10 Analysis of soft drinks and fruit juices

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10.1 Introduction

Although marketing and 'image' have a lot to do with consumers' desire to try a product, if they do not like the flavour they will not purchase it again. In addition, if the quality, odour or flavour of the product is variable, it will not live up to consumers' expectations and it is again likely that they will not be regular consumers of the product. This means that it is critical to control the flavour of the product. This can be done in a number of ways, including sensory assessment and the analysis of certain key ingredients, such as sweeteners, acidity, colour and, in a carbonated soft drink, the level of dissolved carbon dioxide.

If a formulation uses high-intensity sweeteners, preservatives or colours, it is critical to ensure that the levels of these materials in the product do not exceed the statutory limits. Although the fortification of some products has been common practice for many years, the addition of vitamins to soft drinks has only recently become common. In the United Kingdom, this is likely to be attributable in part to the phenomenally successful launch of the Sunny Delight brand, which has prompted the launch of a wide range of 'me too' products. Here again there is an analytical need to ensure that the vitamin levels claimed on the label are met at the end of shelf life.

There is also a growing interest in drinks with associated special characteristics. These products will often contain sugars for energy, caffeine for stimulation and vitamins, amino acids and maybe herbs which impart some unique selling property as a health benefit. A very good example of this type of product is Red Bull, which is described, according to its website, thus: 'Red Bull is much more than a soft drink – it is an energy drink. It was made for moments of increased physical and mental stress and improves endurance, alertness, concentration and reaction speed. In short: it vitalizes body and mind.'

The great success of this brand since its launch has led to the production of a wide range of other products based on the same concept of stimulation and improved alertness. These more complex drinks formulations can pose a significant analytical challenge. This is especially so if the product contains a mixture of herb extracts and there is a requirement to confirm that each one is present.

Thirty years ago there would have been a limited set of equipment in the quality control laboratory of the majority of fruit juice and soft drinks factories. This would probably have consisted of a refractometer, a burette, a spectrophotometer, an instrument to measure the level of carbonation and some equipment for checking the microbiological integrity of the product. However, with the move to larger

and more efficient factories with faster bottling lines, it has become critical to ensure that a bottling formulation is correct before a product is packed. These developments mean that in today's factory if a product's formulation is prepared incorrectly and this is not detected it will be a very expensive mistake to rectify after the product has been packed. Complementary to this is the fact that general analytical equipment, such as high-performance liquid chromatography (HPLC) systems, has become more reliable, requires less-skilled operators to use it and is also relatively cheaper than it was 20 years ago. These developments mean that it is now common to see sophisticated equipment in a factory's quality assurance laboratory so that a bottling formulation can be checked for the basic parameters such as Brix, acidity, high-intensity sweeteners and preservatives before packing.

Since the publication of the first edition of this book, a few more validated methods for the analysis of soft drinks ingredients have been documented. When the first edition was published in 1998, only a handful of methods for the analysis of soft drinks ingredients had been collaboratively tested in the Association of Official Analytical Chemists (AOAC) official methods manual, and only two of these were modern HPLC approaches. At that time, no methods could be found in the British Standards catalogue. Inspection of the British Standards website (http://www.bsi-global.com) now shows that there are two standardised approaches for the analysis of high-intensity sweeteners in soft drinks, both of which use HPLC. This overall lack of standardisation of methods is probably because a soft drink's matrix is relatively straightforward, without many of the problems associated with other areas of food analysis, and so the industry has not felt the need to standardise the test methods.

Although there are only a limited number of methods that have been validated specifically for soft drinks, there are around 80 validated methods available for the analysis of fruit juices, most of which would work equally well for soft drinks. These methods are published in the International Fruit Juice Union (IFU) handbook of analytical procedures, which offers the best reference collection of methods for the analysis of fruit juices in the world, with new methods added on a regular basis (Anon, 2004a). The IFU's collection of analytical methods covers most of the main procedures required to assess the quality and authenticity of fruit juices and nectars. The methods are listed on the IFU's website (http://www.ifu-fruitjuice.com); at the time of writing they cannot be purchased directly from there, but they can be obtained from the Swiss Fruit Union, Zug, and details of how to do this are given on the website. It is possible that at some time the methods will be made available directly from the website.

A number of these IFU methods have provided the basis for those published for fruit and vegetable juices by CEN, the European standardisation organisation, which have been adopted by the member states of the European Union as national standard methods. There are 24 listed on the British Standards website (http://www.bsi-global.com). They can be purchased from this website and should also be available from other standardisation organisations within the European Union and affiliated states such as Switzerland. They are also likely

to be adopted as Codex methods when the standard for juice is finally approved and published. Although some of these IFU procedures, such as titratable acidity in a carbonated product, require modification for use in soft drinks systems, most are equally appropriate for the analysis of soft drinks.

This chapter will cover the analysis of the different key elements of a soft drink formulation or a juice, for example, sweeteners and preservatives, and the procedures for their assessment. When both soft drinks and juices contain a particular ingredient or component, their analysis is discussed together. Where there is no overlap, soft drinks and juices are covered separately.

Although it is beyond the scope of this chapter to discuss in any great detail methods used to assess the authenticity of fruit juices, a brief summary of this area is given at the end. In the first edition of this book, an introduction to HPLC was given to assist newcomers to the field, but it is now felt unnecessary as this has become such a standard procedure.

10.2 Sensory evaluation

The sensoric assessment of soft drinks and fruit juices is discussed briefly here for completeness.

As the flavour and odour of a soft drink or fruit juice are very important elements of a product they should be closely controlled. This is generally carried out by trained panellists who have been screened to ensure that they have an aptitude for this type of assessment; they are often quality assurance personnel or workers from the factory. Sensory assessments should be carried out in surroundings where the panellists can concentrate without distractions.

Every batch of finished product, should be checked to ensure that it tastes ‘normal’ (i.e. is free from off-tastes). Although the tasters should be familiar with the product’s flavour and odour they should always be provided with an approval control for reference purposes. Incoming raw materials such as sugar and water should also be assessed to ensure that they will not impart any off-tastes to the finished product.

10.3 Water

In a soft drink, or a fruit juice reconstituted from concentrate, the quality of the water is an essential element. Checking the water quality includes assessment to ensure that it does not contain any off-tastes or odours. It also involves checking that any water-treatment processes have been effective and have not introduced defects into the water. The water should also be assessed to ensure that it does not contain materials that are likely to precipitate from the product on storage. Such precipitates are often called ‘flocs’.

The most common cause of floc formation in a clear soft drink is microbiological growth (yeasts). If this occurs the yeasts can be identified by

allowing the floc to settle in the container, or precipitating it by centrifugation, and very gently decanting the liquid or syphoning off the product. The precipitate can then be placed on to a microscope slide for examination. The yeast cells will be seen as large (*c.*5 μm) ellipsoids.

Another cause for a floc, particularly in the autumn, can be the presence of algal polysaccharides in the water. Although these polysaccharides are soluble in water at neutral pH values, they will precipitate from solution when the water is made acid. To check for the presence of polysaccharides in the water the following test should be carried out.

To a sample of raw water (200 ml) concentrated hydrochloric acid (2 ml) is added to ensure that the pH is below 2. The water is held at 608C for 12 h and then examined against a dark background under strong illumination to detect any floating particulate materials. The heating period can be extended for a few days to ensure there is no precipitate.

On a less frequent basis, and as part of good manufacturing practice, samples of water should also be analysed more thoroughly for a wider range of components, including the heavy metals (e.g. arsenic, copper, chromium, cadmium, lead, mercury, selenium and zinc) and polyaromatic hydrocarbons (PAHs), some of which are known to be carcinogens. The levels of fluoride and nitrate ions in the incoming water should also be checked periodically. The heavy metals and PAH analyses are both very specialised and are better left to specialist laboratories. However, analysis for fluoride and nitrate levels can readily be performed using ion exchange chromatography linked with conductivity detection. A typical example of this is given in Dionex application note 25 (Anon, n.d.a) and a chromatogram of a standard for this type of separation is given in Figure 10.1.

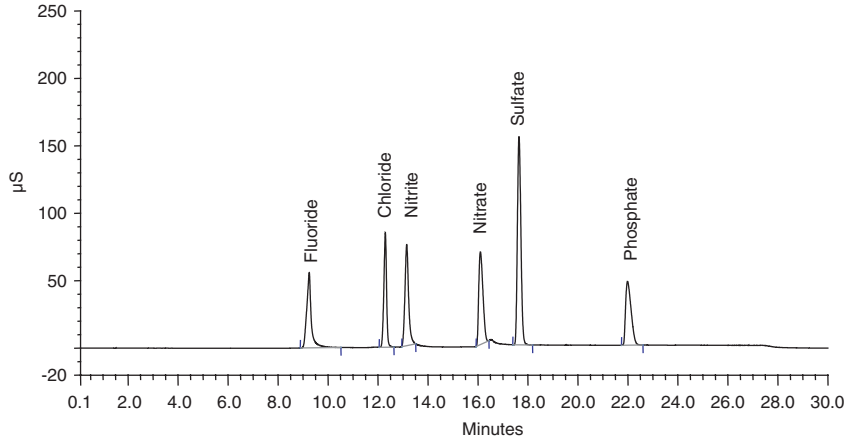

Figure 10.1 Ion-exchange chromatographic separation of main anions found in water using a Dionex HPLC and AS-11 HC column. Conditions: column: Dionex AS-11 HC 250 × 4 mm; solvent Milli-Q water and 3 mM NaOH for 6 mins then to 30 mM NaOH over 15 min; flow rate 1.5 ml/min; suppressed conductivity detection.

10.4 Sweeteners

After water quality, sweetness is probably the most important feature of a soft drink. In fact, until 1995 in the United Kingdom it was essential that a soft drink contained a minimum level of sugars. This level was set at 45 g/l unless the product was listed as a 'low-calorie' soft drink (Anon, 1964; UK Soft Drinks Regulations 1964). These regulations have been revoked (Anon, 1995a), and it is now possible to make a soft drink with or without added sugars, if required, provided the product is appropriately labelled.

The sweeteners used in soft drinks can be divided into two main categories. These are the natural sweeteners, such as sucrose, invert syrups, corn-derived syrups and honey, and the high-intensity sweeteners (artificial sweeteners) such as saccharin, aspartame and acesulfame K. In most fruit juices and many soft drinks, except diet varieties, sugars are a major component of the product.

All fruit juices contain glucose and fructose and some sucrose, but the proportions of these sugars will depend on the actual fruit. In soft drinks the type of sugar used in a formulation will often depend on where the product is being produced. In EU countries, where the price of sugar is adjusted to support local production, the sugar used to prepare soft drinks will often be sucrose or a sugar syrup prepared from beet or possibly cane. However, in the United States most soft drinks are prepared from syrups derived from starch, such as a high-fructose syrup prepared from corn (HFCS), as these syrups are cheaper than sugar derived from cane or beet.

High-intensity sweeteners are used in diet formulations but they also often appear in regular soft drinks. Their use depends on the requirements of the manufacturer and also on the relative prices of sugar and the high-intensity sweetener(s) involved. This substitution was particularly common in the United Kingdom in the 1970s, when part of the sweetness of a product was often provided by the high-intensity sweetener saccharin to control the cost of a soft drink's formulation.

10.4.1 Analysis of natural sweeteners

The natural sweeteners used in soft drinks formulations are generally the same as those found in fruit juices, that is, sucrose, glucose and fructose. As sugars are generally the second largest component in a soft drink or fruit juice, one of the quickest and simplest ways of assessing if a product is within specification is to measure its refractive index. This can be achieved using a simple hand-held refractometer costing a few pounds or a sophisticated temperature-controlled refractometer, which costs several thousand pounds.

The assessment of the refractometric solids content, generally referred to as the Brix value, is one of the basic tests that is carried out in today's soft drinks

and fruit juice factories to assess the soluble solids content of an incoming sugar syrup, a soft drinks bottling syrup before dilution, fruit juice concentrate or a diluted finished product. This measurement of soluble solids content is only an estimate as it assumes that everything in solution has the same refractive index as sucrose. Because of this assumption, it is normal practice to adjust the refractometric solids content of citrus juices for their acidity by application of a correction factor. This correction factor can be calculated using the following equation:

$$\text{acid correction to } ^\circ\text{Brix} = 0.012 + 0.193\,m - 0.0004\,m^2$$

where m is the total acidity obtained by titration to a pH of 8.1 expressed as anhydrous citric acid in g/100 g. In the case of soft drinks this is frequently the limit of analytical testing for sugars. However, it is common to check the levels of the individual sugars in a juice to assess the authenticity and quality of the product. Today most practitioners in this field use either HPLC or enzymatic methods to achieve this.

