Chemistry and Technology of Flavors and Fragrances

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Edited by

David J. Rowe
De Monchy Aromatics Ltd
Poole, UK

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Contributors

David Baines	22 Elizabeth Close, Thornbury, Bristol, BS12 2YN, NJ
Neil C. Da Costa	International Flavors and Fragrances Inc., 1515 Highway 36, Union Beach, NJ 07735
Mark L. Dewis	International Flavours & Fragrances Inc., 1515 Highway 36, Union Beach, NJ 07735
Sanja Eri	International Flavors and Fragrances Inc., 1515 Highway 36, Union Beach, NJ 07735
John Grigor	Chemical and Biological Sciences, University of Teesside, Middlesbrough, TS1 3BA
Stephen J. Herman	AFF International, 130 Clinton Road, Fairfield, NJ 07004, USA
Simon B. Jameson	Oxford Chemicals Limited, North Gare, Seaton Carew, Hartlepool, TS25 2DT
Jack Knights	3 Cottage Gardens, Great Billing, Northampton, N3 9YW, UK
Philip Kraft	Givaudan Schweiz AG, Fragrance Research, Überlandstrasse 138, CH-8600 Dübendorf, Switzerland
John Margetts	Ungerer Limited, Sealand Road, Chester, CH1 4LP, UK
Liam O'Hare	Chemical and Biological Sciences, University of Teesside, Middlesbrough, TS1 3BA
David J. Rowe	De Monchy Aromatics Ltd, Blackhill Road, Holton Heath Industrial Park, Holton Heath, Poole, Dorset, BH16 6LS, UK
Luca Turin	Flexitral, Inc., 4313 Walney Rd, Chantilly, VA 20151, USA
Chris Winkel	Quest International, Huizerstraatweg 28, 1411 GP Naarden, The Netherlands
Michael Zviely	Frutarom Ltd, 25 HaShaish Street, Haifa, Israel 26110

Preface

The aim of this book, as I expressed in a letter to the contributors, is to have a work that could be given to a new chemist or technologist joining the industry with the statement 'read this and you'll know what this industry is about'. Several contributors corrected this to '. . . a little of what this industry is about!' Such comments, and those of the anonymous referees whose remarks were passed on to me, helped to crystallise my thinking on the concepts behind the book. One referee referred to Aroma Chemicals as the core of the book; this is true, and as an organic chemist I make no apologies for this. In this context, the structure could be described as Part I: The origins and identification of the important aroma chemicals of nature, Part II: The chemistry and synthesis of aroma chemicals and Part III: The applications of aroma chemicals. This 'chemical' approach is also aimed at demystifying the industry to a neophyte; whilst no-one would wish to deny the essential creativity of perfumers and flavourists, it is also important to realise that, to quote Steve Herman in his Chapter 'Applications II: Fragrance', '. . . the fundamental key to dealing with the technical aspects of fragrance is to treat them as mixtures of chemicals'.

The content of the book, in general terms, was my decision, and hence any complaints should be addressed to me rather than, the authors! A book that would please everyone would run into many volumes, and it was necessary to be focused, even at the risk of omitting topics that others might see as crucial. The omission of essential oils was a conscious decision, as these have been extensively covered elsewhere; the exception is as part of the chapter on natural aroma chemicals where these are isolated from essential oils. With this in mind, each author was asked to ensure that the key essentials, as they saw it, were covered. However, I suggested, where appropriate, a section of the chapter could be devoted to an area, that, whilst firmly within the remit of the chapter, was of especial interest to the author; a 'hot topic', if you will. Beyond that, the choice of the material within a chapter was at the discretion of the authors. Ultimately, however, an editor is responsible for his or her book, and hence the sins of omission and commission are mine, and mine alone.

There are those within the industry who treat the two F*s* of the Flavour and Fragrance industry as substantially different topics. Obviously there *are* differences; the specific functions of the perfumer and the flavourist are different, and of course, their palettes, as it were, are different. The markets for a musky flavour or a garlic perfume are somewhat limited! However, both areas ultimately have the same aim; to use aroma chemicals, whether as naturally occurring mixtures or as individual compounds, to create an effect that our chemical senses, taste and smell, find desirable. In this context, the aim of this book is to overcome The Great Divide, or at worst, to ignore it!

Thanks go to the many people with whom I have worked over the past dozen years. Chemistry is a 'social' discipline, and I have learned from many people; I hope that, in a way, this book is giving something back. A particular mention goes to Mark Dewis, Simon Jameson, John Heffernan, Kevin Auty, Peter Setchell, Lee Morgan (the co-designer of 'The devil's flavour wheel'), Gareth Jones, John Johnson, Tracy Brown, Peter Cannon, Rob Gregory, and others who I have now offended by not mentioning, and finally to Duncan Mullis, who first employed me in the Flavour and Fragrance Industry; in a way, Duncan, this book is your fault!

Chapter 1

Introduction

David J. Rowe

> The earliest recognisable chemists were women, the perfume-makers of Babylon, who used the earliest known stills to produce their wares. The first individual chemist known to history was "Tapputi, the perfume-maker", who was mentioned on a cuneiform tablet from the second millennium BCE in Mesopotamia
>
> Paul Strathern, *Mendeleev's Dream*, Penguin Books, London, UK, 2001

Thus, it is not an exaggeration to say that the roots of modern chemistry lie in the flavour and fragrance industry! As an area of the modern chemical industry, it is low profile compared with the 'big boys' of pharmaceuticals and petrochemicals, the areas that the 'general public' recognise as industrial chemistry. Yet it is a multi-billion dollar, global industry that impacts on everyone's life in the developed world.

