NATURE AND ORIGINS OF SENSORY CHARACTER IN SCOTCH WHISKY

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A thesis submitted in partial fulfilment for the award of the degree of Doctor of Philosophy

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November 2000

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Total dedication to, the absence of, my two loving sons:

SUNG-RYONG and IL-SUNG.

ACKNOWLEDGEMENTS

First of all,

Thank to all the sensory assessors from University of Strathclyde and Scotch whisky industry for their commitment.

I would like to express a great acknowledge to my supervisor ALISTAIR PATERSON for his support and encouragement throughout this research and beyond. Without the friendship, I never be the one who I am today. Much appreciation to JOHN R. PIGGOTT for his advice and thought me the principal of reliability. An industrial supervisor, GRAEME R. RICHARDSON, his friendship can not be missed from my appreciation. I must also thank Lorraine Allan and John Conner for help chromatography.

Thanks for the donation of whisky samples from Scotch distilleries especially JOHN ROSCROW from Invergordon Distillers, RICHARD PATERSON from JBB plc, KEN FINN from United Distillers, DENNIS WATSON from Chivas Glenlivet Ltd and SHINJI FUKUYO from Suntory Ltd. I also must thank FlavorActiV Ltd. for supplying of the flavour reference standards.

Lastly, but by no means least, Eurtis Morrison, Keith B. Stephen and my family for personal strength are thanked.

ABSTRACT

Scotch whisky production is central to the local economy, with an interesting history, and dominates international markets for brown spirit products. Researchers have characterised whisky congener compositions, behaviour and the nature of wood maturation of whisky. However there remain difficulties in understanding the nature and origins of sensory character in Scotch whiskies, a central issue being development of a consistent flavour terminology.

A key aim was revision of the Scotch whisky flavour wheel, to optimise sensory analysis and training of assessors in the distilling industries, and for consumer studies. The nature of flavour perception in Scotch whisky was then explored, investigating flavour terms currently in use among industrial sensory assessors. This language was revised using standard reference compounds in 23% abv grain whisky to anchor individual flavour attributes. The wheel incorporates only positive attributes to meet current needs for interaction between sensory, marketing and technical professionals.

Concepts of flavour sensation and perception were reviewed with a separation into the reductionist and consideration of the holistic nature of perception. Sensation and perception are from different levels of human reliance, *microscopic* and *macroscopic*. The former is important in sensory assessment and the latter, in understanding consumer choice.

Flavour thresholds and 95% recognition for standard flavour reference compounds were established in 23% grain spirit. The relationships between attribute weighting and product category in blends was explored using 40 retailed blends of four categories - Deluxe, Standard, Retailer and West Highland. Relationships between product category and headspace concentration of important congeners from solid phase micro-extraction (SPME) were explored to clarify the basis of discrimination.

This latter study has shown clear discrimination of two blend categories (Deluxe and Retailer), with West Highland products intermediate between these categories. The dispersion of Standard blends across the product spaces suggested product formulation varied between blenders. Although discrimination of product categories was similar in product spaces derived from sensory and headspace congener composition data using a single SPME, relationships between the two product spaces was not on the whole modelled successfully.

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INTRODUCTION

1.1. WHISKIES

1.1.1. DEFINITION OF SCOTCH WHISKY

Whisky is legally defined under European Community Council Regulation No. 1576/89 and Scotch whisky in the UK under the Scotch Whisky Act 1988 and Order 1990 (Table 1.1). 'Whisky' derived from Gaelic *uisge beatha* (pronounced 'ooshkie bayha'), 'water of life', abbreviated to *uiskie* in the 17th century became *whiskie* by 1715 with modern spelling - whisky - uniform as late as 1736 (MacLean, 1997).

1.1.2. HISTORY OF SCOTCH WHISKY

The technology of distilling was brought from Ireland to Scotland. The first formal record has been found from the Scottish Exchequer Rolls in 1494. This records provision of 'eight bolls of malt to Friar John Cor wherewith to make aquavitae' by order of the King James IV. Aqua vitae a traditional name for any distilled spirit, still current in Nordic languages, is an exact Latin equivalent of the Gaelic *uisge beatha,* and the French *eau de vie*). Distillation had been one of the fundamental operations of alchemy.

Alchemy, *the forerunner of chemistry,* (from the Arabic: *'Al khem'- the art of Egypt)* can be traced back to ancient China, India, Greek and Roman times. The art travelled with the Moors into Andalucia in Spain (Broom, 1998). Potable spirits were first created in Kemi – an early name for Ancient Egypt – before 3000BC, probably from grapes or flower, rather than grain. Early equipment was primitive - for example, sweet water was collected from boiling sea water by hanging sponges in the steam. In the first century AD, the Elder Pliny mentions hanging of fleeces over boiling resin to catch the vapours and make turpentine. The earliest European distillers were generally monks with a medicinal interest. Between 1050 and 1150, a monk at the medical school of Salerno in Italy discovered alcohol (Nicol, 1997). In 1505, King James IV of Scotland (Lyons, 1999), granted the Guild of Surgeon-Barbers of Edinburgh, a monopoly for manufacture of aqua vitae within the burgh in connection with medical uses (preservation of corpse parts prior to dissection) (MacLean, 1997).

TABLE 1.1. Definition of Scotch Whisky (The Scotch Whisky Order, 1990, No. 998)

"Scotch whisky" means whisky

(a) which has been produced at a distillery in Scotland from water and malted barley (to which only whole grains of other cereals may be added) all of which have been –
  (i) processed at that distillery into a mash;
  (ii) converted to a fermentable substrate only by endogenous enzyme systems; and
  (iii) fermented only by the addition of yeast;
(a) which has been distilled at an alcoholic strength by volume of less than 94.8 per cent so that the distillate has an aroma and taste derived from the raw materials used in, and the method of, its production;
(b) which has been matured in an excise warehouse in Scotland in oak casks of a capacity not exceeding 700 litres, the period of that maturation being not less than 3 years;
(c) which retains the colour, aroma and taste derived from the raw materials used in, and the method of, its production and maturation; and
(d) to which no substance other than water and spirit caramel has been added.

Minimum alcoholic strength of Scotch whisky

4. For the purposes of section 2 (1) (b) of the Act there is hereby specified the alcoholic strength of 40 per cent by volume (being also the minimum alcoholic strength by volume prescribed in relation to *whisky/whiskey* by Article 3(1) of Council Regulation (EEC) No1576/89 laying down general rules on the definition, description and presentation of spirit drinks (a).

In witness whereof the Official Seal of the Minister of Agriculture, Fisheries and Food is hereunto affixed on 27th April 1990.

The discovery of distillation was crucial to potable spirits production. In 1560, with dissolution of Scottish monasteries, numerous monks moved from cloister into the community bringing distilling to the laity. Wherever suitable cereals were grown, domestic distilling became part of the farming year.

Fynes Moryson, a late Elizabethan travel writer, recorded distillation of three kinds of spirit in the Western Isles: graded for strength and quality on number of times distilled. 'Usquebaugh' (distilled twice), *a distilled spirit made in Ireland and Highlands of Scotland also called whisky*, was defined as *a compounded* (i.e. mixed with herbs, sugar and spices) *distilled spirit, being drawn on aromaticks; and the Irish sort is particularly distinguished for its pleasant and mild flavour.* 'Trestarig' (distilled three times) was from the Gaelic for 'triple strength', defined as *a kind of ardent spirits distilled from oats.* 'Usquebaugh-baul' was distilled four times. Spirits, all from oats, were also discriminated as *simplex*, *composita* and *perfectissima* on the basis of number of distillations used. Such factors stressed medical values of spirit at that time.

By the early 17th century, whisky production was a part-time cottage industry, part of Scottish social life and economy. Domestic stills were exempt from duty. It was illegal to sell whisky, but despite this it became a currency in the Highlands, often used in payment of rents. However by 1644, production was sufficient to interest the government in imposing an excise tax. The first distillery mentioned in an official document was that at Ferintosh, Black Isle, of Duncan Forbes of Culloden in about 1670. Distilling privileges compensated for loss of a farm, and 'Ferintosh' was so successful (almost two-thirds of legal whisky in Scotland) the name became synonymous with quality and in 1784, the Government terminated the privilege with a lump sum.

The Malt Tax (1725, Appendix I) and the Gin Act (1736, Appendix I) increased small and medium-sized distilleries over Britain but a cereal crop failure in 1757 led to a ban putting most registered distilleries out of business. The subsequent illicit trade yielded better quality whisky as legal distillers used minimal malt contents to reduce malt tax liability. Illegal distillation was easy in the impassble Scottish Highlands - remote glen and hillsides. Condition were ideal (such as lots of water) for distillation of malt whisky. In 1820, the Duke of Gordon, a powerful landowner in the North East, urged the House of Lords to reduce duty and moderate attitudes towards legal distillers, in return for assisting Excise officers in putting down smuggling. An early licence was of George Smith of Glenlivet, a remote district of Speyside, where 200 illicit stills operated in the early 1820s. Legal distilleries were often built on former illegal still sites for water supply with supplies of grain from farms, that could also utilise draff. Distilleries were often built near drovers' inns, a ready source of custom.

In 1784, Wash Acts (Appendix I) made duty chargeable on still capacity not spirit strength, favouring small-scale Highland distillers, producing spirit of better quality than Lowland whisky. The 1829 depression enhanced the appeal of continuous distillation of grain whisky, with processes such as that invented in 1827 by Robert Stein and Aeneas Coffey. Such stills were designed for further spirit rectification for gin or non-beverage use. Although the Corn Laws tied British distillers to barley, the Coffey still could be adapted for maize. The Stein stills used malted barley to produce 'silent malts'.

Grain whisky was consumed by the Scottish poor and transported to England for rectification into gin. Spirits merchants, grocers or 'Italian Warehousemen', also sold tea and coffee and general provisions and became the great names in modern whisky: the Big Five - James Haig, John Dewar, James Buchanan, Alexander Walker

(Johnnie Walker) and Peter Mackie (White Horse); Matthew Gloag (Famous Grouse), and Charles Mackinlay. Other distillers were George Ballentine in Edinburgh, Arthur Bell in Perth, and Thomas Sandeman (VAT 69).

To enhance the popularity of light bodied grain whisky in 1853, blending of whiskies from a single distillery, vatting, was permitted. Blending of malts with cheaper, blander grain whiskies was pioneered by Andrew Usher, Charles Mackinlay and W.P. Lowrie and in 1860, Gladstone's Spirit Act (Appendix I) allowed blending under bond. The three-year maturation of Scotch whisky did not become compulsory until World War I. This made it possible for the first time to produce blended whisky in substantial volumes. The product had a broader appeal than the strongly flavoured, smoky malts or fiery grain whiskies, and was consistent and cheap to produce. The railway networks in Europe and the US, and the Navigation Acts in 1845 (Appendix I), opened export market in the colonies and dominions, and blended whisky had become a international drink and by the early 1900s was dominant. In the late 1970s, around 99% malt whisky went for blending. The blending of whiskies put Scotch onto the world stage.

Development of blended whisky markets was assisted by energetic commercial activity and brilliant salesmanship, and a natural disaster, the *Phylloxera vastatrix* attack on vines. From the mid-1860s French vineyards were devastated, and Grande Champagne vineyards could not supply the Cognac and brandy favoured by the English middle classes so "Brandy and soda" was replaced by Scotch and soda.

As blending become popular, adulteration became attractive to reduce cost and enhance acceptance. There were no regulations in place to control this. In 1870s Charles Cameron, a physician and editor of *The North British Daily Mail,* and Dr James Gray, an analytical chemist, collected samples of whisky from numerous establishments in the Edinburgh; further samples were analysed for the Inland Revenue by the chemist R.R. Tatlock. Despite inconclusive results the Government, in the Licensing Act of 1872, prohibited adulteration and in 1879 an amending Act included a definition of Scotch whisky. In branding and marketing, distillers were aware of consumer fears and the entrepreneurial developed descriptors such as 'pure', wholesome' and 'fine old'.

High productivity in continuous stills brought problems of over-production and market instability. The Distillers Company Limited (DCL), formed by the principal grain whisky producers, achieved self-regulation and this prevented competition. The notorious failure of Robert and Walter Pattison, Elder & Co. of Leith demonstrated the danger of over-production and encouraged the DCL policy of amalgamation. DCL distilleries included Port Dundas, Carsebridge, Cameron Bridge, Glenochil, Cambus and Kirkliston. Malt distillers claimed only malt whiskies met definitions of true Scotch but their legal failure enhanced markets for blended whisky.

In 1914 with the outbreak of World War I, the Government sought to increase efficiency of arms production by reducing consumption of strong liquors. The Central Control Board (Liquor Traffic) reduced pot still production by 30% and eventually distilling was banned and exports forbidden. In 1920, the US prohibited importation of alcoholic beverages except for medicinal purposes, a ban lasting for 13 years. Paradoxically, this prohibition laid foundations for the phenomenal success of Scotch whisky, stimulating demand for quality spirit. Most Scotch was purchased in illegal bars and "speakeasies" using importers such as Captain Bill McCoy, of Berry Bros & Rudd, agents for Cutty Sark: 'The Real McCoy' became synonymous with good whisky.

Illegal imported Scotch in US was diluted heavily to meet demand. Bootleggers preferred dark-coloured, strongly flavoured and heavily malted whiskies such as Campbeltown, once the 'Whisky Capital'. Demand for heavily peated malts led to building of high, pagoda-roofed kilns allowing more ventilation, now an architectural motif of malt whisky distilleries. Unfortunately, spirit quality deteriorated through increasing demand and most distilleries had short lives.

President Roosevelt in 1933 lifted prohibition, and further raised duty on the outbreak of war in 1939. The loss of foreign grain supplies, due to the German U-boat cordon, forced closure of all grain distilleries in 1941. However, the War Cabinet used the export value of whisky to minimise war debts to the US. In a memo of 1945, Winston Churchill wrote: *On no account reduce the barley for whisky. This takes years to mature and is an invaluable export and dollar producer.* As matured stocks decreased, distilleries resumed production with continued expansions until 1960s.

However by the mid-1950s bond stocks of whisky were at all-time high and single malt whisky was promoted, with a subsequent steady market growth. Over the last decade, blended whisky sales have declined in traditional markets.

'Proof spirit' (Appendix I), *spirit of standard and approved strength,* was originally determined by dampening gunpowder with spirit and then applying a flame to assess ignition. American and Britain have different definitions of this term. The term 'proof' was defined in the sixteenth century as *of tried strength of quality.*

1.1.3. PRODUCTION OF SCOTCH WHISKY

1.1.3.1. Water

Peat is acidic, decayed vegetation of composition varying with paleobotany – successive layers and plant components. Lowland peats contain more vegetable matter, have a looser, softer texture, burn rapidly and give off more dust. In Orkney, peat is graded into 'fog', rooty top layer, 'yarphie', the small roots and 'moss' the deepest, darkest layer (MacLean, 1997). Peat bogs close to the sea become saturated with salt spray, and seaweed confers flavour characters to whiskies. Soft *moss-water*, which rises through peat and runs over granite, is considered best for malt whisky production (MacLean, 1997, Cribb *et al.*, 1998). Soft waters, with reduced mineral contents, are good solvents for malt extractives during mashing and reduces spirit yield, i.e. alcoholic strength at 48 - 51% abv, rather than 75%, but enhance flavour (MacLean, 1997). Waters for dilution at bottling should have low calcium and iron contents to minimize discolouration or precipitation in retailed whiskies. Additional minerals (e.g. calcium, magnesium and zinc) impart *spiciness* to finished whiskies. Lime- and sandstones yield waters rich in carbonates or sulphates, neutral or slightly alkaline, and hard.

1.1.3.2. Barley/cereal

Quality distilling barley is high in starch (high fermentability), of low protein/nitrogen content (1.5 +/- 0.1%) and with large corns (>2.2 mm) (Paterson & Piggott, 1989).

TABLE 1.2. Chemical composition of cereals (% dry weight): (Palmer, 1997)

	starch	sugar	protein	lipid	β-D-glucans	pentosan	fibre	minerals
Barley	64	3.0	10.0	3.5	3.5	9.0	5.3	3.0
Wheat	65	2.0	11.0	3.0	0.4	7.0	2.5	1.8
Maize	74	2.0	11.0	5.0	0.3	4.0	3.5	2.0

Certain barley cultivars yield more of certain congeners (e.g. high esters from summer barley) but variety choice is thought to have little impact on overall flavour (MacLean, 1997). Major grains in production are maize (*Zea mays*), barley (*Hordeum polysticum*), wheat (*Triticum vulgare*) and rye (*Secale montanum*) (Table 1.2).

Within cereal endosperms, individual starch granules are embedded in protein matrices (70 - 90% as hordein and glutelins) contained within cell walls: 70 - 75% glucan and 20 - 25% pentosan (Fig. 1.1). Barley lipids (mainly triglycerides) are primarily from linoleic (57%) acid but also saturated palmitic (23%), and unsaturated oleic (11%) acids.

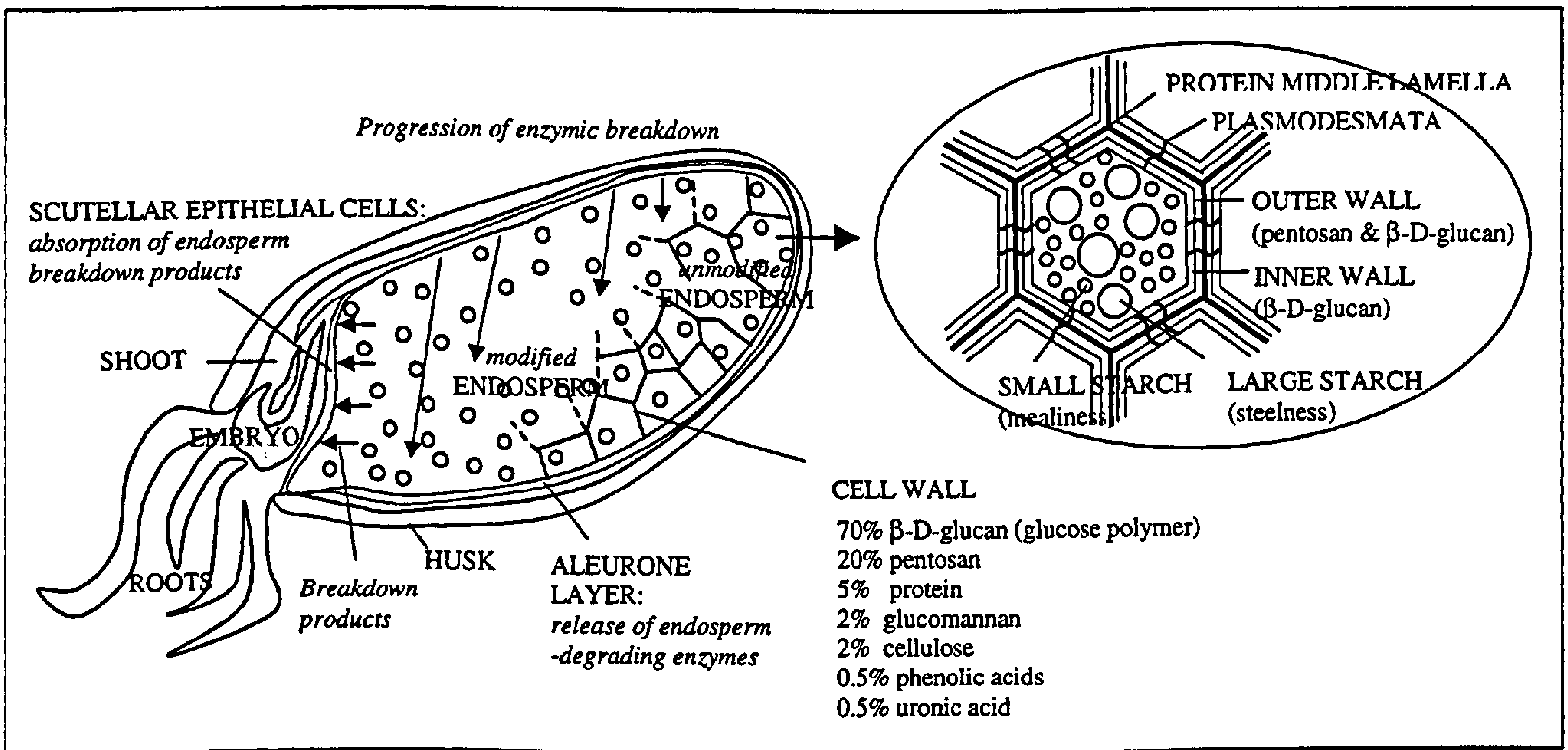


FIG. 1.1. Structure and composition of malt barley

1.1.3.3. Kilning

Kilning stabilises malt at specific degrees of modification, and introduces *peaty* characters. The process reduces microbial contamination of barley. Although traditional peat as primary fuel has been replaced with natural gas or heavy oils, peat smoke, *reek*, is still introduced to impart *smoky* characters into whisky. Rock sulphur or gaseous sulphur dioxide (SO_2) introduced in malt kilning reducing nitrosodimethylamine (NDMA) contents.

Peat well dried, at 25 - 60% moisture contents, is combusted at high temperature in a short-time intensive smoky period (*reek*). In kilning, drying proceeds until malt moisture contents are about 20 - 25% in a *free-drying* phase. Then in the second, *diffusion*, until 10 - 12%, with less water released and increasing barley temperatures. The final *curing* phase, start higher malt temperatures (80 - 100 °C). Final moisture contents should be < 5%.

1.1.3.4. Mashing

Ground malts (grist), mixed with hot (<70 °C) water and transferred to a mash tun, a circular vessel with a stainless steel or cast iron lid to conserve heat. The maltose-rich wort is filtered and cooled (~20 °C) then pumped into a washback for fermentation by yeast.

1.1.3.5. Fermentation

Fermentation is important for formation of congeners contributing to character. Flavour compounds are released from yeast cells, in autolysis, and react with other compounds. Yeast strain and pitching influence yields of flavour-active congeners and ethanol. Pitching yeast can be mixtures of primary distillers' yeast, and secondary brewers' or bakers' yeasts. In whisky production a primary *Saccharomyces cerevisiae* is used for maximum utilisation of carbohydrate, rapid fermentation and high ethanol yield and tolerance. Typical distillers' yeasts ferment <46 °C at pH 3 - 10 in the presence of <15% ethanol and between 0.1 and 25% total sugars. Secondary yeasts, for flavour formation, are slower fermenting, less ethanol tolerant and give lower yields (Berry, 1984), but remain more active near at the end of fermentation (Korhola *et al.*, 1989; Paterson & Piggott, 1989). There is a loss of *estery* characters through replacement of spent brewers' yeast with cultured distilling yeasts (e.g. Lal D) (Hay *et al.*, 1994).

The pitching rate, or inoculum level, of a typical whisky fermentation (at 2 x 10^7 cells/ml) is likely to maximise formation of medium chain fatty acid and ester congeners (Ramsay & Berry, 1983). Increasing pitching rate yields more alcohol but decreased fatty acids and esters (Berry & Watson, 1987).

In fermentations, greater than 33 °C promotes death and lysis of yeast. At the end of a typical fermentation lactic acid bacteria in worts increase (Berry, 1984; Lavery *et al.*, 1986). Laboratory experiments reported 98% viability of yeast and low bacterial contamination ($<10^3 ml^{-1}$) at 30 °C (Ramsay & Berry, 1983). Industrial scale experiments at 36 °C showed yeast viability decreased to 60% and high levels of bacteria (Lavery *et al.*, 1986).

The initial growth (acceleration) phase (typically <12 h) is characterised by exponential increase in yeast cells (2 x 10^8 cells ml^{-1}) and a parallel rise from ambient temperature to 33 °C through catabolism of sugars: maltose, maltotriose, maltotetraose, maltopentaose and higher oligosaccharides together with some monosaccharides (glucose and fructose) and disaccharide (sucrose) (Korhola *et al.*, 1989). Sucrose is hydrolysed by yeast invertase (*β*-fructofuranosidase) (Korhola *et al.*, 1989). In the subsequent stationary (linear) phase, ethanol content increases linearly with no further increase in yeast numbers. In the decline phase yeast decreases and bacteria increase. Wash bacteria produce lactic acid, decreasing pH. At higher temperatures and ethanol concentrations there is yeast autolysis (Berry, 1984). Typically at the end of fermentation, 85% mash fermentables have been converted to ethanol, carbon dioxide and yeast biomass.

Typical, barley grain microbial loads are 10^7 bacteria g^{-1}, in germination 4 x 10^9, and 2 x 10^8 during kilning (Berry, 1984). Bacterial species isolated from barley include *Pediococcus cerevisiae*, *Streptococcus lactis* and *Leuconostoc sp.*. Most bacteria are killed during mashing and early in the fermentation (Berry, 1984). Secondary yeasts and unsterilised worts are potential sources of wort contamination by *Lactobacillus* and *Clostridia* that can impart off-notes in distillates (e.g. *pungent-peppery, creamy, buttery* or *rancid*). Decreased pH also promotes formation of furfural (Berry, 1984; Williams *et al.*, 1984).

1.1.3.6. Distillation

Distillation is a fractional separation of volatile flavour compounds from fermented worts (wash). Final distillate temperature should be <20 °C, to minimise evaporation losses. Seasonal variation in water temperature influences condenser cooling, changing congener concentrations (Nicol, 1989). There are two alternative distillation processes: pot for malt whisky (Fig.1.2) and continuous for grain (Fig. 1.3).

Pot Distillation: First, wash distillation: wash (5 - 8% abv) is distilled over 5 - 6 h to yield low wines at 23% abv, with termination when wash is ca. 1% abv. The residue, pot ale, is discharged for animal feed. Frothing (foaming) during wash boiling is enhanced by unfermented dextrins, yeast and husk residues and dissolved gases. A still is filled to two-thirds capacity (Nicol, 1989) and operators use sight glasses to minimise foaming that may carry less volatile components, and off-notes, into low wines and final spirit (Goodhall *et al.*, 1999). Distillation of a "immature" wash (<35 h) may confer *green* notes so distillers prefer a 44 h wash (Nicol, 1989), encouraging *Lactobacillus* activity: to a certain extent longer fermentations yield spirit of greater sensory quality.

The second spirit distillation, yields initially *fore-shots,* or *heads,* rich in low boiling compounds. This 80 - 75% abv fraction is rejected until distillate is 72 – 75% abv, and distillates should remain clear. In demisting, clarity of distillate diluted to bottling strength (40% abv) is assessed as haze from long chain ethyl esters (Nicol, 1989). This spirit, or middle, fraction is collected until a cut-off point of typically 69% abv for light- and 60% for heavily flavoured whiskies. The third or final fraction is the *feints* (or *tail*). *Feints* and *heads* are generally recycled in distilleries.

Still design is important for flavour - capacity, height, heating and angle of lyne arm. Tall stills collect lighter and more volatile congeners, with higher molecular weight fractions being refluxed. Area of copper contact is important in influencing sulphur contents in spirit particularly with smaller stills. A narrow lyne and neck tends to increase vapour velocity and reduce time of contact with copper.

***Continuous Distillation* (*Coffey still*)**: The original Coffey still was a single column with a stripping section (18 - 22 plates) at the base and rectifying section (50 - 75 plates) at the top. Heads compounds, mainly fusel oils such as *iso*-amyl alcohol, were continually removed via a side stream column (Whitby, 1992). A major disadvantage was liquid discharged from the analyser base was pumped to the top (Panek & Boucher, 1989). In modern stills there are two, *rectifier* and *analyser,* columns (Fig. 1.3), for plant height reductions, and treatment of hot feints. Wash, fed to tops of rectifier, flows to the top of the analyser then under gravity to the base. Ethanol and congeners are steam stripped from the analyser bottom pass to the rectifier and are recovered on the basis of volatility. The heads, mainly fusel alcohols, are collected at plates 7 - 10, spirit at plate 32. Vapour (feint or tail) above plate 32 is trapped by a cooling water coil, condensed and recycled to the analyser. The wash flows in cross-flow pattern a across each plate. Sieve plates have holes for vapour passage mixing with liquid to enrich final volatile congeners. Analyser plate holes must be designed to avoid blockage from grain solids (Panek & Boucher, 1989), entrapment of liquid and ensure adequate mixing of liquid and vapour (Panek & Boucher, 1989).

1.1.3.7. Maturation

New spirit (*ca.* 70% abv) is diluted with demineralised water to 63.5%, considered the most balanced extracter of cask congeners. In maturation of Scotch, distillates are matured in reused (mainly ex-Bourbon or sherry) casks, traditionally originating in Scots parsimony. Bourbon casks (hogsheads; 200 – 250 litres), 93 % total in 1997, are re-assembled from staves with 25% of new wood, including heads. Sherry casks (butts, puncheons; 500 litres) previously used for sherries: dry oloroso, fino, and amontillado. Different sherry styles will impart varying flavours. Cask demands for ex-sherry in Scotch industry are increasing and the current price (£250) is ten-fold higher than ex-Bourbon.

As only volatile congeners are present prior to oak maturation (Singleton, 1995), less volatiles, generally wood-derived (e.g. vanillin, and other lignin derived, aldehydes and acids and tannins) are introduced, at levels influenced by toasting and charring processes that degrade polymers. The oak cask and its treatment are central to maturation but oxidation also plays a major role.

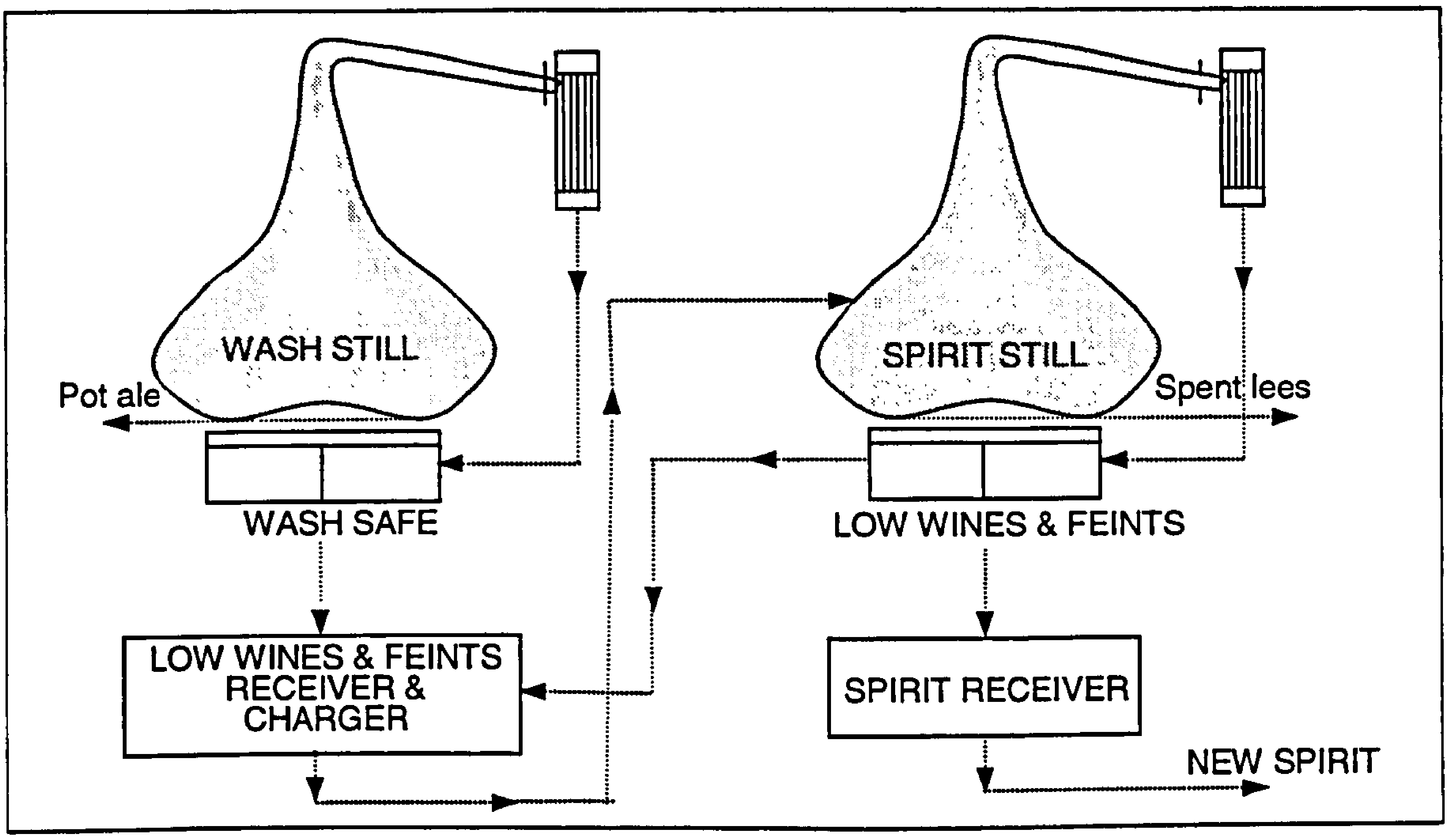


FIG. 1.2. Production of new spirit from pot distillation

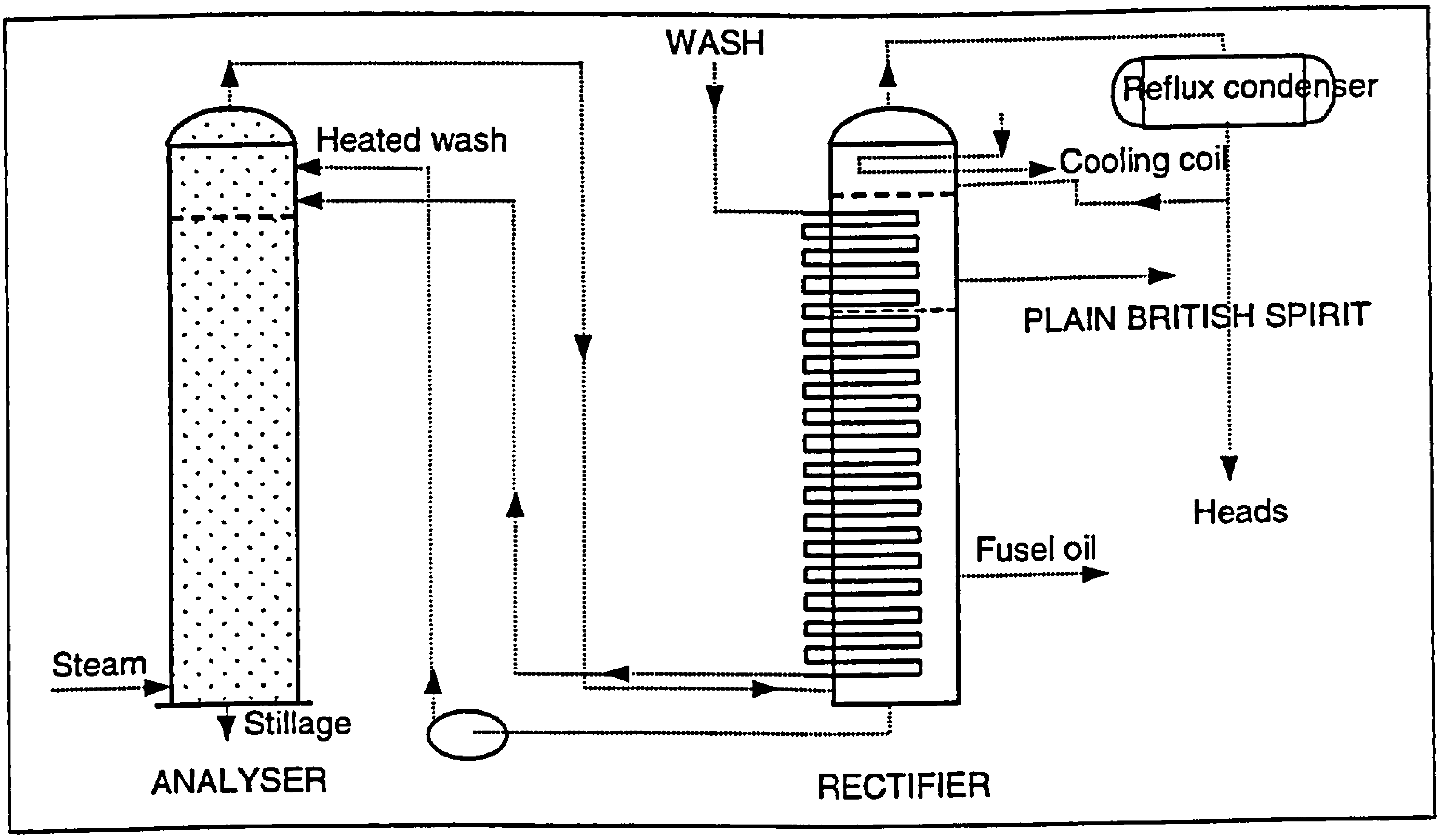


FIG. 1.3. Production of plain British spirit by continuous distillation

Oak wood

Traditionally, white oak is preferred for maturations because of its tough, mouldable wood with high extracts that influence flavour and inhibit rot organisms (Singleton, 1995). Oak casks do not leak partly through the presence of tyloses, occluding structures in vessels from adjacent parenchyma cells, associated with conversion of sap- into heartwood (Mosedale, 1995). Trees for staves should be >80 years old.

American white oak (*Quercus alba*) wood consists of cellulose (49 - 52%), lignin (31 - 33%), and hemicelluloses (*ca.* 22%) and a range of extractables including volatile oils, volatile and non-volatile acids, sugars, steroids, tannic substances, pigments and inorganic compounds (Nishimura *et al.*, 1989). Lignin-derived congeners have greatest influence on flavour, contributing *vanilla* and *spicy* characters. Hemicelluloses, form a heteropolymer matrix rich in pentose sugars, dominated by xylose, that is caramelised in charring adding *caramel* and *sweet* notes and colour. Cellulose, the framework of wood, makes little contribution to flavour. Wood polysaccharides are considered hydroscopic and lignin itself is almost water repelling (Singleton, 1995).

American casks are primarily from white oak whereas 24 different oak species and hybrids are used in Europe, only eight of are economic importance. The two dominant, *Quercus robur* and *Q. petraea* (Mosedale, 1995), differ in anatomy and composition from each other and American (*Quercus alba*) oak. Such differences confer varying flavours (e.g. new and extractive) to spirit but in practice selection of casks is limited. Cask reuse depletes flavour congeners and although charring and rejuvenation can enrich congener precursors not to levels in new casks. Bourbon and Scotch casks differ significantly in extraction of non-volatile such as lactones and coumaric, ferulic and ellagic acids (Piggott *et al.*, 1993a).

Warehouse

The traditional bonded warehouses for maturations, *dunnage*, are low and stone-built with an earth floor and humid circulating air. Three-high racking is used in contrast to twelve in larger, modern racked-warehouses with mechanically controlled temperatures. Casks lose ethanol and water through staves at *ca.* 2% of volume year^{-1} (*the angel's share*). Traditional environments are considered to produce more *mellow* whisky than a modern warehouse.

Cask location and geographical factors influence maturation process. A steady maturation is thought desirable. Coastal bonds, subject to gales and damp salty air, produce whiskies with different characters than inland where seasonal temperatures vary more widely.

1.1.4. SCOTCH WHISKY REGIONS

The Scotch whisky region was initially divided for tax purposes under the Wash Acts (1784), into Highland and Lowlands. Until 1900, region of origin made a discernable contribution but as distilling to character has been modernised influences have been reduced. Canaway *et al.* (1983) found that sensory character of whiskies of four regions (Speyside, Lowland, Islay and Other), only Islay were distinctive. The following division of regions are adapted from a key monograph of whisky (MacLean, 1997) (Fig. 1.4).

Geologically, Scotland can be divided by notable features that influence of water quality (Fig. 1.5) (Cribb *et al.*, 1998). The *Southern Upland Fault*, separates Ordovician and Silurian rocks, grey slates and sandstones from the Midland riff Valley, floored by fertile plains on Devonian and Carboniferous rocks. The *Highland Boundary Fault* is followed by the folded and metamorphosed Dalriadan rocks of the Grampian

Highlands and Argyll Islands regions. The *Great Glen Faults* separates Dalriadan *schists* (intensively metamorphosed mudstone) of Grampian block from the older Moinian rocks, pushed over Lewisian rocks, as old as 2900 million years. The Far North consists of Devonian Old Red Sandstones of the Orcadian (marine) Basin.

1.1.4.1. North Highland region

Most distilleries, including those in Orkney, located in coastal *marine basin* (Cribb *et al.*, 1998) have a distinctive saltiness in their whisky (MacLean, 1997). Regional whiskies benefit from a complete final year of sherry-cask maturation, developed at Glenmorangie. Only Clynelish and Invergordon grain distilleries are in operation currently (MacLean, 1997).

1.1.4.2. East Highland

Speyside region has 55 malt distilleries, 23 built between 1886 and 1899 (MacLean, 1997), producing two thirds of Scottish malt. Water from extensive metamorphic sandstones, mudstones and limestones (Dalriadan rock) has increased alkalinity and hardness. This region is very suitable for barley-growing with ample supplies of peat. Generally, whiskies are lighter (*sweet* and *estery*) than from other regions (MacLean, 1997) with unique histories and distinctive flavour characters.

Forfarshire and ***Aberdeenshire region*** had at one time 36 distilleries, but in 1997 only Glencadam was in production. Central Highlands whiskies tend to be *lighter* bodied and *sweeter* than other Highland malts (MacLean, 1997).

FIG. 1.4. Scotch whisky regions (MacLean, 1997)

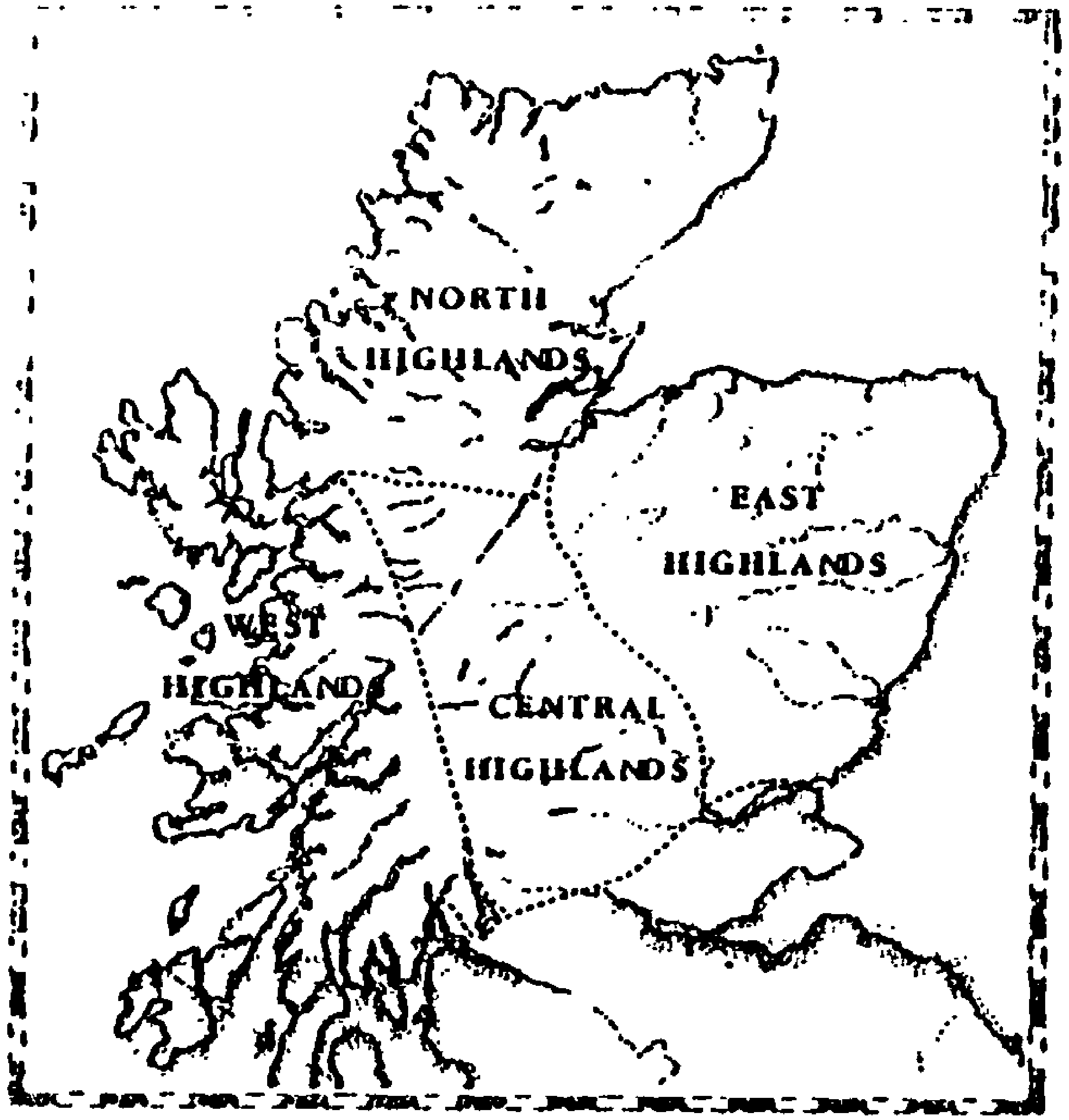


FIG. 1.5. Geographical division of Scotch whisky regions (Cribb *et al.*, 1998)

1.1.4.3. West Highland

There are two well known (Ben Nevis and Oban) distilleries operating. ***Campbeltown*** was well suited for distilling, away from centres of government and with abundant barley and peat. The heavy whiskies were less favoured by blenders than lighter and more fragrant Speyside malts and in 1998 there were only two distilleries (Springbank - *Longrow* and *Glen Scotia*).

Islay malts are famed for *smokiness*, attributable to *phenolics*, and *dark, peaty* island waters. Distilleries are divided into two groups, the Northern and Southern, with Bowmore between. Water can be acidic, but of low mineral content (Cribb *et al.*, 1998). Southern distilleries (Ardbeg, Lagavulin, Laphroaig and the moth-balled Port Ellen) produce heavily peated whiskies. Of the northern distilleries, only Bunnahabhain and Caol Ila are in production. Bruichladdich, the most westerly distillery, with water percolated through the oldest rocks (Rinns gneisses), ceased distilling in 1995.

1.1.4.4. Lowland or Midland regions

Distilleries are divided geologically into two groups: those drawing waters from Dalraidan rocks to the north of the Highland Fault and those with a source to the South (Cribb *et al.*, 1998). Distilling depends on availability of cereals - wheat and oats, as well as barley – and in developments in agricultural technologies (notably fertilisation and drainage). Highland distillation depended on grain surpluses where farming was predominantly pastoral with livestock. In the Lowlands, suited to arable farming, distilling become larger-scale and more industrialised through the effects of the Wash Act (1784) and advent of continuous distillation. Availability of a range of cereals also encouraged mixed mashes with largely unpeated malts. Lowland malt whiskies are lighter in colour and flavour (*green, grassy, herbal, grainy* and *floral* notes), with characters considered by some suitable for aperitifs.

1.1.5. CLASSIFICATION OF OTHER WHISKIES

1.1.5.1. Irish whiskey

The art of distillation was known in Ireland by the 14th century, and widespread by the 16th. Subsequently, illicit distilling (poteen-making), in which potatoes and molasses were often used instead of malted barley (Murray, 1997), was ubiquitous in the countryside. On Christmas day, 1661, the English government charged tax at four pence per gallon on whisky. Duty was further raised to support the Napoleonic Wars. Barley was dried for short periods with intensive peat smoke so that whisky produced in Ireland had a *peaty* aroma and "poteen-flavoured" character. Four big Dublin distillers were successful: John Power, John and William Jameson, and George Roe. The continuous still was not attractive to the Irish distillers, who wanted to keep their whisky light but full flavoured. During prohibition (1920 - 1933) Scotch blended whisky was more successful and Eire suffered civil wars (e.g. the Irish War of Independence and civil war). By 1933 the Irish whisky distiller had lost most of their export markets.

Irish whiskey character is different from Scotch. In the late 19th and early 20th centuries, five cereals were in common use: malt (30-50%), barley (30-40%), oat (20-30%), wheat (5-10%) and rye (3-6%). Mashing takes longer, at lower temperatures than Scotch, adding greatly to *oiliness* (Murray, 1997). However unmalted barley has become the mash substrate in the two remaining Irish distilleries (Bushmills and Middleton). The major process difference between Irish and Scotch whiskies is the number of distillations (three times) in medium-sized pot stills. In theory, Irish maturation should be slower, as casks have less circulated with air stacking upright top of each other, but in practice is faster due to warmer climate in Middleton. Premium character in Scotch can be achieved after 30 to 40 years, but in Ireland rarely beyond 20 years (Murray, 1997). Irish whiskey maturation uses more sherry casks than Scotch.

1.1.5.2. American whiskey (Bourbon)

Definitions by American Bureau of Alcohol, Tobacco and Firearms: 'Bourbon', 'rye', 'wheat', 'malt' or 'rye' whiskey are spirits produced, at not exceeding 160 degrees proof, from a fermented mash of not less than 51% maize, rye, wheat, malted barley, or malted rye grain, respectively. Distillate is matured, at not more than 125 degrees proof, in newly charred oak containers. Corn whisky, from a 80% maize mash matured in used or uncharred new oak barrels, may include a mixture of other whiskies. To be labelled 'Tennessee' Whisky must be produced and aged in wood in the State of Tennessee. All whiskies (Section 5.22, BATF regulations) aged a minimum of two or more years can be designated 'straight'. Light whisky, a US category, is distilled at >160 ° proof but <190; aged at least two years in used or uncharred new oak barrels.

The first commercial US distilleries distilled rum - whisky production only began in 1733 when the Molasses Act imposed a duty on non-British imports and molasses smuggling from Spain and France increased costs. Distilling was imported by Presbyterians (mainly the Scotch-Irish and German) who started to distil with rye. As whisky became important in social life, there were prospects of a tax war and in 1794, George Washington (himself a rye distiller) put down a Whiskey Rebellion (Appendix I) of distilling farmers of Pennsylvania, Pittsburgh, Maryland and Ohio.

Certain distillers fled from Pennsylvania to Kentucky, where a cottage industry was already established by the early 1790s. However, in Bourbon: Jim Beam, Wild Turkey and Heaven Hill are dominant brands and in California the Anchor distillery, the smallest distillery in America, produces a single rye malt whiskey (Murray, 1998).

In Kentucky, a distinctive lighter style of whiskey, Bourbon, was produced from maize, initially by Jacob Beam who sold 'Jim Beam' in 1795 (Travis, 1998). Most leading brands are still produced in this state (Travis, 1998). Two factors have contributed to the success of Bourbon: the accidental discovery of cask charring effects (Ralph, 1999); and in 1790 the continuous distillation process of the Philadelphian Colonel Alexander Anderson. After prohibition, new regulations defined the production of bourbon, rye, corn and blended whiskies and other alcoholic beverages.

American whiskies maintain standards for six production factors: grain proportion in mash, mashing technique, yeast strain, fermentation environment, still type and operation parameters, barrel specification and maturation process. A typical Bourbon has a mash of maize (70%), rye and malt (15% each). Tennessee whiskey with maize (80%), rye and malt (10% each); rye whisky with rye (51%), maize (39%) and malt (10%) (Ralph, 1999). Maize is pressure-cooked to gelatinise starch, malted barley added for saccharification, and rye added on cooling. Traditionally, spent grains (stillage) from a previous distillation are used to sour (control acidity) in mash and limit bacterial infections (Murray, 1998). Using backset, stillage (20 - 30% total) is screened or centrifuged to reduce solids before reuse (Ralph, 1999). However, the process can be potential contamination source and inconsistent flavour. In modern distilling, lactic souring is practised as follow: small grains, rye and malted barley are cooked to about 63 °C, and pH adjusted to 3.8 with lactic acid bacteria grown in a mash is mixed with yeast (Ralph, 1999). Even hops may be used for natural bacteriostatic function against Gram- positive bacteria (Travis, 1998). Most American whiskies are from continuous distillation, although certain have a second 'doubler' or 'thumper' (Ralph, 1999). Two re-opened distilleries (Labrot and Graham) use triple pot distillations (Murray, 1998). Bourbon distillers cannot include artificial colouring and blending materials. Through dramatic seasonal variations, production is in spring and autumn.

In American blended whiskey, grain neutral spirits (GNS), with no age specification, can form up to 80% retail product and this can be matured in new or used uncharred barrels. There are many technical terms and production strategies specific to Bourbon production reviewed in Travis (1998).

1.1.5.3. Canadian whiskey

Canadian distilling was established in the 18th Century by Irish and Scottish settlers (Wright 1998), and whisky has been produced since the late 1940s using rye, abundant and cheap (Morrison, 1999). Canadian whiskies are defined as blends of distillates (at under 95% abv) from cereal grains, aged for not less than three years in small (<681 litre) wooden vessels. Blends are of lighter column distilled whiskies with more flavoured column or pot still whiskies. Lighter base (grain) whiskies, 90 - 95% abv, are from maize mashes, saccharified with microbial enzymes and/or malt. Flavoured heavy-bodied whiskies are from mixtures of small grains, including rye and malts barley.

Canadian whiskies may contain <9.0% of special flavourings or blenders (e.g. wines and sherries, rum, brandy, bourbon, or malt whiskies), to yield niche products. In mashing, most distillers use microbial enzymes instead of malt (Wright, 1998;

Morrison, 1999). Lactic-yeasting is used for all-rye grain mashes (Morrison, 1999). Maturations are in new, charred or uncharred white oak wood, ex-whisky, -sherry, -bourbon or -rum barrels in two strategies: with post-blending as in Scotch; or pre-blending of selected distillates prior to maturation. Generally, Canadian whiskies have characteristic *spicy* or *minty* notes originating in use of rye, but no specific impact compounds were identified (Piggott & Conner, 2000).

1.1.6. CURRENT WHISKY MARKETS

There was a 5% increase in the UK alcoholic drinks market in 1998 to £29 billion: beer (52%), wine (23%), spirits (21%) and cider (4%). Of the 21%, dark spirits (whisky, dark rum, brandy and liqueurs) formed 61% and light (vodka, gin and light rum) 39%. The largest dark spirits product category (46%) was whisky with 93% sales (by volume) Scotch products: blended (85%), malt (7%) and imported (8%) (The UK Food and Drinks Report, 2000). Malt whisky sales are limited, but at the high value end of the market confer prestige on producers (The Global Drinks Industry, 1997). A new prestigious niche is extra premium malts whisky (e.g. Johnny Walker Highland malt). A study of repertoire drinking in consumers (Mintel, 1997) has ascertained that although malt drinkers are less likely to drink other (Deluxe and Standard blended) whiskies, the inverse is not true. Blends are popular with an adaptability and light body important in perceptions of American drinkers

Links between drinks and social activities are strong in younger adults (Mintel, 1997): home consumption, notably of spirits, increases with age. Scotch whiskies especially malts, are consumed heavily by empty-nester adults (>65s); other (Bourbon, Canadian and Irish) whiskies are largely consumed by young adults (<25s), and especially in London (Key Note, 1999). Vodka was the most popular spirit in pubs or restaurants (Key Note, 1999).

The value of the UK spirit market has declined steadily, 25% (6% for domestic, 19% for exporting) since 1994, and this is predicted to continue over the next 5 years (Key Note, 1999). Traditional spirits markets are falling but consumption by the young is being stimulated by the launch of spirit- base mixture products (Mintel, 1997).

1.1.7. AUTHENTICITY OF WHISKIES

Brand authentication is normally carried out by reference comparison of alcoholic strength and higher alcohol (such as methanol, *n*-butanol, *iso*-butanol and *iso*-amyl alcohol) profiles (Aylott *et al*, 1994). *Iso*-amyl alcohol is limited in Scotch grain whisky (removed in continuous distillation) but abundant in malts, rich in cask-extractive congeners. Discrimination of Irish whiskey from other whiskies can also be from congener profiling of higher alcohol (1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol) contents (Gonzalez-Arjona *et al.*, 1999). Higher alcohols were high in Bourbon and sour mash whiskeys, compared to Scotch whisky (Wilson *et al.*, 1991). Colour, also utilised for authentication analysis, shows slow fading on exposure to light and heat (Aylott *et al.*, 1994). As a requirement of straight Bourbon, whiskey is aged in a freshly charred oak barrel for a minimum 2 years, addition of caramel is not allowed. However, in blended whiskey the loss of colour can be compensated by addition of caramel that only imparts 5-hydroxymethyl-2-furaldehyde. The ratio of furfural and 5-hydroxymethyl-2-furaldehyde from freshly charred oak barrel maturation can be used for authentication (Jaganathan & Dugar, 1999).

1.1.8. ORIGINAL WHISKY FLAVOUR WHEEL

A whisky flavour wheel was proposed by the Blending and Bottling Committee of Pentlands Scotch Whisky Research Ltd (presently Scotch Whisky Research Institute) (Shortreed *et al.*, 1979). The vocabularies in the original whisky flavour wheel were from 26 persons involved in distilling, blending and R & D, and specifically designed for use by distillers and blenders. Brewing terms were not suitable for distilling (e.g., *grainy* and *malty*). Unique vocabularies for distilling included objective terms (14) and abstractive terms (22) expressing overall quality. Principal terms at the wheel centre and descriptive terms at the third tiers could be spontaneously "keyed-in and-out" within the system. Various chemical and technical terms were included. *Woody* lyes extended the subtier for various stages of maturation.

Sensory analysis in the whisky industry required extensive use of vocabularies to train panels. The necessity of modification of terminology especially technical and chemical attributes was apparent. Revision of the original wheel was also demanded for commercial practice since some of negative and specific attributes were not applicable for consumer use. In the revised flavour wheel, the original wheel was split into two for industry and consumer purposes by omitting negative terms. MacLean (1997) published a whisky flavour wheel with 8 primary tier terms (*cereal, estery, floral, peaty, feinty, sulphury, woody* and *winey*), but second and third tier terms were extremely subjective (e.g. *exotic smoke, industrial*), specific (e.g. *Dundee cake*) and mixed with chemical attributes. Moreover, the panel used was limited (the Scotch Malt Whisky Society) and used a small number of selected members and regular tasters.

1.2. SENSORY MECHANISM

1.2.1. Sensation and perception

Intrinsic drink attributes are assessed using vision, aroma, taste (gustation), touch and hearing (Land, 1983). Initially sensation is followed by subsequent perception and attribute integration into a mental image of the product. Such differentiation is supported by positron emission tomography (PET) showing specific regions of brain surface with different functions during olfactory processing. Visual and mental image processing were also associated with semantic olfactory processing (Royet *et al.*, 1999). Hopfield (1999) subdivided processing of stimuli into: aroma memory and recognition; background elimination; aroma component separation.

No discrimination was made between a sensation and its perception until at the end of 19th century and these were considered one operation (Harper, 1972). Now *sensation* is thought an unmediated response to the totality of input stimuli; *perception* as elaboration of sensational representations using stored experience i.e. knowledge. Harper (1972) concluded sensations as the basic elements or components on which a reductionist approach could be adopted. Perceptions, holistic, are complex, arising from subjective organisation and integration of sensations (Sheen & Drayton, 1987).

Gestalt psychology was a distinctive contributor of concepts and a main precursor of contemporary cognitive neuroscience. In the theory, establishing cognitive order-formation derived from self-organization (*macroscopic level*), the importance of elementary neurone work units (*microscopic level*) was not appreciated. It was difficult also to comprehend perceptual experiences, except as abstract concepts. Neither descriptions of sensation nor phenomenal experience of transformation from sensational to perceptual representations were available.

Flavour sensation arises from integration (or interplay) of signals produced as a consequence of neural data: aroma, taste and chemesthesis (chemical sense such as *irritating* or *pungency*) (Laing & Jinks, 1996). Heymann *et al.* (1993) has defined flavour as the psychological interpretation of a physiological response to a physical stimulus (Noble, 1996). From psychophysics theory *sensation* arises stimuli whereas *perception* is psychophysical interpretation. Similarly, *meaning* is considered primitive and directly perceived as *a part* of the 'behaviour' or 'perceptual' environment. *Value* is the synthetic result of learning or 'physiognomic character' through interactions of intrinsic attributes with experience of consumption.

Koffka (1935) and Köhler (1944) defined a current view. An assessor obtains *meanings* and *value* from stimuli in three distinct processes. Firstly, the assessor (the ego, *self*) must recognise, on the basis of experience, a glass of whisky as an object of perception. Secondly, detection of perceived meanings (sensation) are from further organisational processes. The assessor will judge the stimuli against his mental map of whiskies. Physical forces (microscopic level) also provide a precise dynamic counterpart to such process, without ontological priority (Kelso *et al.*, 1991). Perceived values (perception) are the interaction between object and brain field (*synergetics*). However, a commonality between physical (brain process – *microscopic level*) and mental (cognitive phenomena - *macroscopic level*) will only emerge if appropriate and commensurate levels of description and conceptual vocabulary are adopted. A logical format during sensory assessments (Harper, 1972) is as follows: is there something can be detected or a detectable difference (differential); how large is the difference; and what is the nature, character or description of attributes present? (Land, 1983). A drink product will have specific intrinsic and extrinsic attributes, the integration recognised as that product. One will not deconstruct this, unless focusing on specific attributes for acceptance, rejection or analysis. Once focused for action (e.g. in formal analysis), the process is a co-operation between cognitive (*perception*) and physical (*sensation*) factors. Both can be defined or condensed forms of attribute constructs.

A basic tenet of cognitive psychology is that humans have physiological limitations in discrimination and identification of aromas (Laing, 1994). To avoid overload, incoming stimuli are grouped on base of perceived similarity, creating mental sets that form frames of reference for future stimuli (expectation, belief). Such mental references are stored in memory in the brain as *perception* or *values*. Marketers examine sequential processing of sensory stimuli to yield positive expectations that influence future behaviour. New sensory information (*sensation* or *meaning*), is integrated with existing mental reference sets. Before new perceptual stimuli are stored, there may be distortion of multidimensional fields of information. Mental processing in food acceptability is clear from placebo effects (White *et al.*, 1985), neurophysiology providing some anatomical evidence (Mishkin & Appenzeller, 1987) and influence of brand labels (Sheen & Drayton, 1987). Cognitive neuroscience developed by Kelso *et al.* (1990), was inspired by theory of pattern formation and self-organisation concepts of synergetics (Stadler & Kruse, 1994) related to pattern formation theory in olfactory systems from spatial coding (Laing & Jinks, 1996) and temporal filtering (Laing, 1994).

1.2.2. Olfactory neurone

Aroma active components are volatiles limited to < 400 Da (Laing & Jinks, 1996) and <1 kDa (Bell, 1996), and heavy enough interact with receptor proteins in cell membranes (~ 40 kDa) (Bell, 1996).

Reviews of the olfaction (Buck & Axel, 1991; Goodenough, 1998) suggest three cell types dominate the epithelium (Fig.1.6a): the olfactory sensory neurone, the sustentacular or supporting and basal stem cells that generate olfactory neurones. Olfactory cells, as primary receptor cells with unique axons (*filum olfactorium*), are regenerated every 30 - 60 days (Buck & Axel, 1991). The olfactory sensory neurone is bipolar; a dendritic process extending to the mucosal surface and an axon to the olfactory bulb and brain to sub-cortical and cortical regions where higher level processing of olfactory information allows the discrimination of aromas (Buck & Axel, 1991).

1.2.2.1. Reception

Mucosal surfaces have extensive capture systems of cilia with specific binding of aroma compounds, directly correlated with the olfactory loss from Bowman's gland (Fig. 1.6a) (Sengupta *et al.*, 1996; Goodenough, 1998). The aroma response is mediated by proteins in receptor cells membrane, sited in an 1 - 2 cm^2 area in the nasal cavity posterior roof (the olfactory reception). The olfactory mucus interacts with water molecules and large glycoproteins, mucins. Mucal saccharides may be crucial to 'dressing up' aroma-active molecules into specific complexes that fit receptor active sites. Other nasal mucus proteins such as odourant-binding proteins, lipocalins (OBPs), and odourant-degrading enzymes (ODEs) play no role in receptor stimulation (Bell, 1996).

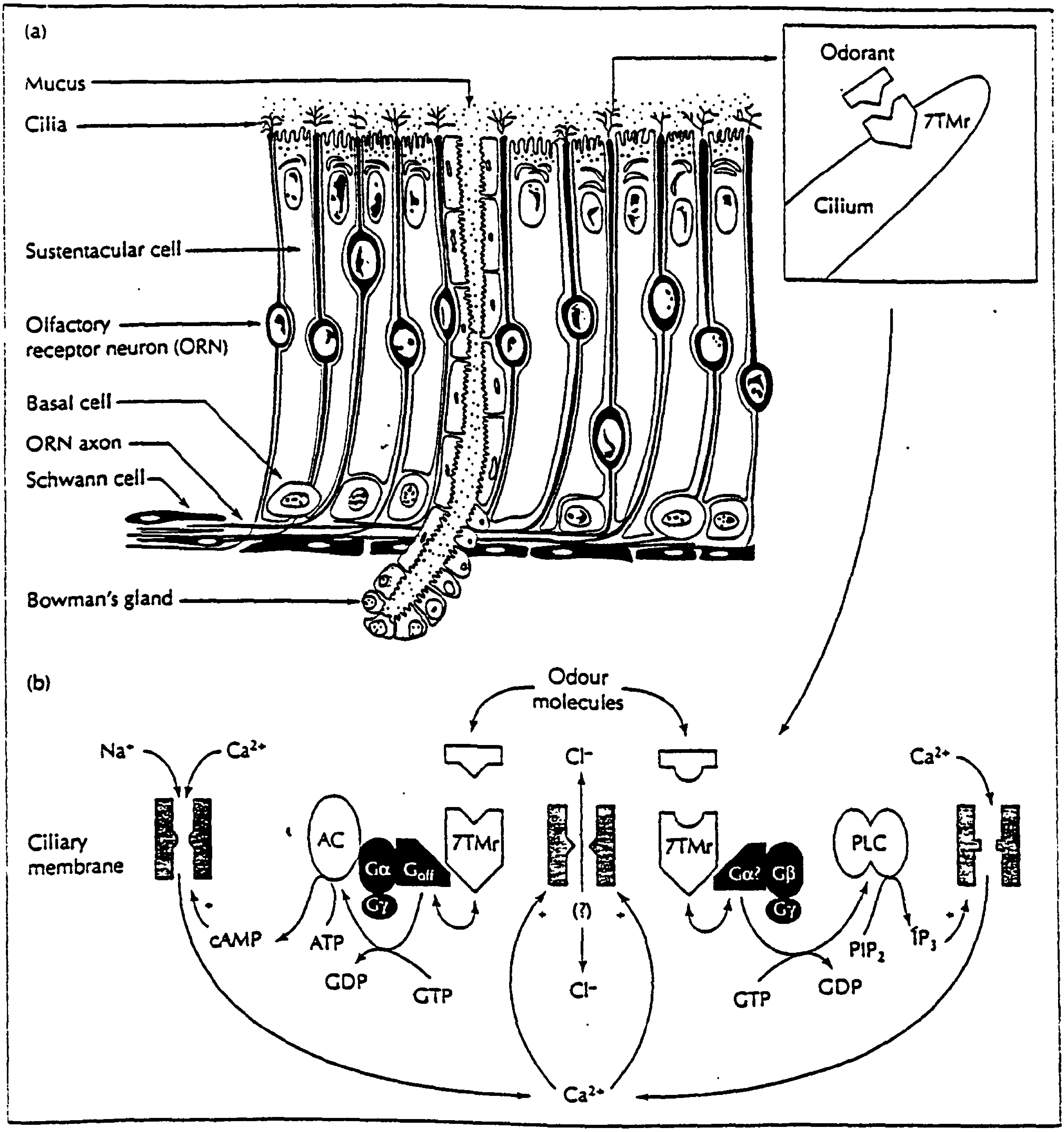


FIG. 1.6(a), Schematic illustration of the olfactory epithelium (reception); (b), A receptor protein with seven transmembrane domains (7TMr) (Bell, 1996)

1.2.2.2. Transduction

Aroma compounds are trapped in a cell-surface receptor protein, a seven transmembrane segment (7TMr) (Fig. 1.6b). This initiates a conformational change (as yet undefined) that starts an enzymatic cascade (transduction), chain of molecular events. Each seven transmembrane protein interacts with one or a small number of compounds. Such a hypothesis accommodates the diversity of aroma perception. The event in the physical environment becomes electrical activity in a nerve cell. Two different G-proteins are coupled: one activating adenyl cyclase (AC) producing second messenger cyclic AMP (cAMP); the other activating phospholipase C (PLC) to produce inositol triphosphate (IP_3). The second messengers (cAMP and IP_3) open the channels admitting ions (Ca^{2+}, Na^+ and Cl-) into the cell, leading to membrane depolarisation (i.e. a sudden flow of charged ions across the cell membrane) and an 'action potential' (a unique series of electrical charges - nerve impulses) (Bell, 1996). These data from many receptor cells converge on a limited number of bulbar cells at glomerular layer (Fig.1.7a) producing a *spatial map* (Fig.1.7b) of activated cells characteristic of that aroma (Laing & Jinks, 1996). This *spatial map* is then transported to other brain olfactory structures (Fig.1.7a), including memory, and triggers specific motor responses. Number of cells contributes to each map is a direct relationship with concentration (Laing & Jinks, 1996).

1.2.2.3. Olfactory bulb to brain

Each olfactory neurone has a single unbranched axon: these together form the olfactory nerve. Neurones corresponding to a specific receptor are randomly distributed within the epithelium, but axons arrive at a single or very few discrete glomeruli within the olfactory bulb. Specific glomeruli are bilaterally symmetrical.

Sub-populations of axons converge on specific glomeruli, a ratio of receptors:glomeruli of < 4:1. Discrimination of aroma arises from specific spatial patterns of glomerular activity within the olfactory bulb (Hopfield, 1999, Goodenough, 1998). Glomerular types are independently assigned binding constants for each aroma-active compound (Hopfield, 1999).

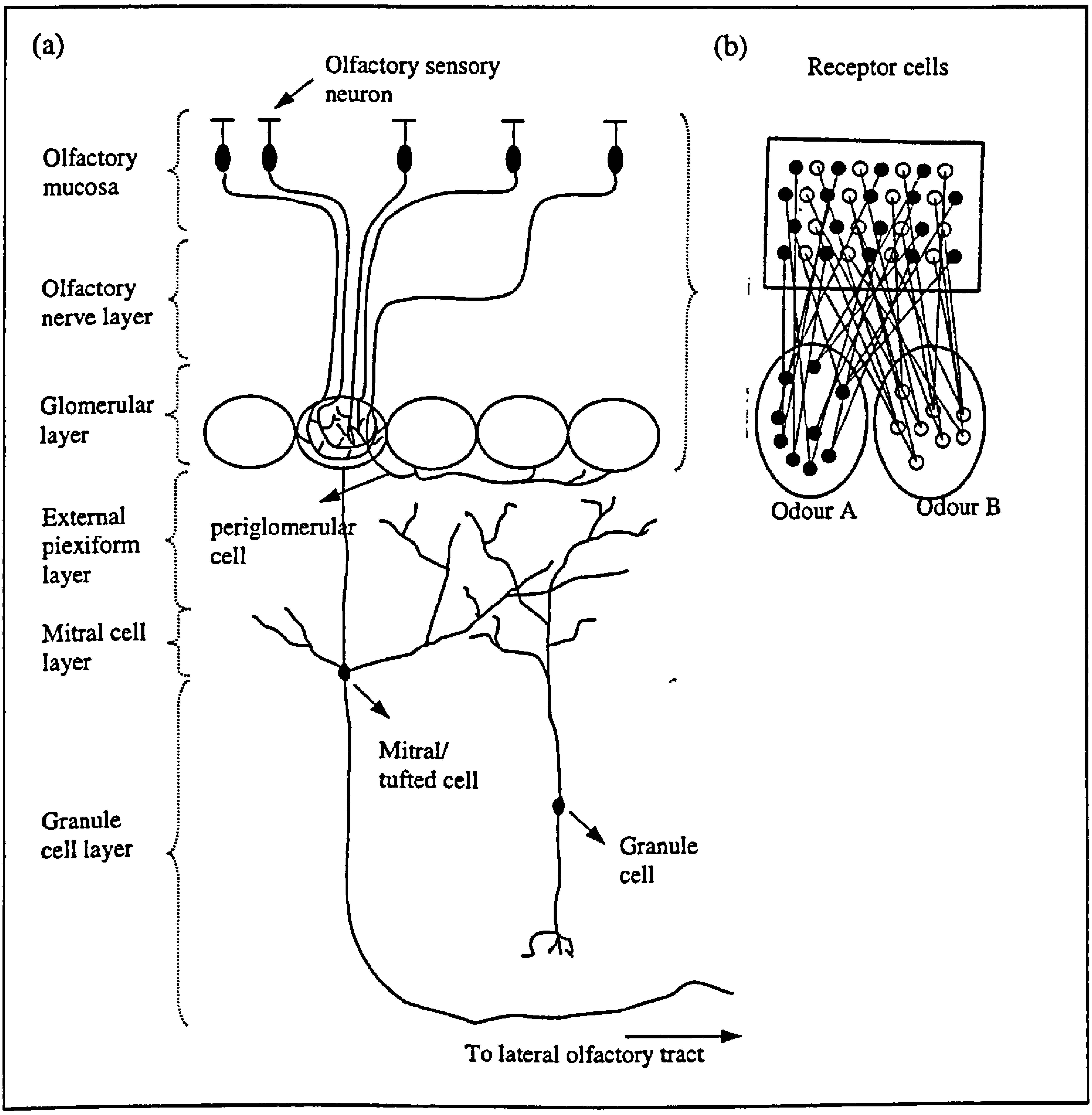


FIG. 1.7 (a), Sensory neurons in the olfactory neuroepithelim. The sensory neurons synapse with the bulb mitral and tufted cells, which in turn project axons to the cortex via the olfactory tract (Goodenough, 1998); (b), Spatial map (Laing & Jinks, 1996).

1.2.2.4. Odour recognition

Recognition or discrimination of aroma involves high level brain processing at the olfactory bulb neurone (Buck & Axel, 1991), depending on spatial maps (Fig. 1.7b). In humans, the olfactory bulb is connected to the primary olfactory cortex by fibres: mitral and tufted cells which are connected to higher cortical centres via the olfactory tract allowing spatial segregation of sensory input from peripheral epithelium to olfactory cortex (Fig 1.7a). Temporal and frontal areas of prepyriform cortex receive direct projections from the olfactory cortex for discrimination, recognition and identification of aroma processing (e.g. BA 47 for semantic association; BA 9 for memory and attention; BA 17 for visual processing, and generate mental image) (Royet *et al.*, 1999).

The convergence of information from primary aroma fibres (receptor axons), produces a spatial map (Laing, 1994), increasing the probability that a stimulus is detected at the secondary neurone. Due to convergence, detection in the olfactory system occurs at concentrations far below one, the recognition threshold (Holley & Døving, 1977). In mixtures of compounds, human can only identify *ca.* seven (Murphy, 1987) or three or four aroma notes (Laing, 1994). Latencies, the time required to stimulate receptor cells, are inversely related to concentration, and influence identification of any aroma compound. Number of receptor cells involved in detection and identification of an aroma-active molecule depends on synergistic, suppression or additive effects between aroma-active compounds (Laing, 1994). Thus, perception of aroma-active mixture is *synthetic* - different aroma notes combine to form new aromas such that individual components can not be perceived *analytically*.

1.3. THRESHOLD

Thresholds have been considered fundamental to food flavour by chemists and certain psychologists. Threshold values are generally specified in physical units, e.g. moles per litre of compounds in a specified base product (Lawless & Heymann, 1998). The values have been utilised as a primary tool, or *iso*-intense reference point (Lawless & Heymann, 1998), to quantify contribution of congeners to flavour. Various strategies have evaluated, including: odour units (o.u) = threshold/concentration (Salo, 1970; Salo *et al.*, 1972); and intensity index (Perry, 1989).

1.3.1. Definition

A definition of threshold is a concentration at which a stimulus is correctly responded (*detection* or *recognition*) to by 50% of assessors. Thus, the definition is similar to concepts used in toxicology and pharmacotherapy: ED_{50} (effect-dose), LD_{50} (lethality-dose) and TD_{50} (toxicity-dose) - all magnitude of effect being subject to biological variation (Ariëns *et al.*, 1976). However, in sensory science, thresholds are dose-effect concepts, and a proportion of detection or recognition level depends on strategy (Morrison, 1982). For two (2-AFC)-, three (3-AFC)-alternative forced choice and triangular tests, this is at 75%, 67% and 50%, respectively with a probability ($0 \leq p \leq 1.0$), of being detected at any given instant. The p is a monotonic non-decreasing function with a threshold at $p = 0.5$ (Morrison, 1982). There is a possibility of error in interpolation from non-linearity to approximate linearity: concentration data is transformed to a logarithm and group distribution to a probability unit - probit (or normal deviates, Z). Accuracy is a function of: accuracy of each individual p value; number and interval of observations (concentrations) (Morrison, 1982); and non-sensory variables - intrinsic variable of assessors, motivation, *Rosenthal effect* and psychophysical interpretations. Thus it can be argued that thresholds are statistical constructs with no conceptual sense (Lawless & Heymann, 1998). Group average thresholds are thought reliable (Brown *et al.*, 1978, Punter, 1983), providing information related to biological activity of flavour-active compounds (Lawless & Heymann, 1998). Generally thresholds are *absolute* for both detection and recognition (Stahl *et al.*, 1978). Differences, in trained assessors, between recognition and detection should be minimal but in untrained assessors it can be larger (Brown *et al.*, 1978).

TABLE 1.3. Terminology of thresholds

Thresholds	Synonyms	Definition (British Standard: 5098: 1975)
Absolute	*Limen*	Minimum intensity that elicits a response a specified percent of the time
Detection	*Stimulus*	Lowest intensity at which a stimulus is perceptible
Difference	*Just noticeable difference* (*JND*)	Smallest change in intensity perceptible, or smallest difference detectable
Recognition		Lowest intensity correctly identified, or lowest concentration for identification
Supra	*Supraliminal*	Above a threshold
Terminal		Intensity above which no changes perceptible, or saturation level

1.3.2. Methods for estimating thresholds

Single-sample test is economical (Brown *et al.*, 1978), but subjective (Morrison, 1982). ***Paired sample tests*** are less dependent on assessor memory, and easy to interpret but require trained assessors. These are time-consuming, expensive, and generate no individual threshold values (Brown *et al.*, 1972). However it is regarded as an objective strategy that allows separation of sensitivity and bias (Morrison, 1982). ***Ascending (or descending) series tests*** are a technically flawed approach using serial dilutions (x2 or x3). Assessors may be subject to significant fatigue and adaptation with errors of habituation or anticipation especially for descending series (Morrison, 1982, Lee *et al.*, 2000b). A variation, ***semi-ascending paired difference*** (Lundahl *et al.*, 1986), is a comparison of three samples with a control, minimising adaptation effects and ensuring independence in judgements but requires care in experimental design. ***Latin square presentations*** (Chapter 3) are compromises between single samples and ascending/descending series, minimizing errors of habituation or anticipation. ***Triangular, Duo-trio and n-Alternative forced choice*** increase detection rate and reduce effects of bias and maximise sensitivity. These may be expensive, with difficulties in interpolation of results. Thresholds are geometric means of last negative (not detected/recognised) and first positive (detected/ recognised) concentration or simply the last positive. A group threshold can be calculated as geometric mean of individual thresholds (Lawless & Heymann, 1998). ***Difference rating tests*** ***(Difference to control, DTC)*** a number of samples, including a hidden control, are scored in relation to a declared control. This can be unreliable but rapid, providing threshold values in a single experiment, and suitable as a preliminary method to determine range and suitable for training people (Brown *et al.*, 1972). ***Signal detection methods*** discard threshold concepts on the basis that sensations emerge continuously from a background with no discernible inflection point. Using differences (d') between two concentrations, a threshold can be defined as only an arbitrary level (Lawless & Heymann, 1998).

1.4. SENSORY ANALYSIS

Sensory evaluation of food employs humans as instruments. Perceptions of flavour can be considered interactions between concentrations of flavour-active molecules in a food or drink and an individual assessing, or consuming the product (von Sydow, 1971). A response to a stimulus is therefore neither the property of the product nor of the assessor but the result of their interaction (Williams, 1994). Thus, flavour quality can be considered an objective set of mental mechanisms: that can be investigated by testing hypotheses. Relationships between instrumental estimates of stimulation patterns and observed variations in human behaviour in word or actions can be analysed and interpreted (Booth, 1994). Since cognitive psychology impact on estimation of food quality, sensory methodology must contain a level of assumption of behavioural effects. These can be either from assessors, data processing and/ or sensory analyst. Examples of this include: use of interval scales, averaging data across whole panel and emphasis on statistical evaluations of results (Piggott *et al.*, 1998).

1.4.1. DISCRIMINATION TESTS

Discrimination tests are used in industry to test if a product differs when ingredients or process have been changed: accept a null hypothesis (sample A = B), or reject (sample A ≠ B).

A-Not-A test: two samples are served sequentially with serving order (AA, BB, AB, BA) randomised. Assessor must be trained to retain the concept of the previous sample. This is used when samples differ but insufficient to influence a specific attribute assessment. ***Paired comparison tests*** use presentation of two samples and selection of product which for more of a designated character such as *fruitiness* or *sweetness* ($p = 0.5$). In an ***2-Alternative forced choice test*** (2-AFC), the nature of the difference is known. A serving sequence (AB, BA) should be balanced to ensure assessors discriminate only on a known attribute (unvariate axis). If assessors are informed nature of difference, power of discrimination can be higher. In ***a Difference paired comparison test,*** with no declared nature, a sequence (AA, BB, AB, BA) must be randomised through assessors and balanced in frequency. ***Duo-trio tests*** use three samples with one marked as reference and assessors are asked which of two products are similar to the reference. The nature of difference is not given and difference is not perceptible probability is $p = 0.5$. In ***constant reference Duo-trio tests,*** a single reference (R_1) is served with two orders (R_1, BA; R_1, AB). In ***balanced reference Duo-trio tests,*** two reference (R_1, R_2) are used with the possible four orders (R_1, BA; R_1, AB; R_2, BA; R_2, AB). The former method is preferred but the latter can be used when products are prototypes (unfamiliar to assessors) or there are limited reference samples (Lawless & Heymann, 1998).

