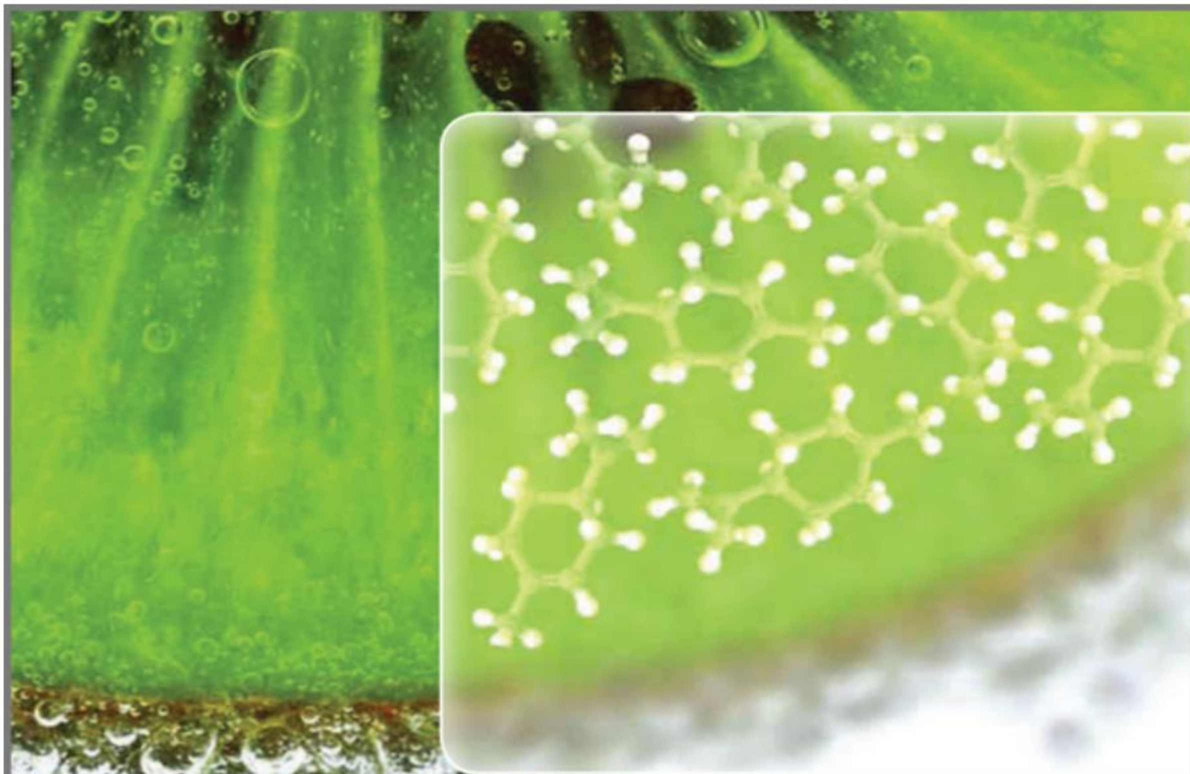

Editors

KEVIN GOODNER

RUSSELL ROUSEFF

Practical Analysis of Flavor and Fragrance Materials

WILEY

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Practical Analysis of Flavor and Fragrance Materials

Edited by

Kevin Goodner and Russell Rouseff

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Preface

Practical Analysis of Flavor and Fragrance Materials

Flavor is one of the most important factors in consumer purchases and long term consumption. However, flavor is not easily quantified as the factors that impact flavor are almost always trace components. So from a chemical point of view, flavor analysis is essentially trace organic analysis. The human factor is essential to understanding flavor because humans have different genetic and cultural backgrounds which will alter their perception of flavor. Therefore all flavor analysis should be guided by human sensory panels. For too many years the study of flavor was conducted by analytical chemists who measured what they could measure using traditional analytical techniques rather than quantifying those trace impact compounds which should be measured. For many years the use of human assessors (sensory analysis) was conducted without interest in determining what was producing flavor changes in products being evaluated. Because sensory panels are impractical for routine quality control purposes, most food and fragrance manufacturers have chosen a middle ground where sensory panel data is used to guide chemists as to which compounds should be monitored to maintain quality or a specific sensory profile.

This book is an attempt to demonstrate how to develop this hybrid approach to flavor analysis. The few books that exist for flavor analysis have exclusively detailed either chemical analysis with sensory input or exclusively sensory analysis without regard to chemical composition.

This book is aimed at the practical side of analytical analyses. We attempt to produce a book as a reference book or as a primer for analytical chemists who are starting out in the flavor and fragrance industry with useful chapters on some of the major topics that someone new to the industry might encounter, including some of the basic tests one might see in the labs such as °Brix, water activity, turbidity, and similar tests.