1.1 History: in the beginning

In a sense the origins of the industry lie in prehistory. At some point our ancestors found there was pleasure in the aroma of a flower, and that mixing certain herbs with food added relish; when this took place will never be known, but it could be said that at that point we became truly human. Certainly our earliest ancestors had uses for aromatic plants; since burial sites have been well preserved, we know that they used aromatic plants as votive offerings to the gods and the supernatural world. By the time that writing developed in the fertile crescent of the Indus, Nile and Tigris valleys, we have many references to the use of herbs and spices. A crucial point is that the uses of flavourings and fragrances, in whatever form, are a feature of a society where at least one social stratum has the 'disposable income' to obtain them; however important they may be to the quality of life, flavours and perfumes are not (despite the efforts of marketing departments to convince us to the contrary) essential to the continuance of life. Thus throughout history we can see the uses of flavours or fragrances as reflecting either the success of a society, or its decadence, depending on the prejudices of the individual. David Pybus [1] and Paul Jose Teisseire [2] have written excellent and amusing accounts of the development of the uses of fragrance, and the interested reader is directed there for more detailed accounts.

1.2 The classical world

The classical world of ancient Greece and Rome was familiar with many oils, spices and perfumes. Some of the key techniques common to the flavour and fragrance industry and the wider chemical industry had been developed, in particular distillation and the concept of extraction. The key concept of separation of materials was introduced to the world. Philosophers such as Aristotle were able to introduce some of the ideas which we might recognise today as science. With the rise of the Roman Empire, the resources of the Mediterranean world became available to the privileged few who could live in perfumed luxury surrounded by slaves. Spices from across the empire and beyond were used to flavour food, both for their inherent flavour and to mask the somewhat dubious taste of meat in a pre-refrigeration age. Pliny the Elder wrote a treatise on 'Natural history' which included observations on aromatic plants and their uses. However, throughout this period there was another feature we frequently see in relation to the uses of flavours and fragrance; for some, such as Pliny, they are an 'honest pleasure', whereas for others they represent decadence and vanity. Those in the latter camp were presumably tutting and saying 'told you so' when, as David Pybus [1] puts it, 'Rome succumbed to the barbarian hordes, the lights went out in all the incense burners in Europe, and the rose petals went out with the bathwater'.

1.3 The mediaeval world

The fall of the Roman Empire in the West ushered in the period commonly known as Western Europe's Dark Ages. The 'one world' of the Roman Empire, stretching from Northern England to the Arabian Peninsula, was broken, and instead we had a period of what might be termed 'localisation', in an analogy to today's globalisation. Petty fiefdoms arose in what were earlier merely provinces of the empire. Nowhere is this clearer than in England, where seven kingdoms, the Heptarchy, arose in what was merely part of a single minor province of the Roman Empire. The end result of this was the loss of raw materials and eventually, the knowledge of what to do with them. The foundations of chemistry were lost into the mysticism of alchemy. Some techniques remained in use, especially distillation, but this also became a mystical affair. The classical world used distillation to refine a crude material into a more valuable one, such as an aromatic plant into an essential oil, a basic concept that we are still familiar with today. In the mediaeval mind this became the search for the Philosopher's Stone, to refine base metal into gold or to confer eternal life. Herbs were still used for flavours, but the breakdown of transport meant that these were restricted to what could be grown in local gardens, the 'parsley, sage, rosemary and thyme' of the English folk song. In parallel with this, the powerful mediaeval church frowned on the use of fragrance as earthly vanity. Cleanliness was not next to godliness, rather 'insanitary' was next to 'holy', from the hovels of the early monks to Thomas Beckett's lice-infested hair shirt. Of course, official disapproval has never prevented those with the finances from doing as they will. Spices and oils still made their way into darkened Europe from the Orient and the Arab world. The latter, in particular, kept some of the flame of the classical world burning, which is reflected in the number of words whose origin is Arabic, including alcohol and chemistry itself. The great trading cities of Venice and Genoa controlled much of this trade, with the Silk Road to the East carrying precious spices and oils via Constantinople, the capital of Byzantium, the Eastern Roman Empire. But disapproval there still was; one of the many things held against England's King John, a man held to be so awful that no other monarch has taken the name in 800 years, was his insufferable vanity – he was said to bathe every month, whether he needed to or not.

1.4 From the Renaissance to the Enlightment

In 1453 Constantinople fell to the Ottoman Turks. The end of the Byzantine Roman Empire, which for a millenium had been the great junction between East and West, had a major impact on trade routes to India and the Orient, the source of the most prized spices. This drove adventurers, especially from the great trading cities of Genoa and Venice, to seek alternative routes to the East. In brief, flavours and fragrances were a major driving force in the European discovery of the Americas (these had been discovered before, of course, by the people who actually lived there, but as they didn't wear trousers, they didn't count). This exposure to new cultures, together with the rediscovery of classical science, often via Arabic texts, led to a new intellectual flowering in Europe. The concepts of science, based on the principles of observation and measurement, were laid down by Galileo. In this context, perhaps the single most important development for the flavour and fragrance industry was the development of the thermometer by Celsius and Fahrenheit. The essentials of modern chemistry were established by the studies of Lavoiser, Davy, Dalton, Priestly, Scheele and others, and the mystical systems the alchemists left behind. It is difficult to overestimate the importance of this change. Though the early chemical laboratory would have looked like an alchemist's workshop with its pots, pans and alembics, chemistry was based on causality; mixing sulfuric acid with sodium chloride always generates hydrogen chloride (or, to use the terminology of the day, mixing oil of vitriol with salt always generates muriatic acid gas) – irrespective of what the operator is wearing, has eaten or has chanted during the process. The difference between alchemy and chemistry is that between astrology and astronomy. Of course, progress is rarely straightforward (witness the number of astrologers in the world); Sir Isaac Newton (1642–1727) believed that his most important work was not on gravity and the calculus but rather his alchemical studies, and in 1794 Antoine Lavoisier found that the Revolution had no need of learned men.

