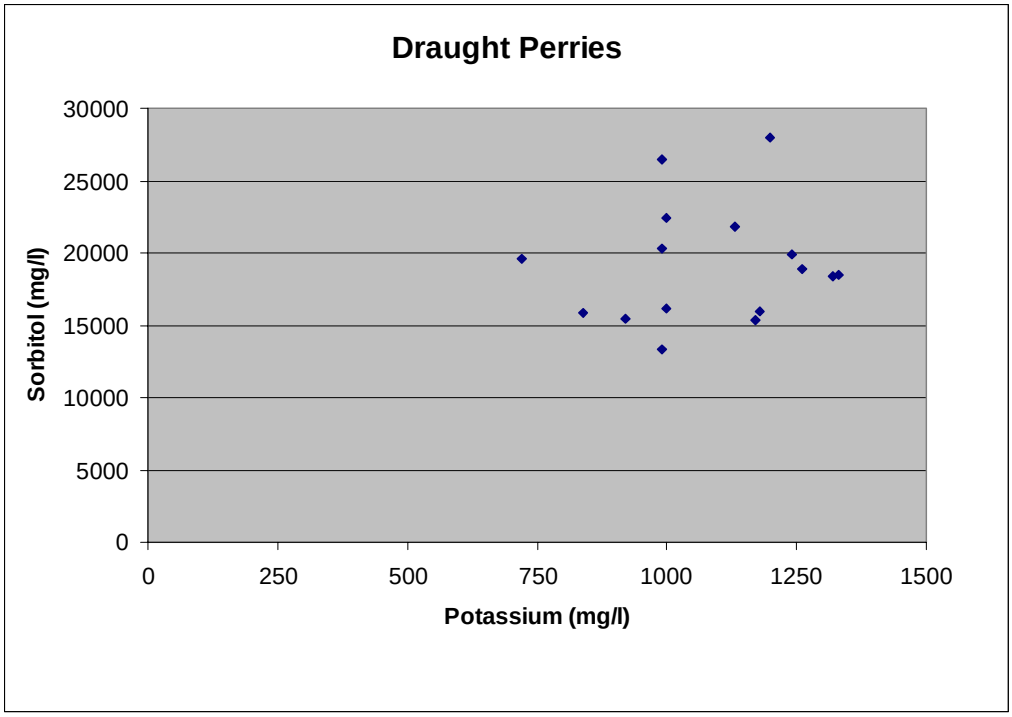
Sample Code	K mg/l	Sorbitol mg/l	Brix	K mg/l	Sorbitol mg/l	**Predicted Juice Content**	**Actual Juice Content**
	As measured			*Recalculated to base value of 11.2 Brix*			
Apple conc + glucose (1+1)							
301	1200	1834	22.5	597	913	**60%**	**48%**
301x1	610	887	22.5	304	442	**30%**	**24%**
301x2	310	442	22.5	154	220	**15%**	**12%**
Apple conc alone							
302	2260	4010	21	1205	2139	**121%**	**97%**
302x1	1120	1781	21	597	950	**60%**	**49%**
302x2	570	912	21	304	486	**30%**	**25%**
Apple conc + pear conc (3+1)							
303	2190	9456	20	1226	5295	**123%**	**100%**
303x1	1110	4627	20	622	2591	**62%**	**50%**
303x2	550	2619	20	308	1467	**31%**	**25%**
Pear conc + glucose (1+1)							
304	1230	13927	22.9	602	6811	**60%**	**49%**
304x1	620	7346	22.9	303	3593	**30%**	**25%**
304x2	320	3305	22.9	157	1616	**16%**	**13%**
Pear conc alone							
305	2270	24828	21.6	1177	12874	**118%**	**101%**
305x1	1130	13464	21.6	586	6981	**59%**	**51%**
305x2	580	6456	21.6	301	3348	**30%**	**25%**
Pear conc + apple conc (3+1)							
306	2400	20866	22.1	1216	10575	**122%**	**102%**
306x1	1200	11625	22.1	608	5891	**61%**	**51%**
306x2	610	5315	22.1	309	2694	**31%**	**25%**

The results given in Table 3.4 confirm that the proposed method based primarily on potassium analysis would be fully applicable to ciders which are 'chaptalised' and then diluted – the juice content predictions are within the ± 25% confidence interval discussed in the literature review. Despite the fact that we have little primary data on potassium levels in pear juices, it seems it would also be generally applicable to perries and to ciders to which the maximum permitted 25% of pear juice has been added. In this case, the sorbitol level is considerably elevated above the baseline apple value, from *ca* 2000 mg/l to 5000 mg/l in the current example shown in Table 3.4. This would provide some evidence that such an addition has taken place. The addition of apple to pear, however, would not be evident in this fashion, since the sorbitol levels in apple are so much lower. Another marker such as phloridzin would have to be used in this case.