David Rowe summarized much of the descriptive information from his recent book on *Chemistry and Technology of Flavour and Fragrance* into the first chapter. Sample preparation techniques are described by Russell Bazemore in the next chapter. It provides a detailed description of classic and cutting edge sampling techniques that ultimately determine the success of any flavor analysis. Traditional analytical techniques that have been used to measure the quality of raw flavor materials and finished products are presented next. Gas chromatography-mass spectrometry is included in this chapter as it is the most common technique employed by flavor chemists.

Gas chromatography-olfactometry, GC-O, is a hybrid technique employing the separation power of high resolution gas chromatography with the particular selectivity and sensitivity of human olfaction. This chapter written by Kanjana Mahattanatawee and Russell Rouseff, covers the hardware, software, and various techniques used for GC-O, along with selected applications, and benefits.

Vanessa Kinton wrote the next chapter on multivariate techniques which are commonly used for data analysis. This chapter describes the mathematical background and theory behind these techniques. The focus is to provide a basic understanding of the theory behind these mathematical approaches knowing that in practice the procedures are handled as a “black box”. These techniques are used extensively in many areas of analysis (electronic nose, MS chemsensor, sensory analysis, etc.) and this chapter provides the basics while the other chapters provide the application examples.

Chapters 5 and 6, by Marion Bonnefille and Ray Marsili respectively, employ many of the multivariate data treatments for two very different sensor types. Chapter 5 concerns the metal oxide based electronic nose while chapter 6 is on the MS-based chemical sensor. Although both techniques employ pattern recognition software from instrumental sensors to mimic human olfaction, they differ profoundly in the types and number of sensors used to obtain the data arrays.

The chapter on sensory analysis by Carlos Margaria and Anne Plotto is likely to be an area in which most chemists have little familiarity. This chapter provides a wealth of practical information about conducting sensory panels both trained and untrained with many anecdotes from their own experience.

The last chapter describes the ever changing regulations that affect flavor analysis in the industry and is written by Robert Kryger. This is an extremely important issue that is rarely taught in schools or universities. He discusses many of the basic terms and regulations as well as some of the complications in interpreting these regulations which vary from country to country.

The editors hope that this compilation will benefit those scientists beginning their careers in the area of flavor. Finally, and most importantly, we wish to thank each contributor for their time and efforts they put into their respective chapters. This book was a long time in the making and we are most appreciative of individual

authors for their dedication and expertise in making this book possible.

About the Editors

Kevin L. Goodner received both a B.S.Ch. in Chemistry and a B.S. in Mathematics from the University of Memphis in 1992 and a Ph.d. in Analytical Chemistry from the University of Florida working with Fourier Transform Mass Spectrometry. His focus changed to flavor chemistry after a 1.5 year post-doctorial position at the University of Florida with Russell Rouseff. Kevin then worked for 9 years at the USDA Citrus and Subtropical Products Laboratory researching flavor and quality aspects of many products. In January of 2008, Kevin switched to industry at Sensus, LLC working on tea, coffee, and other products where he currently is the Director of Research and Development. Kevin has over 50 peer-reviewed publications.

Russell Rouseff is a professor at the University of Florida's Citrus Research and Education Center specializing in flavor and color chemistry. He has 35 years experience in the Florida citrus industry, first with the Department of Citrus and then the University. He has written or edited five books, 37 book chapters and over 108 referred journal articles. He has mentored scores of domestic and international students, 8 post docs and numerous visiting scientists. He has worked with aroma volatiles in fruits, coffee, wine, flowers and foliage and bitter nonvolatiles. He is a Fellow of the American Chemical Society's Agricultural and Food Chemistry Division, recipient of the IFT's Citrus Products Division Research and Development Award and received the American Chemical Society's Award for the Advancement of Food and Agricultural chemistry in 2009. Hobbies include salt water reef aquariums, tennis and motor cycles.

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Chapter 1

Overview of Flavor and Fragrance Materials

David Rowe

Riverside Aromatics Ltd, Poole, UK

The nature of this chapter must be that of an overview as the alternative would be a multivolume series! The difficulty is not a shortage of material but rather a surfeit, and a second issue is how to give a rational coverage; should the materials be classified by chemistry, by odor or by application? The approach here is a combination of all three, and is based in part on a précis of *The Chemistry and Technology of Flavours and Fragrances* [1].

There is, of course, a massive overlap between flavor and fragrance; for example, *cis*-3-hexenol, discussed below, has a 'green', cut-grass odor, and hence contributes freshness to both flavors and fragrances. The division between the two Fs is itself not always a natural one!