1.5 The industrial age

From the early nineteenth century developments in chemistry, mostly in Great Britain and Germany, began to create the flavour and fragrance industry as we know it. Purification of natural materials, especially essential oils, led to the identification of aroma-active materials such as benzaldehyde, cinnamaldehyde and vanillin. The increased knowledge of organic chemistry, beginning perhaps with the discovery of Wöhler that 'inorganic' and 'organic' materials could be interconverted (i.e. that there was no 'vital force' responsible for creating the complexities of organic materials), meant that these isolated materials could now be synthesised in the laboratory. The great cycle of the chemical industry – identification, laboratory synthesis, large-scale synthesis and commercialisation with falling costs and prices – had now begun. At this time the events and discoveries of importance come so thick and fast that the best way to show them is the timeline (Figure 1.1).

1820	Foundation of Roure by Claude Roure in Grasse under the company name Roure Bertrand
1834	Isolation of cinnamaldehyde
1837	Isolation of benzaldehyde
1852	Cinnamaldehyde synthesised
1852	W.J. Bush established
1858	Microbial resolution of (−)-tartaric acid from a racemic mixture using *Penicillin glaucum*
1859	Synthesis of methyl salicylate
1859	Vanillin purified
1860	Foundation of Fritzsche Brothers
1868	Synthesis of coumarin
1870	Foundation of Haarmann & Reimer
1871	Structure of vanillin determined
1872	Vanillin synthesised
1873	Roure wins its first gold medal at the Vienna International Exposition
1878	Synthesis of cinnamic acid
1879	Saccharin is discovered
1880	Lactic acid, the first known optically active compound to be produced by industrial fermentation
1883	Synthesis of phenylacetaldehdye
1884	Synthesis of cinnamaldehdye
1886	*Bacterium zylinum* identified as cause of vinegar production
1886	Nitromusks discovered
1887	Chirality and taste; enantiomers of asparigine found to have different tastes
1888	First determination of an odour threshold. Ethyl mercaptan by Fischer & Penzoldt
1889	Polak & Schwarz's founded
1889	'Jicky' by Houbigant
1891	Synthesis of the ionones
1892	Allyl disulfide identified in garlic
1894	Fischer's 'Lock & Key' Principle
1896	Foundation of Givaudan by Leon and Xavier Givaudan in Zurich
1898	Foundation of Chuit & Naef by Philippe Chuit & Martin Naef in Geneva
1900	Saccharin first used as a sweetener
1908	MSG identified
1912	Maillard reaction described
1913	Foundation of Fries & Fries in Cincinnati, Ohio by Robert G. Fries and his brother George
1914	Foundation of Polak's Frutal Works (PFW) in the Netherlands by Joseph, Jacob and Henri Polak
1918	A.L. van Ameringen formed
1919	Dragoco founded by Carl-Wilhelm Gerberding
1920	Ruzicka introduces the concept of odourant design
1921	'Chanel No. 5' by Coco Chanel
1922	Takasago founded
1926	Furfuryl mercaptan identified in coffee
1931	Amadori products recognised in the Maillard reaction
1933	Naef, Chuit et Cie. becomes Firmenich & Cie
1937	Non-nutritive sweetener cyclamate discovered
1939	Ruzicka wins Nobel Prize for chemistry
1946	Pauling theorises on the influence on odour of molecular shape and size
1950	Polycyclic musks introduced
1952	GC introduced by James & Martin
1952	Hodge scheme for the Maillard reaction
1954	Roure opens its perfumery school in Grasse
1959	International Flavors & Fragrances formed from van Ameringen-Haebler inc. and Polak & Schwarz NV
1964	GC-Olfactometry introduced
1965	BBA formed from WJ Bush & Co Limited, A. Boake Roberts & Co Limited and Stafford Allen & Sons Limited
1968	Amoore's description of the molecular basis of odour
1969	Cyclamate removed from GRAS list by FDA
1969	Hydroxydimethylfuranone identified in cooked beef
1970	Cyclamate banned in the USA
1970	Damascones identified in rose oil
1972	Sacharin removed from GRAS list by FDA
1981	Aspartame gains FDA approval
1982	Grapefruit mercaptan identified
1989	Solid phase micro-extraction (SPME) introduced
1991	Merger of Givaudan and Roure
1998	Sucralose approved by FDA
2000	Spin off of Givaudan Roure from Roche as Givaudan with a listing on the Swiss Stock Exchange
2001	Bush Boake Allen acquired by IFF

Figure 1.1 Discoveries and foundations from the beginning of the Industrial Age to today.

1.6 The post-war world

> Things are different today, I hear every mother say,
> Cooking fresh food for her husband's just a drag
> So she buys an instant cake and she burns a frozen steak...
>
> The Rolling Stones, *Mother's Little Helper*, 1966

Since the end of Second World War the flavour and fragrance industry has expanded greatly. Several interlocking factors have caused this.