3.4.5 Potassium and sorbitol data from draught ciders and perries

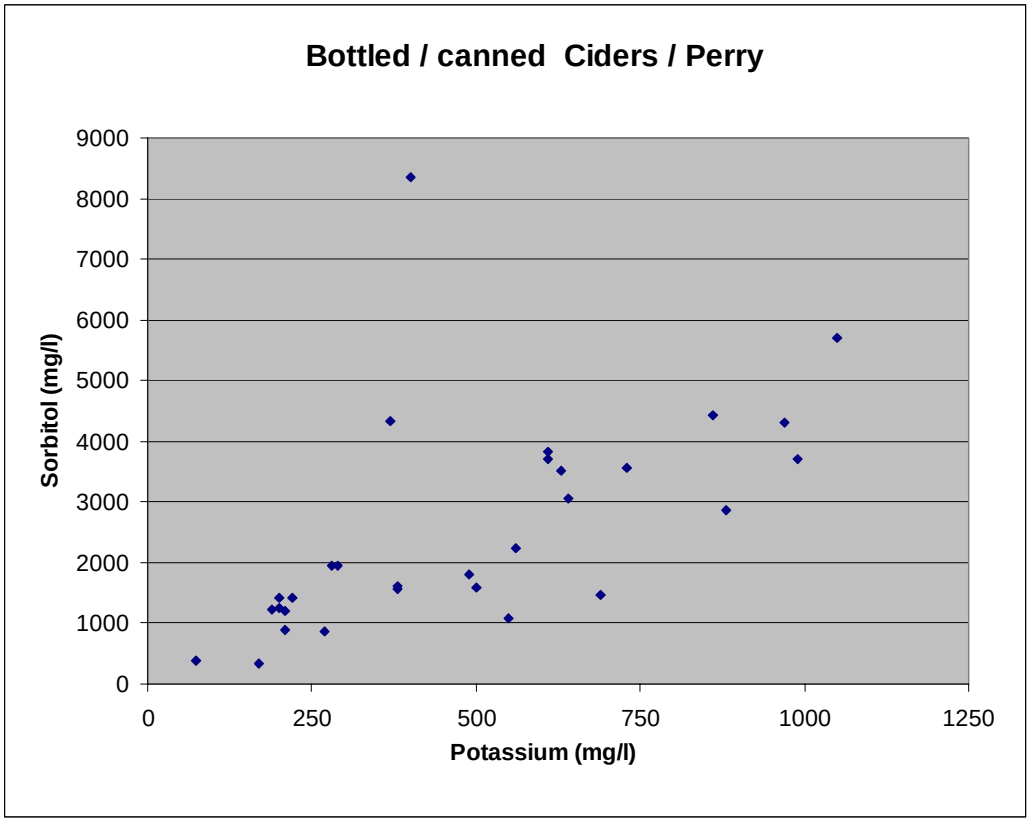
Plots of potassium *vs* sorbitol data for the draught ciders and perries are shown below. They are shown separately for ease of understanding since the sorbitol levels are much higher in the perries. The majority of the ciders are in excess of 750 mg/l potassium and therefore are within the expected limits for 100% juice products (1000 ± 250 mg/l). However, it is evident that a significant minority are below that level and are therefore not made from pure juice. The sorbitol levels in those samples are also low and confirm this observation. All the perries are in excess of 750 mg/l and are probably within the limits for pure pear juice (the pear database is limited).





