inactive phosphate solution of 10 p.p.m. P indicated that the ^{32}P was not present as a contaminant that could be easily lost, although it was not determined how it had entered the tissues of the mites.

To find the direction of movement of mites after emergence from infested buds, galled buds were injected with ^{32}P solution, cut off after 12 hours, and attached to healthy plants with small wire hooks. The mites emerged rapidly from the infested buds but it proved impossible to detect their presence by a monitor after dispersal. They could, however, be detected by taking tissues from the plant, digesting them in nitric acid, making the digest to a standard volume and counting the radioactivity in a liquid M6 type G.M. tube. By this procedure the mites were found to move preferentially upwards from the point of application and to tend to congregate in the leaf axils of the host plant.

TABLE I.

PERCENTAGE DISTRIBUTION OF ^{32}P ON SHOOTS AFTER ATTACHMENT OF INJECTED GALLED BUDS.

Interval from attachment to sampling	Shoot above bud	Shoot and leaf axil tissue below bud	Leaf axil tissue above bud
1 day	64	36	—
3 days	76	22	2
6 days	54	—	46
8 days	68	—	32

The technique has helped to show the direction of movement of mites (1,2), but a close correlation was not obtained between the radioactivity recorded and the numbers of mites determined by dissection of the tissues, probably because of variations in the radioactivity associated with individual mites. The radioactivity appeared not to affect the behaviour of the mites during migration or their penetration of axillary buds and subsequent reproduction.

REFERENCES.

(1) SMITH, B. D. (1960). *Ann. Rep. Long Ashton Res. Sta. for* 1959, 137–138.
(2) SMITH, B. D. (1960). *Ibid*, 130–136.

"NON-FERMENTABLE" SUBSTANCES IN CIDERS AND PERRIES.

By G. C. WHITING.

Introduction.

Both cider-apple and perry-pear juices are now usually fermented to dryness, that is until all the fermentable sugars have been used by the yeasts present. Of the many substances that occur in ciders and perries some are present in the original juice while others are products of microbial or chemical action. In some years, many perries still show high specific gravity when fermentation has apparently ceased. Although it has been shown that the presence of sorbitol can account for some of this high residual gravity, the exceptionally high values found in 1959 required further investigation. The substances present after fermentation of ciders and perries were therefore studied to determine their identity, the amounts present, and their contribution to the specific gravity. Non-volatile organic acids have been previously described (1, 2), but for completeness the contributions of malate and citrate towards specific gravity have also been measured.

Experimental Methods.

The specific gravity of each juice, cider and perry was determined by using an accurate hydrometer, while the gravity of other solutions was obtained by weighing a known volume. All determinations were made at 20°C. To conform with convention, the absolute specific gravities are here multiplied by 1,000 and the units in excess of 1,000 are referred to as "degrees of S.G." Titratable and total acidities were determined as previously described (1). "Tannin" was determined by direct titration with potassium permanganate, using indigo-carmine as indicator (see page 143).

For many of the determinations it was necessary to remove acids and basic materials by passing the solution first through a column of Amberlite resin IR-120 (hydrogen form) and then through a column of Amberlite resin IRA-400 (carbonate form).

Qualitative Determination of Carbohydrates.

The cider or perry, after ion-exchange resin column treatment as described above, was treated with lead acetate solution, which removed a high proportion of "tannin". The clear solution was subjected to paper chromatographic examination using Solvent A : *n*-butanol-ethanol-water (10-1-2). The spray reagents used were aniline hydrogen phthalate, *p*-anisidine hydrochloride (for the detection of sugars), borax-boric acid with bromocresol purple (for the detection of hexitols) (3), and Trevelyan's silver nitrate dipping reagent followed by ethanolic sodium hydroxide spray (for the detection of sugars, polyols and glycerol) (4). Authentic specimens were run on the same chromatogram for comparison of R_F values and spray reactions.

Quantitative Determination of Carbohydrates.

A paper chromatographic method similar to that previously described for acids (1) was used to determine quantitatively glycerol, hexitols and sugars.

Glycerol was determined after spotting 4 μl of cider or perry, after ion-exchange resin treatment, together with standard spots of 10 μg, 20 μg, 30μg, 40 μg and 50 μg of glycerol on the starting line, and running the chromatogram overnight in Solvent A. The glycerol spots were revealed by the use of Trevelyan's reagent (4).

A similar method was used for hexitols with standards of 10 μg-50 μg of sorbitol. The chromatogram was run for two days and sprayed with borate-bromo-cresol purple (3). The solvent used does not separate sorbitol, mannitol and dulcitol but in the absence of a malo-lactic change the hexitol has been assumed to be sorbitol. Fructose was determined using Trevelyan's reagent while, for glucose and xylose, *p*-anisidine was used.