1.6.1 Technical factors

1.6.1.1 Analytical

Development of analytical techniques such as Fourier transform nuclear magnetic resonance spectroscopy (NMR), infra-red spectroscopy and, in particular, gas chromatography (GC) and mass spectrometry (MS) has enabled the key odorants of fragrant flowers and foodstuffs to be identified at ever lower levels; the so-called hyphenated techniques of GC-MS and GC-olfactometry, in conjunction with 'trapping' methods such as GC trapping and solid phase microextraction (SPME), means that we can now identify materials present well below the part per billion level and at quantities down to the picogram level. This means that the trace components, which are often the character impact chemicals of a material, can now be identified. Perhaps someone should calculate how many cows would have to be distilled to prepare sufficient 2-methyl-3-furanthiol, the character impact chemical in roast beef, to be identified by the classical techniques of the nineteenth century.

1.6.1.2 Synthetic

The increase in the 'armoury' of synthetic techniques available to organic chemists has made available to the perfumer or flavourist an enormous number of materials. In particular, it has enabled materials of ever-greater complexity to be available to the applications specialists at a price that, even if the cost per kilo is high, still adds value to the end formulation. The cycle of identifying a potentially valuable material, synthesising it in the laboratory and then developing a commercially viable process (at gradually decreasing cost and sales price) has gone on since the nineteenth century, but the cycle time in today's commercial culture is much lesser.

1.6.2 Social factors

After the end of Second World War, and especially from the 1960s onwards, the developed world, especially North America, western Europe and Japan, has seen a major increase in the standard of living. This has given the 'disposable income' that we have seen is a key factor in the uses of flavours and fragrances. On top of this these regions have seen an unprecedented increase in the social and geographical mobility of their populations.

This has meant that a wide range of people have been exposed to a wider range of tastes and aroma than previous generations, and this has created a market that the industry has expanded to fill; of course, the industry has been a factor in exposing people to these factors anyway. In short, the public palette, if not more sophisticated, is certainly wider. Much of this change has taken place in the author's (born 1958) lifetime. For example, the simple ersatz flavours of the author's childhood, where soft drinks were defined by their colour rather than their flavour ('raspberry cream soda' was 'red pop' as it did not remotely resemble raspberries; no one could work out what 'green pop' was meant to taste of), and potato crisps (= potato chips for Americans and other non-English speakers) were 'ready salted', or for special gourmet occasions, 'cheese and onion'; 'salt and vinegar' flavour was introduced with great fanfare at some point in the 1960s. We can see this in our potato crisp timeline (Figure 1.2).

The other great change, reflected in the words of Dr Jagger and Professor Richards quoted above, is that 'time is money'; much more prepared and processed food is now eaten than ever before. Again, it is no longer acceptable for this to be the peacetime equivalent of powdered egg and coffee made from acorns. People want, and are willing to pay for, high-quality prepared food, as good as, or better than, they could make themselves if they spent time on it. We can see this in the range of ready-meals and cooking sources now available. Health factors have also played a part; vegetarianism is no longer considered a 'cranky' lifestyle and, as more people have opted for this approach, there has been a rise in the market for vegetarian food that does not taste vegetarian. Hence there is a major demand for savoury flavours for vegetable protein such as soya and mycoprotein.

A similar shift has taken place in the market for fragrances. In the 1960s personal care fragrances were limited to women's perfume and the annual bottle of after-shave that

Prehistory		
1960	Ready-salted cheese and onion	**Then:** Bag of ready-salted and a bottle of red pop
1970	Salt and vinegar	
1980	Smoky bacon Roast chicken Prawn cocktail Roast ox	
1990	Roast beef and mustard	
2000	Sea salt and balsamic vinegar Salsa and mesquite	**Now:** Pack of Monterey Jack kettle chips and a mango and passionfruit smoothie

Figure 1.2 The potato crisp story.

all children bought their father for Christmas. Most household items such as washing powder, detergents and bleach were unfragranced. By the start of the new millennium the situation is reversed; almost everything that *can* be fragranced *is* fragranced, with unfragranced materials carrying a premium price for the specialist market. In personal care, the use of fragrances by both sexes is now the norm; the taboo of 'men wearing perfume' was broken in the 1970s' Britain by an advertising campaign for after-shave where sporting figures such as the boxer Henry Cooper, whose masculinity could not be questioned (at least by anyone not wishing to have their face rearranged), exhorted us to 'splash it all over'. Fragrance is now all around us, from air-fresheners in our cars (though not in mine as it helps to cause my motion sickness) to the bleach in the toilets. A cynic might perhaps suggest we have become afraid of smelling human.

1.7 The future

Does the flavour and fragrance industry have a future? It would be surprising if the editor of a book on flavour and fragrance chemistry did not say yes! Perhaps I should back that up by saying we have used flavours and fragrances for at least five millennia, so we are not likely to stop now. Synthetic chemistry is still developing new methodologies, so materials that are at present interesting, but too expensive to use, may become available at an 'accessible' price due to the discovery of a commercially viable synthetic route. Similarly, analytical work on the examination of new 'exotic' materials (such as IFF's 'Living Flower®' work and Givaudan's TasteTrek™ project) may lead to the identification of exciting new compounds as targets for organic chemists. The other key factor is the development of the global economy, and especially that of the developing world. At present the majority of the world's population has no 'disposable income'; returning to the theme of the market for flavours and fragrance as a mirror of the affluence of a society, we can only hope that these billions can eventually share the living standards of the developed world and, in so doing, will open a market for the industry, even larger than the one it has at present.