A wide range of HPLC methods has been used to detect sugars, and the literature includes a long list of papers on the separation and quantification of sugars using HPLC. As the sugars used in soft drinks formulations, except fructose, do not exhibit strong light absorbance in the ultraviolet region they are generally detected using a differential refractometer, which is a non-specific detector with a relatively low sensitivity (typically in the region 0.01–0.05%). This technique is ideally suited for the analysis of sucrose, glucose and fructose at the levels detected in soft drinks and fruit juices, which are usually in the region of 0.5–5%. The separation of the sugars can be performed on a range of different columns; only three are discussed below and any one of these would be appropriate for soft drinks analysis.

The first method employs the use of an amino-bonded silica column. On this column the free hydroxyl groups on the silica particles have been reacted and replaced by amino groups. This type of column, sold by most column manufacturers, will separate sucrose, glucose and fructose in about 10 min, as illustrated in Figure 10.2.

As with all methods, this approach has some limitations: it uses acetonitrile, which is toxic, and the separation of glucose from fructose can sometimes be problematic after extended use of the column. However, sample preparation is easy since it requires only dilution to the required level (often 1 : 10) and filtration prior to analysis to remove particulate materials, which protects and extends the useful life of the column. The degradation of the resolution between glucose and fructose is caused by the partial inactivation of the column by materials in the matrix, but this resolution can be recovered by reducing the acetonitrile concentration in the solvent. The same column can also be used to assay the level of ascorbic acid (vitamin C) in a soft drink or fruit juice, although different detection and solvent systems are used.

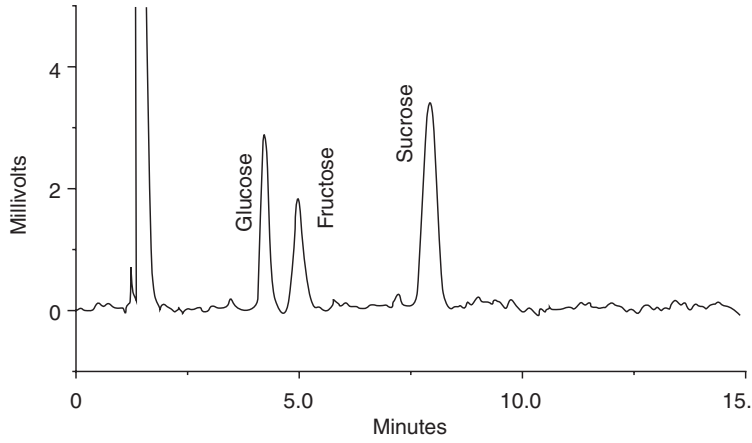

Figure 10.2 Separation of the main sugars found in orange juice using an amino-bonded silica column. Conditions: column: Highchrom 5 μm 250 × 4.5 mm amino-bonded silica; solvent = acetonitrile/water (8:2); flow rate 2 ml/min; refractive index detection.

The second method, using refractive index (RI) detection, is carried out using a resin-based polymer column. Sucrose elutes first from this column, followed by glucose, fructose and then sorbitol. This type of column is generally more robust than the amino-bonded column and if handled well will last much longer; however, it is around three times more expensive. The method has been collaboratively tested for the analysis of sugars and sorbitol in fruit juices by the IFU. The HPLC conditions are given below.

HPLC conditions: column: benzene divinylstyrene sulphonated resin in the calcium form 300 × 7.5 mm; solvent = 0.1 mM Ca EDTA in HPLC-grade water at 0.6 ml/min; at 80°C using RI detection.

The third HPLC method for analysing sugars uses a very different detector system, a pulsed amperometric detector (PAD). If sugars are analysed using a conventional electrochemical detector they very soon 'poison' the electrode and reduce its sensitivity. This problem is overcome with the PAD system, where the electrical potential applied to the cell is varied to automatically clean and recondition the electrode. This type of detector was pioneered by the Dionex Corporation in the 1980s for the quantification of sugars and other oxidisable materials. Figure 10.3 shows a chromatogram of an adulterated apple juice using this approach; the four major peaks that are visible are, in order of elution, sorbitol, glucose, fructose and sucrose (Anon, 1992). The column used for this analysis is a mixed-bed resin column containing both sulfonated and amino-bonded resin beads (PA-10). From the size of the sorbitol peak it is clear that this apple juice contains undeclared pear juice.

Sucrose (Bergmeyer & Brent, 1974a), fructose (Bergmeyer & Brent, 1974b) and glucose (Bergmeyer *et al*., 1974) can also be quantified by the use of an

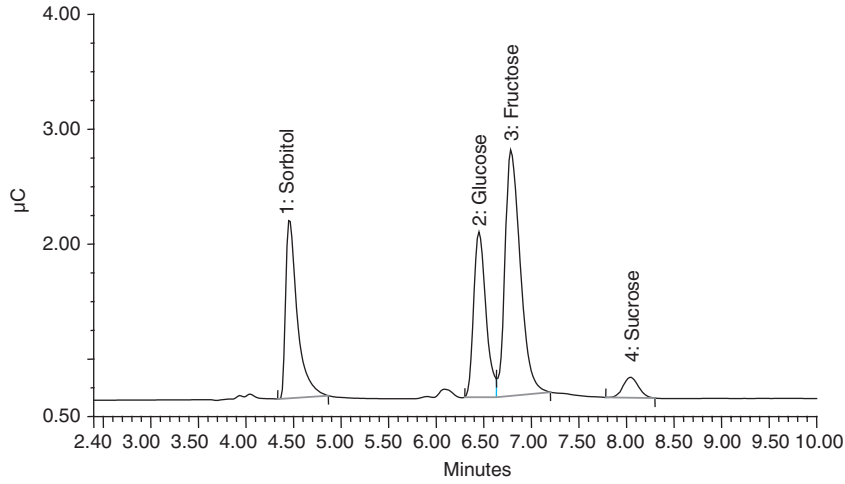

Figure 10.3 HPLC trace of a suspect apple juice run on Dionex HPLC using PA-10 column and PAD detection. Conditions: column: Dionex PA-10 250 × 4 mm; solvent = 100 mM sodium hydroxide in HPLC water; PAD detection; sample diluted 1:100 in water prior to filtration and analysis.

enzyme-linked assay. This type of approach is most commonly used in some areas of Europe (e.g. Germany) for the analysis of sugars in fruit juices or in laboratories that have a spectrophotometer but no HPLC equipment. The kits, developed by Boehringer Mannheim, for the enzymic analysis of sucrose, glucose and fructose are now sold by r-Biopharm. It is also possible to buy the individual chemicals and enzymes to carry out the assay (Anon, 1987a). These methods have been collaboratively tested for the analysis of fruit juices (IFU compendium of methods – see Anon, 1985, 1998a, b).

Generally, in fruit juices the enzymatic methods are slightly more reproducible for glucose and fructose than the HPLC procedure (results from IFU analytical commission ring tests). However, the HPLC methodology gives slightly better quantification of sucrose because the enzymic procedure uses a two-stage process that introduces more analytical variation. In the absence of an automatic enzyme analyser, it is quicker to use HPLC to quantify the sugars in fruit juices. If glucose, maltodextrin or inulin syrups are being used in a soft drinks formulation the total sugars may not add up to a value close to the soluble solids. This is because the higher oligosaccharides that these syrups contain will not be quantified in these procedures. If this is an important feature, then Dionex HPLC can be used to quantify these higher oligosaccharides. However, in this case a sodium acetate gradient is used to elute off the higher sugars from the column (Dionex application note 67 (Anon, (n.d.b)). A typical example of a soft drink containing a maltodextrin syrup is given in Figure 10.4. Maltodextrin

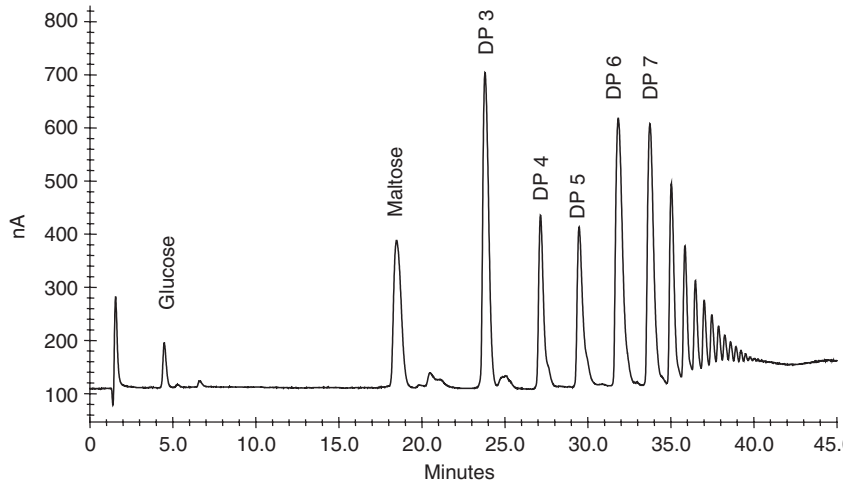

Figure 10.4 Separation of oligosaccharides by Dionex HPLC. Conditions: column: PA-100 5 μm 250 × 4 mm; solvent = 0.002 M sodium acetate in 0.1 M sodium hydroxide; flow rate 1 ml/min; solvent B1M sodium acetate in 0.1 M sodium hydroxide; flow rate 1 ml/min; using pulsed amperometric detection, time 0 = 100% A then a gradient to 46% B over 50 min using curve 9.

syrups are often added to soft drinks to control the osmolality of the product, such as in 'sports' drinks. As the proportion of the different oligosaccharides varies from one manufacturer's product to another's it is impossible to determine the exact level of addition of the maltodextrin syrup unless the actual material used in the formulation is available to the analyst.

10.4.2 Analysis of high-intensity sweeteners

Although sugars are found in juices, the high-intensity sweeteners are not. However, they are often used in juice-based drinks or nectars, and here it is important to ensure that there is resolution of the sweeteners of interest from the compounds naturally present in fruit juices. This can be a particular problem in drinks with a high juice content as naturally occurring polyphenolic materials in the juice can disturb the analysis.

It is common to use one or more high-intensity sweeteners together with sugar in a soft drink's formulation. If some or all of the sugar is replaced with a high-intensity sweetener it becomes important to check whether it has been added at the correct level. This is important not only to produce a consistent product for the consumer but also because in most countries legal maxima are set for these materials. This applies not only to the high-intensity sweeteners but also to the preservatives, bittering agents and other additives that will be

discussed below. In the United Kingdom, these limits are given either in the Sweeteners in Foods Regulations 1995 (Anon, 1995b and its various amendments), which were introduced into law in 1996 to enact EC directive 94/35/EC (Anon, 1994), or the Miscellaneous Food Additives Regulations 1995 (Anon, 1995c and its various amendments), which were introduced into law in 1996 to enact EC directive 95/2/EC as corrected (Anon, 1995d).

Saccharin is a high-intensity sweetener that has been commercially available for about 100 years and has probably been one of the most widely used low-calorie sweeteners. The traditional approach to quantifying saccharin involves its extraction into diethyl ether from a strongly acidic solution, removal of the solvent and quantification of the saccharin by titration against sodium hydroxide using bromothymol blue as an indicator (Egan *et al*., 1990a). Although this is a reliable procedure, it is rather time-consuming and is unlikely to be used extensively today.

With the wider use of HPLC, it is now much more common to assay most sweeteners and some preservatives. A wide range of procedures has been published for the analysis of saccharin in soft drinks using this technique. There is one validated method for the analysis of saccharin in soft drinks in the AOAC manual and also one in the British Standards catalogue. The AOAC procedure separates saccharin, caffeine and benzoic acid using a reverse-phase column and was published as AOAC method number 978.08 (Woodward *et al*., 1979). A reverse-phase C18 column is used with acetic acid/water/propan-2-ol as the mobile phase and detection at 254 nm. The level of propan-2-ol is set between 0 and 2% (v/v) to ensure that there is an appropriate resolution between saccharin, caffeine, benzoic acid and any other compounds, such as sorbic acid or colours, in the soft drink's formulation. The British Standards method uses a phosphate buffer and acetonitrile mixture as the solvent with a C18 column (Anon, 1999a). This method actually allows the separation of a range of other components in soft drinks and foods, that is, preservatives plus the degradation products of aspartame, although the chromatographic resolution of the method between acesulfame K and phenylalanine, which elute very early in the run, is not baseline.

There are a large number of other published procedures for the separation of a number of sweeteners and preservatives at one time; these are all based on reverse-phase HPLC. Perhaps one of the most startling is the method published by Williams (1986). This uses a small particle size (3 μm) C8 column and allows the separation of a range of colours, sweeteners and preservatives in less than 5 min. The materials separated were amaranth, quinoline yellow, quinine sulphate, sunset yellow, caffeine, aspartame, saccharin, vanillin, sorbic acid, benzoic acid and green S.

HPLC conditions: column: 3 μm Spherisorb RP8 100 × 4.6 mm, solvent = 17.5% acetonitrile, 12.5% methanol, 70% buffer (0.85% sulphuric acid in HPLC-grade water containing 17.5 mM KH_2PO_4 at pH 1.8); flow rate 1.35 ml/min; UV detection at 220 nm.

