Sulphite binding in ciders

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Summary The extent of sulphite binding was measured in commercial ciders, and experimentally observed binding curves were compared with theoretically derived curves based on assessment of the levels of individual sulphite binding compounds determined in the ciders. Subsequently, experimental ciders were fermented under various controlled conditions using nine different strains of cider yeasts. The results indicated considerable differences both in the levels of sulphite binding compounds produced, and in the ability of different yeast strains to produce SO_2 in the cider. Juice concentration and the presence of cloud in juice had little or no effect on sulphite binding. Other factors which affected sulphite binding included the type and condition of juice (especially the effect of pectinase treatment) and, in some instances, the use of added yeast nutrients. Significant sulphite binding was also attributable to unaccountable components, probably derived from poor quality fruit, which were present in the apple juice prior to fermentation.

Keywords Cider, fermentation, sulphite, sulphite binding compounds, sulphur dioxide, yeasts.

Introduction

The use of sulphur dioxide in the production of wines and cider has a long and proven history and has been claimed to date back to Homeric times. Certainly it has been in use for cider making since the 17th century, when its application in the form of sulphur candles was strongly recommended by Dr Beale writing in Evelyn's 'Pomona' (1664). It came into standard usage in the English cider industry during the 1950s, both in its application before fermentation to control the natural microflora of apples and to minimize oxidation of apple juice constituents, and in its use at bottling to prevent oxidative changes and to inhibit secondary infection. The chemistry and biochemistry of sulphur dioxide in fermented beverage systems is complex, and has been authoritatively reviewed on a number of occasions (see for instance Hammond & Carr, 1976; Beech *et al*., 1979; Beech & Davenport, 1983; Würdig, 1989) . This paper deals only with its use after fermentation, and in particular with its efficacy in the presence of competitive binding components.

The phenomenon of sulphite binding has been identified previously (Burroughs & Sparks, 1964, 1973a, b, c). Sulphur dioxide in ciders can exist in both free and bound forms, which are in equilibrium between each other to an extent depending on the concentration both of sulphite and of the binding components. These relationships are shown in Fig. 1(a) for a typical cider (based on data obtained during this study). The binding components may originate from the juice itself (e.g. galacturonic acid) or as a consequence of fermentation (e.g. acetaldehyde). For anti-microbial activity, only the free form of SO_2 is effective, and only a very small portion of that, the so called 'molecular SO_2' can actually enter susceptible micro-organisms and disable them at levels *ca* 0.5–1 mg L^{-1} (Hammond & Carr, 1976; Würdig, 1989). The distribution between the molecular SO_2 and the bisulphite anion is pH dependent, following the equilibrium $SO_2 + H_2O \leftrightharpoons HSO_3^- + H^+$, and is shown diagrammatically in Fig. 1(b) (Würdig, 1989). In practical terms, to achieve a target level of 50 mg L^{-1} *free* SO_2 after bottling,

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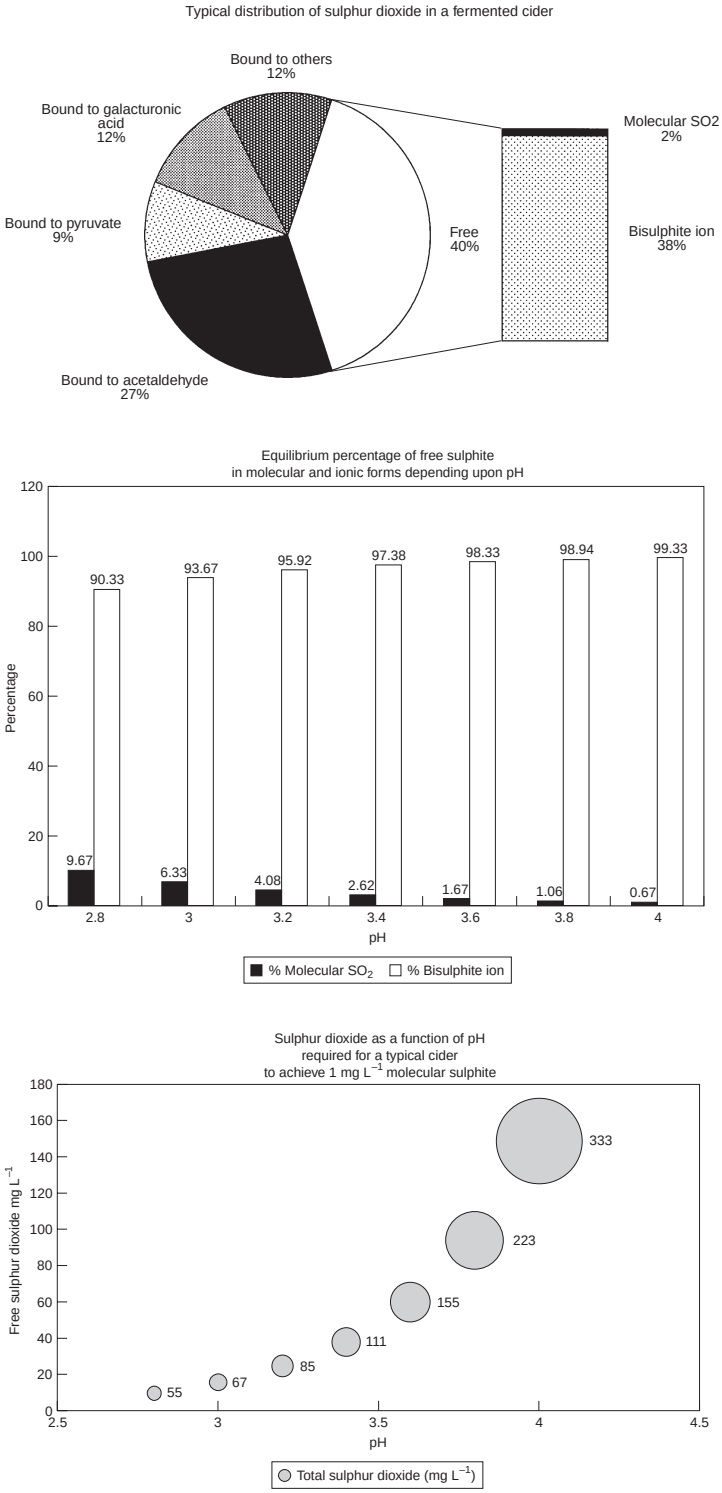