An approximate value for the sorbitol content of perries was obtained, without preliminary ion-exchange treatment, after running the chromatogram with standards of 50 μg-200 μg for two days. The paper was then sprayed with 1% *p*-anisidine hydrochloride in water-saturated *n*-butanol, heated ten minutes at 100°C and then dipped in a mixture of one volume of an aqueous saturated solution of sodium metaperiodate and four volumes of acetone. Sorbitol was revealed as a white spot on a purple background (5).

Volumetric Determination of Sorbitol.

Sorbitol was accurately determined in perries by titration of the formic acid produced on oxidation with periodate. An aliquot of perry was passed through ion-exchange resin columns as described above and finally diluted to 100 times its original volume with distilled water. 2 ml. of 0·1 M sodium metaperiodate solution were added to 5 ml. of the diluted perry in a flask which was then stoppered and allowed to stand 3 hours at room temperature. 5 ml. of 10% aqueous ethylene glycol were added to decompose excess periodate and the mixture was allowed to stand 10 minutes. The formic acid produced was titrated with 0·01 *N* sodium hydroxide solution to the methyl red end-point using a stream of CO_2-free air ; a blank determination was done simultaneously. Corrections were applied for the glycerol and sugars present ; glycerol gave the theoretical yield of formic acid ; glucose, xylose and fructose gave 61%, 56% and 42% respectively of the theoretical yield. A small correction was applied for the acid produced by the action of periodate on the tannin present in perry. Preliminary treatment with nylon powder, which quantitatively removed leucoanthocyanins, made this correction unnecessary.

Results and Discussion.

Non-fermentable Sugars in Dry Ciders.

The main carbohydrate constituents in most dry ciders were hexitol and glycerol. In sulphited fermentations sorbitol present in the original juice remained unchanged. In juices fermented by the natural flora, mannitol, which would be determined together with sorbitol by the methods used, may be formed by the heterofermentative lactic acid bacteria during fermentation. The range of hexitol contents found was 0·35%-0·75%. In some ciders that had been fermented by the natural juice flora, no glycerol was detected, but amounts up to 0·5% were found in sulphited ciders.

The main " non-fermentable " sugar was D-xylose (6)—about 0·05% in most ciders. But in some ciders fermented with the natural flora xylose was

absent, although detectable in the original juices : in these ciders it had evidently been metabolised, probably by lactic acid bacteria. Other sugars detected in trace amounts and tentatively identified by the paper chromatographic methods used were galactose, arabinose, ribose, rhamnose, sorbose and inositol (about 0·04%) ; in addition several oligosaccharides were detected : the main ones had R_G values (R_F values relative to that of glucose) in Solvent A of 0·17 and 0·40. Arabinose and galactose may have been formed by enzymic hydrolysis of the araban and galactan associated with the pectic substances of the juice. These are demethylated by a methylesterase present in the fruit and then degraded by yeast polygalacturonase (7) to mono-, di- and trigalacturonic acids ; these acids have been isolated from ciders (2). It is possible that some trace sugars may have appeared from hydrolysis of flavonoid glycosides during the fermentation ; galactose, glucose, rhamnose, arabinose and xylose have been found in the glycosides of quercetin or cyanidin in the apple (8). Ribose may have been liberated from autolysed yeast cells.

Many of the trace sugars and the oligosaccharides disappear during storage of the ciders, most probably as a result of bacterial action. The total amount of trace sugars at dryness is usually about 0·1% (excluding sugar acids).

Non-fermentable Sugars in Perries.

The trace sugars are more difficult to detect by paper chromatographic means in dry perries than in ciders because of the higher content of sorbitol in perries ; values from 1·0% to more than 5·0% have been found. Glycerol occurs in amounts similar to those in ciders while xylose has sometimes been found in larger amounts, up to 0·2%. Both glycerol and xylose have been observed to be metabolised, probably by lactic acid bacteria, during storage of perry. Trace sugars tentatively identified were galactose, arabinose, ribose and inositol (about 0·04%), together with an oligosaccharide (R_G 0·05 in Solvent A).

The Contribution of Non-fermentable Substances to the Residual Gravities in Dry Ciders and Perries.

The difference between the specific gravities of juice and dry cider or perry may be used to calculate the amount of alcohol formed (decrease in degrees of S.G. $\div 7{\cdot}5$ = per cent alcohol by volume). The gravity of the corresponding alcohol-water mixture (obtained from tables) subtracted from the gravity of the dry cider or perry then gives the degrees of gravity to be accounted for by other constituents. Table I shows the degrees of gravity found for 1% (w/v) solutions of a number of these constituents ; those asterisked were obtained from tables, the rest by laboratory determination. The citric and malic acid solutions were brought to the specified pH values by adding KOH ; the tannin was a leucoanthocyanin from perry.