Triadic presentation tests (Thurstonian modelling): the ***Triangular test*** has six possible sample combinations (AAB, ABA, BAA, BBA, BAB, ABB) and chooses the odd one, where attribute nature is undeclared, and. In ***3-AFC test,*** with attribute declared to assessor, there can be only three sample combinations (AAB, ABA, BAA). Since a Triangular test requires three inter-comparisons, variability in judgement is bigger (through sequential effects) than actual differences. Power of discrimination become lower in Triangular test than in 3-AFC methods (Frijters, 1981; Ennis, 1993; O'Mahony, 1995; Lawless & Heymann, 1998), as 2-AFC is more powerful than Duo-trio (Ennis, 1993). An assessor adopts different cognitive strategies with unknown (Triangular, a response bias) and known (3-AFC, 'skimming strategy') attributes (O'Mahony, 1995). *β-criterion* (the degree or intensity required for the stimulus being perceived as 'given attribute', e.g. *salty*) is applied for 2- and 3-AFC. Whereas, with an undeclared attribute, for the Triangular and Duo-trio tests, the *τ criterion* (distance-criterion, the degree of difference required for the stimuli being perceived as 'different') is applied (Dessirier & O'Mahony, 1999). In repeated (or warm-up) assessments, learning effects (Dacremont & Sauvageot, 1997) may switch *τ* to *β-criterion* complicating interpretation through overestimation of d' value (Ennis, 1993; Dessirier & O'Mahony, 1999). Thus, 2- and 3-AFC sets are regarded more stable methods (Dessirier & O'Mahony, 1999). Cognitive differences result in a *paradox of the nondiscriminating discriminators* (Byer & Abrams, 1953): certain people get Triangular tests wrong, but still make a correct decision in 3-AFC (Lawless & Heymann, 1998) that lowers test power. Declaring an attribute (3-AFC), reduces sample combinations (sequential effects), eliminates *paradox of the nondiscriminating discriminators* (cognitive strategy) and increases the test power (O'Mahony, 1995). However, a reason for the popularity of Triangular tests is that in many situations, differences are unknown. Traditional Thurstonian modelling use distributions along a univariate axis (e.g. intensity) but changing one attribute influences others as in the *Bezold-Brücke phenomenon* in visual (as a red light becomes more intense, it also changes hue) (Dessirier & O'Mahony, 1999). Thus a specified attribute (univariate axis) may influence other attributes, interfering in momentary sensitivity.

Signal detection theory test has been extensively applied in psychological studies (Green & Swets, 1966) separating background stimuli (*noise*), and higher levels of stimulus intensity (*signal*). Parameters include a cut-off point, and z-scores are obtained from a correct (*hits*) and incorrect YES (*false alarm*) as d' = z (*hits*) - z (*false alarm*). The probability transferred to *z*-scores (Fig 1.8) (Table D.1, Appendix II). Such z-scores are independent of assessor criteria (cut-off) as lax or conservative. Detectability in individual assessors can be defined as a ROC (receiver operating characteristic) curve (Lawless & Heymann, 1998).

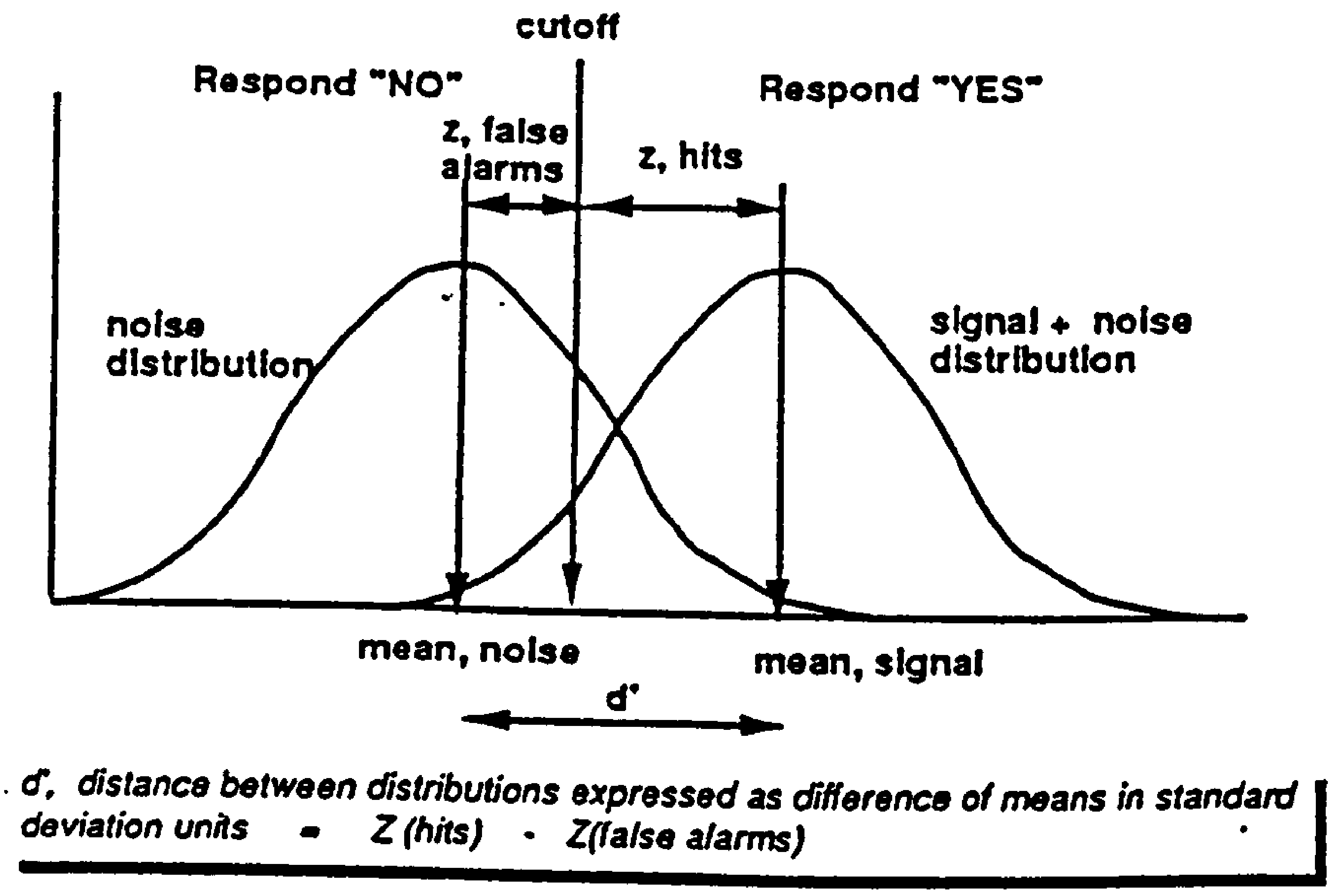


FIG. 1.8. Calculation of sensory difference (d') between *signal* and *noise* stimuli

1.4.1.1. Sequential sensitivity analysis (SSA)

Thurstonian and signal detection models conceptualise two stimuli as independent. This is true for visual and auditory, but not gustatory and olfactory stimuli through sequential sensitivity effects. Bias parameters showed that it only need to be considered one prior stimulus (Ennis, 1995). Variation in d' value influenced by sequential sensitivity analysis (O'Mahony, 1995; O'Mahony &

Goldstein, 1986; Dessirier & O'Mahony, 1999; Dessirier *et al.*, 1999) explaining the increasing discrimination power in *n*-AFC over Duo-Trio and Triangular tests, and 2-AFC over 3-AFC (Dessirier & O'Mahony, 1999). Similarily, in a triad, an odd strong stimulus is superior and a strong after strong is detected least (O'Mahony, 1987, 1995).

1.4.1.2. Warm-up session

Under the definition of Sensory Evaluation I (O'Mahony & Goldstein, 1987), *trained assessors under controlled conditions, with minimal cross-sensory interference, can be used as analytical tools to distinguish differences between samples of foods*, 'warm-up' improves performance in Triangular tests (O'Mahony *et al.*, 1988). The technique as "attention mechanism", has been widely applied in psychology experiments to describe the short-term effect whereby performance rapidly improves at the beginning of experiments, then rapidly dissipates after cessation (O'Mahony *et al.*, 1988). This is different from 'practice' - permanent improvement that takes time to achieve.

1.4.1.3. Conventional data analysis

Significance test: discrimination data are analysed employing binomial tables (Roessler *et al.*, 1978) (Appendices, Table D.2), chi-square (χ^2) (Amerine & Roessler, 1983) (Appendices, Table D.3) and application of normal distributions (Lawless & Heymann, 1998) (Appendices, Table D.4). True discriminators can be calculated as follow:

$C = D + m (N - D)$; where, C = the proportion of correct answers, D = the proportion of true discriminator, N = number of tests (assessors), *m* = the probability of chance (1/3 for triadic, ½ for two sample test).

However, the conventional interpretation (significance level) using probability of a correct response (*Pc*) does not assess true sensory difference between two stimuli, even from true detectors (*Pd*), directly related to *Pc* (Ennis, 1993), ignoring Type I (erroneous difference, α) and Type II (erroneous no difference, β) errors.

1.4.1.4. Advanced data analysis

Sensory difference (d' value): Thurstonian (1927) model measures d' value a direct conversion from probability of correct response (Pc) (Frijters, 1981; Ennis, 1993) using the tables (Appendices, Table D.5). Same d' value can be obtained for varying methods (Ennis, 1993) but there is the possibility of perfect discrimination where d' reaches infinity distorting t-test analysis (Dessirier & O'Mahony, 1999). This can be avoided by various modifications of data (Hautus, 1995; Bi & Ennis, 1998).

Confidence interval: A d' value is a difference between two samples on the assumption of normal distribution with equal standard deviations and estimates of real sensory difference (δ). To analyse how close the estimated d' is to the real value (δ), a confidence interval is calculated using B value as follow (Bi & Ennis, 1997) (Appendices, Table D.6):

Variance of d' ($S_d'^2$) = B value/N (number of assessors used), *Standard deviation* (S_d') = $\sqrt{\text{variance of } d'}$. *Confidence interval*, 95% (Z = 1.96) = (1.96 x standard deviation) ± d' value and 99% (Z = 2.575) = (2.575 x standard deviation) ± d' value.

Defining confidence intervals by MacRae (1995) using grid lines, requires only one factor (α level), but has a limited application in Triangular tests. Bi and Ennis (1997) also introduced assessments of significance between two and multiple d' values.

Minimum number of tests required for an estimation of d' (Bi & Ennis, 1997, Table D.7, Appendices) and Schlich (1993) (Appendix, Table D.8) using predefined Type I (α) and Type II (β) error risks have been introduced.

Replication in discrimination tests: Often a limited number of *in-house* assessors are employed more than once to improve detectability (Dacremont & Sauvageot, 1997). Even 150 Triangular tests provide very little reassurance that the products are identical through chance levels of correct responses (MacRae, 1995). Replications are often industrial practice to achieve an assigned discrimination power level. If the test is performed only once, the data can be analysed in normal procedures (e.g, Ennis, 1993; Schlich, 1993; Bi & Ennis, 1997). In replicated tests, data for each assessor are not independent and probabilities vary. Many researchers (Harries & Smith, 1982; Priso *et al.*, 1994; Brockhoff & Mηller, 1997, 1998; Bi & Ennis, 1998, 1999; Kunert & Meyners, 1999; Schlich *et al.*, 2000) sought to solve the problem of heterogeneity (overdispersion) in data. Kunert and Meyners (1999) disagreed with the Brockhoff and Schlich (1998) in relation to method that models binomial distribution, the sum of all correct assessments and summed over all assessors. This can be only applicable when actual differences exist and no randomisation is employed. Such information is not obtained prior to experiments and samples are randomised.

Beta-Binomial (BB) model has been introduced (Harries & Smith, 1982; Ennis & Bi, 1998) to estimate noted parameters in replicated data, with a table for minimum number to achieve a significance levels (Bi & Ennis, 1999). Spread parameter, γ (variation between tests) is determined: $\gamma = \frac{nS}{\hat{\mu}(1-\hat{\mu})k(n-1)} - \frac{1}{n-1}$, where S = $\sum_{i=1}^{K}\left(\frac{x_i}{n}-\hat{\mu}\right)^2$, where, $\hat{\mu} = \frac{\sum_{i=1}^{K} x_i}{nK}$, where, i = i^{th} assessors, k = number of replicates, n = number of assessors, x = number of successes. Bi and Ennis (1999) also explaining the power of discrimination using the BB tables.

Overdispersion model: Brockhoff and Schlich (1998) also introduce a strategy to handle replicates and experimental design in discrimination tests (SAS®macro REPRISKS in: schlich@arome.dijon.inra.fr, Schlich, 1993). In spite of its generality and simplicity, this method has not solved session effects, order presentation and unbalanced number of replications in a session. If number of replications is too large for a single session, these should be spread over several sessions. Schlich *et al.* (2000) concluded that at least 10 replicates were a safe option in a session to estimate proper heterogenity level, with five assessors performing a test once replaced by one doing the test ten times.

1.4.2. DESCRIPTIVE ANALYSIS AND RELATED STATISTICS

1.4.2.1. Conventional flavour profiling

Descriptive analysis uses trained assessors (or subjects) to provide quantitative data on attributes of food of importance to humans. Early descriptive profiling techniques, Flavour Profile (FPM) and Texture Profile Methods (TPM), employ extensive training and discussions with assessors to obtain a consensus in attributes and of profile products. These are time and effort-intensive sensory methods (Powers, 1988). Harper reduced the subjectivity of flavour description by creating a glossary of commonly used aroma stimuli (Harper *et al.*, 1968a, b). This approach led to use of line scale and statistical methods such as principal component analysis. Quantitative Descriptive Analysis (QDA), unlike FPM or TPM, includes strategies to account for behavioural effects (Piggott *et al.*, 1998) but selection and training of assessors are still important. The spectrum method (Powers, 1988; Einstein, 1991) is similar to FPM and TPM by providing detailed characterizations of profiled products using reference products to provide attribute intensity. However, QDA and Spectrum methods differ from earlier methods in how responses are obtained from assessors and panel averages are calculated. In essence FPM and TPM provided a consensus view of products through panel discussions (Piggott *et al.*, 1998) whereas QDA provides data on individual variation in assessors. Principal component analysis (PCA) (Piggott & Sharman, 1986) is often central to conventional profiling.

New variants of descriptive analysis are Free Choice Profiling (FCP) and Repertory Grid Methodology (RGM) (McEwan & Thomson, 1998; Piggott & Watson, 1992). The former allows assessors to generate unique attributes and the latter elicits terms discriminating between samples in pairs or triplets. Both are particularly suitable for development of terminology for quality management (ISO 6564: 1985). However, a potential disadvantage is that product spaces can only be expressed in terms of multivariate equations, requiring interpretation by a panel leader. Generalised Procrustes Analysis (GPA) (Arnold & Williams, 1986) is employed to analyse FCP data on the basis of the (questionable) hypothesis that individuals share similar sensory perceptual experiences but differ in description of these experiences.

1.4.2.2. Generalised Procrustes Analysis (GPA)

In Generalised Procrustes analysis (GPA) (Arnold & Williams, 1986), individual product configurations are related by mathematical transformation to common origin, with rotation/reflection of axes and isotropic scale changes. Variations between assessors in overall score level, ranges of scores and use of differing terms in same product can be accommodated. The consensus configuration is the mean of transformed configurations, and replaces the panel mean of the un-transformed configurations. It also provides both sample and assessor information.

1.4.2.3. Principal component analysis (PCA)

This method is now a basic tool in sensory multivariate data analysis (Piggott & Sharman, 1986) because relationships between samples are explained in multi-dimensional spaces. The first set of principal components explains the maximum correlation with all variables (*i.e.* highest variance explained). With PCA the data matrix is divided into *scores* (of samples or products) and *loading* (variables or attributes). Plot of loadings can be related directly to that of scores for interpretation. There is, in addition, an *residuals* (error matrix) for unexplained variance in data. A central issue is the determination of the number of valuable components, depending on inter-correlations between samples. Methods for selection of principal component (PC) includes: significance level (p) from ANOVA; rate of improvement in calibration; and validation variances from cross-validation. This latter, validation variance, is thought the most conservative prediction (Wold, 1989).

1.4.2.4. Partial least squares regression (PLS)

The method utilises a systems analysis approach. The basic concept of the application, developed by Wold (1982) for the social sciences, is optimisation of several 'partial' (separate) sub-models, each minimising lack-of-fit residuals by the principle of least squares (Martens & Martens, 1986). This method, *soft modelling*, can be used to model relationships between single variables and a block of data (PLS1) or between two blocks of data (PLS2). A typical analysis of PLS2 in flavour science might use a compositional *X*-matrix (*regressors*: predicting) and sensory *Y*-matrix (*regressands*: predicted) data. Choice of *X*- or *Y*- variables depends on quality of data. An earlier strategy was multiple linear regression (MLR) where regressions of *y* is performed on individual *X*-variables. In contrast, in PLS the regression of *y* is on orthogonal 'latent variables' from multiple *X*-variables. A problem in MLR 'collinear' data is overcome by PLS; MLR also cannot be used if there are more variables than samples (Martens & Martens, 1986). In PLS, cross-validation (Wold, 1989; Kvaal & McEwan, 1996) is used routinely to guard against over-fitting of data. In cross-validation, modelling is repeated, each time using only certain samples for training (predicting *Y* from *X*) and predicting *Y* for the residual samples. Modelling must be possible in the great proportion of samples.

An important tool is PLS1, identical to ANOVA of a single response variable (Martens *et al.*, 1983; Aastveit & Martens, 1986), and also similar to principal component regression (PCR). This is essentially multiple linear regression on the largest of principal component from the regressor *X* matrix. However PLS1 is more efficient than PCR in extracting only relevant regressand *Y* data from the regressor *X* matrix (Martens *et al.*, 1983). In modelling of flavour attributes from congener composition of whiskies, PLS has been successfully employed (Swan & Howie, 1984;

Canaway *et al.*, 1984; Piggott *et al.*, 1993a; Delahunty *et al.*, 1996; Withers *et al.*, 1995, 1996).

A number of other forms of PLS have also been utilised, notably: the PCA-based SIMCA and Discriminant PLS (Martens & Martens, 1986). SIMCA relies on *X*-*Y* relations instead of only intra-*X* and classification is obtained by calculations of distances from each object to every class model. Reliability of this strategy has been reviewed (van der Voet & Coenegracht, 1986).

A further strategy useful for identifying contribution of attributes to different product categories is Discriminant PLS (DPLS; Wold, 1989; Martens *et al.*, 2000). In DPLS, loading plot gives the discrimination power of individual *X*-variables (sensory attributes or compositional factors). Key *X*-matrix (which may be compositional or sensory data) is regressed on to a binary category *Y* variable or matrix, set as '1' for class members and '0' for non-members. Analysis with DPLS2 projects all category variables into a single product space whereas in DPLS1, the individual product categories are modelled separately. Factorial design with different levels of discriminant PLS has been described by Martens and Martens (1986).

1.4.2.5. Concept of the product space

Product space display, in two dimensions, the complexity of inter-relationships between sample distance according to differences. Similarity or dissimilarity between products can be observed as clusters or polar positions along axis and extreme samples can be detected as outliers. However, various factors need to be considered for successful modelling of data. Experimental design should minimise biases to isolate variables of interest. Interpretation of the generated product spaces could be subjective view of the sensory analyst. Knowledge of samples could be beneficial to support interpretation of product spaces (Martens *et al.*, 2000). No method can be eliminate potential behaviour error effects but minimisation by careful selection of method and standardisation or randomisation of experiment design is required to yield precise, accurate and valid sensory data (Piggott *et al.*, 1998).

1.6. RESEARCH AIMS

The purpose of the research was exploration of sensory character in Scotch whiskies, concentrating on the blends that form the bulk of production.

Distinct aims were to determine flavour threshold concentrations for potential reference standards and examine how attributes evoked by reference compounds were described by distilling professionals. Finally weightings of these sensory attributes could be assessed in different categories of blends and related to headspace congeners concentrations. Established sets of references could then be exploited in study of discrimination tests.

A clear outcome of this study would be a revised flavour wheel of greater potential value to the Scotch whisky industry. The existing flavour wheel (Shortreed *et al.*, 1979) appeared to have problems that limited its industrial value. A primary difficulty was that many flavour attributes used in the industry for quality assurance were of limited interest and the vocabulary lacked descriptors that could form a comprehensive language for describing whisky character. Secondly, without a vocabulary backed by flavour references, it was difficult for distilling professionals to discuss whisky character in similar concepts.

It also became desirable to clarify the nature of perception of whisky flavour character during sensory assessment by supporting psychological and physiological theories. Discriminant PLS modelling was exploited to understand the nature of character differentiation in Deluxe, Standard, Retailer, and West Highland blends in terms of weighting of sensory attributes and important headspace congeners.

The chapters forming this thesis are presented in a similar manner to a European doctoral thesis, in that each chapter of the experimental section, with the exception of 1 chapter (this introduction), embodies a manuscript submitted to a peer-reviewed publication.

CHAPER 2:

ORIGINS OF FLAVOUR IN WHISKIES AND A REVISED FLAVOUR WHEEL: A REVIEW

Results of this study have been accepted for publication in *Journal of Institute of Brewing* (Lee *et al.*, 2001)

2.1. ABSTRACT

The nature and origins of flavour in whiskies are reviewed with the aim of developing a revised flavour wheel for training of sensory assessors. Scotch whiskies are perceived as having distinctive characters, generally recognised in pattern recognition (perception, macroscopic brain processing), rather than being subjected to a deconstruction process of evaluating attributes (sensation, microscopic brain processing). Although consumers use simple recognition judgements on whisky flavour in categorical assimilation, industry has a requirement for monitoring spirit quality that necessitates a more reductionist approach. Whisky flavour wheels identify attributes, specific components of flavour character, which can be demonstrated to sensory assessors using reference standards. The advent of cyclodextrin bound reference standards has enabled communication of information on flavour character in training of assessors, as exploited in the brewing industry. A revised flavour wheel, with characters illustrated by reference standards, is proposed to assist assessor training on attributes of whisky flavour character.

Key Words: *Whisky flavour character, whisky flavour reference standards, flavour perception and sensation, sensory assessor training, quality evaluation.*

2.2. INTRODUCTION

Improved analysis of congener composition has not yielded a better understanding of flavour in whisky (Conner *et al.*, 2000). A central factor is that flavour is an interaction between individual assessors and both volatile and non-volatile flavour components (von Sydow, 1971): a dynamic process (Piggott *et al.*, 2000). Quantities of volatile strawberries flavour components differ in the mouth space of experimental assessors (Linforth *et al.*, 1996). Despite this, agreement on product character is obtained. The varying perceptions of flavour notes, termed ***attributes***, are translated into language (Harper, 1972; Shortreed *et al.*, 1979) but are more commonly used by a human brain in pattern recognition, as has been argued for wine (Ripley, 1996). Certain psychologists have argued that most products are recognised holistically, defined as *the perception of the whole over the parts* (macroscopic brain processing) (Fig. 2.1) (Kanizsa, 1994; Stadler & Kruse, 1994).

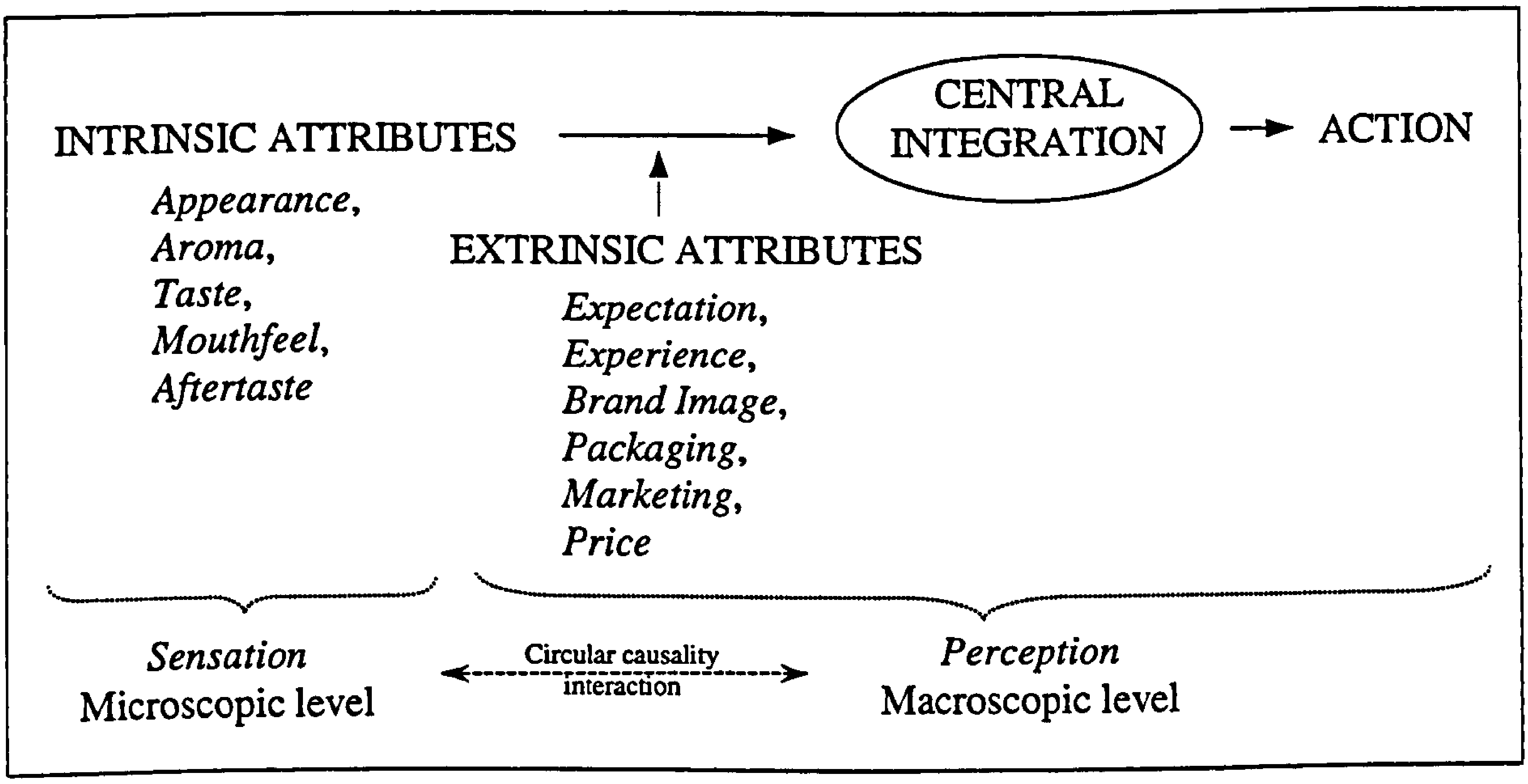


FIG. 2.1. Intrinsic and extrinsic attributes

Such perceptual organisation is complex - utilising pattern recognition (Ripley, 1996), or Gestalt principles (Harper, 1972; Sheen & Drayton, 1987; Mishkin & Appenzeller, 1987; Kanizsa, 1994; Stadler & Kruse, 1994; Epstein & Hatfield, 1994). However, in an industrial sensory assessment situation, specific perceptions have to be evaluated and integrated as single components (sensations). Flavour data is collected from specific sub-groups of neurons, olfactory receptors (microscopic brain processing) and then processed in specific a region of the brain. A circular causality interaction (*slaving effect*) exists between perceptual and sensation levels with influences postulated between the semantic (meaning of word; verbalisation) macro-level to the energetic (sensation) micro-level (Fig. 2.1). Experimental evidence has shown the circular motion is directed by semantic cues (Kruse *et al.*, 1991). In other words, the human mind has an influence over the brain (Stadler & Kruse, 1994). In summary, people generally perceive a product on the basis of expectation, based on prior experience of consumption of that class of product. Sensory appreciation of flavour character in whisky can thus be regarded as a synergistic and holistic perceptual process. Extrinsic attributes (branding, labelling, marketing and packaging) have central roles in choice decisions and other consumer behaviour, especially when evaluating whisky products (Fig. 2.1). Industrial sensory assessors can be considered to provide data related to consumer judgements on whisky character. However, these are not identical, since they utilise different forms of mental processing.

On tasting a whisky, even in the absence of extrinsic attributes, consumers will seek to match their perceptions against prior experience, based on patterns of intrinsic attributes. If a mismatch with expectation is apparent, the individual will then move to a deconstruction process in which small numbers of intrinsic attributes are evaluated in a sequential manner. Such a mismatch of the mental state can be considered as a *perceptual instability.* This is resolved by an adjustment of perception in a multidimensional process, often through semantic processing - evaluation of specific attributes - with creation of a further mental image to regain stability (Stadler & Kruse, 1994). Such a multidimensional perceptual process can be practised during training of panellists for descriptive sensory analysis. A typical industrial sensory training progression for whisky may be evaluation of attributes relevant to appearance, aroma, taste and mouthfeel (after-taste). Each will be a cluster of individual assessments in which stimulus levels of specific attributes are quantified.

Experimental data from psychophysics suggests that the human has a physiological limitation in its ability (Sheen & Drayton, 1987) and is unable to discriminate or identify more than three or four flavour components in any mixture (Laing & Francis, 1989; Laing & Glenmarec, 1992; Laing & Livermore, 1992). Thus the summed spatial response pattern of a mixture of flavour components need not resemble that of any individual flavour note (Laing, 1994). A further problem central to the study of sensory flavour is that the concepts defining only a few (2 - 4) aroma attributes are retained in short-term memory whereas a larger number are held in long-term memory (White, 1998).

The beer flavour wheel (Meilgaard *et al.*, 1979) was developed specifically for industrial implementation, serving to concentrate assessor attention on specific flavour attributes. This wheel was successfully integrated into sensory analysis in the brewing industry. A subsequent revision (Langstaff & Lewis, 1993) incorporated *mouthfeel* attributes, regarded as important components of beer flavour. However, it is clear from studies of both wood-matured ports (Cristovam *et al.*, 2000) and whisky (Piggott & Jardine, 1979) that both expert and novice assessors also use visual data to judge flavour character (*slaving effect*). This may be a reflection of human reliance on visual information. Visual attributes (*e.g.* colour) can be manipulated to change perceptions of product quality such as aroma, taste and flavour (Hall, 1958; Pangborn, 1960; Pangborn & Hansen, 1963; Lawless & Heymann, 1998). The semantic process of odour recognition is now also known to involve activity in the primary visual cortex (BA 17), involving evaluation of the consumable nature, or comestibility, of products (Royet *et al.*, 1999).

The presentation of pre-defined attributes on a flavour wheel serves a useful function in directing sensory assessors towards specific features of flavour character, and can also form the basis for discussions on flavour character. A flavour wheel establishes formal flavour attributes thus defining a vocabulary for whisky flavour perception that facilitates information transfer between individuals. Such information could be related to flavour origins in raw materials or in the production process. Specific reference standards facilitate conceptualisations (knowing what an attribute is) and the development of parallel mental representations of concepts between assessors. This forms an important component of sensory assessor training (ISO 8586-1:1993, 8586-2:1994). Visual representation or symbol in sensory assessment may aid assessor recognition or memory (Royet *et al.*, 1999). Symbols are utilised in commercial beer flavour standards kits (FlavorActiv, 2000); and could be employed more effectively in training of sensory assessors.

2.3. WHISKY MATURATION

The ageing in wooden casks of a new distillate to yield a matured whisky is central to flavour character development. This process has given most whiskies their current consumer appeal. Maturing of whisky can be viewed as replacement of *pungent*, *soapy*, *sour* and *harsh* notes of new distillates with *smooth*, *matured* and *balanced* attributes of retailed whiskies. Such assessments are largely obtained by pattern recognition (Ripley, 1996). In determining maturity, assessors collect information from a number of sensory systems: visual, olfactory, gustatory and chemesthesis. These stimuli are integrated in specific regions of the brain (sensation, microscopic level) to form a mental representation (Sheen & Drayton, 1987) of the whisky (perception, macroscopic level) (Fig 2.1).

It has become clear through work on model and malt whiskies (Conner *et al.*, 1994a,b; 1999a; 2000) that maturation largely proceeds by changing partitioning of a number of important flavour components with less desirable flavour notes (Conner *et al.*, 1996; 1999b). Flavour components conferring immature notes are retained within the liquid phase of the matured whisky. Their concentration in the headspace above the whisky is diminished, reducing stimuli at nasal aroma receptors. Such behaviour has been simulated recently in the "Strathclyde Simulated Mouth" (Margomenou *et al.*, 2000).

It has been concluded that fatty acid ethyl esters are important in stabilising spirit headspace compositions (Piggott *et al.*, 1996). Esters are amphiphilic compounds with central polar groups and peripheral hydrophobic aliphatic carbon chains. This influences ester solubility in the distillate aqueous ethanol. Excess esters, released from solution, form agglomerates in distillates (Tanford, 1980) yielding microemulsions. This limited solubility of esters has major influences on headspace concentrations of flavour-active components of whiskies (Conner *et al.*, 1996). Agglomerates are removed through the chill filteration process prior to bottling (Piggott *et al.*, 1996).

In sensory assessment of spirits at 23% (abv), agglomerate diameters are observed to increase (Paterson *et al*, 1995) forming hazes (Hardy & Brown, 1989;

Perry, 1989). Whisky agglomerates are dominated by ethyl dodecanoate (laurate), tetradecanoate (myristate) and hexadecanoate (palmitate). Agglomerate contribution is determined by ester aliphatic chain length (Conner *et al.*, 1994a, 1994b). In industrial practice, chill filtration serves to stabilise appearance by removing excess esters by physical separation and adsorption. The sensory quality of whisky was not changed by filtration although gas chromatographic analyses showed differences in congener composition (Piggott *et al.*, 1996).

During wood maturation, non-volatile components, including tannic acids, are extracted from staves. Such components appear to stabilise esters in the agglomerate phase (Conner *et al.*, 1994a; Paterson *et al.*, 1995). The influence of temperature on behaviour of agglomerates and distribution of flavour-active congeners between the solution and headspace phase has been studied (Conner *et al.*, 1996). Since at oral temperature, agglomerates suppress the volatile congeners more efficiently (Conner *et al.*, 1996), the difference between nosing and tasting would be small (Piggott & Jardine, 1979). The contribution to agglomerate formation of other flavour-active components such as alcohols, aldehydes and acids, important in matured whisky, has also been studied (Conner *et al.*, 1994b). Alcohol, aldehyde, acid and ester congeners all contribute additively in a whisky. Solubilities are finite and excess solutes form agglomerates, with a function of activity coefficients that are increased by non-volatile components of wood extracts.

The effects of alcohol strength on headspace concentrations have also been studied in detail (Conner *et al.*, 1998). The composition of the spirit matrix changes as ethanol strength changes. Below 20% abv, alcohol molecules are mono dispersed in water (aqueous solution); between 20 and 57% abv, a progressive aggregation of alcohol molecules reduces the hydrophobic hydration of the alkyl chain; above 57%

abv, the solution becomes ethanolic due to the loss of the hydrogen bonded network of the water. Increasing ethanol concentration lowered the interfacial tension between the aqueous phase and ethyl esters. Therefore, their aroma thresholds would be expected to increase (Conner *et al.*, 1998). Reducing bottling strength (40% to 30 - 35% abv) would increase headspace partition coefficients especially of longer chain *soap-like* associated esters. Therefore, reduced bottling strength would result in a reduction in perceived spirit quality (Conner *et al.*, 1998). Dilution of a whisky to 23% abv prior to sensory assessment would be the appropriate procedure to obtain maximum volatile release from the spirit solution.

2.4. WHISKY PRODUCTION

Malt, grain and blended Scotch whiskies differ in production process (Fig 2.2). In malt whiskies (7% market sale by volume – The UK Food and Drinks Report, 2000) parameters in batch (pot) distillations of washes from barley malts influence final character (Nicol, 1989; Whitby, 1992; Goodall *et al.*, 1999). Grain whisky is a product of continuous fractional distillation of fermented wheat and maize (Panek & Boucher, 1989), saccharified by lightly-kilned barley malts (Piggott & Conner, 1995). Dominant (85% market) are blends of grain (60 - 80%) and malt whiskies (20 - 40%) with the lighter grain (2 - 3) providing a flavour background, and single malts (up to 40) the majority of character. Selection of primary (*top-dressing*) and secondary malts has significant impacts (Murray, 1999). Maturations influence final flavour in blends or single whiskies with cask management ensuring product consistency (Mosedale, 1995; Martinez *et al.*, 1996; Chatonnet & Dubourdieu, 1998a).

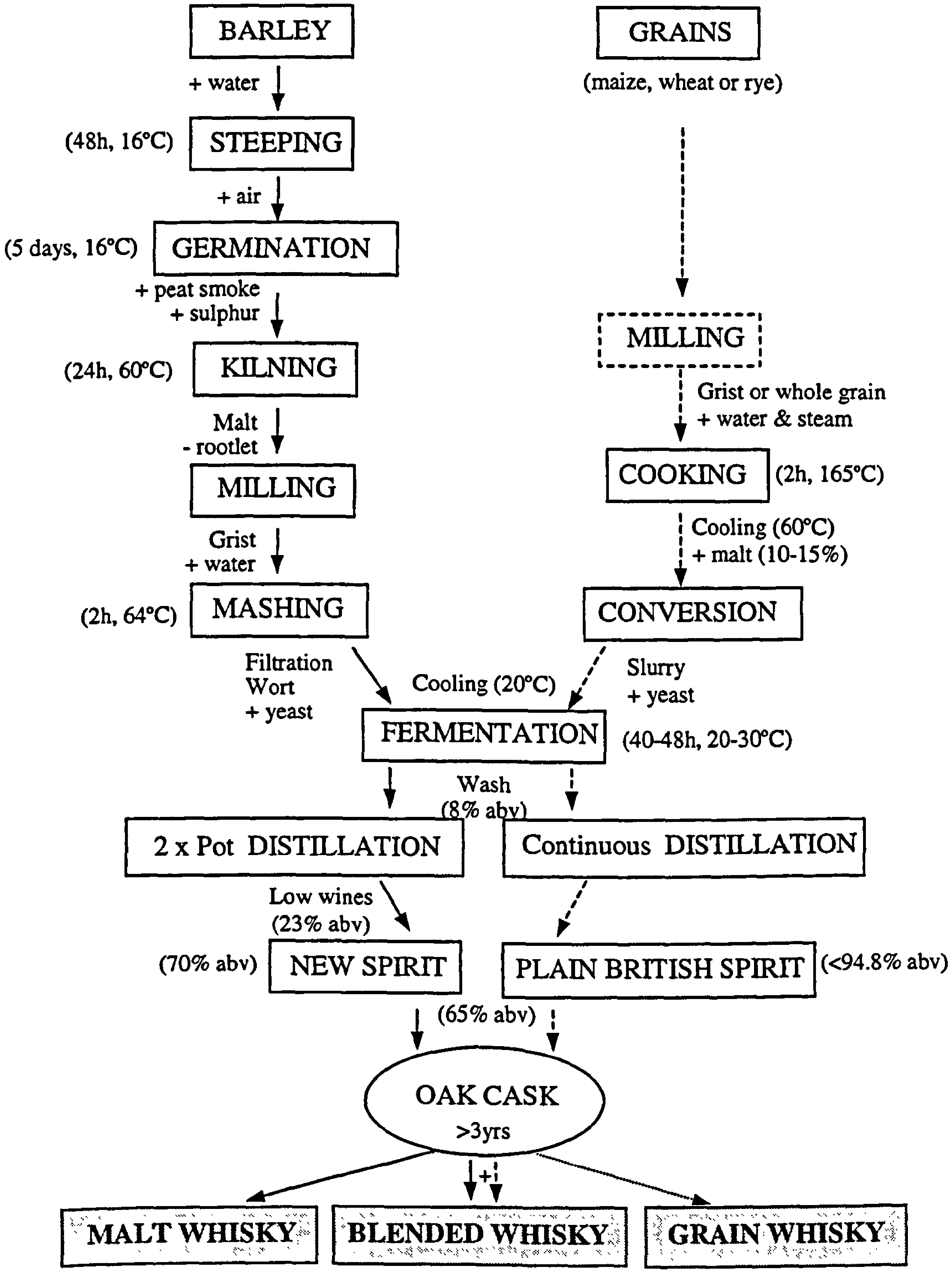


FIG. 2.2. Production of Scotch whisky

2.5. A REVISED FLAVOUR WHEEL FOR INDUSTRIAL SENSORY ANALYSIS OF WHISKY

To meet industrial needs, flavour attributes of whiskies can be ordered in a classification based on presumed origins in the production process, leading to flavour wheels such as that proposed by Shortreed and his coworkers (1979). Hybrid structures in which production and flavour perception contribute to classification are likely to be potentially of more value, demonstrating interactions between assessor and composition inherent in flavour character.

A revised whisky flavour wheel is presented in Figure 2.3. The format is similar to other flavour wheels for alcoholic drinks (beer: Meilgaard *et al.*, 1979: wine: Noble, 1987). The new flavour wheel is structured in a hierarchy of three tiers: primary, a classification based on production origin or generality of nature; secondary, showing more specific sensory or conceptual descriptors; and tertiary with highly specific terms, some of technical importance. This hierarchy is employed in the following description of flavour characters and their component concepts, presented in clockwise order round the wheel. The aroma notes clustered on the right side of the wheel arise under the normal production of whisky; these may also be used for promotional and marketing purposes (Fig. 2.4). In contrast, off-notes, likely to be of more interest to technical functions, are listed on the left side of the wheel as four groups.

In the wheel, technical and chemical terms such as *phenolic*, *aldehydic* and *estery* in the primary tiers were substituted with *peaty/smoky*, *grassy*, *fruity* and *floral*, respectively. However, *feints* was still retained as a generic term since it was the most difficult aromatic group (MacLean, 1997) to describe and no alternative term could be found to represent this group of varying attributes. The terms chosen in subtiers were those most frequently used by sensory professionals (both expert blenders and sensory panellists) in the Scotch whisky industry (Lee *et al.*, 2000b). For sensory training, recommended reference compounds are proposed (Table 2.1): these could be formulated with cyclodextrins to achieve consistency, or parallel conceptualisation, in sensory training.

2.5.1. Nasal effects

N.1. *pungency - ethanolic, peppery, prickle*
N.2. *drying*

Pungency is regarded as a primary sensory process (common chemical sense or irritation, chemesthesis) that initiates a taste or aroma sensation (Laing & Jinks, 1996). Such attributes originate in stimulation of trigeminal nerve endings (Withers *et al.*, 1995) and have been described as "a sharp, stinging or partial sensation of flavour or odour" (Amerine *et al.*, 1965). The characters are generally related to delocalised nasal stimuli, rather than specific flavour attributes. Individuals are more sensitive to aroma than to *pungency* (Shortreed *et al.*, 1979).

Loss of *pungency* can result in *smoothness*, a character important in matured whiskies (Guy *et al.*, 1989; Piggott *et al.*, 1993a). *Pungency* is often reported to originate in the ethanol (*ethanolic-pungency*) of the whisky. Its perception, however, is not solely dictated by ethanol content or spirit strength, but other effects such as the activity of ethyl esters in the headspace (Withers *et al.*, 1995) can contribute. Thus *ethanolic-pungency* characters should not be treated as 'off-notes'.

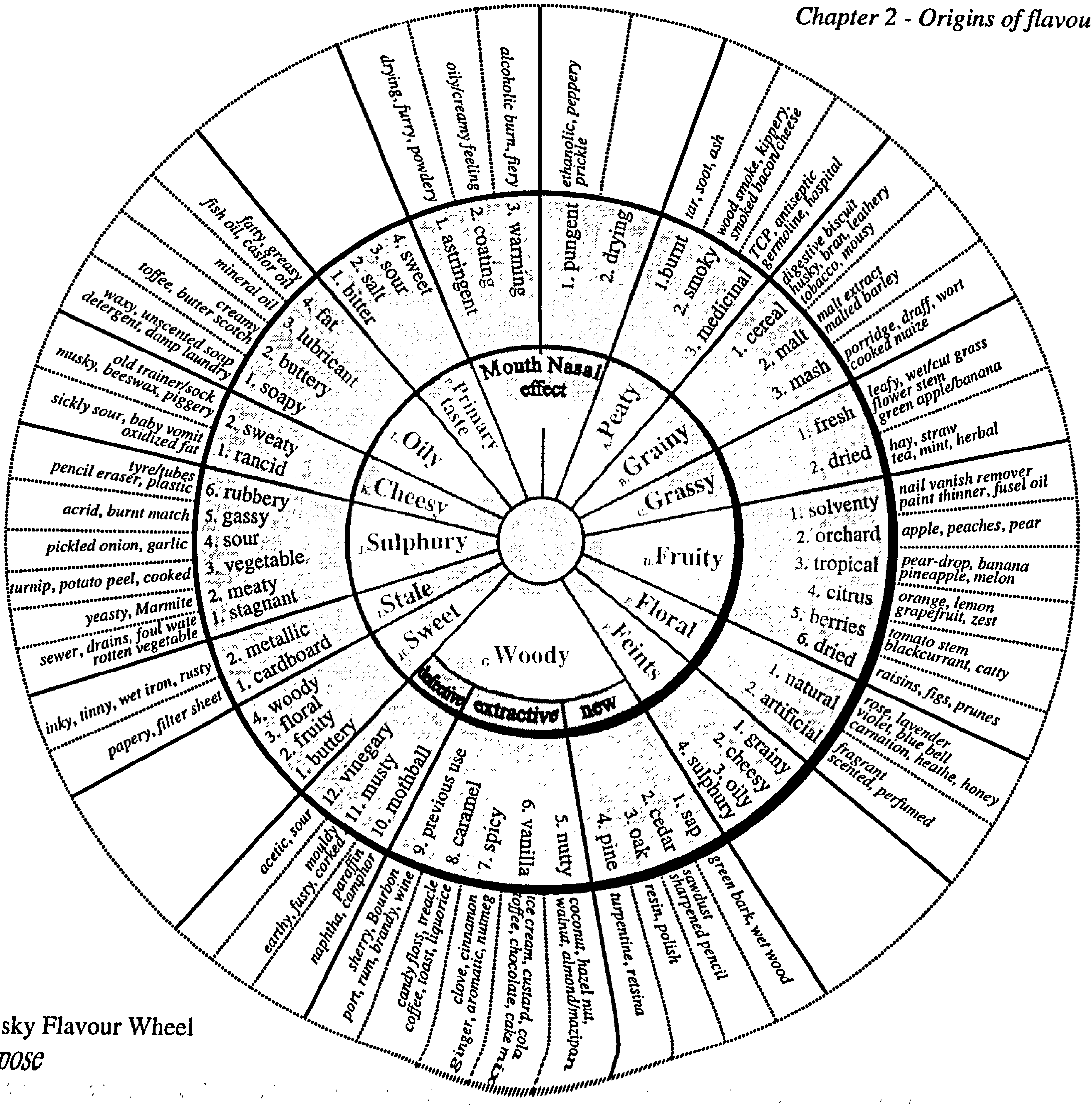


Fig. 2.3.
Revised Scotch Whisky Flavour Wheel
for the industrial purpose

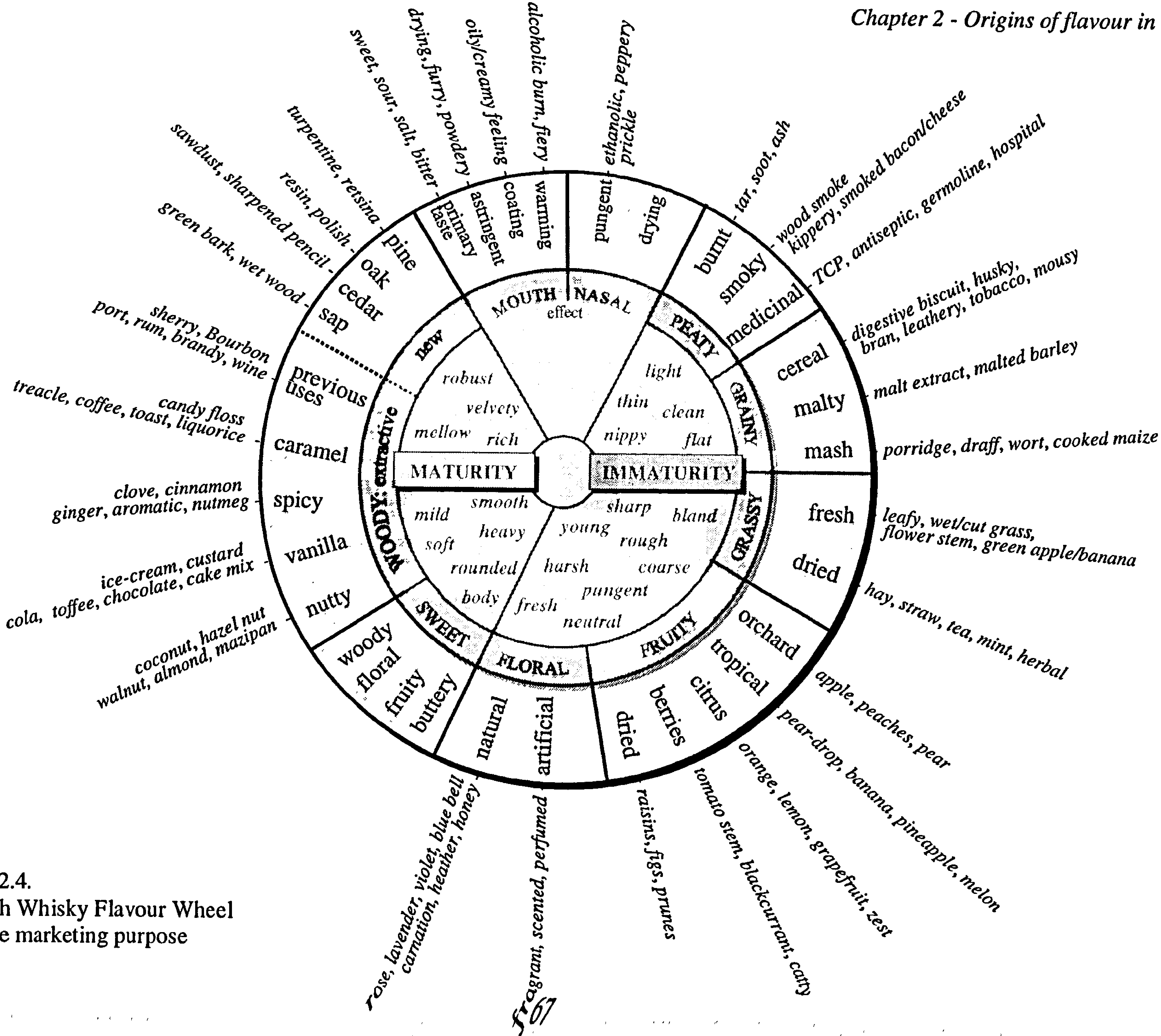


FIG. 2.4.
Scotch Whisky Flavour Wheel
for the marketing purpose

TABLE 2.1. Whisky descriptors and reference compounds

Code	Attributes	Reference compounds	Concentration (mg/L)
N.1	*Pungent*	Formic acid[199]	10 x 10^3
A.1, 2	*Burnt/smoky*	Guaiacol[129]	27
A.3	*Medicinal*	*o*-Cresol[130]	1.75
B.2	*Malty*	Malted barley[199]	-
		2- and 3-Methyl butanal[13,193,194], 4-Hydroxy-2(or 5)-ethyl-2(or 5)-methyl-3(2H) furanone[94], 4-hydroxy-2,5-dimethyl-3(2H) furanone[94]	0.6 (2-methyl butanal)[155], 1.25 (3-methyl butanal)[155]
C.1	*Grassy*	Hexanal[129]	5
		cis-3-Hexen-1-ol[199]	1.00 x 10^3
D.1	*Solventy*	Ethyl acetate[130]	1.12 x 10^3
		2-Methyl propan-1-ol[199]	1.00 x 10^3
D.2	*Fruity* (*appley*)	Ethyl hexanoate[129]	2
D.3	*Fruity* (*banana, pear-drop*)	*iso*-Amyl acetate[129]	7
D.5	*Berry*	Thiomenthone[130]	3x 10^{-3}
	Catty	Thiomenthone[130]	1.26
		Sodium sulphide + mesityl oxide[199]	100 each
E.1	*Floral* (*Natural - rose*	Phenyl ethanol[129]	1.52 x 10^3
	- violet)	α-, β-Ionone[54]	>0.003
	Floral (*Artificial - scented, perfumed*)	Geraniol[129]	19
G.5	*Nutty* (*coconut*)	Whisky lactone[129]	266
	marzipan	Furfural[129]	839
G.6	*Vanilla*	Vanillin[129]	43
G.7	*Spicy*	4-Vinyl guaiacol[129]	71
	Spicy (*clove*)	Eugenol[199,129]	1 - 55
G.8	*Caramel* (*candy floss*)	Maltol[129]	1.14 x 10^3
G.10	*Mothball*	Naphthalene	>0.008
G.11	*Mouldy*	2,4,6-Ttrichloroanisole[199]	10
	Earthy, musty	Geosmin, 2-methyl *iso*-borneol[237,69]	-
G.12	*Vinegary*	Acetic acid[129]	5.32 x 10^3
I.1	*Cardboard*	2-Nonenal[130]	0.08
J.1,6	*Stagnant, rubbery*	Dimethyl tri-sulphide (DMTS)[128]	3
J.2	*Yeasty*	Hydrogen sulphide (H_2S)[128]	> 0.02
	Rotten egg	Hydrogen sulphide[128]	> 0.14
	meaty	Methyl (2-methyl-3-furyl) disulphides[27]	-
J.3	*Vegetable* (*sweet corn, cooked cabbage*)	Dimethyl sulphide (DMS)[128]	> 0.6
J.5	*Gassy*	Ethanethiol[128]	> 0.072
		3-Methyl-2-butene-1-thiol[128]	> 7.2 x 10^{-4}
K.1	*Rancid*	*n*-Butyric acid/ ethyl buryrate[26]	>2
	Sweaty	*iso*-Valeric acid[129]	2
L.	*Oily*	Heptanol[199]	1
L.1	*Soapy*	Ethyl laurate[129]	12
		1-Decanol[199]	100
L.2	*Buttery*	Diacetyl[129]	0.1

199 (Piggott, 1991) – in 23% ethanol solution; 129 (Lee *et al.*, 2000b) - in 23% grain whisky; 128 (Lee *et al.*, 1999) - in lager; 26 (Carter-Tijmstra, 1989), 27 (Carter-Tijmstra, 1990), 54 (de Rijke & ter Heide, 1983), 69 (Evan *et al.*, 1997), 94 (Hayashida & Slaughter, 1997), 130 (Chapter 3), 237 (Simpson, 1990) - > threshold; 155 (Meilgaard, 1981) - threshold in beer

Peppery-pungency notes in whiskies can originate in acrolein from bacteria participating in whisky fermentations, especially *Lactobacillus* strains (Mills *et al.*, 1954; Kahn *et al.*, 1968; Lyons & Rose, 1977; Sponholz, 1993). Such characters are regarded as off-notes. Although acrolein-producing bacteria occur naturally in most cereals, only small numbers have been found in alcoholic fermentation, and require exacting conditions for acrolein production (Mills *et al.*, 1954; Kahn *et al.*, 1968). These bacteria catabolise glycerol produced by yeast into β-hydroxy propionaldehyde at elevated temperatures or in extended fermentations (Sponholz, 1993). This β-hydroxy propionaldehyde degrades to toxic, lachrymatory acrolein during subsequent distillations (Circle *et al.*, 1945) inducing *pungent*, *burnt* and *peppery* notes in whisky (Hardy & Brown, 1989), known as 'red eyes' (Mills *et al.*, 1954). A major source of bacterial contamination is wooden washbacks. Other factors such as pH, oxygen tension, agitation, presence of yeast cells and/or mash residue, fermentable sugars and glycerol are important in influencing acrolein production (Mills *et al.*, 1954). Such off-flavour notes can disappear after 2 or 3 years of wood maturation because acrolein reacts with ethanol to yield 1,1-diethoxy-2-propene (Circle *et al.*, 1945; Lyons & Rose, 1977), 1,1,3 triethoxypropane, 3-ethoxypropionaldehyde and propene (Kahn *et al.*, 1968, 1969; Nishimura & Matsuyama, 1989). None of these compounds possesses either the unpleasant aroma note or its lachrymatory effect. In sensory assessments, this *peppery* attribute should be discriminated from *spicy-peppery* character, which originate from certain wood extracts.

2.5.2. Phenolic characters

A. *Peaty* character

A.1. *burnt - tarry, sooty, ash*

A.2. *smoky - wood smoke, kippery, smoked bacon/cheese*

A.3. *medicinal - TCP, antiseptic, germoline, hospital*

Major contributors to *smoke* aroma notes are wood carbohydrates – cellulose, hemicelluloses and lignins (Chen & Maga, 1993). *Peaty* attributes originate primarily from phenolic compounds in peat smoke (Withers *et al.*, 1996). Other flavour components originating in peat smoke, include sulphur- and nitrogen-containing congeners such as pyridines and thiazoles. Quantitatively important phenols in whisky are phenol, cresols (*m*-, *o*-, *p*-), xylenols, and *p*- and *m*-ethylphenol. However, low concentrations of guaiacol with a low threshold (3 µg/L in 10% spirit, Eriksson & Lehtonen, 1981; detection, 0.09 µgm/L and recognition 3 µgm/L; Lee *et al.*, 2000a) and substituted methoxy phenols (Williams & Tucknott, 1972) have significant flavour impacts. The phenolic and basic subfractions contain the vast majority of the most important volatiles associated with desirable smoke flavour: acidic and neutral subfractions are of secondary importance (Chen & Maga, 1993).

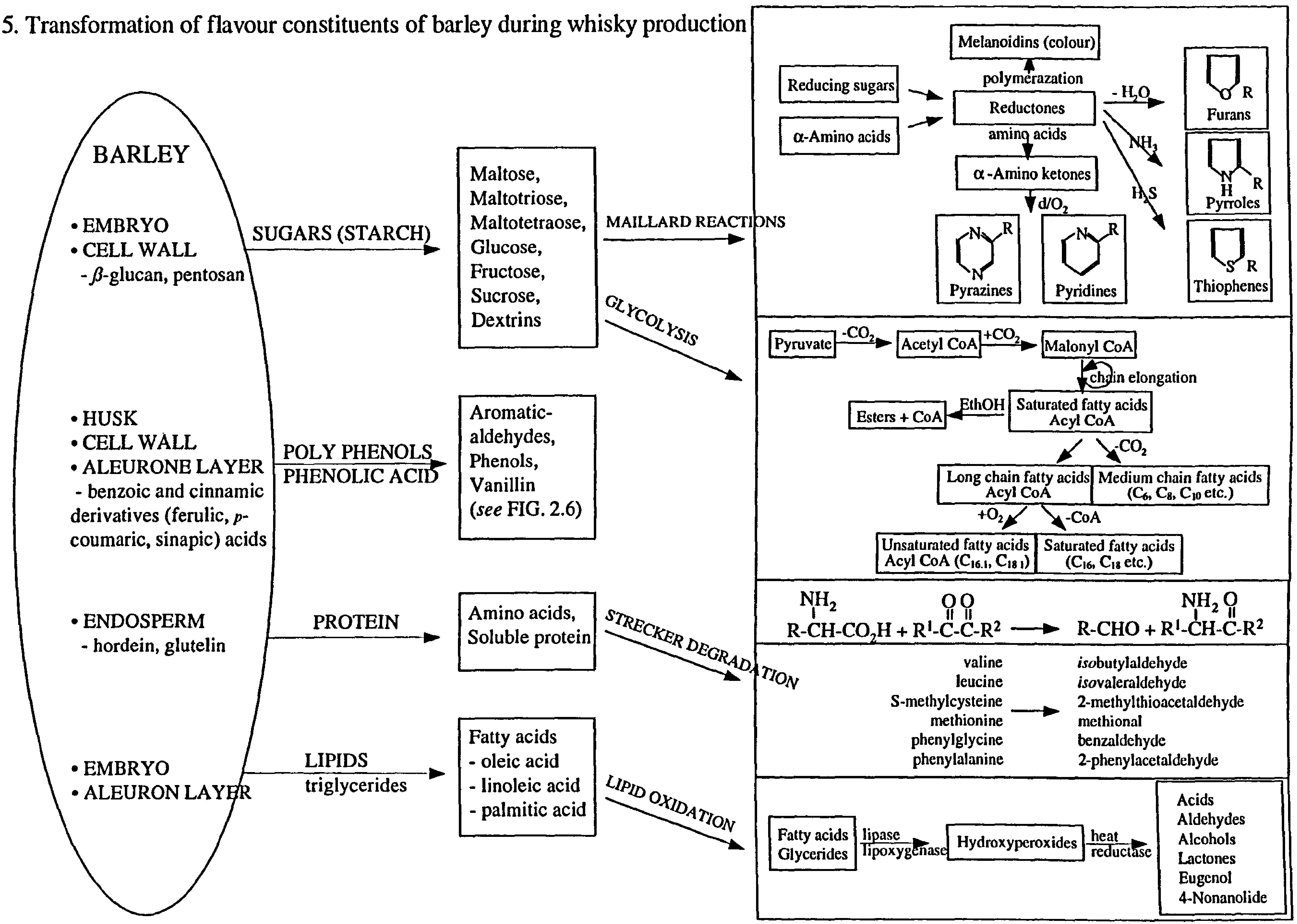
Flavour-active compounds in peat smoke are introduced into the airflow during the kilning processes (Bathgate & Taylor, 1977; Bathgate & Cook, 1989). Peating is a secondary operation that introduces *smoky* flavour characters into final distillates. Currently, gas or fuel oils are used in kilning as a primary fuel but inclusion of a short-time intensive peat combustion yields a characteristic *reek*. Smoke absorption reaches a maximum when malting barley has 15 - 30% moisture content (Bathgate & Cook, 1989). Raising kilning temperatures from 400 to 750 °C yields increased phenol and cresol contents several-fold, whilst reducing guaiacol (Bathgate & Cook, 1989). Thomson (1983) has reviewed relationships between flavour-active phenols and peat composition and malt kilning conditions. Certain *smoky* attributes are also introduced into maturing spirit through the extraction of thermally degraded lignin components (Fig 2.6) and also ethanolysis of Braun's lignin (Nishimura *et al.*, 1983). The resulting aromatic aldehydes induce *sweetness* in addition to *smoky* attributes (Conner *et al.*, 1992, 1993). Other phenolics, such as benzoic, cinnamic, ferulic (4-hydroxy-3-methoxycinnamic) (Humberstone & Briggs, 2000), *p*-coumaric (4-hydroxycinnamic) and sinapic acids which were originated from malted barley (Fig 2.5) are transformed to phenols by thermal decarboxylation during kilning (Tressl *et al.*, 1983). Ferulic and *p*-coumaric acids are converted to 4-vinly guaiacol and 4-ethyl phenols respectively (Fig 2.6) through enzyme-catalysed decarboxylation by certain yeasts (Steinke & Paulson, 1964; Eriksson & Lehtonen, 1981; Chatonnet *et al.*, 1993; Donaghy *et al.*, 1999). Such enzymic activity is observed during whisky fermentations, especially in the production of rye spirits where raw materials are high in phenolic acid content (Paterson & Piggott, 1989).

However, many phenols are retained in feints due to their hydrophilicity hence their concentration in the final spirit is limited (Howie & Swan, 1984; Paterson & Piggott, 1989). Although phenol itself is dominates (46 - 67%) in peated barley malt, cresols can be more abundant (58 - 61%) in final spirits. Both components, with origins in peated malts, have a strong impacts on the character of Scotch, Spanish and Japanese whiskies (Eriksson, 1978). *O*-cresol is quantitatively the most abundant in Scotch whisky with a threshold of 31 mgL^{-1} in 10% spirit (Eriksson & Lehtonen, 1981) and 30 µg/L and 120 µg/L (detection and recognition threshold) in 23% grain whisky (Lee *et al.*, 2000a). Although phenol contributes *circa* 7% of odour units (Salo, 1972; Berry, 1984), the related attributes can play major roles in whisky character, especially in Scotch (Withers *et al.*, 1996). Whisky notes are generally described as *medicinal* and *iodine* (Brander, 1995). In Speyside malts whisky is described more as *peaty*. Strong phenolic character such as *medicinal* is related to mainly *o*- and *m*-cresols from kilning of barley malt, whereas *peaty* may be related to eugenol (Swan & Howie, 1983). Bourbon and Canadian whiskies use unpeated, green malts from barley, corn or rye, and phenol and cresols have less impact on flavour characters. In these whiskies *smoky* attributes are more likely to originate from lignin breakdown components such as eugenol, 4-ethyl phenol and 4-ethyl guaiacol, derived from new oak staves by charring and ethanolic extractions (Fig. 2.6) (Lehtonen, 1983a) or from cereal cell walls. Eugenol has a low threshold value of 11 µg/L (Erikssson & Lehtonen, 1981), 0.5 µg/L and recognition 5 µg/L (Lee *et al.*, 2000a) and may have a significant impact on Bourbon whisky flavour (Eriksson & Lehtonen, 1981). Adding a mixture of phenols to Bourbon whisky results in enhancement of characters typical of Scotch whiskies, specifically *oily* and *woody* notes, but did not influence *estery* and *sweet* attributes (Eriksson & Lehtonen, 1981).

Humic and fulvic acids in mashing waters (Watson, 1983; Nicol, 1990) influence *peaty* characters. Congeners including halogens of marine origins (Nicol, 1990) and microbial activity also generates highly flavour-active compounds such as chloroguaiacols that at extreme dilutions yield distinctive off-notes, *e.g. Rio* character in coffee (Spadone *et al.*, 1990; Decarvalho *et al.*, 1994; Maier, 1999).

Discrimination of the different phenolic attributes is contentious in the original whisky flavour wheel (Shortreed *et al.*, 1979) with clustering of *medicinal, peaty* and *kippery* characters in the secondary tier. The relationship between *medicinal,* of stronger intensity, and *peaty* character is not clear (Bathgate & Taylor, 1977; Swan & Howie, 1983). No relationship between *dryness* of Scotch and total phenol content has been demonstrated (Nettleton, 1913; Bathgate & Taylor, 1977; Swan *et al.*, 1981). Interestingly, a specific volatile phenol anosmia - partial odour blindness - is reported in 15% of the U.K. population for suggesting inconsistent flavour influences (Howie & Swan, 1984).

FIG. 2.5. Transformation of flavour constituents of barley during whisky production



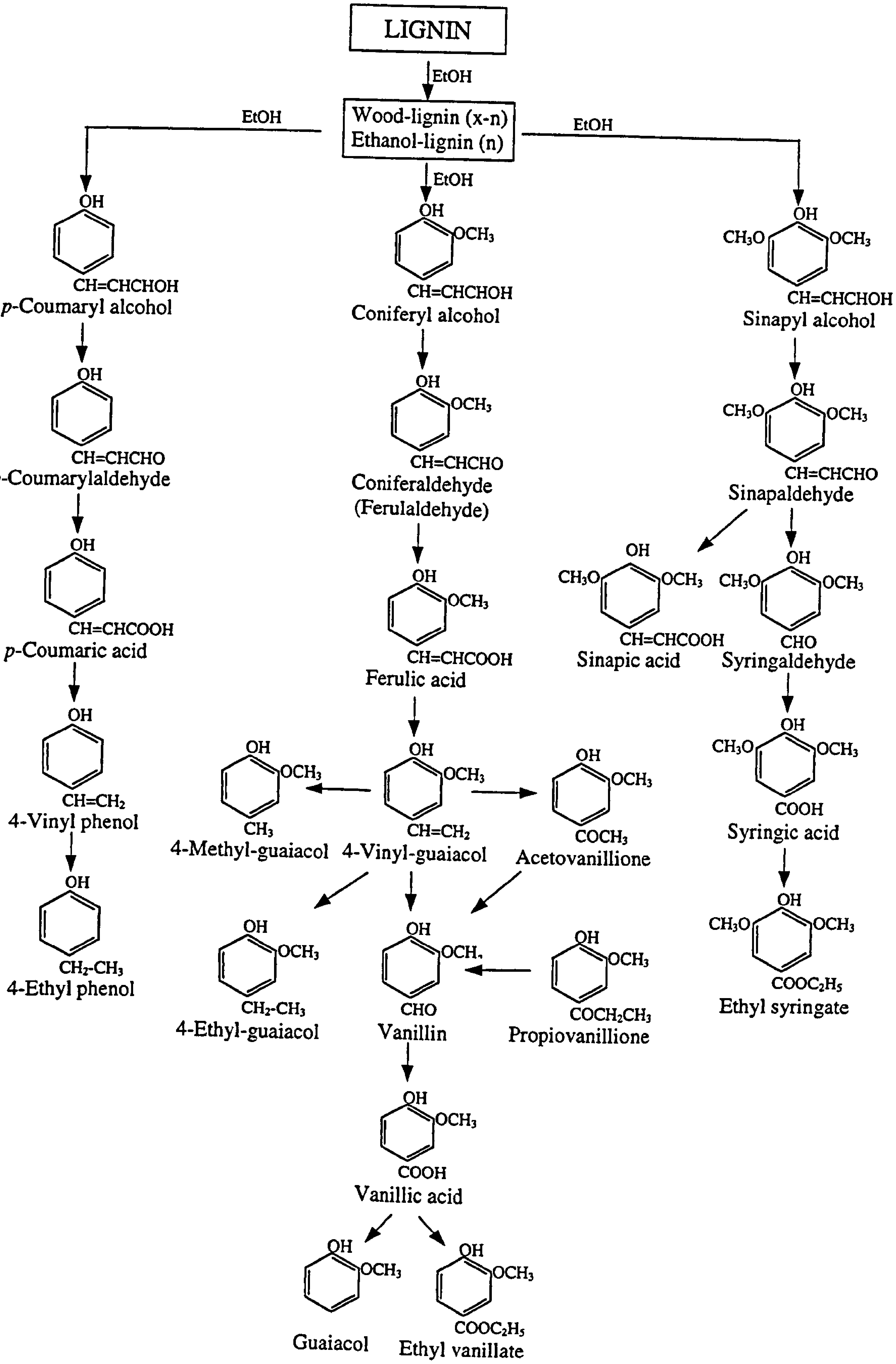


FIG. 2.6. Lignin derived aromatic aldehyde transformation in whisky

2.5.3. Fermentation characters

B. *Grainy* characters

B.1. *cereal - (digestive) biscuity, husky, bran, leathery, tobacco, mousy*

B.2. *malt - malt extract, malted barley*

B.3. *mash - porridge, draff, wort, cooked maize*

The flavour attribute *grainy* has not been linked to any specific chemical compounds but distillers regarded this aspect of character as important in whiskies. Sensory analysts resort to raw materials such as grains or cooked grain (*mash*) as reference standards. Green malt has been described as having *fruity*, *hay-like*, and *damp-straw* notes. As kilning temperatures are increased, these are replaced by *burnt*, *bready*, *malty* and *chocolate-like* notes (Beal & Mottram, 1993). Such notes originate in Maillard browning reaction intermediates or are the result of interactions between these and the products of cereal lipid oxidations (Fig. 2.5).

Furfural may have an aroma note of *grainy* at 20 - 30 mg/L in Scotch whisky (Lyons & Rose, 1977), and may contribute to *hotness* in spirits (Guymon & Crowell, 1972; Singleton, 1995). However, the aroma note associated with furfural at 90% recognition threshold (839 mg/L) (Lee *et al.*, 2000b) was only described by a minority of distillers (<10%) as *grainy*, greater numbers used *marzipan* (*coconut, cake mix, almond, nutty, walnut oil* and *coumarin-like* - 54%), *sweet* (26%) and *oily* (15%) as descriptors (Lee *et al.*, 2000b). The attribute *marzipan* can also be linked to benzaldehyde (Conner *et al.*, 2000; Jellinek, 1985).

Pentose sugars from the breakdown of cell walls in cereal husks yield furfural during pyrolysis processes, both malting and distillation (Bathgate & Taylor, 1977; Berry & Ramsay, 1983; Nishimura *et al.*, 1983; Singleton, 1995) (Fig. 2.5). Furfural yield appears to be a function of pH: when wash contained a high number of lactic acid bacteria, furfural concentration increased (Berry & Ramsay, 1983).

In recent studies (Seitz *et al.*, 1999), volatile compounds collected from whole and ground grains were dominated by aldehydes, enals, 2,3-butanediols (dl and meso forms), acetic acid, chloro- and bromomethoxybenzenes, and aldehydes and pyrazines from roasted barley and malt extract (Kim *et al.*, 1998). The last compound is also associated with *musty* odours in sorghums. *Malty* notes are associated with 2- and 3-methylbutanal in malts (Beal & Mottram, 1994) and linked to *worty* characters in alcohol-free beer (Perpète & Collin, 1999a,b). However this *worty* note is primarily associated with 3-methylthiopropionaldehyde (Perpète & Collin, 1999a,b). Other compounds reported responsible for *malty* or *cereal-like* notes are ethylmethylpyrazines, maltol (Beal & Mottram, 1994) and hydroxydimethylfuranone from 2-methylpropanal (Fickert & Schieberle, 1998). Fermented malt extracts typically contain 4-hydroxy-5(or 2)-ethyl-2(or 5)-methyl-3(2H)-furanone (HEMF) and 4-hydroxy-2,5-dimethyl-3(2H) furanone (HDMF). These compounds with *sweet, malty* and *caramel* notes are produced by *Saccharomyce cerevisiae* in beer fermentations (Hayashida & Slaughter, 1997).

2.5.4. Aldehydic characters

C. *Grassy* character

C.1. *fresh - leafy, wet/cut grass, flower stem, green apple/banana*

C.2. *dried - hay, straw, tea, mint, herbal*

Grassy notes have been considered synonymous with *aldehydic, green* and *leafy* characters in the whisky flavour wheel of Shortreed *et al.* (1979). However, the technical term - *aldehydic* - is better understood as *fresh* or *dried grassy* characters (Lee *et al.*, 2000b). *Grassy* seems a more useful term than *green,* since the term is widely used to imply a lack of maturity in wines (*young*) and associated with use of green malt in whisky (Shortreed *et al.*, 1979). In perfumery, there are also various connotations for *green* (e.g. *trans*-2-hexenal; fruit green especially apple; *cis*-3-hexenal; crushed strawberry and grape leaves; *trans*-2-*cis*-6-nonadienal; cucumber green) (Jellinek, 1985). Interestingly, *grassy* notes associated with hexanal are also perceived as *almond* by a minority (12%) of assessors (Lee *et al.*, 2000b).

Many compounds have been related to *grassy* characters in whisky (Kahn, 1969; Suomalainen & Nykänen, 1970; Suomalainen *et al.*, 1974; Williams & Tucknott, 1972; Swan *et al.*, 1981; Carter-Tijmstra, 1989). Lower aldehydes such as hexanal, *trans*-2-hexenal, 2- and 3-hexenol are perceived as *green leaves*, *grassy* and even *fruity* notes. Flavour characters becomes less pleasant with increasing chain length, with *cardboard-like* and *bitter* notes (Meilgaard, 1975). These compounds originate in barley lipids, dominated by the poly-unsaturated 9,12-octadecadienoic (linoleic), 9,12,15- octadecatrienoic (linolenic) and 9-octadecenoic (oleic) acids. Linolenic and 6,10,14,18-eicosatetraenoic (arachidonic) acids are attacked by lipoxygenases in malting (Fig. 2.5), yielding 9- and 13-hydroperoxides and further aldehydes: hexanal, *trans*-2-hexenal (Paterson & Piggott, 1989), 2- and 3-hexenol (leaf alcohol) (de Rijke & ter Heide, 1983; Jellinek, 1985) and three unsaturated methyl ketones; 6-tridecen-2-one, 6-pentadecen-2-one and 6-heptadecen-2-one (Nishimura & Masuda, 1984).

2.5.5. Estery characters

Characters derived from esters include *solventy*, *fruity*, *floral*, and *feinty*, a function of aliphatic chain length in the aroma-active compound (Ribereau-Gayon, 1978). In distillations, *solventy* and *fruity* characters are related to the heads (foreshot) and the characters pass to *floral* and *feints* (tails) at the late distillation stages (Eriksson, 1981; Paterson & Piggott, 1989) (Fig. 2.7). The cut-off point between the fractions is of primary importance to *estery* character of a final product (Nicol, 1989; Paterson & Piggott, 1989). These differ between distilleries. This order is retained in the revised flavour wheel. Although individual esters are not abundant whisky congeners, the total esters form a key flavour component (Salo, 1972), contributing particularly to *roundness* in whisky (Swan *et al.*, 1981).

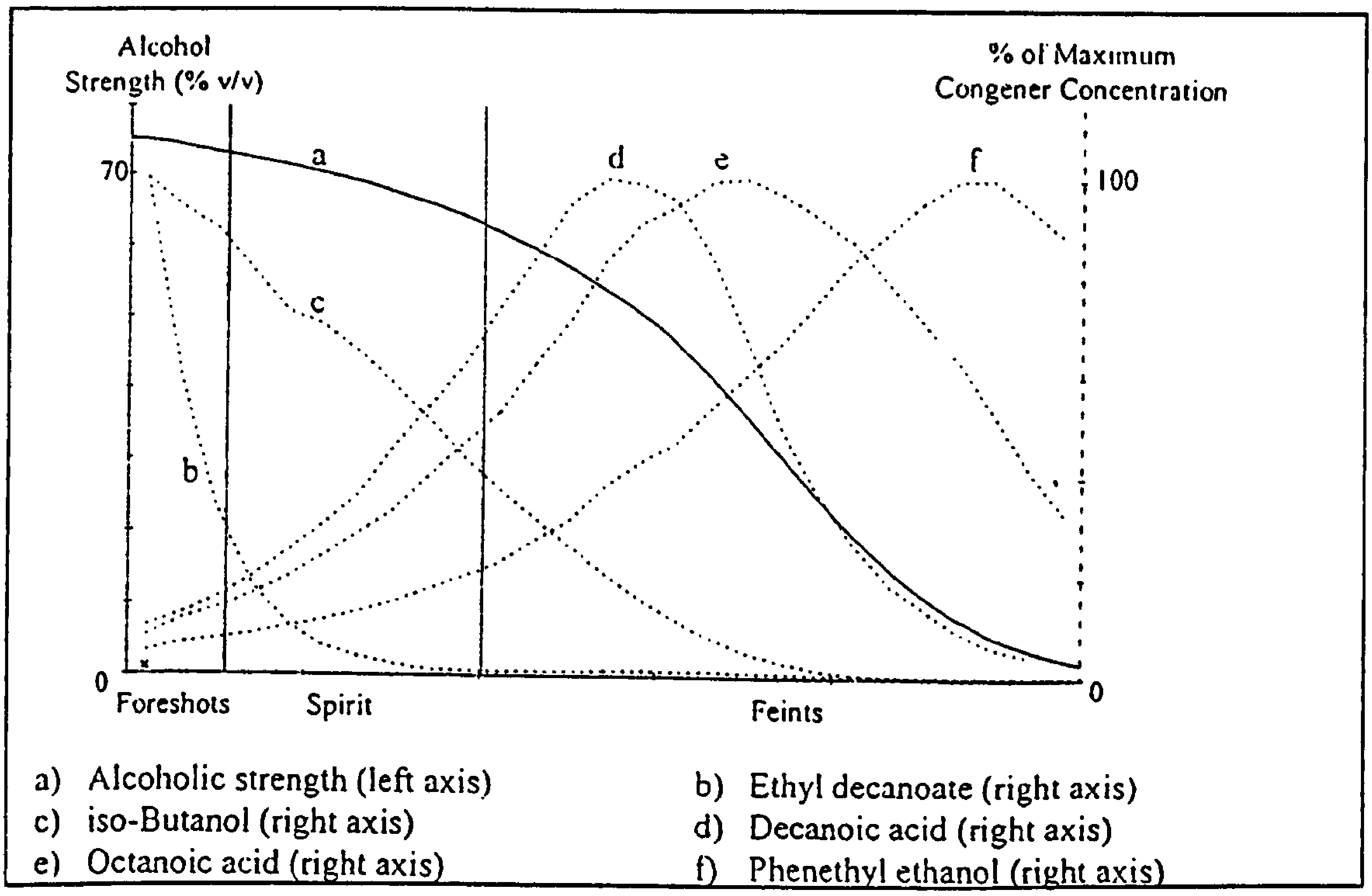


FIG. 2.7. Fractional distillation – foreshots, spirit, feints – and profiling of alcoholic strength and five whisky congeners during a laboratory spirit distillation (Goodall *et al.*, 1999; reproduced with permission from *Institute of Brewing*, 1999, p 105)

In fermentations, esters are formed when acetyl CoA reacts with free alcohols. The rates are inversely related to aliphatic chain length and the ratio is influenced by fatty acids concentrations (Paterson & Piggott, 1989) (Fig. 2.5). Effective concentrations vary because ethyl hexanoate (caproate) is released into wash, whilst ethyl dodecanoate (laurate) is largely retained within the yeast cell (Nykänen & Nykänen, 1977). Formation of esters in fermentations is also influenced by factors such as wort gravity (Ramsay & Berry, 1984), choice of yeast strains (Hay *et al.*, 1994), pitching rate (Ramsay & Berry, 1983; Korhola *et al.*, 1989), unsaturated fatty acid concentration in wort (Lyons & Rose *et al.*, 1977; Thurston *et al.*, 1982; Berry & Watson, 1987), aeration (Thurston *et al.*, 1982; Berry & Watson, 1987) and temperature (Merritt, 1966) of fermentation. Mass transfer of substrates and yeast growth rate appear key factors determining the levels of medium-chain fatty acids in wort (Berry & Watson, 1987).

Further ester formation occurs during maturation through esterification of spirit fatty acids (Reazin, 1981), primarily as ethyl acetate (Nishimura & Matsuyama, 1989). Acetic acid is generated through hydrolysis of side chain acetyl groups in oak cell wall hemicelluloses (Nishimura *et al.*, 1983) and through oxidation of ethanol. Acetaldehyde in spirit is oxidised to acetic acid that reacts with ethanol to yield ethyl acetate in equilibria with acetaldehyde. Acetic acid is also produced as a by-product of wood charring (Chen & Maga, 1993). Certain higher fatty acids, notably ethyl hexanoic (caproic) and tetradecanoic (myristic) acids, react only slowly during maturation whereas other esters - ethyl decanoate (caprate), hexadecanoate (palmitate) and 9,12,15-octadecatrienoic (linoleate) - show significant reverse reactions (Reazin *et al.*, 1981, Nishimura & Matsuyama, 1989).

2.5.5.1. The first-light fraction (foreshots)

D. *Fruity* characters

D.1. *solventy – nail vanish remover, paint thinner, fusel oil*
D.2. *orchard – apple, peaches, pear*
D.3. *tropical – pineapple, melon, banana (pear-drop)*
D.4. *citrus – orange, lemon, grapefruit, zest*
D.5. *berries – blackcurrant, tomato plant, catty*
D.6. *dried – raisins, figs, prunes*

Esters, such as ethyl acetate, are often perceived as *solvent-like*. Of these esters, ethyl acetate is the most abundant at (typically) 175 mg/L in whisky (Berry & Watson, 1987) but has little overall flavour impact with a high threshold (33 mg/L, Meilgaard, 1975; 74 mg/L, Perry, 1989; detection and recognition threshold in grain spirit 14 and 100 mg/L, respectively, Lee *et al.*, 2000a). However, The compound showed synergistic and suppression effects with other esters which could alter overall perceived aroma character (Piggott & Findlay, 1984).