1.1 Flavor Aroma Chemicals

1.1.1 Nature Identical

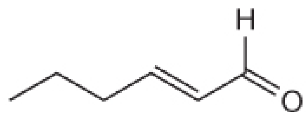
The vast majority of the aroma chemicals used in flavor are nature identical (NI), that is, they have been identified as occurring in foodstuffs in the human food chain. This is a key method of identifying the most important components which create a flavor, and until recently, there were also regulatory implications. European Council Directive 88/388/EEC defined these as “flavouring substances identical to natural substances”, with the alternative being “artificial flavouring substances”, with the latter leading to the stigma of “artificial flavors”. The newest regulations, REGULATION (EC) No 1334/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL, no longer differentiates between Nature Identical and artificial, but the concept is still important – as a guide to flavorists, knowing a material is NI is important, and it can be especially so the context of “from the named food” type of flavours. Regulation 1334/2008 now only differentiates between “flavouring substances” and “natural flavouring substances”, which harmonizes to an extent with the USA, where the NI classification has never been used. Even there, though, the NI concept has value, as materials have to be on the FEMA GRAS list, that is they are “Generally Recognized As Safe”, and the vast majority of such substances are found in Nature.

1.1.1.1 Alcohols

It should be noted that ethanol **1** itself is a flavor component of ‘alcoholic drinks’ as anyone tasting alcohol-free drinks will report! In fact it may considered as a solvent (especially in fragrances), as a flavour substance (FEMA 2419) or an additive (E1510)! *cis*-3-Hexenol **2**, mentioned above, is produced in nature as a ‘wound chemical’, that is, when plant tissue is damaged, ingressing oxygen is ‘mopped up’ by reaction with linoleic acid, which generates the unstable *cis*-3-hexenal, which is enzymatically reduced to the alcohol. Also formed are *trans*-2-hexenal **3**, which has a harsher, more acrid greenness and *trans*-2-hexenol **4**, which is rather sweeter:

1

2



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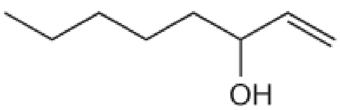
1-Octen-3-ol, ‘mushroom alcohol’ **5**, has the earthy note characteristic of mushrooms. The ‘terpenoid’ alcohols, C10 derivatives, include geraniol **6** and its isomer nerol **7**, citronellol **8** and linalool **9** [2]. Cyclic terpenoid alcohols include α-terpineol **10** and menthol **11**:



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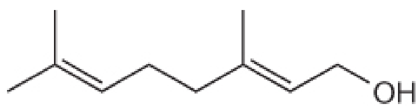

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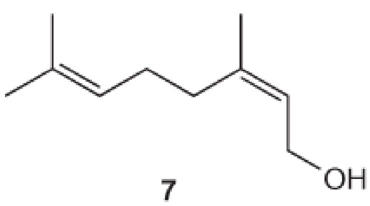

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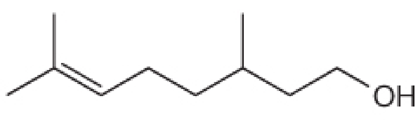

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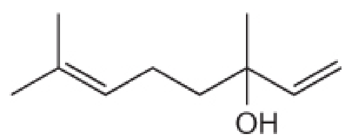

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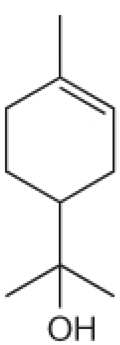

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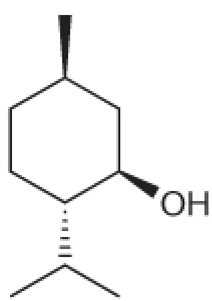
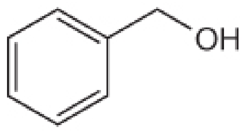
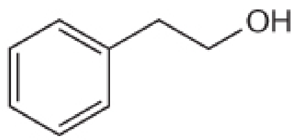
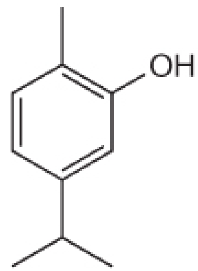
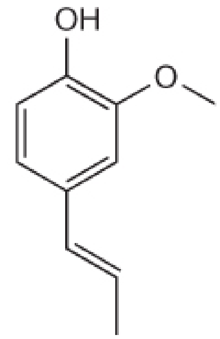


11

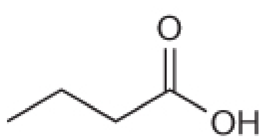
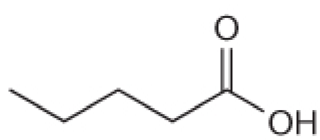
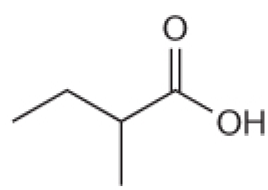
Benzyl alcohol **12** has relatively little odor and is more commonly used as a solvent in flavors; phenethyl alcohol **13** is a component of rose oil and has a pleasant rose-like aroma. Two important phenols are thymol **14** and eugenol **15**, which are also major components of thyme and clove oils respectively:

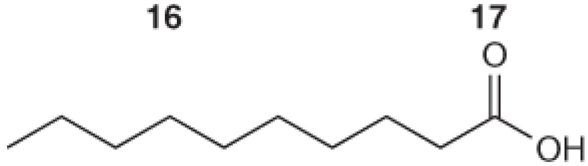
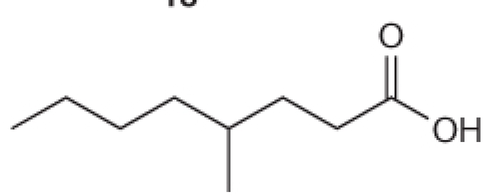
12 **13** **14** **15**

1.1.1.2 Acids

Simple acids contribute sharp notes which often become fruity on dilution. Butyric acid **16** is indisputably 'baby vomit' in high concentration; valeric acid **17** is cheesy, whereas 2-methylbutyric acid **18** is fruitier. Longer chain acids such as decanoic **19** are fatty and are important in dairy flavors. 4-Methyloctanoic acid **20** has the sharp fatty character of roasted lamb:

16 **17** **18**

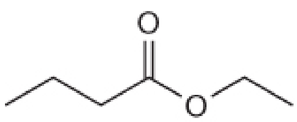



19 **20**

1.1.1.3 Esters

Numerous esters are used in flavors, so it is almost a case of any flavor alcohol combined with any flavor acid!

Important simple esters include the fruity ethyl butyrate **21** and 2-methylbutyrate **22**; allyl hexanoate **23** has a familiar pineapple aroma and isoamyl acetate **24** is ‘pear drops’. Phenethyl 2-methylbutyrate **25** is ‘rose bud ester’ and the warm sweet aroma of methyl cinnamate **26** makes it valuable in strawberry flavors. Methyl salicylate is the main component of wintergreen oil **27** and methyl N-methylanthranilate **28** is found in mandarin, which differentiates this from the other citrus oils:



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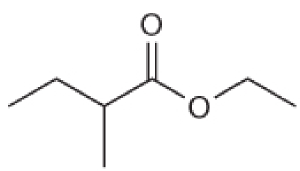

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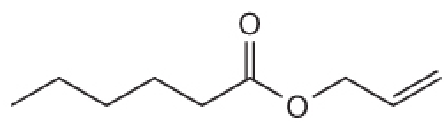

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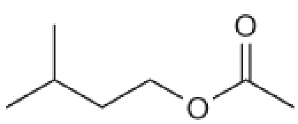

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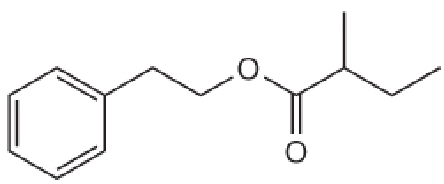

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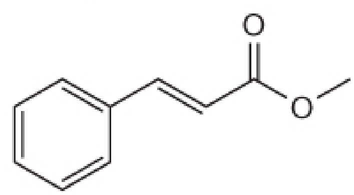

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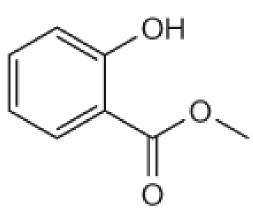

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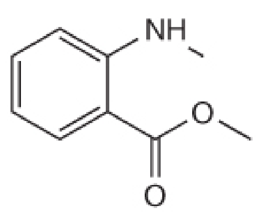
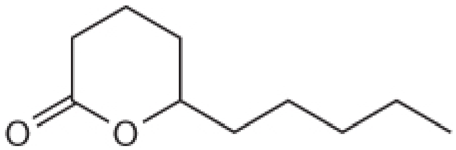


28

1.1.1.4 Lactones

These cyclic esters are usually found as gamma-lactones (five-membered rings) and delta-lactones (six-membered). Like their acyclic cousins they are used in fruit flavors and also for dairy, especially the delta-lactones such as delta-decalactone **29**. Gamma-nonalactone **30**, also misleadingly known as Aldehyde C18, has a powerful coconut odor:



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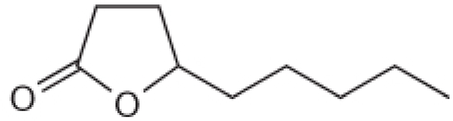
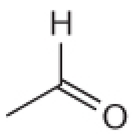