1.8 The structure of the flavour and fragrance industry

At the time of writing, the industry is in a state of flux, similar to that in the pharmaceutical industry in the 1990s, and to an extent, any comments I make here may be out-of-date by the time this is published. There have been a number of mergers and acquisitions, which have taken out 'names' that would once have been considered inviolate, most notably the takeover of Bush Boake Allen by International Flavours and Fragrances, and the merger of Haarmann & Reimer with Dragoco to form Symrise. Somehow it is sad to lose the names of Haarmann & Reimer, whose work on Vanillin was such a landmark in the industry. In terms of value, the industry has an estimated value of $15 billion, with an approximately even split between flavours and fragrances. The top five companies, Givaudan, IFF, Firmenich, Symrise and Quest International each have a turnover in excess of $1 billion. Most interesting is the geographical distribution of sales: North America 32%, Asia-Pacific 26% and western Europe 25%, with 'the rest', eastern Europe, South America, the Middle East and Africa, constituting less than 20%. Here we can see again the principle that the flavour and fragrance industry is a consequence and a measure of 'disposable income'; the developed world accounts for 80% of the sales. Since this is necessarily a changing set of figures, readers are directed to the Leffingwell website (www.leffingwell.com) from which these figures were taken in June 2003. Rather than listing further figures and companies here, we can perhaps take a different approach in terms of breaking down the industry. There are four key groupings:

(1) The Big Players, e.g. IFF, Givaudan, Takasago, Firmenich. These are multi-national companies active in both flavours and fragrances. Apart from formulating the final fragrance and flavour they also manufacture aroma chemicals, which they may sell on the open market or keep as 'captive' for internal use only.

(2) The large consumer groups, which may be further subdivided into:

    (a) food groups, e.g. Kerry, Danisco, and Nestle.
    (b) household and consumer goods, e.g. Proctor & Gamble, Unilever, L'Oreal.

These are unlikely to manufacture aroma chemicals, but have active flavourist and perfumer groups, besides utilising flavours and fragrances supplied by the Big Players.

(3) Specialists, e.g. Frutarom, Duckworths, Flavor & Fragrance Specialities. Smaller in total turnover than the big players, these may still exceed them in key areas, e.g. the manufacture of aroma chemicals, domestic fragrances, savoury flavours and others.

(4) 'Mom and Pop Companies' – This common (if faintly derogatory) term covers the large number of companies who, as individuals, are small, but who constitute a large and often neglected area of the industry.

(5) Mainstream chemical companies who manufacture some aroma chemicals as part of their business, e.g. BASF (*cis*-3-hexenol).

Those working in the industry will be an employee of one of these, and will interact with the others as a customer, supplier or both; one peculiarity of the industry is the number of occasions that a customer for one group of materials is a competitor in another area. There is a tendency to 'take in each other's washing'.

1.9 A note on regulations

The flavour and fragrance is subject to a range of regulations, above and beyond those, such as local environmental and safety laws, applied to the chemical industry in general; this is appropriate, given its proximity to the consumer. In this area there is a fundamental divide in the two Fs. In the fragrance area, uses of raw materials and finished products are regulated through two bodies: the Geneva-based International Fragrance Association (IFRA) and the US-founded Research Institute for Fragrance Materials (RIFM). These two organisations, though formally independent of each other, work together closely to ensure the safety of fragrances. The RIFM is responsible for the testing and monitoring of fragrance materials, commissioning and carrying out tests, setting out protocols, and collating data from basic research and consumer studies. If there is concern over a material, for example sensitisation, a particular concern in recent years, the RIFM can review its use. IFRA is the final arbiter, with its Code of Practice representing the 'best practice' in the industry. Steve Meakins has given a good overview of this [3].

In the flavours area the regulations are less international, with more localised legislation. In the European Union there is the so-called white list of approved aroma chemicals and related substances, but the most important world-wide is the US-based Flavor and Essence Manufacturers Association (FEMA). The FEMA GRAS is a list of over 4000 materials that are Generally Recognised As Safe, i.e. they may be used in foodstuffs at set levels, under which circumstances they need not be declared as individual substances; note that flavour materials are not 'additives' or the dreaded E-numbers of EU legislation. The GRAS list is updated on a roughly biannual basis with new materials. In the USA only materials which are on the FEMA GRAS list may be used.

A second concept divides the USA and Europe, and that is the nature identical/natural/artificial definitions. Both regions recognise materials of 'recent biological origin' as natural, but in Europe there is the concept of nature identical, i.e. 'synthetic' ethyl butyrate, made by the reaction of 'synthetic' ethanol (e.g. from ethylene) and 'synthetic' butyric acid (e.g. from the C4 stream from petroleum cracking), is the same as the ethyl butyrate found in nature, and hence the consumer labelling on a product in which this material is used will simply be 'contains flavouring'. In the USA, a pre-Wöhler view seems to hold and such a material is considered to be artificial, i.e. consumer labelling will be 'contains artificial flavouring'. In Europe, the term 'artificial' is reserved for those materials which are not found in nature, and which now are rarely used; in this region, the labelling 'contains artificial flavouring' is a strong negative from the marketing viewpoint. Ironically, since 'contains artificial flavouring' is more commonly seen in the US, it is less 'negative' there, and materials which would be considered artificial in Europe are more widely used in the USA.