Esters of short-chain alcohols (foreshots) (Fig 2.7) - ethyl, *iso*-butyl and *iso*-amyl esters and *iso*-amyl alcohol are perceived as *fruity*, mainly as *banana* or *apple.* Inclusion of such components in the spirit will alter spirit quality due to increasing contents of fusel oils, ethyl acetate and short chain fatty acid esters, imparting *pear-drop* characters in whisky (Nicol, 1989).

Dried-fruity has been linked with 2-methylpropanal, and 2- and 3-methylbutanal, in dried bell peppers (Luning *et al.*, 1995), though as noted above they have also been linked with *malty* and *worty* notes (Beal & Mottram, 1994; Perpète & Collin, 1999a,b).

2.5.5.2. The middle fraction (spirit)

This mainly consists of ethyl hexanoate (caproate, C6), octanoate (caprylate, C8), decanoate (caprate, C10), lactate and dodecanote (laurate, C12). These are regarded as important contributors to aroma in whisky (Salo, 1972). Ethyl hexanoate imparting *fruity* (*appley*) character in whisky. As chain length increase *soapy*, *oily* and *sour* notes, which are considered immature characters in whisky (Piggott *et al.*, 1992; Clyne *et al.*, 1993), arise as common characters through middle to late distillation stages.

2.5.5.3. The tail fraction (feints)

Ethyl hexanoic (caproic), octanoic (caprylic), decanoic (capric) and dodecanoic (lauric) acids and esters of dodecanoic, tetradecanoic (myristic), hexadecanoic (palmitic) and hexadecenoic (palmitoleic) acids in distillates may give *soapy, oily, sour* and *feints* notes. They are usually discarded as tails (Guymon, 1974) especially the higher fatty acid esters ($>C_{16}$) since excess concentrations cause chill haze problems when the spirit is reduced to bottling strength (Nicol, 1989; Piggott *et al.*, 1996). They are, therefore, present at lower concentrations in new distillates (Cole & Noble, 1995; Goodall *et al.*, 1999) (Fig. 2.7). Their concentration is related to the presence of yeast in wash during distillation (Suomalainen & Nykänen, 1966). Although propanol, *iso*-butanol, active amyl and *iso*-amyl alcohols were common in head and feints fraction, *feints* aromas are rarely reported in the early portion of the spirit fraction (Muller, 1992). Therefore, several fatty acids and sulphur containing compounds (e.g. dimethyl trisulphide) in the tail could be responsible for *feints* character (Muller, 1992).

E. *Floral* characters

E.1. *natural - rose, lavender, violet, blue bell, carnation, heather, honey*
E.2. *artificial - fragrant, scented, perfumed*

Phenylethyl ethanol from the tail fraction (feints) is mainly responsible for *floral, rose-water* and *fragrant* character. A congener with a similar behaviour is β-damascenone which imparts *fragrant* notes on dilution of whisky to 23% (abv) (Perry, 1989). This compound, a major contributor to character in Damascus rose oil, has a high odour unit value of 2500 in whisky, but is not readily detected due to a low intensity index (Perry, 1989).

Autoxidation of vitamin A or lipids, from yeast or barley (de Rijke & ter Heide, 1983), and breakdown of oak norisoprenoids (Cole & Noble, 1995) yields α- and β-ionones with *violet-like* notes in whisky.

F. *Feints* characters

F.1. *grainy*: see section B
F.2. *cheesy*: see section K
F.3. *oily*: see section L
F.4. *sulphury*: see section J

An excess of *feints* character in spirit can affect new distillate quality (Nicol, 1989). Other feints notes are *leathery* or *cereal-like* (*cooked mash, biscuity*) passing to *sweaty* (*piggery*) and into *stale fish* characters (Shortreed *et al.*, 1979). *Sweaty* however has also been related to spirit *iso*valeric acid content (Muller, 1992). Feints, with distinctive *stale* aroma notes and *metallic* aftertaste (Bathgate & Cook, 1989), also contain high concentrations of phenols from malted barley, and DMTS (Carter-Tijmstra, 1990).

2.5.6. Maturation characters

G. *Woody* character

White oak wood (e.g. *Quercus alba*) is composed of cellulose (49 - 52%), lignin (31 - 33%), hemicellulose (22%) and extractable compounds such as volatile oils, volatile and non-volatile acids, sugars, steroids, tannic substances, pigments and inorganic compounds (Nishimura & Matsuyama, 1989). Composition can vary between and within species: different analyses have been published (e.g. Chen & Maga, 1993). Heartwood contained triglycerides of C_{18} unsaturated and C_{16} saturated fatty acids, sterols and a ferulic acid ester with a C_{40} wax alcohol (Singleton, 1995). All these compounds possess a great potential flavour impact as spirit gradually getting matured. Sterols produce haze in barrel-aged spirits as they are reduced in proof with water for bottling (Singleton, 1995).

Cellulose (a glucose polymer) is considered the framework of the wood, hemicelluloses as the matrix and lignin as the encrustant (although 70% of the lignin is in the cell walls). Hemicelluloses are heterogenous polymers including xylose and other sugars especially pentoses. Lignin is a three dimensional polymer of phenylpropane derivatives of guaiacyl (2-methoxyphenol) and syringyl (2,6-dimethoxyphenol) units substituted in the four-position with the aliphatic side chain and further cross-linked by oxidation. Owing to additional linking, lignin, tannins and the carbohydrates are attached to each other to some degree, making precise selective separation difficult. Unless fragmented, none of these major classes of compounds can be directly significant to flavour due to their insolubility. The extractives, however, have immediate potential to affect beverage flavour (Singleton, 1995) by conferring *smoky* (Chen & Maga, 1993) and *woody* characters. Lignins require the highest temperature for destruction while cellulose decomposes at the lowest temperature (Chen & Maga, 1993). Hemicelluloses are insoluble in distillate but slowly decompose in the acidic conditions (~ pH 4.5) of maturation (Nishimura *et al.*, 1983).

Woody characters are complex in both whisky and wines (Noble, 1978). Important compounds extracted into spirit are mainly whisky lactones, and lignin breakdown compounds – vanillin, and other aromatic aldehydes and their acids, esters, tannins and sugars. Pérez-Coello *et al.* (1997) regarded *cis*- and *trans*-lactone, eugenol, vanillin and syringaldehyde as the most important volatiles present in oak woods from a sensory standpoint. The optimum temperature for extraction of vanillin and syringaldehyde was proposed as 165 to 215 °C (Martinez *et al.*, 1996) and the proportion of syringaldehyde/vanillin 1.4/2.5 suggested for a balanced decompositon of lignin (Puech & Joure, 1982). Important interactions between certain lactones and vanillin, at concentrations present in Scotch whisky have been reported to enhance aroma impact of vanilla 50-fold (Swan 1999, IOB seminar). Certain wood-extractive phenolics, notably vanillin (3-formy-2-methoxyphenol), syringaldehyde and 5-hydroxy methyl-2-furfuraldehyde have been regarded as a marker of good quality in spirit. Good staves have a high proportion of early-wood with a high number of annual rings/cm (maximum 12) (Reid *et al.*, 1993). Recently, the most suitable extraction method for a large number of compounds (96) of different polarity from oak wood has been reported (Pèrez-Coello *et al.*, 1998).

Nykänen (1984) concluded that hydrolysis is more important than ethanolysis in maturation, as caskwood absorbs water in preference to ethanol. However, ethanol activity is at a maximum in water-ethanol mixtures at cask strength (60% abv) enhancing solubility of important flavour components, notably acids and phenolics. These, together with their oxidation products, confer *woody* characters - *maturity*, *roundness*, *well-balanced* and *smooth* into distillate (Koga & Yoshizumi, 1977). These *woody* characters have been divided broadly into three categories: new wood, extractives and defective stave characters.

2.5.6.1. New wood characters

G.1. *sap – green bark, wet wood*
G.2. *cedar – sawdust, cardboard, sharpened pencil*
G.3. *oak – resin, polish*
G.4. *pine – turpentine, retsina*

Fresh sawdust or *sap* characters are observed in spirits matured in certain new barrels, a function of wood origin, and are eliminated with a second use (Chatonnet & Dubourdieu, 1998b). Unpleasant *sawdust* notes have been linked to (E)-2-nonenal, 3-octen-1-one, (E)-2-octenal and 1-decanal in wines (Chatonnet & Dubourdieu, 1998b). (E)-2-nonenal has also been linked to *rancid* aroma notes in beer (Tressl *et al.*, 1979; Barker *et al.*, 1983). These compounds are also associated with *cardboard* characteristic in whisky and many food products (Widder & Grosch, 1997; Petersen *et* *al.*, 1999). These compounds originate in the oxidation of unsaturated barley lipid fatty acids such as linoleic acid (Badings, 1970). However, toasting processes reduce (E)-2-octenal, eliminating associated aroma notes in matured wines (Chatonnet & Dubourdieu, 1998b).

Filling at higher alcoholic strengths reduces extraction of wood-derived components and associated flavour notes (Singleton, 1995). In 8-year old whisky filled at 59% abv its character was described as *flavoured*, whereas at 63% it was *less matured* and *weaker* and at 77% abv as *green oak* (Nishimura & Matsuyama, 1989; Singleton, 1995). In the case of Cognac, the flavour compounds: phenolic acid, aromatic aldehydes, acetals, ethyl butyrate, medium chain fatty acid esters (C8, 10 & 12), are preferentially extracted at a filling strength of 60 – 70% abv. However, more sugars, polyols, acetic acid, ethyl acetate and short chain fatty acids (C3, 4 & 5) are extracted at lower strength at 40 – 50% abv (Cantagrel *et al.*, 1995). For the balanced extraction of all flavour constituent, the optimum equilibrium has been found at the intermediate alcohol strength at 50 – 55% abv (Cantagrel *et al.*, 1995).

2.5.6.2. Wood extractive characters

G.5. *nutty - coconut, hazel nut, almond/marzipan, walnut*

Nutty is associated with a product of oak lipid oxidation, described as "whisky lactone", "3-methyl-4-octanolides", "β-methyl-γ-octalactone", "5-butyl-4-methyl-dihydro-2(3H)-furanone", or "Quercus lactone". Associated flavour character is *coconut* at high concentrations (>5.3 mg/L) and *oak wood-like* at lower (0.1 mg/L) (Maarse & van den Berg, 1994; Sharp, 1983). This lactone, together with 4-nonanolide and eugenol (Masuda & Nishimura, 1971; Eriksson & Lehtonen, 1981), are major volatile congeners derived solely from oak (Maga, 1989; Mosedale, 1995).

A possible lactone precursor is 2-methyl-3-(3,4-dihydroxy-5-methoxybenzo)-octanoic acid (Otsuka *et al.*, 1980; Masson *et al.*, 1995). Four lactone isomers are, *cis-(3R,4R)*, *cis-(3S,4S)*, *trans-(3S,4R)* and *trans-(3R,4S)*, differing in flavour character (Table 2.2) (Günther & Mosandl, 1986). Oak contains only *cis-(3S,4S)* and *trans-(3S,4R)* (Masuda & Nishimura, 1981; Masson *et al.*, 1995): other isomers indicate synthetic lactones in flavouring/ageing agents (Maga, 1996; Mosedale & Puech, 1998).

In a racemic mixture of lactone, the detection and recognition thresholds were 0.5 mg/L and 1 mg/L, respectively in 23% grain whisky (Lee *et al.*, 2000a) and 0.05 mg/L (Salo, 1972) in 34% abv grain spirit. Thresholds in white and red wines were 120 and 125 µg/L (Chatonnet *et al.*, 1990), and as a difference threshold 241µg/L in white, 853 µg/L in red wine and 75 µg/L in 12% abv ethanol (Piggott *et al.*, 1995a).

The sensory characteristic of lactone in white wine was perceived as *musty*, in red wine as *harsh* and in 12% ethanol as *coconut*, *woody* and *oaky* (Piggott *et al.*, 1995a) which were more to the *cis* characteristic (Table 2.2). The concentration of *cis* lactone was greater than the *trans* isomer in wines, and the *cis* isomer appears to make a considerable contribution to aroma in wine with also a lower threshold (Piggott *et al.*, 1995a). Threshold of *cis* isomer has been reported to be 0.092 mg/L in white wine, which was 2.5 - 20 times lower than the *trans* form (Mosedale & Puech, 1998).

TABLE 2.2. Taste and odour properties of oak lactones (Günther & Mosandl, 1986; reproduced with permission from *Liebigs Annalen der Chemie*, p 2112)

Compound	Taste	Odour
cis-(3R,4R)	*Creamy, coconut*	*Sweet, woody, coconut*
cis-(3S,4S)	*Spicy, coconut-like*	*Light coconut, musty, hay*
trans-(3S,4R)	*Coconut-like, sweet, Creamy, fatty*	*Spicy, celery, slight coconut, green walnut*
trans-(3R,4S)	*Spicy*	*Coconut, celery*

Significantly higher amount of *cis* form were identified in American white oaks than in Pedunculate or Sessile oaks (Masson *et al.*, 1995). Contradictory results for the ratio of *cis:trans*, and threshold values in alcoholic beverage, have been reported (Otsuka & Zenibayashi, 1974; Maga, 1989; Chatonnet *et al.*, 1989; Mosedale & Ford, 1996) due to misidentification of isomers (Kepner *et al.*, 1972; Otsuka & Zenibayashi, 1974; Marsal & Sarre, 1987; Boidron *et al.*, 1988; Maga, 1989). Oak wood origins can be discriminated clearly by a difference in their ratio (Pérez-Coello *et al.*, 1997) and Günther and Mosandl (1987) reported 77% and 23% of *cis* and *trans* in wood. The ratio in the final product is strongly dependent on the history and treatment of the barrel, but conversion of *trans* to the more stable and flavour-potent *cis* form has been observed during subsequent bottle maturation (Chatonnet, 1991). The ratio may be related to the difference in their location within wood, as *cis* had a maximum concentration (250 mg/kg of wood) at 5 mm below the stave surface whereas *trans* had a maximum (48 mg/kg) at 15 mm (Conner *et al.*, 1993). The shallower maximum concentration, by facilitating easy access for extraction, could allow higher amounts of *cis* lactone in final products (Maga, 1996). However, Waterhouse and Towey (1994) concluded that the same approximate ratio was found among similar oak species (European versus American oak), and that white oak contains a fixed ratio of *cis* to *trans* oak lactone that is genetically determined. Since the conclusion disregards all previous data related to the roles of the isomers, their hypothesis will required additional investigation before it can be accepted (Maga, 1996).

Abbott *et al.* (1995) also measured thresholds of *cis*- and *trans*-lactone in gaseous medium as 0.001 and 0.02 mg/L, respectively, which were much lower compared to liquid media. The isomers had an additional *rose-like* note to *coconut* characteristic. Piggott and Melvin (1995a) also detected a low intensity of *fruity* note in a peak before the main *coconut* odour during purification of oak lactone, which could be an impurity. Flavour characteristic of lactone also had a strong “carrying ability” which lasted up to 10 days in a covered standard-size wine glasse (Abbott *et al.*, 1995).

Extraction of oak lactone differs according to origin, trunk location and depth of wood. American white oak (*Quercus alba*) heartwood contains five-fold more lactone than sapwood due to the high concentration of the precursor lipids (Maga, 1989). American oak is distinctive, with the high content of lactones and low content of extractable polyphenols, and the presence of two 3-oxo-retro-α-ionol isomers could serve as a marker. In new Bourbon casks extractable lactones are ten-fold higher at 0.047 - 0.254 µg/kg than a stave used for Scotch whisky. Their flavour impact in Scotch whisky would be negligible on successive uses of a cask (Conner *et al.*, 1993). An “standard” quality of Scotch whisky contained 0.96 mg/L, while a “premium” had as 1.16 mg/L, and a “high” had 2.17 mg/L of total lactone (Otsuka & Zenibayashi, 1974). A similar correlation between concentrration of lactone and product quality grade has been observed in Cognac (Otsuka & Zenibayashi, 1974).

Eugenol and vanillin concentrations are similar in American and European oaks (Chatonnet & Dubourdieu, 1998a). European pedunculate oak (*Quercus robur*), with a low aromatic yield and high ellagitannin content is best suited to ageing spirits; European sessile (*Quercus petraea*) and American white oak are suitable for wine (Chatonnet & Dubourdieu, 1998a). Eastern European oaks, which are becoming commercially important, share many properties with other European oaks and require an air seasoning due to high tannin contents (Swan 1999, IOB seminar).

To restore lactone content for reuse of casks, a charring process increases lactone yields as much as three-fold through thermal oxidation of lipids (Maga, 1989). No lactone was detected in heavily toasted wood, possibly due to volatilization or thermal degradation of lactone by the high temperature (Chatonnet *et al.*, 1989; Maga, 1996). However, lactone was presummably still present at deeper levels in the wood, and below the char layer in charred wood. Careful toasting operation is required since changes in the *cis/ trans* ratio will influence their overall impact on spirit flavour due to their threshold difference (Maga, 1996). Green oak is mainly described as *vanilla, buttery, nutty, caramel, cedar, cococnut, raisin* and *dill*, and is especially high in *spicy* characteristic (Sefton *et al.*, 1993a,b). However, seasoning increases characters such as *cedar* and *nutty* notes and decreases *raisin* notes (Francis *et al.*, 1992; Sefton *et al.*, 1993a,b).

Recently, γ-nonalactone (C9L), γ-decalactone (C10L) and γ-dodecalactone (C12L) have been found in both mature and immature malt whisky, imparting *fatty* and *sweet* characteristics. C10L and C12L especially contribute a matured *sweet* characteristic in malt whisky (Wanikawa *et al.*, 2000). Other lactones are produced by yeasts, notably *Sporobolomyces* in certain wines and flor sherries, excreting 4-decanolide and *cis*-6-dodecen-4-olide (Muller *et al.*, 1973; Berry & Watson, 1987).

G.6. *vanilla - ice cream, custard, toffee, chocolate, cake mix, cola*

The attribute *vanilla* is described by certain assessors as having *chocolate* and *cola* flavour notes through 'circle minded' associations with flavourings in these products (Jellinek, 1985). As new wood characters are undesirable, new casks are heat treated (toasting); the optimum temperature range for this is 165 - 215 °C (Martinez *et al.*, 1996). In Bourbon whiskey production casks are generally sufficiently charred to eliminate *new-wood* notes and *astringent* taste. This charring process has been correlated with higher contents of vanillin, vanillic acid and related compounds - acetovanillone and propiovanillone, other aromatic aldehydes such as coniferaldehyde, sinapaldehyde, acetosyringone and their acids (Nishimura & Matsuyama, 1989). The aromatic aldehydes are further oxidised to acids or to vanillin and syringaldehyde thus contributing to *vanilla* notes in whisky (Fig. 2.6) (Nishmura *et al.*, 1983; Nishimura & Matsuyama, 1989). They have marked effects on sensory quality in the final spirit (Reazin, 1983; Nishimura *et al.*, 1983; Clyne *et al.*, 1993; Conner *et al.*, 1996) with their synergistic effect (Table 2.3) (Maarse & van den Berg, 1994). Clyne *et al.* (1993) detected significantly higher levels of vanillin and syringaldehyde in whisky from charred casks with a sensory character described as *smooth, vanillin, sweet, malty, spicy, fruity* and *floral* that was reminiscent of Bourbon whiskey. The threshold values of vanillin were defined as 2 mg/L in water, 0.5 and 0.1 mg/L in 10 and 40% respectively ethanol solution (Maga 1984; Singleton, 1995). Whisky from uncharred casks had higher levels of coniferaldehyde, sinapaldehyde and vanillic acid and was described as *pungent, grainy, sour, oily, sulphury, catty, meaty,* and *fishy* and resembled immatured whisky characters. Such compounds are released into spirit in larger quantities after filling and more slowly during subsequent maturation by hydrolysis, ethanolysis and oxidation mechanisms, at rates depending on solubility at the filling strength used (Conner *et al.*, 1992; Singleton, 1995).

Although gallic acid from hydrolysis of tannins is the most abundant extractive of unheated wood, the major flavour impact in Scotch whisky is presented by syringaldehyde (50% of total aldehydes) and vanillin (24%) (Reazin, 1983; Lehtonen, 1983a,b; Conner *et al.*, 1993; Martinez *et al.*, 1996). Increased dissolved oxygen yields higher concentrations of vanillin, syringaldehyde, conniferaldehyde, vanillic and syringic acids (Nishimura *et al.*, 1983; Reazin, 1983; Maarse & van den Berg, 1994; Withers *et al.*, 1995). Conner *et al.* (1993) confirmed that maximal concentration of the sensorially-important phenols were observed at 5 mm below the char in new wood with concentrations of syringaldehyde and syringic acid markedly higher than vanillin and vanillic acid.

TABLE 2.3. Synergistic effects of aromatic aldehydes in 40% abv (Maga, 1984; reproduced with permission from *Elsevier Science*, p. 409)

Compounds	Taste threshold (mg/L)
Vanillin (V)	0.1
Syringaldehyde (Y)	15
Sinapaldehyde (I)	50
Ferulic acid (FA)	30
Vanillic acid (VA)	25
Syringic acid (YA)	10
Sinapic acid (IA)	100
V/Y	2
FA/VA/YA/IA/V	4
FA/VA/YA/V/Y/I	2

Methods of drying oak wood (air or kiln) may also effect the release of vanillin, coniferaldehyde and syringaldehyde (Ward *et al.*, 1998). Air seasoning results in an increased mycoflora attacking cell wall lignin and polysaccharide (Ward *et al.*, 1998) and increasing the extraction of the compounds which are associated with the positive maturation characters (Swan *et al.*, 1992).

G.7. *spicy - clove, cinnamon, ginger, 'aromatic', nutmeg*

The major *woody spicy* attribute is from components of oak wood extracts, particularly eugenol. Eugenol is derived from oxidation of oak lipids (Fig. 2.5) (Maga, 1989; Mosedale, 1995; Mosedale & Puech, 1998), and is found in high concentration in Bourbon whisky (Eriksson & Lehtonen, 1981; Piggott *et al.*, 1993a). The compound has a threshold range 2 - 34 µg/L in beer (Meilgaard, 1993), 11 and 50 µg/L in 10 and 20% ethanol respectively (Singleton, 1995) and detection and recognition thresholds of 0.5 mg/L and 4.9 mg/L respectively at 23% abv in grain spirit (Lee, unpublished data). However lignin thermal degradation products such as vinyl-, allyl- and ethyl guaiacols, guaiacol (2-methoxyphenol), cinnamaldehyde and related phenolic acids also contribute *sweet, smoky* and *spicy* notes to whiskies (Fig. 2.6) (Williams *et al.*, 1984; Singleton, 1995). The yeast enzyme ferulic acid decarboxylase (Donaghy *et al.*, 1999) also converts cell wall ferulic acid to 4-vinyl guaiacol. The attribute *clove* is related and its perception linked with *oakiness* (Singleton, 1995). However *spicy* notes are common to clove, cinnamon bark oils, and other spices (Jellinek, 1985) and a single meaning may be difficult to achieve (Lee *et al.*, 2000b). Relating this to wood character may be difficult and require training with appropriate standards prior to sensory assessments.

G.8. *caramel - candy floss, treacle, coffee, toast, liquorice*

Caramel - *sweet*, *burnt* and notably *smoky* have been associated with products of thermal destruction of lignins, dominated by phenols such as guaiacol (2-methoxy phenol), 4-acetyl-guaiacol and syringol (2,6-dimethoxy phenol), homologues and derivatives. Polymeric lignins are generally degraded only in the presence of strong acids, limiting their contribution to character in spirit after maturation (Conner *et al.*, 1992). Lower molecular weight components such as free guaiacyl and syringyl acids and their aldehydes are extracted directly from caskwood into spirit. Such compounds decrease with repeated cask use. The character notes are also derived from from 5-

hydroxymethyl-2-furfuraldehyde and hydroxymethyl-pyranones (Reid *et al.*, 1993) during ageing in a freshly charred oak cask. Caramel added to enhance colour in blended whiskies also contains 5-hydroxymethyl-2-furfuraldehyde (Jaganathan & Dugar, 1999). In Bourbon whiskey, 2-hydroxy-3-methyl-2-cyclopentenone and 3-hydroxy-2-methyl-4-pyrone (maltol) are important, as they are associated with *sweet* and *burnt* notes (Nishimura, 1983; Nishimura & Matsuyama, 1989).

Other contributors to the character, derived from Maillard reactions (Fig, 2.5) that take place during stave pyrolysis, are furans: furfural, 2-methyl furfural and 5-hydroxyfurfural, and heterocyclic nitrogen compounds: pyrazines, pyridines, thiazoles, aliphatic amines, quinolines and pyrans (Nishimura & Matsuyama, 1989; Maga, 1989; Clyne *et al.*, 1993; Piggott *et al.*, 1993b; Cole & Noble, 1995). The most abundant nitrogen compounds imparting *burnt*, *roasted* and *nutty* characteristics in malt are methylpyrazine and 4-methyl-5-vinylthiazole and they are present at higher levels in charred wood surface layers than in plain wood shavings or deeper layers of charred wood (Piggott *et al.*, 1993a). Their concentrations in whiskies were significantly higher than odour threshold values (Ough, 1984). Pyrazines are mainly associated with roasted foods and they are generally regarded as pleasant flavour notes such as *burnt, toasted, medicinal, nutty, fruity, woody* and *earthy* (Vernin & Vernin, 1982; Piggott *et al.*, 1993b), and *phenolic, nutty* and *green.* Character is enhanced when methoxyl groups are present on pyrazine derivatives (Herent *et al.*, 1995). Unlike pyrazines, pyridines are perceived as less pleasant as *astringent, bitter, buttery, caramel, roasted, green, earthy, rubbery*, and *fatty*, and *pungent, solvent* and *fishy* (Maga, 1981; Delahunty *et al.*, 1993). Viro (1984) reported that reduced pyridine concentrations in Finnish whiskies improved the taste and odour of the whisky. However, they are less likely to be detected in the headspace since pyridines are ionized at low pH (4.0 - 4.5) in matured whisky, and therefore their flavour impact in whisky is reduced (Delahunty *et al.*, 1993; Piggott *et al.*, 1993b).

G.9. *Previous use - sherry, Bourbon, port, rum, brandy, wine*

Scotch whisky has traditionally been matured in casks previously containing other materials and in some cases these may flavour the subsequent fills of whisky. Sherry casks in particular, have been sought after for their effects on whisky but others have also been occasionally used (Madeira, Port). Trend of changing the use of cask from ex-sherry to ex-Bourbon barrels (Mosedale & Puech, 1998; Chatonnet & Dubourdieu, 1998a), some Scotch whisky manufacturers may require an additional treatment prior to the use of ex-Bourbon casks. The cask may be treated with white wine or allowed to absorb a very sweet, dark sherry under pressure to simulate previous sherry use. This has been reported to raise total levels of esters and sugars and claims to produce *mildly flavoured* matured whisky (Mosedale & Puech, 1998).

2.5.6.3. Other wood extractive characters

Mellowness, roundness & smoothness

Mellowness and *lingering* aftertaste of matured whiskies have been related to changes in hydrogen bonding between water and ethanol, with formation of stable cluster structures during maturation (Akahoshi, 1963; Akahoshi & Ohkuma, 1984, 1985; Nishimura & Matsuyama, 1989). The presence of such clusters can be inferrred from the results of differential scanning calorimetry (Fig. 2.8) (Koga & Yoshizumi, 1977, 1979). Stable clustering has also been shown to be increased by non-volatile oakwood extracts (Fig. 2.8) (Koga, 1981; Nishimura & Matsuyama, 1989), and parallel changes in perception of *mellowness* and *roundness* are closely related to non-volatile compounds (Piggott *et al.*, 1992, 1993a; Clyne *et al.*, 1993). A part of such changes is associated with dissolution of monosaccharides from wood cell wall components (pentose, hexose), and glycerol (Reazin, 1981) generated by thermal breakdown of wood triglycerides and also the gradual degradation of wood polymer such as lignin through ethanolysis.

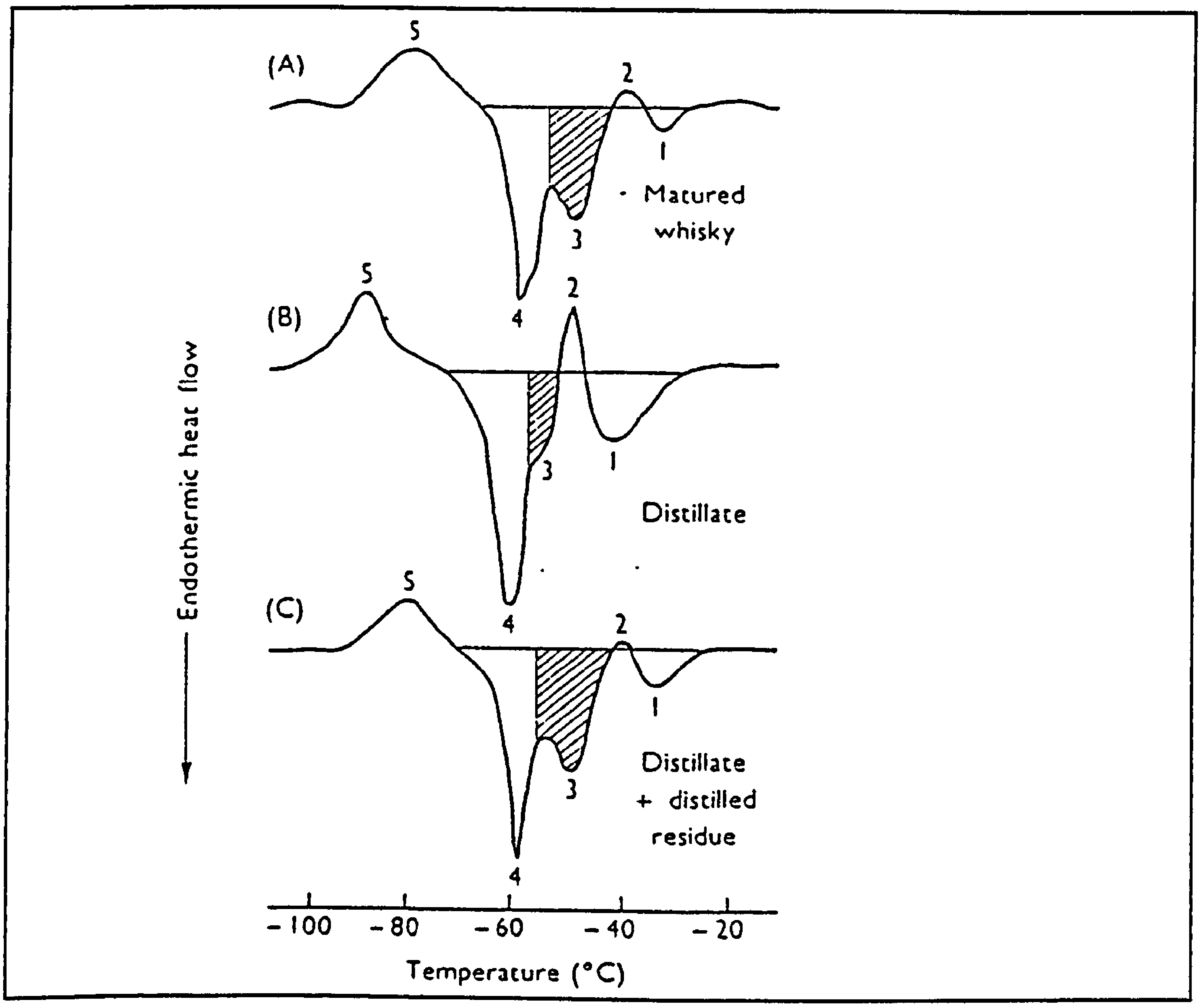


FIG. 2.8. DSC thermograms of the melting of rapidly frozen matured whisky, distillate of matured whisky and restored whisky (Nishimura *et al.*, 1983; reproduced with permission from Piggott, J.R. Ed) *Flavour of Distilled Beverages: Origin and Development*, Chichester, Ellis Horwood, p 252)

2.5.6.4. Defective wood characters

G.10. *mothball - paraffin, naphtha, camphor*
G.11. *musty - mouldy, earthy, fusty, corked*
G.12. *vinegary - acetic, sour*

Mouldy and *earthy* flavour notes have been attributed to components originating in fungi and actinomycetes on malt (Watson, 1983), defective casks (Philp, 1989) and cork closures. A culprit contaminant of wine corks is *Armillaria mellea* (Simpson, 1990; Simpson & Veitch, 1993; Rocha *et al.*, 1996). These aroma notes have been associated with methyl thiopyrazine, 2-acetyl piperidine and its isomer 2-acetyltetrahydropyridine, 1-octen-3-one (*mushroom*), 3-octen-1-one (*musty, mouldy, earthy, mushroom*) (Chatonnet & Dubourdieu, 1998b). In contrast 2-methyl-isoborneol (2-MIB, *earthy*), geosmin (*mildew*) and 2,4,6-trichloroanisole (2,4,6,TCA) are associated with *musty*, or *cork taint* notes in wines (Simpson, 1990; Evans *et al.*, 1997). Other potential compounds are 2,3-diethyl-5-methylpyrazine and 2-isopropyl-3-methoxypyrazine (*musty/mouldy*) (Land *et al.*, 1975; Jagella & Grosch, 1999). Corks contaminated with pesticide chlorophenols such as 6-chlorovanillin, 4-chloroguaiacol, 4,5-dichloroguaiacol and veratrole can yield chloroanisoles (e.g. 2,4,6-trichloroanisole) with *corked taint* and *musty* notes after mould contamination. Such compounds are associated with off-notes in tainted wines (Casey, 1990, 1996; Fuller, 1995; Kugler & Rapp, 1997) and other alcoholic drinks (Duncan *et al.*, 1997) but can also be transferred from woods, including wood shavings (Land *et al.*, 1975). In wine, the threshold showed a bimodal distribution, and can be very low at 1.4 ng/L (Duerr, 1985) and 17.4 - 210 ng/L (Suprenant & Butzke, 1996) and some assessors are particularly sensitive to these flavour notes (Simpson & Veitch, 1993). Corks prior to a surface treatment are described as *woody* and *green cork* and after the treatment as less *woody* with additional *oily* notes from coating materials (Simpson & Veitch, 1993). To reduce off-notes from cork volatiles of microbial origin, autoclaving has been recommended (Rocha *et al.*, 1996).

Geosmin, and 2-methyl-isoborneol are common compounds introduced by water (Lloyd *et al.*, 1998; Ridal *et al.*, 1999) and also have low detection thresholds (ng/L; Lloyd *et al.*, 1998). It has been suggested that these compounds are unstable in the acidic environments of wines and distilled drinks.

2.5.7. *Sweet* characters

H.1. *woody-sweet*: see section G
H.2. *floral-sweet*: see section E
H.3. *fruity-sweet*: see section D
H.4. *buttery-sweet:* see section L

An important component of flavour in whisky is *sweet* character - elicited from different origins in 23% abv grain whisky: diacetyl (47% of respondants), maltol (38%), vanillin (28%), furfural (26%), ethyl hexanoate (24%), *iso*-amyl acetate (15%), whisky lactone (10%) (Lee *et al.*, 2000b). Some assessors used only the generic description *sweet* for these compounds, and did not discriminated between them. This could be either lack of verbal ability or the *sweet* properties of the compound itself.

As *woody-sweet,* the wood derived compound vanillin was described as *vanilla* (58%), *toffee* (35% - *toffee, caramel, fudge* and *chocolate*) and *sweet* (28%). Whisky lactone is mainly described as *coconut* (84% of respondants), but may also introduce a *sweet-almond* character (*nutty*, 11%, *marzipan*, 7% and *almond*, 4%) (Lee *et al.*, 2000b). *Nutty* is also related to *sweet* taste (Jellinek, 1985). 4-Vinyl guaiacol also contributes *sweet-woody* character in whisky. Although *smoky* (32%) appeared as a common descriptor for phenolic compounds, wood derived phenols are mainly perceived as having *sweet-smoky* character: *spicy* (17% - *spicy, nutmeg, ginger, clove* and *aromatic*), *vanilla* (13%) and *woody* (7%). Maltol evoked *candy floss* alone in a high proportion (44%) of respondents, and related terms such as *sweet* (39% - *sweet* and *sugary*) and *caramel* (35% - *caramel, burnt sugar, toffee*) were also relatively frequent. As *floral-sweet*, geraniol was described as *floral* (36%), *lemony* (*citrus* and *washing-up liquid*, 25%), *scented soap* (21%), and also evoked *sweet* (17%) character (Lee *et al.*, 2000b). As *fruity-sweet*, *iso*-amyl acetate was described as *pear-drop* (67%), and other *fruity* terms, mainly *banana* and *pear* (22%) were reported in 23% abv grain whisky (Lee *et al.*, 2000b). As *buttery-sweet*, difficulty was observed in discrimination of perceptions of *sweet* and *buttery* with diacetyl at 90% recognition threshold in 23% grain whisky (Lee *et al.*, 2000b). Diacetyl was mainly described in sweet-related terms (47%), with *buttery* 31% of respondents (Lee *et al.*, 2000b).

2.5.8. *Off-aroma* characters

2.5.8.1. *Stale* characters

I.1. *cardboard - papery, filter sheet*

I.2. *metallic - inky, tinny, wet iron, rusty*: see the section F (feints)

Cardboard-like notes in many foods (e.g. boiled potato and butter oil) are due to the products of lipid oxidation (e.g. unsaturated fatty acids). These or similar compounds in whisky might arise from lipid oxidation in cereals, or as products of yeast metabolism (Berry & Watson, 1987; Dufour & Malcorps, 1995). Oxidation of 9,12-octadecadienoic (linoleic) and 9,12,15-octadecatrienoic (linolenic) acids by a lipoxygenase give rise to pentanal, hexanal, nonanal, (E)-octenal, 2,4-heptadienal, (E)-2-nonenal, (E,E)-2,4-nonedienal and 2,4-decadienal. These compounds are responsible for *cardboard* off-note in boiled potato (Petersen *et al.*, 1999). Autoxidation of hexadecenoic (palmitoleic) acid give rise to (E)-2-nonenal, (E)-2-

octanal and (Z)-2-nonenal and they are responsible for *cardboard* note in butter oil (Widder & Grosch, 1997). The flavour characteristic of trans-2-nonenal in 23% abv grain whisky was described as *cardboard.* Thresholds of detection and recognition were 3 and 8 µg/L respectively (Lee, unpublished data).

2.5.8.2. *Sulphury* characters

J.1. *stagnant - sewer, drains, foul water, rotten vegetable*
J.2. *meaty - yeasty, Marmite, rotten egg*
J.3. *vegetable - turnip, potato, cooked vegetable* (*sweet corn, boiled cabbage*)
J.4. *sour - pickled onion, garlic*
J.5. *gassy - town gas, burnt match, acrid*
J.6. *rubbery - tyre/tubes, pencil erase, plastic*

Sulphur compounds are regarded as major sources of off-flavours influencing spirit quality (Masuda & Nishimura, 1981; Leppänen *et al.*, 1983; Fujii *et al.*, 1992), although in beers low concentrations can enhance acceptability (Berry & Watson, 1987). Their impact is heightened by water dilution due to their hydrophobicity, though volatility in whisky appears to be supressed by wood-derived materials (Piggott *et al.*, 1995b). They have low thresholds; dimethyl sulphide (DMS) and diethyl sulphide at 30 - 50 and 0.4 µg/L, respectively in lager and thiols at <2 µg/L (Meilgaard, 1975; Anness & Bamforth, 1982; Berry & Watson, 1987). A concentration of DMS above 100 µg/L in beer imparts *cooked sweet-corn, spicy* and *malty* (Lee *et al.*, 1999) or *blackcurrant-like* (Anness & Bamforth, 1982). However, when their concentration below the normal level in beer, their flavour impact is not significant (Anness & Bamforth, 1982). Ethanethiol has a much stornger sulphur characteristic described as *gunpowder* and *acrid* in lager (Lee *et al.*, 1999). In 23% grain whisky detection and recognition thresholds of dimethyl trisulphide (DMTS) have been defined as 4 and 20 µg/L respectively (Chapter 3), and 3 - 6 µg/L flavour was mainly described as *garlic* (*onion, cabbage*), *drains* and (*struck*) *match* in beer (Lee *et al.*, 1999). *Rubbery* (21% of respondent), *sour* (21%) and *gassy* (15%) were also employed by minorities (Lee *et al.*, 2000b). The threshold for H_2S is 5 (Thorne *et al.*, 1971) and 6 µg/L (FlavorActiV Ltd). Higher molecular weight sulphur containing compounds have been described as *heavy*, medium weight with *lightly bitter* and *roasted tinge* and lower weight as *light* and *neutral* (Leppänen *et al.*, 1983). *Meaty*, *burnt* and *thiamine-like* attributes are associated with methyl-(2-methyl-3-furyl)-disulphides (MMFD), bis(2-methyl-3-furyl)-disulphides, and methyl (2-methyl-3-furyl)-sulphides and 2,5-dimethyl-3-methyldithiofuran. MMFD has been identified in both grain and malt whiskies and has very low thresholds of 0.005 µg/L in rectified spirit and 0.10 µg/L in grain spirit (Carter-Tijmstra, 1990). The other compounds also have relatively low thresholds, below 1 µg/L, and are probably derived from thiamine and sulphur-containing amino acids. They are also often found in roasted coffee (Tressl & Silwar, 1981) and yeast extracts (Ames & Leod, 1985).

It has been proposed that the use of malathion for pest control on barley can influence the concentrations of sulphur compounds but experimental work has suggested no influence on the final flavour (Thomas, 1987). Spirit sulphur compounds also originate in barley hordein amino acids (cystein, methionine). The major origin of these sulphur compounds (e.g. DMDS and DMTS) in cysteine has been questioned with the absence of influence of cysteine on formation of DMDS, DMTS and methional during distillation (Furusawa, 1996). Metabolic products such as S-methyl-methionine are thermally degraded to methional (Strecker degradation) (Fig. 2.5) followed by methanethiol in wash (Furusawa, 1996). The amounts of dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) in new spirit have linear correlations with methional concentration in the wash, but DMTS concentration can be modulated by still operation (Prentice *et al.*, 1998; Fujii *et al.*, 1992).

Rock sulphur or gaseous sulphur dioxide (SO_2) is added as a sulphur source during kilning of malt to reduce the nitrosodimethylamine (NDMA) level. NDMA is formed through a reaction between barley amines (especially hordeins at the rootlet) and NO_2 from fuel gas. Most nitrosamines (NDMA) are unstable and evaporate before the 'break point' in the kilning. Removal of the rootlet before kilning reduces NDMA formation, but the level of hordein is still high enough to form NDMA (Slack & Wainwright, 1981). SO_2 at 10 - 30 mg/L reduces final malt contents of fungi and bacteria (Flannigan, 1983; Sim, 1983; Bathgate & Cook, 1989) and lightens colour of malt. Concentrations of SO_2 and sulphuric acid lower wort pH and influence breakdown of sugars, glyco-proteins and glycolipids, increasing wort concentrations of protein, α-amino nitrogen, lipids and fatty acids (Sim & Berry, 1996).

DMS is derived from S-methyl-methionine (SMM) which originates in malted barley. SMM is absent from raw barley but increases steadily during gernmination (Anness & Bamforth, 1982). Therefore, the kilning process, especially temperature and moisture levels in barley, greatly influence the conversion of SMM to DMS. Since DMS possesses a low boiling point (38 °C), it is highly volatile. Most DMS evaporates during the kilning and mashing process. However, the released DMS is further oxidised to dimethyl sulphoxide (DMSO) and dimethyl sulphur dioxide (DMSO2) (Anness & Bamforth, 1982; Fujii *et al.*, 1992). Other major sulphur compounds formed during mashing are carbonyl sulphide, methanethiol, carbon disulphide, small amounts of hydrogen sulphide, sulphur dioxide and dimethylsulphide (Ronkainen, 1973).

DMSO can also be reduced to DMS by sulphydryl compounds during fermentation. Bacteria (*Enterobacteriaceae*) are capable of producing DMS from DMSO, which is utilised as the electron acceptor to support their anaerobic growth during fermentation (Anness & Bamforth, 1982). Alternative origins of DMDS, DMTS and certain other sulphur compounds is from yeast autolysis (Suomalainen & Lehtonen, 1979; Berry & Watson, 1987; Prentice *et al.*, 1998). Their levels are related to the duration and intensity of wort heating. Yeasts secrete malic and citric acids during growth (Lyons & Rose, 1977) which promote the excretion of hydrogen sulphide and sulphur-containing compounds by lactic acid bacteria (mainly *Lactobacillus brevis*, and *L. fermentum*) (Lyons & Rose, 1977). Malt whisky mashes are not boiled and are thus susceptable to microbial infection by yeasts. Contaminating yeasts can produce not only sulphides but also further metabolic products, e.g. ethanethiol, through reaction of hydrogen sulphide with ethanol (Berry & Watson, 1987). Strecker degradation (Fig. 2.5) of cysteine with diketone during fermentation also yields hydrogen sulphide (H_2S) which is converted to ethanethiol and diethyl disulphide during distillation (Suomalainen & Lehtonen, 1979). Formation of sulphur compounds also related to yeast metabolism and storage of yeast at 5 °C yields less sulphur compounds than at 20 °C (Morimura *et al.*, 1998). H_2S (typically at 9 mg/L) released from yeast may interact with residual maltotriose (0.8 - 1.4 mM) during distillation, influencing the formation of DMDS from methionine in the presence or absence of copper ions (Furusawa, 1996). H_2S was predominantely described as *rotten-egg* at high concentrations (ca 140 µg/L) (Lee *et al.*, 1999) and *yeasty* at 50 µg/L (Thorne *et al.*, 1971). Aerobic or anaerobic distillation conditions had little effect on the formation of sulphur compounds as foam and carbon dioxide replace air in wash headspaces (Furusawa, 1996).

Copper is capable of the elimination or formation of sulphur compounds (Leppanen, 1983) by reaction with copper ions and is regarded as an essential element in producing a clean spirit (Nicol, 1989; Whitby 1992). Copper is dissolved gradually during wash distillation reaching 15 mg/L after distillation (Quinn *et al.*, 1982; Furusawa, 1996). Copper ions (cupric) react with methional to produce DMDS. However, as the DMDS level decreased with an increase in reaction time, and is susceptible to conversion to other compounds as distillation progresses (Furusawa, 1996). Since this conversion is faster than the formation reaction (Furusawa, 1996), the total DMDS concentration in spirit is reduced.

The addition of copper sulfate was found to remove volatile sulphur compounds by transforming them into non-volatile copper sulfides and mercaptides (Goodall *et al.*, 1999). MacKenzie *et al.* (1990) analysed still deposits and found compounds such as copper cyanides, thiocyanates, oxides and sulfates which may be of importance to an understanding of spirit quality (Goodall *et al.*, 1999). Thiol reacts with a surface layer of copper oxide to form copper thiolates and excess thiol forms copper thiolate complexes which can build up layers (self-assembled layers or chemisorption) (Keller *et al.*, 1994). Such self-assembled sulfur layers can react with flavour congeners (e.g. thiophene) and improve the sensory quality of whisky (Goodall *et al.*, 1999). However, the amount of chemisorbed thiol is limited (<4% of offered thiol concentration) (Keller *et al.*, 1994). Therefore, the excess thiol and corresponding disulphide can reach the spirit and affect quality. However, most sulphur compounds are removed during distillation especially in predistillate (heads) and feints (tail).

The use of a copper still can remove up to 70% of dimethyl disulphide compared with glass distillation (Swan & Burtlers, 1978; Berry, 1984). However, Masuda and Nishimura (1981) quantified DMDS in distillates and showed a ten-fold reduction in glass over copper stills in contrast with other results. Copper also organoleptically improved sugar cane spirit by reducing total sulphur compounds about 25% compared with stainless steel (Faria *et al.*, 1993). However, copper distillation increased concentrations of other compounds such as aldehyde, higher alcohol and ester but not carboxylic acids (Nascimento *et al.*, 1998). Masuda's results are in conflict with all others. It appears that, in practice, copper reduces the level of sulphur compounds in the distillate sufficiently to alter its character.

The oxidation of DMS to dimethylsulphoxide (DMSO) and dimethyl sulphur dioxide (DMSO2) is continued during maturation and 50% of DMS is oxidized to DMSO and DMSO2 after 96 hours in a new cask (Fujii *et al.*, 1992). They also investigated the cause of the reduction using DMS in a 60% alcohol solution and found that charcoal was most significant in the reduction of the DMS concentrations. Gallic acid was less effective. Therefore, there is , at most, a small amount of DMS in whisky after one year maturation depending on maturation conditions (Fujii *et al.*, 1992). DMDS decreases rapidly, within a year, and DMTS decreases more slowly (Masuda & Nishimura, 1981; Leppänen, 1983). Thus the ratio of DMDS/DMTS decreases with ageing in whiskies and may be used as an indicator of maturity (30 for new-filling, 15 after 3 years) (Leppanen, 1983). It is thought that DMDS either evaporates (Fujii *et al.*, 1992) or is converted to DMTS in spirits until its concentration falls below threshold (Leppanen *et al.*, 1983). It has been suggested that it is possible to differentiate commercial whisky products on the basis of their content of sulphur compounds - particularly, thiophenes and polysulphides. It is thought that cask surface to volume ratio is an important factor in the reduction of low molecular weight sulphur compound concentrations (e.g. DMS and DMDS). In contrast, the content of aromatic sulphur compounds such as thiophene and thiazole derivatives is not related to simple evaporation (Masuda & Nishimura, 1981). Nishimura *et al.* (1983) has proposed that the addition of oak wood (chips) and air decrease levels of sulphur compounds especially methionyl acetate and ethyl methionate.

2.5.8.3. *Cheesy* characters

K.1. *rancid - 'sickly sour', baby vomit, oxidized fat*

K.2. *sweaty - old trainer/sock, musky, piggery*

N-Butyric acid and ethyl butyrate (Carter-Tijmstra, 1989) are responsible for a *rancid* (*sickly*) *sour* character and are formed by bacterial action at low mashing temperatures or if the wash is allowed to stand in cast iron vessels (Paterson & Piggott, 1989). Propionic, butyric, *iso*-butyric and *iso*-valeric acids have been reported to have similar character in whisky and are the predominant fatty acids in rum and brandy (Nykänen *et al.*, 1968). *Iso*-valeric acid is more abundant compared with butyric, *iso*-butyric and propionic acids in whisky (Nykänen *et al.*, 1968). Their occurrence in distillates is very much dependent on the yeast used and fermentation conditions (Nykänen *et al.*, 1968).

Rancio, a descriptor of Cognac character, could be easily misunderstood and confused with *rancid* for whisky. The *rancio* characteristic is reminiscent of *musty walnuts*, and used as a mark of an old, well-stored Cognac which is affirmed with age. The Cognac acquires more *fineness*, *mellowness* and *rancio* character (Cantagrel *et al.*, 1995). The gradually acquired *rancio* taste of Cognac is related to hydrolysis of fatty acid esters and their oxidation to ketones (Marché & Joseph, 1975). The four most important methylketones in Cognac were 2-heptanone, 2-nonanone, 2-undecanone and 2-tridecanone. Their concentration is dependent on ageing conditions and peroxidase concentration and the volume of the barrel during maturaiton (Vidal *et al.*, 1993). Long chain aldehydes such as methyl ketones (C_7, C_9 and C_{11}), glyoxal and methyl glyoxal increases with ageing and are responsible for the long-lasting palate of *rancio* characteristic in old wine-spirits (e.g. Armagnac, Cognac) (Vidal *et al.*, 1992, 1993; Bertrand, 1995). Glyoxal has been idientified in whisky but the concentration was too low to be quantified (Puputti & Lehtonen, 1986). *Rancio* character does not appear to have been explicitly identified in whisky, but a similar character could presumably develop on extended maturation. N-(3-methylthiopropyl) acetamide and 3-methylthiopropionic acid derived from methionol are responsible for a *butter/rancio* note in wine (Beloqui *et al.*, 1995).

2.5.8.4. *Oily* characters

L.1. *soapy - waxy, unscented soap, detergent, damp laundry*; see section F (*feints*)
L.2. *buttery - creamy, toffee, butterscotch*
L.3. *lubricant - mineral oil*
L.4. *fat - fatty, greasy, fish oil, castor oil*

Buttery characters are mainly due to diacetyl (2,3-butanedione), and are regarded as the most common off-note in lagers (Takahashi *et al.*, 1995). Diacetyl is produced by strains of citrate-metabolising lactic acid bacteria (Swindell *et al.*, 1996; Aymes *et al.*, 1999). In yeast fermentations, diacetyl is formed from α-acetolactate, and eventually metabolised to acetoin. To control the diacetyl level in beer, the acetolactate decarboxylase (ALDC) gene has been isolated and transferred to brewer's yeast to produce acetoin directly from α-acetolactate without diacetyl production (Vogel *et al.*, 1995). Diacetyl has thresholds of detection of 0.02 mg/L and recognition of 0.04 mg/L by nosing in 23% grain spirit, and 0.2 mg/L for taste threshold (Lee *et al.*, 2000a; Vogel *et al.*, 1995). The oxygen level during fermentation has a substantial effect on diacetyl production (Boumerdassi *et al.*, 1996). Diacetyl and 3-hydroxy butanone are also important in wines, especially sweet sherries (Williams *et al.*, 1984; Martineau & Henick-Kling, 1995).

2.5.9. Primary taste

T.1. *Sweet*

An increase in maximum intensity and duration of sweet taste has been reported in Time Intensity (TI) studies of Brazilian sugar cane spirit (cachaça) matured in American oak for four years (Cardello & Faria, 1999). For sweet taste there was relatively little increase in intensity after 2 year but a substantial increase in duration. It appeared therefore that a change in the spirit was causing an increase in the persistence of the sweet taste, without substantially increasing its maximum intensity (Piggott *et al.*, 2000).

Sugar components in wood are extracted mostly within 3 years (Sato *et al.*, 1994; Reazin, 1981). Monosaccharide sugars found in matured whisky arise from wood cell wall components (pentoses and hexoses) and glycerol is generated by thermal breakdown of wood storage triglycerides (Reazin, 1981). Glycerol may also be the product of trans-esterification of triglycerides and ethanol, yielding fatty acid ethyl esters and free glycerol (Reid *et al.*, 1993). It is thought that monosaccharides (arabinose, glucose, xylose, galactose and rhamnose) are derived from stave hemicellulose and acid-catalysed hydrolysis of hydrolyzable tannins in Bourbon whisky maturation (Sato *et al.*, 1994). Although most sugars show concentration increases in the early stages of wood maturation, fructose and glycerol continue to be formed late in maturation (Fig. 2.9).

Glucose, fructose, proto-quercitol and arabinose are the most abundant sugars in one-year-whisky distillates (Nykänen, 1984; Charrier, 1992; Mosedale, 1995). Compositional analyses of one year old distillate showed similarities in saccharide composition of Scotch and Irish whiskies whereas Bourbons contained less arabinose and greater amounts of xylose through new wood extractions (Fig. 2.9). Glucose and mannose can not be compared since they also derived from caramel (Nykänen, 1984). Greater content of sugars in Bourbon than Scotch and Irish (Table 2.4) showed the use of new casks. Sugar concentrations in whisky are too low to be perceived as *sweet* (Lyons & Rose, 1977; Piggott *et al.*, 2000) compared with their detection threshold of approximately 5g/L in water (Amerine *et al.*, 1965).

Ethanol also decreases *sweetness* intensity and persistence for sugars (Hoopman *et al.*, 1993) since ethanol acts as a water structure enhancer, probably because of its hydrophobic effect (Franks & Ives, 1966; Serghat *et al.*, 1992; Kappatos *et al.*, 1996). Therefore, it is unlikely that sugar content can make any direct contribution to flavour (Piggott *et al.*, 2000). However, the perception of other flavour compounds such as vanillin, whisky lactone and maltol, and *fruity*, *floral* and *buttery* characters can enchance *sweet* character in whisky (Lee *et al.*, 2000b). Recently, Swan has also suggested that products of hemicellulose degradation add *fudgey* and *caramel* notes and colour to whisky, whereas cellulose products (e.g. glucose) have little impact on character (Swan 1999, IOB seminar).

T.2. *Sour*

Wood-derived acids and esters in whiskies are thought to influence flavour little (Nykänen, 1984). Wood-derived non-volatile congeners in matured whiskies include: oxalic, fumaric, succinic, methyl- and methoxy succinic, mesaconnic, adipic, phthalic, azelaic, sebacic and trimethybenzoic acids. New distillate has a fixed acidity of zero (Liebmann & Scherl, 1949) but over 12 months the extraction of acids, oxidations of ethanol and other congeners reactions produce an acidic environment (Liebmann & Scherl, 1949). A typical matured whisky is pH <4.5 (Delahunty *et al.*, 1993).

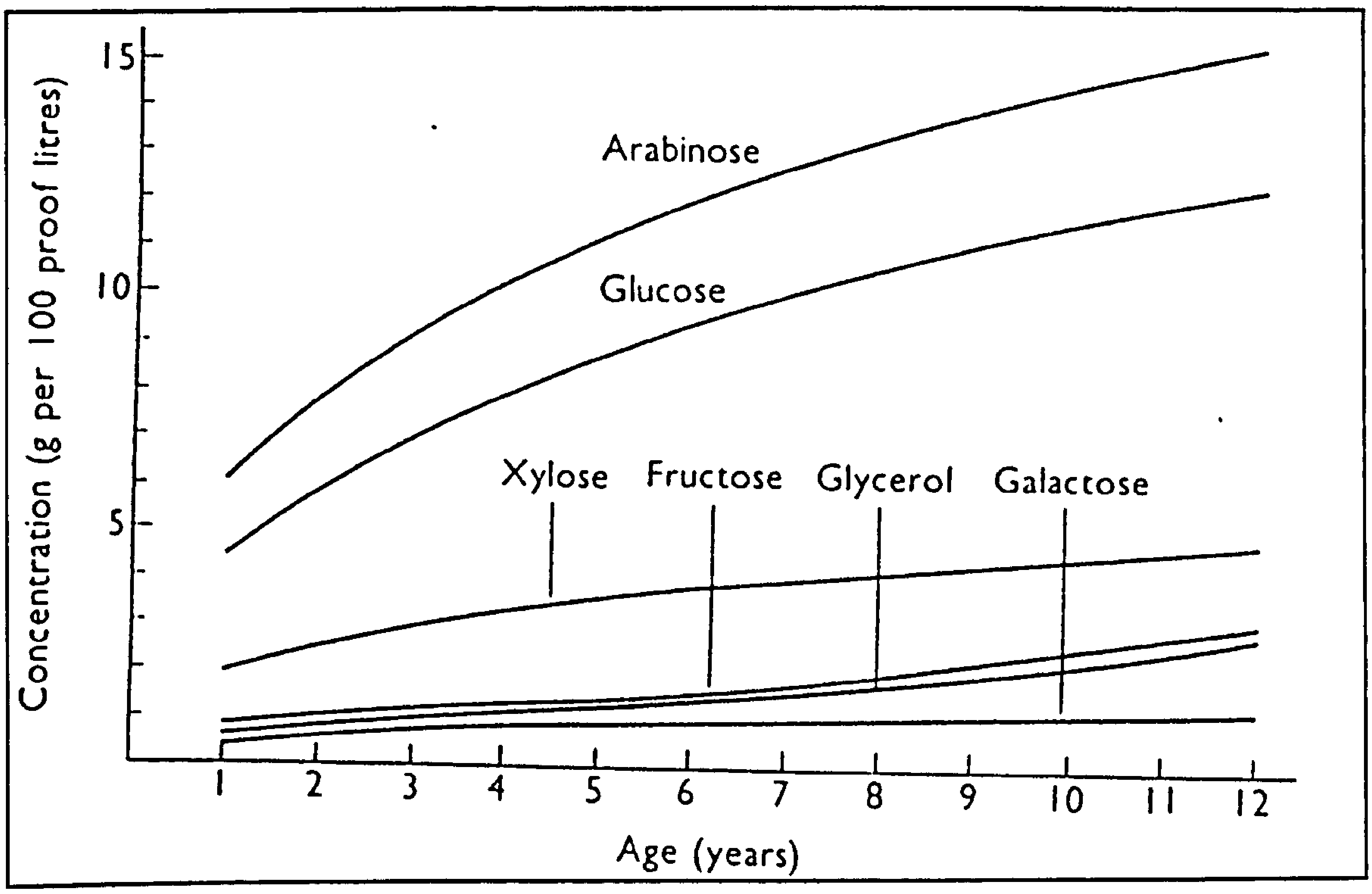


FIG. 2.9. Changes in sugars and glycerol during maturation (Reazin, 1981, reproduced with permission from *American Journal of Enology and Viticulture* 32, p 284)

TABLE 2.4. Contents (mg/L) of sugars in some whiskies (Nykänen, 1984; reproduced with permission from *Helsinki Foundation for Biotechnical & Industrial Fermentation Research*, 1984, p 141)

Sugar	Scotch (6 y)	Scotch (12 y)	Irish	Bourbon
Glucose	106.0 - 248.3	170.5 - 181.6	114.1	85.1
Proto-quercitol	10.4 - 16.5	28.9 - 34.4	8.6	90.9
Arabinose	14.4 - 21.1	35.6 - 42.9	12.4	82.5
Xylose	6.2 - 8.2	18.4 - 20.1	5.3	82.1
Mannose	31.3 - 69.6	35.3 - 35.5	6.8	19.2
Galactose	1.5 - 3.0	5.4 - 6.2	0.7	16.2
Rhamnose	1.5 - 2.2	4.0 - 4.5	1.5	10.6
Inositol	1.2 - 2.2	4.4 - 5.6	1.2	13.1

T.3. *Salt*

This character is not relevant to whisky flavour from a composition point of view. However, the term is often adopted in non-technical flavour evaluation, in connection with peaty water (moss-water) used for production and dilution. Peat bogs close to a sea become saturated with salt spray, and in some cases contain strands of seaweed, relics of a time when they were under water (MacLean, 1997).

T.4. *Bitter*: see section M (moutheffect) - *astringent*

2.5.10. Mouth effect (mouthfeel)

M.1. *warming - alcoholic, burn, fiery*
M.2. *coating - oily, creamy feeling*

A highly intense *biting* sensation is described as *burning* and can increase to a pain sensation. *Burning* also includes a *warm* sensation (Jellinek, 1985).

In many products the term *creaminess* is employed in evaluation of appearance, flavour or texture (Elmore *et al.*, 1999). Understanding of the character is limited. It has been defined as a mouthfeel characteristic 'possessing the textural property producing the sensation of the presence of a miscible, thick, smooth liquid in the oral cavity' (Jowitt, 1974) and also described as 'length in mouth' (Gelin *et al.*, 1996). The perception could be predicted by perceived *thickness*, *smoothness* (Kokini & Cussler, 1983) and *fatty mouthfeel* (Civille & Lawless, 1986), and is closely related to *buttery* (diacetyl) in many foods and whisky (Koeferli *et al.*, 1996; Gelin *et al.*, 1996; Hort *et al.*, 1997; Lee *et al.*, 1999). For whisky, the character may be used by consumers to represent maturity of whisky, described as *smoothness*, *roundness*, *body*, *richness* and *mellowness* (Guy *et al.*, 1989). Ethyl lactate, from esterification of lactic acid from bacterial metabolism in fermentations, is reported to be the major component of many spirits that contributes to *creamy* character (Williams *et al.*, 1984). Ethyl lactate formation is time dependent during fermentation (Geddes & Riffkin, 1989). However, this *creamy* character is discriminated from *creamy* related to *smoothness*.

M.3. *Astringent - drying, furry, powdery*

Astringent is a trigeminal tactile sensation and is defined as "complex of sensations due to shrinking, drawing, or puckering of the epithelium as a result of exposure to such as tannins" and results in *drying*, *roughing*, *puckering* or *drawing* sensations in the mouth (Lawless & Heymann, 1998). The sensation arises from the results of tannins binding to salivary proteins and mucopolysaccharides or directly to the lining of the mouth. Saliva thus loses its lubricating effect giving rise to *roughness*. These effects build up with repeated exposure (Lawless & Heymann, 1998).

New distillates have very low concentrations of compounds contributing to gustation but such flavour components are acquired by extraction of wood and by oxidation processes. The most obvious compounds are wood-derived tannic substances contributing *bitter* and *astringent* character, and which add *colour* and *delicate fragrance* in whisky (Mosedale, 1995).

The tannins, water-soluble plant polyphenols, are commonly divided into two groups: condensed tannins (derivatives of flavonols) and hydrolysable tannins (gallotannins and ellagitannins). Ellagitannins have a monosaccharide polyol (normally D-glucose) as the basic structural unit, with hydroxyl groups which have been esterified by either gallic or hexahydroxydiphenic acid (HHDP acid). These tannins are easily hydrolysed either enzymatically or in acid or base conditions, to form free gallic or HHDP acid, with the latter spontaneously lactonizing to give ellagic acid (Mosedale, 1995). White oak heartwood extracts contain a range of polyphenols including gallic and ellagic acids, gallo- and ellagi-tannins (Nishimura, 1989). Such tannins oxidise slowly but also polymerise as heart wood ages, reducing solubility (Mosedale, 1995). The lower solubility of tannins, due to polymerization, is thought to cause the loss of *astringency* as fruit ripens (Hagerman & Butler, 1991). Cole and Noble (1995) suggested that these extraction products also promote ethanol oxidation to acetaldehyde and diethyl acetal and ester formation from acids (e.g. acetic acid) and alcohols. Tannic substances also contribute to the removal of sulphides (Chatonnet, 1991; Mosedale, 1995) and nitrogen compounds as does the char layer (Fujii *et al.*, 1992). Ellagitannin is most abundant in raw wood and give rise to undesirable flavour characters; to eliminate these, cask wood is treated with hot water or charred (Maga, 1989; Ford & Done, 1991). Since ellagitannin hydrolyses to yield gallic and ellagic acids, these compounds are not found in spirit (Mosedale & Puech, 1998). In malt whisky, gallic and ellagic acids begin to increase after 2 years maturation (Sato *et al.*, 1994). Gallic acid extracted from macerated oak by wine spirit has a maximum after heat treatment of the wood at ca 165 °C (Martinez *et al.*, 1996). Although such compounds (gallic and ellagic acids) can be detected in spirit and used as indicators of maturation (Nishimura & Matsuyama, 1989) their impact on whisky flavour is not clearly understood (Mosedale, 1995).

2.6. FUTURE RESEARCH

This review treats flavour attributes of whiskies, defined in a revised flavour wheel, as if perceived at a unified time point. However, perceptions on ingestion of whisky form a temporal progression and the limited understanding of time intensity features suggest a fertile area for research.

Sensory assessors and panel are not analytical instruments. In assessing products, psychophysics and psychology interplay. Definition and number of attributes and scale useage are important in assessor training; order and session effects should be considered in industrial sensory panel assessments.

Understanding of the fundamental nature of whisky maturation is also required. Little is known about detailed physical structures of the aqueous ethanol liquid phases of new distillates and matured whiskies. High-resolution analysis strategies, such as neutron scatter, combined with headspace congener studies will contribute to an understanding of sensory quality that will benefit whisky distillers.

However perhaps greatest priority should be given to understanding perceptions of flavour character in whiskies. Products are perceived holistically by consumers but analysed by sensory assessors through quantifications of deconstructed attributes. Relationships between these two, fundamentally different, forms of mental processing is an urgent requirement for understanding whisky quality.

2.7. CONCLUSIONS

On drinking a glass of whisky, consumers employ pattern recognition processes, using sensory data to develop a holistic mental image in specific regions of the brain. Flavour recognition involves matching of information from long-term, short-term and sensory memories. Whisky maturation influences volatile congener release into headspaces through modifications of spirit liquid phases, and agglomerates. Complex changes in congener partitioning replace immature notes in new distillates with matured whisky characters.

The revised flavour wheel specifies a vocabulary that defines consensus of deconstructed attributes of whiskies to meet industrial needs. Each attribute is demonstrable by a flavour standard and terms suitable for training of sensory assessors for quality assurance, new product development and similar purposes.

CHAPTER 3:

MEASUREMENT OF THRESHOLDS FOR REFERENCE COMPOUNDS FOR SENSORY PROFILING OF SCOTCH WHISKY

Results of this study have been published in *Journal of Institute of Brewing* (Lee *et al.*, 2000a).

† A portion of this paper was presented as a poster by K-Y.M. Lee *et al.* at the 5th Aviemore Conference on Malting, Brewing and Distilling in May 1998 at Aviemore, Scotland.

3.1. ABSTRACT

Sensory profiling of Scotch whiskies, important for research and product analysis, requires training of assessors including the identification of important whisky aroma characters. Seventeen compounds were selected as potential reference standards for common whisky sensory characteristics. Nineteen assessors determined detection and recognition thresholds for each reference standard dissolved in 3 year old grain whisky at 23% (v/v) ethanol using both ascending dilution series and Latin square presentations. With the former, order effects and errors of habituation and anticipation were apparent. However, the Latin square presentation was satisfactory for threshold measurements in whisky, yielding good correlations and reproducibility, and enabling outliers to be reassessed independently. Slopes obtained from plotting probit against concentration for detection and recognition data, psychometric functions of aroma perception, varied between reference compounds and 90% recognition concentrations were obtained for each aroma standard. Sixteen of the compounds assessed were suitable for use as reference flavour standards for training of whisky assessors.

Key Words: *Sensory analysis, whisky flavour, aroma thresholds, flavour reference compounds, ascending series presentation, Latin square presentation.*

3.2. INTRODUCTION

Monitoring of sensory quality is an important component of whisky production. Traditionally, distillers make personal judgements on the basis of product experience. However, standardised assessment of whisky character is desirable for research and facilitates communication between production, technical, marketing and new product development functions of companies.

Systematic development has yielded a flavour wheel (Shortreed *et al.*, 1979) of 43 terms, a language for Scotch whisky. Other vocabularies have been reported (Eriksson & Lehtonen, 1981; Piggott & Canaway, 1981; Canaway *et al.*, 1984) but used less extensively. Although comparison of vocabularies (Canaway *et al.*, 1984) has shown rather contradictory results, each vocabulary describes similar types of variation in the samples, notably differences between and within new and mature spirits. However, systematic flavour terminologies (*e.g.* the whisky flavour wheel) require agreed flavour descriptors. These should ideally be demonstrable to assessors using reference compounds (Piggott, 1991). Little consideration has been given to availability of single reference compounds that could be used to align concepts between whisky assessors, proven important in training beer assessors (Meilgaard, 1975, 1989; Meilgaard *et al.*, 1979, 1982). Although the Strathclyde terminology has recommended reference compounds (Piggott, 1991), their use was only validated within that limited group alone. An agreed whisky terminology with practical, objective reference standards is a prerequisite for improved sensory analysis. Assessors can then be expected to develop similar concepts (mental references) for flavour characteristics.

For industrial use, specification of references should include single compounds that can be added to a base spirit at a concentration sufficient to demonstrate flavour characteristics. A number of categories of threshold have been defined (Brown *et al.*, 1978): detection - lowest concentration detectable; and recognition thresholds - the lowest concentration identifiable (BS 5098: 1975). Recognition thresholds, higher than detection thresholds (Pangborn, 1981), are perhaps more valuable in industry as the sensation and its description are both presented to assessors (Lawless & Heymann, 1998).

A 'threshold' may be an abstract concept, but it has also a concrete meaning being a specific value derived from experimentation. Specification of any threshold for a particular flavour substance must detail the experimental method employed (Morrison, 1982). The 'statistical threshold' can be defined as a measure of the momentary concentration at which the stimulus is detected a specified proportion of the time, since the sensitivity of any subject must vary. The level of detection required depends on the applied model. Threshold values may be estimated at 75%, 67% or 50% of correct responses depending on the experimental method (Morrison, 1982). Threshold variability arises from different sources *e.g.* mode applied, statistical interpretation (Morrison, 1982), and sensory and non-sensory factors (Brown *et al.*, 1978). Misinterpretation of correct response levels for thresholds has been critically reviewed (Morrison, 1982) and common errors arising from data treatment and non-sensory variance defined (Brown *et al.*, 1978). In any method, accuracy and precision will ultimately be limited by intrinsic variability in the process.

Ascending series presentations are a common and logical method for obtaining threshold data (Brown *et al.*, 1978; Meilgaard, 1989; Analytic-EBC IV, 1987). However, biases are observed, as assessors tend to report 100% detection at the end of a concentration series. Conversely, at the beginning of the sample series, assessors have a tendency not to detect character differences between a control and test sample even if a forced choice test is employed (Morrison, 1982; Lundahl *et al.*, 1986). Such errors of anticipation can be regarded as assessor misbehaviour during an ascending series presentation, reducing value of data. The alternative is that sample presentation order may be randomised or balanced *e.g.* in Latin square sequences. This minimises carry-over effects from previous samples and also assessor pre-judgements. It seemed likely that Latin square presentations could yield adequate threshold data for industrial practice without the need to resort to triangle tests or forced choice decision experiments. These are both expensive and tedious and thus limited in industrial applicability.

The aim of this experiment was to determine whether a simple and economical Latin square presentation provided an adequate method of measuring thresholds as a preliminary stage in definition of aroma standards for Scotch whisky. Thresholds, or strictly 'just noticeable differences', were determined in grain whisky at 23% (v/v), a strength commonly used for sensory work within the Scotch whisky industries. This alcoholic strength enhances perception of volatile whisky aroma characters by reducing the ethanol pungency from higher alcohol strengths.

3.3. MATERIALS AND METHODS

3.3.1. Materials

Scotch grain whisky (59% v/v), matured for 3 years, was filtered to remove solid particles and diluted to 23% ethanol (v/v) using demineralised water. Seventeen potential reference standards (with appropriate descriptors and estimated threshold values, T ppm) were selected on the basis of published literature, purchased, and dilutions prepared: acetic acid, *sour*, 60 ppm in 20% (v/v) ethanol (Richardson, unpublished), BDH/Merck (Hunter Boulevard, Magna Park, Lutterworth, Leics, LE17 4XN), glacial 99.8% pure; diacetyl (butane-2,3-dione), *buttery*, 0.0025 ppm in 9.4% (w/w) grain spirit (Salo, 1970), FlavorActiV (Cara House, Wiremill Lane, Lingfield, Surrey, RH7 6HJ, UK); di-methyl tri-sulphide (DMTS), *sulphury*, 0.0001 ppm in 23% (v/v) ethanol (Perry, 1989), FlavorActiV; ethyl hexanoate (ethyl caproate), *fruity-*

appley, 0.2 ppm in 20% (v/v) ethanol (Richardson, unpublished), FlavorActiV; ethyl laurate (ethyl dodecanoate), *soapy*, 2 ppm in 20% (v/v) ethanol (Richardson, unpublished), Sigma (The Old Brickyard, New Road, Gillingham, Dorset, SP8 4XT, England), 99% pure; furfural (2-furfuraldehyde), *grainy*, 60 ppm in 20% (v/v) ethanol (Richardson, unpublished), Fluka (The Old Brickyard, New Road, Gillingham, Dorset, SP8 4XT, England) 99% pure; geraniol (*trans*-3,7-dimethyl-2,6-octadien-1-ol), *floral*, 0.002 ppm in ppm in beer (Meilgaard *et al.*, 1982), Fluka, 96% pure; guaiacol (2-methoxy phenol), *smoky*, 0.08 ppm in 20% (v/v) ethanol (Richardson, unpublished), Aldrich (The Old Brickyard, New Road, Gillingham, Dorset, SP8 4XT, England), 98% pure; hexanal (capronaldehyde), *grassy*, 0.05 ppm in 20% (v/v) ethanol (Richardson, unpublished), Aldrich, 98% pure; *iso*-amyl acetate (*iso*-pentyl acetate; 3-methyl butyl acetate), *fruity*, 1.5 ppm in 20% (v/v) ethanol (Richardson, unpublished), FlavorActiV; *iso*-valeric acid (3-methyl butyric acid), *sweaty*, 0.9 ppm in 20% (v/v) ethanol (Richardson, unpublished), FlavorActiV; maltol (3-hydroxy-2-methyl-4-pyrone), *sweet*, 30 ppm in 20% (v/v) ethanol (Richardson, unpublished), Fluka, > 98% pure; phenyl ethanol (2-phenyl ethanol), *floral*, 0.6 ppm in 20% (v/v) ethanol (Richardson, unpublished), Sigma; vanillin (4-hydroxy-3-methoxy benzaldehyde), *vanilla*, 0.1 ppm in 23% (v/v) ethanol (Perry, 1989), Aldrich, 99% pure; 4-vinyl guaiacol (2-methyl-4-vinyl-phenol), *spicy*, 0.3 ppm in 20% (v/v) ethanol (Richardson, unpublished), FlavorActiV; whisky lactone (mixture of *cis* and *trans* 3-methyl-4-octanolides), *coconut*, 0.3 ppm in 20% ethanol (Richardson, unpublished), Aldrich, > 98% pure; and ethyl-D-lactate, *creamy*, 14 ppm in 9.4% (w/w) grain spirit (Salo, 1970), Fluka, 99% pure. All spirit samples were nosed in individual sensory booths (ISO 8589). Samples (30 ml) were presented in wine tasting glasses (ISO 5494: 1978) covered with watch-glasses.

3.3.2. Sensory methods

Two variations of the 'Ten Sample Test' method (Brown *et al.*, 1978) were used: initially test samples were presented as an ascending series all to be compared with an open control. In the second form the test samples were presented as Latin square presentation (MacFie *et al.*, 1989). For ascending series presentation (ASP), two dilution series were prepared for each compound relative to its estimated threshold (T), at 0.01T, 0.1T, T, 10T and 100T and 0.05T, 0.5T, 5T, 50T and 500T. Each dilution series of a compound was tested on a different day. Three different compounds (15 samples and 3 hidden controls) were tested in a session. Tests were duplicated on different days. In each session, strict instructions were given to carry out the assessments in ascending order. The Strathclyde University whisky panel was used (17 - 22 assessors). Experienced assessors were instructed to nose 'untreated' control and then experimental sample, and to report whether a difference was detected. If the answer was affirmative, assessors were asked to define the difference.

For Latin square presentations (LSP) (MacFie *et al.*, 1989), each assessor was given a different order of samples at each session. Five concentrations were taken from middle ranges of ascending dilution series presentations, where the threshold values were obtained. Ethyl-D-lactate was omitted since no aroma character had been perceived. Samples (3 sets of 5 and 3 hidden controls per booth per session) were selected arbitrarily, ensuring that low, middle and high concentrations were balanced in a session so that assessors sniffed the complete range of concentrations. Duplicate tests were carried out on different days and the same panel used.

3.4. DATA ANALYSIS

Response percentages for detection and recognition by assessors were calculated as sums of response rates. Only correct or justifiably similar character descriptions of the tested compounds were regarded as successful recognitions. Percentage response data were transformed into probits (Finney, 1971). From the two separate regressions lines (detection and recognition), threshold values were calculated as concentration at 50% response (probit = 5.0). Also 90% recognition thresholds (probit = 6.28) were calculated for evaluation of perception of reference compounds. From regression equations 95% confidence interval ranges of individual compounds were also determined (Finney, 1978).

3.5. RESULTS AND DISCUSSION

Generally detection (DT) and recognition (RT) thresholds from both strategies had similar values (Table 3.1 - 3.4), and although there were some slight variations (acetic acid, DMTS, guaiacol, *iso*-amyl acetate, *iso*-valeric acid, phenyl ethanol, 4-vinyl guaiacol and whisky lactone), 95% confidence intervals overlapped. However, geraniol in detection and recognition and whisky lactone in recognition thresholds showed large differences between ascending series (ASP) and Latin square (LSP) presentations. For geraniol, ASP showed higher values (DT = 6 > 0.2 and RT = 16 > 1 ppm) than LSP. However, LSP showed a higher correlation coefficient (r = 0.97 > 0.96). For whisky lactone, detection thresholds were similar (0.5 and 0.3 ppm, r = 0.95 and 0.91) but recognition thresholds showed a large difference (1 and 7 ppm) and 95% confidence intervals did not overlap (0.5 - 2 and 4 - 16 ppm). Maltol also showed large differences especially in detection (DT = 21 and 8 ppm, RT = 20 and 30 ppm), but again 95% confidence intervals overlapped. However, regression for the detection threshold from ASP was not significant ($p = 0.089$). Thresholds of diacetyl for the two methods also differed (DT = 0.02 and 0.002, RT = 0.04 and 0.006 ppm), with LSP giving higher correlation coefficients (DT = 0.91 < 0.98 and RT = 0.92 < 0.98). 4-Vinyl guaiacol showed the poorest correlation coefficient in detection from both presentations ($r = 0.80$ and 0.89). However, values obtained from both methods were similar (D.T = 0.7 and 0.6, R.T. = 5 and 2 ppm) and the 95% confidence intervals overlapped (0.3 - 2 and 0.09 - 0.6 ppm).

Although thresholds of ethyl lactate, described as *artificial raspberry* and *perfume-like* odour, have been determined in 9.4% (w/w) grain spirit (14 ppm) (Salo, 1970) and in beer (250 ppm) (Meilgaard, 1989), no aroma character was detected in this experiment (Fig. 3.1) at concentrations up to 1400 ppm. Explanations might be that either concentration tested was too low; that the different isomers (ethyl-D-lactate and ethyl-L-lactate) give differing responses; or that an impurity was present in reference compounds used previously. The isomer used in previous work was not specified. On the basis of these present results, ethyl-D-lactate would not be appropriate as a standard aroma reference.

Phenyl ethanol showed a slight difference in detection thresholds between the two methods (DT = 8 and 2 ppm), and big differences in recognition thresholds (246 and 80 ppm). However, confidence intervals overlapped. Nonetheless, once more the ASP recognition (246 ppm) gave a poorer correlation coefficient ($r = 0.89$) than LSP ($r = 0.98$). Recognition slopes from both methods were the lowest ($b = 0.5$ and 0.6) (Table 3.3 & 3.4) among the 16 compounds. Consequently, highest 90% recognition threshold (15188 ppm) and the widest 95% confidence interval ranges (0.01 – 951 and 34 – 190 ppm) were obtained. In a previous study employing grain whisky at 9.4%

ethanol, the threshold (unspecified category) was defined as 7.5 ppm (Salo, 1970). For the recognition of *floral* character, an approximate concentration of 100 ppm was recommended (Piggott, 1991) and threshold quoted as 120 ppm (Meilgaard, 1981), similar to the recognition threshold level (80 ppm) defined here. Similar behaviour of phenyl ethanol in grain whisky (at 9.4% ethanol) has also been observed (Salo, 1972) in the formulation of an artificial model of whisky, requiring highest odour units for recognition.