30

1.1.1.5 Aldehydes

Acetaldehyde **31** is ubiquitous in fruit aromas, though its volatility (b.p. 19 °C) makes it difficult and dangerous to handle as a pure aroma chemical. Unsaturated aldehydes such as the previously mentioned *trans*-2-hexenal (leaf aldehyde) **3** are very important. *trans*-2-*trans*-4-Decadienal **32** is intensely ‘fatty-citrus’; *trans*-2-*cis*-6-nonadienal **33** is ‘violet leaf aldehyde’. ‘Citral’, a mixture of the isomers geranial **34** and neral **35**, is intensely lemon; it is a key flavor component of lemon and to a lesser extent other citrus oils:



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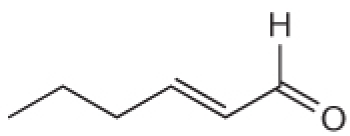

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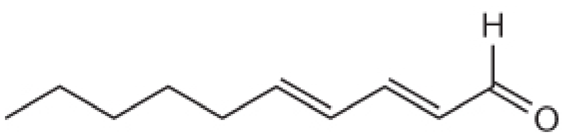

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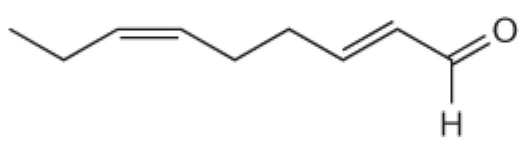

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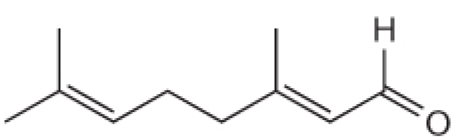

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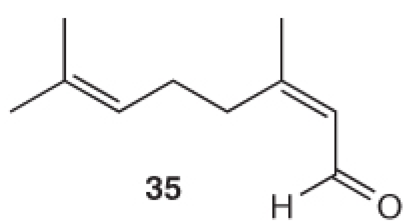
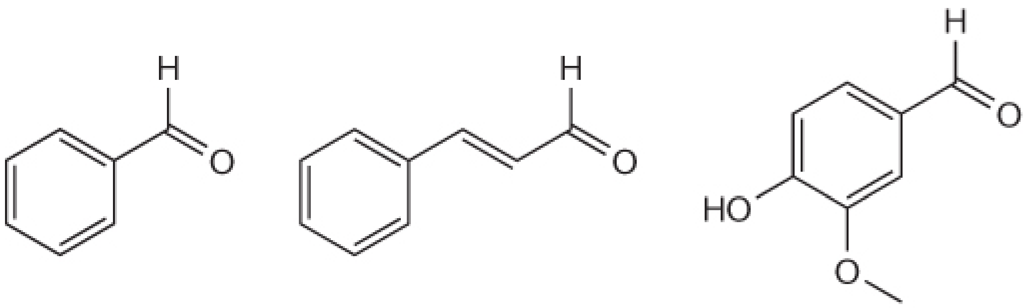


35

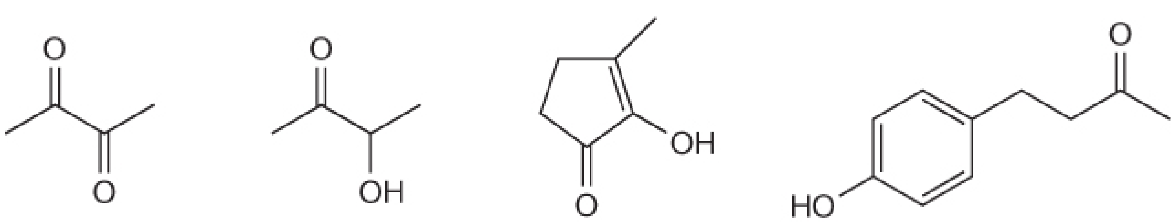
Benzaldehyde **36** is widely used in fruit flavors, especially for cherry, though in fact it is not a key component of cherries. Cinnamaldehyde **37** is found in cassia and cinnamon oils. The most important aromatic aldehyde, and one of the most significant of all aroma chemicals, is vanillin **38**:



36 37 38

1.1.1.6 Ketones

The C4 ketones diacetyl **39** and acetoin **40** are used in butter-type flavors for margarines and other dairy products and hence are used in very large quantities. The former is very volatile and is believed to have led to respiratory damage amongst people exposed to large quantities of its vapor. The cyclic diketone ‘maple lactone’ **41** occurs as the enolic methylcyclopentenolone (MCP) and has the characteristic sweet, caramel odour of maple syrup. Raspberry ketone **42** is unusual in the bizarre world of flavor and fragrance trade names in that it is actually found in raspberries, tastes of raspberries and is a ketone!