Figure 1(a) Typical distribution of sulphur dioxide in a fermented cider, **(b)** equlibrium percentage of free sulphite in molecular and ionic forms depending on pH, **(c)** free and total sulphur dioxide required for a typical cider to achieve 1 mg L^{-1} molecular sulphite, as a function of pH.

equivalent to 1 mg L^{-1} molecular SO_2 at pH 3.5, it may be necessary to have a *total* level as high as 135 mg L^{-1}. At higher pH, more SO_2 is required. These relationships are shown in the 'bubble plot' of Fig. 1(c) which is based on typical data obtained during this study − in this representation, the radius of each 'bubble' is proportional to the *total* amount of SO_2 present, for each level of *free* SO_2 which is shown on the y-axis. Since there is regulatory pressure to minimize the total quantity of sulphite used in foods, it is important to minimize the sulphite binding capacity of ciders as far as possible in order to have an adequate amount of free SO_2 in the packaged product (Beech & Jarvis, 1989).

The sulphite binding components of cider

It is well established that the dominant sulphite binding components of cider are those given in Table 1, ranked in order of known binding power (Burroughs & Sparks, 1964). The binding power of individual components varies widely, and the equilibrium constant (*K*) gives a measure of the intensity of sulphite binding, a smaller value indicating tighter binding and vice versa. Thus, although there is typically much more glucose in ciders than acetaldehyde, acetaldehyde makes a much greater contribution to sulphite binding than glucose. It is important to note that the binding power of acetaldehyde is so great that, in practice, no free SO_2 can exist in a cider until all the acetaldehyde is bound. The other binding compounds exist in equilibrium between their bound and unbound forms. The individual calculations for sulphite binding are complex and are described in detail by Lea *et al*. (2000).

In general, the three major sulphite binders are those resulting from normal yeast metabolism during fermentation. These are acetaldehyde, pyruvate and α-keto glutarate, which are referred to in this paper as 'metabolic carbonyls'. This is fortunate since potentially it allows us to manipulate a relatively controlled part of the cider making process. However, the importance of the other binders should not be forgotten. Galacturonic acid has classically been regarded as a minor binder and indeed its levels in traditional cider apple juice are low. It is present at much higher levels in apple juice concentrates, where it results from deliberate enzymic depectinization, so its contribution to modern ciders is greater than in the past. L-xylosone results from the breakdown of naturally occurring ascorbic acid in apple juice in the presence of SO_2 during the prefermentation stage (Whiting & Coggins, 1960) − little is known of its

Table 1 The key sulphite binding compounds likely to occur in cider

Compound	Origin	Equilibrium constant* (K)	Typical concentration in cider (mg L^{-1})	Percentage bound to SO_2 (at 50 mg L^{-1} free SO_2)	SO_2 bound (mg L^{-1}) (at 50 mg L^{-1} free SO_2)
Acetaldehyde	Fermentation	1.5×10^{-6}	25	99.8	35
Pyruvate	Fermentation	1.6×10^{-4}	20	83	12
α-Keto glutarate	Fermentation	5.6×10^{-4}	15	58	4
L-Xylosone	Ascorbic acid breakdown	1.4×10^{-3}	20	36	4
Galacturonic acid	Pectin breakdown	1.8×10^{-2}	1000	4	15
Glucose	Sweetening sugar	6.4×10^{-1}	7000	0.1	8
5-keto fructose (D-threo 2,5 hexodiulose)	*Fungal and bacterial activity in mouldy and poor quality fruit*	3.4×10^{-4}		70	
2,5 di-keto gluconic acid 5-keto gluconic acid 2-keto gluconic acid D glucuronic acid		4.4×10^{-4}		64	

* Equilibrium constants are composites of various values taken from the literature (Würdig, 1989).

typical levels in modern cider making. The five carbonyls produced by microbial activity in mould damaged fruit are known to contribute significantly to sulphite binding but should not be present at all in good quality fruit. Nothing is known of their presence in modern concentrates but their potential contribution should not be ignored. Methods for the measurement of sulphite binding compounds and the total sulphite binding capacity of ciders are described by Lea *et al*. (2000).

Improved understanding of those factors which affect the binding of sulphur dioxide might enable changes to be made to the formulation of cider juice bases and fermentation processes, to minimize the level of occurrence of sulphite binding compounds. The defined objectives of this study were to identify methods which could enable cider makers to reduce, or largely eliminate, sulphur dioxide binding compounds in juices and ciders, in order to reduce the usage of sulphur dioxide in cider making.

Methods and materials

Analytical methods

Juices, cider bases and fermented ciders were analyzed for the following: pH value, total and free sulphur dioxide, total and volatile acidity, residual sugar, residual gravity and alcohol using standard methods (NACM, 1996).

The levels of individual sulphite binding compounds, and the total sulphite binding capacity of the ciders, were determined using the methods described by Lea *et al*. (2000).

Commercial ciders

Twelve commercial ciders, typical of the types of product on the UK market, were purchased and assayed for their sulphite binding powers and for their content of carbonyls. The data were used to construct experimental and predicted sulphite binding curves.

Yeast cultures

Fermentations were undertaken using yeast strains from the National Association of Cider Makers (NACM) Culture Collection which is lodged with the National Collection of Yeast Cultures (NCYC) at the Institute of Food Research, Norwich. The strain reference numbers cited are those allocated by the NCYC.

Yeast propagation

Yeasts were propagated in a fortified apple juice medium containing 630 mL of commercial apple juice concentrate (AJC), 8 g di-ammonium phosphate and 5 g citric acid diluted with distilled water to 5 litres. After sterilization of the base medium by autoclaving, 100 mL of a filter sterilized solution containing 0.4 g sodium metabisulphite, 2 mg thiamine hydrochloride and 1 mg calcium pantothenate was added. Each yeast was inoculated into 10 mL complete base medium and grown for 24 h at 25 °C while shaking; 1 mL of the culture was then added to 100 mL of the base medium and incubated for 48 h at 25 °C while shaking, followed by inoculation into 1L of base medium which was incubated without shaking for 48 h at 25 °C. This inoculum was then added to 5 litres base medium and sparged with sterile air for 20 min; the cultures were grown for 4 days at 25 °C before harvesting by centrifugation. Purity and viability checks were made on the harvested yeast paste. In the third stage of fermentation studies the inoculum was prepared as a single batch of 20 litres. After harvesting by centrifugation, the yeast cells were 'acid-washed' at <5 °C immediately prior to pitching. Yeast viability ranged from 80 to 99% in the different fermentations.