Two methods have been published which were designed to analyse a range of sweeteners and preservatives in one run. The first method, published in German by Hagenauer-Hener *et al*. (1990), describes the analysis of aspartame, acesulfame K, saccharin, caffeine, sorbic acid and benzoic acid in soft drinks and foods. The method relies on a similar system to that given above but with a less complex solvent system (Figure 10.5). The solvent system has been modified to include a gradient portion to elute the preservatives more quickly.

The second method was specifically developed for the analysis of diet soft drinks and food products by Lawrence and Charbonneau (1988) at Health Canada in Ottawa. The method describes a procedure for the separation of a broad range of sweeteners including cyclamate, alitame, sucralose and dulcin. This technique again uses reverse-phase HPLC with an acetonitrile/phosphate buffer system but in this case, as the compounds have a wider range of polarities, a gradient elution is used. Aspartame, saccharin, alitame, acesulfame K and dulcin were detected using ultraviolet absorbance at 210 or 200 nm. Sucralose, which is becoming more popular as a high-intensity sweetener, was measured using RI detection.

HPLC conditions column: C18 250 × 4.6 mm; solvent = 0.02 M phosphate buffer/acetonitrile from 97 : 3 at pH 5.0 to 80 : 20 at pH 3.5 using a linear gradient.

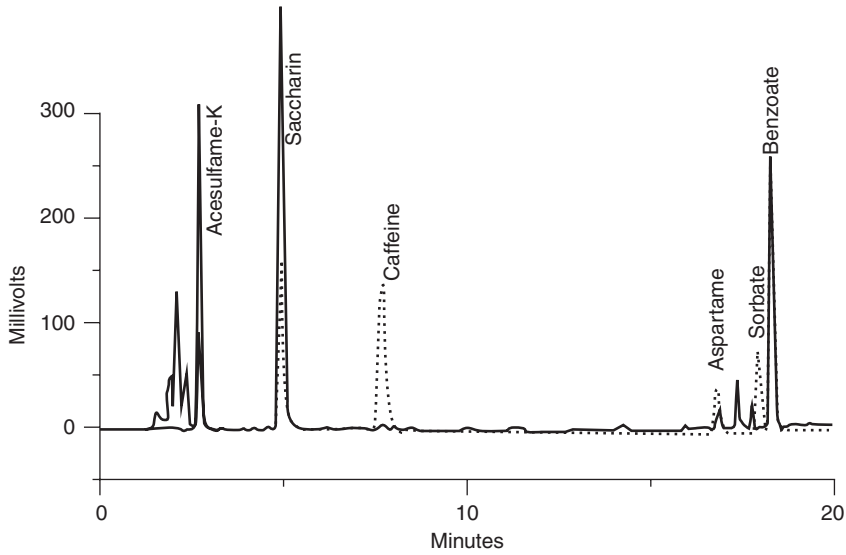

Figure 10.5 Separation of soft drinks ingredients using the method of Hagenauer-Haner *et al*. (1990). Solid line is orange squash; dotted line is mixed standard. Conditions: column PR8 5 μm 150 × 4.6 mm; solvent = 0.02 M phosphate buffer/acetonitrile (90 : 10); flow rate 1 ml/min; using UV detection 220 nm, time 10: 10% to 70% acetonitrile over 15 min.

Aspartame was introduced as a high-intensity sweetener for use in foods in the mid-1980s and has been widely adopted for use in soft drinks. It does have one limitation in that it is not very stable at the pH of normal soft drinks and it is gradually lost by hydrolysis and side-reactions. However, analysis of aspartame and its decomposition products is quite straightforward using HPLC. Tsang *et al*. (1985) used a reverse-phase separation for the analysis of aspartame, whereas Argoudelis (1984) used a strong cation exchanger. The European Norm (EN) method (EN12856:1999) will also separate aspartame and its decomposition products (Anon, 1999a).

Acesulfame K was introduced as a high-intensity sweetener at around the same time as aspartame. It too is much sweeter than sucrose but is also stable under the low pH conditions of soft drinks. Its analysis in a soft drink is relatively straightforward and an HPLC procedure is given by Grosspietsch and Hachenburg (1980).

Although the use of cyclamate was banned in the United Kingdom in the late 1960s, after concerns about its safety, it was re-approved for use in foods in the European Union in 1996 after re-evaluation of toxicological data. A number of the traditional methods for its analysis are given in *Pearson's Analysis of Foods* (Egan *et al*., 1990b) and a number of these involve the oxidation of the molecule to give sulphate ions, which are then measured gravimetrically or colorimetrically.

As cyclamate was banned in a number of countries before HPLC techniques were fully developed, there have not been many methods published for its analysis using modern procedures. The substance offers a challenge to the analyst as it does not have a useful chromophore in the ultraviolet region and its detection by a change in refractive index would be difficult at the levels used in soft drinks (a maximum of 400 ppm).

One paper published in the mid-1980s described the analysis of cyclamate using indirect ultraviolet detection (Herrmann *et al*., 1983). Here the solvent is chosen so that it has a strong ultraviolet absorbance and as the substance elutes from the column it reduces this absorbance and so becomes detectable as a negative peak. A further paper described the analysis of cyclamate using capillary electrophoresis (Zache & Gruending, 1987). There is also a European standard method for its analysis using HPLC (Anon, 1999b). In this case the cyclamate is reacted to form a derivative, *N*,*N*-dichlorocyclohexylamine, prior to its analysis by HPLC using UV detection at 314 nm. The separation is conducted on a C_{18} column and the analysis run takes about 12 min. The European Parliament has recently reviewed the status of cyclamate and reduced the level at which it can be used in soft drinks, which may reduce its usage in beverages.

10.5 Preservatives

There are three main preservatives used in soft drinks in the United Kingdom and Europe: benzoic and sorbic acids and sulphur dioxide. In some countries

para-hydroxybenzoates are also used. The analysis of these ingredients will be covered in two sections: the first will examine the analysis of benzoic and sorbic acids and the *para*-hydroxybenzoates and the second will discuss the analysis of sulphur dioxide.

10.5.1 Benzoic and sorbic acids and para*-hydroxybenzoates*

One of the older methods used to detect the presence of preservatives in soft drinks and juices is thin-layer chromatography (Woidich *et al*., 1967). This provides a useful method to detect benzoic and sorbic acids as well as the substituted benzoic acids. The first stage involves the extraction of the preservatives with diethyl ether prior to their chromatographic separation on polyamide plates. Although it is difficult to use this procedure to quantify the level of these preservatives in a sample, it is not impossible. This approach can still be used today by a laboratory that does not have access to HPLC.

Extraction using diethyl ether has also been used in another traditional method to determine the level of preservatives in a sample. In this case, benzoic acid can be extracted from a product at low pH using diethyl ether. By adjusting the pH of the product, and hence the ionisation of the acids themselves, it is possible to quantify benzoic acid in a soft drink in the presence of saccharin. After extraction the benzoic acid can be assayed spectrophotometrically or by titration (Egan *et al*., 1990c).

Benzoic and sorbic acids are now normally assayed using HPLC. As discussed in the section on the analysis of sweeteners, some of the HPLC methods developed for soft drinks actually allow the separation of both sweeteners and preservatives in one run, for example, Williams (1986), Hagenauer-Hener *et al*., (1990) and the EU method for sweeteners (Anon, 1999a), although the preservatives were not included in the collaborative trial of the method. The separation of benzoic and sorbic acids can sometimes be difficult and care should be taken that the system will actually resolve these two preservatives if they are present; otherwise spurious results can be obtained. The pH of the solvent is a critical feature that allows the separation of these two preservatives.

A validated method (No. 63) for the analysis of benzoic acid, sorbic and *para*-hydroxybenzoic acids in fruit juices has been published in the IFU handbook (Anon, 1995e). In this method the resolution takes place on a C8 reverse-phase column. With this solvent system (methanolic/ammonium acetate, pH 4.55) sorbic and benzoic acids elute quite quickly and the less polar *para*-hydroxybenzoate esters elute much later. The method does stipulate that caution has to be taken by orange juice due to possible interferences by natural materials in the juice that elute close to benzoic acid. As sorbic and benzoic acids elute quite early in the chromatogram using this method, it would

probably not be very useful for soft drinks containing some of the high-intensity sweeteners as they may present problems with interfering peaks.

HPLC conditions: column: 5 μm 250 × 4.6 mm RP8; UV detection at 235 nm; solvent = 0.01 M ammonium acetate and methanol (50 : 40) adjusted to pH 4.55 with acetic acid; flow rate 1.2 ml/min.

There is also a method in the AOAC manual for the analysis of benzoic acid in orange juice (994.11). However, this method was not designed to detect benzoic acid as a preservative. In Florida, when pulpwash was prepared benzoic acid used to be added as a marker, so that if the pulpwash was added to a juice it would be detected. However, it is believed that this practice has now been stopped.

10.5.2 Sulphur dioxide

Sulphur dioxide is a widely used preservative for foods and soft drinks, particularly dilutables, and in principle is quite easy to detect and quantify. Owing to the importance of sulphur dioxide as a preservative an extensive review of its chemistry in foods was undertaken in the mid-1980s (Wedzicha, 1984).

There is a very simple and quick method that can be used to detect the reducing power of sulphur dioxide, developed in the last century and often called the Ripper titration (Ough, 1988). In this method, sulphur dioxide is titrated against iodine or potassium iodate/potassium iodide solution in the presence of starch. When all the sulphur dioxide has been oxidised, a blue colour is produced by the reaction of free iodine with the starch. This is a very quick method but will give only an estimate of the level of sulphur dioxide as other reducing substances, such as ascorbic acid, will interfere; consequently, this method is not particularly appropriate for juices with high ascorbate levels.

To assess the level of sulphur dioxide in a fruit juice, or a soft drink containing juice, a steam distillation procedure is generally used. Here sulphur dioxide is driven out of acidified solution by heating. Acidification displaces any sulphur dioxide that was bound to other food materials. As sulphur dioxide is carried over by the steam it passes through a trap containing a hydrogen peroxide solution, where it is oxidised to sulphuric acid. The acidity contained in the trap is then measured by titration against sodium hydroxide.

There have been many modifications for different applications to the original method published by Monier-Williams (1927). However, for fruit juices it is best to use the Tanner modification (Tanner, 1963), which uses phosphoric acid rather than hydrochloric acid for the acidification as this method is less liable to interferences. The method has also been published in the IFU handbook method no. 7a (Anon, 2000) with collaborative ring test data.

Although some chromatographic methods have been published for the analysis of sulphur dioxide in solution (the sulphite anion), these have often involved a steam distillation to ensure that all the sulphur dioxide is liberated. However, as the distillation is a time-consuming stage rather than a simple acid/base titration, there is little point in using these procedures in systems that do not liberate large amounts of other acidic or sulphurous materials, such as garlic and cabbage, which interfere with the titration method.

Some newer methods have also been developed which will assay both free and total sulphur dioxide. These procedures rely on the separation of the sulphite anion from the other materials in the food using ion-exchange chromatography and measurement with an electrochemical detector. There is an AOAC approved method using direct current (DC) amperometric detection (Anon, 1995f). This employs an alkaline extraction medium (20 mM phosphate buffer containing 10 mM mannitol at pH 9) so that free and total sulphur dioxide can be detected. After extraction the sample is injected directly on to an HPLC system with an ICE-AS 1 column and DC electrochemical detection. The AOAC method suggests cleaning the electrode at the end of each run to maintain optimal detector sensitivity. However, Dionex has improved the original AOAC method by using pulsed amperometric detection (Anon, n.d.c). Using this approach, the detector response is much more stable, as the electrode is constantly cleaned during the analysis rather than at the end of each run as suggested in the AOAC method. Although at the time of publication of the first edition of this book it was thought that this modification might be collaboratively tested, formal verification is still awaited.

HPLC conditions: column: 250 × 4 mm Ion Pac ICE-AS1; solvent = 20 mM sulphuric acid; PAD detection using a platinum electrode.

10.6 Acidulants

After sweetness, the second most important feature of a soft drink is acidity and the balance of the sweetness to acidity (sourness), commonly called the Brix to acid ratio. An acceptable range for this ratio is often laid down in juice specifications. For European tastes in orange juice, a Brix to acid ratio might be set around 15 and 16.

The normal method used to assess the acidity of a soft drink or fruit juice is titration using sodium hydroxide. Traditionally, phenolphthalein was used as an indicator to detect the end-point of the titration. However, with the ready availability of pH meters, testing is now carried out to a final pH of 8.1. A major advantage of this technique is that it allows the titration to be carried out automatically and avoids problems with coloured products, such as blackcurrant juices, where the phenolphthalein indicator end-point, colourless to pink, can be

difficult or impossible to detect. The ability to carry out the titrations automatically is also more time- and cost-effective.