3.4.6 Potassium and sorbitol data from bottled and canned ciders and perries

A plot of the potassium *vs* sorbitol data for all the bottled and canned products is shown below. Two of the samples are known to be perries and these appear as evident 'outliers' due to their high sorbitol values. Samples that are above the threshold level of 750 mg/l are within the confidence levels for pure juice – the majority are variously below that level and are likely to be progressively diluted. The sorbitol levels again confirm that observation. In general, the sorbitol levels are well correlated with the potassium levels – for the bottled and canned ciders, the correlation coefficient $r^2 = 0.75$. Since we have previously established that there is no significant correlation between potassium and sorbitol levels for pure apple juices, the correlation observed here is indicative of a secondary dimension of data (sorbitol), which is truly and independently supportive of the primary dimension (potassium) as regards dilution and juice content. This is confirmed by the slope of the regression line for ciders (Sorbitol = 4.5* Potassium), which is in close agreement with the value of 4.2 predicted previously from the apple juice database values.



3.4.7 Phenolic levels *vs* potassium content

In the literature review it was suggested that phenolic markers, even the most stable ones, were unlikely to be good markers of juice content. The correlation coefficients observed between potassium *vs* sorbitol and various phenolic components for the complete cider data set are as follows:

Potassium vs…	Correlation coefficient
Sorbitol	0.68
Phloretin Derivatives	0.17
Chlorogenic Acid	0.19
p-coumaroyl quinic	0.13

It is evident that the correlation coefficients between potassium and phenolics are far poorer than those for potassium *vs* sorbitol over the same sample set. The data here therefore supports the belief that phenolic levels would be of no value as markers for juice content.

A very limited amount of reverse-phase HPLC work with diode-array detection was undertaken on samples of commercial pear concentrates diluted and injected directly to investigate the presence of iso-rhamnetin glycosides. If present, they were at very low levels - in some samples no flavonol glycosides at all were detectable. Schieber *et al* (2001) found it necessary to use a pre-concentration step with ethyl acetate extraction to achieve adequate sensitivity. Clearly this is necessary but it was not pursued as part of this investigation.

PART 4

OVERALL CONCLUSIONS

The literature review which forms the first part of this report indicates that the majority of ciders on the UK market are likely to contain only 30 – 50% apple juice equivalent. In examining in detail the analytical parameters which might be used to verify juice content, measurements of potassium and sorbitol appear to be the most robust markers, based on considerable published data from authentic apple juices. The analysis of phenolics was shown to be of no value.

Samples prepared and fermented in the laboratory, followed by analytical 'snapshots' of commercial UK ciders and perries using potassium and sorbitol as markers, have confirmed that these measurements appear to be valid, and that they yield data which is fully consistent with the literature indicators. Data on the apparent juice content of a range of bottled or canned ciders and perries which were evaluated during this exercise is given in Table 4.1, based on a value of 1000 mg/l potassium representing 100% juice. The data presented in this table span an apparent range of juice content from 7% – 100% (with a confidence interval ± 25% of the measured value, based on published data for potassium in apple juice).

Potassium, supported by sorbitol, is evidently a very useful marker of juice content. Some possible drawbacks to its usefulness may result from the permitted addition of potassium salts during cidermaking. As previously discussed, potassium content can be slightly elevated by the use of potassium sorbate or metabisulphite as preservatives, the highest predicted level being 140 mg/l potassium when added at the legal maximum of 200 mg/l SO_2. This would represent the equivalent of 14% juice.

The addition of potassium carbonate to reduce the acidity of a cider would cause a more significant distortion, however. To drop the acidity by 0.1% could raise the potassium level by 500 mg/l, which is the equivalent of 50% juice. It is not clear that such an addition is a practice permitted by Customs and Excise, nor whether it is a widespread practice or one that is likely to

become so. But to make the best use of potassium as a marker for determining juice content in this and other fruit-based drinks, further work exploring the isotopic composition of potassium from plant and mineral sources is recommended, in the hope that these sources could be distinguished from each other.

The permitted addition of 25% pear juice to a cider could raise the sorbitol level by *ca* 3000 mg/l, the apparent equivalent of 75% apple juice. In this case the potassium level would remain virtually constant and so the high sorbitol level would be obvious as an 'outlier' (as has been demonstrated). The addition of apple juice to perry could not be determined by sorbitol analysis, however, but the detection of phloridzin by HPLC would provide an appropriate route. It would not be possible to provide an accurate quantitative determination of pear in apple nor of apple in pear by either method, however, since the CV's of the marker components are too wide.