A second regulatory area which concerns flavour materials, which reminds us that they are going to be consumed as part of a foodstuff, is that they must conform to dietary regulations. These may be based on faith (Kosher, Halal), health (allergen-free), ethics (vegetarian, vegan) or fashion (GMO-free, 'Organic'). Of these, the longest-established is the Kosher regulations, where for many years Rabbinical bodies have looked at the interaction of Mosaic Law with the modern flavour and chemical industries. Of course, the term Kosher has entered the English language, especially British English, as a general term for the 'real thing', especially as opposed to anything 'hooky', i.e. obtained by dubious and probably illegal means. In this context, it is not surprising that when the author joined the flavour and fragrance industry and saw a file labelled 'Kosher Certificates' he assumed that they were simply certificates of analysis or conformity, i.e. to prove that they were the 'real thing'.

Uwe-Jens Salzer and Kim Jones have given an excellent overview of the complex field of legislation in the flavour industry [4].

1.10 A note on quality

All the materials produced by the flavour and fragrance industry, whether aroma chemicals or finished perfumes and flavours, are ultimately performance chemicals, and the function they perform is to have the correct taste and/or smell. With this in mind, a fundamental quality aspect is their organoleptic properties; above and beyond the chemical specifications of purity, density, refractive index, etc., the material must smell right. Hence the final and defining test which is carried out on a material is an odour assessment; whilst flavour materials may also be tasted (especially final products), the close relationship between taste and smell (see Chapter 9) means that odour is usually used as the test. This assessment is carried out by trained individuals in quality control, usually by smelling the material on a blotter or strip of stiffened paper and by sniffing the material in a bottle or other containers; the latter gives the aroma of the 'head space', the saturated air above the product, which is usually the first impression a customer gets when opening the container of a product. A reference standard of the material is assessed at the same time in the same way in order to get a comparison. The first criterion is that there must be no 'off-notes', i.e. obvious aromas due to different aroma chemicals, either as impurities or contaminants. This is a serious problem, especially for the manufacturers of aroma chemicals, as traces of impurities, well below the level of standard GC detection, may be detectable on odour if the odour threshold of the impurity is lower than that of the desired material; for example a trace of furfuryl mercaptan (odour threshold 0.003 ppb) will dominate the odour of an ester such as ethyl laurate (odour threshold >1000 ppb) even at 0.001%, and will be detected as an off-note well below that. In addition, traces of odorous by-products will have the same effect in creating off-notes. Figure 1.3,

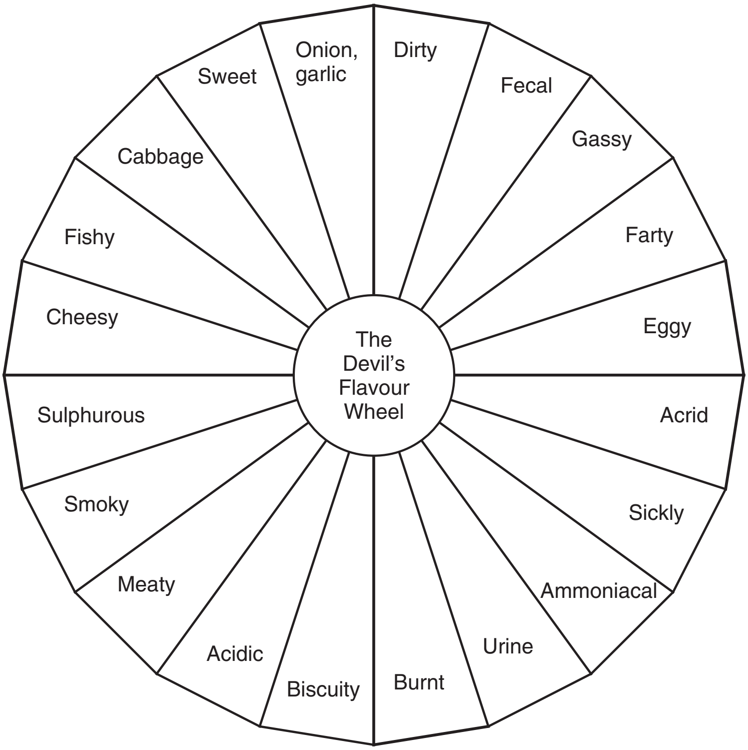

Figure 1.3 The Devil's Flavour Wheel.

'The Devil's Flavour Wheel' [5] summarises just some of the off-notes detected in flavour aroma chemicals.

All this means that cleaning regimes must be rigorous to avoid cross-contamination, and often during a distillation, the odour of individual fractions is assessed and blending carried out accordingly. A second point is that the product must match the reference standard; 'better' does not mean correct in this case. A final point is to refute the claim that odour assessment, as carried out by a trained nose, is 'subjective'. Whilst it can never have the same objectivity as a number coming from a refractometer or from a GC trace, it is as objective as a human assessment can be, and the evidence for the success of this approach is shown in the reliability and consistency that we all expect, and get, from the flavoured and fragranced items that we buy each day.

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Chapter 2
Identification of Aroma Chemicals

Neil C. Da Costa and Sanja Eri

2.1 Introduction

The characterisation of aroma compounds in natural products and foods is still a challenge, despite the sophisticated techniques now available to analysts. Flavour and fragrance compounds are usually present in these matrices at very low concentrations (ppm or even ppb). In addition, they have a wide range of polarities, solubilities, volatilities, and thermal and pH stabilities. The matrices that contain them may be very complex and cause interference with the isolation techniques. Therefore, there is no single, simple method for the identification of aroma compounds. Instead, analysts must first ask themselves what they wish to accomplish with the analysis and then choose an analytical method or combination of methods that they think would work best. Typical analytical goals include obtaining a complete aroma profile of the sample, looking for specific compounds or comparing samples.