Experimental fermentations

Three sets of controlled fermentations were undertaken. In the first study, nine yeast strains representative of those used by UK cider makers were assessed in parallel fermentations using a standard cider fermentation base. For practical reasons, these were run as three sequential batches each of three separate yeast fermentations.

In the second stage, a standard juice was fermented with two yeast strains which, in the first stage, had given the highest (NCYC 5169) and one of the lowest levels of binding (NCYC 3627), respectively. Fermentations were conducted using a juice base with and without added nutrients at temperatures of 15, 20 and 25 °C, thus giving a matrix of 12 different fermentations. These were run as three sequential

batches of four parallel fermentations at a single temperature.

The third stage evaluated the difference between fresh and concentrated juices, prepared from bittersweet or culinary apple cultivars, clear (clarified) or cloudy, all fermented under identical conditions at 20 °C. A matrix of eight systems was therefore investigated as two runs each of four parallel fermentations. The strain of yeast used (NCYC 5169) was that shown previously to produce the highest sulphite binding power.

Controlled fermentations with nine cider yeast strains

The base medium was prepared from a single supply of cider apple juice (previously stored frozen) to which was added malic acid (1.0g L^{-1}) and di-ammonium phosphate (1.6g L^{-1}). The base was pasteurised for 15 min at 70 °C and after cooling 15 litres were transferred through sterile pipes to each of three sterile 20 litre cylindro-conical fermenters. The level of SO_2 was adjusted to a total sulphite level of 150 mg L^{-1} and the base was left overnight to stabilize. Thiamine hydrochloride (0.42 mg L^{-1}) and calcium pantothenate (0.2 mg L^{-1}) were added as sterile solutions. The base was sparged with air to give a dissolved oxygen level of 10 mg L^{-1}. Each fermenter was pitched with a different yeast to give a cell count of 10^7 cells mL^{-1} and the fermentation was allowed to proceed to dryness at 20 °C. Samples were taken daily for chemical and microbiological analyses. At the end of fermentation, the cider was chilled to 3 °C, racked off from the lees and stored at 0 °C prior to filtration through a sheet filter. Samples of the cider were bottled and pasteurized at 20PU prior to analysis. The experiment was performed on three occasions to provide a total of nine yeast fermentations.

Controlled fermentations with two yeast strains at three temperatures in the presence and absence of added nutrients

The fermentation base was prepared from a blend of apple juice concentrate and glucose syrup, stored at 0 °C until required for use. The composition of the base medium (SG 1.045°) was apple juice concentrate (5.12 L), HSS glucose syrup (3.60 L), citric acid (6.8 g) and water to 60 L. Sodium metabisulphite was added to achieve a target level of 30 mg L^{-1} free SO_2; after standing for 24 h further sulphite was added to the base medium to achieve the free SO_2 target level. The base medium was then heated to 80 °C, immediately cooled in line to the target temperature (15, 20 or 25 °C) and 15 litres were transferred to each of four 16 litre fermenters and aerated to achieve dissolved oxygen levels of 10 mg L^{-1}. In those experiments requiring yeast nutrients the following additions were made to 15 litre base medium prior to inoculation, to a target yeast cell count of 10^7 cells mL^{-1}: Fermaid 9 g; Di-ammonium phosphate 24 g; Thiamine chloride 4.5 mg; and Calcium pantothenate 2.25 mg.

Fermentations were stopped on attenuation or after 14 days. The ciders were chilled, filtered and packaged as described above, except that they were fined with bentonite prior to filtration. The fermentations were undertaken on 3 occasions, a different fermentation temperature (15, 20 or 25 °C) being used on each occasion.

Controlled fermentation of bright and cloudy, fresh and concentrated culinary and bittersweet apple juices

Bittersweet and culinary apple juices were taken direct from commercial pressings. Half of each volume was filtered to give a bright juice and half of each bright and cloudy juice was concentrated by evaporation under controlled low temperature conditions in the pilot plant at the Leatherhead Food RA. All juices and concentrates were held at 0–2 °C until required for fermentation. The fermentation bases were prepared as shown in Table 2.

All base media were adjusted by addition of distilled water to an original gravity of 1.045, heated to 80 °C and then immediately cooled to 20 °C. Sodium metabisulphite was added to give a free SO_2 level of 30 mg L^{-1} and the base media were

Table 2 Fermentation bases

Juices and concentrates	Fresh base	Concentrate base
Fresh apple juice	83.5 litres	-
Apple juice concentrate	-	7.6 litres
Glucose syrup	5.4 litres	5.4 litres
Malic acid	to pH 3.3	to pH 3.3
Deionized water	-	to 90 litres

transferred aseptically to sterile fermenters. Yeasts were inoculated after 24 h to give a level of 10^7 yeast cells mL^{-1}. All fermentations were done at 20 °C and gravity was monitored daily. When attenuated, the fermented ciders were cooled to 4 °C and held for 2–3 days prior to racking off, fining, filtration, packaging and pasteurization at 40 PU's.

Results

Occurrence of sulphite binding compounds in commercial ciders

Table 3 gives the results of the carbonyl analyses, together with the predicted and experimental binding of sulphite at a nominal value of 50 mg L^{-1} free SO_2. This value is chosen to allow easy comparisons across a wide range of samples. The detailed sulphite binding curves for two contrasting ciders are shown in Fig. 2.

In eight out of the 12 samples, agreement between the experimental and predicted curves was satisfactory. For instance in product 12, shown in Fig. 2, the discrepancy between the actual and predicted values of bound SO_2 did not exceed 10 mg L^{-1}. In three cases (products 7, 9 and 10) the predicted curve lay considerably below the experimental curve and predicted only half the actual binding. In one case (product 5) both predicted and experimental curves were completely awry owing to the extremely high levels of acetaldehyde and pyruvate which were present. These were later attributed to the presence in this product of extremely high levels (*ca* 500 mg L^{-1}) of ascorbic acid added before fermentation. It is not clear what effect this has on yeast metabolism but it is evidently dramatic. Although L-xylosone could not be analyzed at the time, retrospective evaluation of the chromatograms indicated a large peak running in the position of L-xylosone which may have been produced by ascorbate breakdown.