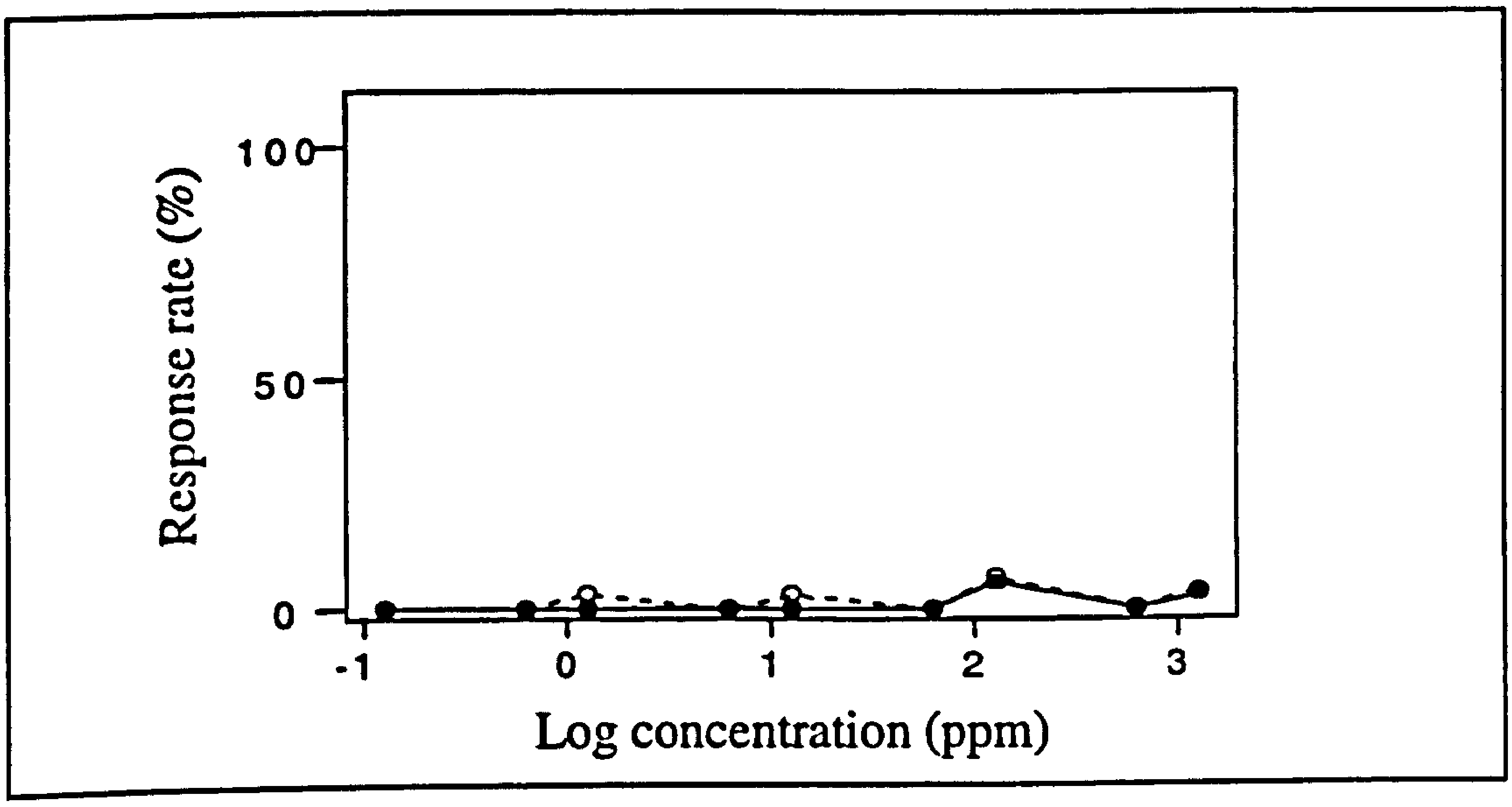


FIG. 3.1. Detection (-o-) and recognition (-●-) threshold measurements of ethyl - D-lactate in 3-year old grain whisky determined from ascending dilution series

TABLE 3.1. Detection thresholds (DT) from ascending dilution series presentation

Compounds	DT (ppm)	Confidence interval (95%)	Slope (b)	Intercept (a)	*p*	R
Acetic acid	28	16 - 51	1.6	2.8	0.078	0.92
Diacetyl	0.02	0.008 - 0.04	0.9	6.7	0.072	0.91
DMTS	0.03	0.02 - 0.07	1.1	6.6	0.005	0.94
Ethyl hexanoate	0.07	0.04 - 0.2	0.8	6.0	0.013	0.86
Ethyl-D-lactate	-	-	-	-	-	n.s.
Ethyl laurate	0.7	0.4 - 1.3	1.1	5.2	0.010	0.92
Furfural	-	-	-	-	-	n.s.
Geraniol	5.6	3 - 12	0.9	4.3	0.036	0.96
Guaiacol	0.09	0.03 - 0.3	0.6	5.6	0.011	0.91
Hexanal	0.07	0.03 - 0.2	0.8	5.9	0.043	0.83
iso-Amyl acetate	0.1	0.05 - 0.3	0.6	6.0	0.098	0.81
iso-Valeric acid	0.2	0.09 - 0.4	0.8	5.6	0.003	0.93
Maltol	21	9 - 47	1.0	3.7	0.089	0.82
Phenyl ethanol	8	0.3 - 18	0.9	4.2	0.000	0.96
Vanillin	-	-	-	-	-	n.s.
4-Vinyl guaiacol	0.7	0.3 - 2	0.7	5.1	0.018	0.80
Whisky lactone	0.5	0.2 - 1.2	0.8	5.2	0.003	0.95

DT = detection threshold were calculated: (probit, 5.0) = b Log conc. + a. Where, a = intercept, b = slope. Log threshold (DT) = 5.0 – a/b. 95% confidence interval ($z_{0.025}$ = 1.96) = log DT. ± z * se. Standard error (se. log DT) = $1/b\sqrt{nd\bar{w}}$, Where, b = slope, n = No. of subjects (assessors), d = No. of dilutions, $\bar{w}$ = mean weighting coefficients for expected probit (Fits) at each dilution point. n.s. = Regression not significant (p > 0.10).

TABLE 3.2. Recognition thresholds from ascending dilution series presentation

Compounds	RT (ppm)	Confidence interval (95%)	Slope (b)	Intercept (a)	*p*	R
Acetic acid	102	48 - 216	1.2	2.5	0.046	0.89
Diacetyl	0.04	0.02 - 0.07	0.9	6.4	0.009	0.92
DMTS	0.1	0.05 - 0.2	1.3	6.3	0.007	0.93
Ethyl hexanoate	0.8	0.4 - 1.8	0.8	5.1	0.002	0.91
Ethyl-D-lactate	-	-	-	-	-	n.s.
Ethyl laurate	2	0.9 - 3.6	0.9	4.8	0.000	0.93
Furfural	-	-	-	-	-	n.s.
Geraniol	16	7 - 34	0.9	4.0	0.001	0.96
Guaiacol	3	1 - 9	0.53	4.8	0.001	0.95
Hexanal	2	1 - 4	1.07	4.7	0.006	0.97
iso-Amyl acetate	2	0.7 - 4	0.8	4.9	0.006	0.94
iso-Valeric acid	1	0.4 - 2	0.8	5.1	0.000	0.95
Maltol	20	10 - 41	1.1	3.5	0.010	0.92
Phenyl ethanol	246	0.01 - 951	0.5	3.8	0.010	0.87
Vanillin	-	-	-	-	-	n.s.
4-Vinyl guaiacol	5	2 - 15	0.6	4.6	0.001	0.91
Whisky lactone	1	0.5 - 2	0.9	5.0	0.001	0.96

RT = recognition threshold were calculated: (probit, 5.0) = b Log conc. + a. Where, a = intercept, b = slope. Log threshold (RT) = 5.0 – a/b. 95% confidence interval ($z_{0.025} = 1.96$): log RT (probit = 5.0) ± z * se. Standard error (se. log RT) = $1/b\sqrt{nd\bar{w}}$, where b = slope, n = number subjects (assessor), d = number of dilutions, $\bar{w}$ = mean weighting coefficients for expected probit (Fits) at each dilution point. n.s. = Regression not significant (p > 0.10).

TABLE 3.3. Detection thresholds (DT) from Latin square presentation

Compounds	DT (ppm)	Confidence interval (95%)	Slope (b)	Intercept (a)	*p*	r
Acetic acid	24	12 - 47	0.7	4.0	0.037	0.90
Diacetyl	0.002	0.001 - 0.003	1.4	8.9	0.000	0.98
DMTS	0.004	0.002 - 0.01	0.7	6.7	0.004	0.98
Ethyl hexanoate	-	-	-	-	-	n.s.
Ethyl laurate	0.8	0.6 - 1	1.3	5.1	0.010	0.96
Furfural	39	23 - 65	1.0	3.4	0.023	0.93
Geraniol	0.2	0.08 - 0.3	0.6	5.5	0.002	0.97
Guaiacol	0.03	0.02 - 0.05	1.0	6.4	0.001	0.99
Hexanal	0.1	0.08 - 0.2	0.9	5.8	0.024	0.98
iso-Amyl acetate	0.3	0.2 - 0.4	1.4	5.8	0.034	0.91
iso-Valeric Acid	0.1	0.07 - 0.1	1.9	6.9	0.000	0.99
Maltol	8	3 - 16	0.6	4.5	0.009	0.96
Phenyl ethanol	2	1 - 4	0.6	4.8	0.006	0.97
Vanillin	0.6	0.3 - 1	0.8	5.2	0.004	0.98
4-Vinyl guaiacol	0.2	0.09 - 0.6	0.6	5.4	0.042	0.89
Whisky lactone	0.3	0.2 - 0.7	0.7	5.3	0.031	0.91

DT = detection threshold were calculated: (probit, 5.0) = b Log conc. + a. Where, a = intercept, b = slope. Log threshold (DT) = 5.0 – a/b. 95% confidence interval ($z_{0.025} = 1.96$): log DT (probit = 5.0) ± z * se. Standard error (se. log DT) $=1/b\sqrt{nd\bar{w}}$, Where b = slope, n = number subjects (assessor), d = number of dilutions, $\bar{w}$ = mean weighting coefficients for expected probit (Fits) at each dilution point. n.s. = Regression not significant ($p > 0.10$)

TABLE 3.4. Recognition thresholds (RT) from Latin square presentation

Compounds	RT (ppm)	90% RT	Confidence interval (95%)	Slope (b)	Intercept (a)	*p*	r
Acetic acid	233	5322	146 - 543	0.9	2.8	0.011	0.99
Diacetyl	0.006	0.1	0.004 - 0.008	1.4	8.1	0.001	0.98
DMTS	0.02	3	0.01 - 0.06	0.7	6.7	0.003	0.98
Ethyl hexanoate	0.4	2	0.3 - 0.5	2.7	6.2	0.014	0.93
Ethyl laurate	3	12	2 - 4	1.8	4.3	0.020	0.93
Furfural	210	839	148 - 272	2.1	0.1	0.002	0.97
Geraniol	1	19	0.6 - 2	1.0	5.0	0.001	0.97
Guaiacol	0.7	27	0.3 - 1	0.8	5.1	0.011	0.96
Hexanal	0.7	5	0.5 - 1	1.5	5.2	0.039	0.96
iso-Amyl acetate	1	7	0.9 - 2	1.8	4.9	0.005	0.98
iso-Valeric Acid	0.3	2	0.2 - 0.4	1.6	5.9	0.008	0.97
Maltol	36	1135	18 - 71	0.9	3.7	0.002	0.99
Phenyl ethanol	80	15188	34 - 190	0.6	3.9	0.004	0.98
Vanillin	3	43	1 - 5	1.0	4.6	0.001	0.99
4-Vinyl guaiacol	2	71	0.8 - 4	0.8	4.8	0.008	0.96
Whisky lactone	7	266	4 - 16	0.8	4.3	0.009	0.96

RT = recognition threshold were calculated: (probit, 5.0) = b Log conc. + a. Where, a = intercept, b = slope. Log threshold (RT) = 5.0 – a/b. 95% confidence interval ($z_{0.025}$ = 1.96): log RT (probit = 5.0) ± z * se. Standard error (se. log RT) $= 1/b\sqrt{nd\bar{w}}$, Where b = slope, *n* = number subjects (assessor), *d* = number of dilutions, $\bar{w}$ = mean weighting coefficients for expected probit (Fits) at each dilution point.

Since the number of samples differed between ascending series (10) and Latin square (5) presentations, the significance levels (p values) of regressions can not be directly compared. In some cases, the number of data points was less than 10 in ascending series presentations, because higher concentrations were not assessed in the second test series after a 100% response had been obtained from the first test. Data points were disregarded (Finney, 1971) when response rates were 0% (probit = - ∞) or 100% (probit = + ∞). This gave rise to non-significant results for detection of acetic acid (p = 0.078), diacetyl (p = 0.072), *iso*-amyl acetate (p = 0.098) and maltol (p = 0.089) from ASP and ethyl hexanoate (p = 0.37) from LSP (Table 3.1).

Since assessors failed to detect the lower concentrations in the series, and showed a sudden subsequent increase in detection and recognition for furfural and vanillin (Fig. 3.2a & c), it was not possible to calculate regressions from ASP data reliably. Fluctuations in ASP data illustrated variation between the two series assessed on different days. It would be expected that detection and recognition regression lines would be approximately parallel. Where this was not the case (e.g. Fig. 3.2g, 3.4e & g) it was presumably due to random variations in response rates by assessors. Such results suggested an ascending series presentation was susceptible to response bias (O'Mahony, 1995; Piggott *et al.*, 1998) and errors of anticipation (Lawless & Heymann, 1998) and habituation - a tendency to respond at the same point in each series irrespective of the compound (Amerine *et al.*, 1965). Sensitivity variation between days was also more apparent.

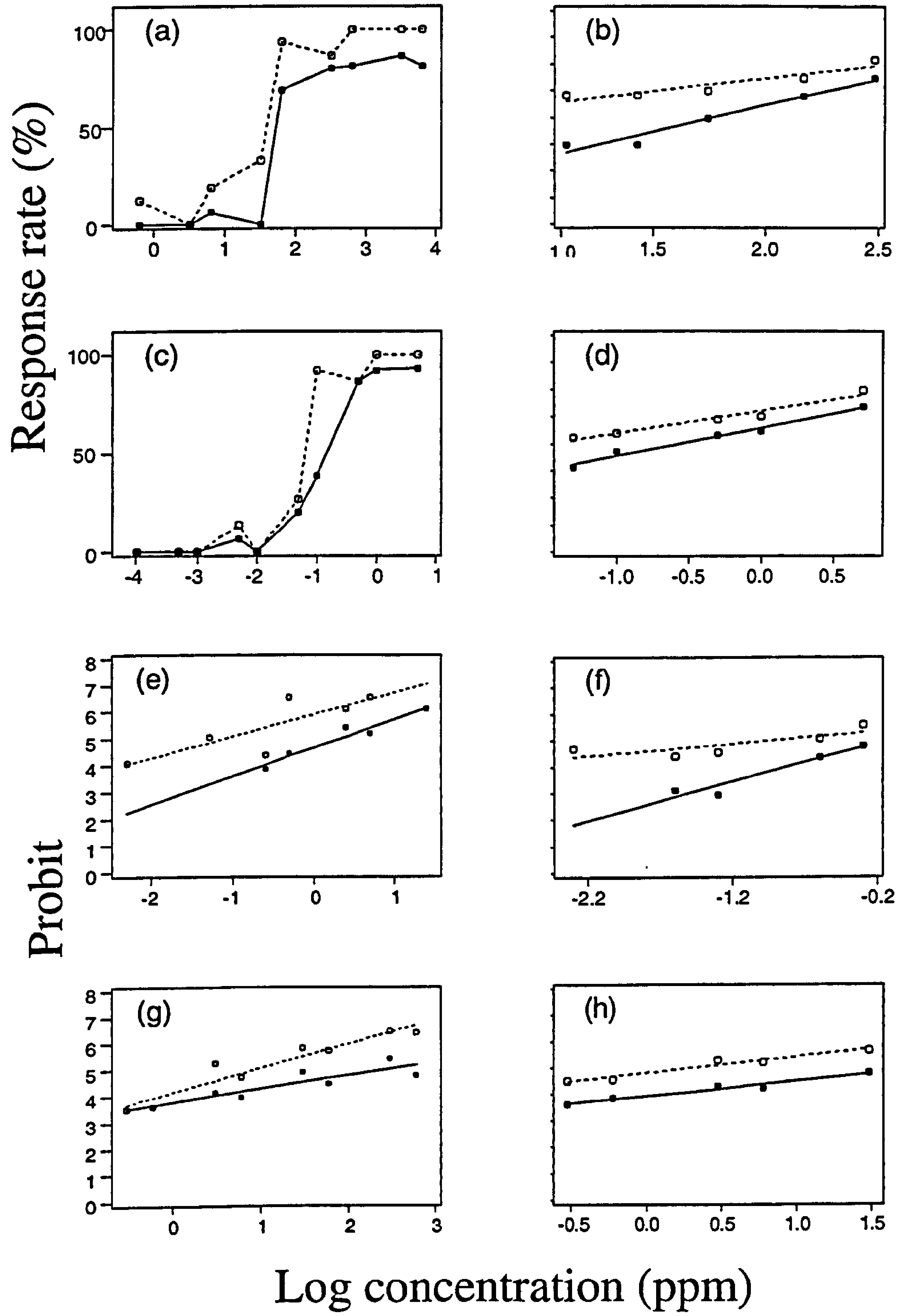


FIG. 3.2. Detection (- -O-) and recognition (-●--) threshold measurements of furfural (a, b), vanillin (c, d), hexanal (e, f) and phenyl ethanol (g, h) in 3-year old grain whisky determined from ascending dilution series (a, c, e, g) and Latin square (b, d, f, h) presentation.

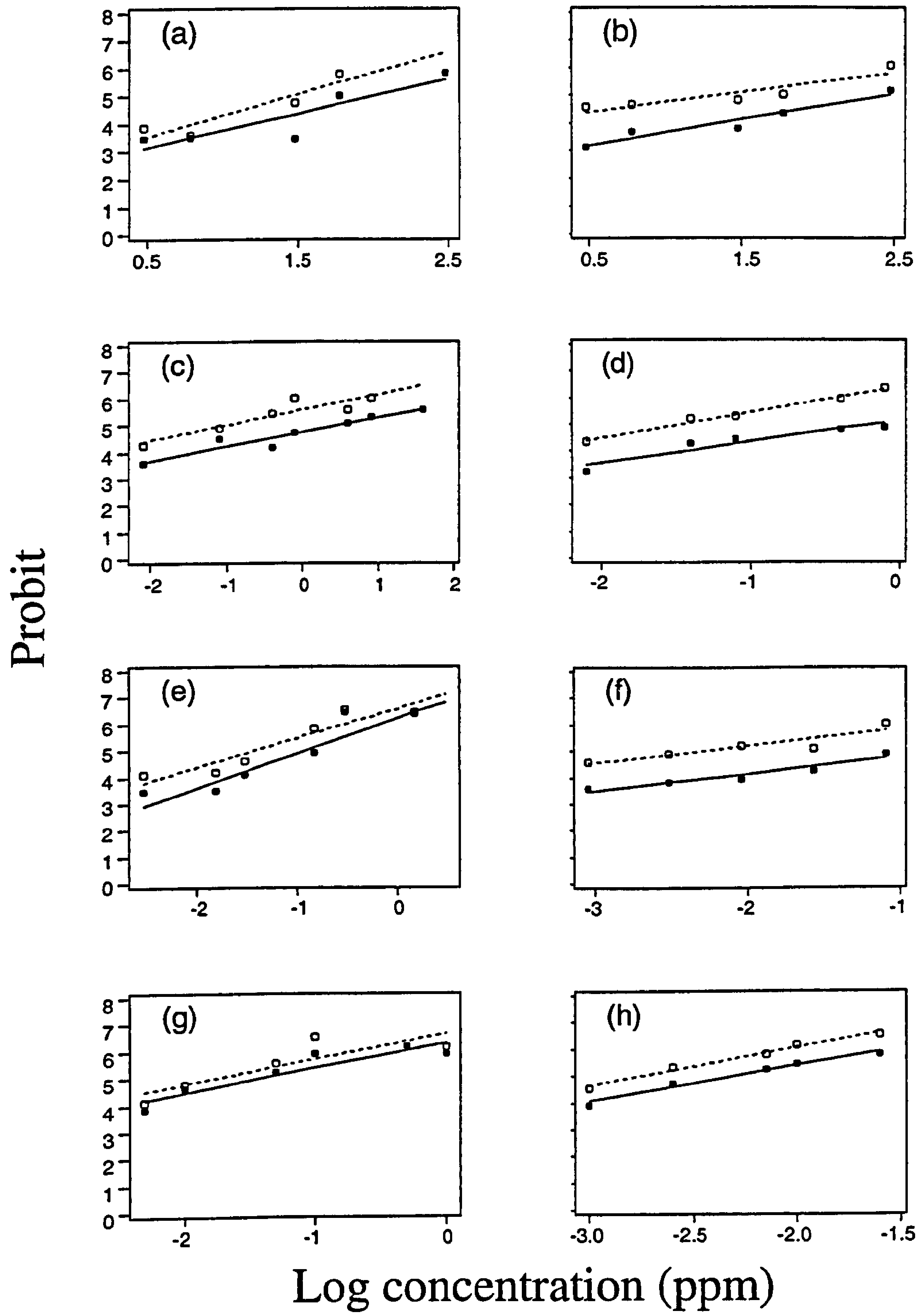


FIG.3.3. Detection (-O -) and recognition (-●-) threshold measurements of acetic acid (a, b), guaiacol (c, d), DMTS (e, f) and diacetyl (g, h) in 3-year old grain whisky determined from ascending dilution series (a, c, e, g) and Latin square (b, d, f, h) presentation.

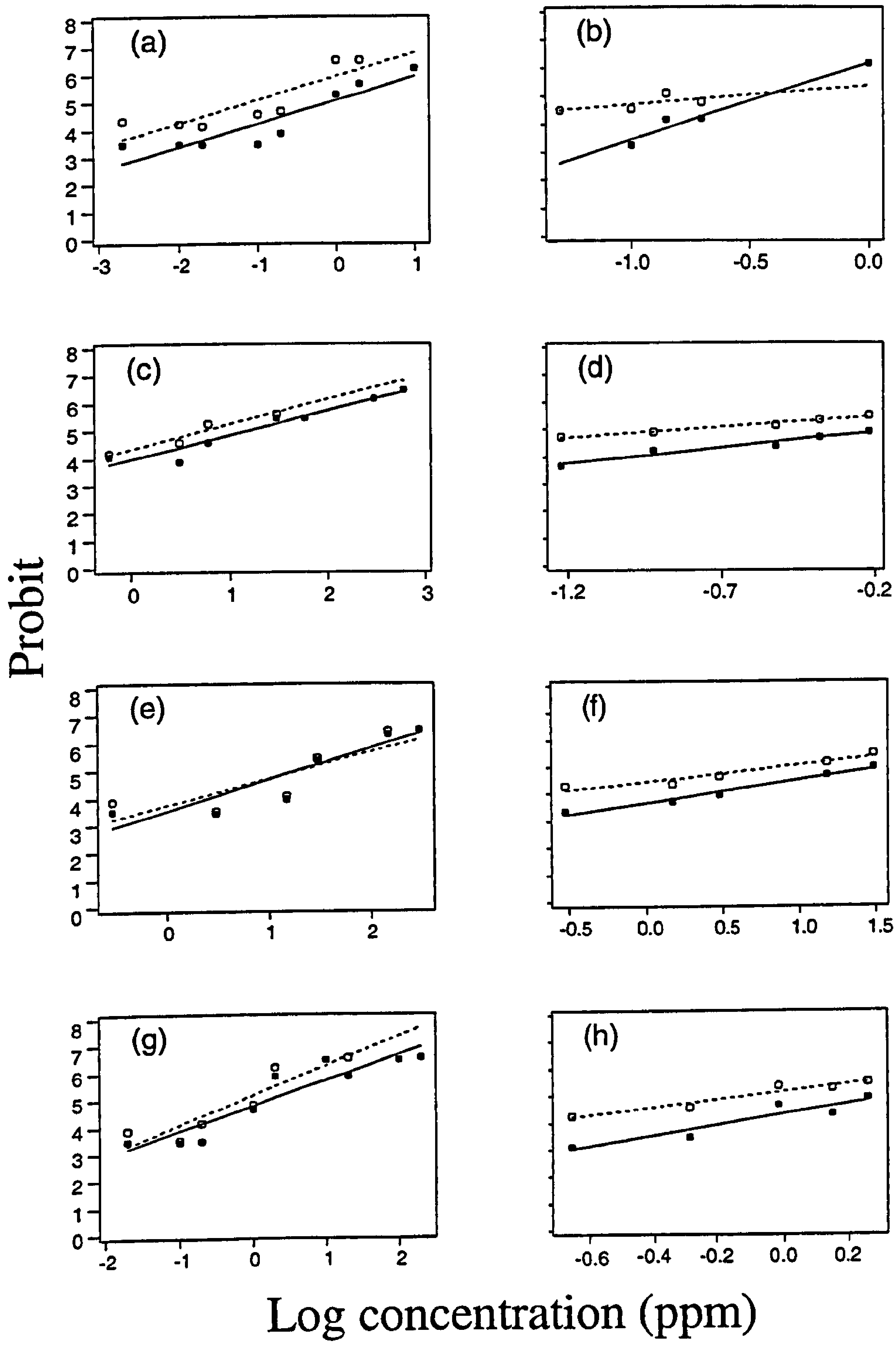


FIG. 3.4. Detection (-○ -) and recognition (-●--) threshold measurements of ethyl hexanoate (a,b), geraniol (c, d), maltol (e, f) and ethyl laurate (g, h) in 3-year old grain whisky determined from ascending dilution series (a, c, e, g) and Latin square (b, d, f, h) presentation.

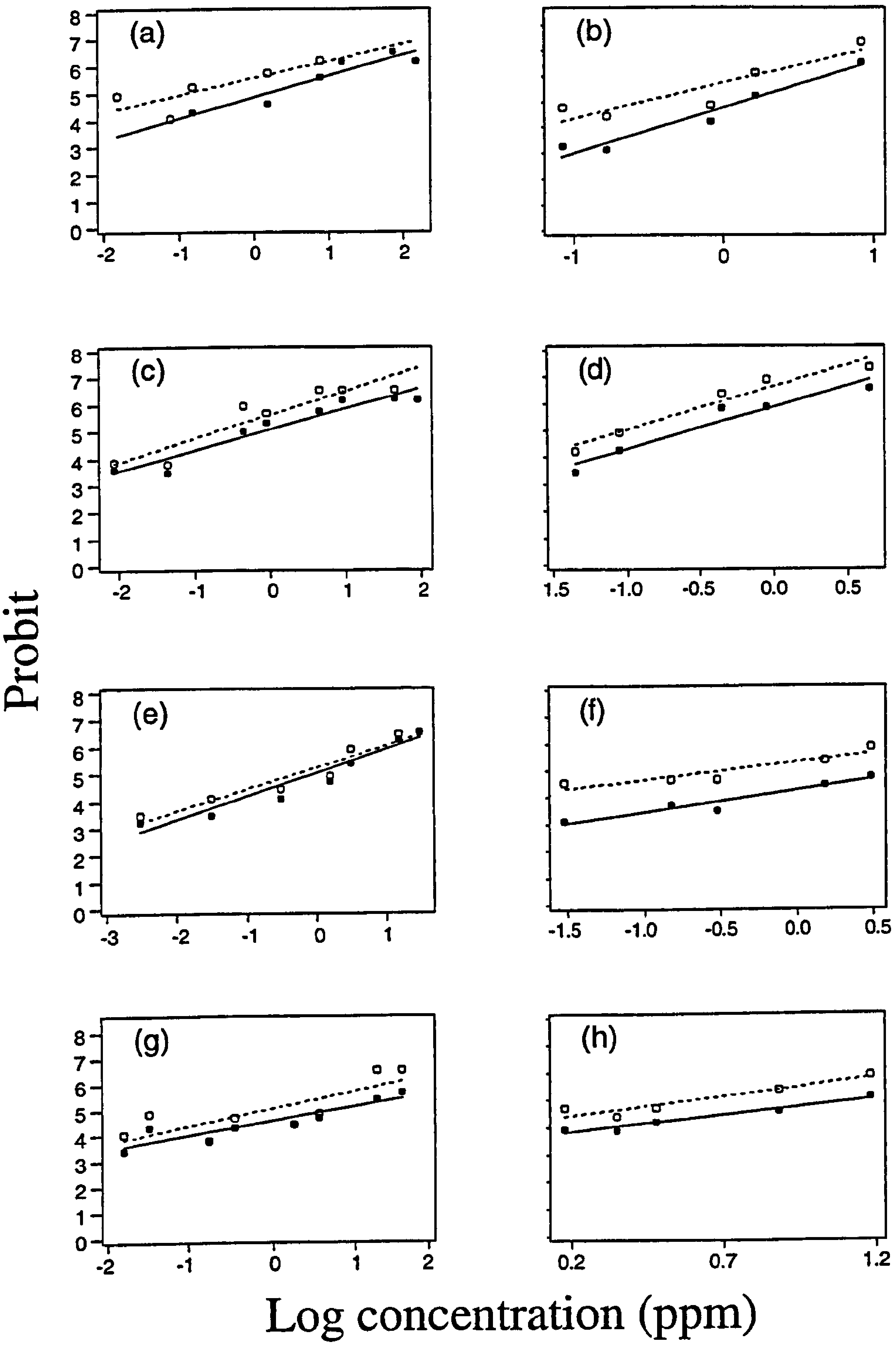


FIG. 3.5. Detection (- o-) and recognition (--●--) threshold measurements of *iso*-amyl acetate (a, b), *iso*-valeric acid (c, d), lactone (e, f) and 4-vinyl guaiacol (g, h) in 3-year old grain whisky determined from ascending dilution series (a, c, e, g) and Latin square (b, d, f, h) presentation

For threshold measurements, both sensory and non-sensory factors have to be considered carefully to reach maximum accuracy (Brown, 1978). In detecting fine sensory differences in stimuli, one of the major problems is response bias in assessors when asked if two samples differ. Interference by 'region of uncertainty' on a correct judgement can easily alter the truth of the measurement. Since setting criteria is an individual cognitive factor and is independent of the sensitivity of the measured character, it can change arbitrarily (O'Mahony, 1995) or habituate (Lawless & Heymann, 1998). However, response bias can be eliminated by a simple strategy of forcing a choice between samples (O'Mahony, 1995; Piggott *et al.*, 1998). However, slight change in instructions or an assessor's interpretation may reintroduce bias (Piggott *et al.*, 1998).

The ascending series presentation was proposed as a 'Ten Sample Test' (Brown *et al.*, 1978) in an attempt to reduce the amount of testing involved in Guadagni's Multiple Pairs Test (Guadagni *et al.*, 1973), which itself involved substantially fewer samples and was therefore more economical than any triadic presentation. However, effects of adaptation were experienced among assessors. To minimise the major problem of the ascending series presentation, a Latin square presentation (MacFie *et al.*, 1989) was used to balance the presentation order in the second test. The same total number of samples was presented in this experiment, but it was thought that presenting different characters (different compounds) and different concentrations within a session would introduce less adaptation. Use of different olfactory receptors to produce a spatial 'map' (response pattern) should induce less adaptation than continual use of specific receptors (Laing & Jinks, 1996).

A large measure of agreement was observed between the two methods, although the effect of presentation order was apparent in the ascending series, and the response bias was not controlled. The Latin square presentation showed good correlations ($r > 0.90$) between concentration and response rate in most of the compounds. Since the experiment was designed to determine flavour perception thresholds (at 90% recognition) for potential whisky reference compounds, it could be argued Latin square presentation is not completely satisfactory as a standard threshold measurement method. However, it is the most economical - estimating thresholds with few samples, and thus a practical way of estimating thresholds for sensory training in Scotch whisky and similar industrial applications.

The test-retest correlation for an olfactory threshold measurement over most studies is about + 0.4, leaving 84% of the variance unaccounted for (Punter, 1983). The sensitivity variation of an individual can be significant over a period of time (Lawless *et al.*, 1995). Generally, within an individual, threshold values decrease with practice (Engen, 1960; Canaway *et al.*, 1984) and may increase if an assessor is given an unexpected taste or odour (Marks & Wheeler, 1998). However, with Latin square presentations, variations (between and within assessors and days) were distributed equally and treated in a similar manner throughout the experimental period, as the varied concentrations of aroma standards were assessed on different days. Therefore any odd results could be reassessed independently to achieve good linearity. Most parameters were thus more reliable and reproducible.

3.6. CONCLUSION

With Latin square presentation, most threshold values yielded higher correlation coefficients. Therefore, Latin square presentation seems adequate for routine threshold measurements of potential aroma standards and is economical, whilst yielding good correlation coefficients and enabling outliers to be reassessed independently. Confidence intervals were found useful in comparing the results of the two methods. Since a threshold quantifies a momentary sensitivity (Morrison, 1982), presenting a threshold within a confidence interval is more realistic and flexible. Sixteen out of the seventeen compounds assessed may be suitable for use as flavour reference standards in training of whisky sensory panellists.

CHAPTER 4:

PERCEPTION OF WHISKY FLAVOUR REFERENCE COMPOUNDS BY SCOTTISH DISTILLERS

Results of this study have been published in *Journal of Institute of Brewing* (Lee *et al.*, 2000b).

† A portion of this paper was presented as a poster by K-Y.M. Lee at the 5th Aviemore Conference on Malting, Brewing and Distilling on May 25th, 1998 in Aviemore, Scotland.

4.1. ABSTRACT

A set of 16 compounds was selected from the literature as potential flavour standards for whisky profiling: acetic acid (*sour*), diacetyl (*buttery*), dimethyl tri sulphide (*sulphury*), ethyl hexanoate (*fruity-appley*), ethyl laurate (*soapy*), furfural (*grainy*), geraniol (*floral*), guaiacol (*smoky*), hexanal (*grassy*), *iso*-amyl acetate (*fruity-banana*), *iso*-valeric acid (*sweaty*), maltol (*sweet*), phenyl ethanol (*floral*), vanillin (*vanilla*), 4-vinyl guaiacol (*spicy*) and whisky lactone (*coconut*). Each compound, at 90% recognition threshold concentration, that at which 90% assessors recognise the flavour character, was added to 3 year old grain whisky diluted to 23% v/v. The solutions were assessed by 72 distilling professionals (blenders, quality control and technical functions) and flavour attributes suggested without, and subsequently with, a prompt list were recorded. Descriptors with a frequency of > 10% were examined. Only limited agreement was found across the industry. Agreement on reference standards and commonality in procedures for training of both blenders and sensory assessors would be of benefit to the whisky industries.

Key words: *Whisky flavour terminology, flavour reference compounds, flavour perception, 90% recognition threshold, sensory analysis.*

4.2. INTRODUCTION

Quantitative descriptive analysis (QDA) is an important sensory analysis technique as it allows communication of data on flavour characters in a specific product. As laboratory procedures become more closely specified and standardised (*e.g.* ISO standards 4121, 5429, 6564, 6658, 8586), it becomes important to make sure that good practice can be established in the whisky distilling industries. Such conventional sensory profiling relies upon assessors within a panel and the industry having similar understandings of meanings of descriptive terms. In Scotch whisky distilleries, personal judgements of flavour are used extensively (Lang, 1983). Flavour concepts and terminology have developed in the context of company culture and needs.

One of the basic principles of sensory analysis is that it must be possible to illustrate the meaning of terms using readily available reference standards (Meilgaard *et al.*, 1979; Piggott & Canaway, 1981). A reference standard has been defined as "any chemical, spice, ingredient or product which can be used to characterise or identify an attribute or attribute intensity" (Rainey, 1986). However, the application in practice of raw materials - spice, ingredient or product - is limited by the nature of the materials with respect to stability, availability and preparation procedure.

The use of multiple reference standards for a single concept has also been reported to enhance learning and the use of a descriptor particularly in the case of complex attributes (Homa & Cultice, 1984; Ishii & O'Mahony, 1991). Thus although it could be useful to obtain a number of reference standard materials for a single concept, this could also easily mislead assessors.

Advantages in obtaining reference standards include: increasing the efficiency of communication (Piggott, 1991) by stabilising the use of vocabulary and improving the quality of panel data; assisting in the training of new panellists for sensory analysis; and providing a medium for demonstrating aspects of Scotch whisky flavour to consumers and marketing professionals. Descriptive terms and corresponding candidate reference materials are available in the literature, but the potential users have not adequately validated these in most cases. Some validation was needed for optimisation of the terminology by defining and clarifying the perception of each reference compound by a range of different professionals in the Scotch whisky industry. The aim of the work described here was, therefore, to assess the suitability of a proposed set of whisky flavour reference compounds for adoption by the Scotch whisky industry.

4.3. METHOD AND MATERIALS

A 3 year old grain whisky 59% (v/v) was filtered to remove solids and diluted with demineralised water to 23% v/v. 16 reference compounds were used at 90% recognition threshold concentration (Table 4.1). 72 assessors were self-selected from the Scotch whisky industry after initial written or telephone enquires and included sensory assessors, sensory analysts and blenders. The samples (30 ml) of whisky, containing a single reference compound, were assessed from each company's standard nosing glasses, covered with a watchglass. Glasses were typically tulip-shaped, similar to wine-tasting glasses (ISO 5494: 1978). Initially, the assessors described the reference compounds using their own terms, but were subsequently prompted from a list of 16 terms (Table 4.1).

4.4. RESULTS AND DISCUSSION

Terms used to describe the aroma character from the addition of whisky reference compounds to grain whisky varied both between individuals, and notably between companies. Certain respondents supplied more than a single descriptor in response to each compound (sum % values >100%).

TABLE 4.1. Compounds and concentrations used

Compounds	Suggested term	90% RT* (ppm)	Sources
Acetic acid	*sour*	5322	BDH/Merck[1], glacial 99.8% pure
Diacetyl (butane-2,3-dione)	*buttery*	0.1	FlavorActiV[2]
DMTS (di-methyl tri-sulphide)	*sulphury*	3	FlavorActiV[2]
Ethyl hexanoate (ethyl caproate)	*fruity-appley*	2	FlavorActiV[2]
Ethyl laurate (ethyl dodecanoate)	*soapy*	12	Sigma[3], 99% pure
Furfural (2-furfuraldehyde)	*grainy*	839	Fluka[4], 99% pure
Geraniol (*trans*-3,7-dimethyl-2,6-octadien-1-ol)	*floral*	19	Fluka[4], 96% pure
Guaiacol (2-methoxy phenol)	*smoky*	27	Aldrich[5], 98% pure
Hexanal (capronaldehyde)	*grassy*	5	Aldrich[5], 98% pure
iso-Amyl acetate (*iso*-pentyl acetate)	*fruity-banana*	7	FlavorActiV[2]
iso-Valeric acid (3-methyl butyric acid)	*sweaty*	2	FlavorActiV[2]
Maltol (3-hydroxy-2-methyl-4-pyrone)	*sweet*	1135	Fluka[4], > 98% pure
Phenyl ethanol (2-phenyl ethanol)	*floral*	15188	Sigma[3]
Vanillin (4-hydroxy-3-methoxy benzaldehyde)	*vanilla*	43	Aldrich[5], 99% pure
4-Vinyl guaiacol (2-methyl-4-vinyl-phenol)	*spicy*	71	FlavorActiV[2]
Whisky lactone (3-methyl-4-octanolides)	*coconut*	266	Aldrich[5], > 98% pure

*90% RT = threshold which 90% population recognise an added character[9]

[1] BDH/Merck - Hunter Boulevard, Magna Park, Lutterworth, Leics, LE17 4XN

[2] FlavorActiV - Cara House, Wiremill Lane, Lingfield, Surrey, RH7 6HJ, UK

[3] Sigma, [4] Fluka, [5] Aldrich - The Old Brickyard, New Road, Gillingham, Dorset, SP8 4XT, England

Acetic acid (Fig. 4.1) *Sour* is used to describe acetic and lactic (butyric) acid character (Shortreed *et al.*, 1979). Since the favoured descriptor was *vinegary* (56%), this could be preferred to *sour* for acetic acid character. Oakwood components are broken down, and ethanol oxidised during storage to form acetic acid as the most abundant organic acid in matured spirits (Ohnishi *et al.*, 1977; Nishimura *et al.*, 1983). However *woody* related characters (*oak lactone, spicy, sawdust, sweet* and *resinous*) and *rancid* have previously been recorded as related to defective cask character (Nishimura *et al.*, 1983; Reazin, 1983).

Diacetyl (Fig. 4.1) The majority of assessors described the character as *diacetyl* (21%) or *buttery* (31%) whereas a similar total of *sweet* (47%) terms (*sweet, caramel, toffee, fudge, hot tablet, chocolate* and *burnt sugar*) were also recorded. Assessors often confused *buttery* with *vanilla* character but after discussion were able to discriminate between the two characters. The prompted rate for *buttery* was 42%, and for *vanilla* was zero. The chemical term *diacetyl* appeared familiar to 22% of assessors. As insensitivity and a specific anosmia to diacetyl has been recorded (Meilgaard *et al.*, 1982; Meilgaard, 1993; Lawless *et al.*, 1994), assessors responding *like-control* (4%) might have been anosmic.

DMTS (Fig. 4.1) The general concept of *sulphury* (44%) was evoked extensively, but *rubbery* (21%), *sour* (21%) and *gassy* (15%) were also employed by significant minorities. The chemical term *DMTS* was used by 14%.

Ethyl hexanoate (Fig. 4.1) was described as *fruity* (39%) although no specific fruit character was specified. The initial perception as *fruity* was reduced by interference from *aniseed* (14%) character. *Aniseed* was regarded as a secondary odour that could not be resolved from the primary odour (Meilgaard *et al.*, 1982).

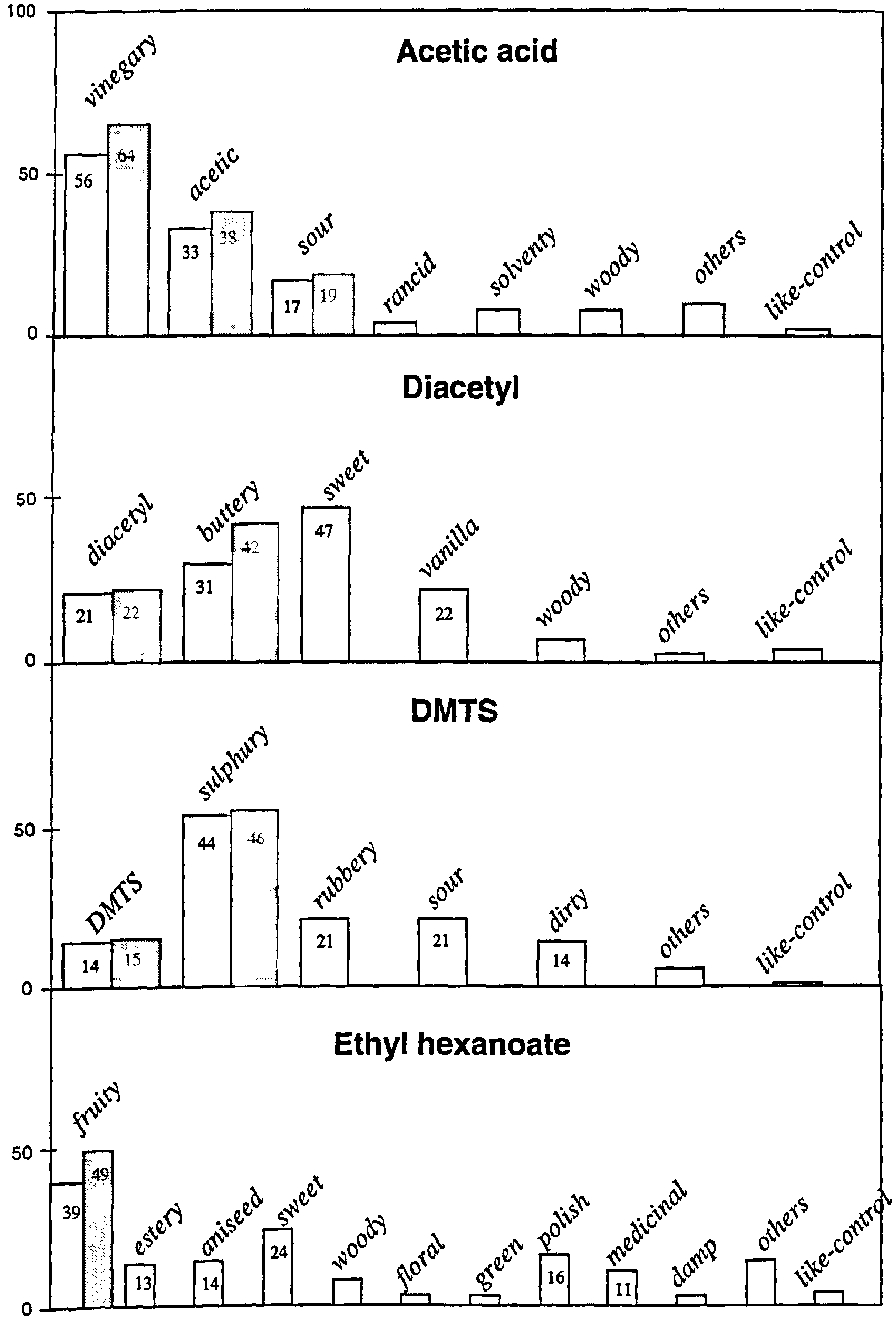


FIG. 4.1. Perception of individual reference compounds
☐: Initial assessment; ☐: Prompted assessment

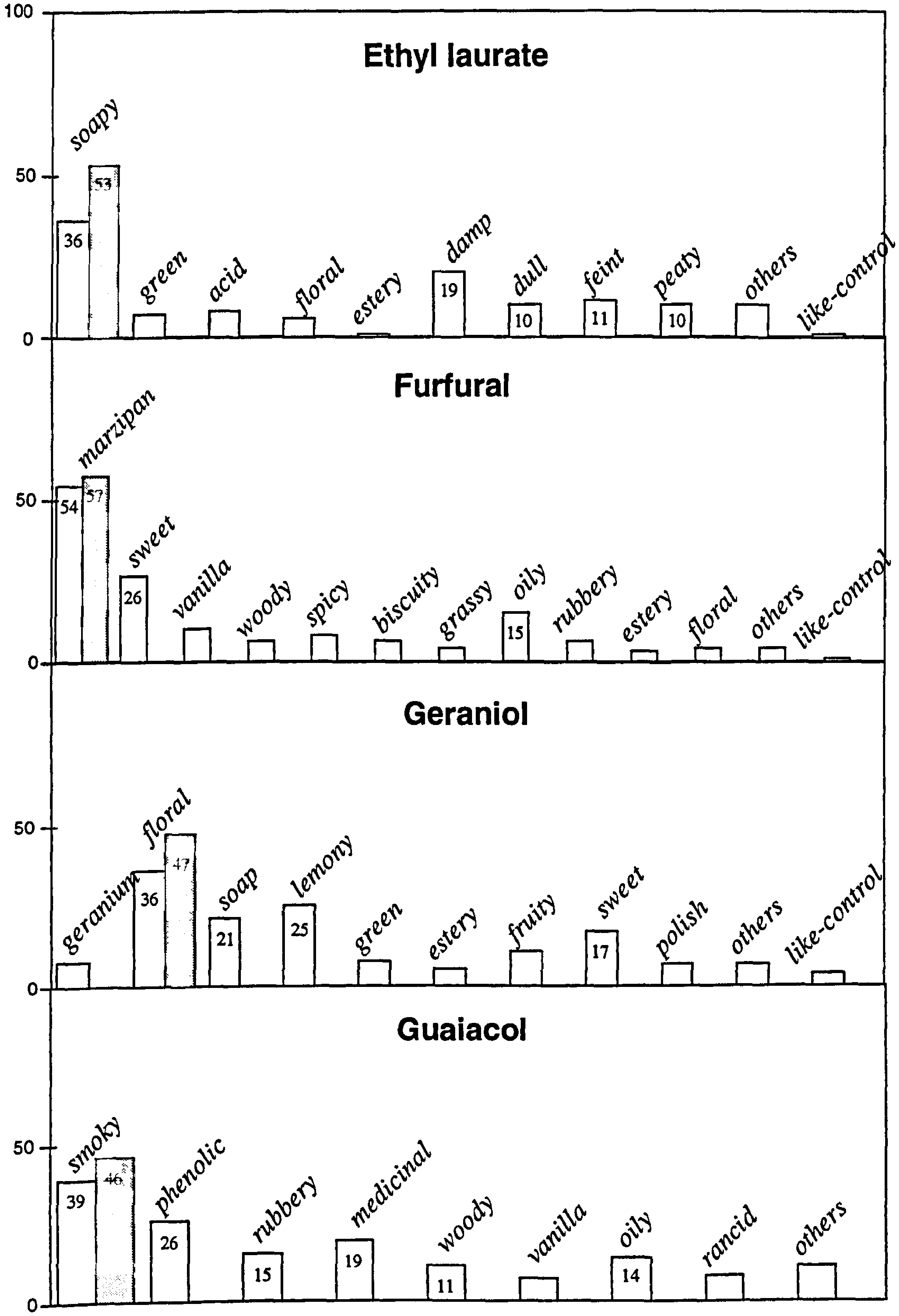


FIG. 4.2. Perception of individual reference compounds
☐: Initial assessment; ▨: Prompted assessment

Background notes such as *polish* (16%), *sour* (14%) and *medicinal* (11%) may suggest either problems with the purity of the compound or that the 90% recognition threshold (2 ppm) was insufficient to give a definite character recognition as *apple fruity*.

Ethyl laurate (Fig. 4.2) was described as *soapy* (36%), *damp* (19%) and *feints* (11%). This suggested a lack of consensus in initial assessment. Terms such as *shampoo*, *green*, *pine*, *fresh*, *chlorine*, *detergent*, *disinfectant* and *cleaning* were employed, suggesting relation to cleaning and soap residue concepts. After discussion with assessors, it was found that *acid* (*citrus*) terms also were used in association with *washing-up liquid* in terms of the *soapy* concept. This interpretation gave rise to a high prompt percentage (53%) for *soapy*. These 'perception linkages' could be important in interpretation of the results.

Furfural (Fig. 4.2) has been described as evoking a *grainy* character (Lyons & Rose, 1977), and contributing to *hotness* of spirits (Guymon & Crowell, 1972; Singleton, 1995), but the character was not defined clearly. Less than 6% of respondents used a term such as *biscuity* or *cereal* that might be related to *grainy* character. Frequent descriptors were *marzipan* (*coconut, cake mix, almond, nutty, walnut oil* and *coumarin-like* - 54%), *sweet* (26%) and *oily* (15%). *Sweet* related terms such as *vanilla, woody* and *spicy* were also recorded. Certain assessors always described the aroma note as *grassy, hay-like* (4%) which seemed incompatible with the dominant furfural character, *marzipan.* The *wood, caramel* or *vanilla-like* character of furfural at 10 ppm can be accentuated by adding lactone (1 ppm) (Reazin, 1981). This suggests that either the concentration presented was insufficient to elicit *almond* character, or that aroma character varied as a function of concentration. Since no alternative reference compound has been suggested for *grainy* character, it might either be more appropriate to use a raw material standard (*e.g.* grains, mash or wort) or discourage the use of a term that cannot be adequately defined.

Geraniol (Fig. 4.2) was described firstly as *floral* (36%); secondly as *lemony* (*citrus* and *washing-up liquid*, 25%); but also as evoking *scented soap* (21%), and *sweet* (17%) characters. The character *lemony* could have resulted from instability of the compound, making it unsuitable as a reference for *floral* (Meilgaard *et al.*, 1982).

Guaiacol (Fig. 4.2) was generally described as evoking a *smoky* (39%) note, also recorded with a similar frequency for 4-vinyl guaiacol (32%). *Rubbery* (15%) and *medicinal* (19%) terms were distinctive characters of guaiacol that could be related to knowledge of the malt peating process. However *rubbery* character was also recorded with DMTS (21%) for a higher proportion of assessors, suggesting a stronger relationship to sulphur than to a phenolic compound. *Peaty* was used less (7%) than expected although accepted terminology.

Hexanal (Fig. 4.3) was generally described as evoking *green* (31%), *grassy* (26%), *fruity* (21%) or *almond* (12%) notes. However, *green* as a descriptor should perhaps be discouraged (Shortreed *et al.*, 1979): it is an oenological concept related to a lack of maturity in wine; and used to describe aroma character in finished whiskies produced from a proportion of green malt. In the context of whisky, *grassy* may be considered a more appropriate descriptor for hexanal and in prompted assessments, 33% of respondents opted for *grassy*. The character of the compound was also perceived as *almond* by a minority (12%). This is difficult to reconcile with *grassy* character.

***Iso*-amyl acetate** (Fig. 4.3), *pear-drop* obtained the highest response frequency (67%), possibly related to average assessor age. The total of other *fruity* terms, mainly *banana* and *pear*, was 22% whereas *estery* was recorded with 25% respondents.

***Iso*-valeric acid** (Fig. 4.3) was described as having *sweaty* (57%) and *cheesy* (51%) characters. The term *musty* (7%) was also used - a character also perceived with low concentrations of diacetyl. The term *fruity* (6%) has been reported (Meilgaard, 1993) and might be derived from a common impurity in the compound (Amoore *et al.*, 1968) or may suggest a possible specific anosmia.

Maltol (Fig. 4.3) evoked *candy floss* alone in a high proportion (44%) of respondents, and related terms such as *sweet* (*sweet* and *sugary*, 39%) and *caramel* (*caramel*, *burnt sugar*, *toffee*, 35%) were also relatively frequent. As with other *sweet* aroma character compounds (diacetyl, vanillin and furfural), 3% of respondents showed insensitivity to the compound.

Phenyl ethanol (Fig. 4.4) was predominantly described as *floral* (69%), a high proportion of respondents but also evoked by geraniol (47%) which had a secondary aroma note (*lemony*). However, a number of *stale* (13%), *oily* (4%) and *medicinal* (3%) responses were recorded that relate to oxidation of phenyl ethanol to phenyl acetaldehyde. Since the recognition psychometric function (slope) of phenyl ethanol was lowest (0.6) (Lee *et al.*, 2000a), the highest 90% recognition concentration (15188 ppm) was used.

Vanillin (Fig. 4.4) was described as *vanilla* (58%), total *toffee* (35% - *toffee*, *caramel*, *fudge* and *chocolate*) and *sweet* (28%).

The term *chocolate* was associated with *vanilla* character especially in young assessors who instantly produced *chocolate* for vanillin. Including the term *chocolate* as a synonym, a high prompted percentage (67%) of *vanilla* was obtained.

4-Vinyl guaiacol (Fig. 4.4) evoked *smoky* (32%) at highest frequency. This term appeared as a common descriptor for notes arising from phenolic compounds, both guaiacol (39%) and 4-vinyl guaiacol. The main discriminative terms were *spicy* (17% - *spicy, nutmeg, ginger, clove* and *aromatic*), *vanilla* (13%) and *woody* (7%). The chemical term *phenolic* was used with a higher frequency for guaiacol (26%) than 4-vinyl guaiacol (7%) and appeared more familiar to assessors than other chemical terms (e.g. *lactone* 1%). *Rancid* related terms (*rancid, sickly, sour, cheesy, dirty* and *stale*) from guaiacol and *musty*, *cork*, *sheep dip* and *sweaty* terms from 4-vinyl guaiacol appeared common descriptors for phenolic characters. The term *spicy* did not show a consensus and was used differently between companies. *Spicy* includes a number of different characters (including *curry*, *ginger*, *minty*, *clove* and *cinnamon*), so may not be appropriate as a general concept to describe wood character. Since 4-vinyl guaiacol is often reported as a wood derived compound (Tressl *et al.*, 1983; Howie & Swan, 1984; Paterson & Piggott, 1989), its application as a reference compound should be associated with more specific terminology (e.g. *clove-like spicy* in beer) (Meilgaard *et al.*, 1982).

Whisky lactone (Fig. 4.4) was predominantly described using the single term *coconut* (84%) including *Malibu.* Similar characters – the terms *nutty* (11%), *almond* (4%) and *marzipan* (7%) were used less extensively. At 5.3 ppm, the *coconut* odour prevailed (Sharp, 1983; Maarse & van den Berg, 1994) and no other terms were used with significant frequency.

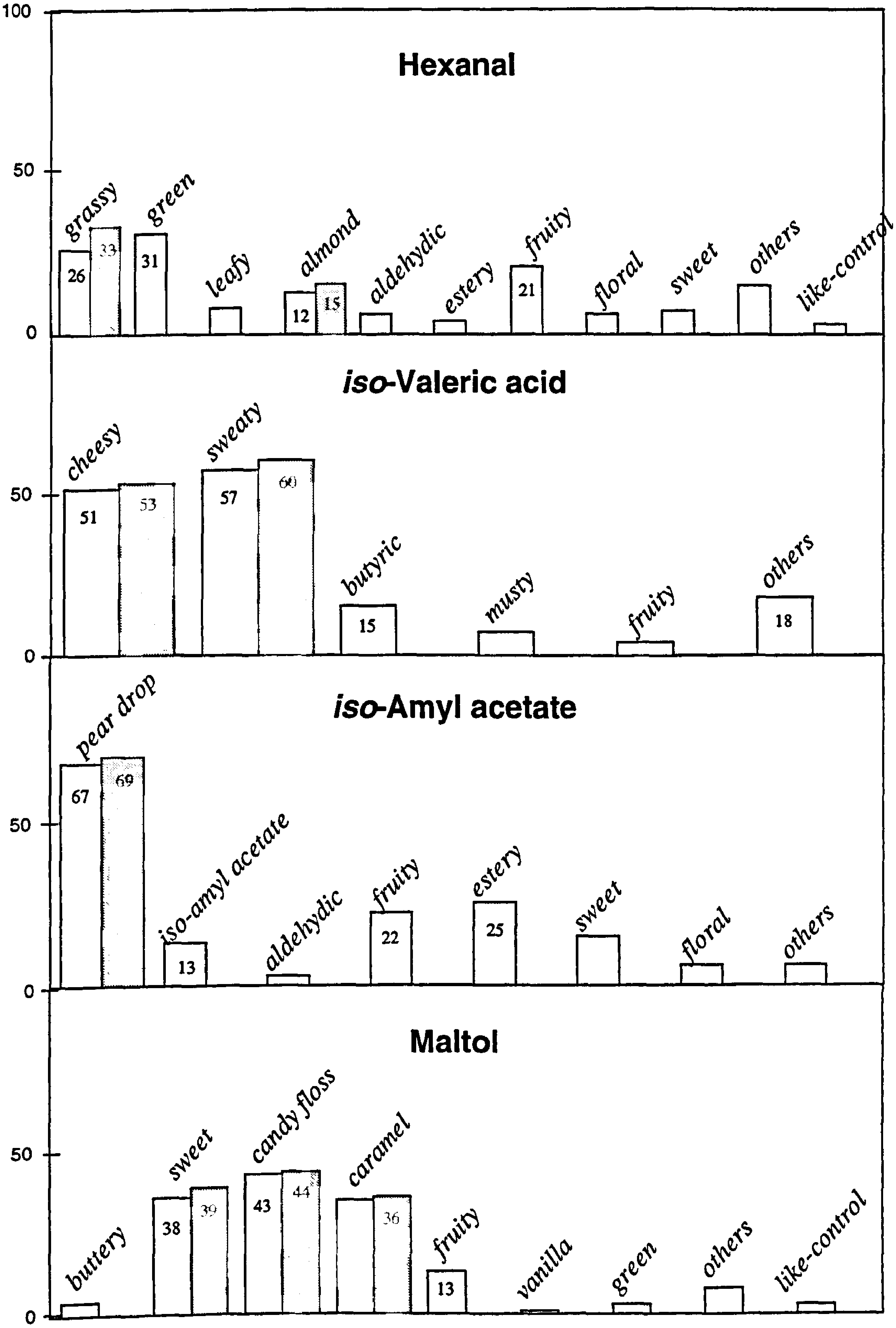


FIG. 4.3. Perception of individual reference compounds

☐ : Initial assessment; ▨ : Prompted assessment

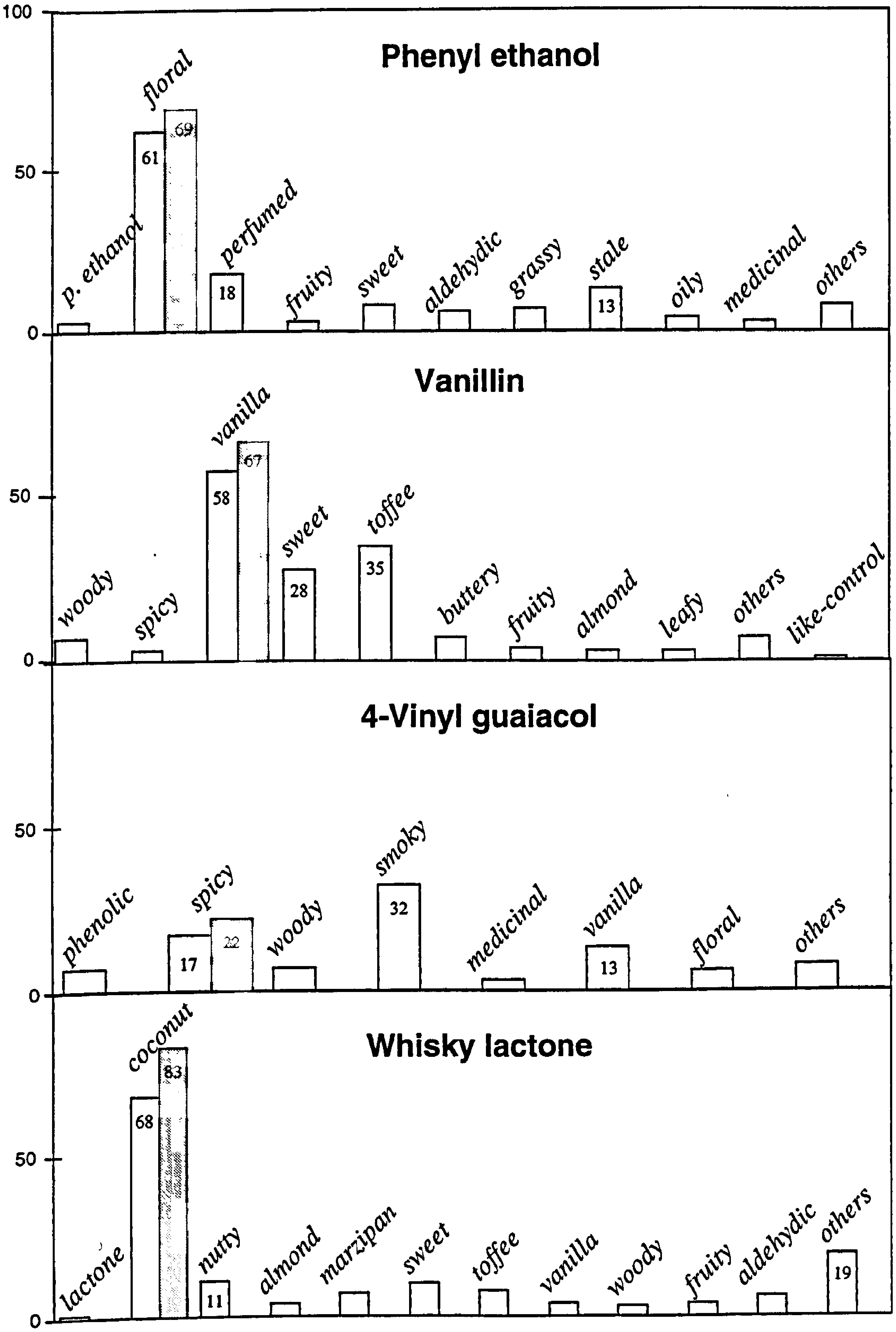


FIG. 4.4. Perception of individual reference compounds
□: Initial assessment; ▨: Prompted assessment

Sensory analysis, in the form of quantitative descriptive analysis, is an important component of quality control. From the results of this study, it was clear that individuals in the distilling industries employ a range of differing terms to describe aroma notes arising from addition of specific compounds. An anchored flavour wheel for whisky could be of greater value as inclusion of suitable reference compounds could illustrate specific individual aspects of flavour character. Three factors need particular attention when establishing a sensory profile

- that assessors differ in their sensitivity and thresholds for sensing individual components of character;
- that assessors may lack awareness or cognizance of certain sample attributes;
- that in most samples there exists a "complex" or "background" of attributes that are not easily identified or separated.

To use sensory analysis in the Scotch whisky industry, significant problems must be overcome before robust analytical procedures for sensory character can be established. Demonstration of sensory attributes in whiskies can be carried out in two ways. Firstly, the panel leader can collect a range of spirit samples that demonstrate the important attributes. However, utilising this approach, specific attributes will be only present in a background of other whisky attributes that may vary between spirit samples. In panel training it is important that an assessor can be focused on specific attributes. Ideally, that attribute should be demonstrated in a neutral base solution such as water or aqueous ethanol. This requires the availability of compounds or flavour complexes (*e.g.* malt extract). The reference compound standards used in this present study were chosen to represent a range of important whisky characteristics. However, the responses of whisky professionals to their addition to grain whisky were unexpectedly diverse. For example, an important component of flavour in a whisky is *sweet* character - elicited after addition to the base spirit of diacetyl (47%), maltol (38%), vanillin (28%), furfural (26%), ethyl hexanoate (24%), *iso*-amyl acetate (15%), whisky lactone (10%), and phenyl ethanol (8%). These *sweet*-related compounds were not easily discriminated by certain assessors. Since addition was at 90% recognition concentration such individuals may fall into the predicted 10% of insensitive assessors. *Sour* was also rather an ambiguous character, elicited from different reference compounds such as *vinegary sour* (56%) from acetic acid, *rancid/cheesy sour* (51%) from *iso*-valeric acid and *sulphury sour* (21%) from DMTS. The term *sulphury sour* was used extensively, especially in one company (43%). *Sour* contained multiple meanings and was applied in different ways between companies. Discrimination between *sour* characters may be necessary to clarify the perception. Similarly acetic acid, an important congener formed during whisky maturation, was described as *sour woody* which related to a defective wood character. Difficulty was also observed with diacetyl in discrimination of perceptions of *sweet* and *buttery*. *Sweet*-related terms (*sweet, caramel, fudge, hot tablet* and *burnt sugar*) was elicited with 47% of respondents, and *buttery* in 31%. However, as opposed to *sweet* and *buttery*, *musty*, *dusty* and *earthy* characters were recorded from a further 13% respondent.

The implications of such findings merit consideration. It is an accepted problem in descriptive sensory analysis that assessors may perceive a flavour in a similar manner but describe it differently (Jack & Piggott, 1993). However, in this experiment, assessors not only described certain flavours differently, but also appeared to perceive their character differently (*e.g. grassy* vs *almond* and *sweaty* vs *fruity*). Since every sensory attribute in a whisky must originate from its congeners, it is important to understand how to harmonise perceptions between assessors.

The descriptors employed to describe the chosen reference compounds seem to be strongly anchored in non-technical language (notably *candy floss*, *pear drops*, *vinegary*, *marzipan*, *soapy*, *smoky*, *floral*, *sweaty* and *cheesy*). The only chemical term frequently used was *sulphury* (44%) for DMTS. Other chemical or technical descriptors (e.g. *phenol*, *diacetyl*, *phenyl ethanol*, *feinty*, *aldehydic* and *estery*) were used less extensively. Therefore, whisky flavour terminology should be understandable to, and in a language for non-technical sensory assessors, and thus also of value in working with consumers and marketing professionals. *Feinty* (in relation with *sweaty* and *stale fish*) has been well known to technical members and was positioned in the first tier of The Whisky Flavour Wheel (Shortreed *et al.*, 1979). However, the term appeared unfamiliar to non-technical assessors (one occurrence in relation to *sweaty* character). *Grainy* (6%) was difficult to define due to the complexity of the character and it may be difficult to avoid using raw materials since no alternative reference compound has been suggested. Definition of different degrees of maturity and *woody* characters may be required for routine consumer assessments of whisky character arising from cask usage, such as new (*sap*, *resinous*, *pine* and *cedar*), American (ex-Bourbon, *vanilla*) and European oak (*sherry* and *walnut-like*).

Flavour reference standards can now be produced in the form of cyclodextrin-linked compounds of high purity. These could be used to establish sensory procedures to ISO standards in the whisky industries although further research and development is required to begin such a process. Training of assessors requires provision of a clear flavour concept that can be demonstrated by a panel leader. Certain concepts may be simple *e.g. coconut* character; others may be relatively complex such as those related to defective wood. To ensure effective communication between whisky professionals and sensory assessors, a level of standardisation of flavour attribute concepts is required. One way of achieving this could be the establishment of standard reference compounds with rationalised and standardised descriptors.

4.5. CONCLUSION

In order to simplify whisky flavour terminology, an appropriate set of reference compounds with standardised terms is required. Thirteen compounds were shown to be adequate as references for illustrating flavour characters in whisky. Clarification of certain aspects of terminology is still required, and compounds illustrating certain attributes remain to be identified.

CHAPTER 5:

SENSORY DISCRIMINATION OF BLENDED SCOTCH WHISKIES OF DIFFERENT PRODUCT CATEGORIES

Results of this study have been published in *Food Quality and Preference* (Lee *et al.*, 2001).

5.1. ABSTRACT

Samples of forty blended Scotch whiskies were analysed in a conventional sensory profiling experiment employing 26 trained assessors, nosing using a consensus vocabulary. Whisky blends were classified into four product categories on the basis of labelling and retail price: Deluxe (11), Standard (22), multiple Retailer (4) and West Highland (3). From the analysis of variance (ANOVA), highly significant attributes for discrimination between blended whiskies were *pungent*, *smoky/peaty*, *smooth*, *woody*, *vanilla*, *sulphury* and *rancid* whereas *catty* and *sour/acetic* were non-discriminating attributes. Principal component analysis (PCA) explained 58% total variance in four significant components. Discriminant PLS2 èxplained 27% total Y-variance in two components, and yielded a similar product space and clustering to that from PCA. Discrimination power of attributes in defining blends categories was determined by DPLS1. Deluxe category was mainly perceived as *fruity*, *buttery*, *malty* and *nutty*, Standard as *oily*, *sulphury*, *rancid*, *grainy*, *smooth*, *soapy*, *vanilla*, *solventy* and *sweet*, Retailer as *solventy*, *soapy* and *rancid*, and West Highland as *woody*, *smoky/peaty*, *spicy* and *medicinal*. Standard category blends were dispersed across the product spaces by sharing similarities in characters with Retailer and also, less extensively, with Deluxe blends.

Keywords: whisky; sensory flavour profiling; blended whiskies categories; PCA; discriminant PLS; validation variance

5.2. INTRODUCTION

The origins of sensory characters in Scotch whiskies have been clarified by increased understanding of wood maturation processes (Piggott *et al.*, 1993a; Conner *et al.*, 1994a,b, 1996, 1999). In essence, malt whiskies are aqueous ethanol microemulsions with continuous and dispersed phases (Conner *et al.*, 1994a). Wood maturation changes the partitioning of flavour components between these two liquid phases, altering the distribution of flavour components in the glass - between the liquid, and its headspace. Sensory character changes because immature aroma notes from volatile flavour components in the headspace of new distillate are retained within the liquid of the matured whisky. Thus limited differences in congener composition may not reflect key quantitative differences in headspace composition, which determine the sensory character in whiskies (Conner *et al.*, 1998, 1999).

Malt whiskies are central to the Scotch whisky industry, both as premium products and in blending, but the incorporation of grain whiskies has been the key to product popularity (Lang, 1983; Burns, 1995). Blends currently form the major part of production and market (85% by volume, The UK Food & Drink Report, 2000). Blended Scotch whiskies are generally produced by blending between four and fifty (often 20) malt spirits (Booth *et al.*, 1989), matured for typically 4 - 5 years, and up to five grain whiskies. Selection of primary malt whiskies and secondary spirits has significant impact on final flavour character in blends (Murray, 1999). Grain distillates, introduced into blends for economy and to reduce heavy malt whisky flavour characters, are considered important for introducing *smooth* characters, a key sensory character in whiskies (Schoeneman & Dyer, 1973).

Relatively little is understood about discrimination between blended whiskies. For retailing, Scotch whiskies can be conveniently divided into Deluxe, Standard and secondary (often Retailer own brand) blends. Classification depends upon proportions and to an extent character of malts in the blend, specified maturation period, and final retail price. These can be subject to a "flexible" approach (Schoeneman & Dyer, 1973; Lyons & Rose, 1977); there have been concerns over insufficient supply of blend ingredients (Hay *et al.*, 1994). In recent years blended whiskies, with high contents of peated malts (Withers *et al.*, 1996), have become speciality products (here referred to as West Highland blends).

Few studies of sensory discrimination between blended Scotch whiskies have been published in the 20 years since the advent of descriptive sensory analysis of whiskies (Piggott & Jardine, 1979; Shortreed *et al.*, 1979; Piggott & Canaway, 1981). Initial conventional profiling reports (Piggott & Jardine, 1979) showed that sensory data acquired by whisky nosing was suitable for studies of product discrimination. Subsequently Piggott *et al.* (1989) and Guy *et al.* (1989) studied consumer whisky discrimination using free choice profiling: a restricted product range and inherent limitations of the profiling strategy (Lawless & Heymann, 1998) limited interpretation of data.

Relationships between congeners and sensory differentiation of blended Scotch whiskies are unclear. Recent studies have suggested differences between ambient and oral temperatures may yield important quantitative differences in volatile headspace flavour components (Conner *et al.*, 1999). A simulated mouth (Margomenou *et al.*, 2000) should facilitate study of relationships between flavour component distribution and sensory character.

The aim of this study was to characterise sensory discrimination between blended Scotch whiskies as a precursor to modelling relationships between headspace concentrations of volatile flavour components and character.

5.3. MATERIALS AND METHODS

5.3.1. Whisky blends

Blended whiskies (40: Table 5.1), donated by industrial collaborators or purchased retail, were stored at ambient temperature. Whiskies were diluted to 23% (abv) ethanol with de-ionised water immediately prior to assessments.

TABLE 5.1. Blended Scotch whisky products in study with category codes

Category	Product code	Ethanol (abv)	Age (yr)	Category	Product code	Ethanol (abv)	Age (yr)
Standard	Sa	40%	>3	Deluxe	Da	40%	>3
	Sb	40%	4		Db	43%	12
	Sc	43%	>3		Dc	43%	12
	Sd	40%	>3		Dd	40%	5
	Se	40%	>3		De	43%	15
	Sf	40%	>3		Df	40%	12
	Sg	40%	>3		Dg	43%	18
	Sh	43%	>3		Dh	43%	21
	Si	40%	>3		Di	43%	>3
	Sj	40%	>3		Dj	40%	12
	Sk	40%	>3		Dk	43%	>3
	Sl	40%	5	Retailer	Ra	40%	>3
	Sm	40%	>3		Rb	40%	>3
	Sn	40%	>3		Rc	40%	>3
	So	40%	8		Rd	40%	>3
	Sp	40%	5	West Highland	Wa	40%	>3
	Sq	40%	>3		Wb	40%	>3
	Sr	43%	>3		Wc	40%	>3
	Ss	40%	>3				
	St	40%	>3				
	Su	40%	>3				
	Sv	40%	>3				

5.3.2. Sensory profiling

Assessors (26; 7 females, 25 - 52 years of age; 19 males, 25 - 55 years of age) were recruited from staff and students at the University of Strathclyde. Assessors were trained to employ a consensus vocabulary (Table 5.2) using reference standards. This vocabulary was developed from earlier versions (Piggott & Canaway, 1981; Piggott, 1991) substituting non-specialist terms such as *fruity*, *floral*, *smoky* for certain chemical terms (e.g. *estery*, *phenolic*) (Lee *et al.*, 2000a).

In each session, eight samples were profiled under red lighting to minimise bias from appearance. Spirit (30 ml) was nosed in a standard wine tasting glass (ISO 5494: 1978) using Latin square presentations to minimise order effects (MacFie *et al.*, 1989). Duplicate samples were assessed on different days. Attributes were scored on line scales, anchored at 10% with *not much* and at 90% with *very much* using the PSA system.

5.4. DATA ANALYSIS

For each attribute, the following were calculated from raw data across the 40 products (Table 5.2): mean, minimum, maximum and standard error of the mean (SEM). Analyses of variance (ANOVA) were carried out to assess attributes significantly different between whiskies and categories using the GLM command in Minitab (v11.11). Data were then averaged across assessors and principal component analysis (PCA: Piggott & Sharman, 1986) was performed, treating replicates separately, using Unscrambler v 7.01 (CAMO A/S, N-0115 Oslo, Norway). Significance in principal components (PCs) was examined using one-way ANOVA (Minitab v11) and replicate scores were averaged before plotting mean product spaces.

Modelling of relationships between flavour components and sensory character has been transformed by partial least squares regression (PLS; Wold *et al.*, 1983; Martens, *et al.*, 1983, 2000; Martens & van der Burg, 1984; Martens & Martens, 1986). In this experiment, discriminant partial least squares regression (DPLS; Martens & Martens, 1986; Martens *et al.*, 2000; Wold, 1989) was used to investigate group (cluster) patterns within samples. In DPLS2, the sensory data as *X*-matrix (regressors: predicting) was regressed on to four blend category *Y*-variables (regressands: predicted), formulated '1' for class members and '0' for all other objects, allowing all specified design variables in a single product space. In DPLS1, the individual product categories were modelled separately, to determine the discrimination power of each attribute. Full cross validation was applied to assure optimal interpretation of data (Wold, 1989; Kvaal & McEwan, 1996).

5.5. RESULTS

5.5.1. Selection of discriminating descriptors

Low scoring attributes for the products were *catty*, and *rancid/cheesy*, with means of 17%. The highest mean scores were 43% for *pungent* and 33% for *smoky/peaty* (Table 5.2). The largest score ranges (between minimum and maximum) were for *mould/musty* (26%), *smoky/peaty* and *solventy* (each 25%) and the narrowest *buttery*, *spicy* and *soapy* (each 9%). The latter might suggest the products were perceived similar (Table 5.2). A high mean score was recorded for *smooth* (34%), important in whiskies (Guy *et al.*, 1989) (Table 5.2).

Results of ANOVA for attribute scores are summarised in Table 5.2. Sources of variation were whiskies, blend categories and assessors. Effect of assessor was significant ($p<0.000$) for all attributes indicating lack of agreement on the concept of the descriptors used. However, ANOVA for individual assessors (except three) showed consistent and significant use of attributes. The scores for 5 attributes - *floral, oily, catty, spicy* and *acetic sour* - were not significantly different between whiskies and categories. However, *floral, oily* and *spicy* were retained in further analyses as key attributes in whisky flavour (Shortreed *et al.*, 1979; Piggott & Jardine, 1979; Piggott & Canaway, 1981; Lee *et al.*, 2000b). *Catty* and *acetic-sour* scores were eliminated from subsequent analyses.

Highly significant ($p<0.002$) attributes for discrimination between whiskies were *pungent, smoky/peaty, smooth, woody, vanilla, sulphury* and *rancid.* Two of these related to whisky wood maturation (*vanilla, woody*) (Paterson & Piggott, 1989). Significant ($0.002<p<0.01$) attributes for blend discriminations were *medicinal, grassy, solventy, sweet* and *nutty.* The attributes *grainy, fruity, buttery* and *mouldy/musty* were less significant ($0.01<p<0.05$) in discrimination. ANOVA identified three assessors using no attribute significantly ($p>0.05$) in discrimination between products; these assessors were omitted in subsequent data analysis.

5.5.2. Determination of attributes for individual blend categories (DPLS1)

To determine attributes important for each product category, DPLS1 regression analysis was carried out using sensory data as the X-variable and single product categories as the Y. Total variance explained for Deluxe, Standard, Retailer and West Highland were 31, 41, 22 and 23%, respectively. For the first three categories only PC1 was recommended by full-cross validation. No component was recommended for West Highland.

TABLE 5.2. Attributes (23) of 40 blended Scotch whisky products: mean, minimum, maximum, Standard error of the mean (SEM) and analysis of variance (ANOVA) of raw data

	Raw data				ANOVA		
	Mean	Min	Max	SEM	p value		
Attribute					Whiskies	Categories	Assessors
Pungent	43	32	50	2.27	0.000^{***}	0.001^{***}	0.000
Smoky/Peaty	33	25	50	2.20	0.000^{***}	0.000^{***}	0.000
Medicinal	26	20	34	2.09	0.003^{**}	0.089^{ns}	0.000
Malty	32	22	38	1.83	0.181^{ns}	0.017^{*}	0.000
Grainy	32	24	38	1.97	0.023^{*}	0.466^{ns}	0.000
Grassy	25	19	37	2.08	0.003^{**}	0.381^{ns}	0.000
Floral	27	19	31	1.82	0.141^{ns}	0.284^{ns}	0.000
Fruity	28	16	32	1.78	0.049*	0.006^{**}	0.000
Solventy	32	25	50	2.25	0.013^{**}	0.008^{**}	0.000
Oily	20	17	28	1.50	0.172^{ns}	0.302^{ns}	0.000
Smooth	34	21	42	2.42	0.001^{***}	0.132^{ns}	0.000
Sweet	32	19	38	2.59	0.004^{**}	0.384^{ns}	0.000
Buttery	22	18	27	1.58	0.050^{*}	0.042^{*}	0.000
Nutty	22	16	29	1.36	0.005^{**}	0.165^{ns}	0.000
Woody	31	23	46	1.87	0.000^{***}	0.000^{***}	0.000
Vanilla	25	15	33	1.84	0.000^{***}	0.012^{**}	0.000
Spicy	22	19	28	1.34	0.870^{ns}	0.174^{ns}	0.000
Mouldy/musty	24	19	45	2.64	0.015^{*}	0.456^{ns}	0.000
Sulphury	21	16	36	1.94	0.001^{***}	0.007^{**}	0.000
Rancid/cheesy	17	13	36	2.14	0.000^{***}	0.069^{ns}	0.000
Soapy	21	17	26	1.42	0.208^{ns}	0.033^{*}	0.000
Catty	17	13	37	2.39	0.242^{ns}	0.973^{ns}	0.000
Sour/acetic	21	16	27	1.55	0.312^{ns}	0.227^{ns}	0.000

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns = not significant

Ranking of attribute loading weights from DPLS1 was presented (Table 5.3; column a) and ANOVA defined significant terms for each category (column b). Important attributes for Deluxe whiskies were *fruity, buttery, malty* and *nutty*, and less important were *vanilla, floral, pungent, sweet* and *smooth.* From ANOVA result, *sulphury* and *oily* were significant in discriminating between products but they were negatively related (by loading weights) to the category character. For Standard blends, *oily, sulphury* and *rancid* were highly and positively related, but they were non-significant (p>0.05) from ANOVA. In contrast, *pungent, fruity, spicy, floral* and *nutty* were negatively related but showed significant impact to the category character. Retailer blend character was dominated by - *solvent, soapy, rancid, grassy, mouldy, pungent, grainy, oily* and *sulphury.* Only the first three were significant. Similarities between Standard and the Retailer blends were observed in a commonality of *solventy, grainy, rancid, oily* and *mouldy* attributes. It was however clear that Standard could be discriminated from Retailer blends on the basis of *sweet, smooth, vanilla* and *buttery* attributes. West Highland blends did not yield any valid components but they were (as would be expected) dominated by *woody, smoky/peaty, spicy, medicinal, suphury, mouldy, pungent, soapy, malty* and *floral.* The first three attributes were highly significant (p<0.02) from ANOVA result. *Spicy* was a non-discriminating attribute from ANOVA results (Table 5.2), it was however important to describe West Highland blends character.