In this chapter, a reader will find an overview of the techniques now available to the analyst interested in characterising aroma chemicals. Each technique's practical advantages and disadvantages will also be indicated where relevant. Numerous papers and books have been published on this subject and this overview by no means covers all of them, but it should be a good starting point to a newcomer in the world of flavours and fragrances and a useful reference to those who are already involved in it.

2.2 Isolation of aroma chemicals

To the early organic chemists the only approach available was isolation of pure chemicals and identification by classical means. The development of chromatography, especially gas chromatography (GC), and in particular gas chromatography-mass spectrometry (GC-MS), made identification of compounds in mixtures much easier. However the quality and usefulness of the GC and GC-MS analyses, or any other technique, largely depend upon the method used to prepare the sample. If the sample is concentrated enough it can be injected directly into the GC, such as essential oils or natural extracts. In this case, the only preparation recommended is dilution of the sample with a solvent to obtain better chromatographic results and to reduce the introduction of the contaminants into the GC. Very concentrated aqueous samples can also be injected directly, although the injection volume and split ratio have to be chosen carefully due to the formation of steam in injection port. Problems such as extinguishing the flame ionisation detector (FID), contamination of the mass spectrometer or degradation of non-bonded polar phases may occur.

If, however, the aqueous sample is too dilute or the aroma compounds are contained within a matrix, they need to be isolated. It is beneficial to the analyst to know as much as possible about the matrix (whether it is a fruit, vegetable, flower, thermally processed food, fermentation product, flavour, fragrance, etc.) as well as the aroma chemicals that they expect to find in it, especially the compounds that are the most significant contributors to the aroma. Unfortunately, these are not necessarily the components of highest concentration. Very often, these important components are at trace levels, making isolation and identification more challenging. Once we understand the scope of the problem, we can choose the most appropriate isolation method that will give us the 'truest' extract with minimum loss or distortion of the original aroma and minimal generation of artefacts. Ideally, the isolation method should not discriminate between polar and non-polar compounds, not cause thermal degradation of aroma compounds, oxidation, reduction, pH changes or loss of highly volatile compounds. In addition, if we know that the material we are going to extract has high molecular weight or non-volatile content, we can choose a method that reduces the appearance of these components in the extract, thus reducing the problems of dirty liners, columns and subsequently poor chromatography.

Very often, to achieve the most complete aroma profile, the analyst may have to use more than one extraction technique. For example, headspace techniques obtain a profile of the highly volatile components, which may not have been seen in a liquid extract, as some of them may be too volatile to be captured and others could be obscured by the solvent peak. This kind of approach is illustrated later in Section 2.5.

This latter point is of great importance as solvents may contain impurities, which when concentrated may give misleading results. Aromatic hydrocarbons and phthalates occur almost everywhere; especially if the sample has come into contact with plastic containers, where phthalates are used as plasticisers. Silicones are common impurities as well, arising from oil or grease used in the extraction apparatus, anti-foaming agents and from GC column bleed.

There are many techniques for isolation of aroma chemicals that are currently available and many are still being developed. Once a new method is introduced, it is subsequently modified to increase its efficiency, reproducibility and range of matrices for which it can be used. Many studies that compare the results obtained with different methods using either model solutions or real samples have been published [1–5]. Based on the principle of aroma compound isolation, sample preparation methods for GC analysis can be broadly grouped into solvent extraction methods, steam distillation methods, headspace techniques and sorptive techniques.

2.2.1 Solvent extraction

These techniques are the most widely used and originate from classic organic chemistry extraction work, the concept being the transfer of the aroma chemicals from the sample to the organic solvent. The extract is obtained by mixing and agitating a liquid or solid sample with organic solvent, allowing separation and collection of the solvent phase. The extraction can be done manually or automatically and be in batches or a continuous flow extraction. In any case, if the extraction process is conducted successfully, the analyst should end up with an extract whose sensory properties closely resemble those of the original sample.

The choice of solvent is very important, as it must fulfil certain criteria. It must have a low boiling point so that it can be easily removed from the extract without a significant loss of sample volatiles and so it does not obscure other components during chromatography. It must be able to extract polar and non-polar components. Below are listed some of the most popular solvents used, the top two being most favoured in flavour extraction work.

Solvent	Boiling point (°C)	Miscibility with water
Dichloromethane	45	Immiscible/denser than water
Pentane/Diethyl ether	35	Immiscible/less dense than water
Freons (halogenated mixture)	<45	Immiscible/denser than water
Hexane – concretes extraction	69	Immiscible/less dense than water
Ethanol – absolutes extraction	78	Miscible with water
Acetone – some fragrance bases	56	Miscible with water

In addition, a new breed of solvents has been used for the extraction of aroma compounds; namely supercritical fluids such as carbon dioxide and nitrogen. Supercritical fluid extraction (SFE) will be covered later in this chapter.

Sometimes, the material from which we are planning to extract aroma compounds may require additional care before we mix it with a solvent (homogenising, grinding, milling, degassing, enzyme deactivation, etc.). For example, in the case of fresh fruits, enzyme deactivation can be easily effected using fresh saturated calcium chloride solution or methanol.

Advantages

- Good recovery of aroma compounds; the most quantitative of all isolation methods.
- Relatively easy to perform.

