Flavour Impact of Aged Beers*

By David J. Evans, Diederik J. M. Schmedding, Arnold Bruijnje, Theo Heideman, Bonnie M. King and Niek M. Groesbeek

Quest International Nederland BV, Huizerstraatweg 28, 1411 GP Naarden, The Netherlands

Using a gas chromatography olfactometry (GCO) technique, it has been possible to identify a number of flavour impact components of fresh, naturally aged and forced aged lager. One of the benefits of this technique is that a wide range of volatile flavour components, for example esters, aldehydes, sulphur compounds and lactones can be examined within a single analysis. Together with appropriate conventional chemical analysis for aldehydes and sensory analysis, it has also been possible to relate perceived changes in overall flavour balance to some specific changes in aldehyde levels during ageing. In this way, time dependent changes in the levels of methional, phenylacetaldehyde, 4-methoxybenzaldehyde, octanal, (E)-2-nonenal have been indicated.

GCO allows for the convenient detection of flavour components whose contribution may remain undetected by current conventional chemical analysis but which may play a key role in determining the flavour impact of aged beers.

INTRODUCTION

The rate at which aged or stale off-flavour forms in beer has presented a problem to brewers for many years. With the trend towards an increasing number of international beer brands, the problem of beer flavour staling is ever more evident as brewers strive to assure the quality of their product in the global marketplace.

Flavour staling of beer is first and foremost a problem of off-flavour formation. Beer stale flavour, however, is additionally complex relative to other off-flavour problems, in that flavour perception is not only time dependent, but also represents the net effect of a series of chemical changes to the flavour impact components of beer. The application therefore of experimental techniques devised to identify and analyse complex flavours can provide a valuable insight into this problem.

In brewing research, sensory analysis has been widely used to evaluate the flavour of aged beers[6] together with a range of chemical analyses to identify off-flavours. These chemical analyses include gas chromatography[5], liquid chromatography[17], mass spectrometry[19] and more recently electron spin resonance[14] to name but a few. A technique, however, which has not been so widely used in the brewing environment but which is very effective in flavour analysis, is gas chromatography-oflactometry (GCO)[3].

In very simple terms, this technique uses chromatography to separate complex flavours into individual components and uses the sensitivity of the human nose to identify the odour character of the components. In combination with other analytical procedures e.g. mass spectrometry, it is a very powerful tool in the characterisation of complex flavour matrices. Although there are some examples of the use of GCO to evaluate beer flavour[1,27], the application of this technique to characterise stale flavour is rare[27]. The aim of this work therefore has been to try and identify flavour impact components of aged beer using a combination of GCO with electron capture detection (GC-ECD) and sensory evaluation.

EXPERIMENTAL

MATERIALS

All beer samples used in this study have been of one Pilsner-type lager brand and have been obtained from the country of origin.

GCO and sensory study

Four samples were used in this series of experiments:

A Fresh beer <1 month old from date of packaging

B Naturally aged by storing in the dark at 20°C for 11 weeks

C   Naturally aged by storing in the dark at 20°C for 16 months

D   Forced aged by storing in the dark at 40°C for 6 weeks.

Samples A, B and D originated from the same production batch. Samples were extracted for GCO and stored under nitrogen at -18°C until required. For the sensory study, all four samples were tasted within a week period of each other. The fresh and forced aged beer samples (A and D) were placed in a refrigerator at -4°C for periods of approximately 11 and 5 weeks respectively, to minimise further flavour deterioration, in order that they could be tasted alongside samples B and C.

Carbonyl study

Four samples were used in this series of experiments:

E   Fresh beer <1 month old from date of packaging

F   Naturally aged by storing in the dark at 20°C for 6 months

G   Naturally aged by storing in the dark at 20°C for 12 months

H   Naturally aged by storing in the dark at 20°C for 18 months.

All four samples were from different production batches. Samples were derivatised and extracted immediately (see carbonyl analysis) after they had reached the required degree of ageing, and were stored under nitrogen at -18°C until required for analysis.

METHODS

Gas chromatography olfactometry

Beer (20 ml) was adsorbed onto an Extrelute 20 extraction column (Merck, cat. no. 111737) under a steady flow of nitrogen to exclude oxygen. At all times during the extraction the sample was kept in darkness. Extraction of the adsorbed beer from the column was achieved by passing pentane (100 ml) through the column, again under a steady flow of nitrogen. Pentane was used as a selective extraction solvent to prevent the extraction of the very polar components (i.e. shorter chain fatty acids) which disturb "sniffing". Although extraction with pentane is not quantitative for the medium polar components, it is reproducible. The eluted pentane plus extracted material was concentrated to ca 35 mg by distilling off the excess solvent followed by microdistillation, when the extract volume was reduced to ca 5 ml. An aliquot of the resultant concentrate was injected directly into the gas chromatograph.