The outcomes obtained from DPLS1 and ANOVA, as predicted by Martens and Martens (1986) (*i.e.* ranking of loading weights in DPLS1 and order of significance from ANOVA), were identical. However, in Table 5.3, the attribute order was prioritised in only positively related terms, omitting negatively loaded attributes.

TABLE 5.3. Ranking of attributes for blend categories: (a) by the loading weights on the first component from DPLS1; and (b) p values from analysis of variance (ANOVA).

Deluxe		Standard		Retailer		West Highland	
a	b	a	b	a	b	a	b
Fruity (0.367)	Fruity** (0.004)	Oily (0.149)	Pungent** (0.003)	Solventy (0.435)	Solventy** (0.004)	Woody (0.483)	Woody** (0.006)
Buttery (0.337)	Buttery** (0.009)	Sulphury (0.138)	Fruity** (0.008)	Soapy (0.410)	Soapy** (0.008)	Smoky/peaty (0.473)	Smoky/peaty (0.007) **
Malty (0.332)	Malty** (0.01)	Rancid (0.009)	Spicy* (0.031)	Rancid (0.353)	Rancid* (0.024)	Spicy (0.416)	Spicy* (0.018)
Nutty (0.323)	Nutty* (0.013)	Grainy (-0.029)	Floral* (0.034)	Grassy (0.235)	Smooth (0.063)	Medicinal (0.314)	Medicinal (0.080)
Vanilla (0.239)	Sulphury* (0.015)	Smooth (-0.035)	Nutty* (0.050)	Mouldy (0.227)	Malty (0.141)	Sulphury (0.225)	Buttery (0.206)
Floral (0.235)	Oily* (0.039)	Soapy (-0.082)	Malty (0.072)	Pungent (0.226)	Grassy (0.141)	Mouldy (0.206)	Sulphury (0.214)
Pungent (0.204)	Vanilla (0.073)	Vanilla (-0.083)	Woody (0.146)	Grainy (0.203)	Mouldy (0.155)	Pungent (0.206)	Mouldy (0.256)
Sweet (0.196)	Floral (0.076)	Solventy (-0.087)	Buttery (0.184)	Oily (0.179)	Pungent (0.156)	Soapy (0.151)	Pungent (0.257)
Smooth (0.189)	Soapy (0.101)	Sweet (-0.092)	Smoky/peaty (0.188)	Spicy (0.107)	Grainy (0.204)	Malty (0.147)	Soapy (0.408)
Spicy (0.073)	Pungent (0.126)	Mouldy (-0.094)	Grassy (0.197)	Floral (0.052)	Oily (0.263)	Floral (0.104)	Malty (0.419)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

TABLE 5.4. (a) Validation variance and (b) root-mean-square-error of prediction (RMSEP) of individual categories from DPLS2

	Total	Deluxe		Standard		Retailer		West Highland	
	a	a	b	a	b	a	b	a	b
PC0	1.19	0	0	0	0	0	0	0	0
PC1	1.14	17.56	0.42	5.07	0.50	2.17	0.31	-2.01	0.27
PC2	1.09	17.24	0.42	17.48	0.46	7.66	0.30	-2.20	0.28

5.5.3. Sensory differences between products and blend categories

Initial PCA of the full data set explained 44% variance, with three significant components from ANOVA (1, 2 and 4; p = 0.026, 0.023 and 0.000; 22, 14 and 8% variance, respectively). The two non-discriminating attributes (*catty* and *acetic-sour*) were eliminated, and PCA (Fig. 5.1a-c, Fig. 5.3) yielded 4 significant principal components (p = 0.006, 0.035, 0.036 and 0.000, respectively) explaining a total of 58% variance (23, 16, 11 and 8%, respectively), a 14% improvement. Although PCA in Unscrambler suggested 7 components, the final three components accounted for a low % of variance (5, 5 and 4%, respectively), and ANOVA revealed these final 3 components were non-significant. Not only the PCs 5, 6, and 7 showed low % calibration variance but also their validation variances (cross-validation) (41, 41 and 44%, respectively) were hardly increased after the PC4 (41%) (data not shown here). Thus, the PC 4 would be an ideal cut-off point. Aastveit and Martens (1986) have suggested various strategies can be employed for optimisation of selecting number of principal components.

Fig 5.1a is a biplot showing PC1 vs PC2, with mean scores for all (40) products and loadings for attributes. In this product space PC1 is defined by Deluxe blends with negative scores, and Retailer products with positive. In PC2 a single West Highland blend showed a high positive and majority of Standard blends negative scores.

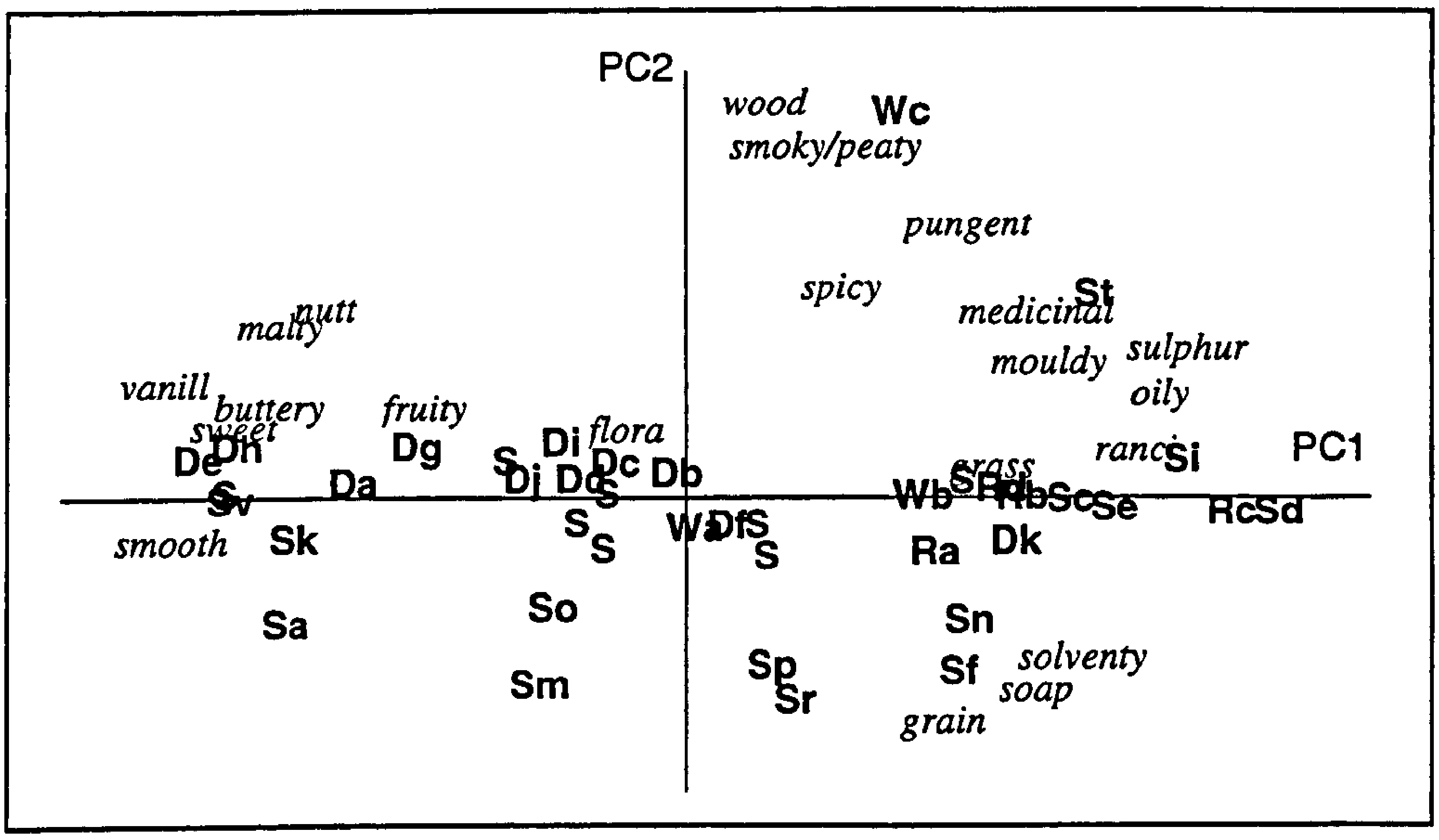


FIG. 5.1a. PCA product space - biplot of 40 blended Scotch whiskies: PC1 vs PC2

The edited data set (excluding outlier assessors and non-significant attributes) was then subjected to DPLS2 with sensory data regressed on to product categories: Deluxe, Standard, Retailer and West Highland. In this DPLS2 space (Fig. 5.2a-c), only two components were significant - explaining 27% of *Y*-variance (category) and 40% of *X*-variance (sensory data). Residual *Y*-validation variances for PC0, PC1 and PC2 were 1.19, 1.14 and 1.09%, respectively (Table 5.4). Individual categories within this product space: Deluxe, Standard, Retailer and West Highland were explained total of 33, 41, 16 and 15% variance, respectively (data not shown here). Although the total validation variance of Deluxe and Standard categories were similar from two components (PC1 and 2) (17.2 and 17.5%), Deluxe category was best discriminated on component 1 with higher validation variance (17.6%) compared with component 2 (17.2%). This reduced validation variance suggested that no more additional information had been explained by the second component for Deluxe, whereas Standard category was better explained in the component 2 with higher validation variance (17.5%) compared with component 1 (5.0%). West Highland category was least explained, with negative validation variance in both components, and the lowest RMSEP (0.28). This was also reflected by no yielding of valid component for West Highland category from DPLS1. This may reflect small number of products (3) in that category or the products were perceived differently in sensory character.

Figure 5.2a is the DPLS2 product space showing 4 product categories and attribute loadings. Although the overall product space structure was slightly rotated since PCA tended to show more systematic variation with the samples (Martens *et al.*, 2000) whereas DPLS2 elicited relationships between product categories and their attributes. However, the distributions of loadings and cluster structures were shown similar between two methods. In the DPLS2, since the validation variance for *grassy* (-11.1 and -3.8), and *grainy* (-9.6 and -22.2) were negative on the both components (data not shown), they were eliminated for the interpretation. In ANOVA (Table 5.2) they were non-discriminating attributes for product categories.

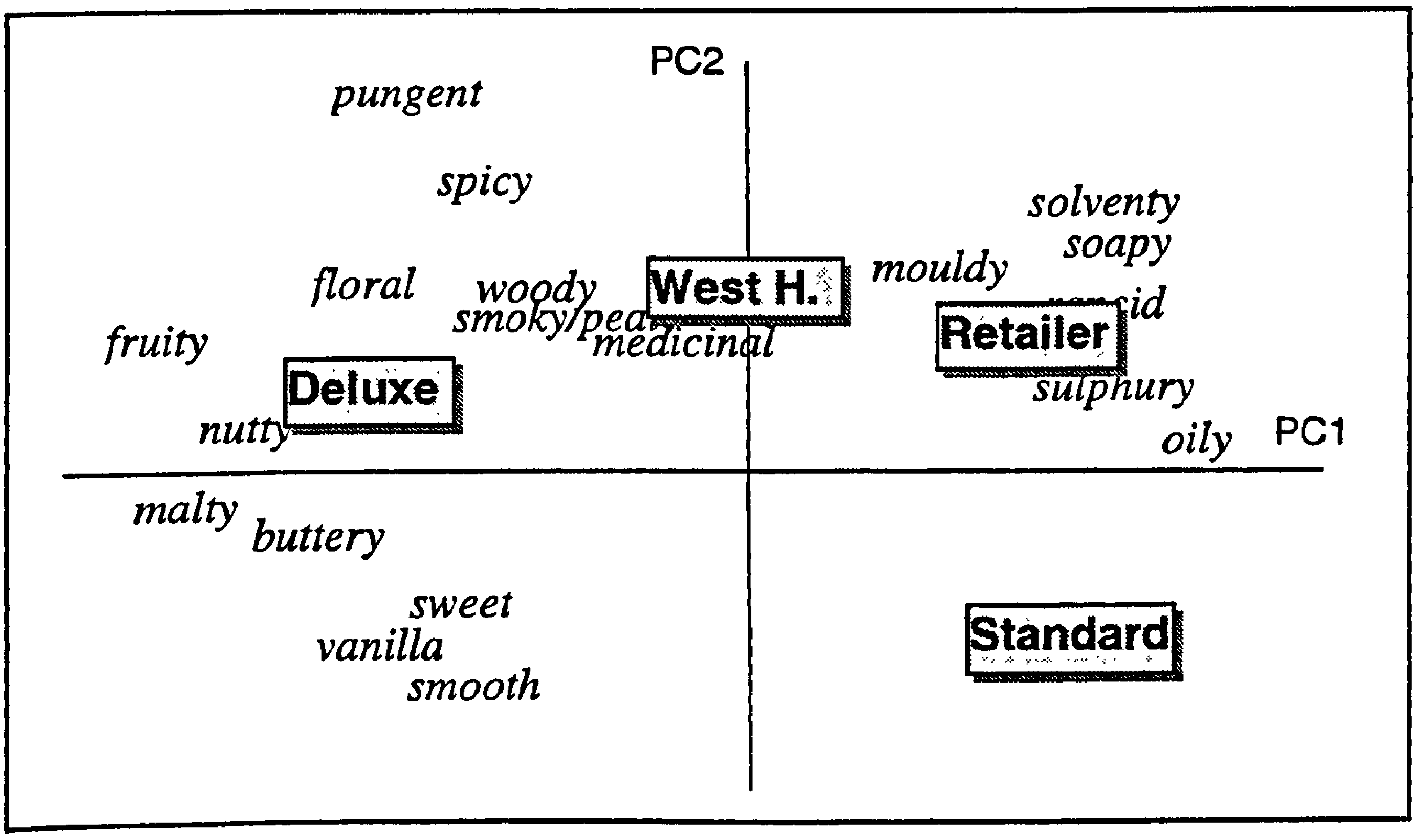


FIG. 5.2a. DPLS2 product space - biplot loadings of 40 blended Scotch whiskies: component 1 vs component 2

To simplify interpretation, Fig. 5.1b & 5.2b show only scores for Deluxe and Retailer blends. Character of Deluxe blends could be related to the following attributes - *vanilla*, *buttery*, *sweet*, *malty*, *nutty*, *fruity* and *smooth*. In contrast attributes linked to the cheaper Retailer blends had notes - *sulphury*, *oily*, *rancid*, *solventy*, *soapy* and *mouldy*.

Deluxe blends were differentiated into three main clusters (Fig. 5.1b & 5.2b). **Dh** and **De** had the most matured attributes - *sweet*, *vanilla*, *buttery*, *malty*, *nutty* and *smooth* since these blends had the longest declared maturation time, 21 and 15 years, respectively. The second Deluxe cluster (**Dg** and **Da**) had *fruity/estery* characters. The third cluster (**Dj**, **Di**, **Dd**, **Dc** and **Db**) was linked with *floral* characters (Fig. 5.1b). The internal patterns within the cluster were similar in PCA and DPLS2 except for **Dd**, the blend matured least (5 yrs). Blends **Df** and **Dk** were outliers from major clusters. The former appeared to be more related to *spicy*, *smoky/peaty* and *woody* characteristics and the latter had been formulated differently for export to the Asian market, and was not discriminated from Retailer blends. The 12 year-old products (**Db**, **Dc**, **Df** and **Dj**) were perceived as *floral*, among them **Df** was differed from the other as being described above. Of blends matured longer, **Dh**, **Dg**, **De** and **Df** (21, 18, 15 and 12 year-old blends, respectively) were all from one blender and had marked similarities on PC2, but were discriminated on the first components on the basis of *smoothness* and wood-related characters. The Retailer blends were discriminated into a cluster and an outlier on PC1 from both product spaces. Blends **Ra**, **Rb** and **Rd** were perceived similarly as *grassy*, *mouldy*, *medicinal*, *solventy*, *soapy* and *grainy*. **Ra** was also blended for export to Asia, and perceived similar to the other export product (**Dk**). The outlier **Rc**, a multiple Retailer product in domestic UK market, showed *rancid*, *oily* and *sulphury* characters.

Fig. 5.1c (PCA) & 5.2c (DPLS2) show the product spaces for Standard and West Highland blends. On both PC1 and component 1, West Highland blends showed commonality in character as *medicinal*, *smoky/peaty*, *woody*, *spicy*, *mouldy* and *pungent*. However, on PC2 **Wc** was more *woody*, *smoky/peaty*, *pungent* and *spicy* whereas **Wb** and **Wa** were more *floral*, *grassy*, *rancid* and *oily* (Fig. 5.1c), and also *mouldy* and *sulphury* (Fig. 5.2c). Standard blends could be differentiated into four clusters by PC1, but product distribution within clusters was slightly different between PCA and DPLS2 (Fig. 5.1c & 5.2c). The first cluster (**Sq**, **Sv**, **Sk** and **Sa**), with negative scores on PC1, had mature characters similar to the Deluxe blends, perceived as *smooth*, *sweet*, *vanilla*, *buttery*, *malty*, *nutty* and *fruity*. The second (**Sb**, **Sj**, **Su**, **Sh**, **So** and **Sm**) also had high scores for mature character but were more *floral*. The third cluster (**Sl**, **Sg**, **Sp** and **Sr**) was not linked to any specific attribute. In contrast, the fourth cluster (**Sn**, **Sf**, **Sc**, **Se**, **Ss** and **Si**), with positive scores on PC1, was perceived as *grainy*, *soapy*, *solventy*, *grassy*, *rancid* and *oily*, all immature character notes dominated in the cheaper Retailer blends. Blend **Sd** (a 8 yrs, popular in domestic markets) was an outlier in both product spaces, and was the most *sulphury*, *oily* and *rancid* in character. With positive score on PC2 (Fig. 5.1c), product **St** was an outlier, and perceived as *medicinal*, *smoky/peaty* and yet *sulphury*, and with negative score, **So**, **Sm**, **Sp**, **Sr**, **Sn** and **Sf** were also perceived *smooth*, *vanilla* and *sweet*, similar with part of Deluxe blends characteristics.

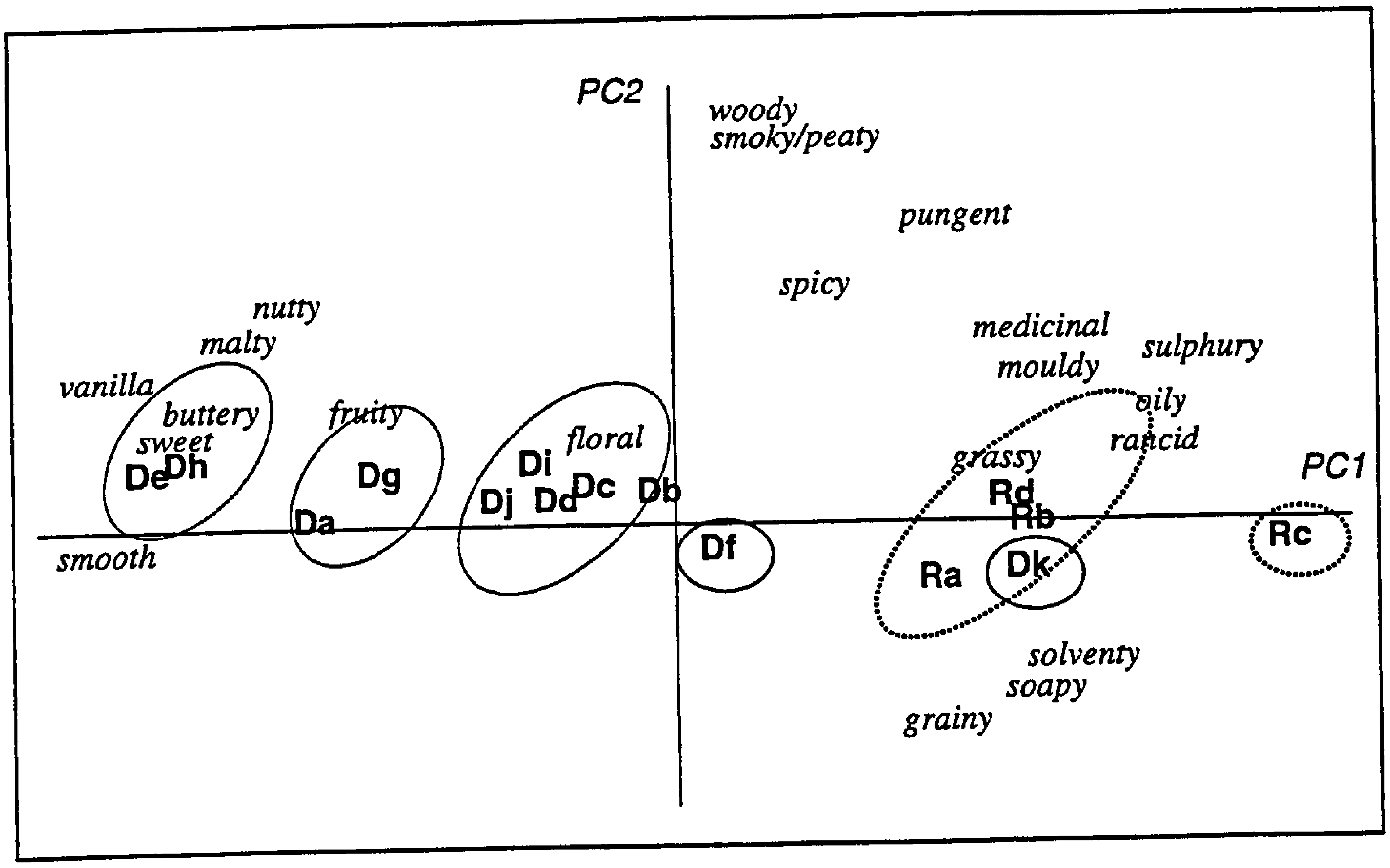


FIG. 5.1b. PCA product space (PC1 vs 2): biplot of Deluxe & Retailer blends only

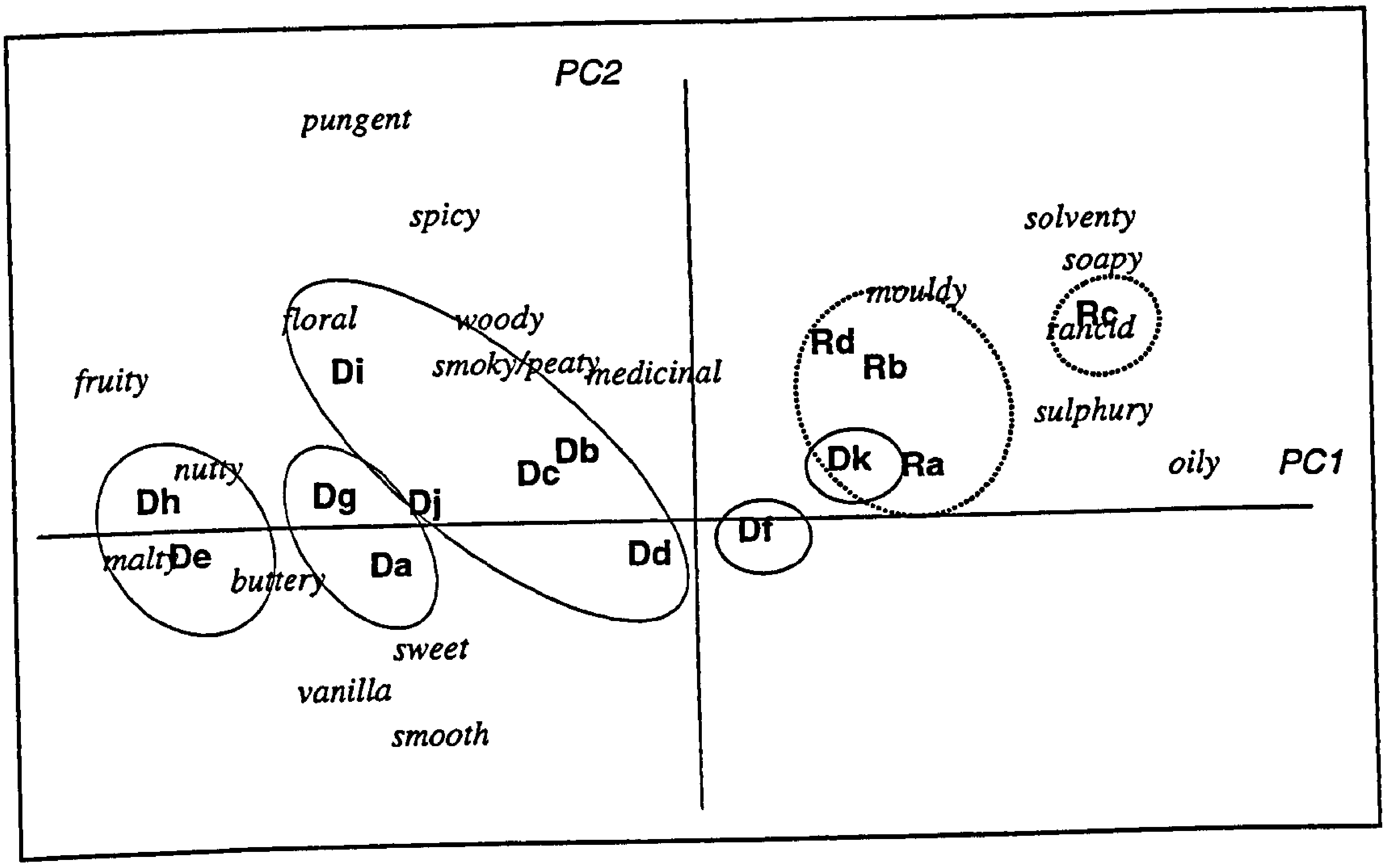


FIG. 5.2b. DPLS2 product space (component 1 vs 2): biplot of Deluxe & Retailer blends only

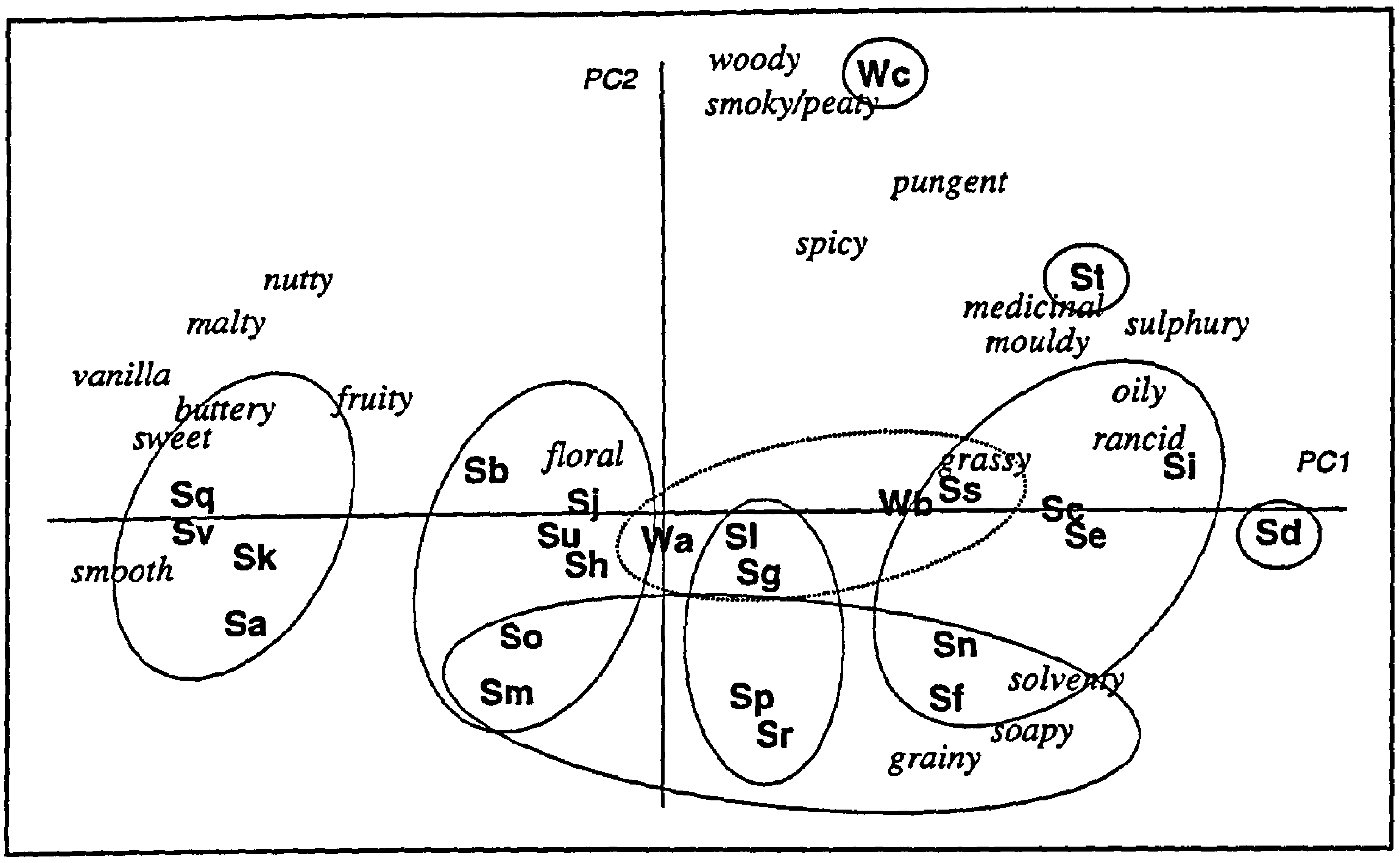


FIG. 5.1c. PCA product space (PC1 vs 2): biplot of Standard & West Highland blends only

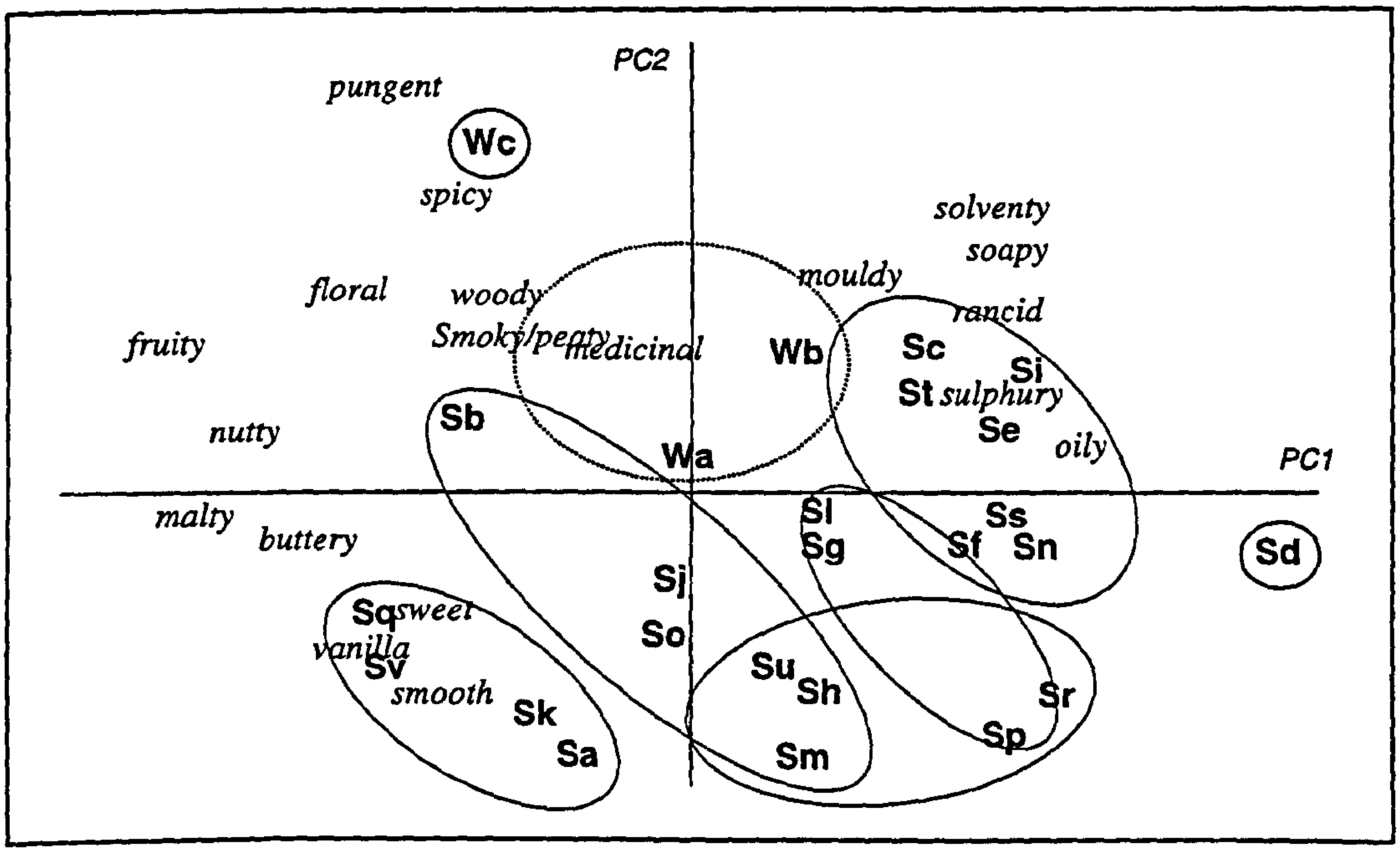


FIG. 5.2c. DPLS2 product space (component 1 vs 2): biplot of Standard & West Highland blends only

5.5.4. PCA product space (principal component 3 and 4)

Fig. 5.3 shows the PCA product space explaining a total of 19% variance (PC 3 and 4: 11 and 8%, respectively). No distinct clustering could be related to product categories. This was expected, as third and fourth components in DPLS2 analysis were not valid. Attributes which showed a decreased validation variance - *smooth, vanilla, spicy* and *soapy* - were eliminated since they were most explained on PC 1 and 2, and provided no additional information but noise on these components.

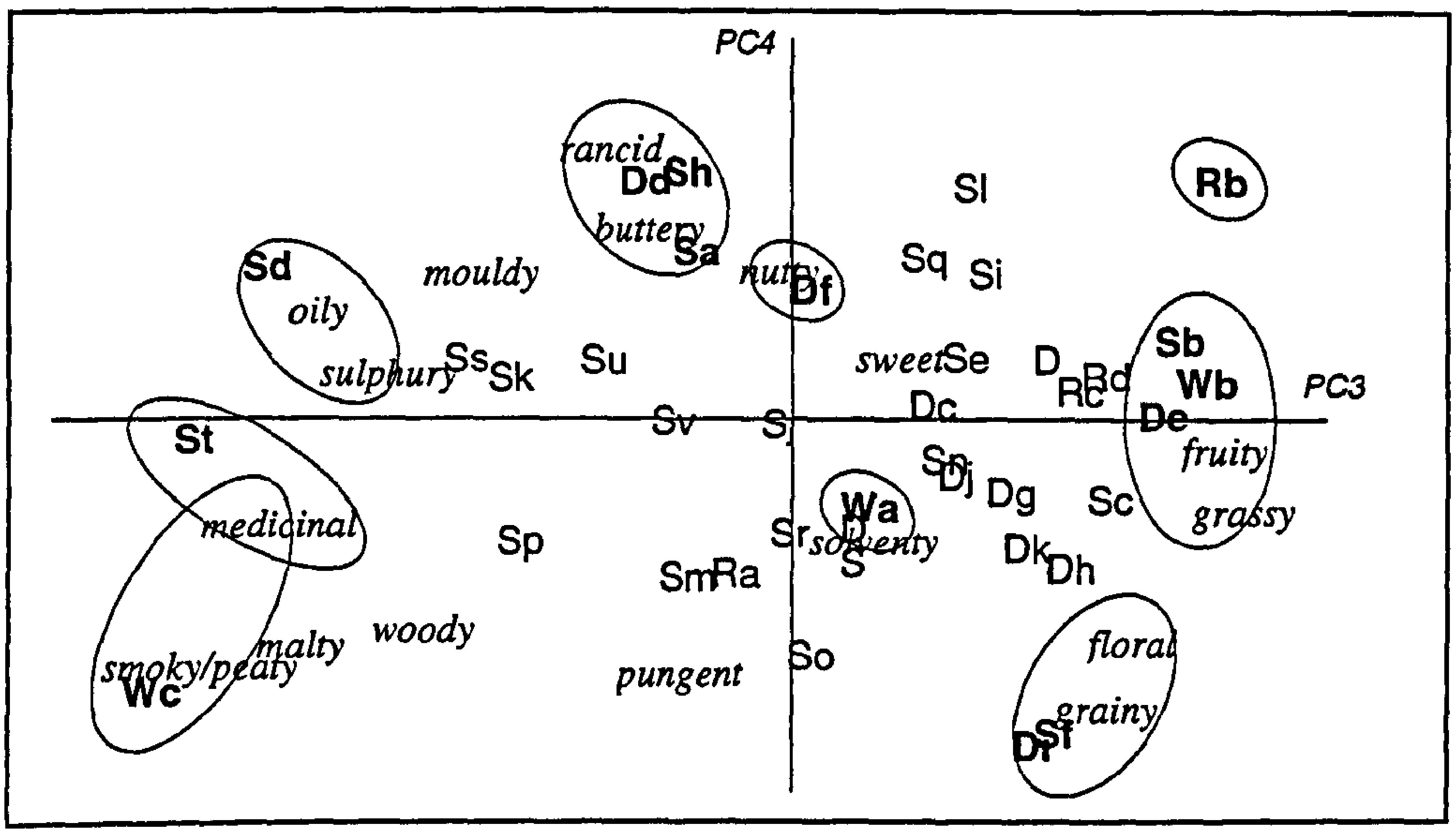


FIG. 5.3. PCA product space (PC3 vs PC4): biplot of 40 blended Scotch whiskies

The PC3 and 4 thus provided more detailed information on individual product characters. On PC3, with positive loading, blends were differentiated on the basis of *grassy, fruity, floral, grainy* and *sweet*, and with negative, *smoky/peaty* and *medicinal.* Three West Highland whiskies were clearly differentiated on PC3. **Wc** was again clearly linked to *smoky/peaty* on the negative score, **Wb** was *fruity* and *grassy* on the positive, and **Wa** was intermediated as *solventy* and *sweet.* Blend **St** was perceived as the most *medicinal* and *smoky/peaty* among Standard blends, and **Sd** as the most *oily, sulphury* and *mouldy.* On the PC4 with positive score, **Dd** (5 yrs), the youngest

Deluxe, showed as *rancid* and *buttery* characters. **Df** as an outlier from 12 years Deluxe group due to *spicy*, *woody* and *smoky/peaty* character also related to *nutty* character on this component. On PC4 with negative score, **Di**, a member of *floral* Deluxe group in Fig. 5.1b and 5.2b, was clearly differentiated as *floral*, and **Sf** who showed nearest vicinity in Fig. 5.1c, was also distinctively recognised as *grainy*. **Rb** was perceived as *grassy* and *fruity* on PC 3 and *rancid* on PC4. Blends **Sa** and **Sh** were previously (Fig. 5.1c) linked to the positive attributes of *sweet*, *fruity* and *floral*, but they were also associated with less desirable attributes *rancid* and *buttery*.

5.6. DISCUSSION

Combining various data analyses and discriminant PLS (DPLS1 and 2), was beneficial, specifically for selecting pertinent terms (e.g. *floral*, *spicy* and *oily*) which were identified as important in DPLS1 in definition of specific product categories, but were not significant for discriminating between whiskies from ANOVA. Comparison between PCA and DPLS2 was also useful in examining clustering in product spaces, internal cluster structures (e.g. **St**), and relating these to specific attributes.

In the PCA and DPLS2 product spaces, close relationship between *woody* and *smoky/peaty*, and *medicinal* characters suggest that further training in use of *woody* terms may be appropriate in whisky profiling. Alternatively the inclusion of peated malts, with abundant phenolic compounds, brings groups of correlated attributes into blend character. Such complexities of perceptual discrimination and similarities in use of the attributes have been observed previously in whiskies (Swan & Howie, 1984; Lee *et al.*, 2000b). Training could optimise interpretation of appropriate *woody* concepts (e.g. *smoky-woody*, *sweet-woody* and *spicy-woody*).

Pungency in whisky is generally believed to be related to ethanol content (*ethanolic-pungency*) and immature characteristics of spirit (*harshness*, *sharpness*). However, the intensity of this attribute is not solely dictated by ethanol content, but is related to activity of ethyl esters in whisky headspaces (Withers *et al.*, 1995). However, in these product spaces, the attribute *pungent* was closely related to *smoky/peaty*, *medicinal*, *woody* and *spicy* characters (Fig. 5.1a, PC2) likely from use of peated malts and cask charring practices. Polarisation of attributes *pungent* and *smooth*, loading weights: -0.33 and 0.13 (Fig. 5.1a), and -0.22 and 0.42 (Fig. 5.2a), supports the hypothesis that *smoothness* can also be understood as a loss of *pungency* with development of *mature* character (Guy *et al*, 1989; Piggott *et al.*, 1992). *Smoothness* appeared to be most closely related to *vanilla* and *sweet* character in whisky (Fig. 5.1a & 5.2a).

From ANOVA (Table 5.2), *woody*, *vanilla*, and *smooth*, *sweet* and *nutty* ($p<0.005$) appeared to be key attributes defining matured whisky character. *Spicy* ($p = 0.870$) was not used significantly in blend discriminations other than for West Highland products ($p = 0.018$) (Table 5.3). Complexity of definition of flavour characteristics of this attribute in whisky is discussed elsewhere (Lee *et al.*, 2000b). *Spicy* includes a number of different aroma notes - *curry*, *ginger*, *minty*, *clove* and *cinnamon*, ubiquitous aroma notes in daily life. Thus, there seems a clear need for reference standards to define and stabilise whisky flavour concepts.

Interpretation of product space is complicated (Martens *et al.*, 2000) especially when large number of samples and variables are involved. Use of loading weights to define how much impact each individual attribute has on a specific principal component is commonly practised in sensory analysis. Validation variance shows how well an individual attribute or principal component is validated in that model calibration. For instance, in this experiment, selection of the optimal number of principal components by deciding a cut-off point (no further increase or at maximum validation variance), and the reason why the West Highland category did not obtain any significant component in DPLS1 was reflected by the negative validation variance obtained from DPLS2. It was also useful to simplify interpretation of product spaces by eliminating attributes, which were poorly validated (negative or decreased validation variance).

5.7. CONCLUSION

Forty blended Scotch whiskies of four product categories (Deluxe, Standard, Retailer and West Highland) were analysed by conventional sensory profiling to clarify how assessors discriminate flavour characters and to investigate relationships with product category. Each product category (except West Highland) was well validated. From DPLS1, Deluxe blends were dominated by *fruity*, *buttery*, *malty* and *nutty* notes and with lesser *vanilla*, *floral*, *pungent*, *sweet*, and *smooth* characters. Standard blends were dominated by *oily*, *sulphury*, *rancid*, *grainy*, *smooth*, *soapy*, *vanilla*, *solventy* and *sweet* attributes. The less expensive Retailer blends were perceived as *solventy*, *soapy*, *rancid*, *grassy*, *mouldy*, *pungent*, *grainy*, *oily*, and *spicy* in character. West Highland blends were described as *woody*, *smoky/peaty*, *spicy* and *medicinal*, with lesser contributions from *sulphury*, *mouldy*, *pungent*, *soapy* and *malty* attributes. However, Standard category blends were dispersed across the sensory product spaces by sharing marked similarities in characters with Retailer and also, less extensively, with Deluxe blends. This reflects, to an extent, the rather arbitrary commercial differentiation of blends into Deluxe, Standard and Retailer product categories. Certain Standard blends could equally have been classed as Deluxe or Retailer products, as appropriate, on the basis of sensory character.

CHAPTER 6:

HEADSPACE CONGENERS OF BLENDED SCOTCH WHISKIES OF DIFFERENT PRODUCT CATEGORIES FROM SPME ANALYSIS

Results of this study have been accepted for publication in *Journal of Institute of Brewing* (Lee *et al.*, 2001, in press).

6.1. ABSTRACT

Flavour congeners in headspaces of 40 blended Scotch whiskies, of 4 different product categories (Deluxe, Standard, Retailer and West Highland), were collected by solid phase micro extraction (SPME) over 10 ml diluted (23% abv) whisky. Fibres (100 μm diameter) were thermally desorbed for 5 min in a split/splitless injection port and congeners quantified by high resolution gas chromatography. Two bonded SPME phases (polydimethylsiloxane and polyacrylate) were evaluated: exposure time was varied between 5 and 30 min; with whisky at either nosing (25 °C) or tasting (37 °C) temperature. The optimal analysis was with polyacrylate fibres at 37 °C for 15 min but extraction at 25 °C generated data relevant to sensory (nosing) data. Principal component (PCA) and discriminant partial least square (DPLS) analyses of mean % peak areas for all 57 resolved HRGC components and congeners (38) common to all whiskies gave product spaces showing clear separation of Deluxe, Retailer and West Highland blends, but dispersed Standard blends. In DPLS2 product clustering was similar whether relative (%) or absolute peak area was used in analyses but congeners loadings differed. From product spaces it was possible to assess contributions of flavour congeners, mainly esters and alcohols, to headspaces of individual blends and product categories.

Key Words: *Blended whisky, whisky flavour, solid phase micro extraction (SPME), whisky headspace congeners, PCA, DPLS, validation variance*

6.2. INTRODUCTION

The basis of flavour discrimination by nosing of whisky must relate to behaviour of volatile congeners and their headspace concentrations. Activity coefficients and solution concentrations of congeners determine equilibrium headspace concentrations with relationships important for whisky flavour, and particularly maturation processes (Conner *et al.*, 2000). Key congener activity coefficients are influenced by ethanol concentration, and ambient temperature and pressure (Conner *et al.*, 1999a). Maturation, influences activity coefficients (Piggott, *et al.*, 1992) largely through dissolution of wood components but also through accumulation of organic acids from ethanol oxidation and breakdown of cask hemicelluloses (Conner *et al.*, 1999b). In maturation headspace concentrations of congeners with immature aroma notes in new distillates are reduced by retention in the liquid phase of matured whisky. Analyses of spirit congener composition thus may not reflect the headspace differences that determine perception of aroma character (Conner *et al.*, 1998, 1999b).

Sensory discrimination in blended Scotch whiskies has been studied by Piggott and his colleagues (Piggott & Jardine, 1979; Piggott & Canaway, 1981; Piggott *et al.*, 1989; Guy *et al.*, 1989) and earlier at the Scotch Whisky Research Institute (Swan *et al.*, 1981). There have also been a number of authentication (Aylott *et al.*, 1994) and analytical studies (e.g. Schoeneman & Dyer, 1973). However, relationships in blended Scotch whiskies between headspace concentrations of congeners and sensory differentiation remains unclear.

Solid phase micro extraction (SPME) has provided a simple quantification for volatile congeners in headspaces concentration of alcoholic drinks (Arthur *et al.*, 1992; Steffen & Pawliszyn, 1996; Llompart *et al.*, 1998; Conner *et al.*, 2000; Ebeler *et al.*, 2000). Adsorption in SPME is influenced by such factors as: fibre bonded phase and surface area; liquid phase temperature and volume (Vergnais *et al.*, 1998; Hayasake & Bartowsky *et al.*, 1999); non-volatiles; and liquid phase agitation (Yang & Peppard, 1994; Hayasaka & Bartowsky, 1999). Vapour phased diffusion rates may vary (Zhang & Pawliszyn, 1993) but under specified conditions, concentrations on the bonded phase will attain equilibria determined by congener structure and properties (Yang & Peppard, 1994). Either liquid or gas phase may be sampled by SPME, the latter being more rapid (Yang & Peppard, 1994). In Cognac, it is known that bonded phases exhibit selectivity for specific congeners (Ebeler *et al.*, 2000): and headspace congener quantitations can be a problem. In summary SPME shows different yields from other extraction methods (Yang & Peppard, 1994), is more sensitive to esters than fatty acids (Yang & Peppard, 1994), and has higher sensitivity to less volatile compounds than static headspace sampling (Yang & Peppard, 1994). Such domination of SPME analyses by congeners with large Henry's constants (high volatility and hydrophobicity and large partition coefficients), especially esters, may be undesirable (Yang & Peppard, 1994). However the analyst can select polar (e.g. polyacrylate) bonded phases, sensitive to molecules such as methanol and ethanol (Steffen & Pawliszyn, 1996; Miller & Stuart, 1999); or polydimethylsiloxane (PDMS) that binds effectively both polar and apolar molecules (Steffen & Pawliszyn, 1996; Moens *et al.*, 1997; Lespes *et al.*, 1998).

In modelling of relationships between flavour components and sensory character (Williams, 1994), partial least squares regression analysis (PLS; Wold *et al.*, 1983; Martens *et al.*, 1983, 2000; Martens & van der Burg, 1984; Martens & Martens, 1986) has proven particularly useful. Discriminant partial least squares regression (DPLS; Wold, 1989; Martens *et al.*, 2000), a related strategy, has been used less but can be useful in understanding factors differentiating products or product categories. In DPLS experimental data (*X*-matrix - regressors: predicting) is regressed on to a product category (*Y*-variable - regressands: predicted): formulated '1' for class members and '0' for non-members (Aastevit & Martens, 1986). From such analyses it is possible to derive product spaces suggested by Williams (1994) to be the key to understanding pertinent features of group of products. Discriminant PLS2 analysis plots all categories into a single product space, showing relationships. In contrast, DPLS1 models each category separately by PLS regression, allowing assessments of contributions of individual congeners to a headspaces of a specific blend category.

The aim of this study was to determine whether groups of blended Scotch whiskies could be discriminated according to headspace congeners, as a precursor to modelling relationships between congener headspace concentrations and sensory character.

6.3. EXPERIMENTAL METHODS

6.3.1. Whisky blends

Blended whiskies (40: Table 5.1) were donated by industrial collaborators or purchased from retailers. Whisky, stored at ambient temperature, was diluted to 23% (abv) ethanol with de-ionised water immediately prior to headspace analyses.

6.3.2. Optimisation of SPME for headspace flavour congeners

Fibres with two bonded phases were evaluated: polyacrylate (PA, 100 μm) and polydimethylsiloxane (PDMS, 100 μm) (Sigma-Aldrich Ltd, Supelco UK, Fancy Rd., Dorset, BH12 4QH). In each analysis a fibre was introduced through a septum into a 25 ml glass vial, containing of 10 ml of whisky, and exposed to the headspace. In initial optimisations of exposure time, a randomly selected whisky (**Da**) was used.

Phases were exposed for 5, 10, 15, 20 and 30 min at 37 °C. Then, chromatograms from the two phases were compared using two randomly selected whiskies, **Da** and **Dc**, at constant time (15 min) with whisky at 37 °C. Finally the influence of temperature was studied, again using **Da** and **Dc**, with whisky at: 25 °C, to represent nosing; and 37 °C, simulating ingestion. Each day, a control (**Da**) SPME was chromatographed to ensure consistency between days. Duplicate extractions were analysed.

Fibre phases were desorbed for 5 min at 50 °C in a Carlo Erba HRGC 4200 (Thermoseparations Ltd, Crawley, UK) in split/splitless injector and congeners resolved on CP Wax 52 CB (30 m x 0.25mm i.d. x 0.25 μm). The carrier gas was helium at 1.8 ml min^{-1} and a temperature ramp was employed: 50 °C for 5 min, increasing to 220 °C at 7 °C min^{-1} and finally to 240 °C at 6 °C min^{-1} held for 5 minutes. Duplicate headspaces were chromatographed.

6.3.3. Chromatographic data analysis

In quantitations of congeners, flame ionisation detection was used. Raw chromatographic data was converted (ChromPerfect v.2, Justice Innovations, Mountain View, Ca., USA) to relative (% total) and absolute peak areas. Analyses of variance (ANOVA) were carried out in Minitab (v11.11) for product and categories to assess discriminating power of specific congeners. Principal component analysis (PCA: Piggott & Sharman, 1986) (Unscrambler v 7.01, CAMO A/S, N-0115 Oslo, Norway) was used to generate product spaces (Williams, 1994). Significance of principal components (factors) was examined using one-way ANOVA, and means of replicates plotted only after PCA.

Discriminant PLS (DPLS1 and DPLS2) were used to investigate the relationships between congeners and product (Deluxe, Standard, Retailer and West Highland) category. In DPLS2 either absolute or relative (%) peak areas (Roberts *et al.*, 2000, Jacobsen *et al.*, 2000) were used to form *X*-matrices; the four blend categories were the *Y*-data (Wold, 1989; Wold *et al.*, 1983; Martens & Martens, 1986; Marten *et al.*, 2000). Initially all 57 resolved GC components were included in two *X*-matrices: absolute and relative (%) peak area. Only the 38 components common to all blends were considered. Again two *X*-matrices were considered: absolute and relative (%) areas. Finally congeners not significant in discriminations between categories were eliminated from the *X*-matrices: leaving 28 congeners. In DPLS2, full cross validation was applied to ensure optimal interpretation of data (Wold, 1989; Kvaal & McEwan, 1996). Discriminant PLS1, in which individual product categories were considered separately, was carried out to determine congeners important for each product category.

6.3.4. Identification of headspace congeners

Individual chromatographic components were identified as congeners from calculation of retention indices from FID data and comparison of GC-MS spectra, obtained on a Varian HRGC with Finnegan-MAT ion-trap ITS40 integrated benchtop GC-MS (Thermoseparations Ltd, Hemel Hempstead, UK).

6.4. RESULTS

6.4.1. Optimisation of SPME extraction time

A typical SPME chromatogram (whisky **Da**; phase PA; 15 min extraction at 25 °C) is shown (Fig. 6.1).

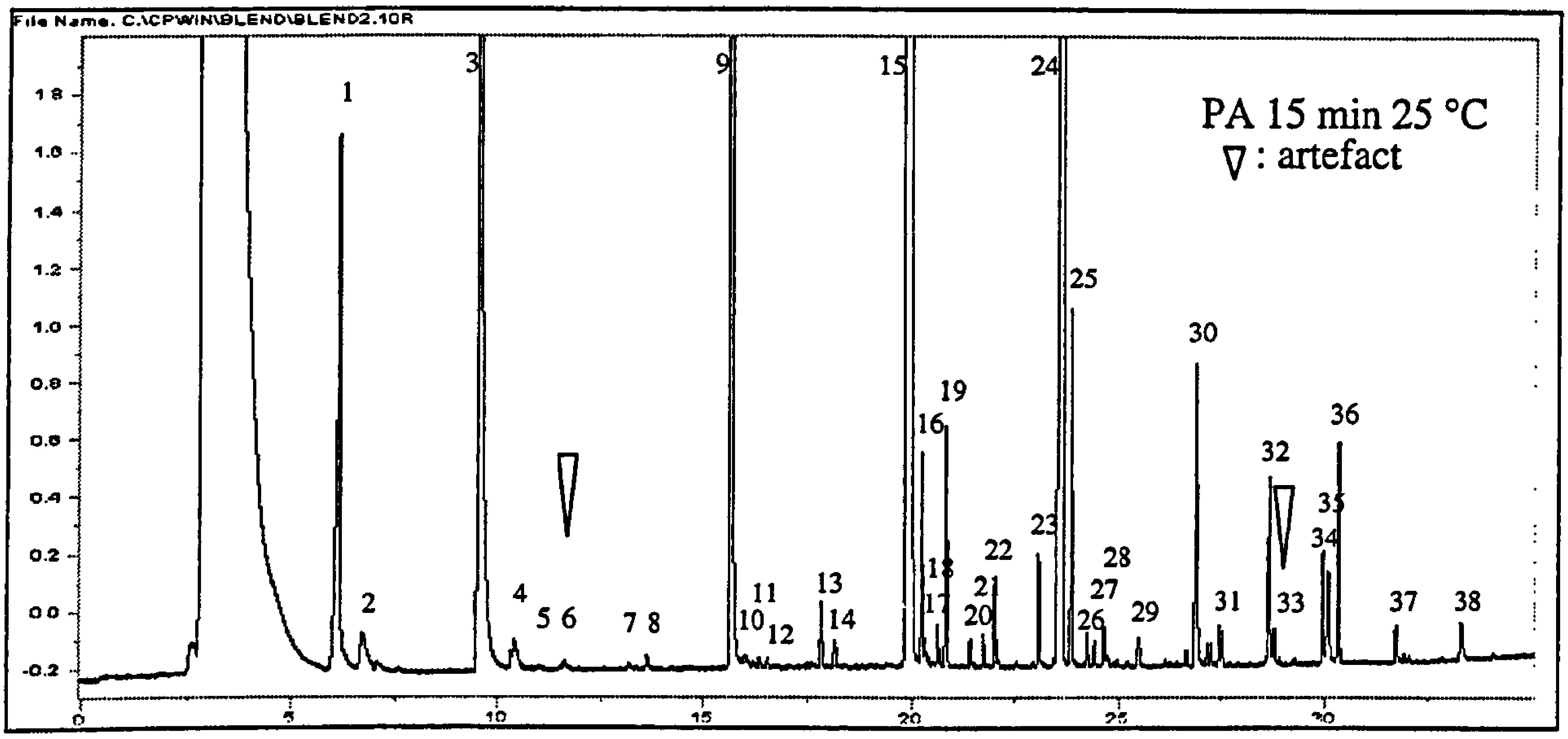


FIG. 6.1. Typical SPME gas chromatogram of a Scotch blended whisky (**Da**) indicating the 38 common chromatographic components.

In optimising extraction time, absolute peak area chromatographic data on the 38 peaks common to both phases were considered - determining coefficients of variation (CV) for each time of extraction (Table 6.2) and total absorbed congener (Fig. 6.2). With polyacrylate (PA) greatest absolute peak area and CV minima for the maximum of resolved components (20) was obtained at 15 min. With the PDMS siloxane phase, CV minima for only 11 components was reached after 20 min. and total peak area showed a plateau at 5 and 10 min with a subsequent linear increase with time (Fig. 6.2).

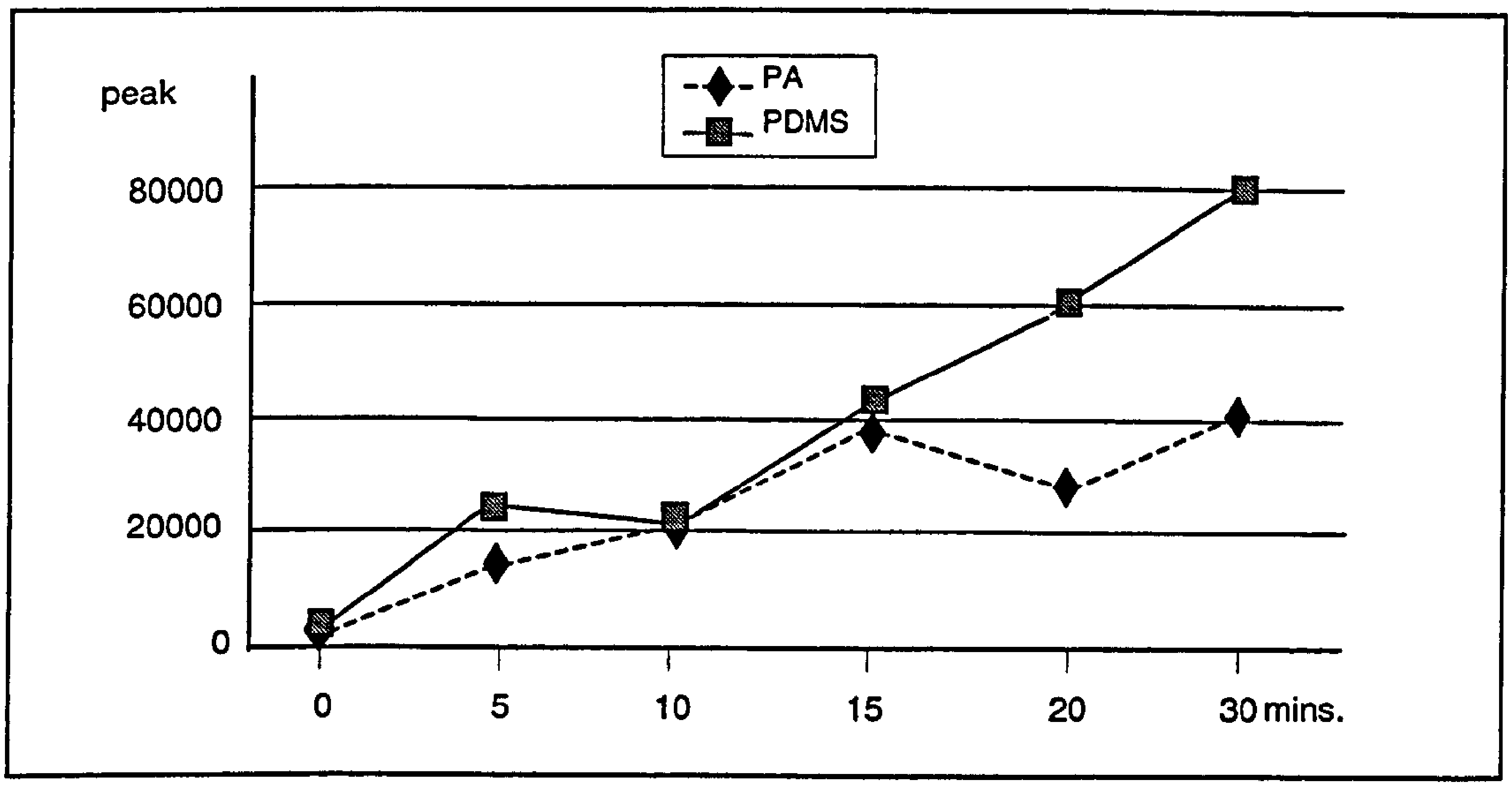


FIG. 6.2. Relationship between total absolute peak area and extraction time in PA and PDMS phases

TABLE 6.3. Influence of SPME bonded phase from PCA of GC components.

		Number of significant peaks ($p < 0.05$)	Total variance (%)	*p* value (PC 1)	Δ PC1 score of replicate 1 & (2)	
					Whisky 1	Whisky 2
Fibre	PA	29	71	0.006	-5.79 (-5.67)	4.84 (6.62)
	PDMS	22	76	0.031	-3.87 (-8.08)	6.59 (5.47)
Temperature	25 °C	29	78	0.010	4.47 (6.84)	-5.88 (-5.43)
	37 °C	52	86	0.008	6.70 (5.00)	-6.45 (-5.26)

TABLE 6.2. Comparison of coefficients of variation (CV) for extraction times and SPME bonded phase.

	Polyacrylate (PA)					Polydimethylsiloxane (PDMS)				
Peaks	5min	10min	15min	20min	30min	5min	10min	15min	20min	30min
P1	0.126	0.055	0.080	0.057	**0.002**	0.111	0.369	0.028	0.053	**0.011**
P2	0.206	0.013	**0.007**	0.047	1.272	**0.070**	0.167	0.125	0.460	0.116
P3	0.092	**0.015**	0.074	0.027	0.294	**0.003**	0.306	0.041	0.021	0.067
P4	0.187	0.154	**0.008**	0.147	0.310	0.112	0.217	0.114	**0.006**	0.129
P5	1.414	nd	**0.081**	1.414	1.414	1.414	0.644	1.414	1.414	**0.378**
P6	0.260	0.174	nd	1.414	**0.127**	nd	nd	nd	nd	0.078
P7	0.320	nd	**0.026**	nd	0.194	nd	nd	nd	nd	0.400
P8	**0.122**	0.166	0.188	0.206	0.222	0.105	0.631	0.235	0.096	**0.089**
P9	0.118	0.052	**0.020**	0.067	0.080	0.028	0.098	0.080	**0.026**	0.099
P10	0.227	**0.025**	0.131	0.766	0.071	nd	nd	0.629	0.655	**0.264**
P11	1.414	**0.051**	nd	nd	0.318	nd	nd	nd	nd	0.365
P12	**0.131**	nd	0.213	0.317	nd	**0.004**	0.477	0.289	0.131	0.385
P13	0.096	0.049	0.007	**0.001**	0.092	0.053	0.076	**0.014**	0.016	0.089
P14	0.172	0.040	**0.022**	0.050	0.146	0.063	0.150	0.072	0.120	**0.061**
P15	0.056	0.023	0.010	**0.009**	0.022	**0.005**	0.075	0.012	0.003	0.012
P16	0.089	0.036	0.066	**0.020**	0.022	**0.005**	0.047	**0.005**	0.069	0.009
P17	0.548	**0.072**	0.588	0.260	0.101	0.075	0.669	**0.054**	0.602	0.116
P18	ns	**0.079**	0.134	0.210	0.192	0.067	0.167	**0.031**	0.101	0.085
P19	0.112	0.035	0.024	**0.013**	0.073	0.051	0.119	0.009	**0.000**	0.008
P20	ns	0.275	**0.053**	0.108	0.144	0.266	0.108	**0.075**	0.130	0.099
P21	0.013	0.310	0.272	0.120	**0.010**	0.042	0.154	**0.004**	0.035	0.144
P22	0.080	0.125	**0.041**	0.109	0.114	**0.019**	0.066	0.032	0.070	0.063
P23	0.283	0.173	**0.027**	0.091	0.226	0.097	**0.003**	0.015	0.016	0.010
P24	0.067	0.067	**0.016**	0.028	0.179	0.044	0.043	0.051	**0.032**	0.080
P25	0.034	0.127	0.013	**0.012**	0.184	0.089	0.056	0.046	**0.042**	0.093
P26	0.239	0.050	0.062	0.122	**0.096**	0.153	0.339	0.105	**0.153**	0.049
P27	nd	0.026	**0.000**	0.127	0.091	0.357	0.064	0.064	**0.016**	0.031
P28	0.129	**0.055**	0.206	0.075	0.134	0.596	0.061	0.389	0.033	**0.289**
P29	0.504	0.085	**0.049**	0.149	0.210	0.452	0.119	0.108	**0.035**	0.142
P30	0.162	0.124	**0.004**	0.036	0.030	0.075	0.208	0.040	**0.008**	0.154
P31	0.233	0.093	**0.007**	0.138	0.014	0.495	0.272	0.061	**0.021**	0.211
P32	0.302	0.095	**0.026**	0.220	0.242	**0.019**	0.231	0.026	0.059	0.165
P33	0.420	0.422	**0.096**	0.210	0.134	0.485	0.382	0.430	0.435	**0.277**
P34	0.338	0.153	**0.011**	0.199	0.208	0.009	0.344	0.077	**0.007**	0.202
P35	0.221	0.080	**0.028**	0.057	0.234	0.027	0.397	0.277	0.060	**0.000**
P36	0.361	0.143	**0.074**	0.115	0.164	0.026	0.324	**0.024**	0.025	0.218
P37	0.245	0.056	**0.051**	0.087	0.229	0.063	0.532	**0.003**	0.097	0.020
P38	0.104	0.117	0.134	0.067	0.305	0.152	0.338	0.152	0.166	0.122
Total	2	6	20	5	4	7	1	8	11	8

nd = not detected, number in bold; minimum values of CV

6.4.2. Selection of SPME bonded phase

From ANOVA of relative (%) peak area data at 37 ° C, for phase PA 29 out of the total of 62 components were significant in discrimination between blends; for PDMS 22 of 67. The relative peak area data were then subjected to PCA, including only components significant in discrimination. The first factor (PC1) was significant for both PA (p = 0.006) and PDMS (p = 0.031) (Table 6.3). Reproducibility of PA was better than for PDMS, from distance between scores of the replicates (Table 6.3).

6.4.3. Selection of SPME temperature

With phase PA, the higher temperature (37 °C) yielded larger peaks for individual chromatographic components and a greater total peak area (data not shown). Again, in PCA, only the first factor was significant at both 25 (p = 0.01) and 37 °C (p = 0.008) (Table 6.3). Better discrimination of the two whiskies was obtained at 37 °C, with greater variance explained, higher significance, and 52 discriminating components as opposed to 29 at 25 °C. Reproducibility at 37 °C was also better (Table 6.3). However, to generate headspace congener quantitation that could be related to sensory data for these blends (Lee *et al.*, 2001), collected for whisky by nosing, 25 °C was selected for further quantitation of headspace congeners.

6.4.4. Chromatographic components and whisky congeners

A total of 36 chromatographic components (Table 6.4), excluding two artefacts peaks, were common to the complete set of 40 whiskies and from ANOVA all were significant ($p<0.006$) in discrimination between blends (Table 6.4). Using relative (% area) data, mean, minimum and maximum, and standard error of mean (SEM) were calculated for each component over the 40 whiskies. Each chromatographic component was then identified (Table 6.4), predominantly as ester and alcohol congeners. Two artefactual components were identified (peaks *6* and *33*, Table 6.2): the first in PA - peak 6 (retention time, 13:1-2 min) - was an artefact from styrene, possibly fibre glue (Conner, private communication), with major ions at m/z 103 and 78, and minor at 41, 51, 60 and 88; the second peak 33 (retention time, 28:7-8 min) in both phases had a major ion at m/z 100. These components, present in blank runs, were deleted from subsequent data matrices, together with one present on blank runs.

For the daily control (18 injections), the mean coefficient of variation (CV) was 0.18 for the total of absolute peak area, and the CV for individual components (38) was 0.24 for relative (%) and 0.36 for absolute peak area. However, the mean CV between replicates of the 40 whiskies using the total of absolute peak area at 0.07 showed good reproducibility (data not shown).

6.4.5. The initial PCA product space

In the initial product space the total (57) of chromatographic components in all blends were included in the data matrix. The PCA product space for relative (%) peak area (Fig. 6.3) showed an extreme outlier - **Sr** - with four unique components (data not shown). This product and the unique chromatographic components were eliminated from further analyses.

The PCA product space was then recalculated (Fig. 6.4) with 53 chromatograph components. The initial 6 factors explained a total 60% of variance (27, 10, 8, 6, 5 and 4%, respectively). However, only the first two factors were discussed to consider similarity of clusters in the product spaces.

TABLE 6.4. The 36 chromatographic components common to the 40 Scotch blended whiskies: mean, minimum and maximum values (% area), and Standard Error of the Mean (SEM) from the relative (%) peak area data and analysis of variance (ANOVA) (omitting artefacts *6* and *33*).

			Raw data				ANOVA (p value)	
Peaks.	Compound	*Abbre.*	Mean	Min	Max	SEM	Whiskies	Categories
P 1	*Iso*-butanol	*iBut*	2.05	1.04	3.52	0.41	0.000***	0.013*
P 2	3-Methyl butyl acetate	*3MeButAct*	0.53	0.14	0.97	0.12	0.000***	0.000***
P 3	*iso*-Amyl alcohol	*iAmyl*	7.28	3.53	11.28	0.95	0.000***	0.020*
P 4	Ethyl hexanoate	*EtHexa*	0.54	0.00	0.94	0.11	0.000***	0.381^{ns}
P 5	Hexyl Acetate	*HexylAct*	0.10	0.00	0.64	0.07	0.000***	0.193^{ns}
P 7	Ethyl heptanoate	*EtHepta*	0.06	0.00	0.15	0.02	0.000***	0.013*
P 8	1-Hexanol	*1Hex*	0.05	0.00	0.15	0.01	0.001***	0.077^{ns}
P 9	Ethyl octanoate	*EtOcta*	13.31	5.77	21.19	1.71	0.000***	0.000***
P 10	*iso*-Amyl hexanoate	*iAmylHexa*	0.10	0.00	0.26	0.03	0.001***	0.070^{ns}
P 11	Furfural	*Furfur*	0.08	0.00	0.16	0.02	0.000***	0.000***
P 12	Octyl acetate	*OctylAct*	0.05	0.00	0.11	0.02	0.006**	0.230^{ns}
P 13	Ethyl nonanoate	*EtNona*	0.21	0.13	0.38	0.03	0.000***	0.000***
P 14	*iso*-Butyl octanoate	*iButOcta*	0.12	0.09	0.20	0.01	0.010**	0.001***
P 15	Ethyl decanoate	*EtDeca*	55.17	48.02	67.58	2.54	0.000***	0.008**
P 16	3-Methyl butyl octanoate	*3MeButOcta*	0.56	0.39	0.78	0.06	0.000***	0.000***
P 17	Ethyl benzoate	*EtBenz*	0.10	0.00	0.25	0.03	0.000***	0.011*
P 18	Decyl acetate	*DecylAct*	0.11	0.04	0.18	0.02	0.000***	0.000***
P 19	Ethyl 9-decenoate	*Et9Dece*	0.45	0.15	0.75	0.10	0.000***	0.050*
P 20	Propyl decanoate	*PrDeca*	0.08	0.05	0.13	0.01	0.006**	0.022*
P 21	Ethyl undecanoate	*EtUnDeca*	0.07	0.05	0.12	0.01	0.000***	0.357^{ns}
P 22	Propyl decenoate	*PrDece*	0.23	0.12	0.43	0.04	0.000***	0.000***
P 23	Phenyl ethyl Acetate	*PhEtAct*	0.24	0.08	0.72	0.06	0.000***	0.001***
P 24	Ethyl dodecanoate	*EtDoDeca*	15.75	8.81	31.63	2.50	0.000***	0.000***
P 25	3-Methyl butyl decanoate	*3MeButDeca*	0.60	0.23	1.16	0.12	0.000***	0.000***
P 26	Dodecyl acetate	*DoDecAct*	0.07	0.03	0.12	0.01	0.000***	0.000***
P 27	Ethyl dodecenoate	*EtDoDece*	0.06	0.00	0.11	0.01	0.000***	0.146^{ns}
P 28	2-Phenyl ethanol	*PhEt*	0.11	0.06	0.19	0.02	0.000***	0.389^{ns}
P 29	Dodecanol	*DoDec*	0.11	0.06	0.20	0.02	0.000***	0.000***
P 30	Ethyl tetradecanoate	*EtTetraDeca*	0.44	0.18	0.81	0.07	0.000***	0.000***
P 31	Ethyl tetradecenoate	*EtTetraDece*	0.05	0.02	0.14	0.02	0.000***	0.006**
P 32	Tetradecanol	*TetraDec*	0.19	0.06	0.39	0.04	0.000***	0.000***
P 34	Ethyl hexadecanoate	*EtHexDeca*	0.06	0.00	0.18	0.02	0.000***	0.000***
P 35	Decanoic acid	*DecAcid*	0.29	0.11	0.60	0.05	0.000***	0.905^{ns}
P 36	Ethyl 9-hexadecenoate	*Et9HexDece*	0.15	0.03	0.43	0.04	0.000***	0.000***
P 37	Phenyl ethyl *n*-octanoate	*PhEtOcta*	0.04	0.00	0.12	0.02	0.000***	0.049*
P 38	Ethyl octadecanoate	*EtOctaDeca*	0.10	0.05	0.27	0.02	0.000***	0.059^{ns}

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns = not significant

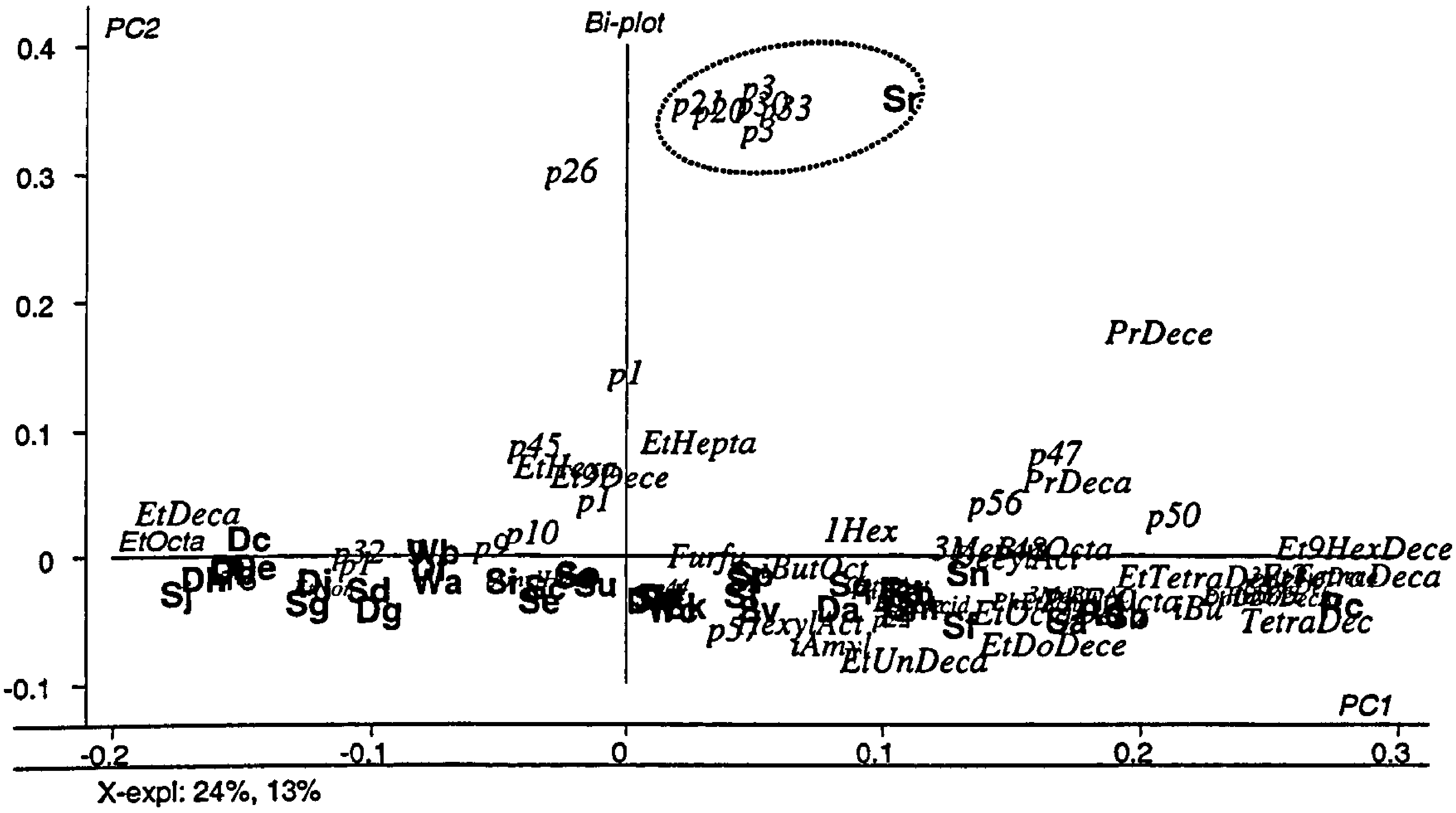


FIG. 6.3. PCA product space - biplot of all 40 blended Scotch whiskies from relative (%) peak area of all 57 chromatographic components: factor 1 vs factor 2

6.4.6. The edited PCA product space

Excluding two artefacts peaks and outlier product Sr, a matrix of 36 common components was obtained and variables identified as congeners. This matrix, by PCA, yielded initial 6 factors that contributed to interpretation of the product space (Fig. 6.5a-c), explaining a total of 71% (33, 12, 10, 6, 5 and 5%) variance. The software suggested 20 valid factors from total calibration variance in PCA and ANOVA ($p \leq 0.001$). However validation variances (by full-cross validation) after initial 6 factors showed minimal increases in explained variance factors (e.g. 1 and 2% for factors 7 and 8; data not shown). Validation variance and loading weights of the individual congeners on PC1 to PC6 are shown in Table 6.5.