In a carbonated product, carbon dioxide has first to be removed by boiling, as sonication alone is not enough to ensure that all the gas has been liberated from a soft drink. It should be noted that if dealing with cola products, which contain phosphoric acid, a titration taken to an end-point of pH 8.1 will not affect the final ionisation of the hydrogen phosphate ion (HPO_4^{2-}) to the phosphate ion (PO_4^{3-}). As the pKa of HPO_4^{2-} is 12.67, this final dissociation will not take place under normal titration conditions and should be allowed for when the acid content of cola products is being determined; for example, the acid only 'appears' to be divalent.

In the analysis of fruit juices, it is important to determine the levels of the individual acids to assess authenticity and quality. A range of these acids can be determined using an enzyme-linked assays and these procedures have been collaboratively tested and published in the IFU compendium of methods (citric no. 22, isocitric no. 54, D-malic no. 64, L-malic no. 21 and D-and L-lactic acids no. 53). r-Biopharm now distributes the Boehringer Mannheim kits to assess the levels of these acids. Similar kits are available from other suppliers.

Most acids can also be assessed with HPLC using either ultraviolet or conductivity detection. The following procedure was developed to assess the organic acid profile of cranberry and apple juices but has been found to work well for other organic acids (Coppola & Starr, 1986). It has been published as AOAC method no 986.13. A typical trace for cranberry juice run using this method is given in figure 10.6. The method recommends separation of the acidic materials from the sugars by prior treatment with an ion-exchange resin.

Separation of the organic acids is also possible using an ion-exclusion column, such as a Bio-Rad HPX-87H. However, in this case it is essential that the acids are separated from the sugars; otherwise a number of peaks co-elute, thus distorting the quantification. This method is particularly good for the analysis of fumaric acid in apple juice because no pre-treatment is required and the acid elutes late in the chromatogram, well separated from other components (Figure 10.7).

Fumaric acid has been found to be a good marker to detect the addition of D,L-malic acid to apple juice (Junge & Spandinger, 1982). With the advent of the enzymic assay procedure for D-malic acid the method fell out of use. However, in 1995, a number of samples of apple juice in Germany were found to contain elevated levels of fumaric acid, which was attributed to the addition of L-malic acid.

Organic acids can also be quantified using HPLC linked to a conductivity detector. This has one advantage over UV detection in that only charged species are measured, which means that the method is liable to fewer interferences. Depending on the actual approach chosen, it is sometimes possible to detect other anions, such as Cl^-, SO_4^{2-} and PO_4^{3-}, in the same run. If the conditions given in Dionex application no. 21 are used, this allows the organic acids to be separated without interferences from the fully ionised anions such as Cl^-, SO_4^{2-} (Anon, n.d.d).

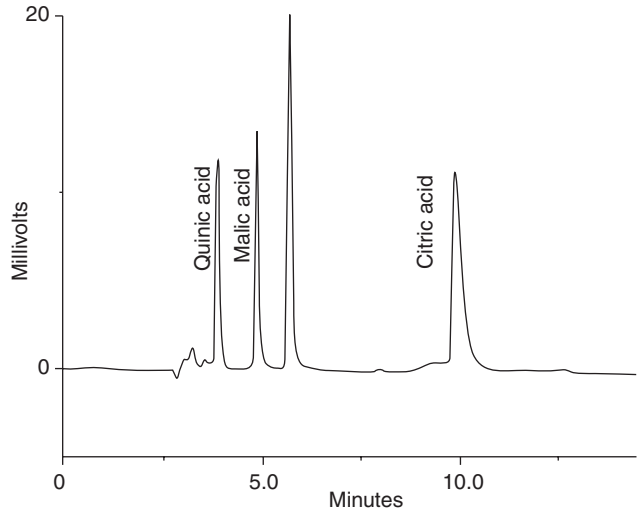

Figure 10.6 HPLC separation of the organic acids in cranberry juice using Coppola's method. Conditions: column: 5 μm ODS2 250 × 4.6 mm; UV detection at 210 nm; solvent = 0.05 M phosphate buffer at pH 2.5; flow rate 1 ml/min.

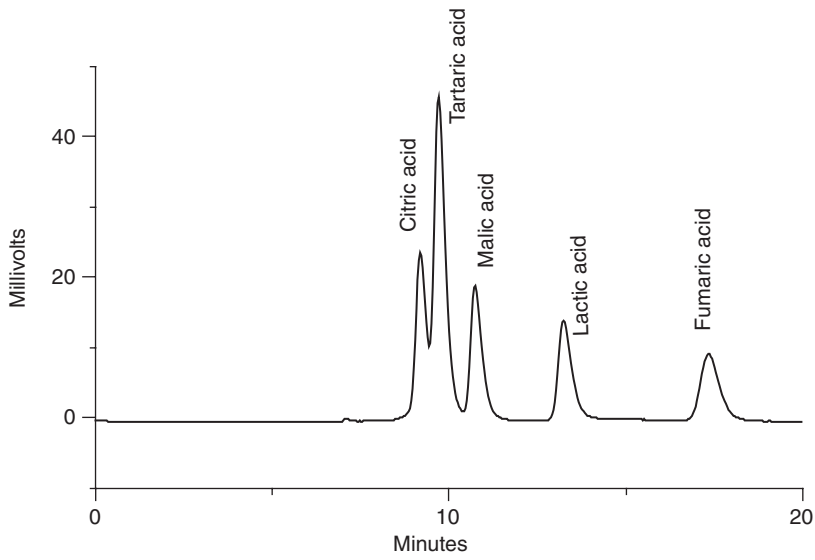

Figure 10.7 HPLC separation of organic acid standard by ion-exclusion chromatography with UV detection. Conditions: column Phenomonex Rezex monos 300 × 8 mm; UV detection at 210 nm; solvents 4 mM H_2SO_4 in Milli-Q water; flow rate 0.6 ml/min.

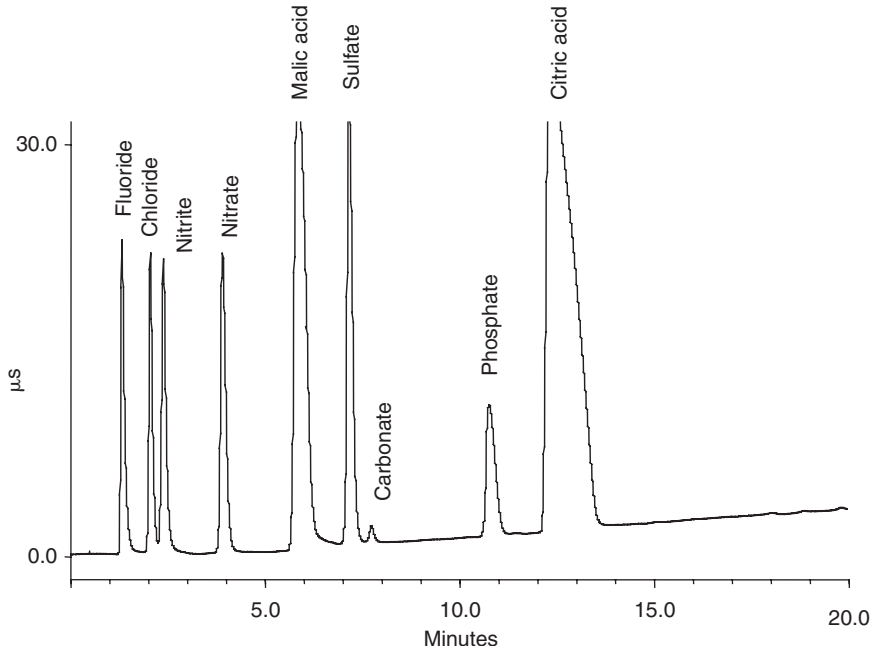

Figure 10.8 HPLC separation of organic acids and anions by ion-exchange chromatography and conductivity detection. Conditions: column Dionex AS-11 250 × 4 mm: suppressed conductivity detection; solvents 18 MΩ water and 0.3 M NaOH; flow rate 1.5 ml/min 2 mM NaOH to 33 mM NaOH over 15 min.

HPLC conditions: column: 2xICE-AS1 300 × 7.9 mm; conductivity detection; solvent = 2 mM octanesulphonic acid in 2% propan-2-ol; flow rate 0.5 ml/min.

If a method similar to Dionex application no. 123 is used, both organic acids and anions can be separated on the AS-11 column (Figure 10.8) (Anon, n.d.e). In fact, care has to be taken with this method as nitrate and malate ions elute very close to each other under these conditions. This can be a particular issue if the column is not operating well and there is a need to determine the level of nitrate in juices which contain high levels of malic acid.

10.7 Carbonation

For carbonated soft drinks the level of carbonation is a key parameter. If the level of carbonation is too low or too high the overall flavour balance of the product will be disrupted. The level of carbonation can be measured in a number of ways and four are discussed here. The first is a manometric method published by AOAC for beer (940.17); the second is also a manometric procedure, but is somewhat less complicated than the AOAC procedure. The final two methods use

specialist equipment: one uses the electric conductance of carbon dioxide gas for the determination and the other method uses ion chromatography.

In the AOAC procedure, a container's cap is punctured with a strong needle. The gas is then shaken out of the product and collected in a gas burette. The pressure is finally adjusted to atmospheric before the volume is read off the burette. Carbon dioxide can also be removed by dissolving it in sodium hydroxide solution; any other gases in the container can also be measured in this way.

The second method is a similar procedure; the cap of the product's container (plastic, glass or can) is punctured with a needle. Carbon dioxide is expelled from solution by vigorous shaking, and the headspace pressure is recorded. From the pressure rise, the volume of carbon dioxide dissolved in the product can be determined. This method is probably one of the most commonly used procedures for measuring carbonation; however, the exact method used varies from one manufacturer to another. One supplier of this type of carbonation tester is Stevenson & Reeves of Edinburgh, which also sells a slide rule to convert pressure rise measurements into carbonation levels. Information about their products can be found on their website (http://www.stevenson-reeves.co.uk).

Caution. *Care has to be taken when measuring the carbonation level of products using this method in glass containers as there is a risk that the container might fracture. To remove this risk, special carbonation testers can be purchased in which the glass bottle is contained inside a shield so that if it does fracture no glass fragments can escape.*

The first of the more sophisticated approaches requires a specialised instrument, the Corning 965 carbon dioxide analyser.

The soft drink is initially cooled to 4°C in a fridge. The container is then opened and an aliquot of concentrated sodium hydroxide (40%) is added to 'quench' the carbon dioxide in the product (typically 10 ml of NaOH is added to 284 ml of product). The carbon dioxide is quenched by reaction with the sodium hydroxide to form bicarbonate and carbonate ions. An aliquot (50 μl) of the quenched product is removed and pipetted into the corning instrument's cell. The cell is closed and the solution acidified to release the carbon dioxide, which is then detected by the change in the thermal conductance of the vapour phase.

This is a rapid procedure and gives reproducible results similar to the pressure rise method and is extensively used in the brewing and soft drinks industries.

The final method uses ion chromatography and shows comparable results to the Corning method (Harms *et al*., 2000). Here the carbon dioxide is again converted to carbonate by the addition of sodium hydroxide. The treated sample is then analysed using HPLC linked with conductivity detection. Harms *et al*., found that the method worked well for both beers and soft drinks and that there were no significant differences between the results produced by this

procedure and the data obtained using the normal Corning method discussed above.

HPLC conditions: column: ICE-AS1 150 × 4 mm; conductivity detection; solvent = 18 MΩ HPLC-grade water; flow rate 0.4 ml/min.

During the carbonation of water, the levels of oxygen and nitrogen need to be reduced to the minimum. This means not only that the overpressure of the carbon dioxide applied to the carbonator can be lower but also that fewer problems are seen with fobbing (rapid out-gassing) during filling. Fobbing causes loss of product when the container leaves the filler head. Oxygen and nitrogen gases are often typically driven out of solution by rapid agitation of the water as it is fed into the carbonator and during carbon dioxide sparging to ensure that the problem is kept to a minimum.

In non-carbonated products, oxygen can also be a problem as it can cause oxidative changes to the flavour and also reduce vitamin retention, particularly of vitamin C. Although headspace oxygen measurements can be made using, gas–liquid chromatography (GLC) this is not a straightforward analysis and requires skilled staff and specialised equipment (Anon, 1997a).

10.8 Miscellaneous additives

Two compounds that are used in some soft drinks formulations for specific purposes are caffeine, used in a range of beverages including colas for its stimulant properties, and quinine, used for its bitter taste. Traditional techniques for the analysis of these two compounds have often involved their extraction from aqueous solution into an organic solvent and then quantification by one of a range of methods.

Over the last 5–10 years, there has been a rapid growth in drinks with certain associated attributes. As highlighted earlier, Red Bull has been a great success based on its energy and stimulant properties. However, there have been a range of other drinks which have focused on different aspects of health, and these often contain green tea extracts, soy extracts and/or extracts of herbs.