The principle of GCO has been described elsewhere[1]. A Carlo Erba 5300 series gas chromatograph equipped with a sniffing port and flame ionisation detection (FID)

was used in this study. Cold splitless injection was used to introduce the sample on to an HP-5, 50m, 0.32mm ID column. The thermal gradient for separation was 40-270°C at 3°C/min and hold at 270°C for 45 minutes to clean the column. The split of the eluant from the column was in the ratio of 10:90 for FID:sniff port. Sniffing was performed for 60 minutes approximately.

In this study GCO was not used to obtain quantitative information about levels of odour components. Aroma peaks were identified on the basis of their odour description and Relative Retention index, to obtain an aromagram. An aromagram is a collection of data, which includes the FID signal, the aroma peak, the odour descriptors, and the Relative Retention indices (see Fig. 1). During the aromagram run, the Flavourist indicates when the odour impression begins and ends with a hand-held push-button switch. This action records a time dependent signal in a computer.
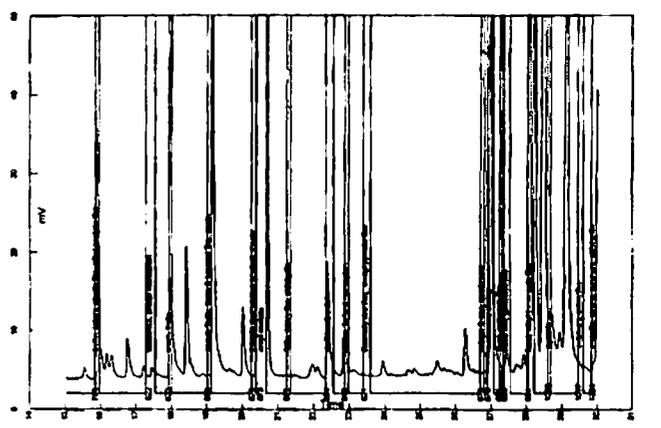

FIG. 1.   Portion of an Aromagram from CG Olfactometry overlaid on GC Chromatogram of the same lager naturally aged for 11 weeks.

After the aromagram has been completed the retention times of these aroma peaks are recalculated to Relative Retention indices. The determination of Relative Retention indices for linear temperature programmed GC has been described elsewhere[8]. The alkane series used for these runs included all the n-alkanes·from C6-C24.

The odour descriptions are recorded on a voice-activated tape recorder. After the aromagram run is complete, they are recorded with the Relative Retention index, alongside the appropriate aroma peak.

Carbonyl analysis

The procedure used for carbonyl analysis has been described elsewhere[11]. The principle of analysis is based on derivatisation of the carbonyls using pentafluorobenzylhydroxylamine followed by detection of the derivatised carbonyl using GC-ECD. A Carlo Erba 5300 series gas chromatograph equipped with electron capture detection was used in this study. Splitless

injection was used to introduce the sample on to an HP-5, 50m, 0.32mm ID column. The thermal gradient for separation was> 120-280°C at 6°C/min.

Sensory analysis and principal components analysis

The procedures used for sensory analysis and principal components analysis (PCA) have been described elsewhere[16].

RESULTS

Figure 1 shows a portion of an aromagram (combined data output of GCO and GC-FID) of a naturally aged lager of 11 weeks. It can be seen that several aroma peaks (labelled according to odour description and Relative Retention index), e.g. peaks 822, 850, 870, 886, 921 and 973 do not coincide with significant GC peaks. Peaks 798 and 904 however, correspond with ethyl butanoate and methional respectively, and here GC peaks can clearly be seen. Typical levels of ethyl butanoate and methional found in beer, range from 0.04-0.2 mg/litre and <0.05 mg/litre respectively[20], thereby illustrating the sensitivity for GCO compared with GC-FID analysis.

Tables I to VIII illustrate some of the aroma peaks of samples of fresh, naturally aged and forced aged lager grouped into categories of flavour impact components, e.g. higher alcohols, esters, etc. The 58 aroma peaks identified in Tables I to VIII have been selected from a total number of 127 aroma peaks identified during the analysis of four samples of fresh, naturally aged and forced aged lager. The peaks are included in the appropriate table on the basis of their odour description or, if a named compound, their chemical identity. Identification of a compound is based upon a combination of odour description, in-house experience of Relative Retention indices of pure flavour compounds and information from GC mass spectrometry databases. Quantitative GCO, either by charm[4] or aroma extract dilution analysis[10] has not been performed in this study. The four beer samples used in the GCO and sensory study, denoted A, B, C and D were found to have a total number of aroma peaks of 80, 77, 72 and 81 respectively. Of these, 55 peaks were found to be common on all four samples.