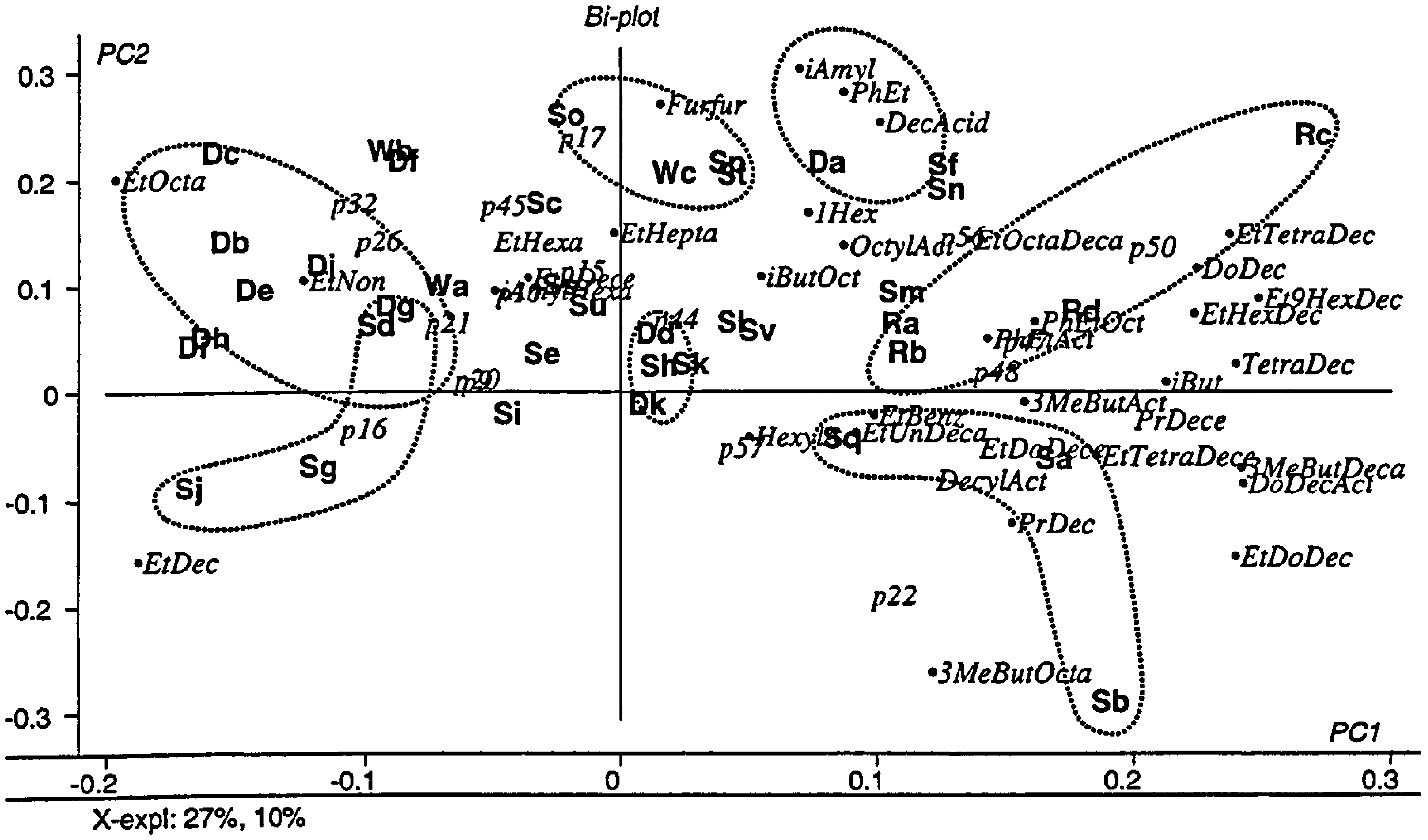


FIG. 6.4. PCA product space - biplot of 39 blended Scotch whiskies from relative (%) peak area of 53 chromatographic components: factor 1 vs factor 2

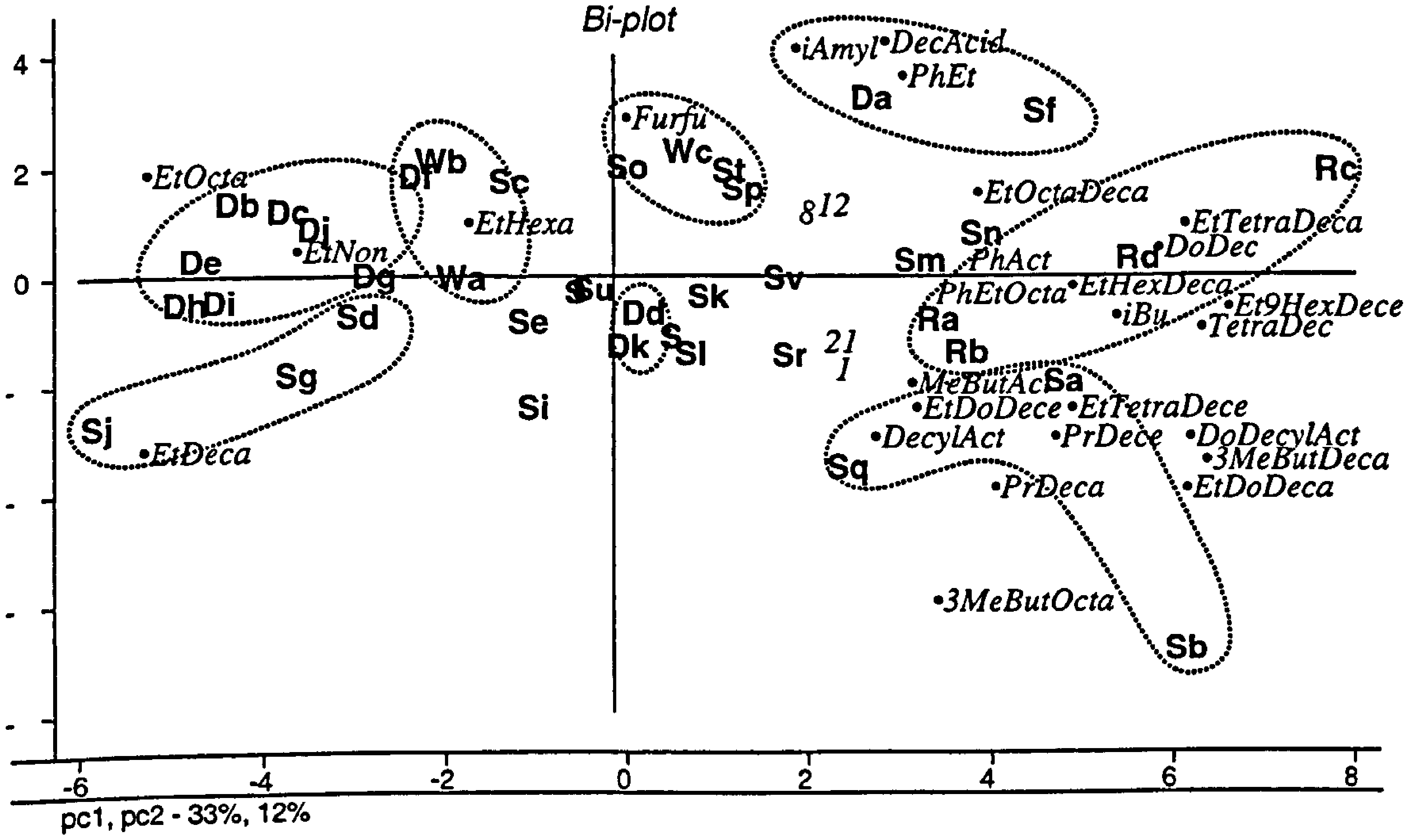


FIG. 6.5a. PCA product space - biplot of 39 blended Scotch whiskies from relative (%) peak area of 36 common congeners: factor 1 vs factor 2

In the first two factors (PC1 vs 2) (Fig. 6.5a), five congeners (*hexyl acetate, ethyl heptanoate, iso-amyl hexanoate, iso-butyl octanoate* and *ethyl 9-decenoate*) had negative validation variances in both factors (Table 6.5), and were thus removed. Negative (or reduced) validation variance suggests no contribution to variance explained by those factors.

On factor 1, Deluxe blends with negative scores were discriminated from Retailer blends with positive scores. Congeners strongly related to a major cluster of Deluxe products (**Dh, De, Di, Db, Dc, Dj, Dg** and **Df**) were *ethyl octanoate* (-0.22), *ethyl decanoate* (-0.22), *ethyl nonanoate* (-0.14), and *ethyl hexanoate* (-0.06) (Table 6.5). Each of these blends had been matured for >12 yrs except **Di** (>3 yrs). Two blends abundant in these congeners **Dh** and **De** were matured for 21 and 15 yrs, respectively. The three outlier Deluxe products were **Dk** (export to the Asian market), **Dd** with a relatively short (5 yrs) maturation, and **Da** differentiated by contents of *iso-amyl alcohol* and *2-phenylethanol*, and *decanoic acid.* Of the Standard blends **Sj, Sg** and **Sd** were most similar to the major Deluxe cluster, but differentiated by *ethyl decanoate* content. There was a small hybrid cluster (**Wb, Wa, Df** and **Sc**) linked to *ethyl hexanoate,* whereas **Wc** was clustered with **So, St** and **Sp** on the basis of *furfural* content.

With positive scores, distinctly linked to Retailer blends, notably **Rc** and **Sb,** were: *ethyl-9-hexadecenoate, 3-methyl butyl decanoate, dodecyl acetate, ethyl dodecanoate, tetradecanoate, tetradecanol, dodecanol, ethyl hexadecenoate, iso-butanol* and *ethyl tetradecenoate* (loading weights ≥2.0). On factor 2, **Da** and **Sf** were outliers (loading weights ≥0.33) as was **Sb** with an extreme negative score, linked to *3-methyl butyl octanoate.* Other Standards blends were dispersed across the product space.

TABLE 6.5. The 36 chromatographic components common to the 40 Scotch blended whiskies: validation variance and loading weights from PCA (omitting artefacts *6* & *33*)

	Loading weights						Validation variance					
	PC1	PC2	PC3	PC4	PC5	PC6	PC1	PC2	PC3	PC4	PC5	PC6
P1	0.23	0.02	0.06	-0.07	-0.14	0.09	58.07	55.81	54.68	53.36	55.40	55.36
P2	0.17	-0.01	-0.22	0.23	0.20	0.19	30.52	21.67	39.62	52.47	60.61	65.22
P3	0.07	0.37	-0.04	0.14	-0.01	0.01	-1.13	49.59	59.15	62.54	60.69	58.33
P4	-0.06	0.17	-0.04	-0.28	0.29	-0.40	-2.53	5.66	-1.48	5.38	9.20	64.86
P5	0.04	-0.06	0.14	0.32	-0.16	0.19	-4.38	-5.21	-15.10	-2.08	-16.24	-5.65
P7	0.00	0.12	0.32	0.27	0.15	-0.22	-6.61	-9.86	28.76	48.11	45.31	55.11
P8	0.08	0.17	0.17	0.20	0.23	-0.12	2.31	6.86	16.88	21.03	24.45	25.05
P9	-0.22	0.23	0.14	-0.09	0.00	-0.08	53.71	77.13	84.48	85.56	84.63	84.19
P10	-0.06	0.12	0.20	0.03	0.14	-0.11	-0.95	-3.57	10.83	3.94	1.65	-3.67
P11	0.02	0.28	-0.09	-0.15	0.25	0.14	-6.63	16.83	25.13	24.81	38.60	37.87
P12	0.09	0.17	-0.17	0.02	0.24	0.25	4.53	4.24	14.72	7.45	18.67	24.30
P13	-0.14	0.12	0.29	0.25	-0.11	0.05	19.58	20.49	49.88	67.87	70.55	69.80
P14	0.05	0.10	0.06	0.32	0.34	0.06	-5.25	-5.95	-14.20	14.81	33.81	32.53
P15	-0.22	-0.15	-0.22	0.06	0.06	0.15	55.24	62.76	81.38	81.31	80.95	84.87
P16	0.12	-0.33	-0.09	0.04	0.10	-0.14	11.75	56.94	59.15	56.77	55.81	59.49
P17	0.10	-0.04	0.12	0.05	0.20	0.22	6.27	-0.29	1.81	-6.15	-3.98	-1.33
P18	0.13	-0.10	-0.27	0.19	0.25	0.09	17.01	13.78	42.60	48.43	60.06	60.18
P19	-0.05	0.10	0.28	-0.23	0.04	0.35	-2.21	-7.00	21.84	28.25	22.80	48.54
P20	0.17	-0.15	-0.01	0.02	0.00	-0.31	28.47	34.24	32.21	27.90	21.85	40.58
P21	0.09	0.00	0.16	0.43	-0.29	0.01	5.01	-1.38	-7.45	38.43	64.29	67.05
P22	0.19	-0.09	0.05	0.08	0.11	-0.32	38.67	39.64	36.52	34.55	32.72	48.70
P23	0.15	0.10	-0.33	0.17	-0.07	0.08	22.76	8.57	63.29	67.87	67.76	66.03
P24	0.25	-0.19	0.08	-0.03	-0.05	-0.09	72.06	83.13	91.14	91.00	90.93	92.60
P25	0.26	-0.13	0.07	-0.04	0.04	-0.13	75.56	81.57	84.55	83.96	83.28	85.81
P26	0.26	-0.12	0.11	-0.05	-0.01	0.01	74.82	78.07	85.24	84.87	84.32	82.97
P27	0.14	-0.06	0.32	-0.12	-0.07	0.24	19.53	4.43	56.58	58.24	56.99	64.71
P28	0.10	0.33	-0.06	0.08	-0.08	-0.18	6.04	51.74	53.09	51.73	49.21	54.49
P29	0.24	0.13	0.02	-0.02	-0.02	-0.05	65.25	70.54	69.46	67.70	65.78	62.44
P30	0.25	0.15	0.03	-0.14	-0.04	0.03	73.95	82.52	82.35	85.93	85.23	84.82
P31	0.20	-0.08	0.20	-0.21	-0.01	0.13	40.51	31.58	54.27	64.06	62.35	62.99
P32	0.25	0.02	-0.06	-0.02	-0.13	-0.08	75.25	73.54	74.23	72.26	74.76	73.11
P34	0.23	0.06	0.01	-0.10	0.10	0.18	60.21	59.50	56.06	54.67	55.83	60.82
P35	0.11	0.34	-0.15	-0.07	-0.27	-0.03	8.19	45.92	63.85	61.54	80.57	80.50
P36	0.26	0.05	0.01	-0.10	0.04	0.07	78.94	78.48	76.50	77.37	76.94	77.08
P37	0.17	0.08	0.15	-0.04	0.18	0.00	31.95	29.07	34.16	28.05	27.94	24.13
P38	0.15	0.19	-0.19	-0.01	-0.34	-0.06	21.47	27.82	44.06	37.59	66.65	66.02

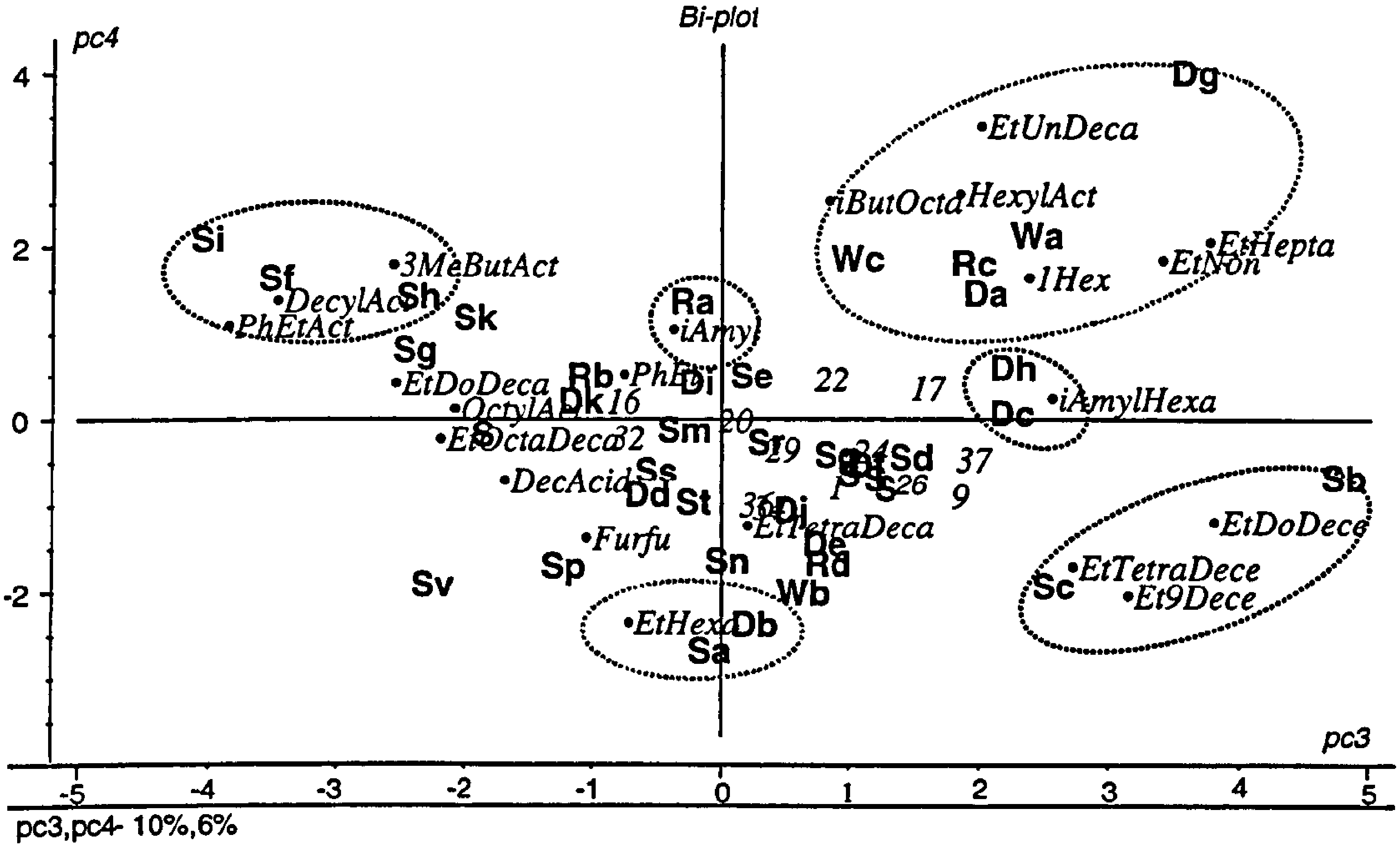


FIG. 6.5b. PCA product space - biplot of 39 blended Scotch whiskies from relative (%) peak area of 36 common congeners: factor 3 vs factor 4

The next two factors, 3 and 4 (Fig. 6.5b) shows no clustering of blends on the basis of product category. Five congeners (*hexyl acetate*, *ethyl heptanoate*, *iso-amyl hexanoate*, *iso-butyl octanoate* and *ethyl-9-decenoate*) previously removed for negative validation variance had high loading weights. Although *hexyl acetate* showed negative validation variance on all factors, it was explained best on factor 4. Factor 3 was defined by a distinct cluster of Standard blends (**Si**, **Sf**, **Sh**, **Sj**, **Sk**, **Sg**, and **Sv**) with positive scores. **Sh** was an outlier on the basis of content of acetate esters (*phenyl ethyl-*, *decyl-* and *3methyl butyl acetates*). Polar to this product was a cluster, **Sb** and **Sc**, differentiated on the basis of unsaturated acid ester contents (*ethyl dodecenoate*, *-tetradecenoate* and *-9 decenoate*). *Iso-amyl hexanoate* was linked to **Dc** and **Dh**. Factor 4 was defined by *ethyl undecanoate* with a positive score and *ethyl* *hexanoate* with negative to which were linked to **Sa, Db** and less extensively, **Wb.** In contrast, **Da, Dg, Wa, Wc** and **Rc** were related to *ethyl undecanoate, iso-butyl octanoate, hexyl acetate, 1-hexanol,* and *ethyl nonanoate* and *heptanoate.* These appeared to be key congeners differentiating **Wb** from **Wa** and **Wc.**

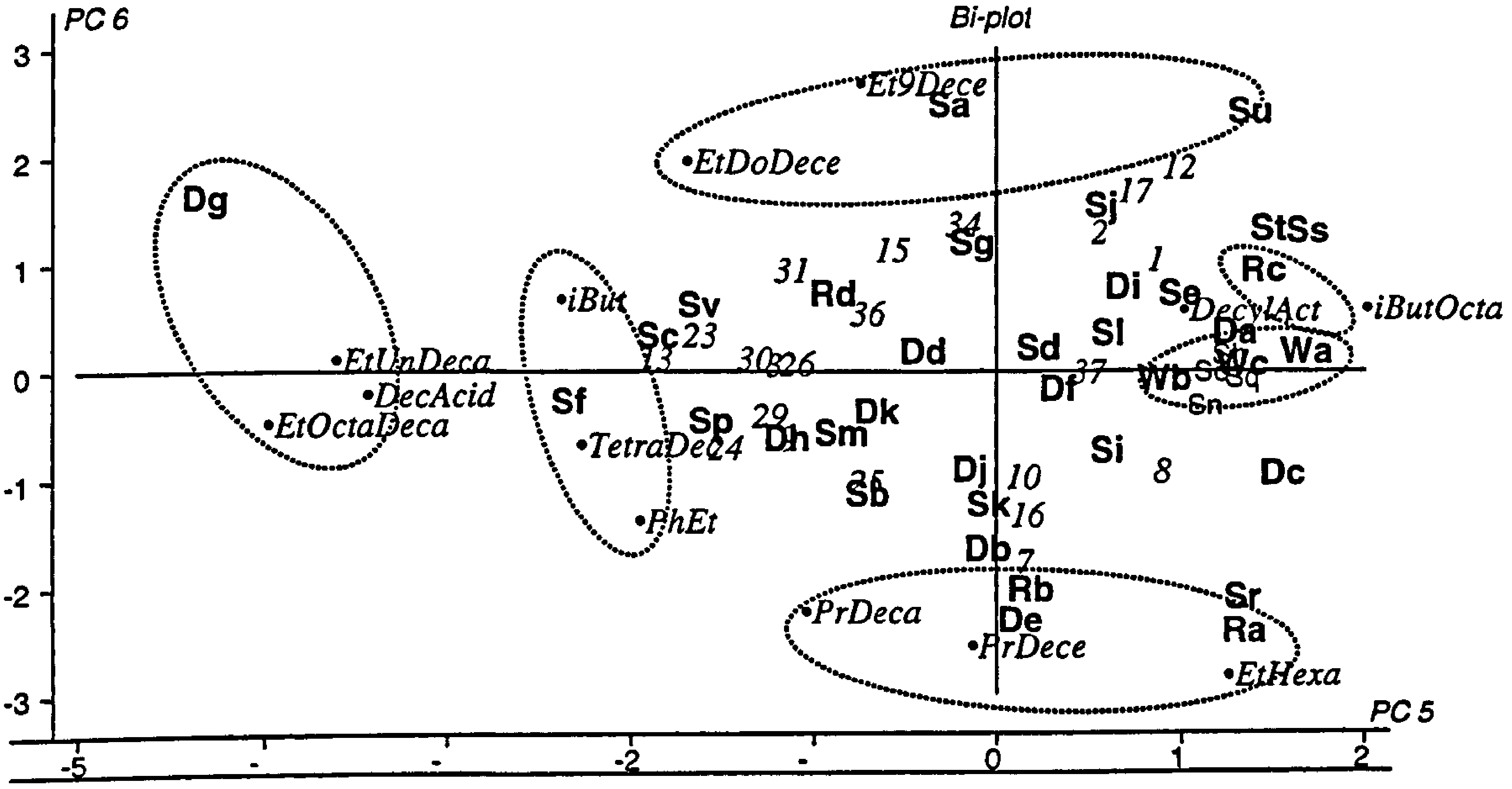


FIG. 6.5c. PCA product space - biplot of 39 blended Scotch whiskies from relative (%) peak area of 36 common congeners: factor 5 vs factor 6

In Fig. 6.5c (PC5 vs 6) factor 5 was defined by *iso-butyl octanoate* with a positive score, linked to **Wa, Wb** and **Wc,** and with negative scores *ethyl octadecanoate, -undecanoate,* and *decanoic acid,* asssociated with **Dg.** Factor 6 was defined by positive scores for *ethyl 9-decenoate* and *-dodecenoate,* linked to **Sa** and **Su**; with negative scores, **Ra** and **Sr** with *ethyl hexanoate,* and **De** and **Rb** with *propyl decenoate* and *-decanoate.*

6.4.7. Discriminant PLS2

Fig. 6.6a & b shows scores for the forty products and loadings for the both the four blends categories and each congener (excluding the ten non-discriminating congeners) (Table 6.4). Three components were valid, explaining a total of 61% (44, 11 and 6%) of *X*- and 47% (16, 16 and 15%) *Y*-variance. Residual *Y*-validation variances for factors 0 to 3 were 1.19, 1.07, 1.00 and 0.97, respectively (Table 6.6). A total 64% variance was explained for Deluxe, 49% for Standard, 42% for West Highland and 36% for Retailer blends (data not shown). In Fig. 6a (factors 1 and 2), only Deluxe, Standard and Retailer categories were plotted: the category West Highland had negative validation variance on both factors (-0.2 and -2.1) (Table 6.6). Similarly *furfural* and *iso-butyl octanoate*, both with negative validation variances, were eliminated (Table 6.7). However, *iso-amyl alcohol* was included as explained (the least negative value, -1.20) on these two factors.

TABLE 6.6. Total explained and residual validation variance and validation variance from individual product categories from DPLS2

variance	Total (*Y*) validation	Residual	Deluxe validation	Standard validation	Retailer validation	West Highland validation
PC0	0	1.19	0	0	0	0
PC1	10.8	1.07	27.1	0.9	17.8	-0.2
PC2	16.4	1.00	33.2	22.7	18.2	-2.1
PC3	19.2	0.97	43.6	23.4	14.1	2.7

TABLE 6.7. Validation variance of individual congeners, common to the 40 blends, significant ($p<0.05$) in discriminations in DPLS2: relative peak areas.

Peaks	Compounds	PC1	PC2	PC3
P1	*iso-Butanol*	50.6	48.8	50.1
P2	*3-Methyl butyl acetate*	33.6	32.4	37.4
P3	*Iso-Amyl alcohol*	-14.1	-1.2	-10.9
P7	*Ethyl heptanoate*	-10.8	43.9	41.5
P9	*Ethyl octanoate*	65.6	77.5	76.0
P11	*Furfural*	-16.0	-24.1	25.1
P13	*Ethyl nonanoate*	30.9	63.9	58.5
P14	*iso-Butyl octanoate*	-13.9	-9.2	14.5
P15	*Ethyl decanoate*	40.6	78.3	78.8
P16	*3-Methyl butyl octanoate*	26.8	29.8	32.5
P17	*Ethyl benzoate*	12.4	4.8	15.7
P18	*Decyl acetate*	30.6	48.1	56.5
P19	*Ethyl 9-decenoate*	-0.1	0.4	1.3
P20	*Propyl decanoate*	43.3	35.8	34.6
P22	*Propyl decenoate*	40.0	40.0	37.3
P23	*Phenethyl acetate*	18.7	26.1	18.4
P24	*Ethyl dodecanoate*	76.1	73.9	77.4
P25	*3-Methyl butyl decanoate*	80.7	79.3	80.1
P26	*Dodecyl acetate*	78.1	78.0	77.6
P29	*Dodecanol*	64.7	73.0	71.3
P30	*Ethyl tetradecanoate*	62.6	72.3	70.1
P31	*Ethyl tetradecenoate*	46.9	47.1	30.4
P32	*Tetradecanol*	68.9	68.9	70.4
P34	*Ethyl hexadecanoate*	53.9	55.4	56.1
P36	*Ethyl 9-hexadecenoate*	73.3	75.5	74.4
P37	*Phenyl ethyl n-octanoate*	31.7	30.8	31.0

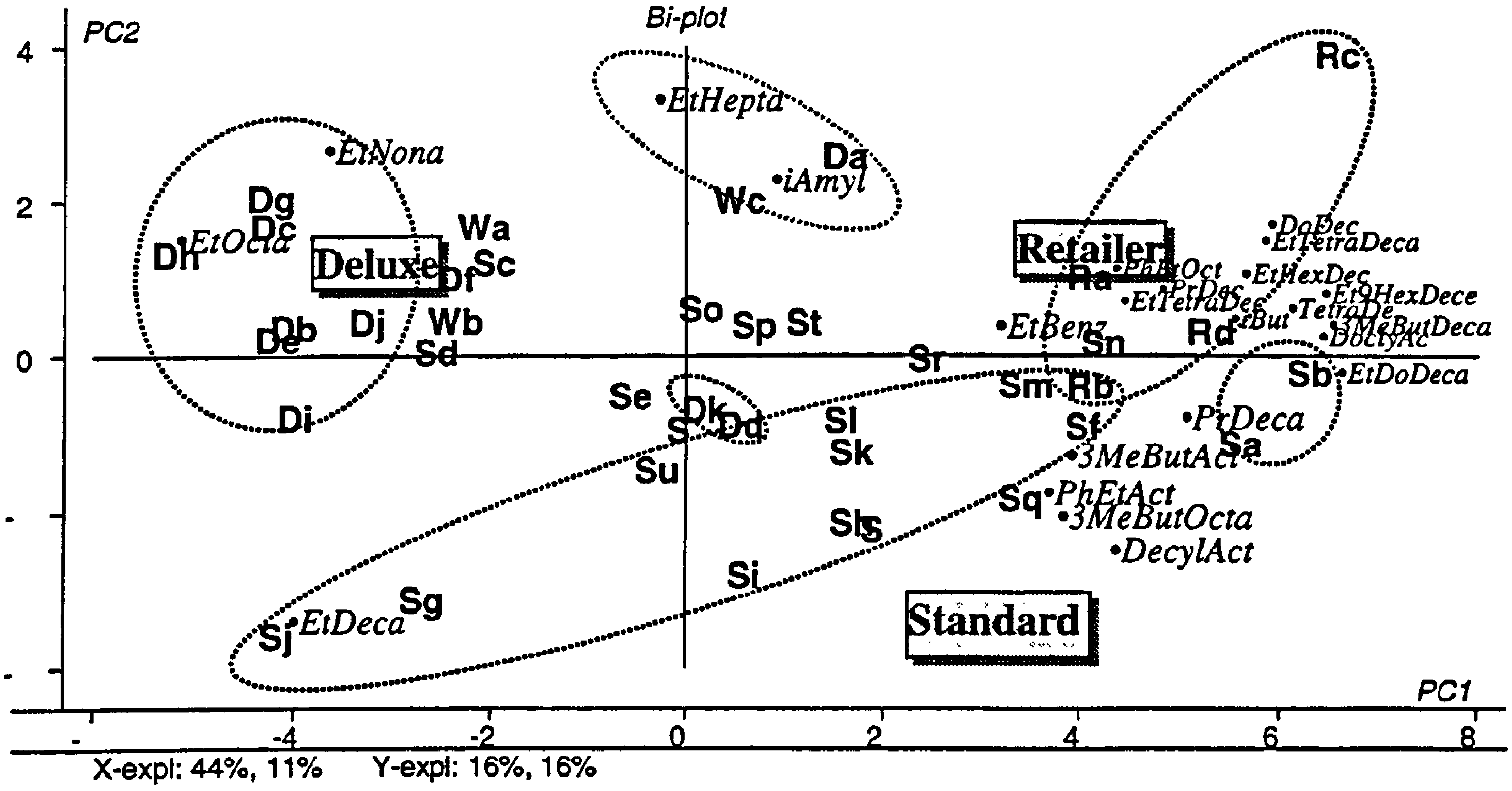


FIG. 6.6a. DPLS2 product space - biplot of Deluxe, Standard and Retailer blended Scotch whiskies from relative (%) peak area of significantly discriminating common congeners: factor 1 vs factor 2

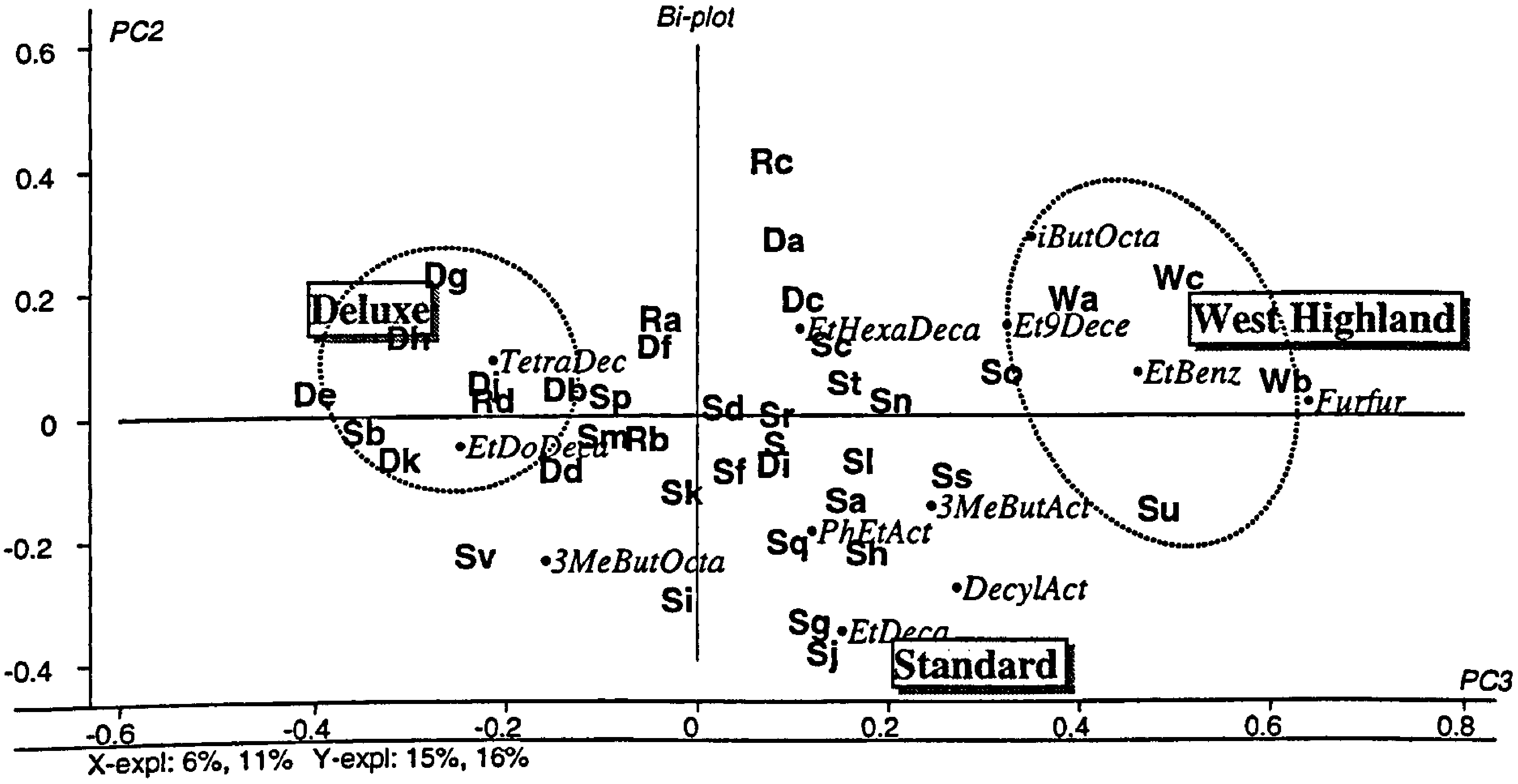


FIG. 6.6b. DPLS2 product space - biplot of Deluxe, Standard and West Highland blended Scotch whiskies from relative (%) peak area of significantly discriminating common congeners: factor 2 vs factor 3

Factor 1 was defined by Deluxe products with negative scores, and Retailer blends with positive. These categories were differentiated with validation variances of 27.1 and 17.8%, respectively (Table 6.6). The two DPLS2 and PCA product spaces were similar in terms of product clustering, relationship with congeners and outlier products. Deluxe blends were linked to the ethyl esters - *octanoate*, *nonanoate* and *decanoate*. As in the PCA space, in factor 1 **Dh** had the highest negative score and **Df** was clustered with **Wa**, **Wb** and **Sc**. Similarly, **Sj**, **Sg**, **Sd** and **Sc** were clustered with Deluxe blends, although the first two were discriminated by *ethyl decanoate* content on factor 2. A cluster **Rc**, **Sb** and **Sa**, were differentiated with positive scores on factor 1, linked to high molecular-weight esters and alcohols, as in PCA.

Factor 2 was defined by *ethyl heptanoate*, *nonanoate* and *iso-amyl alcohol* with positive scores linked to **Wc** and **Da**, and less extensively, **Rc**. With negative scores, were *ethyl decanoate*, *3-methyl-butyl-octanoate*, and ethyl ester acetates (*decyl-*, *phenyl ethyl-* and *3-methyl-butyl acetates*) linked to **Sj**, **Sg**, **Si**, **Sh**, **Sv** and **Sq**.

In Fig. 6b (factor 2 vs 3), factor 3 was defined by *furfural*, *ethyl benzoate* and *-9decenoate* and *iso-butyl octanoate* with positive score associated with **Wa**, **Wb** and **Wc** with **Su**, and *tetradecanol* and *ethyl dodecanoate* with negative linked to certain Deluxe blends. The poorly defined (negative validation) *furfural*, and *iso-butyl octanoate* on factors 1 and 2 have been clearly defined on these factors.

The product categories Deluxe was explained best, with high validation variance (43.6%) and West Highland with 2.7%; that for Standard was increased <2% and for Retailer blends variance was decreased (Table 6.6). Only congeners with positive and increased validation variance were plotted.

6.4.8. Discriminant PLS1

Relationships between individual congeners (Table 6.8) and product categories were clearer in DPLS1 than from product spaces. Although a total variance explained for Deluxe, Retailer, Standard, and West Highland were 37, 31, 30 and 25%, respectively, full-cross validation only confirmed the validity of the first three categories not West Highland blends with negative validation variances in DPLS2 (Table 6.6).

TABLE 6.8. Ranking of congeners in relative peak area contributions to headspaces of blends of different product categories: (a) by loading weights on the first component from DPLS1; and (b) from p values from analysis of variance (ANOVA)

Deluxe			Standard			Retailer			West Highland		
	a	b		a	b		a	b		a	b
Ethyl octanoate	0.000	0.304	*Decyl acetate*	0.012	0.294	*Tetradecanol*	0.000	0.322	*Furfural*	0.008	0.412
Ethyl nonanoate	0.012	0.200	*Phenyl ethyl acetate*	0.029	0.257	*Dodecanol*	0.000	0.294	*Ethyl nonanoate*	0.040	0.327
iso-Amyl hexanoate	0.045	0.161	*3-Methyl-butyl octanoate*	0.060	0.224	*Ethyl-9-hexadecenoate*	0.000	0.281	*iso-Butyl octanoate*	0.041	0.326
Ethyl heptanoate	0.178	0.110	*3-Methyl-butyl acetate*	0.132	0.180	*3-methyl-butyl decanoate*	0.001	0.277	*Ethyl heptanoate*	0.091	0.272
iso-Amyl alcohol	0.420	0.066	*Dodecyl acetate*	0.151	0.173	*Ethyl hexadecanoate*	0.002	0.255	*Ethyl benzoate*	0.171	0.222
Ethyl hexanoate	0.509	0.054	*Ethyl dodecanoate*	0.166	0.166	*Dodecyl acetate*	0.005	0.232	*Ethyl octanoate*	0.215	0.201
Ethyl-9-decenoate	0.547	0.050	*iso-Butanol*	0.200	0.154	*Ethyl tetradecanoate*	0.006	0.229	*Ethyl 9-decenoate*	0.271	0.179
1-Hexanol	0.610	0.042	*Ethyl decanoate*	0.245	0.140	*Propyl decanoate*	0.007	0.224	*1-Hexanol*	0.368	0.147
2-Phenyl ethanol	0.732	0.028	*Ethyl benzoate*	0.245	0.140	*Ethyl dodecanoate*	0.009	0.217	*Ethyl undecanote*	0.380	0.143
Ethyl decanoate	0.748	0.026	*Ethyl hexadecanoate*	0.253	0.138	*iso-Butyl octanoate*	0.014	0.205	*Ethyl hexanoate*	0.389	0.140

It was possible to ranking loading weights of congeners from DPLS1 (Table 6.8); ANOVA defined significant terms for each category, which was identical. Only congeners contributing positively to each product category are shown. Congeners important ($p<0.05$) for Deluxe blends were *ethyl octanoate, nonanoate* and *iso-amyl hexanoate,* for Standard products ($p<0.03$) *decyl* and *phenyl ethyl acetate,* and for Retailer whiskies ($p<0.02$) high molecular weight congeners such as *tetradecanol, dodecanol, ethyl-9-hexadecenoate, 3-methyl-butyl decanoate, ethyl hexadecanoate, ethyl tetradecanoate, propyl decanoate, ethyl dodecanote, dodecyl acetate* and *iso-butyl octanoate.* There were similarities between Standard and Retailer blends but the former could be differentiated by *decyl-*, *octyl-* and *phenylethyl acetates* and *3-methyl butyl octanoate* contents.

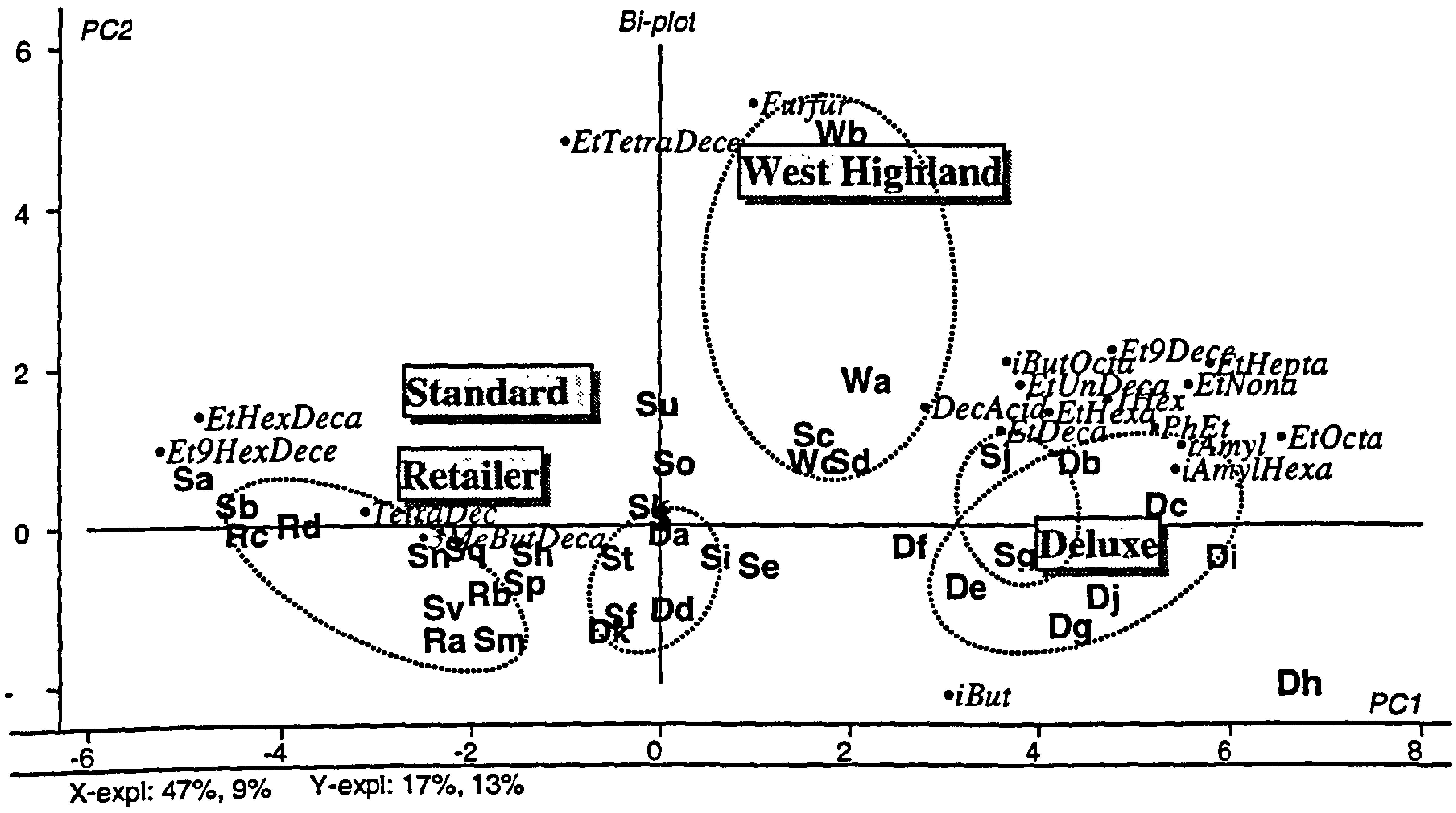


FIG. 6.7. DPLS2 product space - biplot of Deluxe, Standard, Retailer and West Highland blended Scotch whiskies from absolute peak area of significantly discriminating common congeners: factor 1 vs factor 2

6.4.9. The DPLS2 product space from absolute peak areas

Fig. 6.7 shows the first two factors of the DPLS2 space for the 40 blends calculated using absolute peak areas, as opposed to relative (%). Only the first factor was valid explaining 47% of *X*-variance and 17% of *Y*. Only 20 chromatographic components were significant in discriminating between categories.

The significant factor was defined with negative scores by a cluster (**Sa**, **Sb** and Retailer blends) linked to *ethyl hexadecanoate* and *ethyl 9-hexadecenoate*. These cheaper blends also had high contents of *tetradecanol, 3-methyl butyl decanoate*. With positive scores were most of the Deluxe blends, notably (in order) **Dh**, **Di**,,**Dc**, **Dj**, **Db**, **Dg**, **De** and also **Sg** and **Sj**. These were linked to a large number of medium chain (C6 - C10) acid esters and also certain alcohols: *iso-amyl alcohol, hexanol* and *phenylethanol*. The West Highland blends were clustered on factor 1 and linked to *furfural* and *ethyl tetradecenoate*.

6.5. DISCUSSION

Solid-phase microextraction (SPME), which was promoted initially for extraction of organic compounds from solutions, has been successfully evaluated for application in distilled spirit research. In previous study of brandy (Ebeler *et al.*, 2000), SPME was shown to provide a rapid but selective extraction technique for congeners, providing data that could be used to clarify relationships between congener composition and sensory character.

In the product spaces of 40 blends of four categories, cluster structures were similar from PCA and DPLS2. Also DPLS2 product spaces were similar using both relative (%) and absolute peak area chromatographic data. Loadings did, however, show some differences. In addition, in defining contributions to differentiation of product categories, there were identical rankings of congeners in DPLS1 (loading weights) and ANOVA (p value). This supports previous findings in product quality research (Martens & Martens, 1986).

Although certain congeners were not significant in discriminations between product categories in DPLS2, linkage to certain blends can be obtained from PCA. Thus, PCA and DPLS2 provided complementary data. For instance, *ethyl hexanoate*, non-discriminating, was closely linked to **De** and **Dh, Sa, Sv, Sp, Sn, Sc, Sr, Ra, Rd** and **Wb** on PCA product spaces (Fig. 6.5a,b,c): *iso-amyl hexanoate* to **Dh** and **Dc** (Fig. 6.5b), *hexyl acetate, 1-hexanol* and *ethyl undecanoate* to **Da**, **Dg**, **Rc**, **Wc** and **Wa** (Fig. 6.5b), and *2-phenyl ethanol* and *decanoic acid* to **Da** and **Sf** (Fig. 6.5a). Outlier **Dg** had abnormal contents *ethyl undecanoate*, *-octadecanoate*, and *decanoic acid* (Fig. 6.5c).

From PCA, DPLS2 and DPLS1, congeners abundant in Deluxe blends were primarily ethyl (C6 - C10) esters, *ethyl 9-decenoate* and *iso-amyl alcohol. Iso-amyl hexanoate* was also high in Deluxe blends. Such esters derived from middle distillate fractions are key contributors to whisky character (Salo, 1972; Piggott *et al.*, 1992; Clyne *et al.*, 1993).

Iso-amyl alcohol from "heads" contributes *fruity* (*appley*, *pear-drop*, *banana*) and *sweet* notes (Nicol, 1989) was linked to Deluxe blends, especially **Da** (Fig. 6.5a & 6.6a). In contrast, *iso-butanol* abundant in Retailer and Standard whiskies is derived from grain whisky; *iso-amyl alcohol* comes from malt spirits, and is indicative of the proportion of malt whisky in a blend (Aylott *et al.*, 1994). Higher alcohols are used as marker in authenticity in whiskies (Aylott *et al.*, 1994; Gonzalez-Arjona *et al.*, 1999).

Retailer blends had high contents of long aliphatic chain (C12 - C18) esters and alcohols: including unsaturated fatty acid esters - *ethyl 9-hexadecenoate*, -

dodecenoate, - *tetradecenoate* and *propyl decenoate* (Table 6.8, Fig. 6.5a). These, abundant in "feints", confer *soapy*, *oily*, *sour* and *feinty* notes (Guymon, 1974), and haze problems in bottling (Nicol, 1989; Piggott *et al.*, 1996). Unsaturated *ethyl hexadecenoate* is found only in Scotch whiskies (Suomalainen & Lehtonen, 1979) and is more abundant than *ethyl hexadecanoate* (Lyons & Rose, 1977).

Formation of whisky agglomerates (Tanford, 1980), or microemulsions, is promoted by *ethyl dodecanoate*, *tetradecanoate* and *hexadecanoate* together with other alcohols or aldehydes (Conner *et al.*, 1994b). These micelles, influenced by mouth temperature (Conner *et al.*, 1996) and wood non-volatiles (Paterson *et al.*, 1995; Conner *et al.*, 1994a), can suppress release of undesirable volatile congeners into headspaces. This should be central to product category discrimination, especially for Deluxe blends formulated with more and older malt whiskies.

It was predicted that well-matured and Deluxe blend whiskies should show increased agglomerate effects with lower headspace concentrations notably of long chain aliphatic esters, alcohols and acids. In contrast in Standard blends with high contents of acetate esters, there would be less micellar suppression, and more long-chain aliphatic esters in the whisky headspace. This should be most evident in Retailer products. Medium chain aliphatic (C6 - C10) fatty acid esters show the greatest suppression, notably in Deluxe. These predictions were confirmed whether relative (%) (Fig. 6.6) or absolute (Fig. 6.7) peak areas were used in construction of product spaces.

The high *furfural* content of West Highland blends reflects the peated malts were (Ramsay & Berry, 1982) used to meet the product category specification.

In this experiment CV values, based on absolute peak areas, were 0.18 for total adsorption and 0.36 for the mean of individual peaks favouring use of relative %

peak areas (Roberts *et al.*, 2000; Pelusio *et al.*, 1995) in discrimination between blend categories. The replicates of control whisky showed a higher coefficient of variation (CV = 0.18) than between products (CV = 0.07) in a single day.

Although absolute peak areas are commonly used in quantification in gas chromatography SPME analyses do not facilitate use of an internal standard (Ebeler, 2000). As the signal/noise ratio in SPME can low and coefficient of variation high, relative (%) area has been used by other workers (Roberts *et al.*, 2000; Pelusio *et al.*, 1995). There is clear similarity in the clustering in the two DPLS2 product spaces Figs. 6.6a,b and Fig. 6.7. Relative peak areas yielded more (3) valid factors and greater calibration variance (47%) explained than absolute (1 factor; 17 % variance). Relative peak areas reduced dispersion of Deluxe blends in the product space and yielded better discrimination of Standard and Retailer blends in DPLS2 (Fig. 6.6a) compared to absolute peak data (Fig. 6.7). Relationships between congeners and individual products showed differences related to congeners significant in discrimination between the categories. Such observations can be explained by reference to the high coefficient of variation for replicate samples in a study of brandies employing absolute peak area quantitation (Ebeler *et al.*, 2000), at 11 % and 18% in this study. Considering all chromatographic components (Fig. 6.4) and common congeners (Fig. 6.5a), both product spaces showed similar clustering, relationship between products and distributions of loadings. However, using common congeners yielded explanations of greater variance and discriminated successfully between blends categories.

The polyacrylate (PA) fibre exhibited a preference towards polar compounds, notably most esters and certain alcohols (Yang & Peppard, 1994; Steffen & Pawliszyn, 1996). As in the study of brandy (Ebeler *et al.*, 2000) congeners were dominated by esters and alcohols with polar congeners less well represented (Roberts *et al.*, 2000): e.g. vanillin (detection threshold 0.6 ppm in 23% grain whisky, Lee *et al.*, 2000b) was not detected at 1 ppm (Yang & Peppard, 1994). For vanillin there are differences between GC-O analysis and sensory data on odour intensity (Goodner *et al.*, 2000). Such differences in sensitivity would be a problem for lactone and phenolic compounds, important in matured whisky character (Withers *et al.*, 1996; Conner *et al.*, 2000). Thus SPME may not quantify congeners with important roles in blend discrimination.

As SPME is an equilibrium method (Steffen & Pawliszyn, 1996), influenced by solubility of congeners in the liquid phase (Henry's law) and affinity for the SPME phase (Moens *et al.*, 1997), with absorption of only a limited proportion of headspace molecules (Jiménez *et al.*, 1998; Mestres *et al.*, 1998). The use of salt in SPME of brandies (Ebeler *et al.*, 2000) is likely to enhance headspace concentrations but distort values from those experienced in sensory analysis. Reproducibility is greatest at an equilibrium point, due to the limited surface (competition phenomenon), thus short-time sampling to avoid saturation has been recommended (Song *et al.*, 1997). Equilibrium is reached more rapidly in PDMS than PA fibre where a crystalline structure reduces analyte diffusion rates hence influencing adsorption (Steffen & Pawliszyn, 1996). The superior performance of PA over PDMS, observed in this study has also been reported for volatile constituents in orange juice (Steffen & Pawliszyn, 1996), and metabolites produced by *Staphylococci* (Vergnais *et al.*, 1998). Thus, for understanding relationships between sensory and headspace compositional analyses it is important to avoid abnormal temperatures, adjusting the composition of the liquid phase, and to appreciate the limitations in the chosen SPME phase.

The articulation of PDMS plots at 10 and PA fibre at 20 min (Fig. 6.2), where total peak areas were decreased and subsequently increased was also observed previously by Moens and co-workers (1997). This suggests equilibria between adsorption of congeners and ethanol: influencing yields of specific congeners in relation to total number of adsorbed molecules. Ethanol interference has been reported in brandy headspace analysis (Ebeler *et al.*, 2000) and for terpenes (De la Calle Garcia *et al.*, 1996). There is an inverse relationship between ethanolic strength and absolute peak area although ratios of peak areas for a component and the internal standard remained constant (Mestres *et al.*, 1998). Also competition phenomenon could be critical when both compounds of low and high affinity are quantified (Górecki & Pawliszyn, 1996). This makes accurate absolute quantification of volatiles difficult (Roberts *et al.*, 2000). Largely quantitative, as opposed to qualitative differences have been reported between SPME and other headspace methods in apple juice (Song *et al.*, 1997), soft drink (Elmore *et al.*, 1997) and truffle (Pelusio *et al.*, 1995). Such difficulties can be overcome by stable isotope dilution assays (IDA) (Roberts *et al.*, 2000).

Possible artefacts from SPME (Yang & Peppard, 1994), include siloxanes from PDMS and 1-methyl-2,4-diisocyanatobenzene from PA have been reported arising from insufficient conditioning of fibre phases and teflon-coated septa in injection ports (Elmore *et al.*, 1997) and can be minimised by SPME headspace samplings at ambient temperature (Pelusio *et al.*, 1995). Extraction at 25 °C influences both overall headspace and specific congener concentrations, through changing partition coefficients (Llompart *et al.*, 1998). Temperature effects are critical before, but less after equilibrium points (Arthur *et al.*, 1992; Yang & Peppard, 1994). Oral temperatures (37 °C) yielded more congeners in headspaces as reported for diacetyl in wine - a 65% at 33 °C compare with 22 °C (Hayasaka & Bartowsky, 1999).

Both Williams (1994) and Martens *et al.* (2000) have concluded interpretation of product space is complicated by large number of samples and loadings. Loading weights define impact of variables on factors from multivariate data analysis. Validation variance is less popular but shows how an individual variable or factor is validated in that model calibration. Such strategies were important in this study in determining the optimum number of PC factors and ascertaining why DPLS1 yielded no valid factor on West Highland blends, with negative validation variance in DPLS2. Calculations of validation also simplify interpretation of product spaces through eliminating poorly validated congeners, with negative or decreased validation variance.

6.6. CONCLUSION

In this study of 40 blends, both relative (%) and absolute peak area in chromatograms of SPME congeners differentiated products and discriminated between the four categories. Important for Deluxe blends were ethyl (C6 - C10) esters - *ethyl hexanoate*, *-heptanoate*, *-octanoate*, *-nonanoate* and *-decanoate*, and *iso-amyl hexanoate* and *-alcohol.* Standard blends were differentiated by contents of acetate esters (*dodecyl-*, *phenyl ethyl-* and *3-methyl-butyl-acetates*). In contrast, Retailer blends were dominated by high contents of longer (>C10) aliphatic esters, alcohols and the unsaturated ethyl fatty acid esters (*ethyl hexadecenoate*, *-octadecenoate*, *-tetradecenoate*) and *propyl decenoate*. Characteristic of West Highland blends were *furfural*, *ethyl benzoate*, *iso-butyl octanoate* and medium-chain esters common to Deluxe blends notably *ethyl nonanoate*.

However, Standard blends were dispersed across product spaces sharing similarities with Retailer and, less extensively, Deluxe blends. This reflects a rather arbitrary commercial differentiation of blends into Deluxe, Standard and Retailer categories observed from sensory data on these blends (Lee *et al.*, 2001). On these basis of this study certain Standard blends could have been classified as Deluxe and others as Retailer products. Relationships between quantitative differences in headspace congeners and sensory character merit further study.

Recently, the presence of styrene in top-fermented beer has been reported (Daly *et al.*, 1997). Styrene and 4-vinyl guaiacol were both produced by the enzymatic decarboxylation (cinnamic acid decarboxylase) of cinnamic and ferulic acids, respectively, by a *Pof* phenotype in a wild yeast strain (Pof^+), as a result of infection. Rapid accumulation of styrene at the initial phase of fermentation with aerobic growth of yeast cells and then styrene transfer to gaseous phase in the evolved CO_2. As evolution of CO_2 decreases at the end of fermentation, accumulated styrene reached approximately 30 ppb in green beer imparting strong *clove/spicy* and *heavy-solvent* aroma notes (Daly *et al.*, 1997). As present in beer, styrene is likely to be also a whisky flavour component although flavour impact has not been reported.

CHAPTER 7:

RELATIONSHIPS BETWEEN SENSORY CHARACTER AND HEADSPACE CONGENER CONCENTRATIONS IN BLENDED SCOTCH WHISKIES

Results of this study have been submitted for publication in *Journal of Institute of Brewing* (Lee *et al.*, 2001, submitted).

7.1. ABSTRACT

Forty Scotch blended whiskies were profiled by a sensory panel (Chapter 5) and headspace congeners were quantified by GC-SPME (Chapter 6). The relationships between sensory attributes and headspace congeners were modelled using PLS2 with full-cross validation. No correlation was found between the two data sets whether relative or absolute quantification of GC was used, and all (57) or common (38) congeners. From all product spaces, Standard blends were dispersed among other categories. Excluding Standard blends, PLS2 yielded a valid factor (Factor 1) with a total 20% *Y*-variance with clear discrimination of Deluxe and Retailer blends with West Highland intermediate. For Deluxe, *malty*, *smooth*, *vanilla*, *fruity*, *buttery*, *floral*, *sweet*, and *nutty* were linked to *iso*-amyl hexanoate, ethyl-9-decenoate and C7 - C10 esters; for Retailer, *soapy*, *rancid*, *solventy*, *mouldy*, *oily* and *sulphury* were linked to higher molecular weight (C12 - C18) esters, acetates and alcohols. For West Highland blends, *woody*, *smoky/peaty* and *medicinal* were linked to ethyl benzoate, -undecanoate, -heptanoate and *iso*-amyl alcohol. In PLS1, *floral*, *nutty*, *vanilla* and *woody*, only Deluxe related characters showed high r^2 values. *Floral* notes were related to ethyl undecanoate, ethyl oleate, decanoic acid and 2-phenyl ethanol contents; *nutty*, *iso*-amyl hexanoate and ethyl dococenoate; *vanilla*, *iso*-amyl hexanoate, ethyl dodecenoate and undecanoate; *woody*, ethyl benzoate, *iso*-butyl octanoate, and 1-hexanol. *Soapy* associated with Retailer blends have been predicted from high molecular weight unsaturated fatty acid esters and alcohols.

7.2. INTRODUCTION

Modelling of relationships between flavour components and sensory character has been transformed by partial least squares regression (PLS; Wold *et al.*, 1983;

Martens *et al.*, 1983, 2000; Martens & van der Burg, 1984; Martens & Martens, 1986), occasionally used in whisky studies (Canaway *et al.*, 1984; Swan & Howie, 1984). The technique is based on regression on to latent variables to achieve good prediction performance. To establish number of latent variables (components) full-cross validation (Wold, 1989; Kvaal & McEwan, 1996) is used, where samples are used both for model estimation and testing. In this study, single products were removed from the data set one at a time and model calibrated on the residual samples. Predictive performance was quantified by calculation of cross-validated index r^2; varying from 0 (no fit) to 1 (perfect prediction).

7.3. MATERIAL AND METHODS

7.3.1. Whiskies

Forty commercial blended Scotch whiskies (Table 5.1) were used.

7.3.2. Data analysis

Two sets of data were obtained from 21 sensory attributes excluding non-significant attributes (*catty*, *acetic sour*) (Table 5.2) and SPME headspace congener data of the common 36 relative peak area (Table 6.4). Since the separate product spaces showed dispersion of Standard blends, data from these products were removed and the residue were subjected to PLS1 and PLS2 analysis in Unscrambler (v 7.0). Full-cross validation was employed. Headspace data formed as *X*-variables (predicting, regressors) and sensory attributes scores *Y*-variables (predicted, regressands). Duplicate samples were averaged only after PLS, to ease visualisation of product spaces.

7.4. RESULTS

From PLS2 analysis, excluding Standard blends category, only the first factor was validated explaining 45% of *X*- and 20% *Y*-variance for score (Fig. 7.1) and loading (Fig. 7.2). In the product space, Factor 1 and 2 (Fig. 7.1), clusters and outliers products were observed similarly to product spaces for headspace congeners (Fig. 6.5a) and sensory data (Fig. 5.1b; 5.2b). Factor 1 was clearly differentiated with Deluxe blends having negative score and Retailer blends on the positive. West Highland blends were intermediated but closer to Deluxe.

In Fig. 7.2, loadings of attributes *grainy, spicy* and *pungent* were eliminated from the product space with large (> -10%) negative validation variance (data not shown). On Factor 1, negatively loaded attributes were *malty, smooth, vanilla, fruity, buttery, floral, sweet,* and *nutty* linked to *iso*-amyl hexanoate, ethyl-9-decenoate and ethyl C7 - C10 esters. Three attributes *malty, smooth* and *vanilla* were linked to *iso*-amyl hexanoate, ethyl octanoate, -nonanoate and -decanoate and ethyl-9-decenoate and -heptanoate to *fruity, buttery, floral, sweet,* and *nutty.* These were correlated with the Deluxe blends (Fig. 7.1). Positively loaded attributes - *soapy, rancid, solventy, mouldy, oily* and *sulphury* - were linked to high molecular weight (C12 - C18) esters, acetates and alcohols. Propyl decanoate had a close relationship with *solventy, rancid* and *soapy* as well as *grassy.* These attributes and headspace congeners were associated with Retailer blends in the product space (Fig. 7.1). In the centre of the Factor *woody, smoky/peaty* and *medicinal* were linked to ethyl benzoate, *iso*-amyl alcohol, ethyl undecanoate and less to ethyl heptanoate and related to West Highland blends in the product space (Fig. 7.1).

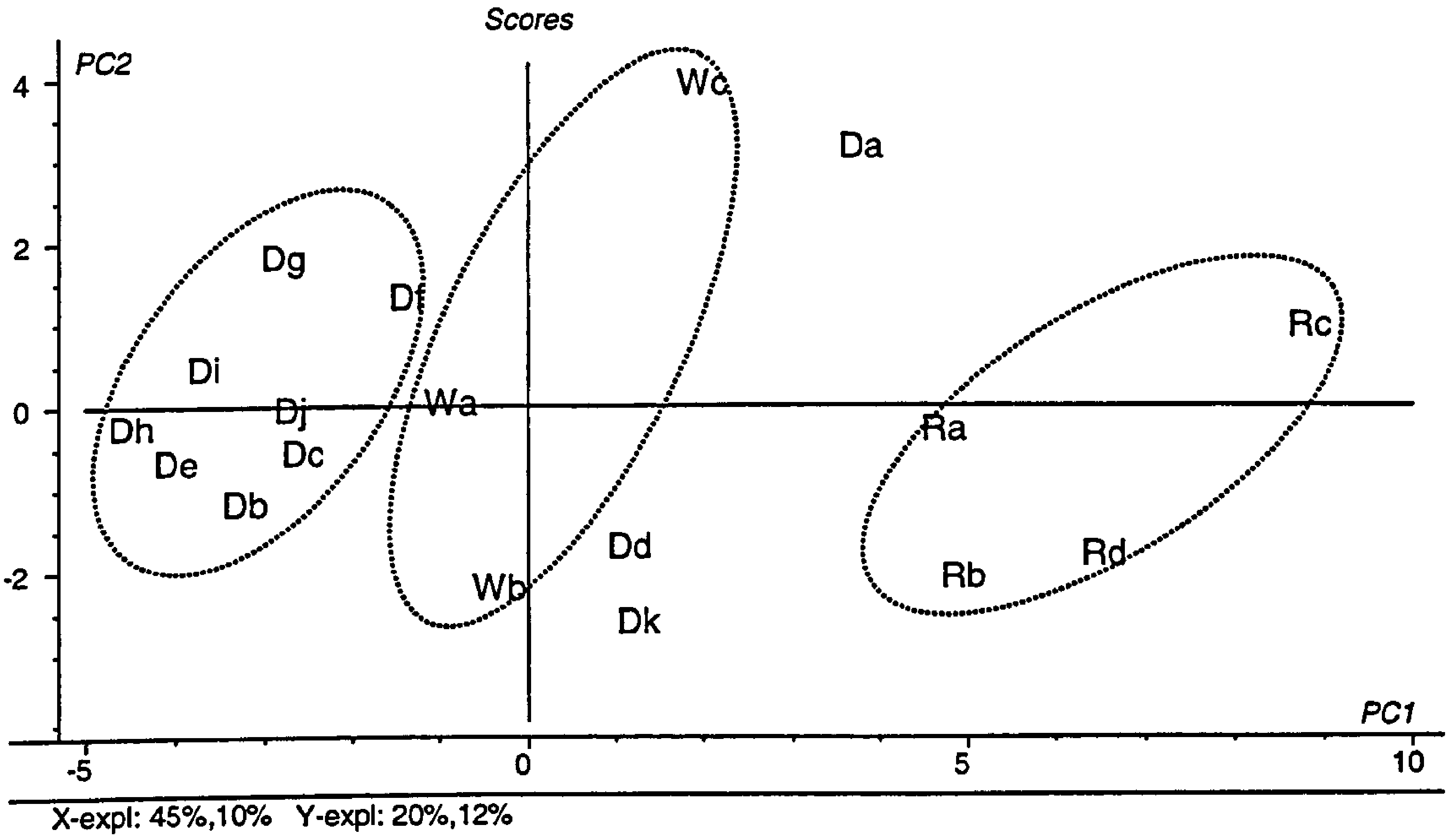


FIG. 7.1. Product scores of the forty blended Scotch whiskies from PLS2 regression

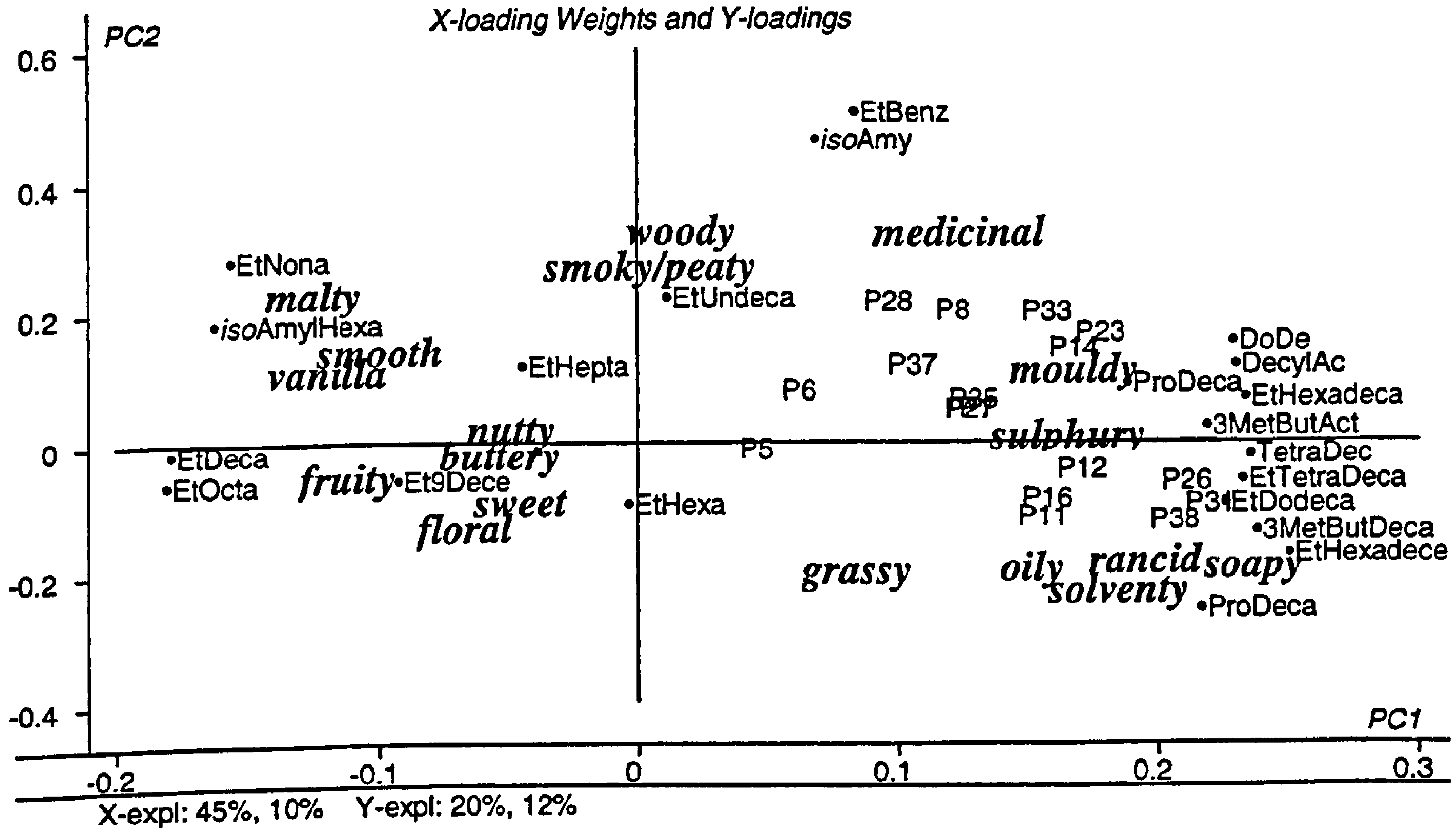


FIG. 7.2. Loadings of the forty blended Scotch whiskies from PLS2 regression

Modelling of PLS1 was performed with all forty blends to predict relationships between individual sensory attributes and headspace congeners. The modelling of *floral*, *nutty*, *vanilla* and *woody*, Deluxe characters, showed good predictions (r^2) values of 0.751, 0.743, 0.707 and 0.698, respectively (Fig. 7.3 - 7.6). Less well modelled were *medicinal* ($r^2 = 0.551$), *soapy* ($r^2 = 0.528$) and *grassy* ($r^2 = 0.504$) (data not shown).

Floral was modelled best ($r^2 = 0.751$) (Fig. 7.3). With these Deluxe blends (**De**, **Dg** and **Dh**) with highest scores being the most matured 15, 18 and 21 years, respectively. Similar results were obtained for *nutty* (Fig. 7.4) and *vanilla* (Fig. 7.5). Blends **Sb**, **Sf** and **Sc** had high scores for *floral* and **Sb** in *nutty* and *vanilla* with strong linearity to the regression line. *Woody* character was intense in **Wc** and **St** (Fig. 7.6).

Regression coefficients (> 0.03) (Table 7.1) summarised relationship between headspace congeners (predictors, *X*-variables) and five attributes (response variables) ($r^2 > 0.500$). Only positive regression coefficients were listed in rank. Congeners associated with *floral* were ethyl undecanoate and oleate, decanoic acid and 2-phenyl ethanol; for *nutty*, *iso*-amyl hexanoate and ethyl docecenoate; for *vanilla*, *iso*-amyl hexanoate, ethyl dodecenoate and -undecanoate; for *woody*, ethyl benzoate, *iso*-butyl octanoate, ethyl nonanoate and 1-hexanol. *Soapy*, associated with Retailer blends, had relationships with high molecular weight unsaturated fatty acid esters and alcohols. Headspace congeners have been shown commonality between Deluxe (*vanilla* and *nutty*), and West Highland characters (*woody* and *medicinal*) (Table 7.1). Interestingly, although *floral* was characteristics of Deluxe blends, there was commonality with *grassy* in certain congeners - ethyl oleate, heptanoate, undecanoate, hexanol and 2-phenyl ethanol.

7.5. DISCUSSION

Many studies have shown correlations between sensory data and chromatographic analysis in whisky flavour mainly in relation with maturation processes particularly for phenols (Swan & Howie, 1984) and wood extracts congeners (Conner *et al.*, 2000). Canaway and his co-workers (1984) studied relationships between sensory and whisky flavour congeners (including number of esters and alcohols) using PLS regression. These authors were able to relate certain congeners - ethyl acetate, *iso*-amyl acetate and *n*-butanol - to *sour* and *solventy* characters, and aldehydes to *peaty* and *headsy*, and 2-phenylethyl acetate to *greasy* notes. Most of these congeners were linked to character in Retailer blends in this present experiment. However, ethyl esters of C8, C10, C12 aliphatic acids and 2-phenyl ethyl acetate have not been clearly related to any sensory attributes. In this present experiment, ethyl esters were discriminated in relation to blends sensory character by PLS1.

Lack of correlation between headspace congeners and sensory data may be caused by: i) the dispersed Standard blends adding much noise to data; or ii) insufficient West Highland blends with distinctively different individual product characters; or iii) dispersion of Deluxe clusters along PC1 in the product space (Chapter 5, Fig. 5.1b); or iv) selectivity of SPME fibre phase to certain congeners (Chapter 6). It also could be related to decreased pH in longer maturation, only partitioning non-ionised molecules into headspaces, whilst retaining ionised flavour congeners in solution (e.g. 3-methyl butanoic acid) (Conner *et al.*, 2000). Thus a signal in GC olfactometry does not guarantee a sensory influence in the original drink product (Conner *et al.*, 2000).

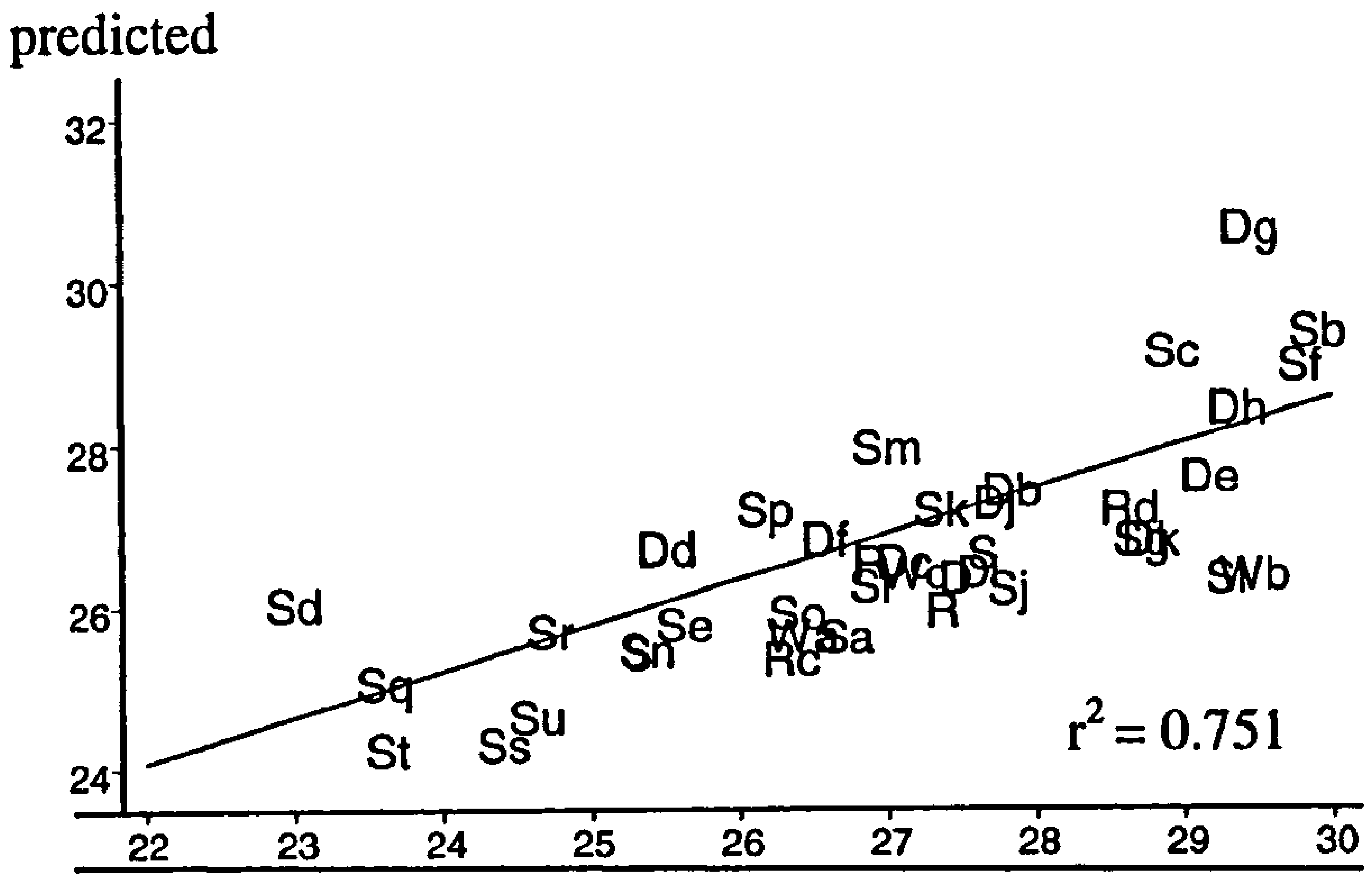


FIG. 7.3. measured *Floral*

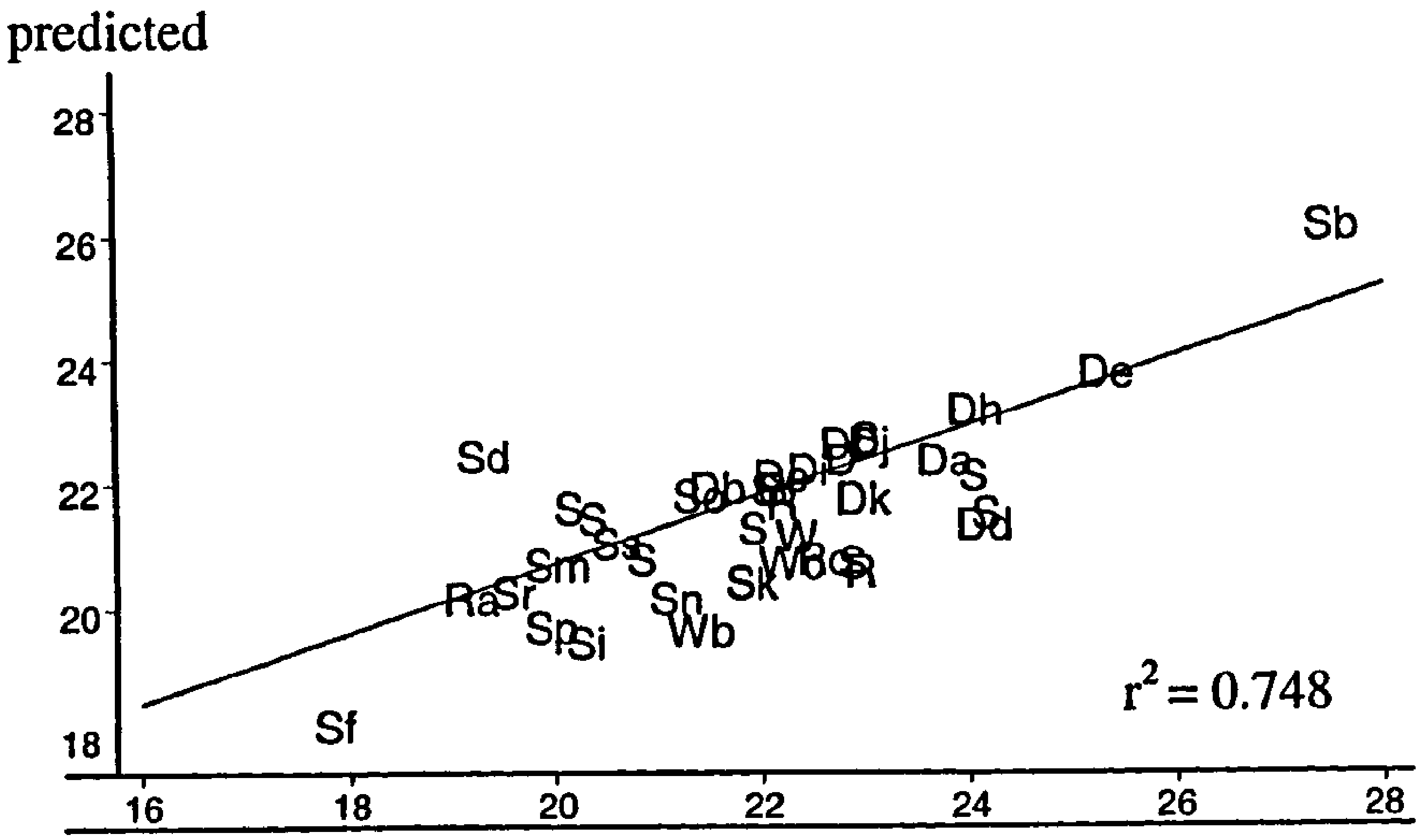


FIG. 7.4. measured *Nutty*

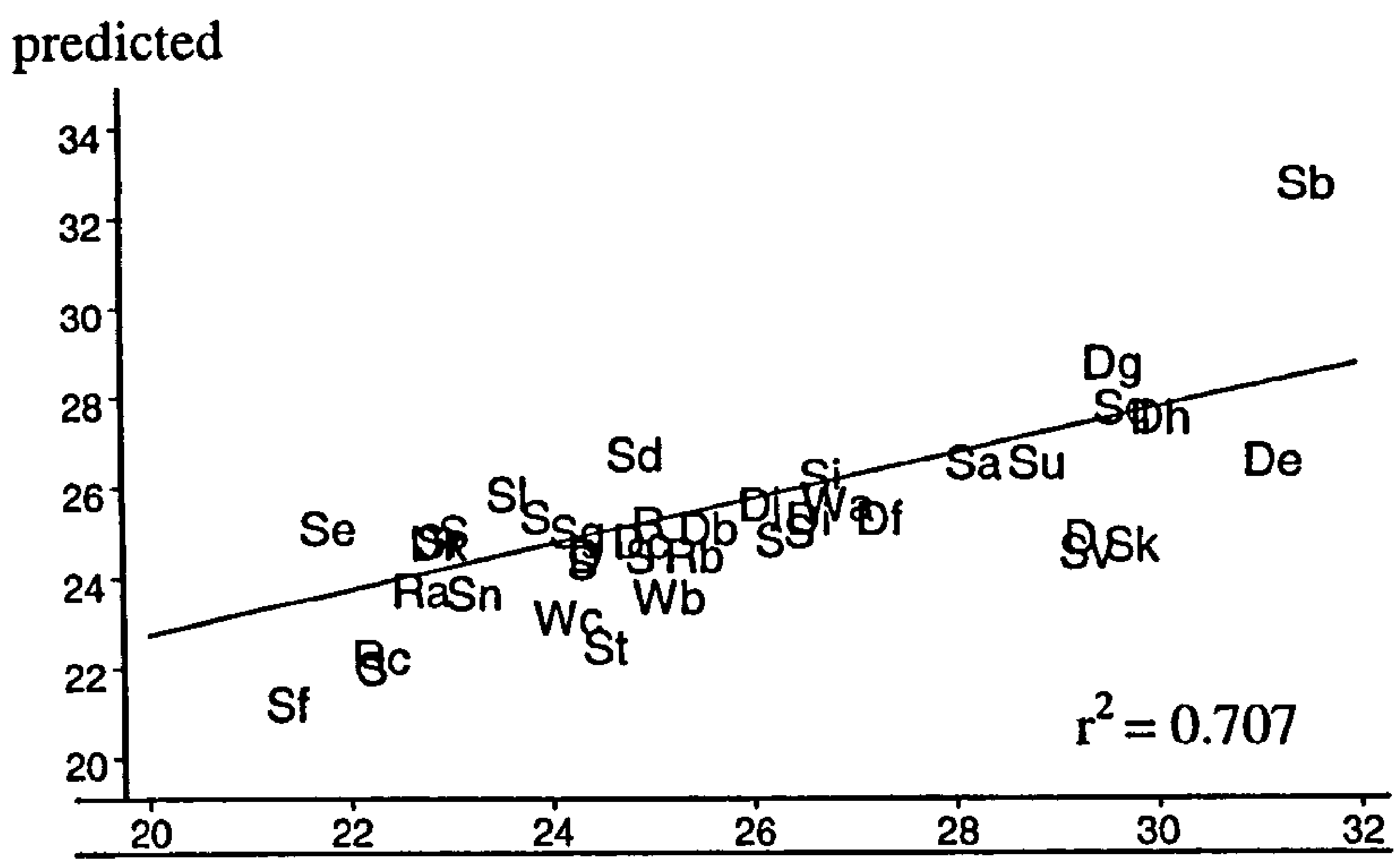


FIG. 7.5. measured *Vanilla*

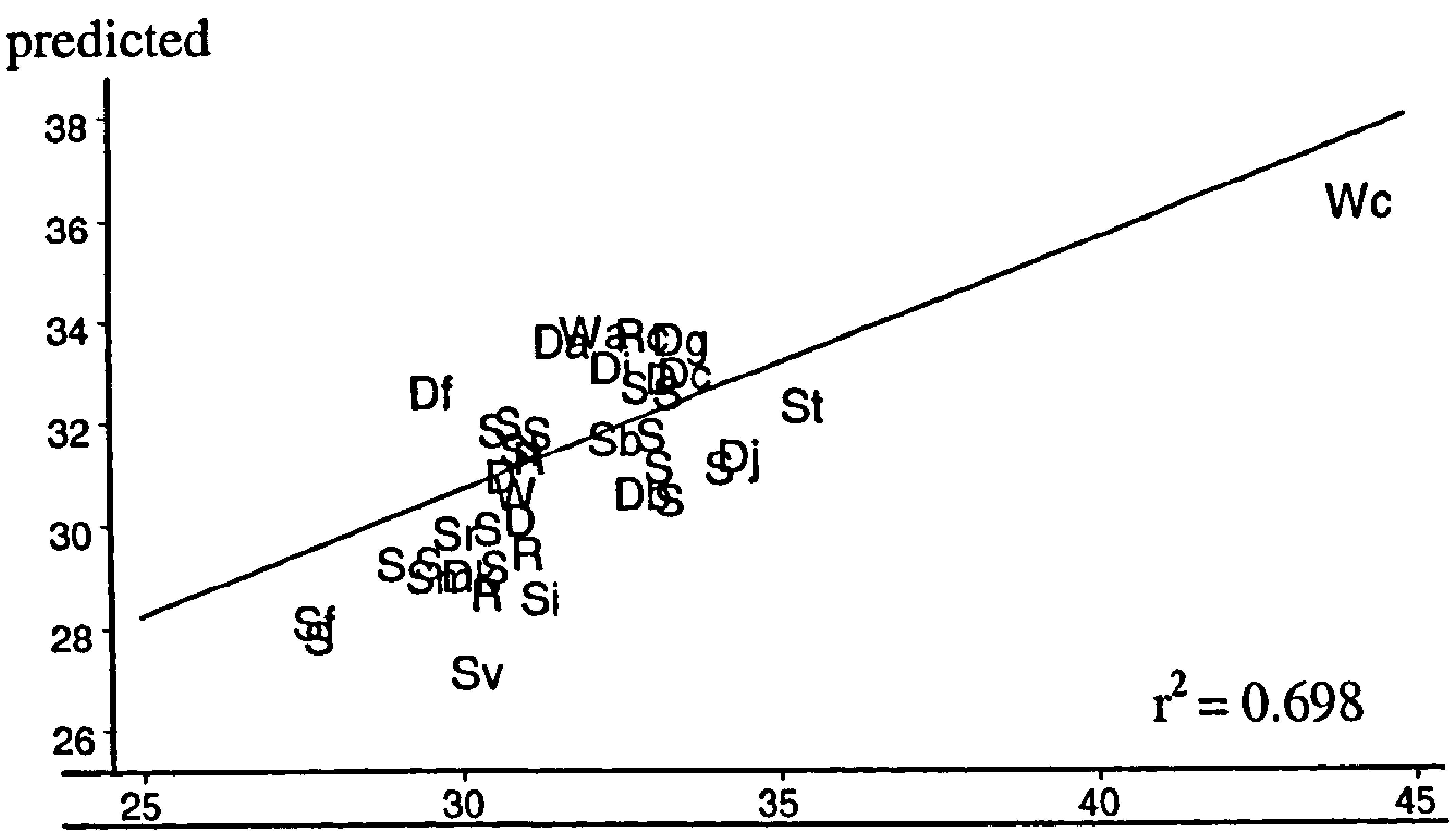


FIG. 7.6. measured *Woody*

TABLE 7.1. Linkage (Regression coefficients) of sensory attributes on Factor 1 from PLS1 model to headspace congeners concentrations

Floral		*Vanilla*		*Nutty*		*Woody*		*Medicinal*		*Soapy*		*grassy*	
Ethyl undecanoate	0.173	*iso*-Amyl hexanoate	0.151	*iso*-Amyl hexanoate	0.195	Ethyl benzoate	0.241	1Hexanol	0.144	Propyl decenoate	0.051	Ethyl oleate	0.173
Ethyl oleate	0.129	Ethyl dodecenoate	0.104	Ethyl dodecenoate	0.186	*iso*-Butyl octanoate	0.157	*iso*-Amyl alcohol	0.140	Ethyl-9-hexadecenoate	0.044	Ethyl heptanoate	0.134
Decanoic acid	0.113	Ethyl undecanoate	0.102	*iso*-Butanol	0.112	1Hexanol	0.129	*iso*-Butyl octanoate	0.112	Ethyl dodecanoate	0.042	1Hexanol	0.109
2-Phenyl ethanol	0.091	Ethyl tetradecenoate	0.078	Dodecyl acetate	0.094	Ethyl nonanoate	0.125	Ethyl benzoate	0.099	Dodecanol	0.041	Ethyl undecanoate	0.092
Ethyl tetra decenoate	0.083	Ethyl dodecanoate	0.070	Ethyl tetradecenoate	0.080	*iso*-Amyl alcohol	0.105	Ethyl nonanoate	0.081	Ethyl tetradecenoate	0.042	2-Phenyl ethanol	0.068
iso-Butanol	0.081	Dodecyl acetate	0.053	1Hexanol	0.071	Ethyl dodecenoate	0.079	Ethyl undecanoate	0.071	Propyl decenoate	0.042	Dodecyl acetate	0.062
Ethyl nonanoate	0.077	*iso*-Butanol	0.053	Ethyl dodecanoate	0.068	Ethyl heptanoate	0.073	Ethyl hexadecanoate	0.064	3-Methyl butyl decanoate	0.041	Decanoic acid	0.058
Dodecanol	0.059	-		3-Methyl butyl octanoate	0.060	Ethyl hexadecanoate	0.064	Phenylethyl acetate	0.055	Dodecyl acetate	0.040	*iso*-Butyl octanoate	0.051
Propyl decanoate	0.053	-		-		Ethyl undecanoate	0.057	-		Ethyl tetradecanoate	0.038	-	
Ethyl dodecanoate	0.052	-		-		-		-		Ethyl hexadecanoate	0.038	-	

Although the Standard blends were excluded from PLS2 analysis, it was possible to obtain information for this blend category from PLS1. Since only Deluxe character attributes - *floral, nutty, vanilla* and *woody* - were well predicted (r^2), the information were limited to only certain number of products (**De**, **Dg**, **Dh** and **Sb**) which had longer maturation period (>15 y). This has been supported by headspace congener analysis of wood extractives after four years maturation showed that only certain compounds - ethyl hexanoate, *cis*-lactone and vanillin - were presented above their odour thresholds (Conner *et al.*, 2000). Differentiating of **Sb** character from other Standard blends, with similarity to certain Deluxe products, was probably because of it had been blended with a single grain whisky probably older and strong characters (e.g. *floral*).