Of the remaining three samples in which discrepancies were evident (e.g. product 9 shown in Fig. 2), there appeared to be no common feature in the high performance liquid chromatography (HPLC) traces. It was suggested that the presence of one or more of the following could be involved: anthocyanin extract (one of these samples was coloured with blackcurrant); caramel colour; synthetic azo dye colours; saccharin; hydroxymethyl furfural and other carbonyls generated in stored concentrate; keto sugars (from fruit in poor condition). It is important to remember that the identification of 'classical' sulphite binders by Burroughs & Sparks (1964, 1973a, b, c) was carried out at a time when these materials were not in commercial use and, apart from the keto sugars, nothing is known of their sulphite binding potential.

An unexpected observation was that the free galacturonic acid levels in commercial ciders could be as high as 2000 mg L^{-1}, and in several cases provided the second highest total binding capacity

Table 3 Carbonyl analysis and sulphite binding power of commercial ciders

Sample code	Concentration of binders (mg L^{-1})					Bound SO_2 (mg L^{-1}) at 50 mg L^{-1} free SO_2	
	Acetaldehyde	Pyruvate	α-keto glutarate	Galacturonic acid	Glucose	Predicted	Actual
1	9	6	40	515	4180	35	48
2	20	3	2	994	14700	50	55
3	23	17	23	876	10700	65	60
4	30	33	49	1010	6520	91	88
5	128	927	223	953	3000	816	∞
6	10	4	12	891	5180	33	40
7	23	10	15	640	6380	54	82
8	15	20	32	1040	7830	58	66
9	15	4	9	379	7390	34	78
10	13	15	17	672	7740	43	75
11	45	45	60	599	5710	117	105
12	38	15	22	2030	7250	99	89

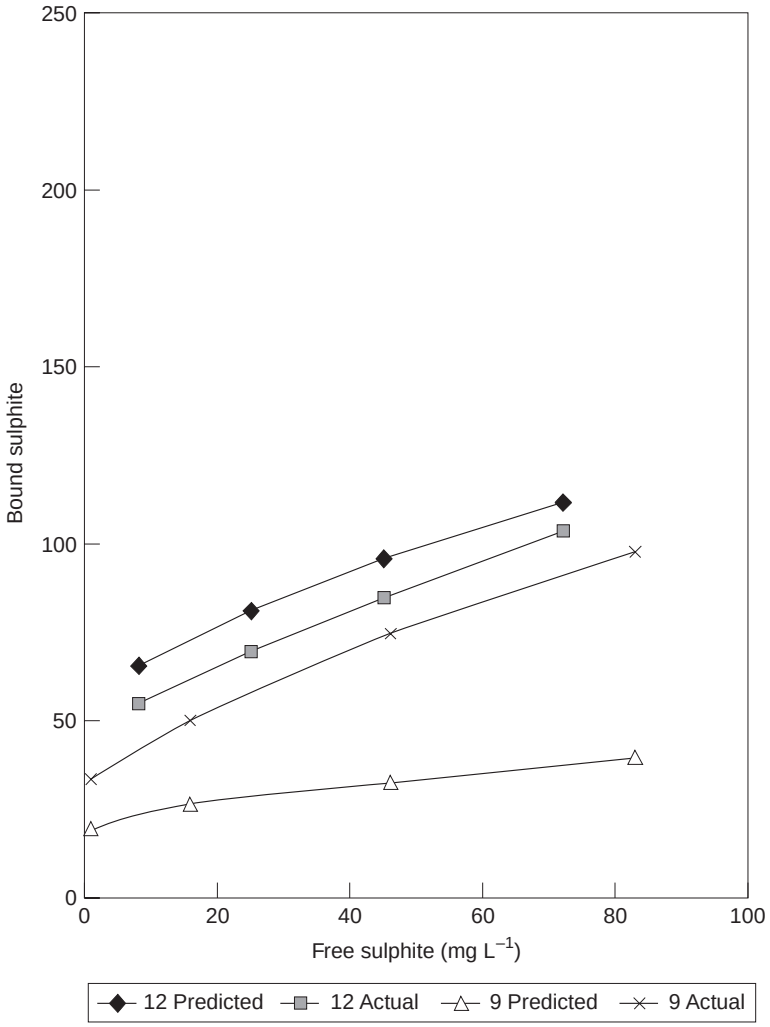

Figure 2 Sulphite binding curves for two contrasting commercial ciders (products 9 and 12).

after acetaldehyde. This reflects directly the use of depectinized apple juice concentrate in modern cider making.

Controlled fermentations using nine cider yeast strains

Fermentation data

Microbiological analyses on the yeast inocula showed no significant evidence of contamination. The cider bases all showed a low level contamination with filamentous fungi and short bacterial rods (probably *Bacillus* spp) which had survived pasteurization. After fermentation, the ciders generally contained low levels of bacteria and wild yeasts.

The fermentations proceeded rapidly, generally attenuating (i.e. fermenting to SG $\leq$ 1.0000) after 7–8 days, and one strain (NCYC 2347) had fermented out within 3 days although strain NCYC 3627 required 12 days to attenuate. At the end of fermentation, the levels of alcohol produced and residual sugar levels varied between strains as did the sulphite binding power of the ciders (Table 4). The production of SO_2 during fermentation also appeared to vary between yeast strains.