10.8.1 Caffeine

Three spectrophotometric procedures are given in the AOAC compendium of methods (960.22, 962.13 and 967.11) for the analysis of caffeine, all of which have an extraction stage followed by a quantification procedure. There is also an HPLC method, discussed earlier, which was designed to measure saccharin, benzoic acid and caffeine at the same time (AOAC, 978.08). Again, the HPLC method, EN 12856:1999 (Anon, 1999a), can be used for the analysis of caffeine, but this analyte was not included in the collaborative study.

In *Pearson's Analysis of Foods* two methods are quoted for the analysis of caffeine. The first is a simple solvent extraction followed by quantification by ultraviolet absorbance at 273 nm (Egan *et al*., 1990d) and the other is a GLC method.

10.8.2 Quinine

The level of quinine in a soft drink can be measured either by determination of the absorbance of an extract of the soft drink after making the product alkaline with ammonia or by detecting it directly spectrophotometrically or fluorimetrically (Egan *et al*., 1990e).

As with the other additives used in soft drinks, caffeine and quinine can be, and often have been, detected using the same HPLC method used for other materials, such as in the method published by Williams (1986). This method separates most of the major additives used in soft drinks in a short time (4–5 min). Although some of the resolutions are not quite baseline, as would be expected in such a short analysis time, and not all of the synthetic colours are separated from each other, this is still a very impressive method.

10.8.3 Taurine

Taurine, or 2-aminoethanesulphonic acid as is it is correctly called using the IUPAC method, is one of the specialised ingredients often included in energy drinks or products that claim to stimulate the mind. It is similar to an amino acid except that the carboxylic acid group is replaced by a sulphonic acid residue. It is involved in a number of functions in the body such as digestion and is thought to be involved in brain development. It can be analysed using the liquid chromatographic method used for amino acids. This involves separation using an ion-exchange column and detection in the visible region after post-column addition of ninhydrin. There is a standardised method for the analysis of amino acids in fruit and vegetable juices (Anon, 1999c) which would be applicable for this type of analysis. However, this approach needs a dedicated machine and is costly to set up if not run on a regular basis.

There are also other pre-column derivatisation methods, such the Waters Pico-Tag (Cohen, 1984) or AccQ-Tag approaches (Anon, 1996a,b). Here the amino acids and taurine are treated with a reagent which gives the derivatised molecules either a UV (Pico-Tag) or fluorescent (AccQ-Tag) chromophore, and these derivatised molecules are separated on special HPLC column designed for the purpose. There is also a new method, recently published by Dionex, which requires no pre- or post-column derivatisation. Here amino acids are separated on an ion-exchange column (PA-10) which happens also to be used for sugars analysis, using a hydroxide ion or hydroxide/acetate gradient (Anon, 2003a).

10.8.4 Fibre analysis

There is a growing interest in the inclusion of soluble fibre into the diet to help improve health. This is extending to drinks as well, with such fibre being added to milk- and fruit-based products such as 'smoothies'. One source of soluble fibre which has attracted attention over the last few years is inulin or oligofructans. Inulin consists of oligosaccharides that are extracted from chicory or Jerusalem artichokes and that are claimed to improve colon function and to have prebiotic properties, enhancing the working of the gut. Inulin is a complex carbohydrate which can be assayed in a number of different ways. However, there are two published methods in the AOAC manual for its analysis (997.08 and 999.03).

In method 997.08, high-pH anion exchange chromatography linked with pulsed amperometric detection (HPAEC-PAD) is used to analyse the product for sugars at various stages of the analysis process. Initially the concentrations of the free sugars in the product are measured. The sugars are then determined after treatment of an extract with an amylase and finally an inulinase. From the levels of the sugars (sucrose, glucose, fructose and galactose if milk is present) found at each stage, it is possible to determine the level of inulin in the product.

Method 999.03 is slightly different and uses two enzymes to hydrolyse any sucrose or starch that the product contains. The glucose and fructose liberated in this step are then reduced with sodium borohydride to their corresponding alcohols. Finally, the borohydride-treated extract is mixed with a fructanase to degrade the inulin, and the free sugars that are liberated in this step are quantified using a colorimetric assay. Once again, from the level of free sugars liberated by the fructanase, the inulin content of the product can be determined.

There are a number of well-documented, validated methods for the analysis of total fibre in food products in the AOAC manual (993.19, 993.23, 991.42, 991.43 and 958.29). Most of these are gravimetric methods where the fibre is precipitated with ethanol and washed and the fibre content calculated after allowances are made for any protein and/or ash that the precipitate contains. These methods rely on precipitation of the fibre with alcohol for their quantification, but as inulin is soluble in mixtures of ethanol and water it cannot be detected in these tests. The most recent method (2001.03) includes an HPLC stage to allow for detection of the presence of maltodextrins, which are poorly digested in the body, but this will not detect inulin.

10.8.5 Herbal drinks

There is a growing interest in the health benefits that can be derived from taking herbal extracts, and this has led to the inclusion of such extracts in soft drinks. Although this is a relatively small and specialist market at present, it is

growing in importance. The analysis of herbal extracts is a very complex issue and outside the scope of this chapter. However, it is incumbent on manufacturers to check the authenticity of the materials they are buying. This can be done in a number of ways. However, as with the authenticity of fruit juices, the analysis is often best left up to specialist laboratories that have a broad range of experience in this field.

There are some useful references in this area. The German Pharmacopoeia (DAB 10) has a large section on the analysis of herbal extracts; many of these analyses use high-performance thin-layer chromatography (HPTLC). Another useful reference is the book edited by Grainger-Bisset (1994) which gives a long list of herbs, the risk of their adulteration, pictures to assist in their authentication and some analytical procedures. There is also a small monograph which details a number of herbal extracts in the British Herbal Pharmacopoeia (Anon, 1996c). There is a growing level of interest in this topic in the United States and an increasing number of HPLC methods are being published in the US Pharmacopoeia.

If it is a challenge to confirm the authenticity of a herbal extract, it is even more of an analytical challenge in a complex finished product that might contain juice and other ingredients. This means that a manufacturer should check incoming raw materials and that they should be delivered with a certificate of analysis from a reputable laboratory that specialises in this area.

10.8.6 Osmolality

Although osmolality is not an additive, it is an important parameter to measure in sports drinks and isotonic products that are being sold for rehydration purposes. Such products require an osmotic pressure similar to serum, which is typically 285–295 mOsm/kg. Osmolality is measured using the colligative properties of solutions, which chemists cover in their first year of physical chemistry at university. These properties are dependent on the numbers of molecules in solution rather than their nature. Osmometers typically use either the depression of freezing point or the elevation of vapour pressure to measure the osmolality of a product. Two manufacturers who supply these types of instruments are Advanced Instruments Inc, Norwood, Massachusetts, USA (http://www.aitests.com) and Wescor Inc, Logan, Utah, USA (http://www.wescor.com). Analysis for osmolality typically requires no sample treatment; simply, a sample is introduced into the instrument and a few minutes later the value is printed out.

10.9 Analysis of colours used in soft drinks

The colours used in soft drinks can be split into two classes. The first class consists of the synthetic food colours that have been used to enhance the

appearance of foods and beverages for many years. They are generally water soluble and include tartrazine, quinoline yellow, sunset yellow and ponceau 4R. However, over the last few years these have become less popular with the consumer because of the bad publicity concerning their possible links with hyperactivity in children. This strong consumer trend led to the reformulation of a wide range of products, particularly orange dilutables, in the United Kingdom in the mid-1980s to remove the azo-dyes such as tartrazine and sunset yellow, and replace them with natural colours or synthetic colours based around the carotenoid molecule, such as annatto, β-carotene and β-apo-8′-carotenal. This switch from the water-soluble colours to β-carotene or β-apo-8′-carotenal caused its own problems as these new formulations showed a tendency to neck ringing, forming unsightly red/orange rings at the top of the container.

Although the anthocyanin and carotenoid pigments are stable at low pH and provide a strong colour, they can in some cases fade due to oxidation. This is a particular limitation with the carotenoid pigments, but is generally overcome by the addition of an antioxidant such as ascorbic acid, which significantly improves their stability.

10.9.1 Assessment of colour

The colour of a soft drink or fruit juice may be assessed in a number of ways. If the product is clear it can simply be carried out by measuring the absorbance of the product at one or more wavelengths. The actual values chosen will depend on the particular colour of the product. For a yellow product, such as apple juice, wavelengths of 465, 430 or 420 nm are often chosen to assess the colour. These values can then be expressed in European brewing convention (EBC) units by multiplication by a factor of 25. The actual Brix value chosen to assess the colour depends on the country; however, levels between 11 and 12 are often taken as the norm. If dealing with a red-coloured product, then the assessment is generally carried out at 520 nm. Absorbance values are sometimes also taken at 420 nm in red or black juices to assess the brownness of the product. The two absorbance values are often used to express a colour ratio, which gives an indication of colour versus brownness:

$$\text{colour ratio} = \text{absorbance at 520 nm/absorbance at 420 nm}$$

Sometimes the absorbance is also assessed at 580 nm and a ratio calculated for the values at 520 and 580 nm, which is called the blue index. With the red/black juices, where the product may have to be diluted, the intensity of the colour is assessed by dilution in an acidic buffer to ensure that the true colour intensity is recorded. If this is not done the colour can be reduced by the shift in pH affecting the apparent colour of the product. The analysis of the colour in juices is detailed in IFU Handbook method No. 78.

In cloudy products, colour assessment is more difficult. The particulate material can be removed by filtration in some cases. However, this does not solve the problem in all cases as some colours, particularly the carotenoids and some anthocyanin pigments, are absorbed on to the particulate material/filters and their removal gives a low colour reading. In addition, the particulate material affects the opacity of the product, which affects the visual perception of the colour. It has been known for a number of years that the simple approach of measurement of a product's absorbance values at 420 or 520 nm does not fully address this issue because some products can have similar absorbance values at these wavelengths but will be perceived visually as very different.

One way in which this has been addressed is by the use of tristimulus colour measurement. In this method the colour is split into three separate primary components, which more closely match the way the eye perceives colour. In this method the absorbance or reflectance of a product is measured at a range of wavelengths in the red, green and blue areas of the spectrum. The values are taken and the components X, Y and Z are calculated. These X, Y and Z values are then transformed to split the colour up into three variables that address the colour or hue (red/green, yellow/blue) of a product, the opacity of the colour (how much light it reflects or absorbs) and finally the depth or intensity of the colour (deep purple/pale purple). This three-dimensional space can be represented by either L, a, b or H, C, L values, which are different conventions used to describe a colour in a conceptually similar manner. In the L, a, b system, used by Hunter Lab, the colour is defined in terms of its lightness (L), which is the extent of black or white it contains (from 0 to 100), and in terms of two variables a and b which define the colour hue and intensity. Red colours are situated in the $+a$ direction; green has $-a$ values. Yellow is in the $+b$ direction and blue has $-b$ values. The value is indicative of the intensity of the colour (i.e. the larger the absolute value, the more intense is the colour). In the CIELAB 76 convention, L again defines the lightness as above, whereas H (hue angle) defines what the colour is (red is situated at 0°, yellow at 90°, green at 180° and blue at 270°) and C (chroma) describes the intensity or depth of colour. This area was reviewed by McKaren (1980).

The different instruments (Hunter, Gardner, Instrumental Colour Systems, etc.) process absorbance and reflectance data in slightly different ways, which means that the values obtained can differ slightly from one instrument manufacturer to another. Therefore, a product's defined colour has to be qualified with a statement indicating the instrument used. Notwithstanding this limitation, it is not uncommon to find a tristimulus 'colour meter' in a manufacturer's quality assurance laboratory so that routine quantitative assessment of a product's colour can be made. This is particularly true for tomato-based products, whose nature makes conventional spectrophotometric assessment meaningless.

The discussion above has addressed the assessment of a product's colour or perceived colour in basic terms. In the next two sections, methods to determine which coloured compounds are present in a product will be addressed. For the purpose of this chapter, the section on synthetic dyes will cover the analysis of the water-soluble dyes, or so-called coal tar dyes, and the section on natural pigments will cover the anthocyanin pigments, such as grape skin extracts, and the carotenoid-based materials, even if they are of synthetic origin.

10.9.2 Synthetic colours

Synthetic colours are materials based around azo-sulphonic acids. They are water soluble and provide strong stable colours. There is an interesting chapter by Wadds (1984) that discusses some of the basic methods, including thin-layer, paper and high-performance liquid chromatography, which have been used in the past and are still used today to analyse for these colours.

When colours are added to a food system, their characterisation is often more difficult due to interferences from other materials in the food or difficulty with their extraction from the food. This is particularly the case for high-protein foods, which bind colours very tightly and can make their quantitative analysis very difficult. However, analysis for azo-dyes in soft drinks is generally straightforward using modern methods. There is less interference than in other food systems and as the colours are already in solution, and not bound to other materials, this makes the analysis easier. In some cases, the colours can be analysed without prior concentration and in others they have to be concentrated by solid-phase extraction methods, for example, C_{18} cartridges followed by elution with a small volume of methanolic ammonia.