7.6. CONCLUSION

The data of three blends categories (Deluxe, Retailer and West Highland) - excluding Standard - sensory attributes and headspace SPME congeners (relative (%) peak areas and common to all whiskies), was correlated using PLS1 and 2 analysis.

From PLS2, the Deluxe category of blends showed close relations with *malty, smooth, vanilla, fruity, buttery, floral, sweet,* and *nutty* and the following headspace congeners: *iso*-amyl hexanoate, ethyl-9-decenoate and C7 - C10 esters. In the Retailer category, *soapy, rancid, solventy, mouldy, oily* and *sulphury* characters were linked to high molecular weights esters (C12 - C18), acetates and alcohols. In West Highland, *woody, smoky/peaty* and *medicinal* were linked to ethyl benzoate, *iso*-amyl alcohol, ethyl undecanoate and -heptanoate. In PLS1, *floral, nutty, vanilla* and *woody*, Deluxe characters were well predicted. *Floral* were associated with the following congeners: ethyl undecanoate and -oleate, decanoic acid and 2-phenyl ethanol; for *nutty*, *iso*-

amyl hexanoate and ethyl dodecenoate; for *vanilla*, *iso*-amyl hexanoate, ethyl dodecenoate and undecanoate; for *woody*, ethyl benzoate, *iso*-butyl octanoate, and 1-hexanol. The *soapy* character, of Retailer blends, was linked to high molecular weight unsaturated fatty acid esters and alcohols.

CHAPTER 8:

ANALYSIS OF DISCRIMINATION TESTS

Results of this study have been published in *Agro-Industrie et Méthodes Statistiques* (Hunter *et al.*, 2000).

8.1. ABSTRACT

Three large triangular test experiments were run at Strathclyde University to supply "real" data for investigating (statistical) replication in discrimination test analysis strategies. In these experiments assessors carried out multiple assessments.

A Randomisation test (Hunter, 1996) was found to be an effective way of identifying when there is reason to allow for assessor effects. The Generalized Linear Model provides one way of analysing assessor totals. Our results show that the confidence intervals predicted from it are much narrower than the intervals found using the alternative strategy promoted by other European workers (Brockhoff & Schlich, 1998).

Much work requires to be done to identify the assumptions made in our Generalized Linear Model approach and approaches from the literature. Only by gaining a better understanding of the issues involved can well founded advice be given to sensory scientists.

Keywords: *Triangle test, Discrimination test, Difference test, Assessor differences, Generalized linear model*

8.2. INTRODUCTION

Discrimination tests, including the triangular test, are the most widely used form of sensory testing in industry. One reason for the widespread use of the triangular test is that an assessor can make successful judgements without knowing how the samples differ. This property makes it useful for assessing the detectability of sensory changes in a product when a new source of raw material or a changed process is introduced. In these circumstances there may be uncertainty about the nature of the alteration (if any) to the finished product (see, for example, MacRae, 1995).

There has been much criticism of the triangular test (e.g. Ennis 1990, 1993). The core of the criticism is the lack of specific instructions, which conversely is the advantage of this test. One of the dangers associated with both triangular and duo-trio tests is that the assessor identifies the nature of the difference between the samples and thereafter converts subsequent tests into the 3-alternative forced choice (3-AFC) format. Ennis (1990) advances arguments for preferring the duo-trio test in which the assessors match either the second or third sample with the first sample. On the basis of equations from the literature, Ennis (1990, 1993) shows that the triangular and duo-trio test should not be used to test for small differences and that 3-AFC tests are to be preferred when they are an option.

On the assumption, that each assessor performs the test only once, the statistical procedures for analysing this type of data are well established and are given in almost all textbooks on sensory methodology (e.g. Stone & Sidel, 1993). Smith (1981) and MacRae (1995) have both suggested that the proportion of discriminators should be estimated using a very simple model of individual differences which classifies assessors into those that guess and thus have a $\hat{p}=0.3333$ (for the triangle test) and those that can always distinguish and thus have a $\hat{p}=1.0000$. This model of assessor differences also underlies the tables of Schlich (1993).

In most laboratory environments using each assessor for only one test leads to experiments with very low power due to shortage of assessors (Schlich, 1993). Uniquely Schlich's calculations are based on exact Binomial probabilities rather than the more common Normal approximation to the Binomial distribution which is not sufficiently precise for small numbers of assessors. Inevitably, tables of critical values lead to a hypothesis testing rather than an estimation mindset. MacRae (1995) tackles the same problem using confidence intervals and presents his results in the form of graphics.

Assessors can be used for several tests in an experiment, in which case it may be incorrect to assume that the test data for each assessor are independent.

Priso *et al.* (1994) show how to fit a mixed model to the data. This model has random effects for assessors and sampling error which are both assumed to be normally distributed.

Harries and Smith (1982), and Ennis and Bi (1998) use the Beta Binomial to model the assessor differences in the proportion correct. This distribution can deal with the case where there are one or more super-sensitive assessors. In particular, Ennis and Bi (1998) give a full account of how to estimate the parameters of the model and to carry out statistical tests. They illustrate their ideas by means of a number of examples drawn from experience.

Brockhoff and Schlich (1998) use the Binomial with a correction for lack of independence determined from calculating the overdispersion of the assessors results. This work is based on a paper in the statistical literature (Brockhoff & Mηller, 1997). They present a practical schema, which can be readily adopted by any laboratory with this kind of data. Kunert and Meyners (1999), however, has been somewhat critical of the philosophy underlying the application of this method. Part of the problem may be that the Brockhoff and Schlich (1998) method is packaged in a hypothesis testing framework.

Although we have not come across examples in the sensory literature, it has been possible for many years to fit a Generalized Linear Model to data from discrimination tests using one of several readily available statistical packages.

Identity, Logit, Probit and Complementary log-log transformations are available with Normal or Binomial error distributions. With a Binomial error distribution, we would expect to find evidence of overdispersion of assessor effects. The distributional assumptions of these models will not be fully met when there are one or more sensitive assessors who correctly identify the "odd" sample in each test. A considerable task remains to be done introducing these methods, which are widely used elsewhere, into sensory science.

Specifically to test the methods from the literature and to develop our own ideas, three large triangular test experiments were run at Strathclyde University. This paper reports the results of the analysis of this data. An empirical statistical viewpoint was adopted.

8.3. EXPERIMENTAL

8.3.1. Materials

8.3.1.1. Experiment 1

Raspberry drink, *Bouvrage*, was purchased from Ella Drinks Ltd. (Alloa, FK10 3SA, Clackmannanshire, UK). *Bouvrage* is a carbonated drink prepared from raspberry juice, sugars, natural flavourings and citric acid. Samples were drawn from two batches prepared from different raspberry cultivars with slight variations in processing enzymes and conditions.

8.3.1.2. Experiments 2 and 3

Grain whisky spirit, 23% v/v, was spiked with 80 μg/ml (experiment 2) or 400 μg/ml (experiment 3) phenyl ethanol to produce two samples for each of these experiments.

8.3.2. Sensory Analysis

The assessors, who had no or little experience of sensory analysis, were recruited from staff and postgraduate students at the University of Strathclyde. In all three experiments, testing was carried out over four sessions (session 1 and 2 – replicate 1, session 3 and 4 – replicate 2) in which three of the six possible “trios” were tested. The “trios” were randomised to session for each replicate, and the order of presentation within each session was balanced over assessors using a William’s Latin square design (MacFie *et al.*, 1989). The sensory evaluation took place in a purpose built sensory laboratory under red light to minimise the possibility that assessors were able to discriminate between samples on the basis of slight colour differences. Samples were carefully coded to ensure that the testing was carried out “blind”.

8.3.2.1. Experiment 1

Ten female and twenty male assessors participated in this trial. The samples (20 ml) were presented in 30 ml disposable polypropylene measuring cups (A.W. Gregory & Co. Ltd, London, SE4 1RY, UK). The assessors were instructed to rinse their mouth between tests.

8.3.2.2. Experiment 2 and 3

In Experiment 2 eight female and sixteen male assessors participated and in Experiment 3 eight female and fifteen male assessors. The samples were presented in standard nosing glasses (ISO 5494:1978) covered with a watch glass. Samples were assessed solely by nose. Assessors were told to take a short break after each test.

8.4. RESULTS

8.4.1. Simple Summary Statistics

Tabulation of the numbers of tests correct showed, for all three experiments, that there were no time trends. There is thus no evidence that the assessors turned the triangular test into a 3-AFC test. There was also no evidence to suggest that the A form of the test (ABB, BAB or BBA) was different from the B form of the test (BAA, ABA or AAB). Likewise there was no evidence that the position of the odd sample influenced the proportion determined correctly.

8.4.1.1. Experiment 1

The proportion of tests correctly classified was $\hat{p} = 0.4722$. On the basis that each test was independent, the standard error was computed to be 0.02631 using the Normal approximation. Thus this proportion was significantly different from 1/3, Student's $t = 5.279$. The 95% confidence interval for $\hat{p}$ was 0.4206 to 0.5238.

8.4.1.2. Experiment 2

The proportion of tests correctly classified was $\hat{p} = 0.4062$. On the basis that each test was independent, the standard error was computed to be 0.02894 using the Normal approximation. Thus this proportion was significantly different from 1/3, Student's $t = 2.520$. The 95% confidence interval for $\hat{p}$ was 0.3495 to 0.4629.

8.4.1.3. Experiment 3

The proportion of tests correctly classified was $\hat{p} = 0.5326$. On the basis that each test was independent, the standard error was computed to be 0.03003 using the Normal approximation. Thus this proportion was significantly different from 1/3, Student's $t = 5.279$. The 95% confidence interval for $\hat{p}$ was 0.4737 to 0.5915.

8.4.2. Distribution of numbers correct for each assessor

When the data are classified by assessor, we get the distribution of numbers correct given in Table1. In all three experiments there is evidence of that some of the assessors have an ability to distinguish between samples but no clear evidence that assessors can be divided into "discriminators" that can correctly identify the odd sample in all tests and "guessers" that score around 4 correct answers. Taking each assessor individually, in order for their data to refute the Null Hypothesis of $\hat{p} = 1/3$, then 8 or more correct answers are required. In experiment 1 three assessors from thirty reach this level of discrimination, in experiment 2 three assessors from twenty four and in experiment 3 six assessors from twenty three. For each experiment the expected distribution of scores has been calculated using a binomial distribution with $\hat{p} = 1/3$ and also $\hat{p} =$ proportion correct. The latter distributions closely match the distribution of the numbers correct for each assessor. A χ^2 goodness-of-fit test gives values of 0.70, 1.87 and 1.22 with 3 df for experiments 1, 2 and 3. There are no grounds for refuting the null-hypothesis that the assessors have equal ability to discriminate between samples. However, this test is not sensitive to values in the tails of the distribution since it requires cells to be amalgamated into groups so that the expected count is 5 or more in every cell.

A simple alternative method of testing for the distribution of the numbers correct for each assessor, advocated by Hunter (1996), is a Randomization test. Given the proportion correct, the Null Hypothesis is that there is no additional effect of assessors beyond the Binomial sampling effect.

TABLE 1. Distribution of correct scores from assessors and "expected numbers" from the Binomial distribution using the proportion expected from the Null Hypothesis (1/3) and from the proportion correct in the experiment.

(a) Experiment 1

<table>
<tr><td colspan="13"><i>Number of correct answers (max 12)</i></td></tr>
<tr><td>0</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td></tr>
<tr><td colspan="13"></td></tr>
<tr><td colspan="13"><i>Number of Assessors with this number of correct answers</i></td></tr>
<tr><td>0</td><td>0</td><td>1</td><td>2</td><td>3</td><td>7</td><td>8</td><td>6</td><td>2</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="13"></td></tr>
<tr><td colspan="13"><i>Expected number of Assessors with</i> $\hat{p}=1/3$</td></tr>
<tr><td>0.2</td><td>1.4</td><td>3.8</td><td>6.4</td><td>7.2</td><td>5.7</td><td>3.3</td><td>1.4</td><td>0.5</td><td>0.1</td><td>0.0</td><td>0.0</td><td>0.0</td></tr>
<tr><td colspan="13"></td></tr>
<tr><td colspan="13"><i>Expected number of Assessors with</i> $\hat{p}=0.4722$</td></tr>
<tr><td>0.0</td><td>0.2</td><td>0.7</td><td>2.2</td><td>4.5</td><td>6.4</td><td>6.6</td><td>5.1</td><td>2.8</td><td>1.1</td><td>0.3</td><td>0.0</td><td>0.0</td></tr>
</table>

(b) Experiment 2

<table>
<tr><td colspan="13"><i>Number of correct answers (max 12)</i></td></tr>
<tr><td>0</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td></tr>
<tr><td colspan="13"></td></tr>
<tr><td colspan="13"><i>Number of Assessors with this number of correct answers</i></td></tr>
<tr><td>1</td><td>0</td><td>1</td><td>5</td><td>5</td><td>3</td><td>3</td><td>3</td><td>1</td><td>2</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="13"></td></tr>
<tr><td colspan="13"><i>Expected number of Assessors with</i> $\hat{p}=1/3$</td></tr>
<tr><td>0.2</td><td>1.1</td><td>3.0</td><td>5.1</td><td>5.7</td><td>4.6</td><td>2.7</td><td>1.1</td><td>0.4</td><td>0.1</td><td>0.0</td><td>0.0</td><td>0.0</td></tr>
<tr><td colspan="13"></td></tr>
<tr><td colspan="13"><i>Expected number of Assessors with</i> $\hat{p}=0.4062$</td></tr>
<tr><td>0.0</td><td>0.4</td><td>1.4</td><td>3.2</td><td>5.0</td><td>5.5</td><td>4.4</td><td>2.6</td><td>1.1</td><td>0.3</td><td>0.1</td><td>0.0</td><td>0.0</td></tr>
</table>

(c) Experiment 3

Number of correct answers (max 12)												
0	1	2	3	4	5	6	7	8	9	10	11	12
Number of Assessors with this number of correct answers												
0	0	2	1	1	4	3	6	3	1	0	1	1
Expected number of Assessors with $\hat{p} = 1/3$												
0.2	1.1	2.9	4.9	5.5	4.4	2.5	1.1	0.3	0.1	0.0	0.0	0.0
Expected number of Assessors with $\hat{p} = 0.5326$												
0.0	0.0	0.2	0.8	2.1	3.8	5.0	4.9	3.5	1.8	0.6	0.1	0.0

A simple test statistic is the sum of squares of numbers correct for each assessor. This is first computed for the data and is 72.67 for experiment 1, 114.6 for experiment 2 and 133.5 for experiment 3. The data for each experiment are then randomized and the computations repeated many times to form reference distributions. By finding where the statistic lies in the reference distribution, the statistical significance of the assessor "statistic" can be determined. For the first experiment it was at the 29th percentile, and for the second and third experiments at the 99th percentile. An alternative statistic is the number of assessors with 8 or more correct answers. For the three experiments this was 3, 3 and 6. The Randomisation test shows these values occur at the 25th, 95th and 48th percentiles of the reference distributions. We conclude that the sum of squares statistic is a more suitable criterion for testing for assessor effects and that a Randomization test is a suitable way of testing this criterion.

8.5. MATHEMATICAL MODEL

The general model for the data in discrimination tests can be written as:

$$y_{ij} = p + a_i + e_{ij}$$

where y_{ij} is the (Binary: 0 = wrong, 1 = correct) response of i^{th} assessor to the j^{th} test. When there is no statistical evidence of assessor effects, each observation can be assumed to be independent and the simple calculations at the start of this section used to determine $\hat{p}$ with an appropriate confidence interval using the Normal approximation for the standard error.

8.5.1. Estimating the Parameters of the Model

In the general case, both a_i and e_{ij} are "error" terms and are constrained to sum to zero. The Binomial and possibly the Beta-Binomial distributions are more appropriate than the Normal distribution to model assessor effects. The aim of the analysis is to determine $\hat{p}$ and to derive a 95% confidence interval. Software is now readily available to fit the generalised linear model to data of this form (for example Genstat 5.3, 4th edition, for Windows). The starting point of the analysis is the number correct for each assessor together with the number of tests each assessor performed.

A Generalised Linear Model was fitted with logit link function and Binomial variation to the results from each experiment. Consistent with the results of the Randomisation test, the residual mean deviance from the first experiment was 0.8717 compared to 1.0000 - the theoretical mean deviance if there were no assessor effects. We conclude for this experiment that there is no evidence of an assessor effect. Interestingly, the assessor with only two correct answers was identified as an outlier.

The point estimate for the proportion correct is $\hat{p} = 0.4723$ with a 95% confidence interval of 0.4209 to 0.5242 which is in good agreement with the figures obtained more simply at the head of this section.

For experiment 2 the residual mean deviance was found to be 1.931. A variance ratio test with 23 and infinity degrees of freedom shows this to be significant at the 1% level. In terms of the mathematical model, this was evidence that the a_i were not equal to zero. The two assessors with 9 correct and especially the assessor with 0 correct were identified as being outliers. The point estimate for the proportion correct is $\hat{p} = 0.4064$ and the 95% confidence interval is 0.3511 to 0.4641 ignoring assessor variation and more correctly 0.3305 to 0.4870 after allowing for it. The adjustment was made by multiplying the standard error by the square root of the mean deviance.

For experiment 3 the residual mean deviance was found to be 2.360. The variance ratio test with 22 and infinity degrees of freedom showed this to be significant at the 0.1% level. This was very clear evidence of an effect of assessors. In terms of the model above, the a_i were shown not to be equal to zero. The assessors with 2, 3, 11 and 12 correct were identified as being outliers. The point estimate for the proportion correct is $\hat{p} = 0.5327$ and the 95% confidence interval is 0.4735 to 0.5910 ignoring assessor variation and more correctly 0.4419 to 0.6214 after allowing for it. The adjustment was made by multiplying the standard error by the square root of the mean deviance.

8.5.2. Alternative Approaches

The Brockhoff and Schlich (1998) method was applied to the data from the three experiments. The factor, $\hat{\sigma}^2$, used to adjust for the assessor effects was 1.000, 2.441 and 3.058. The point estimates are the same as derived by the simple statistics but the 95% confidence intervals are 0.4207 to 0.5238, 0.3176 to 0.4949 and 0.4297 to 0.6355. These intervals are much wider for experiments 2 and 3 than the estimates from the Generalized Linear Model.

8.6. DISCUSSION

The problem of identifying when there is statistical evidence of assessor effects in the results from discrimination tests has been tackled by :

- testing the actual distribution of assessor numbers correct against the distribution predicted by the Binomial model using the χ^2 goodness-of-fit test and
- by Randomisation tests.

It is shown that the former approach is relatively insensitive because of the difficulties in handling small number of assessors. A more effective method of tackling this problem is shown to be a Randomisation test using the sum of squares of the number of correct tests for each assessor as the test statistic. Although, asymptotic distributions can be derived the small number of assessors and integral nature of the data make Randomisation tests a more secure option.

The end point of a triangular or other discrimination test is to be able to give a point estimate for the proportion correct together with the 95% confidence interval. We have derived these figures (Table 2) by a number of different routes.

TABLE 2. Point estimates of proportion of tests correct ($\hat{p}$) with a 95% confidence interval (95% CI).

	Experiment 1		Experiment 2		Experiment 3	
	$\hat{p}$	95% CI	$\hat{p}$	95% CI	$\hat{p}$	95% CI
Simple Summaries	0.4722	0.4206-0.5238	0.4062	0.3495-0.4629	0.5326	0.4737-0.5915
Binomial Analysis						
Without Correction	0.4723	0.4209-0.5242	0.4064	0.3511-0.4641	0.5327	0.4735-0.5910
With Correction	-	- -		0.3305-0.4870		0.4419-0.6214
Brockhoff & Schlich	0.4722	0.4207-0.5238	0.4062	0.3176-0.4949	0.5326	0.4297-0.6355

Clearly confidence intervals which ignore assessor effects are misleading when such effects exist. Our initial calculations based on modelling assessor results using a Generalized Linear Model suggest that the Brockhoff and Schlich (1998) method is very conservative.

8.7. CONCLUSION

Given the importance of discrimination tests to food scientists in industry, particularly the triangular test, it is vitally important that statisticians with an interest in this area of application, provide sound advice using up-to-date statistical methods.

Although several authors have tackled this problem only Brockhoff and Schlich (1998) provide detailed advice to sensory scientists.

Initial results from this study suggest that their strategy is extremely conservative. Much work requires to be done to identify assumptions made in this and in other potential strategies from the literature.

CHAPTER 9:

DISCUSSION

9.1. FLAVOUR PERCEPTION OF SCOTCH WHISKY

Scotch whisky dominates (*ca.* 93% sales by volume) the international brown spirits market (UK Food & Drinks Report, 1999), with a distinctive sensory character. The category Scotch has a unique identity that generates an active expectation in consumers based on a perceptual assimilation (Kanizsa, 1994) despite variations in product characters. Retrieval of such active expectations is a typical facet of human experience (Kahneman & Tversky, 1982).

When product quality does not match a consumer's expectations (disconfirmation process), there is a post-exposure evaluation. Holistic conceptualisations of products are general in consumers but on an analysis flavour is deconstructed into single flavour attributes. Often there is a passive expectation, that acts in evaluating whether a product quality, or character, is as expected, or alternatively is satisfactory (positive disconfirmation) or unsatisfactory (negative disconfirmation).

Disconfirmation effects originate in associated cognitive emotional experiences (Oliver, 1977) where there are domains that effect brain processing at the macroscopic level. Relationships in product evaluation between cognitive macroscopic (*perception*) and the energetic microscopic (*sensation*) levels have been extensively studied, primarily in cognitive neuroscience. A guiding principle seems to be that there is linkage between coherent events at the neuronal ensemble level and at the macroscopic behavioural level (Kelso *et al.*, 1991). This is by through shared dynamics, not because any single level has ontological priority over any other (Kelso *et al.*, 1991). Such relationships have been described as slaving processes with: behavioural patterns, percepts and other mental processes represented by order parameters (collective process, central integration). Although such systems are at the macroscopic level and make possible, for instance, human communication, at the same time there is activity at the micro-system level, e.g. the neurones. Such order parameters have features as predicted by Gestalt theory notably a resistance to deformations and damage (Haken, 1990).

Although sensory evaluation of whisky must be related to psychophysics, such relationships have not yet been studied extensively. Cognitive behaviour during evaluation of products by consumers has been studied (e.g. Oliver, 1977; Yi, 1990) but relationships between sensory analysis and cognitive self-organisation processes in terms of assessor, behaviour have not been clearly defined. This process must be relatable to congener data obtained by chromatographic analysis of whisky headspaces. Hence, a greater understanding of the underlying processes determining decision making, which must be self organised during sensory assessment would be predicted to enhance panel performance and yield improved data sets.

9.2. WHISKY FLAVOUR WHEELS

Strategies of product evaluation differ widely between consumers and sensory assessors in terms of attributes (intrinsic or extrinsic) and vocabulary employed. In consumer views, the influences of extrinsic attributes are strong in product perception; whereas intrinsic attributes are central to data from sensory assessors. Uses of vocabularies differ in that consumers employ more abstracted and subjective terms and fewer terms than sensory assessors (O'Mahony, 1990; Andani *et al.*, 2000; Cristovam, 2000). The two terminology categories have been integrated in revised flavour wheels for the whisky distilling industry (Fig. 2.3) and consumers of Scotch whisky (Fig. 2.4). The consumer wheel could be of potential benefit for stabilising product images and relating this to perceptions of quality, producing enhanced mental references. These mental reference frames can also be closely linked to the extrinsic attributes such as advertisement, package, price and social factors (Fig. 2.1).

Language for describing drinks products is flexible (Meilgaard *et al.*, 1979; Piggott, 1991), and the initial terminology of Shortreed and his coworkers (1979) has evolved. This can occur with turnover of assessors in a sensory panel and changes in the industry. When the original wheel was developed, the majority of terms were derived from and assessed by blenders and other technical professionals who possessed a certain level, or knowledge of whisky character and production. However the needs of quality assurance and new product development have been reflected in sensory practice in the Scotch whisky industry. Currently sensory assessors can be technical and also administrative staff, with little product knowledge. Social and cultural changes are also reflected in concept of attributes. For instance, the terms previously familiar are now no longer meaningful (e.g. *coal gassy*, *carbolic*, *new cowhide* and *iodine*), too specific (*graphite*, *putty*, *beeswax*, *mutton fat*) or do not reflect current UK culture (*tallowy*) (Canaway *et al.*, 1984). The revision of flavour wheels substitutes less formal terms for technical and chemical attributes (e.g. *phenolic*, *aldehydic* and *estery* by *peaty/smoky*, *grassy*, *fruity* and *floral*, respectively).

In this study a number of reference compounds were tested as flavour standards. These were initially extracted from the published literature (including sensory and instrumental) and contained flavour components important in whisky character. Flavour terms from individual distillers were compiled and summarised (Appendix I). The terms included in the consumer wheel were based mainly on attributes derived from Guy *et al.* (1989) and Shortreed *et al.*, (1979). Currently, terms extensively used by blenders were listed in Appendix I. Professional blender attributes were found not only too subjective but also possess abstractive underlying meanings: *false* – does not taste as whisky is expected to be; *classic* – typical of best accepted quality; *iodine* – the interpretation of intensity of flavour, not tasting of iodine; *commercial* – a product style that meets a marketplace requirement and will sell.

An updated whisky flavour wheel was recently published by McLean (1997) in *Whisky*. This consisted of terms, largely collected from connoisseurs, but many are esoteric or too specific for objective conceptualisations by assessors in sensory panels. Other terms placed in categories were not relevant to flavour origins. For instance *cooked maize* a typical term derived from a sulphur impact compound (dimethyl disulphide), was placed in the sub-category with *cereal* and it may not be appropriate as malt mash is not boiled. The absence of certain flavour references also limits the value of this wheel for industrial application.

There has long been a need for revision of the flavour wheel for marketing purposes achieved in this study. Important factors were substitution of technical terms for non-technical with inclusion of abstractive terms and reformat of the structure of the wheel so that it would become meaningful to marketing professionals and consumers. Industry comments regarding negative terms were taken on board in separation of the wheel into two sections: the right for positive and the left for negative attributes. Negative attributes were removed for consumer usage. Order of attributes was retained in a similar to reflect origins in production and facilitate understanding of the origins of individual attributes.

Generic terms *sweet – wood, fruity, floral* and *buttery* can be related to consumer abstracted terms for representing maturity. However, *malty*, used by consumers and industry, may represent different concepts. In technical and industry usage it is applied to immaturity and linked to *grainy* in the revised wheel. For consumers *malty* can be related to maturity and included with other abstractive terms linked to premium quality (and price). Further studies are required to clarify this issue.

9.3. EVALUATION OF REFERENCE COMPOUNDS

Individual assessors possess different presages of products, or past experience and expectation thus processing of flavour perceptions also vary. It is an accepted problem in descriptive sensory analysis that assessors may perceive a flavour note in a similar manner but describe it differently (Jack & Piggott, 1993). However, during evaluation of reference compounds, assessors not only described certain flavour notes differently, but also appeared to perceive nature of characters differently (*e.g. grassy* vs *almond* and *sweaty* vs *fruity*) (Chaper 4). Interpretation of major differences can be explained either from the integration in macroscopic level (perceptual factors) of perceived flavour or from the microscopic level differences in detecting flavours (sensations). The clear linkage of the microscopic level to sensory evaluations can be supported in sensations such as an anosmia in *iso*-valeric acid that results in assessors perceiving *sweaty* as *fruity*. Perception of *woody* related *spicy* notes was not clear with the potential reference compound (4-vinyl guaiacol). Other wood extractive compounds such as eugenol could be more appropriate for developing the concept related to the attribute. However, as eugenol is abundant in Bourbon, prior to application as a reference in Scotch whisky the relationship between concentration and flavour note should be explored thoroughly.

The reference compounds (Table 2.1) used were from experimental results (Chapter 3 and 4) or as recommended elsewhere in the literature. Development of a set of reference compound in cyclodextrin formulations, as is successful in the

Brewing industry, could improve a quality of data from sensory panel and of retailed products.

9.4. MEASUREMENT OF THRESHOLDS

Formulation of reference compound in cyclodextrin-linked forms has gained acceptability in the brewing industry. In this present study five cyclodextrin-linked compounds; diacetyl, DMTS, ethyl hexanoate, *iso*-valeric acid, *iso*-amyl acetate and 4-vinly guaiacol, were used in threshold determinations (Chaper 3). In evaluation of 90% recognition threshold in 6 different spirits (Scotch grain, Scotch malt, Bourbon, Canadian, Japanese whiskies and Rum) that the six cyclodextrin complex compounds did not yield 90% recognition level (unpublished results). This suggests values for flavour thresholds for these compounds may be influenced by purity, but more likely by matrix effects (Harrison & Hills, 1997).

9.5. DISCRIMINATION OF SCOTCH BLEND CATEGORIES

Unlike malt whiskies, discrimination of blends has not been extensively studied. The product space drawn from this experiment was similar to those of Piggott and Canaway (1981) in an analysis of twenty Deluxe and Standards whisky blends. Similarities in overlap between Deluxe and Standard category indicated product category could not be related directly to flavour character. In that previous study also showed apparent discrimination of export products from the major cluster of blends for domestic markets. In this study, clear discriminations between Deluxe and Retailer blends were apparent.

Terms from the revised wheel were used in discrimination of Scotch blends of different categories: Deluxe, Standard, Retailer and West Highland blends (Chapter

5). Successful discrimination of blends of Deluxe and Retailer categories based on attributes for mature (*fruity*, *buttery*, *malty*, *nutty*, *vanilla*, *floral*, *sweet* and *smooth*) and also immature characters - *solventy*, *soapy*, *rancid*, *grassy*, *mouldy*, *pungent*, *grainy* and *oily*. There was also clear differentiation between *smooth* and *pungency* reflecting the efficient use of terms. *Buttery*, a sub-category of *oily* characteristic (Fig. 2.3) .in the original wheel, showed close linkage to *sweet* character. The relationship between these attributes was also revealed from evaluation of reference compounds (Table 4.1). It would be advantageous to place *buttery* under generic *sweet* category in the revised wheel.

Pungency is generally known to be related to ethanol strength, and also activity of ethyl esters in whisky headspaces (Withers *et al.*, 1995). However, the relationships of *pungency* to *smoky/ peaty*, *medicinal*, *woody* and *spicy* showed *pungency* related to content of peated malts and cask charring. Previous analysis of *peaty* character in Scotch malt whisky (Withers *et al.*, 1996) also demonstrated that attributes related to phenol compounds were *phenolic*, *pungent* and *woody* but no causative relationship was demonstrated. *Pungency* appeared closely related to *smoky/ peaty* character (phenol concentration) in whisky. Again further work should clarify this issue.

Maturity is evaluated as *smooth* and wood extractive congeners (e.g. vanillin and lactone) inducing *sweet* characters. However, the term *woody* itself was discriminated from characters and perceived similar to *pungent* and *smoky/peaty* characters in product spaces (Chapter 5). Such relationships have also been observed in studies of malt whiskies (Swan & Howie, 1984; Withers *et al.*, 1996).

Classification of blends depends upon proportions and an extent character of malts in the blend, maturation period, and effectively final retail price. Since there is inclusion of a high proportion of malt in Deluxe blends, it would be predicted that Deluxe blends should show more mature characters than cheaper Retailer whiskies. Clear separation of Deluxe and Retailer blends stressed the role of intrinsic sensory attributes in product quality. Negative and immature attributes were mainly *solventy*, *soapy* and *rancid* for Retailer. Positive and matured characteristics were *fruity*, *buttery*, *malty*, *nutty* and *vanilla* in Deluxe blends. In the factors central to the product space West Highland was intermediate linked with *woody*, *smoky/peaty*, *spicy* and *medicinal*. This suggests a well-defined linkage between intrinsic product attributes and proportion of strongly peated malts. However, Standard blends were dispersed and showed *oily*, *sulphury* and *rancid* characters. Since the blend categories were mainly defined by retail price, dispersion of Standards blends reflects a possible mismatch between intrinsic and extrinsic attributes in determining product quality. Even if there are major differences in intrinsic character in Standard blends, all were of the same category suggesting extrinsic attributes such as brand name, advertising, label or packaging may play more important roles (Fig. 1.1) in establishing consumer constructs for products. In future work, it would be interesting to see how blends would be categorised if extrinsic and intrinsic attributes were assessed together.

In soft modelling PLS2 analysis (excluding Standard blends) there was clear separation of Deluxe and Retailer categories in product spaces from both: sensory and headspace congener data. This indicated that attributes related to mature character of Deluxe blends in *fruity*, *buttery*, *malty*, *nutty*, *vanilla*, *floral*, *sweet*, and *smooth*, linked ($p<0.05$) to headspace congeners of ethyl (C6 – C10) esters including *iso-amyl hexanoate*, *ethyl 9-decenoate* and *iso-amyl alcohol*. Attributes describing immature characters in Retailer blends were *solventy*, *soapy* and *rancid*, closely linked ($p<0.02$) and longer (C12 - C18) aliphatic chain esters and alcohols including unsaturated fatty acid esters - *ethyl hexadecenoate*, - *octadecenoate*, - *tetradecenoate* and *propyl decenoate*. Such congeners have been reported as conferring *soapy*, *oily*, *sour* and *feinty* notes to whisky (Conner *et al.*, 1994a,b).

From PLS1, the prediction performance (r^2) of individual attributes showed that characteristic differentiation of ethyl esters and alcohols according to aliphatic chain lengths. Since many whisky flavour studies have related to maturation (Conner *et al.*, 2000) or phenolic compounds (Swan & Howie, 1984) with its related flavour character, individual contributions of esters to whisky flavour and its role to differentiation of products were not studied extensively. It also has been concluded by Canaway *et al.* (1984) that medium-chain alphatic esters (C6 - C10) were not linked to any of sensory attributes. Differentiation of esters in relation to sensory character has been explored by PLS1 (Table 7.1).

There was also a clear linkage between West Highland blends and a specific group of headspace congeners ($p \leq 0.041$) *furfural*, *iso-butyl-octanoate*, *ethyl benzoate*, *ethyl nonanoate* and *9-decenoate*. Such a linkage was observed in PCA, DPLS1 and 2 for *ethyl benzoate* and *iso-amyl alcohol* with *woody*, *smoky/peaty*, *spicy* and *medicinal* notes.

There was an agreement of the three PLS regressions: PLS1 and DPLS 1 and 2. The headspace congeners related to *woody* and *medicinal* attributes in PLS1 (Table 7.1) showed similarity with West Highland blends from DPLS1 (Table 6.8). West Highland blends can be discriminated from other categories with high proportions of *furfural*, *iso-butyl-octanoate* and *ethyl benzoate*. In soft modelling relationships with pertinent sensory attributes, regression coefficients were low at 0.551, 0.471 and 0.498, for *medicinal*, *smoky/peaty* and *spicy*, respectively. These suggested that other congeners might make major contribution to these characters.

9.6. DISCRIMINATION TESTS

A number of different discrimination tests and analyse methods have been described. Commonly, two sample (Duo-trio and 2-AFC) and three sample comparison (triangular and 3-AFC) tests are exercised. Significance tests using the Binomial distribution table (e.g. Roessler *et al.*, 1978) are widely employed in industry to analyse discrimination test results. Since the conventional data methods largely ignore Type II errors (erroneously, no difference) and number of assessors are insufficient to achieve an acceptable level of discriminability. There has also no information on sensory difference between two tested samples as from a d' values.

Since measuring sensory differences (d') is not common, it might be difficult to apply the method for decision-making routinely in industrial situations. A routine procedure would be: calculate d' values and their confidence intervals for each sessions from records, then define, the significance of multiple d' values (Bi & Ennis, 1997). Once a d' value and its confidence interval have been defined - in terms of a tolerable d', this can be included as a product specification. This procedure would make more sense in industrial practice than simple significant triangle tests, with results are very dependent on number of assessors (Piggott *et al.*, 1998). In specific circumstances, number of assessments required can be predefined to achieve an estimation of σ with pre-specified precision (Bi & Ennis, 1997). However, a limited in-house panel with assessors used more than once, requires different approaches to data analysis. In solving the heterogeneity in a data, two methods (Brockhoff & Schlich, 1998; Bi & Ennis, 1999) provide a practical and simple application for sensory analysts.

Even if, an analytical method is strongly supported by a statistic theory, the accessibility to sensory analysts is often limited in industry. Either a paper published can be too difficult to understand at users level, or sensory analysts are not trained or not sufficiently adventurous for changes in practice. Applying advanced experimental and analysis methods focused on the methods using tables and examples should be contemplated in the future. Whichever method - familiar and suitable for a given situation - is adapted, calculation of sensitivity (d') of products should be included in product specifications. The key factor that should be emphased is that the proportion of discriminators is task dependent, while d' values are not.

CHAPTER 10:

CONCLUSIONS AND FURTHER RESEARCH

This research yielded two revised Whisky flavour wheels for current industrial and marketing applications. The formal vocabularies used by sensory panels differed from professionals employed in the distilling industry. The collected consensus terms were mostly non-technical language in daily use. In contrast blenders used terms that were not only subjective but also possessed abstractive (perceptive) meaning. To understand blender meanings of terminology better, the origins of flavour attributes and their *values* can be related to thinking of individual blenders. When terms are subjective, clear understanding of the psychology of perception is required for interpretation. Fundamental understanding of cognitive psychology could reduce the gap in understanding between professional blenders and industrial sensory panels.

Objective sensory assessment is required in the distilling industry at this time. To achieve common conceptualisation of attributes; frequent use of the Whisky Flavour Wheel is required in combination with use of different concentrations of the recommended reference compounds. Trial with reference compounds should be extended and training improved through discussions. This could lead to identification of improved reference compounds for specific attributes. Potential application of the marketing flavour wheel could be setting sensory quality standards for whisky used in labelling to promote products to consumers. Combining of sensory science and consumer perception could also make possible development of flavour standard kits.

This study provides a foundation for developing appropriate training reference compounds in cyclodextrin-complex forms that will facilitate focus on with better, experimental design and effective panel management for sensory analysts.

Discrimination tests in the distilling industry can now be amended on the basis of modern statistical theory. To clarify clear sensory discrimination between products, conventional significant tests should be substituted by d’ value. Other assignment supplementary values such as proportion of true discriminators, confidence interval, power of discrimination and dispersion of heterogeneity in replicated tests should also be considered. Quality management and sensory analysts in industry should promote methods that can easily adapted, based on a more solid theoretical basis. It can also be expected that International Organisation for Standardisation (ISO) should provide a new amendment for discrimination test protocols.

Study of categorisation of blended Scotch whiskies showed wide dispersion of Standard blends in both sensory and headspace congener analysis. Intrinsic attributes not only influence product categorisation but can be used to support extrinsic attributes. Clear discrimination between Deluxe and Retailer blends from both sensory and headspace congener data demonstrated for the first time the critical role of maturation in product discriminations. Soft modelling of the two data sets showed that Deluxe characters - *floral*, *vanilla*, *nutty* and *woody* were predicted effectively with high power (r^2); *soapy* was predicted in Retailer blend character. From PLS1, correlations between sensory attributes and headspace congeners can be explored. The influences of different ranges of aliphatic chain esters and alcohols on sensory character can clarify much of the problems in use of sensory training standards in the distilling industry. Matrix effects can explain good correlations between certain sensory attributes and non-volatile concentrations in solutions (Conner *et al.*, 2000; Cristovam *et al.*, 2000) and failures in predicting character from GC data. However, whisky matrix effects and partitioning of flavour congener into headspace require further study before it will be possible to obtain good correlations between sensory data and whisky congener concentrations. In addition, on ingestion, whisky evokes a temporal progression of flavour notes and the limited understanding of such time-intensity features (Piggott *et al.*, 2000) suggest a fertile area for future research.

CHAPTER 11:
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APPENDICES

Appendix I

Malt Act (1725)

In 1707 with the union between England and Scotland, English officials (Board of Excise) were established in Edinburgh and the English malt tax was imposed in Scotland at 50% of the English rate. This new malt tax caused riots in Glasgow (e.g. Shawfield riot) and Edinburgh. The ultimate effect was to reduce ale quantity, the staple drink of the populace, increasing consumption of whisky hence encouraging the development of illicit sitlls throughout Scotland.

Gin Act (1736)

To reduce consumption of gin, peculiarity English (London) problem, not in Scotland. The duties on spirits distilled in England and Scotland differed. As English distillers objected to the lower Scottish duty the Act of 1751 equalised duty on Scottish spirit exported to England but Scottish rates remained lower until 1855.

Wash Act (1784)

This Act formalised the line between Lowland and the Highlands. In the latter duty was to charge not on amount of whisky produced but on capacity of wash. Highland distillers were favoured thus with lower tax and allowed to use smaller stills (91 litres capacity). These were charged with weak washes and worked off slowly, producing flavour-some whisky. However, distillers were restricted to locally grown grains and forbidden to export spirit outwith the region. Lowland distillers responded to the Act by using stronger, higher grain content washes and developing a new kind of still (continuous still). This further impaired the quality of Lowland whisky. In Lowland the tax was 5d/gallon on the wash whereas the Highland all stills had to pay a license duty of 20s/gallon of still content.

Whisky Rebellion (1794)

The US Government employed a scheme of taxing distillers irrespective of whether stills were used for commercial or personal use in Pennsylvania. In combination with the lack of protection from the Government against continuing Indian raids, the scheme was taken as an insult and not tolerated. Riots between the Excise Officer and distillers, continued for six years before the full resentment of distillers from Pennsylvania, Pittsburgh, Ohio, Maryland and Kentucky lead to erection "liberty poles". Military action by the President, General Washington, used twelve thousand militia men, claimed to be more than used to defeat the British, and put down the Rebellion. The majority of distillers fled Pennsylvania for Kentucky after the Rebellion.

Navigation Acts (1845)

The Act opened up the export market to the colonies and dominions such as Canada, India, New Zealand and South Africa.

Gladstone's Spirit Act (1860)

The Act raised duty 10d/gallon, but allowed blending in bond, which leads to a dramatic increase in blending for the first time.

Proof (Daiches, 1969)

The Custom and Excise Act (1952) gave the following definition. "Spirit shall be deemed to be at proof if volume of ethyl alcohol contained therein made up to the volume of the spirits with distilled water has a weight equal to that of twelve-thirteen of a volume of distilled water equal to the volume of the spirits, the volume of each liquid being computed as at 51° F.

British proof is spirit 57.1% of alcohol by volume (abv) or 49.28% of alcohol by weight.

American proof is spirit contains 50% alcohol by volume at 60°F. British 100° proof is 114.2° or 14.2° over proof American (American 100° proof = British 87.7° proof). A bottle strength of Scotch whisky for American market is 76.2° proof corresponding to American 86.8° proof on the American standard of proof. Most Scotch whisky for domestic market is 70° proof.

FLAVOUR TERMINOLOGY OF WHISKY FROM THE SCOTCH WHISKY DISTILLERIES AND PROFESSIONAL BLENDERS

1. Scotch Whisky Research Institute

1st tier term	2nd tier term	3rd tier term
1. *Nasal effects*	*Pungent*	-
	Prickle	*Prickle*
	Nose-warming	-
	Nose-drying	-
2. *Peaty*	*Medicinal*	*TCP, iodine, carbolic*
	Burnt	*Cresote-like, burned wood, tar-like*
	Smokey	*Wood smoke, smoked cheese, kippers, etc*
	Spicey	*Clove, cinnamon, ginger*
3. *Feints*	*Leathery*	*New cowhide*
	Tobacco	*Fresh tobacco, stale tabacco-ash*
	Sweaty	*Beeswax, musky, piggery, stale yeast*
	Stale fish	*Aminey, scorched plastic*
4. *Cereal*	*Malted barley*	*Grain, husk, chaff, malt dust*
	Malt extract	-
	Cooked mash	*Maize cooker, mash tun draff, digestive biscuit, mealy, porridge, poultry-food*
	Cooked vegetable	*Cooking swede, boiled corn*
	toasted	*Burnt toast, roasted malt, cocoa, coffee*
5. *Aldehyde*	*Hay-like*	*Dry hay, herbal mown hay*
	Leafy	*Green leaves, lawn cuttings, crushed green bracken*
	fragrant	*Geraniums, green, tomatoes, violets, flowering currant, tom-cat*
6. *Estery*	*Floral*	*Perfumed, scented, rose-like, carnation-like*
	Fruity	*Banana, pear drops, apple lemonadey, winey*
	Solvent	*Paint thinners, nail varish remover, fusel oils*
7. *Sweet* associated	-	
8. *Woody*	*New wood*	*Sap-, pine-, cedar-like, resinous newly sharpened pencils, sawdust*
	Developed extract	*Vanilla, oaky, caramel, treacle, coconut*
	Sherry	*Sherry like, walnut-like, burnt rubber, polish*
		Dry-earthy, vanilla-like, scented
	Bourbon	
	Other woods/uses	*Rum-, brandy-like, fruit pulp, orange peel, aromas of other types of wood*
	Cask defects	*Sour associated, musty, fusty, paraffin, naphtha/camphor-like*
9. *Oily*	*Nutty*	*Almond, marzipan*
	Buttery	*Creamy*
associated	*Fatty*	*Unscented soap, tallowy, oiled-wood, mutton fat*

	Rancid	*Oxidised fats and oil*
10. *Sour* associated	*Sickly*	-
	Cheesy	-
	Vinegary	-
11. *Bitter* associated	-	-
12. *Sulphury*	*Stagnant*	*Drainy, rotten vegetable*
	Gassy	-
	Rubbery	*new rubber (tyres), pencil eraser*
	Cabbage-water	-
13. *Stale*	*Metallic*	*Inky, tinny, graphite, boot-polish, wet iron*
	Wet paper	*Filter sheets, cardboard*
	Musty	*Mouldy, damp cellars, old books*
	Earthy	*Damp soil, wet filteraid*
14. *Primary tastes*	*Sweet*	-
	Sour	-
	Salty	-
	Bitter	-
15. *Mouthfeel* effects	*Mouth coating*	*Oily-feel, creamy-feel*
	Astringent	*Mouth drying, furring, powdery*
	Mouth warming	*Mouth and throat prickle, alcoholic burn*

2. Distillery A

acidic, acrid (acrolein), antiseptic, appley, balanced, blackcurrant, bland, Bourbon, bran, bready, bubble gum, burnt, butyric, caramel, cardboard, chalky, chichen, chocolate, coffee, creamy, diry water, DMTS, drains, dry, dull, earthy, emulsion, estery, fatty, feinty, flat, floral, flowery, foul, fresh, fresh green bark, fruity, glue, grainy, green, harsh, heathery, herbal, immature, light, medicinal, metallic, musty, nail polish remover, nippy, nutty, oily, onions, paint odour, peaty, perfumed, peroxide, phenolic, plasticy, potato peelings, pungent, raisins, raspberry, rubbery, sawdust, sherry, sickly, soapy, solventy, sour, spicy, spiritous, stale, strawberry, sulphury, sweet, toffee, vanillin, vegetable like, vinegar like, warm, washy, watery, waxy, wet paper, winey, woody, yeasty, aftertertaste-dry, -sherry like, -sulphurous, -bitter

3. Distillery B

- *cereal, feinty, estery, aldehydes, metallic, earthy, oily, sulphury*

4. Distillery C

- *cereal, estery, ethyl alcohol, floral, leathery, nose drying, pungency, vanilla, woody*

5. Distillery D (grain distillery)

aromatic, biscuity, blackcurrant, burnt, buttery, cabbage water, cardboardy, cereal, citric, cod liver, cold tea, diacetyl, dirty, drainy, dried milk, earthy, estery, feinty, fishy, flat, fresh, fruity, garlic, gassy, green, green leaves, harsh, heady, honey-like, leathery, liquorice, metallic, mousy, nutty, oily, peaty, peppery, perfume-like, pineapple, rotten vegetable, rubbery, soapy, solventy, sour, sour-draff, spicy, stagnant, toffee, tom-cat, turnip, vegetable, wet-grass, woody

6. Distillery E (grain distillery)

- *clean, fruity, green/grassy, green/oily, meaty, metallic, nutty, peaty, perfumed, spicy, sulphury, sweet, vegetable, waxy*

7. Flavour Terminology of Whisky Used by Professional Blenders

Clean – No foul or foreign smells when smelt ("foreign" meaning "unusual and uncharacteristic").

Hard – You can smell hardness. It is not soft or velvety, and strikes one as being "hard", and it attacks the back of the nose.

Neutral - Smells of very little.

Bland – Slightly loose/flabby without much character.

Burnt – whisky is often put in a cask, which has been fired, leaving a slight tast of burt oak.

Aggressive – High acid/tannin content.

Short – This refers to the length of time the taste stays on the palate. The longer the flavours last, the better the quality.

Deep nose – The depth of flavour when you smell it.

Full flavour – Over-powering and fills the nose and mouth.

Typical Skye – An iodine/seaweedy taste, as commonly produced in whiskies from the Isle of Skye.

Rounded – Balanced, ie the components are balanced.

Soft – Doesn't attack the nose or palate when smelt or drunk.

Highly flavoured – A big/hugh flavour, stronger and more pungent.

Concentrated – Relates to the strength of flavour. Flavours all packed in.

Distinctive – A penetrating flavour, individuality.

Rich – Concentrated – richness determines quality concentration.

Far – Similar consistency to oil.

Sour – Acid with a slightly foul taste.

Bung Rag smells – A sour smell of sacking. When a cask is closed, a piece of sacking is put across the opening before the bung is banged on.

Very little – Very little bouquet.

Sweet – Fruity sweet, not sugary sweet.

False – Doesn't taste as whisky is expected to taste – not good.

Smoky – Burnt wood smoke.

Classic – Typical of the accepted best quality.

Light – Not very much palate or pungency.

Vanilla – The smell of vanilla which comes from the oak of the barrel.

Mid flavour – when something hits the palate, the flavour spreads out until it drops off the end. Sometimes there is a gap in the palate where the taste dips. The middle of the extended palate length.

Medicinal – Smells of chemical matter, and not natural matter.

Iodine – The interpretation of an intensity of flavour – not one that tastes of iodine.

Youthful – Tends to attack the nose – fiery.

Firm – Opposite to flabby. Smell gets you instantly – direct.

Apricots – Smell of real apricots.

Feminine – Light, stylish and elegant.

Bouquet – Smell.

Bright – the visible condition.

Fresh – Clean and bright.

Bottle smells – Smells as if it has been in a bottle for a considerable time.

Commercial – A style of product that meets the requirement of the market and will sell.

COMMENTS FOR THE REVISION OF THE WHISKY FLAVOUR WHEEL

– letters from distillery sensory members

1. **Distillery A**

- If the wheel to be revised for a small group of technical experts within the industry, then only the experts may benefit from the increased understanding of whisky chemistry and flavour. There will therefore be no major benefit to the marketers or eventual customers.
- One member prefer to use the vocabulary learned with United Distillers.
- One member utilises the systematic flavour terminology sheet produced by Pentlands which gives first, second and third tier terminology.
- The writer has found some use in the wheel but has not in recent times referred to it when nosing.

2. **Distillery B**

- We are very aware of the whisky wheel and are actively working on modifications to it ourselves. We feel in it's present form it has too many attributes for panellists to score against. Our aim would be to reduce the number of attributes to about nine.
- We hold training sessions with our panellists to encourage common language development, this is time consuming and it is not always easy to get representative characteristic odours. The wheel still provides a very good basis for discussion.

3. **Distillery C**

The main consensus of opinion is exactly what I envisaged and can be broken down into 3 areas:

- Two distinct wheels – one for industry use and one for distributors using commonly understood terms both for new and matured whiskies
- The terminology used to describe aged whisky characteristics is different to standards and there should be provision for this
- This one I disagree with but am passing it on – "Devise a system of comparison where whiskies are rated on their weight of flavour"

4. **Distillery D**

- Our grain distillery does not use the flavour wheel as the basis for the descriptors for the monitoring of our spirit
- Because we only make grain spirit and regularly monitor only the new make, we use fewer descriptors than are necessary for the description of malt whisky and maturing whiskies
- We do not encourage our sensory evaluation panel to use non-speicific terms. Rather we allow only a limited number of chemical terms e.g. *ethyl acetate* or specific terms which describe an odour such as "B" odour
- We feel that the existing wheel employs a number of terms which make it difficult to use when discussing spirit with people from outside the industry. Terms like *sickly, sweaty, stale fish* and so on should be avoided

5. **Distillery E**

- Since I do not have chemical or biochemical background, I approach whisky form the production side. However, I seem to remember the terms unhappy with such as *sickly, sweaty* etc should be noted.
- This is where the conflict arises. Is the whisky wheel to be re-designed to meet the purposes of the trade of the terminology sensitive marketing departments? Are you planning to make the whisky wheel more generally available to the public and encourage its use?
- I would like to offer some constructive thoughts on the *estery* sector of the whisky wheel. My threshold for this sector appears to be quite low and this is the region that I always pick out first. I would suggest that the *fruity* sector could be expanded or

separated into two sections. The first would be the *artificial* aromas that can be readily identified chemically and also descried such as *ethyl acetate, iso-amyl acetate* and *ethyl caproate.* The second section could include (slightly subjective) aromas such as *blackcurrant, melon, kiwi, mango* and so on. I acknowledge that these are abstract terms but they could be demonstrated and learned.

6. Distillery F

- It was always the plan to produce a commercial wheel for particular use in the market place by sales and marketing personnel. As you know some of the descriptions on the wheel like tarry and sweaty etc. would be misleading to the general public.
- A section of the wheel to related to new malt spirit, new grain spirit and blended whisky. I think the wheel at present certainly covers the general spirit quality very well but could be more specific on the above categories.
- Obviously with the wood maturation chemistry being carried out running in parallel with the sensory analysis, there must be additional terms, which can be introduced to the wheel which would give an indication of good versus poor quality maturation in casks.

7. Distillery G

- I use both the sensory training and the wheel on many occasions. May be it is due to the lack of skill on my part but I have found the flavour wheel to be excellent and cannot think of any area for improvement. However, I look forward to seeing the new revised wheel.

QUESTIONNAIRES

Q.1. What other terms, apart from reference compound presented, are necessary to describe Scotch whisky characters?

- *Acetal, biscuity, Boubon, bracken, bready, burnt tyre, butyric, cabbage, candles, caramel, catty, cereal, chocolate, clean, cloves, colour, creasol, earthy, fatty, feints, fish oily, floor polish, ginger, grainy, green, green bark, honey, husky, leafy, level of maturity, Malt, mashy, meaty, metallic, musty, Musty, Napthalene, nutty, oak vanilla, oily, overall flavour balance, pear drop, perfumed, Phenolic, potable, potty, raisins, resin, rubbery, scented soap, sherry, smoky, sour, sour wet wood, spanish oak, swampy, TCP, tomato sauce, tomato stalk, vegetable, washy, waxy, wet wood, wine-like, woody*
- wider vocabulary would be preferable to be able to distinguish subtle differences between samples

Q.2. Any alternative choices of compounds?

- *musty*: mysetylene
- *nutty-almond*: benzaldehyde
- *cheesy*: *n*-butyric acid
- *catty*: 4-methyl thiazole
- *violet*: α-ionone

Table 1. Flavour Perception of acetic acid from industrial consensus

	Attributes	Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
Vinegary	***vinegary***	2	2	4	2	3	2	-	-	6	4	3 (2)	2	9 (1)	1 (2)	56 (64)
Acetic	***acetic /acidic***	2	1	2			1	1	1	1	1	2 (1)	2 (1)	9 (1)	1	33 (38)
	sharp	1														
Sour	***sour***	3			1	2	1 (1)		(1)	1	2		1			17 (19)
	ketchup												1			
Rancid	***sour***				1					1	1					4
	rancid									1						
	sweaty				1						1					
Solventy	***solventy***			1										2		8
	floor wax								1							
	emulsion														1	
	paint															
Woody	***oak lactone***						1					1		1		8
	spicy													1		
	sawdust											1				
	sweet/estery/													1		
	pear drop															
	resinous										1					
Others	***smoky***											1				10
	acetone		1													
	diacetyl													1		
	stale														1	
	cardboard														1	
	dry		1													
	salty													1		
	sulphury					1										
	acetaldehyde						1									
	earthy										1					
Like-control	***like-control***												1			2
	none												1			

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 2. Flavour Perception of *Diacetyl* from industrial consensus

	Attributes	Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D1 4 (3)	14 (72)
Diacetyl	***diacetyl***		1	1			2		1			2 (1)	2	6		21 (22)
Buttery	***buttery***	1		1	(1)	2	2		1	1 (4)	6	1 (1)	2 (1)	5 (1)		31 (42)
Vanilla	***vanilla***	1		1	2	1				3		3		2		22
	creamy	1	1											2		
Sweet	***sweet***	1	1	1	3	2						1	4	3		47
	caramel									1		1		2		
	toffee	1	1	1	1	1	2	1		1	1		1	4	1	
	fudge															
	hot tablet														1	
	chocolate								1							
	burnt sugar							1								
Woody	***bourbon***	1					1									7
	woody									1		1				
	malty			1												
	cedar wood													1		
Others	***musty***	1		1												19
	earthy	1														
	dusty											1				
	metallic					1										
	meaty													1		
	sulphury													1		
	almond															
	marzipan															
	sour															
	feinty														1	
	leathery															
	oily					1					1					
	fruity															
	red wine															
	burnt wood								1							
	unpleasant															
	soapy															
	green / leafy										2					
Like control	***like control***	1														4
	none													2		

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 3. Flavour Perception of DMTS from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
DMTS	*DMTS*											5		5		14 (15)
Sulphury	*sulphury*	1	2	2	1	1	1 (1)		1	2	3	2	4	11	1	53 (54)
	gassy	1	1	2	2									2		
	burnt match					1					1			1		
	cooked veg.						1				1					
	dull/rotten veg.							1		1	1			1		
	eggy									1				1		
Rubbery	*metallic*									1						30
	rubbery			3	1	1	1		1	1	1	1	1	1	2	
	leathery									1						
Sour	*sour*					1				3	1	1	1	1	1	30
	rancid		1													
	acetic acid	1											1			
	stale		1													
	pickled onion								1							
	sharpness	1														
	cheesy					1										
	sweaty										1			1		
	butyric													1		
	sick															
Dirty	*dirty*															14
	musty	1		1			1									
	damp															
	stale	1			1											
	drainage						1							2		
	sewage															
Others	*cardboard*	1														14
	meaty														2	
	acrid							1		1	1					
	papery	1														
	soapy	1														
	biscuity	1														
	phenolic							1								
	oily										1	1				
	smoky													1		
Like-control	*like control*										1					1

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 4. Flavour Perception of *Ethyl hexanoate* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 . (72)
Fruity	*fruity*	2		2	2	1	2			2		3	1	3	1	39 (49)
	apple		2 (1)				1 (1)		(1)	(2)		(2)				
	orange	1						1								
	citrus							1	1					1		
	acid			1												
	pine apple								1							
	pear drop									1						
	cherry liqueur											1				
	winey			1												
Estery	*estery*			1		1	1	1				3		1		13
Aniseed	*aniseed*	1	1	1		1	1				2		1	2		14
	linseed oil									1						
Sweet	*sweet*			3	1	1			2			1	1	3	1	24
	liqcurice	1			1		1							1		
	sharbet						1									
woody	*woody*										1			1		8
	aromatic						1									
	clove													1		
	spicy															
Floral	*floral*						1									3
	cheap perfume										1					
	soap										1					
	violet										1					
Green	*green*														2	3
	pine													1		
	aldehydic	1														
Polish	*polish*									1		1		1		16
	paint									1			1			
	solvent	1												1		
	oily										1					
	fusel oil													1		
	greasy										1					
	TCP										1					
	plastic													1		
Medicinal	*medicinal*						1									11
	cough mixture			1												
	rubbery			1												
	leathery									1						
	phenolic									1						
Damp	*damp wood*									1						3
	musty															
	mushroom		1													
Sour	*stale*														1	14
	cheap white wine													1		
	sour	1														
	feinty													1		
Others	*airy*															
	light									1		1				
Like control	*little difference*													3		4

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 5. Flavour Perception of *Ethyl laurate* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
Soapy	*soapy*		(2)	2	2	1	1	1	2	1	(1)	1	1	7	2	36
					(2)	(1)						(2)	(3)	(1)		(53)
	shampoo					1										
	detergent						1									
	fresh				1											
	clean				1								1			
	perfume													1		
	scented														1	
	chloroform	1														
	disinfectante													1		
Green	*green*					1										7
	grass									1						
	leafy					1										
	pine													1		
	aldehydic	1														
Acid	*acid*			1										1		8
	sour										1	1	1			
	citrus						1									
	fruity						1									
	pear						1									
Floral	*floral*					1								2	1	6
Estery	*estery*										1					1
Damp	*damp*									2						19
	off-smell												1			
	stale	1				1				1		1	1			
	cardboard	2														
	papery	1														
	dirty still			1											1	
	musty											1	1	2		
	fungal															
Dull	*dull*	1												1		10
	flat	1												2		
	bland			1								2		1		
	weak											1		1		
Feint	*feint*		1								1					11
	waxy								1							
	oily										1					
	fatty					1										
	coal									1						
	gassy									1						
	sulphury										1					
	fusel fume											1				
Peaty	*peaty*			1							1					10
	smoky			1									1			
	burnt													1		
	leathery									1						
	plastic				1											
	metallic		1													
Others	*woody*		1									1	1			10
	cereal								1							
	sawdust						1									
	sharp													1		
	acrolein													1		
	peppery													1		
	acrid										1					
Like-control	*little difference*													1		1

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 6. Flavour Perception of *Furfural* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
Marzipan	*marzipan*	3		3	1	3	1	1			1	1	1	4		54 (57)
	coconut		2						1		1			3		
	cake mix												1			
	almond	1		1	1		3	1			2	3	1 (1)	1	1	
	nutty					1						1		1		
	walnut oil						1									
	castor oil											1				
	courmarin-like										1					
Sweet	*sweet*	2				1		1		2	2	1	1	5		26
	caramel													1		
	buttery													1		
	eucalptus								1	1						
Vanillin	*vanilla*	1								1		1		4		10
Woody	*woody*			1							1			2		6
Spicy	*spicy*										1			1		8
	cinnanom			2						1						
	minty								1							
Biscuity	*biscuity*					1					1					6
	cereal									1						
	grainy														1	
	cardboard									1						
Grassy	*grassy*										1			1		4
	hay-like									1						
Oily	*oily*	1				1	1							1		15
	wax polish				2					1				1		
	nail vanish remover	2														
	solvent													1		
	benzaldehyde	1														
Rubbery	*rubbery*													1		6
	plastic													1		
	smoky											1				
	metallic		1													
Estery	*estery*			1												3
Floral	*floral*					1										4
	fragrant												1			
	perfumed												1			
Others	*fruity*												1			4
	sour						1									
	dry											1				
Like-control	*like-control*									1						1

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 7. Flavour Perception of *Geraniol* from industrial consensus

Attributes		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	%total 14 (72)
Geraniol	*geranium*	1	1	(1)	1					1		1		1		8.3
Floral	*floral*	1	2		1	2 (1)			1	3 (1)	3 (1)	1 (2)	2 (1)	1		36 (47)
	fragrant	1				1										
	rose-petal										1	1				
	rose-water										1			1		
	bloosom	1														
	odd fellow									1	1					
	lilac										1					
	lavender													1		
	parmaviolet													1		
	violet													1		
	perfumed		1										1 (1)			
Soap	*scented*										1		1	1		21
	soap				1	1								3		
	fresh									1				1		
	air-freshner						1									
	washing up liquid		1							1				2		
	disinfectant					1										
	floor cleaner								1							
Lemony	*lemony*	1	1				2	1	1	1	1	1		5		25
	citrus				1		1	1	1					2		
	tangy						1									
	fizzy						1									
	cremola drink															
Green	*green*										1	1		2		8
	pine													2		
	aldehydic	1														
Estery	*estery*	1								(1)		1		1		5
Fruity	*fruity*					2	1				2	1 (1)	1	1		11
	banana					1										
Sweet	*sweet*			2		2				1		1		1	1	17
	burnt			1												
	caramel															
	toffee					1										
	sickly					1										
	turkish delight											1			1	
	vanilla										1					
Polish	*polish*									1				1		7
	teak oil													1		
	PVC									1						
	wax wood													1		
	tar			1												
Others	*aromatic*													1		7
	woody			1												
	peaty											1				
	sawdust			1												
like control	*like-control*			1								1	1			4

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 8. Flavour Perception of *Guaiacol* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
Smoky	*smoky*	1	1		1	3				2	2	2		2	1	39
	sooty		1													(46)
	peaty	1		1		1						1			1	
	burnt	1					1						1	2		
	burnt wood													1		
	kippery	1		1												
	fishy	1										1				
	smoked fish			2							1					
	smoked bacon			1							1					
Phenolic	*phenolic*	1			1		1		2	2		2	3	4	1	26
	carbolic							1				1				
	photo fix							1								
	hot bakelite	1														
Rubbery	*rubbery*												1	3	1	15
	plastic	1					1					1		1		
	scorched plastic	1														
	tar													1		
	leathery										1					
Medicinal	*medicinal*		1		1	1				1				1		19
	antiseptic			1						1			1	1		
	disinfectant									1				1		
	mothball										1			1		
	germoline													1		
	TCP		1				1			2						
	chemical												1	1		
Woody	*woody*		1			1								2		11
	new cut wood										1					
	matured spirit												1			
	spicy													1		
	sawdust										1					
	cardboard													2		
Vanilla	*vanilla*										2					7
	creamy													1		
	honey						1									
	buttery										1					
	sweet													1		
Oily	*oily*						1							1		14
	oliver oil									1						
	greasy						1									
	fatty											1		1		
	polish													1		
	solvent													1		
	paint thinner													1		
	meaty			1										1		
Rancid	*rancid*										1					8
	sickly			1												
	sour			1												
	cheesey					1										
	dirty										1					
	stale											1				
	sheep dip						1									
Others	*nutty*		1													11
	green			1												
	furfural						1									
	soap													1		
	linseed											1				
	sharp													1		
	sulphury													1		

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 9. Flavour Perception of *Hexanal* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
Grassy	*grassy*		1	1		1	1		1	2	2	2	2	3	2	26 (33)
	reed											1				
	hay-like									1				2		
Green	*green*		2	1					1	1	1	1		6		31
	green apple	1														
	green banana												1			
	lecttuce salad					1										
	green bracken					1										
	rubarb										1					
	clover													1		
	pine			1												
	fresh / clean				1					2						
Leafy	*leafy*	2	1	1		1						1				8
	crushed leaf			1												
almond	*almond/marzipan*			1		1								1		13 (15)
	nutty	1			1		1							1		
Aldehydic	*aldehydic*	2				1					1					6
Estery	*estery*					1							1	1		4
Fruity	*fruity*					1	1			2			1	2		21
	crab apple			2							1					
	apple juice						1									
	banana									1	1					
	acetal													2		
Floral	*floral*													3		6
	flower stem		1													
Sweet	*sweet*					1								1		7
	vanilla											1				
	woody									1	1					
Others	*rubbery*													1		15
	metallic										1					
	damp													1		
	musty													1		
	oily	1												1		
	papery										1			1		
	sour										1					
	mouth watering									1						
	cereal	1			1											
	oat	1														
	pungent								1							
	waxy													1		
like-control	*like-control*												1			3
	not much different											1				

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 10. Flavour Perception of *iso-Amyl acetate* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
iso-Amyl acetate	*iso amyl acetate*			1			3				1	4			1	13
Aldehydic	*aldehyde*	1									1					3
Fruity	*fruity*	3					1			1	1		1	2		22
	banana	1		1				1	1	1			1	1	1	
	pear									1						
	matured banana												1			
Estery	*estery*	2			1				1	3		1	1	5		25
Pear drop	*pear drop*	2	2	4	3	4	3	1	1	5	5	3	2	8	1	67
	opal fruit						1									(69)
	wine gum			1												
	bubble gum													3		
	angel delight			1												
Sweet	*sweet*	2			1				1	1	1			3	1	15
	fudge										1					
	vanilla												1			
	boiled sweet													1		
Floral	*floral*				1							1		1		6
	geranium									1						
	violet											1		1		
	perfumed															
Others	*woody*		1													6
	wine															
	nail vanish		1							1					2	

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 11. Flavour Perception of *iso-Valeric acid* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
Butyric	*butyric*		1								1	1	1	7		15
Sweaty	*sweaty*	2	1	2	2	3	2		1	1	5	3		3	(2)	57 (60)
	piggery	1														
	dirty sock	1	1	1			1			1			2	2		
	men's locker room												1			
	dung			1												
	stale	1				1	1			1			1			
	putrid													1		
	foul water										1					
	sweage									1						
	sulphury						1							1		
	bad veg.			1			1									
Cheesy	*cheesy*	1	2	2	1			1		2	2	(1)		4		52 (53)
	sour	1			1	1	1				1	3	1	5		
	sour fruit	1														
	vomit											1				
	Sickly										1	1		3		
	rancid								1	1						
	vinegary	1														
	feinty	1														
	yeasty			2												
Musty	*musty*		1		1		1			1						7
	damp				1											
	agricultural smell									1						
Fruity	*fruity*															4
	banana													1		
	sweet		1													
	sweet bread										1					
Other	*beefy*											1				18
	Leathery									1						
	Oily					1										
	floor wax								1							
	nail varnish										1					
	sharp acetal													1		
	Fatty													1		
	Pate														1	
	wall paper														1	
	Wood					1										
	Grainy	1														
	malt whisky														1	

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 12. Flavour Perception of *Maltol* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
Buttery	*buttery*			1									1	1		4.2
Sweet	*sweet*	4		1			2	1	(1)	3	3	3	1	5	1	38
	sugary	3		1			1 (1)							3		(39)
Candy floss	*candy floss*	3	3	1	1	2		1	1	1	2	1	5	8	2	43 (44)
Caramel	*caramel*			1	1			1		1		2				35 (36)
	burnt sugar								1			1		1	1	
	toffee	1		2			1 (1)			1	2	1		3	1	
	fudge									1						
	syrupy				1					1						
	treacle									1						
	jam											1		1		
	honey													1		
Fruity	*fruity*					1	1				1			1		13
	strawberry					1			1	1						
	lemony						1									
	estery										1			1		
Vanilla	*vanilla*						1				1					1
Green	*green*										1					3
	clean				1											
	fresh				1											
Others	*stale*					1										8
	musty					1										
	wood cask					1										
	vanish						1									
	toasted											1				
	coconut															
	nutty										1					
Like-control	*close to control*				1											3
	little differemt													1		

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 13. Flavour Perception of *Phenyl ethanol* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
2-phenyl ethanol		1					1									3
Floral	*floral*	2	1	1	(2)	2		1	1	4	4	1 (1)	2	8		61
	fragrant	2				1							1			(69)
	rose petal		2	1	1 (1)	2	1	1	2		1 (1)	2 (2)		3	1	
	lavender						1							1		
	violet										1			1		
	cut flower			1												
	rose wood						1									
	summer flower									1						
	heather													1		
	sweet pea flowers													2		
	blue bell				1											
	carnation							1								
Perfumed	*perfumed*		1	2								1	3	5		18
	scented													1		
	floral soap														1	
Fruity	*fruity*													1		3
	wine-like									1						
Sweet	*sweet*					1							1	1		8
	toffee										1					
	diacetyl										1					
	vanilla										1					
	heather											1				
	honey															
	woody											1			1	
	malty									1						
	spicy										1					
Aldehyde	*aldehyde*	2								1		1				6
Grassy	*grassy/green*			1					1			2				6
	dead leaves										1					
dull	*dull*				1											13
	stale					1										
	wet grain									1						
	wet mown			1												
	acidic													1		
	sour											1		1		
	sickly									1						
	musty													1		
	cork													1		
Oily	*oily*												2			4
	solvent	1														
medicinal	*medicinal*									1						3
	camphor						1									
	mothball						1									
Others	*beer*													1		8
	nose dry									1						
	dry white wine									1						

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 14. Flavour Perception of *Vanillin* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
Woody	*Woody*			1							1	1		1		6.9
	grown wood						1									
Spicy	*spicy*								1	1				1		3
Vanilla	*vanilla*	2	2	2	2	3	3	1	1	2	(4)	5 (1)	(1)	10	2	58
	ice-cream	1													1	(67)
	custard								1				1			
	creamy											1				
Sweet	*sweet*	3		2	1	1			1	4	1	2	3	6		28
Toffee	*toffee*		1		1			1		1	1		3	1		35
	fudge										1			1		
	caramel	1			1					1				3		
	chocolate					1	1			1	1	1	1	2		
	icing sugar									1						
Buttery	*buttery*												2	2		7
	diacetyl		1													
Almond	*almond*														1	3
	cake-mix					1										
Fruity	*fruity*													1		4
	estery													1		
	fragrant	1														
	bourbon													1		
Leafy	*leafy*					1										3
	green					1										
Others	*coca-cola*													1		7
	powdery									1						
	grainy													1		
	cereal					1										
	old-lino							1								
Like - control	*similar to control*											1				1

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 15. Flavour Perception of *4-Vinyl guaiacol* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	14
		(4)	(3)	(5)	(3)	(4)	(4)	(1)	(2)	(7)	(7)	(8)	(6)	(15)	(3)	(72)
Phenolic	*phenolic*							1			1	1	2			6.9
Spicy	*spicy*	1	(1)				1		1		2	(2)			2	17
											(1)					
	nutmeg										1					(22)
	ginger			1												
	clove	1					1		1		1					
	aromatic						1					1				
Woody	*woody*			1					1			2				7
	wood shaving								1							
Smoky	*smoky*	1		1							2	3	1			32
	soothy			1								1				
	smoked bacon						2									
	kippery	1	1								1					
	fishy	1														
	burnt			1				1					2			
	burnt match			2												
	sulphury			1												
	peaty			1								1	1			
	tarry rope			1												
Medicinal	*medicinal*	1														3
	antiseptic														1	
Vanilla	*vanilla*						1				1	1				13
	chocolate-like						2				1					
	sweet								1			1			1	
	fudge										1					
Floral	*floral*										1					6
	cherry										1					
	bourbon		1													
	flower shop	1														
Others	*oily*											2				8
	musty	1														
	cork											1				
	sheep dip						1									
	sweaty										1					

Numbers in bracket: promted distribution, Total number of assessors: 72

Table 16. Flavour Perception of *Lactone* from industrial consensus

Attributes		Distillery (number of participants)														%total
		D1 (4)	D2 (3)	D3 (5)	D4 (3)	D5 (4)	D6 (4)	D7 (1)	D8 (2)	D9 (7)	D10 (7)	D11 (8)	D12 (6)	D13 (15)	D14 (3)	14 (72)
Lactone	*lactone*													1		1
Coconut	*coconut*	2	1 (1)	3 (1)	1 (1)	4	1	1	2	5 (2)	5 (1)	5 (1)	3 (2)	15	1 (2)	68
	Malibu	1														(83)
Nutty	*nutty*					1	1									11
	walnut		1													
	hazel nut						1									
	nougat										1					
Almond	*almond*						1						1	1		4
Marzipan	*marzipan*											1	3	1		7
Sweet	*sweet*	1			1	2					1	2				10
Toffee	*toffee*				1							1				8
	fudge										1					
	caramel									1						
	tablet														2	
Vanilla	*vanilla*			2												4
	creamy													1		
Woody	*woody*													2		3
Fruity	*fruity*					1										4
	estery							1								
	banana									1						
Aldehydic	*aldehydic*	2														6
	pine	1														
	leaves															
	green					1										
Others	*waxy*	1														18
	beewax	1														
	oily						1							1		
	solventy			1												
	floor							1	1							
	cleaner															
	polish									1						
	musty			1												
	hand			1												
	rolling															
	tabacco															
	dry	1														
	dirty				1											
	plastic															
	linseed oil		1													
	menthol								1							
	minty								1							
	leathery									1						
	curry											1				
	sour										1					
	stale										1					

Numbers in bracket: promted distribution, Total number of assessors: 72

Appendix II

Table D.1.
p and Z-values for calculation of d'

p	.01	.02	.03	.04	.05	.06	.07	.08	.09	.10
Z	−2.33	−2.05	−1.88	−1.75	−1.64	−1.55	−1.48	−1.41	−1.34	−1.28
p	.11	.12	.13	.14	.15	.16	.17	.18	.19	.20
Z	−1.23	−1.18	−1.13	−1.08	−1.04	−.99	−.95	−.92	−.88	−.84
p	.21	.22	.23	.24	.25	.26	.27	.28	.29	.30
Z	−.81	−.77	−.74	−.71	−.67	−.64	−.61	−.58	−.55	−.52
p	.31	.32	.33	.34	.35	.36	.37	.38	.39	.40
Z	−.50	−.47	−.44	−.41	−.39	−.36	−.33	−.31	−.28	−.25
p	.41	.42	.43	.44	.45	.46	.47	.48	.49	.50
Z	−.23	−.20	−.18	−.15	−.13	−.10	−.08	−.05	−.03	−.00
p	.51	.52	.53	.54	.55	.56	.57	.58	.59	.60
Z	+.03	+.05	+.08	+.10	+.13	+.15	+.18	+.20	+.23	+.25
p	.61	.62	.63	.64	.65	.66	.67	.68	.69	.70
Z	+.28	+.31	+.33	+.36	+.39	+.41	+.44	+.47	+.50	+.52
p	.71	.72	.73	.74	.75	.76	.77	.78	.79	.80
Z	+.55	+.58	+.61	+.64	+.67	+.71	+.74	+.77	+.81	+.84
p	.81	.82	.83	.84 .85	.86	.87	.88	.89	.90	
Z	+.88	+.92	+.95	+.99	+1.04	+1.08	+1.13	+1.18	+1.23	+1.28
p	.91	.92	.93	.94	.95	.96	.97	.98	.99	.995
Z	+1.34	+1.41	+1.48	+1.55	+1.64	+1.75	+1.88	+2.05	+2.33	+2.58

Table D.2.
Minimum numbers of correct judgements[1] to establish significance at probability levels of 5% and 1% for paired-difference and Duo-trio tests (one-tailed, p = ½)[2] and the Triangle test (one-tailed, p = 11/3)[3]

Paired-Difference and Duo-trio Tests				Triangle Test	
Number of Trials (n)	Probability Levels		Number of Trials (n)	Probability Levels	
	0.05	0.01		0.05	0.01
7	7	7	5	4	5
8	7	8	6	5	6
9	8	9	7	5	6
10	9	10	8	6	7
11	9	10	9	6	7
12	10	11	10	7	8
13	10	12	11	7	8
14	11	12	12	8	9
15	12	13	13	8	9
16	12	14	14	9	10
17	13	14	15	9	10
18	13	15	16	9	11
19	14	15	17	10	11
20	15	16	18	10	12
21	15	17	19	11	12
22	16	17	20	11	13
23	16	18	21	12	13
24	17	19	22	12	14
25	18	19	23	12	14
26	18	20	24	13	15
27	19	20	25	13	15
28	19	21	26	14	15
29	20	22	27	14	16
30	20	22	28	15	16
31	21	23	29	15	17
32	22	24	30	15	17
33	22	24	31	16	18
34	23	25	32	16	18
35	23	25	33	17	18
36	24	26	34	17	19
37	24	26	35	17	19
38	25	27	36	18	20
39	26	28	37	18	20
40	26	28	38	19	21
41	27	29	39	19	21
42	27	29	40	19	21
43	28	30	41	20	22
44	28	31	42	20	22
45	29	31	43	20	23
46	30	32	44	21	23
47	30	32	45	21	24
48	31	33	46	22	24
49	31	34	47	22	24
50	32	34	48	22	25
60	37	40	49	23	25
70	43	46	50	23	26
80	48	51	60	27	30
90	54	57	70	31	34
100	59	63	80	35	38
			90	38	42
			100	42	45

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[1] Adapted with permission from Roessler et al., 1978.