Sulphite binding

Experimental sulphite binding power was compared with the predicted values from carbonyl analyses. For calculation, levels of the three metabolic carbonyls were taken into account (which varied considerably

Table 4 Analytical data from the nine cider fermentations

Yeast strain (NCYC)	Time to attenuation (hrs)	Residual gravity (degrees Sacch)	Cider analysis					
			Alcohol (%v/v)	Sugar (% w/v as sucrose)	Acidity (mEq/L^{-1} as malic)	Total SO_2 in cider (mgL^{-1})	Sulphite bound (mgL^{-1}) at 45 mg/l free SO_2	
							Predicted	Actual
3627	260	2.7	4.99	0.24	68	43	92	142
9012	240	4.1	5.19	0.31	80	31	82	123
5832	190	2.4	5.15	0.61	74	72	95	158
0862	170	2.2	5.37	0.25	76	51	99	142
2166	168	2.4	5.10	0.31	67	62	87	158
4467	145	1.9	4.93	0.30	72	70	132	178
5169	90	2.4	5.00	0.45	79	104	142	>200
4704	75	2.2	5.33	0.32	83	48	82	157
2347	96	2.2	5.37	0.32	78	72	121	170

between yeast strains), together with those for galacturonic acid and L-xylosone (which were constant at 200 and 55 mg L^{-1} respectively and derived from the original juice). In these fermented unsweetened ciders the level of glucose was trivial.

Three features were immediately evident from the data, presented in Table 4. Firstly, the binding power of the ciders varied nearly twofold across the range of yeasts used, strain NCYC 5169 producing the greatest and strain NCYC 9012 the lowest binding power. Secondly, the shapes of the binding curves, which are presented in Fig. 3 for NYC 5169 and 9012, were much steeper than those obtained for the commercial ciders analyzed previously − i.e. the binding power per unit volume of the experimental ciders was much greater. This reflects the fact that commercial ciders are usually 'broken back' (i.e. diluted) with water after fermentation to achieve the required alcohol level, thus reducing their final sulphite binding power, whereas these pure juice ciders were not diluted. Thirdly, the agreement between predicted and experimental binding was poor and typically predicted only 50% of the experimentally observed value.

To eliminate the possibility of experimental error in the determinations, the HPLC determinations of the metabolic carbonyls were replicated by enzymic methods and similar values were obtained. Since there appeared to be a common discrepancy between all nine samples, suspicion therefore lay with the juice itself. Unfortunately no reference sample of the original juice (1993 season) was available for study and therefore samples of a similar blended bittersweet juice from the following season (1994) were examined. The binding power of this juice did not differ between pasteurized and deep frozen samples, thus eliminating any influence from pasteurization. However, the experimental binding power was much higher than could be accounted for by levels of the only known carbonyls in the juice (glucose and acetaldehyde), and was much higher than previously observed by Burroughs & Sparks (1962) in studies of sound bittersweet cider juices. In practice, virtually no free SO_2 could be obtained even at 200 mg L^{-1} total SO_2. Even when the binding measurements were carried out by direct iodine titration (the Ripper method) similar results were obtained and it was therefore evident that the methodology was sound and that the juice itself truly contained unknown binding compounds.

Inspection of the HPLC chromatograms (Fig. 4) indicated that in the 1994 juice and in the nine ciders fermented from 1993 juice there were two significant peaks preceding acetaldehyde, which had not been observed previously in chromatograms of bittersweet ciders. By inference, these peaks may have been responsible for the additional sulphite binding which was determined. They cannot be attributable to pasteurization, to concentration or to any additives, since none were used. It is therefore possible that they are associated with poor quality fruit, and that these unknown peaks may correspond with the

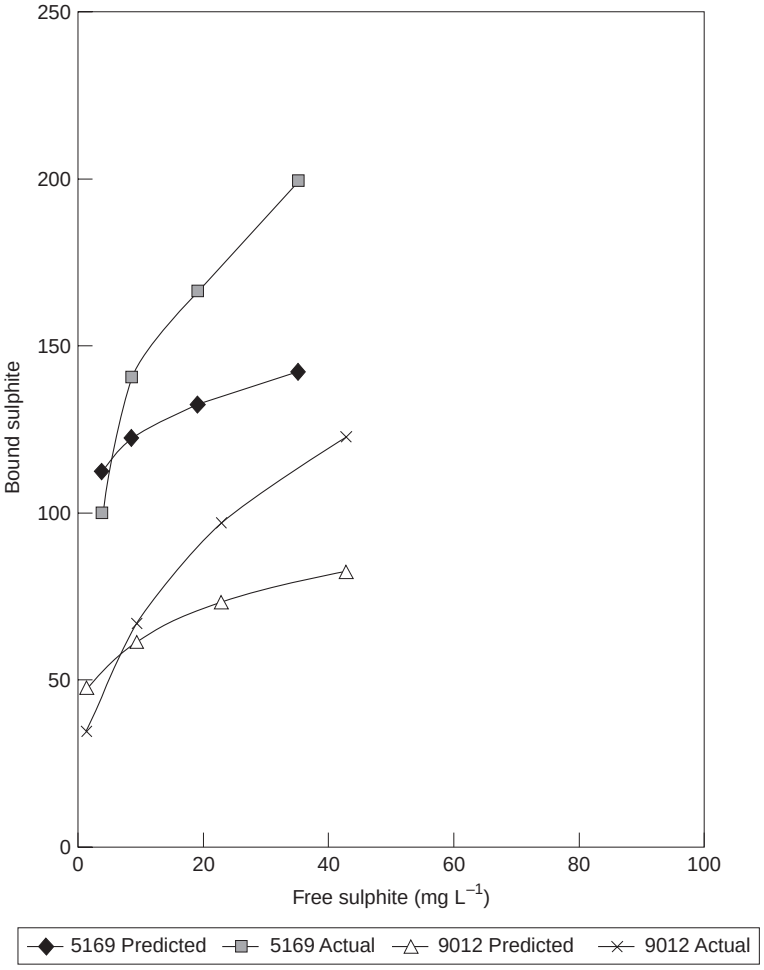

Figure 3 Sulphite binding curves for yeast strains NCYC 5169 and 9012.

known sulphite binders 5-keto fructose and 2,5 diketogluconic acid which are produced by microbial action. In the absence of authentic standards, however, this could not be confirmed.

Despite the presence of unknown binding compounds in the juice, which might reflect the condition of the original fruit, it is clear that the yeast strains investigated were capable of producing widely varying levels of metabolic carbonyls in the cider.