Owing to the varied structures of various food dyes, they can often be differentiated from one another by their characteristic ultraviolet/visible absorbance spectra. Using HPLC coupled with a diode array detector (HPLC-DAD) it is possible to collect a compound's absorbance spectrum as it elutes from the HPLC column, which greatly assists in identification. At Reading Scientific Services Ltd (RSSL) this type of detector is routinely used in a range of analyses of such substances as patulin, a mycotoxin found in apple juice, and in the analysis of colours and vitamins, which allows a more certain assignment of a particular peak to a specific compound to be made.

A number of different approaches, all of which have both advantages and shortcomings, have been proposed for the analysis of water-soluble dyes. The separation of colours has been carried out using ion-exchange resins, reverse-phase HPLC coupled with ion-pair reagents and reverse-phase HPLC at low pH where the ionisation of the dyes is suppressed. The last of these is the technique used at RSSL and is also the method recommended by Wadds (1984). It offers the simplest approach to this type of analysis and a typical HPLC profile of

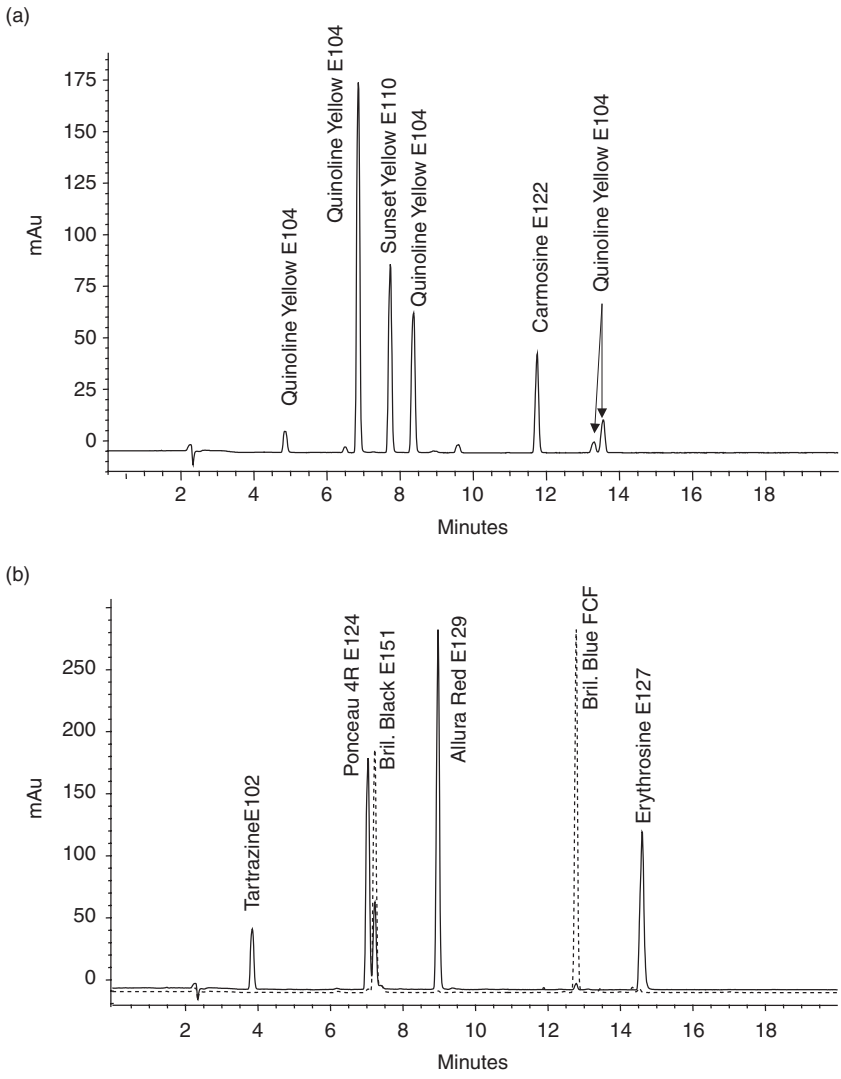

Figure 10.9 HPLC trace of standard pigments using gradient elution system. (a) standard mixture (1) monitored at 415 nm; (b) standard mixture (2) monitored at 490 nm and 590 nm. Conditions: column: 5 μm C18 150 × 4.6 mm, using diode array detection at 450 nm and gradient elution; solvent A = 0.02 M ammonium acetate, Solvent B = acetonitrile; gradient profile: 0 min 95% A, 20 min 50% A, 25 min 95% A; flow rate 1.0 ml/min.

some water-soluble pigments is given in Figure 10.9. This approach allows the identification of pigments and undeclared materials present in a product. However, the quantitative analysis of colours is often more difficult because of the lack of pure standards or pigments of 'known' purity. This area is further

complicated by the fact that some pigments are made up from a mixture of a number of components rather than a single entity: for example, in Figure 10.9(a) several peaks are attributed to a single colour, quinoline yellow, which is made up of five different compounds.

10.9.3 Natural pigments

This section examines the analysis of two major classes of compounds:

(1) anthocyanin-based materials (e.g. grapeskin extracts and highly coloured juices);
(2) carotenoid-based materials (e.g. annatto, β-carotene and β-apo-8′-carotenal).

Whereas the quantitative analysis of the synthetic water-soluble pigments is sometimes difficult, it is often impossible with the natural pigments because of the lack of pure standards or their prohibitive cost. If they are available they can cost several hundred dollars per 100 mg. There is also a large natural variation between one plant extract and another. Quantitative analysis of these natural extracts is further complicated because they generally contain a range of different compounds that make up the 'colour'. A concord grapeskin extract can contain over 30 different monomeric anthocyanin pigments plus some polymeric materials; all of these contribute to the colour but will not be readily quantifiable by HPLC. Quantitative analysis of anthocyanins, however, is often less critical than analysis of the synthetic pigments. In fact, the analysis of anthocyanin pigments is usually carried out to assess the authenticity of red and black fruit juices rather than to quantify the level of added colour. This is changing with the increased interest in the health aspects of polyphenolic compounds such as those seen in grapeskin extracts.

The analysis of the natural carotenoid pigments found in orange juice, for example, is extremely complex and should be left to an expert laboratory. However, the quantitative analysis of added β-carotene and/or β-apo-8′-carotenal to an orange drink or dilutable is much easier as pure standards are commercially available and these compounds can be readily separated using HPLC.

10.9.3.1 Anthocyanin pigments

As extensive research into anthocyanin has been conducted over the years, a large amount of information is available on which pigments are found in a particular fruit. This allows an analyst to assess whether a sample contains the expected pigments and to determine whether there are any added from another source or fruit. A very good reference book which details the anthocyanins found in various plants and also gives details of other phenolic materials found in fruits is Macheix's book on plant phenolics (Macheix *et al.,* 1990).

Most anthocyanin analysis today is carried out using reverse-phase HPLC, often coupled with diode array detection, as this offers the best approach to assess these compounds. The use of reverse-phase HPLC means that predictions can be made about the elution order of compounds from the column, which is based on their polarity and makes the interpretation of the anthocyanin profiles easier. A couple of useful chapters that discuss the analysis of anthocyanin pigments more fully are those of Lea (1988) and Wrolstad *et al*. (1995). Both of these describe more fully the types of methods used and give examples of profiles found in different fruit types. The method used by Wrolstad *et al*., is similar to the procedure that is routinely used at RSSL for this type of analysis.

Another method of analysis that is used around Europe, and given below, was used by Hofsommer in the analysis of red and black juices as part of an EU-sponsored project in the early 1990s on methods to determine the authenticity of fruit juices (Hofsommer, 1994a). This method has been collaboratively tested by the IFU analytical commission and was found to give acceptable results (Anon, 1998b). The procedure does not involve any sample cleanup for juices; the product

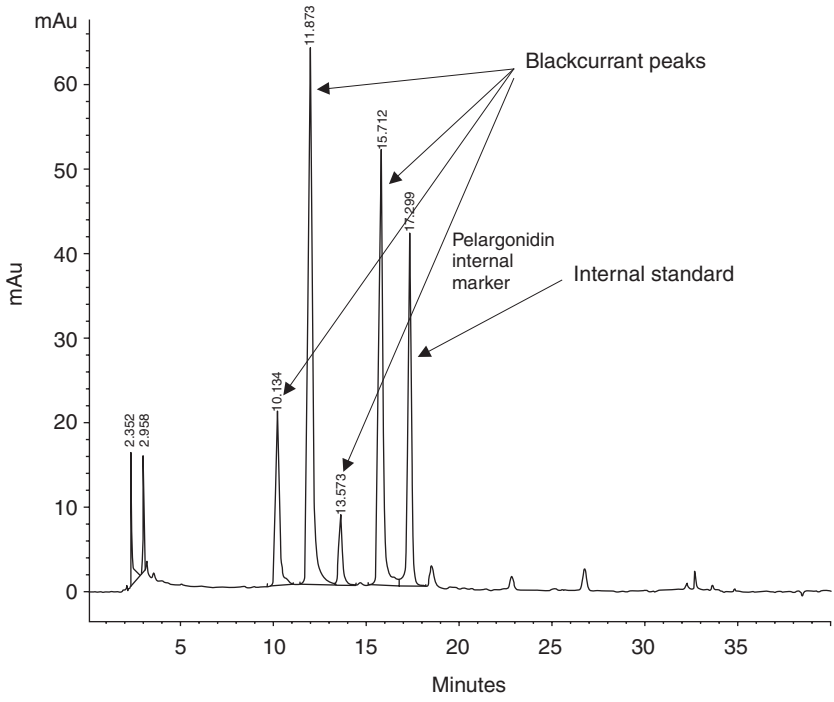

Figure 10.10 HPLC analysis of a blackcurrant juice using the IFU method. Conditions: column: 5 μm ODS2 250 × 4.6 mm; flow rate 1 ml/min; solvent A = 10% formic acid, solvent B = 10% formic acid in 50% acetonitrile and 40% water; gradient 0–1 min 12% B, 1–26 min from 12% B to 30% B, 26–35 min 30% to 100% B, 38 min 100% B, 38–43 min 100% to 12% B; detection at 518 nm using DAD.

is merely filtered and an internal standard added prior to analysis. However, for soft drinks it is often useful to employ a reverse-phase cartridge to concentrate the colours prior to analysis due to their low levels. Two examples of this method for juices are given in Figures 10.10 and 10.11. Figure 10.10 is an HPLC trace for an authentic blackcurrant juice, which shows four peaks for the juice plus the late-running pelargonidin internal marker. Figure 10.11 shows a chromatogram for an adulterated blackcurrant juice that contains elderberry. In this trace there are the same five peaks seen in Figure 10.10, but two others are also present (indicated by an arrow) and the intensity of the third peak of the blackcurrant juice (retention time 13.5 min) is enhanced by the co-elution of a pigment from the elderberry (also indicated by an arrow). This demonstrates the importance of this type of procedure, since adulteration was instantly detectable using this method. However, adulteration is not always as easy as this to detect. The judicious choice of materials can enable adulteration to be hidden. Assessment of the anthocyanin profiles of red and black juices should be part of any routine screening procedure designed to assess the authenticity of these products.

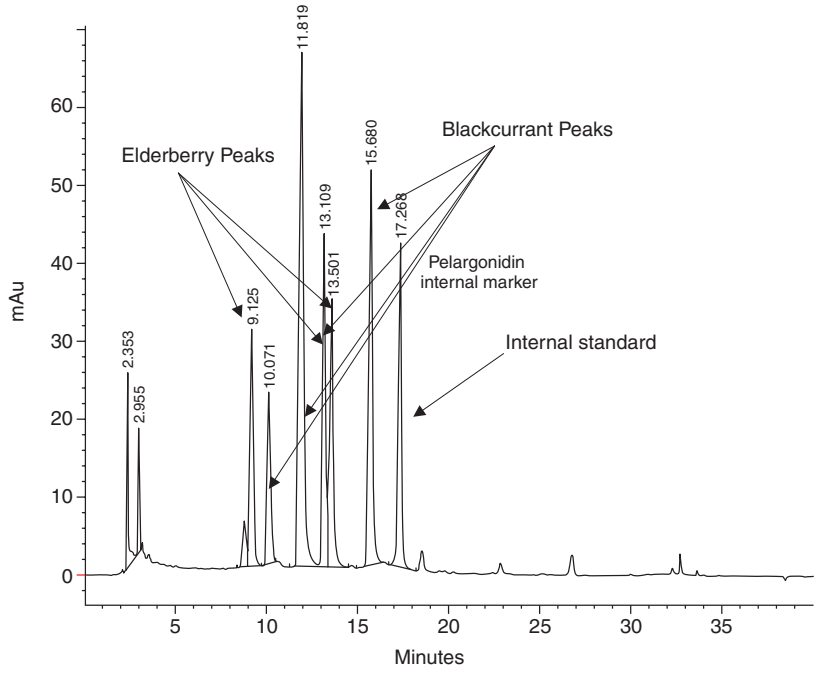

Figure 10.11 HPLC analysis of adulterated blackcurrant juice containing elderberry using the IFU method. Conditions: column: 5 μm ODS2 250 × 4.6 mm; flow rate 1ml/min; solvent A = 10% formic acid, solvent B = 10% formic acid in 50% acetonitrile and 40% water; gradient 0–1 min 12% B, 1–26 min from 12% B to 30% B, 26–35 min 30% to 100% B, 38 min 100% B, 38–43 min 100% to 12% B; detection at 518 nm using DAD.