Disadvantages

- Solvent removal may cause loss of some of the more volatile compounds.
- Use of solvents which may be toxic and/or flammable and which may introduce contaminants.
- A need for large amounts of sample in order to produce a strong extract.
- Obtained extracts may contain high-boiling and non-volatile materials and colour components, which may lead to further problems in analysis and chromatography.
- Emulsion formation during extraction may occur.
- Solvent peak may cover early eluting volatiles in the chromatogram.

If a sample is carefully concentrated, frozen and the top liquid layer decanted, it is possible to reduce or even eliminate very high-boiling, non-volatile or unwanted co-eluting components in an extract (such as fat, preservatives like benzoic and sorbic acids and caffeine).

Other possible ways to reduce contamination of a GC when constantly dealing with samples containing high-boilers and non-volatile materials, are the use of a 'guard' column of deactivated fused silica tubing, or a retention gap (a small length of the normal column) between the injector and the column. The 'guard' column or retention gap can be replaced once they get dirty.

2.2.1.1 Extraction of liquid samples

If the sample is an aqueous liquid, extraction with an organic solvent can be easily carried out in a separatory funnel or commercially available extractor. However, the separation of an aqueous and organic solvent layer may be complicated by emulsion formation. In this case, centrifugation is often required to break down emulsions and aid in layers separation. Using a saturated salt solution (sodium chloride), instead of pure distilled water to dilute the sample, may also prevent emulsion formation. The use of solid phase extraction (SPE) columns [6–8] and solid phase disc extraction (SPDE) methods can also prevent emulsions formation. These last two approaches can also aid in pre-concentration and cleaning the samples of non-volatiles.

2.2.1.2 Extraction of solid samples

If the sample is solid, it can either be extracted as it is or dissolved in water and extracted as an aqueous sample. If the sample is to be extracted as it is (such is usually the case with solid foods, non-encapsulated powders or in the manufacture of concretes), the sample can be simply soaked in the solvent for a fixed period of time, after which the solvent is decanted, filtered and the extract concentrated. In the case of encapsulated samples, it is preferable to use water to release flavour before the solvent is added.

Other techniques available for solid sample extraction are Soxhlet extraction, accelerated solvent extraction (ASE) and SFE.

2.2.1.3 Soxhlet extraction

Soxhlet extraction is a continuous solvent extraction method that can be used for solid, semi-solid or viscous liquid samples. In this method, a sample is placed in a porous thimble and continuously extracted over several hours by allowing a low-boiling organic solvent to flow through it. New automated versions of Soxhlet, which can extract and concentrate the sample in one step, are now available on the market. Semi-solid and viscous liquid samples need to be mixed with a solid support such as diatomaceous earth (e.g. Celite®) and/or sodium sulfate or magnesium sulfate to absorb water and make the sample solid. A disadvantage of this method is the presence of non-volatile components (especially fats) in the resulting extract.

2.2.1.4 Accelerated solvent extraction

A method that has been used more recently for extracting solids and semi-solids is ASE [9]. In this method, the sample is packed into a pressure-tight cell, topped up with solvent and subjected to an elevated temperature and pressure. The cell is then purged using nitrogen gas to send the extract into a collection vial. As in Soxhlet extraction, semi-solid samples need to be mixed with a support. Typical solvents used are methanol, isopropyl alcohol, hexane and water. The advantages of this method are that it uses relatively small amounts of solvent and that extraction times can be as short as 15 min. The disadvantage is that very volatile compounds can be lost during the elevated temperature extraction. Also, on transfer from the cell to the collection vial, it is possible that the sample may precipitate, thus clogging the line.

2.2.1.5 Supercritical fluid extraction

One of the most innovative methods of solvent extraction is SFE, where the solvents used are supercritical fluids [10–14] such as carbon dioxide. By changing the pressure during the extraction, the extracting power of the supercritical fluid can be modified, hence the extract profile tailored. It works best on dry solids or viscous liquids. In practice, SFE has been shown as particularly useful for extracting fragrances from products with high-boiling materials and bases, such as shampoos and conditioners. Liquids again need to be mixed with a solid support and packed inside cells for extraction. The cells are pressurised and extracted with supercritical fluid, and the extract collected in small amounts of solvent in vials.

Advantages of SFE include use of non-toxic solvents, selectivity of extraction, short extraction times, and less reaction of thermally labile and oxygen-sensitive compounds. The main problem with the apparatus is clogging of the transfer line with extraction material. Supercritical solvents tend to discriminate against polar components but this can be overcome by using modifiers like methanol or ethanol. Additional disadvantage is the difficulty in balancing temperature, pressure and flow rate for each type of sample. This makes method development very time consuming. Also, the sample capacity is relatively limited.

2.2.1.6 Fractionation of solvent extracts

The extracts obtained by solvent extraction, especially ones from natural products, can be very complex and the GC chromatogram may contain many co-eluting compounds, which make identification of individual aroma compounds difficult. One way to overcome this problem is to fractionate the extract, and hopefully move co-eluting peaks into the different fractions for better identification. This is particularly important for gas chromatography-olfactometry (GC-O) analysis. In this case, the analyst's goal is to separate co-eluting peaks as best as possible to reveal organoleptically important compounds that may be hidden under bigger, less-odorous peaks. GC-O techniques will be covered later in the chapter.

Fractionation of the extracts can be accomplished in several ways. Manipulation of the extract by washing it with dilute acid, dilute base, and either sodium metabisulfite