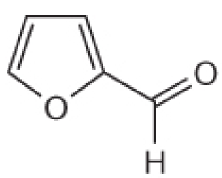


39 40 41 42

1.1.2 Heterocycles [3]

1.1.2.1 Oxygen-containing

The importance of materials containing the five-membered furan ring cannot be overstated [4]. Furfural **43** is formed by the Maillard reaction from pentoses in the cooking process, and 5-methylfurfural **44** from hexoses similarly. The latter has an almond, ‘marzipan’ aroma similar to benzaldehyde but with more naturalistic character. Methyl tetrahydrofuranone, ‘coffee furanone’ **45**, is sweet and caramelic, but the most important flavor furan must be 2,5-dimethyl-4-hydroxy-3[2H]-furanone **46**, an aroma chemical of many names, including strawberry furanone, and pineapple ketone. This has sweet, fruit and caramel notes, making it of obvious importance in fruit flavors, but it is also important in meat flavors, where it seems to function as a flavor enhancer. Its homologue Soy Furanone **47** is also very sweet, whereas its isomer ‘Sotolone’, or fenugreek lactone **48**, has an intense fenugreek tonality, becoming more caramel-like in high dilution. The saturated furan Theaspiran **49** is found in black tea and a number of fruits:



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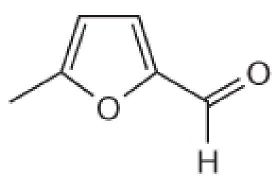

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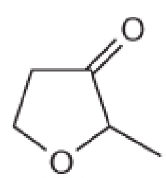

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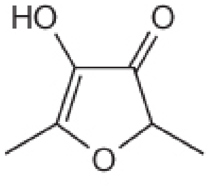

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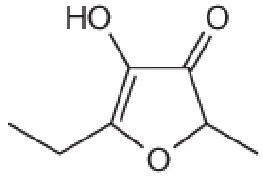

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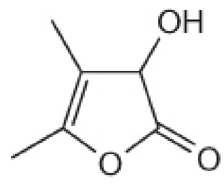

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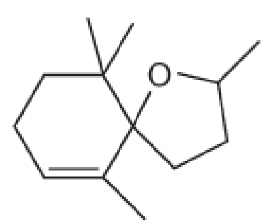
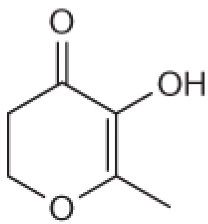


49

The most important pyrans must be Maltol **50** and Coumarin **51**. The former is another caramel compound, with the latter having sweet and spicy notes. The saturated furan 1,8-cineole, or eucalyptol **52**, is the main component of eucalyptus oil as well as being widespread in other oils such as lavender, distilled lime and rosemary:



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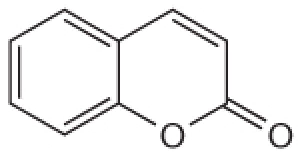

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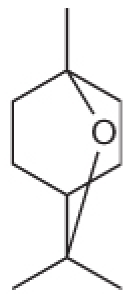

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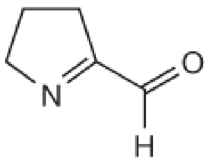
1.1.2.2 Nitrogen-containing

The pyrrole group is relatively unimportant in flavors, though mention should be made of 2-acetyldihydropyrrole **53**, which has the ‘Holy Grail’ aroma of freshly baked bread but is too unstable for commercial use. The most important nitrogenous heterocycles are pyrazines, which are readily formed in the Maillard reaction from amino acids and sugars; simple alkyl pyrazines such as 2,3,5-trimethylpyrazine **54** have roasted, cocoa-like notes making them important for chocolate and roasted notes. Tetrahydroquinoxaline **55** has particularly noticeable roasted notes. 2-Acetylpyrazine **56** has very pervasive roasted, biscuit notes. The alkoxyalkylpyrazines are also found in fresh fruits and vegetables, the intensely odorous 2-methoxy-3-isobutylpyrazine **57** often being known as ‘Bell Pepper Pyrazine’.