10.9.3.2 Carotenoid-type materials

For orange-based drinks, either flavoured or containing juice, carotenoid-type materials such as annatto, β-carotene and β-apo-8′-carotenal are often used as colours. Although the analysis of the natural carotenoid pigments found in orange juice is extremely complex because of the very large number of compounds present, the analysis of soft drinks for added carotenoid pigments is often much more straightforward. If β-carotene or β-apo-8′-carotenal is being used it can be extracted either by solvent extraction (ethyl acetate) or by precipitation of the pigments, using Carraz reagents followed by extraction of the pellet material with acetone. Analysis and quantification of these pigments in products with a low juice content is relatively straightforward because there are few interferences. Often a simple gradient or isocratic system using acetone/water, a reverse-phase column (RP18) and detection at 430 nm can be used.

Another pigment that is sometimes used in soft drinks is annatto. This is extracted from *Bixa orellana* and contains a range of apocarotenoid materials based around a C_{24} dicarboxylic acid from the central portion of the carotenoid backbone, as shown in Figure 10.12. This figure also shows the structures of β-carotene and β-apo-8′-carotenal for comparison. After extraction, the extract is subjected to a basic hydrolysis to cleave some of the ester groups, giving the water-soluble 'annatto' colour. This is a mixture of free dicarboxylic acids and esterified materials and its analysis by HPLC gives a number of peaks because the product is a mixture of a range of closely related compounds.

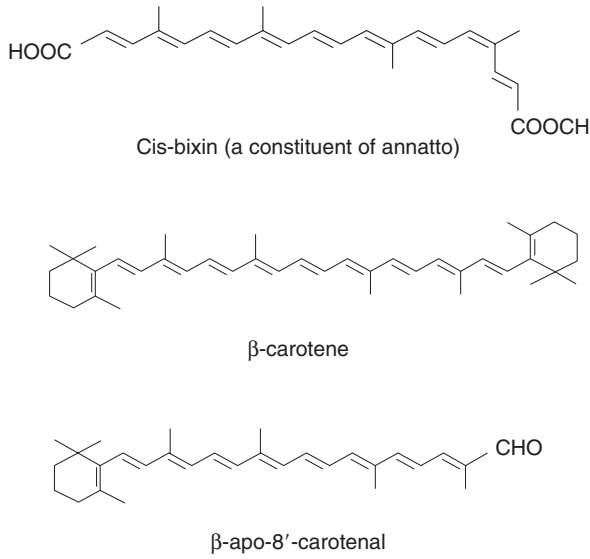

Figure 10.12 Chemical structures for annatto, β-carotene and β-apo-8′-carotenal.

A typical example of an HPLC method used to analyse carotenoids is given below and again comes from work sponsored by the European Union in the early 1990s (Hofsommer, 1994b):

Conditions: column: 5 μm ODS2 250 × 4.6 mm; flow rate 1 ml/min; solvent A = water pH 6.5 (with sulphuric acid), solvent B = methanol, solvent C = acetone; gradient: 0 min 20% A, 25% B and 55% C, 1 min 14% A, 24% B and 62% C, 22 min 7.2% A, 12.8% B and 80% C, 42 min 3% A, 7% B and 90% C, 43 min 100% C, 44 min 100% C, 45 min 20% A, 25% B and 55% C; detection at 430 nm.

As β-carotene also has some vitamin A activity it is sometimes added to products for fortification as well as colourant purposes. If this is the case, the methods outlined above can be used. A more recent reference has used a similar method to look at carotenoid isomers in carrot juices and fortified drinks (Marx *et al*., 2000).

10.10 Vitamin analysis in soft drinks systems

The fortification of some foods has been common practice for years. However, the production of soft drinks containing added vitamins was much less common until about 10 years ago. A few fortified products had been available for a long while, such as Trink 10 in Germany, which is a mixture of ten juices fortified with a range of vitamins, but these were uncommon. Also the fortification of beverages can sometimes offer its own challenges, one being to mask the taste of the added vitamins. However, with the concentration on a healthier lifestyle, fortification is now more common, and the highly successful launch of Sunny Delight, which contains vitamins, in the late 1990s meant that there has been a lot of 'me too' activity. The introduction of the revised Fruit Juice Directive in the United Kingdom in 2003 (Anon, 2003b) has led to the possibility of fruit juices fortified with vitamins and/or minerals, rather than just fortified fruit juice drinks.

If vitamins are added to a product to make a nutritional claim, it is critical that shelf-life studies are undertaken to prove that the overages added are sufficient to ensure that the label claims can be met at the end of product shelf life. This is important as none of the vitamins are fully stable in a soft drink environment and some, for instance vitamin C, are very quickly lost in the presence of oxygen. The addition of the fat-soluble vitamins to a soft drink also offers a formulation challenge to ensure that they are fully dispersed and that there are no problems with neck ringing during storage. Trace metals, particularly the transition elements, can also have a deleterious effect on vitamin shelf life, and sometimes metal scavengers, such as EDTA or phosphate salts, are added to improve the shelf life.

Vitamins are generally assayed in one of two different ways. The first route uses a microbiological technique. Here, a specialist organism is used, the growth of which is dependent on the concentration of the particular vitamin of interest.

These methods are highly sensitive to interferences that limit the growth of the micro-organism and often take rather a long time to perform, as they rely on the growth of micro-organisms under stressed conditions, for example, limited levels of a critical vitamin. However, they do generally offer quite specific methods. Very special skills are required to run these tests effectively.

The second major, and growing, route is the use of HPLC and either UV or fluorescence detection. Here vitamins are extracted from the product and then assayed using HPLC. This is another very specialised area and is often best left to an expert laboratory, as there may be problems with interferences and losses due to such factors as oxidation during extraction.

A few examples of the typical HPLC methods are given below, but if the reader is interested to know more about this area there are two good reviews. One is by Ian Lumley (1993) from LGC in the United Kingdom, who gives good background on the area with information on how the methods have developed. The second is a book which covers all the different types of vitamin analyses using modern chromatographic methods and is edited by Leenheer *et al*. (1992).

It is not uncommon to have to include a hydrolysis step before vitamins are analysed. This can either be to hydrolyse ester groups to liberate the free alcohol, in the case of fat-soluble vitamins, or to remove phosphate groups with some of the B vitamins. There are a large number of references for the analysis of vitamins in foods but few of these were designed specifically for juices or soft drinks. Over the last 5 years, there has been a lot of CEN activity in this area and there are now 10 standard methods published in the British Standards collection for foodstuffs; it is likely that there will soon be more methods published for this type of analysis in foods. The present methods cover vitamins A, B_1, B_2, B_6, C, D, E and K_1.

Although there are a number of citations in the AOAC manual, most of these are once again for foods rather than beverages, but this should not preclude the use of these methods for fortified drinks. There is one reference for vitamin A in fortified milk products (2002.06) and a number of references for a range of vitamins in infant formulations.

10.10.1 Fat-soluble vitamins

If fat-soluble vitamins (A, D and E) are added to a product, the first step in the analysis will often be hydrolysis. In fortified products this converts any esters, for example, vitamin A palmitate or vitamin E acetate, to their free alcohols. The product is then extracted with a hydrophobic solvent, such as hexane or diethyl ethyl or a mixture of these solvents, prior to HPLC analysis often using a reverse-phase (C_{18}) column.

Carotenoids also have vitamin A activity and they can be analysed using the procedures discussed above. Another recent method will separate some of the

carotenoids and tocopherol isomers (Schieber *et al*., 2002) but this is similar to that of Hofsommer described above.

10.10.2 Vitamin B class

There are a number of vitamins in this case, B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), B_6 (pyridoxine) and B_{12} (cyanocobalamine), and their analysis is complex. Chromatograms are often displayed by column manufactures showing the resolution of the majority of these vitamins in one run. However, this is generally only for standards or premixes. When the vitamins are added to a finished product at the normal levels, the analysis of B_5 and B_{12} is very difficult/impossible by HPLC. The presence of colours in the beverage can also interfere with the analysis of these materials and give erroneously high results. This is where diode array detection (DAD) is quite critical for analysis using UV or visible detection, so that the analyst is able to confirm the integrity of the peak and check for co-eluting materials.

10.10.3 Vitamin C

This is one vitamin that most laboratories can measure. There are a number of old-fashioned approaches that use 2,6-dichloroindophenol in a titrimetric method such as AOAC 985.33. This works well in some systems but can give rise to false positive results if there are other reducing substances present. It will not detect dehydroascorbic acid (DHA) and so it may well underestimate the actual vitamin C activity if a product contains a significant level of DHA. However, even with for these shortcomings, it is often used as a quick and rough method. In the AOAC there is also a fluorometric method (AOAC 984.26) where ascorbic acid is oxidised to DHA and this is reacted with *o*-phenylenediamine to give a fluorometric compound which can be detected. This is a robust method that has general applicability.

Vitamin C can also be effectively detected in juices and soft drinks after treatment with a reducing agent such as dithiothreitol or cysteine and then quantified using HPLC on either a reverse-phase or an amino-bonded column. This type of approach was adopted in the recently published EU method (Anon, 2003c). Vitamin C is one of the vitamins that should be routinely assayed in a range of juices such as orange, grapefruit and blackcurrant that are all good sources.

Another vitamin which is of interest in fruit juices is folate, which can be found in low, but significant, levels in some juices, such as orange. However, the analysis of natural levels of this vitamin is very difficult and is best performed using microbiological methods.

Although microbiological and HPLC methods are the most commonly used methods for analysis of vitamins, there are some immunological methods

available for the determination of some of these compounds. These are either a standalone enzyme linked immunosorbent assay (ELISA) method such as the method for B_{12}, folate and biotin which is sold by r-Biopharm (http://www.r-biopharm.de) or the use of a dedicated piece of equipment such as the Biacore. This is a sophisticated instrument that permits immunological assays; instead of using changes in the visible absorbance of a solution, detection is by changes to the response of the surface of a sensor chip. At present there are kits available for the analysis of vitamins which are more difficult to assay by normal HPLC methods (e.g. B_{12}, folate and biotin) and recently a method for B_2 has been launched. For more information on this type of assay go to the Biacore website (http://www.Biacore.com).

10.11 Methods used to detect juice adulteration

Over the past 30 years, extensive research has been carried out to find ways to detect the adulteration of fruit juices. The approaches have developed from simple procedures, such as measuring the potassium and nitrogen contents of juices, to the use of highly sophisticated and expensive equipment to detect the most recent approaches that unscrupulous suppliers may be using to extend their products. Such adulteration often involves the substitution of some of the fruit juice solids by sugars derived from beet, cane, corn or inulin, or the addition of cheaper juices or second extracts of the fruit.

The methods used to detect adulteration of juices have been reviewed by a number of authors and two examples are given here (Fry *et al*., 1995; Hammond, 1996). With the changing methods of unscrupulous suppliers it is now common to use a battery of tests to ensure that a product is authentic. Although this is costly, it is the only way to ensure the authenticity of a product and protect company reputations. The array of tests will often include a number of the procedures described above, such as sugar and acid profiles, along with other methods such as stable isotopic and fingerprinting procedures.

A number of multi-component approaches have been used to assess fruit juices such as the German RSK system and the French AFNOR system. However, it is now common across Europe to use the Association of the Industry of Juices and Nectars from fruits and vegetables of the European Union (AIJN) Code of Practice (COP) (Anon, 2004b), which can be viewed at the association's website for a fee (http://www.AIJN.org). In fact it is mandatory in countries that form part of the European Quality Control System (EQCS) to use these guidelines to 'judge' the quality of 'juices' on sale within that market. The EQCS is a voluntary industry self-control scheme which has gradually grown out from the German system and now covers most of the EU states and also a number of the extension members of the Union. Its principles are to ensure that products on sale within the countries are all authentic and that there is fair and even competition between the various companies. In doing this, it also protects the interests of the

consumer and the good and healthy image that fruit juices have in the eyes of the general public. It offers a number of independent, but interlocking, schemes which control the quality of the products in their markets and a network to alert other schemes to any problems that have been detected in their market.