Controlled fermentations with two yeast strains at three temperatures in the presence and absence of added nutrients

Fermentation data

Microbiological analysis of the yeast inocula and the fermented ciders showed no evidence for any significant microbial contamination. Most fermentations reached attenuation within 14 days although some attenuated much faster than others (Table 5). In the absence of yeast nutrients, fermentation rates were slower, especially at the lower fermentation temperatures; strain NCYC 3627 fermented more slowly in the absence of nutrients at any of the three temperatures than did strain NCYC 5169. Chemical analysis suggested that NCYC 5169 may be a producer of SO_2 especially at the lower temperatures (15 and 20 °C) and in the presence of yeast nutrients. This was confirmed from the total SO_2 data obtained on analysis of the ciders from the previous experiment (Table 4). Because of the need to terminate all fermentations at 14 days, residual gravity was much higher in some fermentations, especially those with NCYC 3627.

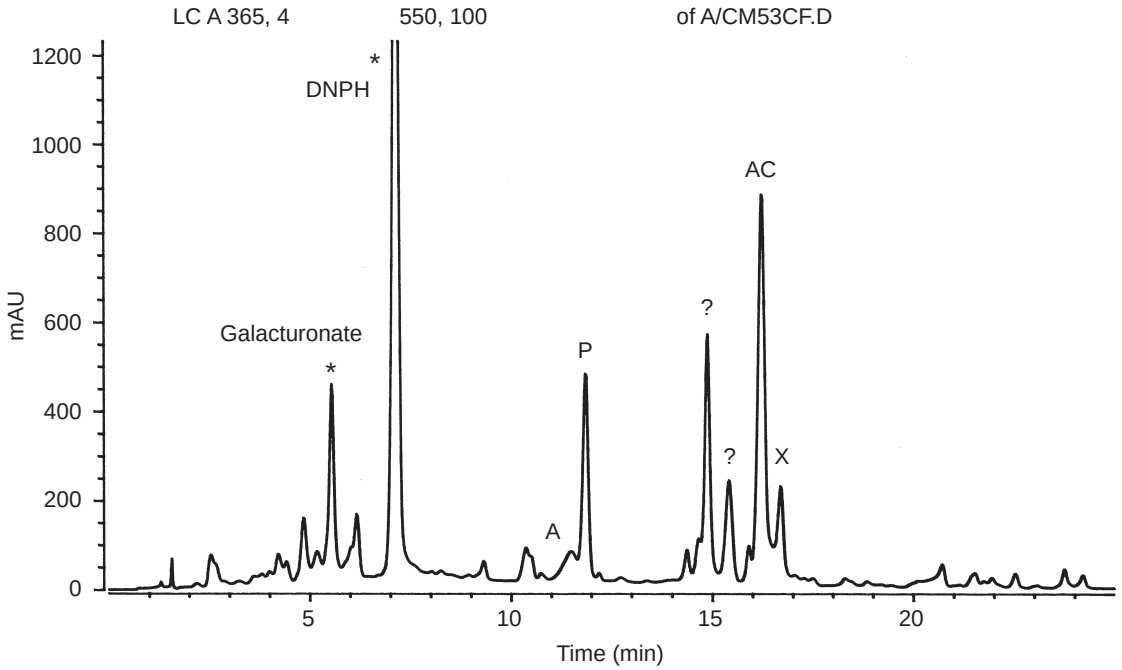

Figure 4 HPLC chromatogram for a fermented cider from 1993 juice. A: α-keto glutarate; P: pyruvate; AC: acetaldehyde; X: L-xylosone; ?: unknown carbonyls (described in text).

Analysis of the bottled ciders (Table 6) showed that, in the presence or absence of yeast nutrients, NCYC 5169 produced a similar level of alcohol at all temperatures as did NCYC 3627 in the presence of nutrients. However, in the absence of nutrients, NCYC 3627 produced considerably less alcohol at all temperatures. This correlated with its slower rate of fermentation and the increased content of metabolic carbonyls − presumably their final conversion to ethanol (where thiamine and pantothenate are known to be cofactors) was not so readily accomplished under these conditions. Both strains produced more acid and had lower levels of residual sugar when grown in the presence of added nutrients.

Sulphite binding data

In this series no measurements of experimental binding power were carried out since attention was focused on the behaviour of the yeast in its

Table 5 Effects of temperature and addition of yeast nutrients on the fermentation performance of two yeast strains

Yeast strain	Parameter	Fermentation in the presence (+) or absence (−) of nutrients at fermentation temperature of					
		15 °C		20 °C		25 °C	
		+	−	+	−	+	−
3627	Attenuation time (days)	12	>14	9	~14	14	9
	Residual gravity	2.2	4.2	1.5	2.7	1.2	2.5
	Δ Total SO_2 (mgL^{-1})*	−6.3	+1.4	+1.7	+4.7	n.a.	n.a.
5169	Attenuation time (days)	9	14	7	14	7	11
	Residual Gravity	2.3	1.5	1.7	1.5	1.3	1.3
	Δ Total SO_2 (mgL^{-1})*	+52.3	+28.8	+33.5	+22.7	n.a.	n.a.

* Difference between total SO_2 level immediately prior to inoculation and at day 14.

Table 6 Analytical data on ciders fermented at 15, 20 or 25 °C in the presence or absence of added nutrients

Yeast strain	Cider analysis	Fermentation in the presence (+) or absence (–) of nutrients at fermentation temperature of					
		15 °C		20 °C		25 °C	
		+	–	+	–	+	–
3627	Alcohol (% v/v)	5.37	5.00	5.53	5.11	5.42	5.00
	Total acidity (g L^{-1})	5.18	4.85	5.15	4.56	5.41	4.55
	Residual sugar (% w/v as sucrose)	0.48	1.10	0.33	0.66	0.25	0.87
	SG (degrees Sacch)	1.8	4.2	2.0	2.9	1.1	3.6
	α-keto glutarate (mg L^{-1})	38	110	44	93	78	157
	pyruvate (mg L^{-1})	44	133	23	73	28	86
	acetaldehyde (mg L^{-1})	23	35	14	20	10	15
	Bound SO_2 at 45 mg L^{-1} free	69	156	45	95	50	110
5169	Alcohol (% v/v)	5.34	5.48	5.54	5.53	5.35	5.43
	Total acidity (g L^{-1})	5.26	5.05	7.08	4.96	6.3	4.96
	Residual sugar (% w/v as sucrose)	0.39	0.48	0.29	0.42	0.29	0.53
	SG (degrees Sacch)	2.4	1.0	2.2	1.8	2.4	1.3
	α-keto glutarate (mg L^{-1})	34	59	42	93	35	108
	pyruvate (mg L^{-1})	87	69	57	73	58	95
	acetaldehyde (mg L^{-1})	53	39	36	20	45	25
	Bound SO_2 at 45 mg L^{-1} free	137	112	96	95	108	119