Table 4.1

Apparent Juice Content in Bottled / Canned Ciders and Perries

Sample Code	Potassium (mg/l)	Sorbitol (mg/l)	Apparent Juice Content (%)
128	73	391	**7**
101	170	346	**17**
122	190	1228	**19**
118	200	1250	**20**
123	200	1411	**20**
115	210	882	**21**
117	210	1205	**21**
131	220	1428	**22**
113	270	860	**27**
121	280	1948	**28**
129	290	1957	**29**
126 (Perry)	370	4340	**37**
102	380	1611	**38**
112	380	1567	**38**
104 (Perry)	400	8361	**40**
103	490	1803	**49**
130	500	1579	**50**
108	550	1087	**55**
114	560	2233	**56**
120	610	3696	**61**
125	610	3837	**61**
124	630	3509	**63**
105	640	3067	**64**
127	690	1478	**69**
107	730	3558	**73**
110	860	4421	**86**
106	880	2867	**88**
116	970	4308	**97**
109	990	3710	**99**
111	1040	1414	**104**
119	1050	5700	**105**

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APPENDIX

This Table is extracted from UK Customs and Excise Notice 162 (1998) and lists all the ingredients and materials permitted in UK Cider Production

Additive	Maximum concentration
General	
Acesulfame-K (E950)	*
Acetic acid	*
Apple esters (natural only)	Limited to restoring the natural aroma of the concentrated apple juice
Apple juice (fresh or concentrate)	Limited to 25% if used in production of perry bittersweet, desert or culinary
Apple wine	No limit but must only contain ingredients permitted in the making of cider and perry
Ascorbic acid and its salts (E300 - E302)	*
Aspartame (E951)	*
Carbon dioxide	*
Cider - out of condition	No limit
Cider vinegar	Limited to the quantity necessary to adjust acidity
Citric acid and its salts (E330 - E333)	*
De-alcoholised concentrated cider (Cidrasse)	No limit but must only be produced from ingredients permitted in the making of cider
Lactic acid and its salts (E270, E325, E326)	*
Malic acids and its salts (E296, E350a, E351b, E352a)	*
Neo-hesperidine	No limit
Nitrogen	No limit
Pear esters (natural only)	Limited to restoring the natural aroma of concentrated pear juice
Pear juice (fresh or concentrate)	Limited to 25% if used in the making of cider
Pear wine	No limit but must only contain ingredients permitted in the making of cider and perry
Perry - out of condition	No limit
Perry vinegar	Limited to the quantity necessary to adjust

	acidity	
Saccharin (and Na, K, and Ca salts) (E954)		*
Sorbic acid and its salts (E200, E202, E203)		*
Sugars and sugar syrups e.g. High fructose corn syrup/high fructose syrup, Fructose, Hydrolised starch/hydrolised starch syrup, Glucose, Liquid sugars, Sucrose, Sugar	No limit	
General (cont)		
Sulphur dioxide and its salts (E220 - E224, E226 - E228)		*
Salt (Sodium chloride)		*
Tartaric acid and its salts (E334 - E336)		*
Water	No limit	

** Limits set by current food legislation.*

Colourings	
Acid brilliant green BS (E142)	#
Caramel (E150a, E150b, E150c, E150d)	#
Carmoisine (E122	#
Cochineal (E120)	#
Indigotine (E132)	#
Ponceau 4R (E124)	#
Quinoline yellow (E104)	#
Sunset yellow (E110)	#
Tartrazine (E102)	#

 # colourings and other substances which may impart colour may only be used to produce cider or perry in the colour range - straw/gold/golden brown.

Processing aids

These are materials used in the processing of cider and perry. There are no restrictions on the use of processing aids provided they do not change or alter the characteristics of the cider or perry. Small residual traces of these aids may remain in the final product provided they do not contribute to the stability, colour or flavour of the cider or perry. Examples of processing aids are:

Decolourizers	Charcoal
Enzymes	Pectinase
Filter aids	Cellulose ; Kieselguhr
Fining Aids	Bentonite; Gelatin; Isinglass;Tannin
Miscellaneous	Anti-foaming agents; Calcium carbonate; Ion-exchange resins; Lactic acid bacteria
	Microbial nutrients other than urea and its derivatives. Yeast & yeast culture.

Report Issued By: Date:

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