The usual range of degrees of gravity to be accounted for in dry cider is 6°–12° ; in practice, if a cider fermentation ceases at a gravity greater than 1005°, fermentable sugar is still present. The residual gravity of a dry cider is accounted for by sorbitol, glycerol, tannin and acid, free and combined.

Perry fermentations may cease at a gravity much higher than 1005°. In

TABLE II.

RESIDUAL GRAVITY IN PERRIES.

Variety	Original Gravity of Juice	Final Gravity of Perry	Degrees Gravity to be accounted for	% Sorbitol	% Tannin	% Fructose	% Glucose	% Xylose	% Glycerol	% Total Acidity	Degrees Gravity accounted for
Rumblers	1070·6	1014·2	20·2	2·75 *9·63*	0·32 *1·70*	0·08 *0·35*	—	0·1 *0·42*	0·63 *1·51*	1·28 *6·91*	*20·5*
Taynton Squash ..	1094·4	1015·4	23·4	3·25 *11·38*	0·24 *1·27*	0·2 *0·84*	Trace	0·1 *0·42*	0·88 *2·11*	1·64 *8·36*	*24·4*
Brown Huffcap ..	1104·2	1025·4	33·4	5·04 *17·64*	0·84 *4·45*	0·03 *0·12*	Trace	Trace	0·83 *1·99**	1·22 *7·56*	*31·8*
Thorn	1088·5	1009·4	17·4	2·97 *10·40*	0·34 *1·80*	0·1 *0·42*	Trace	Trace	0·84 *2·02*	1·09 *5·89*	*20·5*
Oldfield	1088·1	1023·7	30·7	4·40 *15·40*	0·34 *1·80*	0·4 *1·68*	0·2 *0·8*	0·05 *0·21*	0·76 *1·82*	1·84 *9·57*	*31·3*
Rock	1091·1	1017·3	25·3	3·86 *13·51*	0·70 *3·71*	0·1 *0·42*	Trace	0·1 *0·42*	0·66 *1·58*	0·96 *5·95*	*25·6*

Values in italics represent degrees of gravity calculated from Table I.

TABLE I.

DEGREES OF GRAVITY OF 1% SOLUTIONS OF JUICE CONSTITUENTS.

Fructose	4·2*
Sorbitol	3·5
Glycerol	2·4*
Citric acid : pH 3	4·6
Citric acid : pH 4	5·6
Malic acid : pH 3	4·6
Malic acid : pH 4	6·2
Tannin	5·3

the seasons 1955-58, 75 perries were fermented and their final gravities determined ; the final gravity of one perry was 1021°, ten were in the range 1010°–1020° while the remainder were below 1010°. Sorbitol contents of up to 3% were found in the high gravity perries. In the 1959 season the final gravities of 65 perries were determined ; seven were greater than 1020° and fifteen were in the range 1010°–1020°. Six of these high gravity perries were examined in detail. Each of the main perry constituents was determined and its contribution to the gravity calculated from Table I. The results are shown in Table II. The sum of the individual contributions to the gravity (final column Table II) agreed well with the " degrees of gravity to be accounted for " described above. Sorbitol and acid made the main contributions : tannin and glycerol were rather less important. The unusually high sorbitol and acid contents were presumably due to the hot dry growing season ; dehydration of the fruit may also have been contributory.

Summary.

(1) The trace sugars in dry ciders and perries have been tentatively identified.
(2) Methods are described for the determination of glycerol and sorbitol in ciders and perries.
(3) The contribution of sorbitol, leucoanthocyanin, citric and malic acids to the gravity of dry cider or perry have been determined and the high final gravities of certain dry perries have been explained.

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REFERENCES.

(1) Phillips, J. D., Pollard, A. and Whiting, G. C. (1956). *J. Sci. Fd Agric.* **7,** 31–40.
(2) Whiting, G. C. and Coggins, R. A. (1960). *J. Sci. Fd Agric.* **11,** 337–344.
(3) Bradfield, A. E. and Flood, A. E. (1950). *Nature* **166,** 264–265.
(4) Trevelyan, W. E., Proctor, D. P. and Harrison, J. S. (1950). *Nature* **166,** 444–445.
(5) Bean, R. C. and Porter, G. G. (1959). *Anal. Chem.* **31,** 1929–1930.
(6) Whiting, G. C. and Coggins, R. A. (1960). *Nature* **185,** 843–844.
(7) Pollard, A. and Kieser, M. E. (1959). *J. Sci. Fd Agric.* **10,** 253.
(8) Siegelman, H. W. (1955). *J. biol. Chem.* **213,** 647–654.