All the compositional guidelines – RSK, AFNOR and AIJN – define ranges for a number of analytes within which a sample's data should fall. These are valuable approaches but they should not be used to the exclusion of other, newer procedures, which are sometimes more sensitive to the more sophisticated approaches that adulterators now have to use to extend their products with any hope that they will not to be detected.

Individual companies can do some of the analyses, including most of those laid down in the AIJN COP, to safeguard their own name, but very few companies have the time or money necessary to carry out all these tests or some of the isotopic procedures. This should not prohibit them from using such procedures as there are contract laboratories around the world that can carry them out. They should also, if possible, use a list of approved juice suppliers whom they have inspected and with whose production methods they are happy. This is expensive but could limit a company's exposure to bad publicity or prosecution if problems occur in the market, such as happened in 1995–6 when a large number of well-known companies were found to have purchased apple juice adulterated with syrups derived from inulin. This was detected using a new capillary gas chromatographic fingerprinting method (IFU recommendation No. 4, Low & Hammond, 1996; Anon, 2004c).

Another way of tackling raw material supplier audits is to subcontract them to an outside body or use Germany's Schutzgemeinschaft (SGF) International Raw Material Assurance (IRMA) programme, details of which can be found on its website (http://www.sgf.org). The IRMA scheme is a system set up some years ago by a wide range of suppliers who have the same aims as the SGF, for example, to produce high-quality products. These suppliers are open to unannounced audits by the IRMA team at any time to ensure that these standards are being met. Although the use of approved suppliers does not exclude the need for a company to test incoming materials, it does limit the risk and means that a lower level of testing is required than would otherwise be necessary.

Fingerprinting methods such as the anthocyanin methods and the Kirksey method for polyphenols (Kirksey *et al*., 1995) offer good ways to check for the addition of other fruits in a product. As the adulterators have become more sophisticated in the approaches that they use to extend juices, there has been a need for more complex methods of analysis. This means that it is now not uncommon to have to use fingerprinting techniques and isotopic methods to detect the most sophisticated forms of adulteration. These sophisticated analytical methods can even involve detection of the isotope ratios within a class of compounds such as sugars (Hammond *et al*., 1998). Using the RSSL ^{13}C-IRIS approach, which was developed with financial support from the UK Food Standards Agency, it was possible to reduce the detection limit for the addition of C_4-derived sugars to juices by about a factor of two.

Sometimes it is even important to look at the internal isotope ratios seen within a molecule such as malic acid in apple juice. Two groups, Isolab in Germany and Eurofins in France, found it was useful to look at the carbon isotope ratios at the C_1 and C_4 positions of malic acid extracted from apple juice. This allowed them to detect the addition of synthetic L-malic acid to apple juice at much lower levels than would be possible by other means (Jamin *et al*., 2000).

However, sometimes simple methods such as that developed by Low in the early 1990s using capillary gas chromatography, which is readily available in well-equipped laboratories around the world, can expose major problems of adulteration (Low & Hammond, 1996). Here a well-established technique, analysis of sugars by capillary gas chromatography, was able to be applied to detect two routes that were known to be used to extend juices, the addition of HFCS and invert syrups. The method also discovered the adulteration of apple juices with another type of sugar syrup that had only been proposed as a possible adulterant.

10.12 Methods used to assess the juice or fruit content of a soft drink

Under the UK Soft Drinks Regulations 1964 certain products had defined minimum juice contents or minimum fruit contents, for example, a fruit crush had to have a minimum juice content of 5% and a comminuted drink had to contain a minimum level of fruit (10%) before dilution. When these regulations were revoked in 1995 the various statutory descriptions such as ‘drink’ and ‘crush’ were removed but the need to define the juice content of a product was not. Under the EU regulations (Anon, 1997b) covering the labelling, presentation and advertising of foodstuffs, the so-called QUID (quantitative ingredient declaration) amendment, manufacturers have to declare on the label of a food the levels of characteristic ingredient(s); for example, in an orange juice drink a manufacturer will have to label the juice content of the product. The regulations allow the manufacturer to list the juice content based on the level of juice in the product formulation. Although this means that the producer can put a calculated value on the label, it will still be up to the regulatory authorities to check that this value is appropriate and they will have to assess the product to determine whether the label is correct.

A similar situation exists in the United States with the Nutrition Labelling and Education Act (NLEA). When this was introduced in 1990, it placed the onus on the manufacturer to label the product with the individual level of the component juices in, say, a 100% juice blend or the quantity of juice in a soft drink claiming to contain fruit juice on the packaging. The conditions for labelling, together with information concerning how the juice content should be assessed, including the minimum Brix level considered acceptable for a range

of single-strength juices, are given in the Code of Federal Regulations (CFR) 21 CFR.101.30 (Anon, 1990).

The estimation of juice or fruit content is a difficult area and a number of approaches have been suggested. These have involved assessing the ash content, levels of potassium ions, phosphorous content, total level of free nitrogen by measuring the formol value, levels of specific amino acids and a range of other components. The simplest of these procedures, the assessment of the total nitrogen content, phosphorous and potassium levels (N, P, K), was proposed by Hulme *et al*. (1965) as a method to assess the fruit content of comminuted orange drinks. This type of approach can also work for other juices where the levels of these components are well documented. Useful references in this area are the RSK values (Anon, 1987b), *McCane and Widdowson's The Composition of Foods* (Holland *et al*., 1991), Souci *et al*. (1981) and the AIJN Code of Practice (Anon, 2004b), which give compositional information on a wide range of juices that can be used to estimate the juice or fruit content of a product.

However, if only a simple approach to establish the juice content of a drink is used, such as assessing the levels of nitrogen, phosphorus and potassium, it is easy for an unscrupulous supplier to circumvent this method of assessment by the addition of potassium and ammonium phosphate salts, which enhances the nominal juice content of the product.

Alternative testing procedures have been suggested by other authors, such as the use of certain amino acids (Ooghe & Kastelijn, 1985) and organic acids (Wallrauch, 1995). However, because of the wide natural variation of all these components, any calculation of juice or fruit content should really be considered only as an estimate rather than an absolute value. The situation is even more complicated for a mixture of juices in a juice blend or soft drink. In this case, the parameters chosen for analysis, often potassium or phosphate, should be fairly constant between the juices within the blend, and if possible components that are characteristic of a particular juice, such as quinic acid in cranberry juice, or isocitric acid in orange, grapefruit or lemon, say, should be analysed. This type of specific marker can be used to assess the overall level of a particular juice in a blend, but not every juice has a unique marker.

10.13 Conclusions

It is essential for soft drinks companies to assess their products on a batch-to-batch basis to ensure that there have not been errors in the syrup formulation, for example. This is possible with HPLC systems that can be used near to the production line and now require less-skilled staff to operate them than was the case 10 years ago. In the future it may be possible to use in-line monitoring of the product using infrared or near-infrared spectroscopy to ensure proper product formulation. In fact, FOSS offers a system at present that can determine a

wide range of parameters – ethanol, pH, volatile acidity, total acidity, levels of tartaric, malic, gluconic, lactic and acetic acids, reducing sugar content, glucose to fructose ratio and density – in wines and musts from one simple measurement (http://www.foss.dk), which is perhaps an indication of things to come.

Fruit juice companies can themselves do a lot of the testing required to assess whether a product is authentic. However, they may not have the expertise necessary to assess the fine detail and an analyst without sufficient breadth of knowledge in this field might miss some subtle deviations in the data for a sample that would be detected by an expert.

When setting up a scheme to control the authenticity and quality of raw materials, a range of tests should be used to assess samples as this will provide the best chance of detecting any problems that exist. This does not mean that every test conceivable should be carried out on every sample; a range of tests should be applied on a programmatic basis and this will give the best chance of detecting any deviations from normal. One of the most critical features in the authenticity area is to know your supplier; if this is the case you are less likely to have problems.

Cautionary note: *A number of the HPLC methods listed in this chapter use phosphate buffers as solvents. It should always be remembered that phosphate salts are very abrasive to piston seals and pistons. If using these systems, always remember to wash out the aqueous pump thoroughly with water at the end of a run. If this operation is not carried out, the piston seal lifetime will be significantly reduced and in some cases, if left too long, pistons can stick and possibly fracture, which will be expensive to repair/replace.*

In the early part of 2004 there was a problem in the UK caused by low levels of bromate in a branded bottled water. This arose from the presence of low levels of bromide in the water that was then disinfected by treatment with ozone. The bromate ions formed were at levels above the EU and EPA limit of 10μg/l for drinking water. The analysis of this anion at trace levels is demanding and should be left to a specialist laboratory. However, Dionex have published four methods that can be used for the analysis of bromate ions in water and the application notes (81, 101, 136 & 149) are available from the Dionex website (http:/www.dionex.com/)

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11 Microbiology of soft drinks and fruit juices

P. Wareing and R.R. Davenport

11.1 Introduction

Soft drinks and fruit juices represent an important market within the food industry. The increasing variety of products being released at a bewildering rate has altered the potential for spoilage problems. Soft drinks are generally nutrient-poor media that are spoiled by relatively few organisms – usually yeasts, and a few acid-tolerant bacteria and fungi. Carbonation shifts the spoilage flora to those organisms tolerant of carbon dioxide. Soft drinks enhanced by the addition of low levels of fruit juice tend to exhibit similar spoilage flora to fruit juices. The use of ever more exotic raw ingredients may lead to the discovery of unusual spoilage organisms in the future. Yeasts in general, and *Zygosaccharomyces bailii* in particular, remain the key spoilage organisms because of their overall physiology and resistance to organic acid preservatives (Stratford *et al.*, 2000).

Microbial problems within soft drinks and fruit juices can be divided into two groups:

(1) growth in, and deterioration of, the product by general organisms to produce spoilage;
(2) growth in, or contamination of, the product by pathogens to produce food poisoning.

There have been relatively few instances of food poisoning in fruit juices or soft drinks, but microbial spoilage is very common. The previous edition of this book contained an excellent review and practical guide to the identification of spoilage problems in the soft drink industry by the late Professor Davenport (1998). The present review will therefore keep key parts of the previous text, enhancing it with recent data, and simplifying some of the information. For example, some new information on acid-tolerant bacteria such as *Alicyclobacillus acidoterrestris*, and data on the pathogens *Escherichia coli* O157:H7 and *Salmonella* spp. within fruit juices, merit examination, as do some novel processing methods.

11.2 Composition of soft drinks and fruit juices in relation to spoilage

There is a bewildering variety of soft drink and fruit juices for sale, and many methods for their manufacture. Soft drinks can be non-carbonated, carbonated,

with or without added fruit juice, often with the addition of organic acid preservatives. They can be filled on standard or clean fill lines. Fruit juices, fruit juice concentrates and fruit nectars may be fresh, unpasteurised and clean filled, or pasteurised, then hot, aseptic, or clean filled (Stratford *et al.*, 2000; Stratford and James, 2003). Recent technology using ultra-high pressure has been used to produce 'cold pasteurised' fruit juices. These have the advantage of a fresh juice mouthfeel, but with destruction of pathogens and the majority of spoilage agents, enhancing the shelf life of an essentially fresh product (Mermelstein, 1999; Zook *et al.*, 1999).

Simple soft drinks such as orangeade and lemonade are too acidic for the growth of most organisms, so that spoilage is generally by carbonation-resistant species such as *Dekkera anomala* (Stratford and James, 2003). Yeasts usually require a carbon source such as a hexose sugar, a nitrogen source such as amino acids or ammonium salts, simple salts (phosphate, sulphate, potassium and magnesium ions), trace minerals and vitamins. Some yeasts have particular sugar requirements; for example, *Z.bailii* and *Z.rouxii* cannot utilise sucrose (Pitt & Hocking, 1997; Stratford *et al*., 2000).

Sugars have a protective effect on the heat resistance of yeasts and bacteria; this is an important consideration at higher concentrations of sugar. Soft drinks are often nitrogen poor and thus the addition of fruit juice greatly enhances the potential for spoilage. Some yeasts, for example *Dekkera bruxellensis*, can use nitrate. Phosphate levels are often low, trace minerals satisfactory, particularly in hard water areas. The low pH value of soft drinks and fruit juices, pH 2.5–3.8 (Table 11.1), inhibits most bacteria, but leaves yeasts unaffected. In soft drinks

Table 11.1 Examples of fruit and vegetable juice pH and risk organisms

	Approximate pH ranges	Risk organisms
Fruits		
Apples	2.9–3.91	Yeasts
Grapes	3.20–4.51	Yeasts
Oranges	3.20–4.3	Yeasts
Raspberries	3.12	Yeasts
Blackcurrants	2.48–3.60	Yeasts
Pineapples	3.3–3.7	Yeasts and bacteria
Mangoes	3.95–4.50	Yeasts and bacteria
Tomatoes	3.80–4.80	Yeasts, bacteria and moulds/bacteria
Vegetables		
Carrot	4.90–6.44	Bacteria
Celery	5.7–6.1	Bacteria
Cabbage	5.4–6.0	Bacteria
Pea	6.65–6.77	Bacteria