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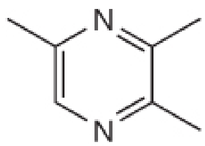

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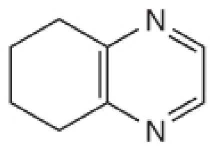

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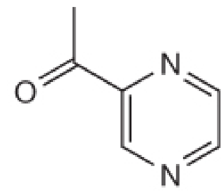

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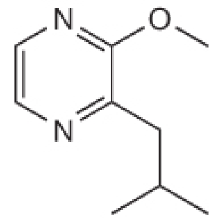

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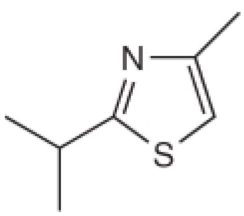
1.1.2.3 Sulfur-containing

Whilst a few simple thiophenes are used, the most important sulfur heterocycles are thiazoles, especially 2-isopropyl-4-methylthiazole **58** and 2-isobutylthiazole **59**, which have peach/tropical and tomato vine character respectively. 4-Methyl-5-thiazoleethanol, Sulfurol **60**, is widely used in dairy and savory flavors.



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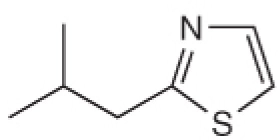

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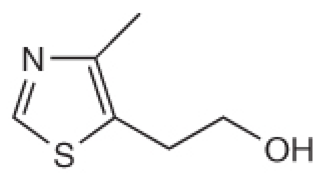

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1.1.3 Sulfur Compounds [5]

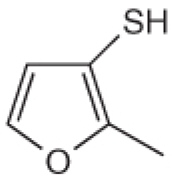
The importance of sulfur compounds reflects their highly odorous character; the most odorous compounds known are sulfur compounds, with odor thresholds down to the 10^{-4} parts per billion level. They are the single largest group of ‘High Impact Aroma Chemicals’, materials which provide ‘character impact’ even at very low levels [6].

1.1.3.1 Mercaptans

These are generally the most odorous of the most odorous, as it were, the *capo di capi* of the flavor industry. Methyl mercaptan **61** is widespread in meat aromas, as is

2-methyl-3-furanthiol (MFT) **62**; the latter is especially important in beef. Furfuryl mercaptan **63** is a character impact aroma chemical of roasted coffee. The latter two are Maillard reaction products formed from cysteine and pentoses. ‘Fruity’ mercaptans include the blackcurrant/cassis materials **64** and thiomenthone **65**, and p-menthene-8-thiol, the Grapefruit Mercaptan **66**. The accurately named Cat Ketone, 4-mercapto-4-methyl-2-pentanone **67**, is also found in grapefruit and wines.

61



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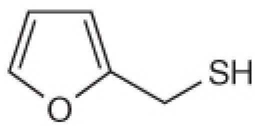

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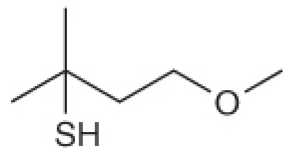

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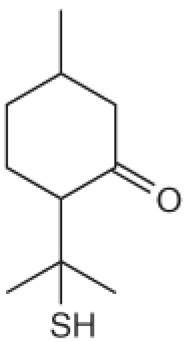

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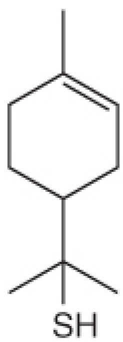

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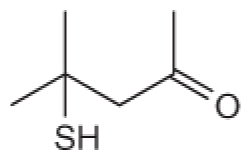

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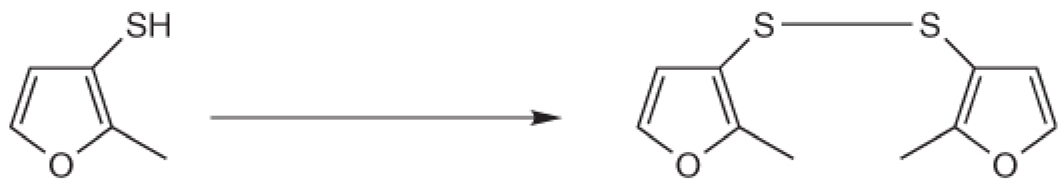
1.1.3.2 Sulfides

The simplest sulfide, dimethyl sulfide (DMS) **68** has a vegetable, sweetcorn odor; sulfides are less odorous than mercaptans, and hence a key aspect of quality is the need to remove all traces of mercaptans; impure DMS is quite repellent. Propyl and allyl sulfides are perhaps the commonest, especially as di- and higher sulfides; allyl disulfide **69** is the major component of garlic oil, with the remainder being mostly higher sulfides. Propyl compounds such as dipropyl trisulfide **70** are found in onion; ethyl compounds are found in Durian fruit, and to human noses other than those raised with the fruit, are at best unpleasant and sewer-like:

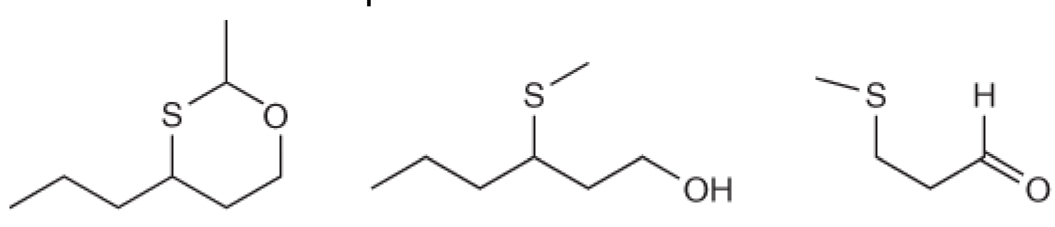
68 **69** **70**

Some mercaptans oxidize very easily to form disulfides, such as the formation of bis(2-methyl-3-furyl) disulfide **71** from MFT **62**:



62 **71**

There are a number of fruity sulfides, often derived in some way from C6 units with an oxygen atom in the 3-position relative to the sulfur; such a grouping is found in the 'tropicals' Tropathiane **72** and 3-methylthiohexanol **73** as well as the potato-like methional **74**:

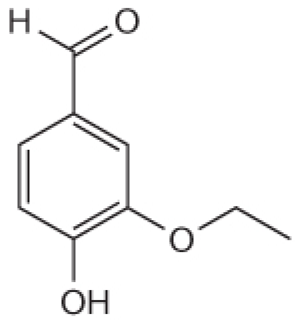


72 **73** **74**

1.2 Flavor Synthetics

There are still a number of important flavor materials which have, to date, not been found in nature. They are often used because they have properties which suitable NI materials lack; for example the so-called Ethyl vanillin

75 has a lower odor threshold than vanillin and is more soluble in organic solvents, making it more suitable for use in oil-based flavors, and 'Ethyl maltol' **76** is more powerful than maltol. Several glycidate esters are used, such as ethyl 3-methyl-3-phenylglycidate **77**, so-called Aldehyde C16, which has a powerful strawberry aroma and is used in flavours as well as fragrances.



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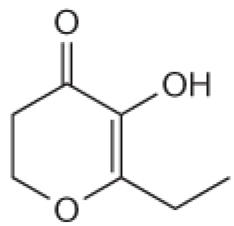

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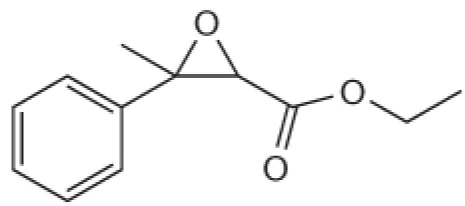

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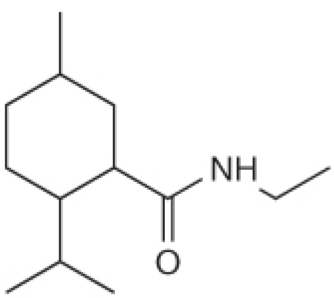
Synthetics have proved especially valuable in the area of what might be termed 'sensates', the molecules of taste and sensation [7]. For example, the carboxamides **78** and **79** are both cooling agents which are longer-lasting than menthol **81**:



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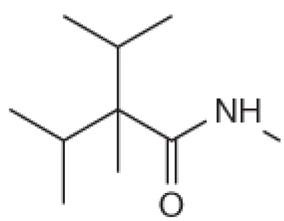

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Over the years a number of 'synthetics' have been found in nature, changing their status, such as the previously mentioned cat ketone and allyl hexanoate, and other materials are surely 'synthetic' simply because they are still hiding in foodstuffs! In addition, the "new" European regulations, EC 1334/2008, removes the "artificial" classification, and gives, potentially, a new lease of life to these materials.

1.3 Natural Aroma Chemicals [8]

The seemingly innocent term 'natural' is, in fact, a more troublesome one than it seems. In essence 'natural' materials are those which are:

(a) obtained by physical means from materials in the human food chain, that is, isolates;

(b) obtained by biological conversions of natural materials, that is, biotechnology; or

(c) obtained by reacting natural materials together in the absence of chemical reagents or catalysts, that is, cooking chemistry or soft chemistry.

These definitions are enshrined in US (CFR 21, 101.22 (a) (3)) and European (REGULATION (EC) No 1334/2008) regulations. As far as aroma chemicals are concerning, "Natural" is a marketing conceit; the marketing departments of flavour and food companies, the supermarkets and other major retailers are unlikely to reverse their policies of promoting their subliminal (and sometimes not so subliminal) formula of Natural = Healthy, especially with the so-called "Clean Label" concept. The importance of the regulations is that they set the criteria which enable a material to be called "Natural".

1.3.1 Isolates

A number of essential oils consisting of high levels of valuable components, make their direct isolation by physical means commercially viable. Examples include citral **34, 35** from litsea cubeba oil, anethole **80** from star