[2] Values (X) not appearing in table may be derived from $X = (z\sqrt{n} + n + 1)/2$

[3] Values (X) not appearing in table may be derived from $X = 0.4714\, z\sqrt{n} + [(2n + 3)/6]$ where n = number of trials, X = minimum number of correct judgements, if X is a whole number, or the next higher integer if X is not a whole number and where z is equal to 1.64 at probability (α) equal to 5% and z is equal to 2.33 at probability (α) equal to 1%.

Table D.3.
Critical values of CHI-SQUARE

	Probability Under H_0 that $x^2 \geq$ chi-square					
df	.20	.10	.05	.02	.01	.001
1	1.64	2.71	3.84	5.41	6.64	10.83
2	3.22	4.60	5.99	7.82	9.21	13.82
3	4.64	6.25	7.82	9.84	11.34	16.27
4	5.99	7.78	9.49	11.67	13.28	18.46
5	7.29	9.24	11.07	13.39	15.09	20.52
6	8.56	10.64	12.59	15.03	16.81	22.46
7	9.80	12.02	14.07	16.62	18.48	24.32
8	11.03	13.36	15.51	18.17	20.09	26.12
9	12.24	14.68	16.92	19.68	21.67	28.88
10	13.44	15.99	18.31	21.16	23.21	29.59
11	14.63	17.28	19.68	22.62	24.72	31.26
12	15.81	18.55	21.03	24.05	26.22	32.91
13	16.98	19.81	22.36	25.47	27.69	34.53
14	18.15	21.06	23.68	26.87	29.14	36.12
15	19.31	22.31	25.00	28.26	30.58	37.70
16	20.46	23.54	26.30	29.63	32.00	39.29
17	21.62	24.77	27.59	31.00	33.41	40.75
18	22.76	25.99	28.87	32.35	34.80	42.31
19	23.90	27.20	30.14	33.69	36.19	43.82
20	25.04	28.41	31.41	35.02	37.57	45.32
21	26.17	29.62	32.67	36.34	38.93	46.80
22	27.30	30.81	33.92	37.66	40.29	48.27
23	28.43	32.01	35.17	38.97	41.64	49.73
24	29.55	33.20	36.42	40.27	42.98	51.18
25	30.68	34.38	37.65	41.57	44.31	52.62
26	31.80	35.56	38.88	42.86	45.64	54.05
27	32.91	36.74	40.11	44.14	46.96	55.48
28	34.03	37.92	41.34	45.42	48.28	56.89
29	35.14	39.09	42.56	46.69	49.59	58.30
30	36.25	40.26	43.77	47.96	50.89	59.70

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Table D.4.
Cumulative probabilities of the standard Normal distribution entry area 1 - α under the standard normal curve from -∞ to z (1 - α)

z	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
.0	.5000	.5040	.5080	.5120	.5160	.5199	.5239	.5279	.5319	.5359
.1	.5398	.5438	.5478	.5527	.5557	.5596	.5636	.5675	.5714	.5753
.2	.5793	.5832	.5871	.5910	.5948	.5987	.6026	.6064	.6103	.6141
.3	.6179	.6217	.6255	.6293	.6331	.6368	.6406	.6443	.6480	.6517
.4	.6554	.6591	.6628	.6664	.6700	.6736	.6772	.6808	.6844	.6879
.5	.6915	.6950	.6985	.7019	.7054	.7088	.7123	.7157	.7190	.7224
.6	.7257	.7291	.7324	.7357	.7389	.7422	.7454	.7486	.7517	.7549
.7	.7580	.7611	.7642	.7673	.7704	.7734	.7764	.7794	.7823	.7852
.8	.7881	.7910	.7939	.7967	.7995	.8023	.8051	.8078	.8106	.8133
.9	.8159	.8186	.8212	.8238	.8264	.8289	.8315	.8340	.8365	.8389
1.0	.8413	.8438	.8461	.8485	.8508	.8531	.8554	.8577	.8599	.8621
1.1	.8643	.8665	.8686	.8708	.8729	.8749	.8770	.8790	.8810	.8830
1.2	.8849	.8869	.8888	.8907	.8925	.8944	.8962	.8980	.8997	.9015
1.3	.9032	.9049	.9066	.9082	.9099	.9115	.9131	.9147	.9162	.9177
1.4	.9192	.9207	.9222	.9236	.9251	.9265	.9279	.9292	.9306	.9319
1.5	.9332	.9345	.9357	.9370	.9382	.9394	.9406	.9418	.9429	.9441
1.6	.9452	.9463	.9474	.9484	.9495	.9505	.9515	.9525	.9535	.9545
1.7	.9554	.9564	.9573	.9582	.9591	.9599	.9608	.9616	.9625	.9633
1.8	.9641	.9649	.9656	.9664	.9671	.9678	.9686	.9693	.9699	.9706
1.9	.9713	.9719	.9726	.9732	.9738	.9744	.9750	.9756	.9761	.9767
2.0	.9772	.9778	.9783	.9788	.9793	.9798	.9803	.9808	.9812	.9817
2.1	.9821	.9826	.9830	.9834	.9838	.9842	.9846	.9850	.9854	.9857
2.2	.9861	.9864	.9868	.9871	.9875	.9878	.9881	.9884	.9887	.9890
2.3	.9893	.9896	.9898	.9901	.9904	.9906	.9909	.9911	.9913	.9916
2.4	.9918	.9920	.9922	.9925	.9927	.9929	.9931	.9932	.9934	.9936
2.5	.9938	.9940	.9941	.9943	.9945	.9946	.9948	.9949	.9951	.9952
2.6	.9938	.9940	.9941	.9943	.9945	.9946	.9948	.9949	.9951	.9952
2.7	.9965	.9966	.9967	.9968	.9969	.9970	.9971	.9972	.9973	.9974
2.8	.9974	.9975	.9976	.9977	.9977	.9978	.9979	.9979	.9980	.9981
2.9	.9981	.9982	.9982	.9983	.9984	.9984	.9985	.9985	.9986	.9986

Selected Percentiles

Cumulative probability 1 − α:	.90	.95	.975	.98	.99	.995	.999
z(1 − α):	1.282	1.645	1.960	2.054	2.326	2.576	3.090

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Table D.5. Probability of a correct response ($x10^4$) as a function of δ for 2-AFC METHOD

δ	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	5000	5028	5056	5085	5113	5141	5169	5197	5226	5254
0.1	5282	5310	5338	5366	5394	5422	5450	5478	5506	5534
0.2	5562	5590	5618	5646	5674	5702	5729	5757	5785	5812
0.3	5840	5868	5895	5923	5950	5977	6005	6032	6059	6086
0.4	6114	6141	6168	6195	6221	6248	6275	6302	6329	6355
0.5	6382	6408	6434	6461	6487	6513	6539	6565	6591	6617
0.6	6643	6669	6695	6720	6746	6771	6796	6822	6847	6872
0.7	6897	6922	6947	6971	6996	7021	7045	7069	7094	7118
0.8	7142	7166	7190	7214	7237	7261	7284	7308	7331	7354
0.9	7377	7400	7423	7446	7469	7491	7514	7536	7558	7580
1.0	7602	7624	7646	7668	7689	7711	7732	7754	7775	7796
1.1	7817	7837	7858	7879	7899	7919	7940	7960	7980	8000
1.2	8019	8039	8058	8078	8097	8116	8135	8154	8173	8192
1.3	8210	8229	8247	8265	8283	8301	8319	8337	8354	8372
1.4	8389	8406	8423	8440	8457	8474	8491	8507	8523	8540
1.5	8556	8572	8588	8603	8619	8635	8650	8665	8681	8696
1.6	8711	8725	8740	8755	8769	8783	8798	8812	8826	8840
1.7	8853	8867	8881	8894	8907	8920	8933	8946	8959	8972
1.8	8985	8997	9009	9022	9034	9046	9058	9070	9081	9093
1.9	9104	9116	9127	9138	9149	9160	9171	9182	9193	9203
2.0	9214	9224	9234	9244	9254	9264	9274	9284	9293	9303
2.1	9312	9321	9331	9340	9349	9358	9367	9375	9384	9393
2.2	9401	9409	9418	9426	9434	9442	9450	9458	9465	9473
2.3	9481	9488	9495	9503	9510	9517	9524	9531	9538	9545
2.4	9552	9558	9565	9571	9578	9584	9590	9596	9603	9609
2.5	9615	9620	9626	9632	9638	9643	9649	9654	9659	9665
2.6	9670	9675	9680	9685	9690	9695	9700	9705	9710	9714
2.7	9719	9723	9728	9732	9737	9741	9745	9749	9753	9757
2.8	9761	9765	9769	9773	9777	9781	9784	9788	9791	9795
2.9	9798	9802	9805	9809	9812	9815	9818	9821	9824	9828
3.0	9831	9833	9836	9839	9842	9845	9848	9850	9853	9856
3.1	9858	9861	9863	9866	9868	9870	9873	9875	9877	9880
3.2	9882	9884	9886	9888	9890	9892	9894	9896	9898	9900
3.3	9902	9904	9906	9907	9909	9911	9912	9914	9916	9917
3.4	9919	9921	9922	9924	9925	9926	9928	9929	9931	9932
3.5	9933	9935	9936	9937	9938	9940	9941	9942	9943	9944
3.6	9945	9947	9948	9949	9950	9951	9952	9953	9954	9955
3.7	9956	9956	9957	9958	9959	9960	9961	9962	9962	9963
3.8	9964	9965	9965	9966	9967	9968	9968	9969	9970	9970
3.9	9971	9972	9972	9973	9973	9974	9974	9975	9976	9976
4.0	9977	9977	9978	9978	9979	9979	9980	9980	9980	9981
4.1	9981	9982	9982	9983	9983	9983	9984	9984	9984	9985
4.2	9985	9985	9986	9986	9986	9987	9987	9987	9988	9988
4.3	9988	9988	9989	9989	9989	9990	9990	9990	9990	9990
4.4	9991	9991	9991	9991	9992	9992	9992	9992	9992	9993
4.5	9993	9993	9993	9993	9993	9994	9994	9994	9994	9994
4.6	9994	9994	9995	9995	9995	9995	9995	9995	9995	9995
4.7	9996	9996	9996	9996	9996	9996	9996	9996	9996	9996
4.8	9997	9997	9997	9997	9997	9997	9997	9997	9997	9997
4.9	9997	9997	9997	9998	9998	9998	9998	9998	9998	9998
5.0	9998	9998	9998	9998	9998	9998	9998	9998	9998	9998
5.1	9998	9998	9999	9999	9999	9999	9999	9999	9999	9999

(Ennis, 1993)

Table D.5. Probability of a correct response ($x10^4$) as a function of δ for 3-AFC METHOD

δ	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	3333	3362	3390	3418	3447	3475	3504	3533	3562	3591
0.1	3620	3649	3678	3707	3737	3766	3795	3825	3855	3884
0.2	3914	3944	3974	4003	4033	4063	4093	4124	4154	4184
0.3	4214	4244	4275	4305	4336	4366	4396	4427	4458	4488
0.4	4519	4549	4580	4611	4641	4672	4703	4734	4764	4795
0.5	4826	4857	4888	4918	4949	4980	5011	5042	5072	5103
0.6	5134	5165	5195	5226	5257	5288	5318	5349	5380	5410
0.7	5441	5471	5502	5532	5563	5593	5624	5654	5684	5714
0.8	5745	5775	5805	5835	5865	5895	5925	5955	5985	6014
0.9	6044	6074	6103	6133	6162	6191	6221	6250	6279	6308
1.0	6337	6366	6395	6423	6452	6481	6509	6538	6566	6594
1.1	6622	6650	6678	6706	6734	6761	6789	6816	6844	6871
1.2	6898	6925	6952	6979	7005	7032	7059	7085	7111	7137
1.3	7163	7189	7215	7241	7266	7292	7317	7342	7367	7392
1.4	7417	7442	7466	7491	7515	7539	7563	7587	7611	7635
1.5	7658	7682	7705	7728	7751	7774	7796	7819	7842	7864
1.6	7886	7908	7930	7952	7973	7995	8016	8037	8058	8079
1.7	8100	8121	8141	8162	8182	8202	8222	8242	8261	8281
1.8	8300	8319	8339	8357	8376	8395	8413	8432	8450	8468
1.9	8486	8504	8522	8539	8556	8574	8591	8608	8624	8641
2.0	8658	8674	8690	8706	8722	8738	8754	8769	8785	8800
2.1	8815	8830	8845	8860	8874	8889	8903	8917	8931	8945
2.2	8959	8973	8986	9000	9013	9026	9039	9052	9065	9077
2.3	9090	9102	9114	9127	9138	9150	9162	9174	9185	9197
2.4	9208	9219	9230	9241	9252	9262	9273	9283	9293	9304
2.5	9314	9324	9333	9343	9353	9362	9372	9381	9390	9399
2.6	9408	9417	9426	9434	9443	9451	9460	9468	9476	9484
2.7	9492	9500	9508	9515	9523	9530	9538	9545	9552	9559
2.8	9566	9573	9580	9587	9593	9600	9606	9613	9619	9625
2.9	9631	9637	9643	9649	9655	9661	9666	9672	9677	9683
3.0	9688	9693	9698	9703	9709	9713	9718	9723	9728	9733
3.1	9737	9742	9746	9751	9755	9759	9764	9768	9772	9776
3.2	9780	9784	9788	9791	9795	9799	9802	9806	9809	9813
3.3	9816	9820	9823	9826	9829	9833	9836	9839	9842	9845
3.4	9848	9850	9853	9856	9859	9861	9864	9867	9869	9872
3.5	9874	9877	9879	9881	9884	9886	9888	9890	9892	9894
3.6	9897	9899	9901	9903	9904	9906	9908	9910	9912	9914
3.7	9915	9917	9919	9920	9922	9924	9925	9927	9928	9930
3.8	9931	9932	9934	9935	9937	9938	9939	9940	9942	9943
3.9	9944	9945	9946	9948	9949	9950	9951	9952	9953	9954
4.0	9955	9956	9957	9958	9959	9960	9961	9961	9962	9963
4.1	9964	9965	9965	9966	9967	9968	9968	9969	9970	9970
4.2	9971	9972	9972	9973	9974	9974	9975	9975	9976	9977
4.3	9977	9978	9978	9979	9979	9980	9980	9981	9981	9981
4.4	9982	9982	9983	9983	9984	9984	9984	9985	9985	9985
4.5	9986	9986	9986	9987	9987	9987	9988	9988	9988	9989
4.6	9989	9989	9989	9990	9990	9990	9990	9991	9991	9991
4.7	9991	9992	9992	9992	9992	9992	9993	9993	9993	9993
4.8	9993	9993	9994	9994	9994	9994	9994	9994	9995	9995
4.9	9995	9995	9995	9995	9995	9995	9996	9996	9996	9996
5.0	9996	9996	9996	9996	9996	9996	9997	9997	9997	9997
5.1	9997	9997	9997	9997	9997	9997	9997	9997	9998	9998
5.2	9998	9998	9998	9998	9998	9998	9998	9998	9998	9998
5.3	9998	9998	9998	9998	9998	9998	9999	9999	9999	9999

Table D.5. Probability of a correct response (x10^4) as a function of δ for TRIANGULAR METHOD

δ	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	3333	3333	3334	3334	3335	3336	3337	3338	3339	3341
0.1	3343	3344	3347	3349	3351	3354	3357	3360	3363	3366
0.2	3370	3374	3378	3382	3386	3390	3395	3400	3405	3410
0.3	3415	3421	3427	3432	3439	3445	3451	3458	3464	3471
0.4	3478	3486	3493	3501	3508	3516	3524	3533	3541	3550
0.5	3558	3567	3576	3586	3595	3604	3614	3624	3634	3644
0.6	3654	3665	3676	3686	3697	3708	3719	3731	3742	3754
0.7	3766	3778	3790	3802	3814	3827	3839	3852	3865	3878
0.8	3891	3905	3918	3932	3945	3959	3973	3987	4001	4016
0.9	4030	4045	4059	4074	4089	4104	4119	4134	4149	4165
1.0	4180	4196	4212	4228	4244	4260	4276	4292	4309	4325
1.1	4342	4358	4375	4392	4409	4426	4443	4460	4477	4494
1.2	4512	4529	4547	4564	4582	4600	4618	4636	4654	4672
1.3	4690	4708	4726	4745	4763	4782	4800	4819	4837	4856
1.4	4875	4893	4912	4931	4950	4969	4988	5007	5026	5045
1.5	5065	5084	5103	5122	5142	5161	5180	5200	5219	5239
1.6	5258	5278	5297	5317	5337	5356	5376	5396	5415	5435
1.7	5455	5474	5494	5514	5534	5554	5573	5593	5613	5633
1.8	5653	5672	5692	5712	5732	5752	5771	5791	5811	5831
1.9	5851	5870	5890	5910	5930	5950	5969	5989	6009	6028
2.0	6048	6068	6087	6107	6127	6146	6166	6185	6205	6224
2.1	6244	6263	6283	6302	6321	6341	6360	6379	6398	6418
2.2	6437	6456	6475	6494	6513	6532	6551	6570	6589	6608
2.3	6627	6645	6664	6683	6701	6720	6739	6757	6776	6794
2.4	6812	6831	6849	6867	6885	6903	6922	6940	6958	6976
2.5	6993	7011	7029	7047	7064	7082	7100	7117	7135	7152
2.6	7169	7187	7204	7221	7238	7255	7272	7289	7306	7323
2.7	7340	7356	7373	7390	7406	7423	7439	7455	7472	7488
2.8	7504	7520	7536	7552	7568	7584	7600	7616	7631	7647
2.9	7662	7678	7693	7709	7724	7739	7754	7769	7784	7799
3.0	7814	7829	7844	7859	7873	7888	7902	7917	7931	7945
3.1	7960	7974	7988	8002	8016	8030	8044	8057	8071	8085
3.2	8098	8112	8125	8139	8152	8165	8179	8192	8205	8218
3.3	8231	8243	8256	8269	8282	8294	8307	8319	8332	8344
3.4	8356	8368	8381	8393	8405	8417	8428	8440	8452	8464
3.5	8475	8487	8498	8510	8521	8532	8544	8555	8566	8577
3.6	8588	8599	8610	8620	8631	8642	8652	8663	8673	8684
3.7	8694	8704	8715	8725	8735	8745	8755	8765	8775	8784
3.8	8794	8804	8813	8823	8833	8842	8851	8861	8870	8879
3.9	8888	8897	8906	8915	8924	8933	8942	8951	8959	8968
4.0	8977	8985	8994	9002	9010	9019	9027	9035	9043	9051
4.1	9059	9067	9075	9083	9091	9099	9106	9114	9122	9129
4.2	9137	9144	9151	9159	9166	9173	9180	9188	9195	9202
4.3	9209	9216	9223	9229	9236	9243	9250	9256	9263	9269
4.4	9276	9282	9289	9295	9301	9308	9314	9320	9326	9332
4.5	9338	9344	9350	9356	9362	9368	9374	9379	9385	9391
4.6	9396	9402	9407	9413	9418	9424	9429	9434	9440	9445
4.7	9450	9455	9460	9465	9470	9475	9480	9485	9490	9495
4.8	9500	9504	9509	9514	9518	9523	9528	9532	9537	9541
4.9	9546	9550	9554	9559	9563	9567	9571	9575	9580	9584
5.0	9588	9592	9596	9600	9604	9608	9612	9615	9619	9623
5.1	9627	9630	9634	9638	9641	9645	9648	9652	9655	9659
5.2	9662	9666	9669	9673	9676	9679	9682	9686	9689	9692
5.3	9695	9698	9701	9704	9707	9710	9713	9716	9719	9722
5.4	9725	9728	9731	9734	9736	9739	9742	9745	9747	9750
5.5	9753	9755	9758	9760	9763	9765	9768	9770	9773	9775
5.6	9778	9780	9782	9785	9787	9789	9792	9794	9796	9798
5.7	9800	9803	9805	9807	9809	9811	9813	9815	9817	9819
5.8	9821	9823	9825	9827	9829	9831	9833	9834	9836	9838
5.9	9840	9842	9843	9845	9847	9849	9850	9852	9854	9855
6.0	9857	9859	9860	9862	9863	9865	9866	9868	9869	9871

(Ennis, 1993)

Table D.5. Probability of a correct response (x10^4) as a function of δ for DUO-TRIO METHOD

δ	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	5000	5000	5000	5001	5001	5002	5003	5005	5006	5007
0.1	5009	5011	5013	5015	5018	5021	5023	5026	5030	5033
0.2	5037	5040	5044	5048	5053	5057	5062	5066	5071	5077
0.3	5082	5087	5093	5099	5105	5111	5117	5124	5131	5137
0.4	5144	5152	5159	5166	5174	5182	5190	5198	5206	5215
0.5	5223	5232	5241	5250	5259	5269	5278	5288	5298	5308
0.6	5318	5328	5339	5349	5360	5371	5382	5393	5404	5415
0.7	5427	5438	5450	5462	5474	5486	5498	5511	5523	5536
0.8	5548	5561	5574	5587	5600	5614	5627	5641	5654	5668
0.9	5682	5695	5709	5724	5738	5752	5766	5781	5795	5810
1.0	5825	5840	5854	5869	5884	5900	5915	5930	5945	5961
1.1	5976	5992	6007	6023	6039	6055	6071	6087	6102	6119
1.2	6135	6151	6167	6183	6200	6216	6232	6249	6265	6282
1.3	6298	6315	6331	6348	6365	6381	6398	6415	6432	6448
1.4	6465	6482	6499	6516	6533	6550	6567	6584	6601	6618
1.5	6635	6652	6669	6686	6703	6720	6737	6754	6771	6788
1.6	6805	6822	6839	6856	6873	6890	6907	6923	6940	6957
1.7	6974	6991	7008	7025	7042	7058	7075	7092	7109	7125
1.8	7142	7159	7175	7192	7208	7225	7241	7258	7274	7291
1.9	7307	7323	7340	7356	7372	7388	7404	7420	7436	7452
2.0	7468	7484	7500	7516	7531	7547	7563	7578	7594	7610
2.1	7625	7640	7656	7671	7686	7701	7717	7732	7747	7762
2.2	7777	7791	7806	7821	7836	7850	7865	7879	7894	7908
2.3	7923	7937	7951	7965	7979	7993	8007	8021	8035	8049
2.4	8062	8076	8090	8103	8117	8130	8143	8156	8170	8183
2.5	8196	8209	8222	8235	8247	8260	8273	8285	8298	8310
2.6	8323	8335	8347	8360	8372	8384	8396	8408	8420	8431
2.7	8443	8455	8466	8478	8489	8501	8512	8523	8535	8546
2.8	8557	8568	8579	8590	8600	8611	8622	8632	8643	8653
2.9	8664	8674	8685	8695	8705	8715	8725	8735	8745	8755
3.0	8765	8774	8784	8794	8803	8813	8822	8831	8841	8850
3.1	8859	8868	8877	8886	8895	8904	8913	8921	8930	8939
3.2	8947	8956	8964	8973	8981	8989	8997	9006	9014	9022
3.3	9030	9038	9046	9053	9061	9069	9077	9084	9092	9099
3.4	9107	9114	9121	9129	9136	9143	9150	9157	9164	9171
3.5	9178	9185	9192	9199	9206	9212	9219	9225	9232	9239
3.6	9245	9251	9258	9264	9270	9276	9283	9289	9295	9301
3.7	9307	9313	9319	9324	9330	9336	9342	9347	9353	9359
3.8	9364	9370	9375	9381	9386	9391	9397	9402	9407	9412
3.9	9417	9423	9428	9433	9438	9443	9447	9452	9457	9462
4.0	9467	9471	9476	9481	9485	9490	9494	9499	9503	9508
4.1	9512	9517	9521	9525	9529	9534	9538	9542	9546	9550
4.2	9554	9558	9562	9566	9570	9574	9578	9582	9586	9589
4.3	9593	9597	9601	9604	9608	9612	9615	9619	9622	9626
4.4	9629	9633	9636	9639	9643	9646	9649	9653	9656	9659
4.5	9662	9665	9669	9672	9675	9678	9681	9684	9687	9690
4.6	9693	9696	9698	9701	9704	9707	9710	9713	9715	9718
4.7	9721	9723	9726	9729	9731	9734	9736	9739	9742	9744
4.8	9747	9749	9751	9754	9756	9759	9761	9763	9766	9768
4.9	9770	9772	9775	9777	9779	9781	9783	9786	9788	9790
5.0	9792	9794	9796	9798	9800	9802	9804	9806	9808	9810
5.1	9812	9814	9816	9817	9819	9821	9823	9825	9827	9828
5.2	9830	9832	9833	9835	9837	9839	9840	9842	9843	9845
5.3	9847	9848	9850	9851	9853	9854	9856	9857	9859	9860
5.4	9862	9863	9865	9866	9868	9869	9870	9872	9873	9874
5.5	9876	9877	9878	9880	9881	9882	9884	9885	9886	9887
5.6	9888	9890	9891	9892	9893	9894	9895	9897	9898	9899
5.7	9900	9901	9902	9903	9904	9905	9906	9907	9908	9909
5.8	9910	9911	9912	9913	9914	9915	9916	9917	9918	9919
5.9	9920	9921	9922	9922	9923	9924	9925	9926	9927	9928
6.0	9928	9929	9930	9931	9932	9932	9933	9934	9935	9935

Table D.6.
The B value for estimation of the variance of d' obtained from 2-AFC METHOD

d'	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	3.1416	3.1417	3.1418	3.1421	3.1425	3.1430	3.1436	3.1444	3.1452	3.1462
0.1	3.1473	3.1485	3.1498	3.1513	3.1528	3.1545	3.1562	3.1581	3.1601	3.1623
0.2	3.1645	3.1669	3.1694	3.1720	3.1747	3.1775	3.1805	3.1835	3.1867	3.1900
0.3	3.1934	3.1970	3.2007	3.2045	3.2084	3.2124	3.2166	3.2209	3.2253	3.2298
0.4	3.2345	3.2392	3.2442	3.2492	3.2544	3.2597	3.2651	3.2706	3.2763	3.2821
0.5	3.2881	3.2942	3.3004	3.3067	3.3132	3.3198	3.3266	3.3335	3.3405	3.3477
0.6	3.3550	3.3624	3.3700	3.3778	3.3857	3.3937	3.4019	3.4102	3.4187	3.4273
0.7	3.4361	3.4450	3.4541	3.4633	3.4727	3.4822	3.4920	3.5018	3.5119	3.5220
0.8	3.5324	3.5429	3.5536	3.5645	3.5755	3.5867	3.5981	3.6096	3.6213	3.6332
0.9	3.6453	3.6576	3.6700	3.6826	3.6954	3.7084	3.7216	3.7350	3.7486	3.7624
1.0	3.7763	3.7905	3.8049	3.8195	3.8343	3.8492	3.8645	3.8799	3.8955	3.9113
1.1	3.9274	3.9437	3.9602	3.9770	3.9939	4.0111	4.0286	4.0462	4.0642	4.0823
1.2	4.1007	4.1194	4.1383	4.1574	4.1768	4.1965	4.2165	4.2367	4.2571	4.2779
1.3	4.2989	4.3202	4.3418	4.3637	4.3858	4.4083	4.4310	4.4541	4.4774	4.5011
1.4	4.5250	4.5493	4.5739	4.5989	4.6241	4.6497	4.6757	4.7019	4.7285	4.7555
1.5	4.7828	4.8105	4.8386	4.8670	4.8957	4.9249	4.9544	4.9844	5.0147	5.0454
1.6	5.0766	5.1081	5.1401	5.1724	5.2052	5.2385	5.2722	5.3063	5.3408	5.3759
1.7	5.4114	5.4473	5.4838	5.5207	5.5581	5.5960	5.6344	5.6734	5.7128	5.7528
1.8	5.7933	5.8343	5.8759	5.9181	5.9608	6.0041	6.0480	6.0925	6.1375	6.1832
1.9	6.2295	6.2764	6.3240	6.3722	6.4211	6.4706	6.5208	6.5717	6.6232	6.6755
2.0	6.7285	6.7823	6.8367	6.8919	6.9479	7.0047	7.0622	7.1206	7.1797	7.2397
2.1	7.3005	7.3622	7.4247	7.4881	7.5524	7.6176	7.6837	7.7508	7.8188	7.8877
2.2	7.9577	8.0286	8.1005	8.1735	8.2475	8.3226	8.3988	8.4760	8.5543	8.6338
2.3	8.7145	8.7963	8.8793	8.9635	9.0489	9.1356	9.2235	9.3128	9.4033	9.4952
2.4	9.588	9.683	9.779	9.876	9.975	10.076	10.178	10.281	10.386	10.492
2.5	10.601	10.710	10.822	10.935	11.050	11.166	11.285	11.405	11.527	11.651
2.6	11.777	11.904	12.034	12.166	12.299	12.435	12.573	12.713	12.856	13.000
2.7	13.147	13.296	13.448	13.601	13.758	13.917	14.078	14.242	14.408	14.578
2.8	14.750	14.924	15.102	15.282	15.466	15.652	15.841	16.034	16.230	16.428
2.9	16.631	16.836	17.045	17.257	17.473	17.692	17.915	18.142	18.373	18.607
3.0	18.846	19.088	19.335	19.586	19.841	20.100	20.364	20.632	20.905	21.183
3.1	21.465	21.752	22.044	22.342	22.644	22.952	23.265	23.583	23.908	24.237
3.2	24.573	24.915	25.262	25.616	25.977	26.343	26.716	27.096	27.482	27.876
3.3	28.277	28.685	29.100	29.523	29.953	30.392	30.838	31.292	31.755	32.227
3.4	32.706	33.196	33.694	34.201	34.718	35.244	35.780	36.327	36.883	37.451
3.5	38.028	38.617	39.217	39.828	40.450	41.085	41.732	42.392	43.063	43.748
3.6	44.447	45.158	45.884	46.623	47.377	48.146	48.929	49.728	50.544	51.374
3.7	52.221	53.085	53.966	54.864	55.782	56.716	57.669	58.641	59.632	60.644
3.8	61.678	62.731	63.805	64.903	66.021	67.163	68.328	69.516	70.729	71.968
3.9	73.231	74.522	75.838	77.181	78.552	79.954	81.384	82.844	84.335	85.852
4.0	87.41	88.99	90.62	92.27	93.96	95.68	97.45	99.25	101.09	102.97
4.1	104.88	106.84	108.85	110.89	112.98	115.12	117.30	119.53	121.81	124.14
4.2	126.52	128.95	131.44	133.97	136.58	139.23	141.95	144.72	147.55	150.46
4.3	153.42	156.46	159.55	162.72	165.96	169.28	172.68	176.14	179.68	183.32
4.4	187.03	190.84	194.72	198.70	202.76	206.92	211.18	215.54	219.99	224.55
4.5	229.20	234.00	238.88	243.91	248.99	254.24	259.65	265.13	270.75	276.51
4.6	282.42	288.43	294.62	301.00	307.42	314.06	320.90	327.80	334.99	342.25
4.7	349.85	357.46	365.30	373.36	381.61	390.07	398.73	407.54	416.72	426.01
4.8	435.55	445.37	455.30	465.61	476.25	486.93	498.06	509.36	521.03	532.78
4.9	545.18	557.67	570.63	583.69	597.26	611.25	625.34	639.87	654.69	670.19
5.0	686.03	702.24	718.81	736.00	752.88	771.18	789.70	808.15	827.37	847.43
5.1	867.8	889.0	910.2	932.1	954.2	978.0	1001.6	1026.1	1051.9	1077.8

(Bi & Ennis, 1997)

Table D.6.
The B value for estimation of the variance of d' obtained from 3-AFC METHOD

d'	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	2.7925	2.7862	2.7801	2.7740	2.7681	2.7624	2.7568	2.7513	2.7460	2.7408
0.1	2.7357	2.7307	2.7259	2.7212	2.7167	2.7123	2.7080	2.7038	2.6998	2.6959
0.2	2.6921	2.6884	2.6849	2.6814	2.6782	2.6750	2.6719	2.6690	2.6662	2.6635
0.3	2.6610	2.6585	2.6562	2.6540	2.6519	2.6499	2.6481	2.6464	2.6448	2.6433
0.4	2.6419	2.6406	2.6395	2.6384	2.6375	2.6367	2.6360	2.6355	2.6350	2.6347
0.5	2.6344	2.6343	2.6343	2.6345	2.6347	2.6351	2.6355	2.6361	2.6368	2.6376
0.6	2.6385	2.6396	2.6407	2.6420	2.6434	2.6449	2.6465	2.6483	2.6501	2.6521
0.7	2.6542	2.6564	2.6587	2.6611	2.6637	2.6664	2.6692	2.6721	2.6751	2.6783
0.8	2.6815	2.6849	2.6884	2.6921	2.6958	2.6997	2.7037	2.7079	2.7121	2.7165
0.9	2.7210	2.7256	2.7304	2.7353	2.7403	2.7454	2.7507	2.7561	2.7616	2.7673
1.0	2.7731	2.7790	2.7851	2.7913	2.7976	2.8041	2.8107	2.8175	2.8244	2.8314
1.1	2.8386	2.8459	2.8534	2.8610	2.8688	2.8767	2.8847	2.8930	2.9013	2.9098
1.2	2.9185	2.9273	2.9363	2.9454	2.9547	2.9642	2.9738	2.9836	2.9936	3.0037
1.3	3.0140	3.0244	3.0351	3.0459	3.0569	3.0680	3.0794	3.0909	3.1026	3.1145
1.4	3.1265	3.1388	3.1512	3.1639	3.1767	3.1898	3.2030	3.2164	3.2301	3.2439
1.5	3.2580	3.2722	3.2867	3.3014	3.3163	3.3314	3.3468	3.3623	3.3781	3.3942
1.6	3.4104	3.4269	3.4437	3.4607	3.4779	3.4954	3.5131	3.5311	3.5493	3.5678
1.7	3.5866	3.6056	3.6249	3.6445	3.6643	3.6845	3.7049	3.7256	3.7466	3.7679
1.8	3.7895	3.8114	3.8336	3.8561	3.8790	3.9021	3.9256	3.9494	3.9735	3.9980
1.9	4.0229	4.0480	4.0736	4.0995	4.1257	4.1523	4.1793	4.2067	4.2344	4.2626
2.0	4.2911	4.3201	4.3494	4.3792	4.4093	4.4399	4.4710	4.5025	4.5344	4.5667
2.1	4.5996	4.6328	4.6666	4.7008	4.7356	4.7708	4.8065	4.8427	4.8794	4.9167
2.2	4.9545	4.9928	5.0317	5.0711	5.1111	5.1517	5.1929	5.2346	5.2769	5.3199
2.3	5.3635	5.4077	5.4525	5.4981	5.5442	5.5910	5.6386	5.6868	5.7357	5.7853
2.4	5.8357	5.8868	5.9386	5.9912	6.0446	6.0988	6.1538	6.2096	6.2662	6.3236
2.5	6.3819	6.4411	6.5012	6.5622	6.6241	6.6869	6.7507	6.8154	6.8811	6.9479
2.6	7.0156	7.0843	7.1542	7.2250	7.2970	7.3701	7.4443	7.5196	7.5961	7.6737
2.7	7.7526	7.8327	7.9141	7.9967	8.0806	8.1658	8.2524	8.3403	8.4296	8.5204
2.8	8.6125	8.7061	8.8012	8.8979	8.9960	9.0957	9.1971	9.3000	9.4047	9.5110
2.9	9.619	9.729	9.840	9.954	10.069	10.186	10.305	10.426	10.549	10.674
3.0	10.801	10.930	11.062	11.195	11.331	11.469	11.609	11.752	11.897	12.045
3.1	12.195	12.347	12.503	12.660	12.821	12.984	13.150	13.319	13.491	13.666
3.2	13.844	14.025	14.209	14.396	14.587	14.781	14.978	15.179	15.383	15.591
3.3	15.802	16.018	16.237	16.460	16.687	16.918	17.154	17.393	17.637	17.886
3.4	18.138	18.396	18.658	18.925	19.196	19.473	19.755	20.042	20.334	20.632
3.5	20.936	21.245	21.559	21.880	22.206	22.539	22.878	23.224	23.575	23.934
3.5	24.299	24.672	25.051	25.438	25.832	26.234	26.643	27.061	27.486	27.920
3.7	28.362	28.813	29.273	29.741	30.218	30.706	31.203	31.710	32.225	32.752
3.8	33.290	33.838	34.397	34.968	35.549	36.142	36.748	37.365	37.996	38.638
3.9	39.295	39.964	40.647	41.343	42.055	42.781	43.522	44.278	45.051	45.837
4.0	46.642	47.463	48.301	49.157	50.031	50.925	51.835	52.765	53.716	54.686
4.1	55.676	56.687	57.722	58.778	59.856	60.959	62.083	63.232	64.406	65.609
4.2	66.835	68.085	69.363	70.679	72.011	73.378	74.776	76.203	77.663	79.153
4.3	80.676	82.231	83.826	85.456	87.122	88.824	90.568	92.347	94.168	96.029
4.4	97.94	99.89	101.87	103.91	106.00	108.13	110.30	112.54	114.82	117.15
4.5	119.54	122.00	124.49	127.06	129.67	132.36	135.11	137.91	140.79	143.72
4.6	146.75	149.83	153.00	156.23	159.55	162.92	166.39	169.94	173.60	177.32
4.7	181.14	185.06	189.07	193.16	197.35	201.65	206.08	210.59	215.24	219.93
4.8	224.81	229.85	234.91	240.13	245.52	251.01	256.65	262.39	268.29	274.38
4.9	280.57	286.97	293.44	300.11	307.05	314.10	321.37	328.68	336.35	344.18
5.0	352.08	360.42	368.70	377.37	386.31	395.30	404.63	414.26	423.99	433.91
5.1	444.34	454.81	465.84	477.06	488.24	500.04	512.35	524.87	537.15	550.34

(Bi & Ennis, 1997)

Table D.6.
The B value for estimation of the variance of d' obtained from TRIANGULAR METHOD

d'	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	*******	65800.	16452.	7314.	4115.	2635.	1831.	1346.	1031.	815.
0.1	661.08	546.88	460.03	392.44	338.81	295.54	260.13	230.78	206.19	185.38
0.2	167.61	152.31	139.06	127.50	117.35	108.40	100.45	93.38	87.05	81.36
0.3	76.236	71.597	67.387	63.554	60.054	56.850	53.910	51.205	48.711	46.406
0.4	44.273	42.294	40.455	38.742	37.146	35.655	34.261	32.954	31.729	30.578
0.5	29.496	28.477	27.517	26.611	25.755	24.945	24.179	23.452	22.764	22.110
0.6	21.489	20.898	20.336	19.801	19.291	18.805	18.341	17.897	17.474	17.069
0.7	16.681	16.310	15.954	15.614	15.287	14.973	14.672	14.383	14.106	13.839
0.8	13.582	13.335	13.097	12.868	12.647	12.435	12.230	12.032	11.841	11.657
0.9	11.479	11.308	11.142	10.982	10.827	10.677	10.532	10.392	10.256	10.125
1.0	9.998	9.875	9.756	9.641	9.529	9.421	9.316	9.214	9.115	9.019
1.1	8.926	8.836	8.749	8.664	8.582	8.502	8.424	8.349	8.276	8.205
1.2	8.136	8.069	8.004	7.941	7.880	7.820	7.762	7.706	7.651	7.598
1.3	7.547	7.497	7.448	7.401	7.355	7.310	7.267	7.225	7.184	7.144
1.4	7.106	7.068	7.032	6.997	6.962	6.929	6.897	6.866	6.835	6.806
1.5	6.778	6.750	6.723	6.697	6.672	6.648	6.624	6.601	6.579	6.558
1.6	6.538	6.518	6.499	6.480	6.462	6.445	6.429	6.413	6.398	6.383
1.7	6.369	6.355	6.342	6.330	6.318	6.307	6.296	6.286	6.276	6.267
1.8	6.258	6.250	6.242	6.235	6.228	6.222	6.216	6.211	6.206	6.201
1.9	6.197	6.193	6.190	6.187	6.185	6.183	6.181	6.180	6.179	6.179
2.0	6.178	6.179	6.179	6.180	6.182	6.184	6.186	6.188	6.191	6.194
2.1	6.198	6.201	6.206	6.210	6.215	6.220	6.226	6.231	6.238	6.244
2.2	6.251	6.258	6.265	6.273	6.281	6.289	6.298	6.307	6.316	6.326
2.3	6.336	6.346	6.356	6.367	6.378	6.389	6.401	6.413	6.425	6.438
2.4	6.450	6.463	6.477	6.490	6.504	6.518	6.533	6.548	6.563	6.578
2.5	6.594	6.609	6.626	6.642	6.659	6.676	6.693	6.710	6.728	6.746
2.6	6.765	6.783	6.802	6.821	6.841	6.861	6.881	6.901	6.921	6.942
2.7	6.963	6.985	7.007	7.029	7.051	7.073	7.096	7.119	7.143	7.166
2.8	7.190	7.214	7.239	7.264	7.289	7.314	7.340	7.366	7.392	7.418
2.9	7.445	7.472	7.500	7.527	7.555	7.584	7.612	7.641	7.670	7.700
3.0	7.729	7.760	7.790	7.821	7.852	7.883	7.914	7.946	7.979	8.011
3.1	8.044	8.077	8.111	8.144	8.179	8.213	8.248	8.283	8.318	8.354
3.2	8.390	8.427	8.463	8.500	8.538	8.576	8.614	8.652	8.691	8.730
3.3	8.770	8.810	8.850	8.890	8.931	8.973	9.014	9.056	9.099	9.142
3.4	9.185	9.228	9.272	9.317	9.361	9.406	9.452	9.498	9.544	9.591
3.5	9.638	9.685	9.733	9.781	9.830	9.879	9.929	9.979	10.029	10.080
3.6	10.131	10.183	10.235	10.287	10.340	10.394	10.448	10.502	10.557	10.612
3.7	10.668	10.724	10.781	10.838	10.896	10.954	11.012	11.072	11.131	11.191
3.8	11.252	11.313	11.375	11.437	11.500	11.563	11.627	11.691	11.756	11.821
3.9	11.887	11.954	12.021	12.089	12.157	12.225	12.295	12.365	12.435	12.506
4.0	12.578	12.651	12.723	12.797	12.871	12.946	13.022	13.098	13.174	13.252
4.1	13.330	13.409	13.488	13.568	13.649	13.730	13.812	13.895	13.979	14.063
4.2	14.148	14.233	14.320	14.407	14.495	14.584	14.673	14.763	14.854	14.946
4.3	15.039	15.132	15.226	15.321	15.417	15.514	15.611	15.709	15.809	15.909
4.4	16.009	16.111	16.214	16.318	16.422	16.527	16.634	16.741	16.849	16.958
4.5	17.068	17.180	17.292	17.405	17.519	17.634	17.750	17.867	17.985	18.104
4.6	18.225	18.346	18.468	18.592	18.717	18.842	18.969	19.097	19.226	19.357
4.7	19.488	19.621	19.755	19.890	20.026	20.164	20.303	20.443	20.584	20.727
4.8	20.871	21.016	21.163	21.310	21.460	21.610	21.763	21.916	22.071	22.227
4.9	22.385	22.544	22.705	22.867	23.031	23.196	23.363	23.531	23.701	23.872
5.0	24.046	24.220	24.397	24.575	24.754	24.936	25.119	25.304	25.491	25.679
5.1	25.869	26.061	26.255	26.451	26.648	26.847	27.049	27.252	27.457	27.665

(Bi & Ennis, 1997)

Table D.6.
The B value for estimation of the variance of d' obtained from DUO-TRIO METHOD

d'	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0		74025.	18508.	8228.	4630.	2964.	2059.	1514.	1160.	917.
0.1	743.52	615.05	517.34	441.30	380.96	332.29	292.45	259.43	231.77	208.35
0.2	188.36	171.16	156.25	143.24	131.83	121.75	112.82	104.86	97.74	91.34
0.3	85.576	80.358	75.622	71.310	67.374	63.770	60.463	57.420	54.615	52.024
0.4	49.624	47.398	45.330	43.405	41.610	39.933	38.365	36.897	35.520	34.226
0.5	33.010	31.864	30.785	29.766	28.804	27.895	27.034	26.218	25.444	24.710
0.6	24.012	23.349	22.719	22.118	21.545	21.000	20.479	19.981	19.506	19.052
0.7	18.618	18.202	17.803	17.422	17.056	16.705	16.368	16.044	15.733	15.435
0.8	15.148	14.872	14.606	14.350	14.104	13.867	13.638	13.418	13.205	13.000
0.9	12.803	12.612	12.427	12.249	12.078	11.911	11.751	11.596	11.446	11.301
1.0	11.160	11.025	10.893	10.766	10.643	10.524	10.409	10.297	10.189	10.084
1.1	9.983	9.885	9.789	9.697	9.608	9.521	9.437	9.356	9.277	9.201
1.2	9.127	9.055	8.986	8.918	8.853	8.790	8.729	8.669	8.612	8.556
1.3	8.502	8.450	8.400	8.351	8.304	8.258	8.214	8.171	8.130	8.090
1.4	8.051	8.014	7.978	7.943	7.910	7.878	7.847	7.817	7.788	7.760
1.5	7.734	7.709	7.684	7.661	7.638	7.617	7.597	7.577	7.559	7.541
1.6	7.524	7.509	7.494	7.480	7.466	7.454	7.442	7.432	7.422	7.412
1.7	7.404	7.396	7.389	7.383	7.378	7.373	7.369	7.365	7.363	7.361
1.8	7.360	7.359	7.359	7.360	7.361	7.363	7.366	7.369	7.373	7.377
1.9	7.382	7.388	7.395	7.401	7.409	7.417	7.426	7.435	7.445	7.456
2.0	7.467	7.478	7.490	7.503	7.516	7.530	7.545	7.560	7.575	7.591
2.1	7.608	7.625	7.643	7.661	7.680	7.699	7.719	7.740	7.761	7.782
2.2	7.804	7.827	7.850	7.874	7.898	7.923	7.948	7.974	8.000	8.027
2.3	8.055	8.083	8.111	8.140	8.170	8.200	8.231	8.262	8.294	8.326
2.4	8.359	8.392	8.426	8.461	8.496	8.531	8.567	8.604	8.641	8.679
2.5	8.717	8.756	8.796	8.836	8.876	8.917	8.959	9.001	9.044	9.088
2.6	9.132	9.176	9.221	9.267	9.313	9.360	9.408	9.456	9.505	9.554
2.7	9.604	9.654	9.705	9.757	9.809	9.862	9.916	9.970	10.024	10.080
2.8	10.136	10.192	10.250	10.308	10.366	10.425	10.485	10.545	10.606	10.668
2.9	10.731	10.794	10.857	10.922	10.987	11.052	11.119	11.186	11.254	11.322
3.0	11.391	11.461	11.531	11.603	11.675	11.747	11.820	11.894	11.969	12.045
3.1	12.121	12.198	12.275	12.354	12.433	12.513	12.593	12.675	12.757	12.839
3.2	12.923	13.007	13.093	13.179	13.265	13.353	13.441	13.530	13.620	13.711
3.3	13.802	13.894	13.987	14.081	14.176	14.271	14.368	14.465	14.563	14.662
3.4	14.762	14.862	14.964	15.066	15.169	15.273	15.378	15.484	15.591	15.698
3.5	15.807	15.916	16.026	16.137	16.250	16.363	16.477	16.591	16.707	16.824
3.6	16.942	17.061	17.180	17.301	17.422	17.545	17.668	17.793	17.919	18.045
3.7	18.173	18.301	18.431	18.561	18.693	18.826	18.960	19.094	19.230	19.367
3.8	19.505	19.644	19.784	19.926	20.068	20.212	20.356	20.502	20.649	20.797
3.9	20.946	21.096	21.248	21.400	21.554	21.709	21.866	22.023	22.182	22.342
4.0	22.503	22.665	22.829	22.994	23.160	23.327	23.496	23.666	23.838	24.010
4.1	24.184	24.360	24.536	24.714	24.894	25.075	25.257	25.441	25.626	25.812
4.2	26.000	26.189	26.380	26.573	26.767	26.962	27.159	27.357	27.557	27.759
4.3	27.962	28.166	28.373	28.580	28.790	29.001	29.214	29.428	29.645	29.862
4.4	30.082	30.303	30.526	30.751	30.978	31.206	31.436	31.668	31.902	32.138
4.5	32.376	32.615	32.857	33.100	33.345	33.593	33.842	34.093	34.347	34.602
4.6	34.859	35.119	35.381	35.645	35.911	36.179	36.449	36.722	36.996	37.274
4.7	37.553	37.835	38.119	38.405	38.694	38.985	39.279	39.575	39.873	40.174
4.8	40.478	40.784	41.093	41.404	41.718	42.035	42.355	42.677	43.001	43.329
4.9	43.660	43.993	44.329	44.668	45.010	45.356	45.704	46.054	46.409	46.766
5.0	47.127	47.490	47.857	48.227	48.600	48.976	49.357	49.740	50.127	50.517
5.1	50.911	51.308	51.709	52.113	52.521	52.933	53.349	53.768	54.191	54.618

(Bi & Ennis, 1997)

Table D.7. Numbers of judgements required for estimation of d'
with 0.90 CONFIDENCE LEVEL

2-AFC $1-\alpha = 0.90$

δ	$\Delta d' = 0.1$	$\Delta d' = 0.2$	$\Delta d' = 0.3$	$\Delta d' = 0.4$	$\Delta d' = 0.5$
0.5	885	220	99	55	35
1.0	1017	253	113	64	41
1.5	1286	320	143	80	51
2.0	1810	451	202	113	72

Note: δ = true value of d'; $\Delta d'$ = allowed error of estimate of δ, $\Delta d' = |d' - \delta|$.

3-AFC $1-\alpha = 0.90$

δ	$\Delta d' = 0.1$	$\Delta d' = 0.2$	$\Delta d' = 0.3$	$\Delta d' = 0.4$	$\Delta d' = 0.5$
0.5	707	176	79	44	28
1.0	745	186	83	47	30
1.5	877	218	98	55	35
2.0	1154	287	129	72	46

Note: δ = true value of d'; $\Delta d'$ = allowed error of estimate of δ, $\Delta d' = |d' - \delta|$.

Triangular $1-\alpha = 0.90$

δ	$\Delta d' = 0.1$	$\Delta d' = 0.2$	$\Delta d' = 0.3$	$\Delta d' = 0.4$	$\Delta d' = 0.5$
0.5	7936	1977	885	496	317
1.0	2690	670	300	168	108
1.5	1824	454	203	114	73
2.0	1662	414	185	104	67

Note: δ = true value of d'; $\Delta d'$ = allowed error of estimate of δ, $\Delta d' = |d' - \delta|$.

Duo-trio $1-\alpha = 0.90$

δ	$\Delta d' = 0.1$	$\Delta d' = 0.2$	$\Delta d' = 0.3$	$\Delta d' = 0.4$	$\Delta d' = 0.5$
0.5	8877	2211	990	554	355
1.0	2959	737	330	185	118
1.5	2071	516	231	129	83
2.0	2018	503	225	126	81

Note: δ = true value of d', $\Delta d'$ = allowed error of estimate of δ, $\Delta d' = |d' - \delta|$.

(Bi *et al.*, 1997)

Table D.7. Numbers of judgements required for estimation of d'
with 0.95 CONFIDENCE LEVEL

2-AFC $1-\alpha = 0.95$

δ	$\Delta d' = 0.1$	$\Delta d' = 0.2$	$\Delta d' = 0.3$	$\Delta d' = 0.4$	$\Delta d' = 0.5$
0.5	1263	316	138	79	51
1.0	1451	362	161	91	58
1.5	1837	459	206	115	73
2.0	2584	646	289	161	103

Note: δ = true value of d'; $\Delta d'$ = allowed error of estimate of δ, $\Delta d' = |d' - \delta|$

3-AFC $1-\alpha = 0.95$

δ	$\Delta d' = 0.1$	$\Delta d' = 0.2$	$\Delta d' = 0.3$	$\Delta d' = 0.4$	$\Delta d' = 0.5$
0.5	1010	252	113	63	40
1.0	1064	266	119	66	43
1.5	1252	313	140	78	50
2.0	1647	412	184	103	66

Note: δ = true value of d'; $\Delta d'$ = allowed error of estimate of δ, $\Delta d' = |d' - \delta|$

Triangular $1-\alpha = 0.95$

δ	$\Delta d' = 0.1$	$\Delta d' = 0.2$	$\Delta d' = 0.3$	$\Delta d' = 0.4$	$\Delta d' = 0.5$
0.5	11328	2832	1269	708	454
1.0	3840	960	430	240	154
1.5	2604	651	292	163	104
2.0	2373	593	266	148	95

Note: δ = true value of d', $\Delta d'$ = allowed error of estimate of δ, $\Delta d' = |d' - \delta|$

Duo-trio $1-\alpha = 0.95$

δ	$\Delta d' = 0.1$	$\Delta d' = 0.2$	$\Delta d' = 0.3$	$\Delta d' = 0.4$	$\Delta d' = 0.5$
0.5	12672	3168	1419	792	495
1.0	4224	1056	473	264	165
1.5	2957	739	331	185	116
2.0	2880	720	323	180	113

Note: δ = true value of d', $\Delta d'$ = allowed error of estimate of δ, $\Delta d' = |d' - \delta|$

Table D.8. Table of Risks (%) for TRIANGULAR TEST

			β		
n	*x*	α	50%	37.5%	25%
5	3	21	21	35	50
6	3	32	10	20	34
	4	10	32	49	
7	3	43	5	11	23
	4	17	17	32	50
	5	5	43		
8	4	26	9	20	36
	5	9	26	44	
9	4	35	4	12	25
	5	14	14	30	
	6	4	35		
10	4	44	2	7	17
	5	21	8	20	38
	6	8	21	41	
	7	2	44		
11	5	29	1	12	27
	6	12	12	28	50
	7	4	29		
12	5	47	2	7	19
	6	18	7	19	39
	7	7	18	38	
	8	2	37		
13	5	45	1	4	13
	6	24	3	12	29
	7	10	10	27	50
	8	3	21	48	
	9	1	45		
14	6	31	2	8	21
	7	15	6	18	40
	8	6	15	35	
	9	2	31		
15	6	38	1	5	15
	7	20	3	12	30
	8	9	9	25	
	9	3	20	44	
	10	1	38		
16	6	45	0	3	11
	7	26	2	8	23
	8	13	5	18	40
	9	5	13	33	
	10	2	26		
	11	0	45		
17	7	33	1	5	17
	8	17	3	12	31
	9	8	8	24	50
	10	3	17	41	
	11	1	33		
18	7	39	0	3	12
	8	22	1	8	24
	9	11	4	17	41
	10	4	11	31	
	11	1	22	49	
	12	0	39		
19	7	46	0	2	8
	8	28	1	5	18
	9	15	2	12	32
	10	6	6	23	50
	11	2	15	39	
	12	1	28		
	13	0	46		
20	8	34	0	3	13
	9	19	1	8	25
	10	9	4	16	41
	11	4	9	30	
	12	1	19	46	
	13	0	34		
21	8	40	0	2	9
	9	24	1	5	19
	10	12	2	11	33
	11	6	6	22	50
	12	2	12	37	
	13	1	24		
	14	0	40		
22	8	46	0	1	7
	9	29	0	3	14
	10	16	1	8	26
	11	8	3	16	42
	12	3	8	28	
	13	1	16	44	
	14	0	29		
	15	0	46		
23	9	35	0	2	11
	10	21	1	5	20
	11	11	2	11	34
	12	5	5	21	50
	13	2	11	35	
	14	1	21		
	15	0	35		
24	9	41	0	1	8
	10	25	0	3	15
	11	14	1	7	27
	12	7	3	15	42
	13	3	7	27	
	14	1	14	41	
	15	0	25		
	16	0	41		
25	9	46	0	1	5
	10	30	0	2	11
	11	18	1	5	21
	12	9	2	11	35
	13	4	4	20	
	14	2	9	33	
	15	1	18	48	
	16	0	30		
	17	0	46		
26	10	36	0	1	8
	11	22	0	3	16
	12	12	1	7	28
	13	6	2	14	42
	14	2	6	25	
	15	1	12	39	
	16	0	22		
	17	0	36		
27	10	41	0	1	6
	11	27	0	2	12
	12	15	1	5	22
	13	8	1	10	35
	14	4	4	19	
	15	1	8	31	
	16	1	15	46	
	17	0	27		
	18	0	41		
28	10	46	0	0	4
	11	31	0	1	9
	12	19	0	3	17
	13	10	1	7	29
	14	5	2	14	43
	15	2	5	24	
	16	1	10	37	
	17	0	19		
	18	0	31		
	19	0	46		
29	11	36	0	1	7
	12	23	0	2	13
	13	13	0	5	23
	14	7	1	10	36
	15	3	3	18	
	16	1	7	29	
	17	0	13	43	
	18	0	23		
	19	0	36		
30	11	42	0	1	5
	12	28	0	1	10
	13	17	0	3	18
	14	9	1	7	29
	15	4	2	13	43
	16	2	4	23	
	17	1	9	35	
	18	0	17	50	
	19	0	28		
	20	0	42		
31	11	47	0	0	4
	12	32	0	1	7
	13	20	0	2	14
	14	12	0	5	24
	15	6	1	10	36
	16	3	3	17	
	17	1	6	28	
	18	0	12	41	
	19	0	20		
	20	0	32		
	21	0	47		
32	12	37	0	1	6
	13	24	0	1	11
	14	14	0	3	19
	15	8	1	7	30
	16	4	2	13	43
	17	2	4	22	
	18	1	8	34	
	19	0	14	47	
	20	0	24		
	21	0	37		
33	12	42	0	0	4
	13	29	0	1	8
	14	18	0	2	13
	15	10	0	5	24
	16	5	1	9	36
	17	2	2	17	50
	18	1	5	27	
	19	0	10	39	
	20	0	18		
	21	0	29		
	22	0	42		
34	12	47	0	0	3
	13	33	0	1	6
	14	21	0	1	11
	15	13	0	3	20
	16	7	1	7	30
	17	3	1	12	43
	18	1	3	21	
	19	1	7	32	
	20	0	13	45	
	21	0	21		
	22	0	33		
	23	0	47		
35	13	38	0	0	4
	14	25	0	1	9
	15	15	0	2	16
	16	9	0	5	25
	17	4	1	9	37
	18	2	2	16	50
	19	1	4	25	
	20	0	9	37	
	21	0	15		
	22	0	25		
	23	0	38		
36	13	42	0	0	3
	14	29	0	1	7
	15	19	0	1	12
	16	11	0	3	20
	17	6	0	7	31
	18	3	1	12	43
	19	1	3	20	
	20	0	6	30	
	21	0	11	43	
	22	0	19		
	23	0	29		
	24	0	42		

n, Total number of responses; *x*, number of correct responses.
α, Type 1 risk; β, type 2 risk for percentage of distinguisher above chance.

(Schlich, 1993)

Table D.8. Table of Risks (%) for TRIANGULAR TEST - *contd-*

n	*x*	*α*	*β*		
			50%	37·5%	25%
37	13	47	0	0	2
	14	34	0	0	5
	15	22	0	1	9
	16	14	0	2	16
	17	8	0	5	26
	18	4	1	9	37
	19	2	2	15	50
	20	1	4	24	
	21	0	8	36	
	22	0	14	49	
	23	0	22		
	24	0	34		
	25	0	47		
38	14	38	0	0	4
	15	26	0	1	7
	16	16	0	1	13
	17	10	0	3	21
	18	5	0	6	31
	19	2	1	11	44
	20	1	2	19	
	21	0	5	29	
	22	0	10	41	
	23	0	16		
	24	0	26		
	25	0	38		
39	14	43	0	0	3
	15	30	0	0	5
	16	20	0	1	10
	17	12	0	2	17
	18	7	0	5	26
	19	3	1	8	37
	20	2	2	15	50
	21	1	3	23	
	22	0	7	34	
	23	0	12	46	
	24	0	20		
	25	0	30		
	26	0	43		
40	14	47	0	0	2
	15	34	0	0	4
	16	23	0	1	8
	17	14	0	1	13
	18	8	0	3	21
	19	4	0	6	32
	20	2	1	11	44
	21	1	2	18	
	22	0	4	28	
	23	0	8	39	
	24	0	14		
	25	0	23		
	26	0	34		
	27	0	47		
41	15	38	0	0	3
	16	27	0	0	6
	17	17	0	1	11
	18	10	0	2	17
	19	6	0	4	27
	20	3	1	8	38
	21	1	1	14	50
	22	1	3	22	
	23	0	6	32	
	24	0	10	44	
	25	0	17		
	26	0	27		
	27	0	38		
42	15	43	0	0	2
	16	31	0	0	4
	17	20	0	1	8
	18	13	0	1	14
	19	7	0	3	22
	20	4	0	6	32
	21	2	1	11	44
	22	1	2	17	
	23	0	4	26	
	24	0	7	37	
	25	0	13	50	
	26	0	20		
	27	0	31		
	28	0	43		
43	15	47	0	0	2
	16	35	0	0	3
	17	24	0	0	6
	18	15	0	1	11
	19	9	0	2	18
	20	5	0	4	27
	21	3	1	8	38
	22	1	1	13	50
	23	1	3	21	
	24	0	5	31	
	25	0	9	43	
	26	0	15		
	27	0	24		
	28	0	35		
	29	0	47		
44	16	39	0	0	2
	17	28	0	0	5
	18	18	0	1	9
	19	11	0	1	15
	20	6	0	3	23
	21	3	0	6	33
	22	2	1	10	44
	23	1	2	17	
	24	0	3	25	
	25	0	6	36	
	26	0	11	48	
	27	0	18		
	28	0	28		
	29	0	39		
45	16	43	0	0	2
	17	31	0	0	4
	18	21	0	0	7
	19	13	0	1	12
	20	8	0	2	19
	21	4	0	4	28
	22	2	0	8	38
	23	1	1	13	
	24	0	2	20	
	25	0	4	30	
	26	0	8	41	
	27	0	13		
	28	0	21		
	29	0	31		
	30	0	43		
46	16	47	0	0	1
	17	35	0	0	3
	18	25	0	0	5
	19	16	0	1	9
	20	10	0	1	15
	21	6	0	3	23
	22	3	0	6	33
	23	1	1	10	44
	24	1	1	16	
	25	0	3	24	
	26	0	6	34	
	27	0	10	46	
	28	0	16		
	29	0	25		
	30	0	35		
	31	0	47		
47	17	39	0	0	2
	18	28	0	0	4
	19	19	0	0	7
	20	12	0	1	12
	21	7	0	2	19
	22	4	0	4	28
	23	2	0	7	39
	24	1	1	12	
	25	0	2	19	
	26	0	4	28	
	27	0	7	39	
	28	0	12		
	29	0	19		
	30	0	28		
	31	0	39		
48	17	43	0	0	1
	18	32	0	0	3
	19	22	0	0	6
	20	14	0	1	10
	21	9	0	1	16
	22	5	0	3	24
	23	3	0	5	33
	24	1	1	9	44
	25	1	1	15	
	26	0	3	23	
	27	0	5	33	
	28	0	9	44	
	29	0	14		
	30	0	22		
	31	0	32		
	32	0	43		
49	17	47	0	0	1
	18	36	0	0	2
	19	25	0	0	4
	20	17	0	0	8
	21	10	0	1	13
	22	6	0	2	20
	23	3	0	4	28
	24	2	0	7	39
	25	1	1	12	
	26	0	2	19	
	27	0	3	27	
	28	0	6	37	
	29	0	10	49	
	30	0	17		
	31	0	25		
	32	0	36		
	33	0	47		
50	18	40	0	0	2
	19	29	0	0	3
	20	20	0	0	6
	21	13	0	1	10
	22	8	0	1	16
	23	4	0	3	24
	24	2	0	5	34
	25	1	0	9	44
	26	0	1	13	
	27	0	2	22	
	28	0	4	31	
	29	0	8	42	
	30	0	13		
	31	0	20		
	32	0	29		
	33	0	40		

n, Total number of responses; *x*, number of correct responses.
α, Type 1 risk, *β*, type 2 risk for percentage of distinguisher above chance.

(Schlich, 1993)

Table D.8. Table of Risks (%) for DUO-TRIO TEST

n	*x*	*α*	*β*		
			50%	37·5%	25%
5	4	19	37	50	
6	4	34	17	28	40
	5	11	47		
7	5	23	24	38	
8	5	36	11	22	35
	6	14	32	48	
9	5	50	5	11	22
	6	25	17	30	45
	7	9	40		
10	6	38	8	17	31
	7	17	22	38	
	8	5	47		
11	7	27	11	24	40
	8	11	29	47	
12	7	39	5	14	27
	8	19	16	31	49
	9	7	35		
13	8	29	8	19	35
	9	13	21	38	
	10	5	42		
14	8	40	4	11	24
	9	21	11	25	44
	10	9	26	46	
	11	3	48		
15	9	30	6	16	31
	10	15	15	32	
	11	6	31		
16	9	40	3	9	22
	10	23	8	21	39
	11	11	19	38	
	12	4	37		
17	10	31	4	13	28
	11	17	11	26	47
	12	7	23	45	
	13	2	43		
18	10	41	2	8	20
	11	24	6	17	35
	12	12	14	32	
	13	5	28		
	14	2	48		
19	11	32	3	10	25
	12	18	8	22	42
	13	8	17	38	
	14	3	33		
20	11	41	1	6	18
	12	25	4	14	32
	13	13	10	27	49
	14	6	21	44	
	15	2	38		
21	12	33	2	9	23
	13	19	6	18	38
	14	9	13	32	
	15	4	26	50	
	16	1	43		
22	12	42	1	5	16
	13	26	3	12	29
	14	14	7	22	45
	15	7	16	38	
	16	3	30		
	17	1	48		
23	13	34	1	7	21
	14	20	4	15	35
	15	11	10	27	
	16	5	20	43	
	17	2	35		
24	13	42	1	4	15
	14	27	2	10	26
	15	13	5	19	41
	16	8	12	32	
	17	3	23	49	
	18	1	39		
25	13	50	0	2	10
	14	35	1	6	19
	15	21	3	12	32
	16	11	7	23	47
	17	5	15	37	
	18	2	27		
	19	1	44		
26	14	42	1	4	13
	15	28	2	8	24
	16	16	4	16	38
	17	8	9	27	
	18	4	18	43	
	19	1	31		
	20	0	48		
27	14	50	0	2	9
	15	35	1	5	17
	16	22	2	10	29
	17	12	5	19	43
	18	6	11	32	
	19	3	21	48	
	20	1	36		
28	15	43	0	3	12
	16	29	1	7	22
	17	17	3	13	34
	18	9	7	23	49
	19	4	14	37	
	20	2	25		
	21	1	40		
29	15	50	0	2	8
	16	36	1	4	16
	17	23	2	9	26
	18	13	4	16	40
	19	7	9	28	
	20	3	17	42	
	21	1	29		
	22	0	44		
30	16	43	0	2	11
	17	29	1	6	20
	18	18	2	11	31
	19	10	5	20	46
	20	5	11	32	
	21	2	20	47	
	22	1	33		
	23	0	49		
31	16	50	0	1	8
	17	36	0	3	14
	18	24	1	7	24
	19	14	3	14	37
	20	7	6	24	
	21	4	13	37	
	22	1	23		
	23	1	37		
32	17	43	0	2	10
	18	30	1	5	18
	19	19	2	9	29
	20	11	4	17	42
	21	6	8	28	
	22	3	15	42	
	23	1	26		
	24	0	41		
33	18	36	0	3	13
	19	24	1	6	22
	20	15	2	12	34
	21	8	5	20	48
	22	4	10	32	
	23	2	18	46	
	24	1	30		
	25	0	45		
34	18	43	0	2	9
	19	30	0	4	16
	20	20	1	8	26
	21	11	3	14	39
	22	6	6	24	
	23	3	12	37	
	24	1	21		
	25	0	34		
	26	0	49		
35	19	37	0	2	12
	20	25	1	5	20
	21	16	2	10	31
	22	9	4	17	44
	23	4	8	28	
	24	2	14	41	
	25	1	24		
	26	0	37		
36	19	43	0	1	9
	20	31	0	3	15
	21	20	1	7	24
	22	12	2	12	36
	23	7	5	21	49
	24	3	9	32	
	25	1	17	46	
	26	1	27		
	27	0	41		
37	20	37	0	2	11
	21	26	0	4	19
	22	16	1	8	29
	23	9	3	15	41
	24	5	6	24	
	25	2	11	36	
	26	1	19	50	
	27	0	31		
	28	0	45		

n, Total number of responses: *x*, number of correct responses.
α, Type 1 risk: *β*, type 2 risk for percentage of distinguisher above chance.

(Schlich, 1993)

Table D.8. Table of Risks (%) for DUO-TRIO TEST - *contd-*

			β		
n	*x*	α	50%	37·5%	25%
38	20	44	0	1	8
	21	31	0	3	14
	22	21	1	6	22
	23	13	2	10	33
	24	7	3	18	46
	25	4	7	28	
	26	2	13	41	
	27	1	22		
	28	0	34		
	29	0	49		
39	21	37	0	2	10
	22	26	0	4	17
	23	17	1	7	27
	24	10	2	13	38
	25	5	4	21	
	26	3	9	32	
	27	1	15	45	
	28	0	25		
	29	0	38		
40	21	44	0	1	7
	22	32	0	2	13
	23	21	0	5	21
	24	13	1	9	31
	25	8	3	15	43
	26	4	5	24	
	27	2	10	36	
	28	1	18	49	
	29	0	28		
	30	0	42		
41	22	38	0	1	9
	23	27	0	3	16
	24	17	1	6	24
	25	11	2	11	35
	26	6	3	18	48
	27	3	7	28	
	28	1	12	40	
	29	1	21		
	30	0	32		
	31	0	45		
42	22	44	0	1	7
	23	32	0	2	12
	24	22	0	4	19
	25	14	1	8	29
	26	8	2	13	40
	27	4	4	21	
	28	2	8	32	
	29	1	14	44	
	30	0	23		
	31	0	35		
	32	0	49		
43	23	38	0	1	9
	24	27	0	3	14
	25	18	0	5	23
	26	11	1	9	33
	27	6	3	16	45
	28	3	5	25	
	29	2	10	36	
	30	1	17	48	
	31	0	26		
	32	0	39		
44	23	44	0	1	6
	24	33	0	2	11
	25	23	0	3	17
	26	15	1	6	26
	27	9	1	11	37
	28	5	3	18	49
	29	2	6	28	
	30	1	11	40	
	31	0	19		
	32	0	29		
	33	0	42		
45	23	50	0	0	4
	24	38	0	1	8
	25	28	0	2	13
	26	19	0	4	21
	27	12	1	8	31
	28	7	2	14	42
	29	4	4	21	
	30	2	8	32	
	31	1	13	44	
	32	0	22		
	33	0	33		
	34	0	45		
46	24	44	0	1	6
	25	33	0	1	10
	26	23	0	3	16
	27	15	0	5	24
	28	9	1	10	35
	29	5	2	16	46
	30	3	5	25	
	31	1	9	35	
	32	1	15	48	
	33	0	24		
	34	0	36		
	35	0	49		
47	24	50	0	0	4
	25	39	0	1	7
	26	28	0	2	12
	27	19	0	4	19
	28	12	1	7	28
	29	7	1	12	39
	30	4	3	19	
	31	2	6	28	
	32	1	11	39	
	33	0	18		
	34	0	27		
	35	0	39		
48	25	44	0	1	5
	26	33	0	1	9
	27	24	0	2	15
	28	16	0	5	23
	29	10	1	8	32
	30	6	2	14	44
	31	3	4	22	
	32	1	7	31	
	33	1	12	43	
	34	0	20		
	35	0	30		
	36	0	42		
49	25	50	0	0	4
	26	39	0	1	7
	27	28	0	2	11
	28	20	0	3	18
	29	13	0	6	26
	30	8	1	10	37
	31	4	2	16	48
	32	2	5	25	
	33	1	8	35	
	34	0	14	47	
	35	0	23		
	36	0	33		
	37	0	46		
50	26	44	0	0	5
	27	34	0	1	8
	28	24	0	2	14
	29	16	0	4	21
	30	10	1	7	30
	31	6	1	12	41
	32	3	3	19	
	33	2	6	28	
	34	1	10	39	
	35	0	16		
	36	0	25		
	37	0	36		
	38	0	49		

n. Total number of responses: *x*. number of correct responses.
α. Type 1 risk; β. type 2 risk for percentage of distinguisher above chance.

(Schlich, 1993)

Table D.8. Minimum total number of responses (n) and associated number of correct responses (x) for a range of type 1 and 2 risks for DUO-TRIO TEST

Risk 1	Risk 2 (1-power)									
	0·200		0·100		0·050		0·010		0·001	
	n	*x*	*n*	*x*	*n*	*x*	*n*	*x*	*n*	*x*
					p_d = 50%					
0·200	12	8	19	12	26	16	39	23	58	33
0·100	19	13	26	17	33	21	48	29	70	41
0·050	23	16	33	22	42	27	58	36	82	49
0·010	40	28	50	34	59	39	80	51	107	66
0·001	61	43	71	49	83	56	107	70	140	89
					p_d = 40%					
0·200	19	12	30	18	39	23	60	34	94	52
0·100	28	18	39	24	53	32	79	46	113	64
0·050	37	24	53	33	67	41	93	55	132	76
0·010	64	42	80	51	96	60	130	79	174	103
0·001	95	63	117	76	135	86	176	109	228	138
					p_d = 30%					
0·200	32	19	49	28	68	38	110	60	166	89
0·100	53	32	72	42	96	55	145	81	208	114
0·050	69	42	93	55	119	69	173	98	243	135
0·010	112	69	143	86	174	103	235	136	319	181
0·001	172	107	210	128	246	148	318	187	412	238
					p_d = 20%					
0·200	77	43	112	61	158	85	253	134	384	201
0·100	113	65	168	93	214	117	322	173	471	250
0·050	158	90	213	119	268	148	392	213	554	297
0·010	252	145	325	184	391	219	535	295	726	395
0·001	386	224	479	274	556	315	731	408	944	520
					p_d = 10%					
0·200	294	155	431	235	618	320	1006	517	1553	795
0·100	461	245	658	346	861	450	1310	679	1905	981
0·050	620	331	866	458	1092	574	1583	825	2237	1158
0·010	1007	541	1301	693	1582	838	2170	1140	2927	1527
0·001	1551	837	1908	1022	2248	1198	2937	1553	3812	2002
					p_d = 5%					
0·200	1152	591	1809	923	2477	1260	4033	2044	6206	3137
0·100	1818	937	2635	1351	3424	1750	5218	2656	7637	3875
0·050	2477	1280	3444	1771	4348	2229	6314	3223	8969	4563
0·010	4027	2088	5209	2689	6312	3249	8668	4443	11 729	5991
0·001	6182	3213	7662	3967	8974	4634	11 736	6036	15 271	7827

p_d. Percentage above chance.

(Schlich, 1993)

Table D.8. Minimum total number of responses (n) and associated number of correct responses (x) for a range of type 1 and 2 risks for TRIANGULAR TEST

Risk 1	Risk 2 (1-power)									
	0·200		0·100		0·050		0·010		0·001	
	n	*x*	*n*	*x*	*n*	*x*	*n*	*x*	*n*	*x*
					p_d = 50%					
0·200	7	4	12	6	16	8	25	11	36	15
0·100	12	7	15	8	20	10	30	14	43	19
0·050	16	9	20	11	23	12	35	17	48	22
0·010	25	15	30	17	35	19	47	24	62	30
0·001	36	22	43	25	48	27	62	33	81	41
					p_d = 40%					
0·200	12	6	17	8	25	11	36	15	55	22
0·100	17	9	25	12	30	14	46	20	67	28
0·050	23	12	30	15	40	19	57	26	79	34
0·010	35	19	47	24	56	28	76	36	102	46
0·001	55	30	68	36	76	39	102	50	130	61
					p_d = 30%					
0·200	20	9	28	12	39	16	64	25	97	37
0·100	30	14	43	19	54	23	81	33	119	47
0·050	40	19	53	24	66	29	98	41	136	55
0·010	62	30	82	38	97	44	131	57	181	76
0·001	93	46	120	57	138	64	181	81	233	101
					p_d = 20%					
0·200	39	16	64	25	86	33	110	52	212	77
0·100	62	26	89	36	119	47	178	68	260	97
0·050	87	37	117	48	147	59	213	83	305	116
0·010	136	59	176	74	211	87	292	117	397	155
0·001	207	91	257	110	302	127	396	162	513	205
					p_d = 10%					
0·200	149	55	238	86	325	116	529	186	819	285
0·100	240	90	348	128	457	166	683	244	1011	357
0·050	325	123	447	166	572	210	828	299	1181	421
0·010	525	201	680	256	824	307	1132	413	1539	557
0·001	803	310	996	379	1165	439	1530	568	1992	730
					p_d = 5%					
0·200	593	208	931	323	1273	439	2074	710	3201	1090
0·100	927	328	1359	476	1761	613	2682	926	3943	1353
0·050	1271	452	1763	621	2223	779	3216	1127	4616	1592
0·010	2059	737	2660	944	3236	1142	4440	1554	6021	2093
0·001	3162	1137	3903	1393	4585	1628	6006	2116	7827	2739

p_d. Percentage above chance

(Schlich, 1993)