production of metabolic carbonyls under different conditions. The HPLC data (Table 6) were used to give curves of predicted binding power. It was immediately evident that NCYC 3627 always gave the lowest binding power (irrespective of temperature) when it was supplied with nutrients. However, in the absence of nutrients, its binding power was twice as high and was similar to that of NCYC 5169 (whose binding power was independent of nutrient supply). These effects were rationalized on the basis of the detailed composition of acetaldehyde, pyruvate and α-keto glutarate in each case. It appeared that NCYC 3627 was unable to synthesize its own cofactors (e.g. thiamin) for the conversion of pyruvate to acetaldehyde (and thence to ethanol) whereas NCYC 5169 accumulated acetaldehyde even when nutrients were supplied. The tendency of this yeast (NCYC 5169) to synthesise sulphite *de novo* may also be a contributory factor.

In practice, the lowest sulphite binding was achieved using strain NCYC 3627 with the addition of nutrients. There was no clear effect of temperature. Samples of part fermented ciders were also analyzed, but no clear differences were discernible except in one set where the results were similar to those already described.

Controlled fermentation of bright and cloudy, culinary and bittersweet juice bases prepared from fresh and concentrated apple juices

Fermentation data

The data are summarized in Table 7. The fresh juice bittersweet (BS) fermentations attenuated more rapidly than did the BS concentrated juice fermentations; by contrast there was no difference between the fresh and concentrated culinary juices. Clear juices all attenuated more rapidly than did the corresponding cloudy juices. In all cases, the residual gravity was lower in the fermented fresh juices than in the concentrates, but the fall in total SO_2 was greater in the concentrate fermentations than in the fresh juice fermentations. There was little difference in the fall in free SO_2 levels in the different fermentations. A

Table 7 Summary of data from cider fermentations using different juices

Parameter	Bittersweet juice				Culinary juice			
	Cloudy		Clear		Cloudy		Clear	
	Fresh	Conc	Fresh	Conc	Fresh	Conc	Fresh	Conc
Attenuation time (days)	12	16	11	14	15	14	9	9
Residual gravity	0.9	2.0	0.9	1.0	0.83	1.0	0.38	0.90
Δ Total SO_2 (mg L^{-1})*	-40	-88	-40	-33	-10	-31	-3	-8
Δ Free SO_2 (mg L^{-1})*	-30	-27	-26.8	-25	-24	-22	-28	-28
Δ Total acidity (mg L^{-1})*	+15	+9	-4	+12.1	-14.4	-9.6	-1.6	-5.3
Δ Volatile acidity (mg L^{-1})*	+0.06	+0.5	+0.04	+0.11	+0.25	+0.26	+0.33	+0.31

* Change in total or free SO_2, total acidity and volatile acidity between time of inoculation and attenuation of the cider

major difference observed was a significant increase in total acidity in 3 of the 4 BS fermentations whereas there was a fall in total acidity in all culinary juice fermentations. Some differences were observed also between the final levels of volatile acidity in the BS fermentations (greater increase in the fermented concentrates) but no differences in the culinary juice fermentations. Analytical data on the fined, pasteurized ciders are summarized in Table 8. The level of alcohol in the culinary ciders (mean 5.85 ± 0.16% v/v) was greater than in the BS ciders (5.70 ± 0.21% v/v) but the difference was statistically insignificant ($P > 0.20$). As would be expected, the total acidity in the culinary ciders (68.3 ± 4.1 mg L^{-1}) was significantly greater ($P < 0.01$) than that in the BS ciders (36 ± 4.1 mg L^{-1}) but the levels of volatile acidity in the culinary and BS ciders (0.39 ± 0.09 mg L^{-1} and 0.28 ± 0.04 mg L^{-1}, respectively) were not significantly different ($P > 0.05$).

Sulphite binding data

The binding curves for the culinary juice fermentations were similar to those obtained previously using the same yeast at the same temperature. At a free SO_2 of 45 mg L^{-1}, the bound SO_2 ranged from 116 to 160 mg L^{-1}; in the BS fermentations the overall binding power was much higher (131–217 mg L^{-1} at 45 mg L^{-1} free SO_2) with the cloudy concentrate giving the highest binding and the clear concentrate giving the lowest. There is no immediate explanation why bittersweet juice fermentations should give generally higher values of binding than those of culinary juice, unless some component of the juice (e.g. polyphenols) influences the way in which the metabolism of the yeast carbonyls takes place.

Discussion and conclusions

The purpose of these studies was to ascertain the

Table 8 Analytical data on ciders produced from the different juices

Parameter	Bittersweet juice				Culinary juice			
	Cloudy		Clear		Cloudy		Clear	
	Fresh	Conc	Fresh	Conc	Fresh	Conc	Fresh	Conc
Alcohol (% v/v)	5.81	5.82	5.77	5.39	5.98	5.68	5.99	5.73
Total acidity (g L^{-1})	37	38	30	39	72	64	69	68
Volatile acidity (g L^{-1})	0.23	0.26	0.29	0.32	0.28	0.36	0.46	0.46
Residual sugar (% w/v as sucrose)	0.84	0.70	2.06	0.57	0.76	0.96	0.84	0.72
α-Ketoglutarate mg L^{-1}	138	100	41	119	140	96	72	70
Pyruvate mg L^{-1}	173	161	139	101	84	70	77	76
Acetaldehyde mg L^{-1}	40	67	60	29	53	35	46	47
Sulphite binding at 45 mg L^{-1} free SO_2	195	217	179	131	160	116	130	130

factors affecting the binding of sulphite by constituents of apple juice and cider. Analytical procedures developed as part of this project by Lea *et al*. (2000) were used to measure the binding power of a range of commercial cider products in two ways. Firstly, through the experimental determination of bound and free sulphite after equilibration with four added levels of sulphite; secondly, through HPLC analysis of the binding carbonyls in the cider followed by calculation of their contribution to the sulphite binding power. Experimental results demonstrated a good agreement between the two methods in eight of the commercial ciders examined. In three ciders the experimentally determined binding power was significantly higher than the levels predicted by the carbonyl analyses; this was considered to be owing, in part, to the presence of L-xylosone in the ciders. However, other possible causes of binding may include the use of anthocyanins, azo-dyes and caramel colouring agents, saccharin, hydroxymethyl-furfural (from poor quality concentrates) and the occurrence of keto sugars from poor quality fruit.

In the remaining case the sulphite binding power was so high that neither method yielded sensible results − this was later attributed to the use of ascorbic acid in the cider which would have blocked the fermentation process with the accumulation of excessive quantities of pyruvate and acetaldehyde. Such an effect is known to occur if sulphite is added to an actively fermenting yeast (Hammond & Carr, 1976), but it is not clear if a similar mechanism operates in this case. Of the eight ciders for which good agreement was achieved, it was possible to explain the binding of sulphite by the presence of the known carbonyls. A critical conclusion was that galacturonic acid may be of much greater significance than had previously been believed, due to increased use of depectinization processes in the preparation of apple juice concentrates.

The study demonstrates unequivocally that the sulphite binding power of ciders is dependent upon the strain of fermentation yeast, one strain (NCYC 5169) producing nearly twice the binding power of the least able strain (NCYC 3627). Hence, in seeking to minimize the production of binding compounds, the selection of yeast strain is of considerable importance. There was a moderate correlation between sulphite binding power in the cider and the rate of fermentation − yeasts with a high fermentative capacity, which reached attenuation quickly (e.g. NCYC 5169), tended to produce ciders with greater sulphite binding power. The extent of binding, as reflected in the shapes of the curves was much greater in the experimental ciders than had been observed in the commercial products − probably reflecting the fact that the experimental ciders were made from 100% juice. The ciders contained significant quantities of unknown binding compounds which could not be attributed to additives, pasteurization or concentration processes since none were used. It is probable that they reflect the presence of sulphite binding compounds derived from poor quality (e.g. mould damaged) fruits.

When ciders were fermented using the strongest and weakest producers of sulphite binding compounds, it was clear that strain NCYC 3627 always produced the lower level of sulphite binding when it was provided with added nutrients, but in the absence of nutrients its binding power was significantly increased and did not differ from that of ciders produced by strain NCYC 5169. This observation is in accord with existing knowledge since the addition of thiamin and/or pantothenate is known to lower the accumulation of pyruvate and acetaldehyde in susceptible yeast strains (Whiting, 1976; Würdig, 1989; Beech, 1993).

Although there was no overall effect of fermentation temperature, examination of the data shows a consistent trend whereby strain NCYC 3627 produces significantly more pyruvate and acetaldehyde at 15 °C and more α-keto glutarate at 25 °C than at the other temperatures, regardless of the presence or absence of nutrients, although sulphite binding levels were consistently higher in the absence of added nutrients than in their presence. The sulphite binding capacity of the NCYC 3627 cider was greater when fermented at 15 °C than at the other temperatures. Similarly, strain NCYC 5169 produced more α-keto glutarate and pyruvate at 20 °C and more acetaldehyde at 15 °C than at the other temperatures. For this strain, sulphite binding power was higher at 15 °C and 25 °C than at 20 °C.

It is of interest that the yeast strains with the highest and lowest sulphite binding capacity were also the highest and lowest producers of sulphite respectively during fermentation (where sulphate is presumably used as a source). Indeed there was a general correlation between sulphite binding and sulphite production across all 12 yeast strains.

Table 9 Causes of high sulphite binding in cider

Possible causal effect	Conclusion derived from current work
Poor fruit quality	By inference only, juice with high sulphite-binding levels which are not attributable to simple carbonyls implies high level of rotting fruit
Type of juice	Results demonstrate that bittersweet juice supports production of higher levels of sulphite binding compounds – but see below
Acidity of juice	Not investigated specifically, but culinary juices had higher acidity and lower levels of binding compounds that did bitter sweet juices
Pectinase treatment of juice	Evidence was found for high levels of galacturonic acid in some juices and ciders. This is presumed to have arisen from deliberate hydrolysis of pectin prior to concentration
Presence of cloud	No evidence for any consistent effect
Effect of concentration	No evidence for any effect in freshly-prepared concentrate produced under low temperature conditions – inference from earlier work that poorly produced or badly stored concentrate may have increased level of binding compounds
Strain of fermentation yeast	Highly significant difference between UK commercial strains.
Temperature of fermentation	No definitive evidence for any effect although suggestion that lower binding produced at higher temperatures (e.g. 20–25 °C)
Use of added nutrients (in concentrated juice fermentations)	Effect varied between yeast strains – whilst it did not affect the production of binding compounds in a high yielding strain, omission of nutrients in a low yielding strain significantly increased the production of binding compounds
Phenolics	Not assessed specifically but could be a further factor in understanding why bittersweet juice binds more than culinary juice
Antioxidants	Not assessed directly – but in a commercial cider fermented in the presence of ascorbic acid, the level of binding compounds was exceptionally high
Production of sulphite by yeast metabolism	Not formally investigated but appears to contribute to increased binding power after fermentation

According to Würdig (1989) this is the same mechanism responsible for the increased aldehyde production following the addition of exogenous sulphite to an active fermentation. The binding of acetaldehyde by sulphite as it is formed, and the consequent blockage of the conversion pathway to ethanol, induces accumulation, rather than metabolism of acetaldehyde.

In the experiments using fresh and concentrated bittersweet and culinary juices fermented at 20 °C the overall binding power of the ciders was similar to that observed previously with the yeast strain NCYC 5169. There was no clear pattern of difference between the juices except that ciders made from bittersweet juice had significantly higher levels of sulphite binding compounds than did the culinary juice ciders. In three of the four sets, the cloudy juice and concentrate ciders had higher binding levels than did the clear juice ciders; similarly, in three of the sets the fresh juice ciders had higher levels of binding compounds than the concentrate derived ciders. However, the differences were not statistically significant.

In the early stages of the project a range of factors was identified which might be expected to increase the level of sulphite binding compounds in cider. For practical and economic reasons it was not possible to investigate all the possibilities. Table 9 provides a summary of these possible factors and the findings from the present series of investigations.

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