Table I shows some if the higher alcohol components of the four beers. These three components were common to all beer samples. 2-Phenylethanol and 3-methylbutanol are well known flavour components of beer. 1-Octene-3-ol is reported to be found in beer at levels of around 0.03 mg/litre[20].

Table II shows some ester components of four beers. The esters, ethyl pentanoate and ethyl octanoate were detected in the fresh sample only, whereas an unknown estery flavour (ester 2, RRI:1035) apparently was formed on extended ageing and appeared only in naturally aged lager of 16 months and forced aged lager. Other work has reported changes in the ester character of beer during ageing[19].

TABLE II. Ester components of lager as detected by GC-olfactometry

Compound	RRI[a]	Odour description	Presence in lager			
			F[a]	NA11w[a]	NA1y[a]	FA[a]
Ethyl Butanoate	798	Sweet, fruity	√	√	√	√
Ethyl 3-methylbutanoate	847	Sweet, fruity	√	√	√	√
3-methylbutyl acetate	872	Isoamyl acetate	√	√	√	√
Ethyl pentanoate	898	Fruity, rhubarb	√	√	√	√
Ester 1	960	Estery	√	√	√	√
Ethyl hexanoate	994	Ethyl hexanoate	√	√	√	√
Ester 2	1035	Estery	√	√	√	√
Ethyl octanoate	1198	Honey-like	√	√	√	√
2-Phenylethyl acetate	1264	Sweet, honey	√	√	√	√

a   see Table I

Table III shows the aldehydic components of the four lagers. Here the aromagram results suggest a decrease in levels of octanal together with the formation of 4-methoxybenzaldehyde and an unknown aldehyde (aldehyde 1, RRI:1067). Figures 2A and 2B show the changes in some named aldehydes during natural ageing of lager. In this analysis, four samples of lager of the same type including fresh, and naturally aged for 6, 12 and 18 months were analysed for changes in levels of carbonyl

TABLE I. Higher alcohol components of lager as detected by GC-olfactometry

Compound	RRI	Odour description	Presence in lager			
			F	NA11w	NA1y	FA
3-Methylbutanol	728	3-Methyl butanol	√	√	√	√
Octene-3-ol	976	Metallic, mushroom	√	√	√	√
2-Phenylethanol	1120	Phenyl ethanol	√	√	√	√

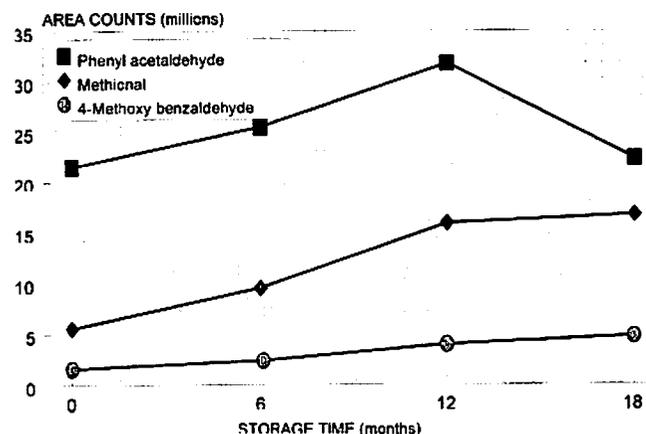|			
F	Fresh Beer	NA 11w	Naturally Aged 11 weeks
NA 1y	Naturally Aged> 1 year	FA	Forced Aged
RRI	Kovats Index	ND	Not Detected

FIG. 2A. Changes in levels of some aldehydes during natural ageing of lager.
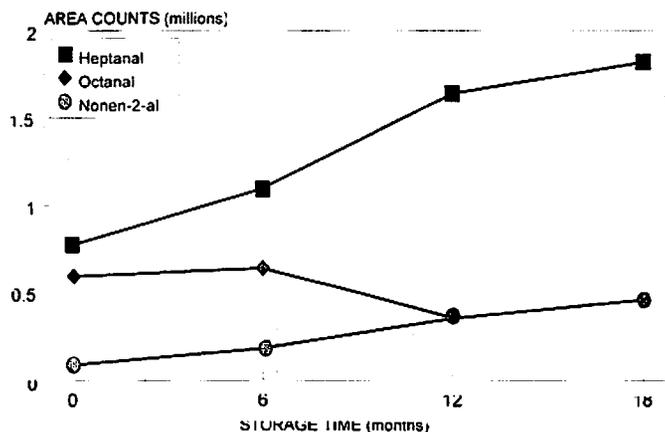
FIG. 2B. Changes in levels of some aldehydes during natural ageing of lager.

compounds. The named components listed in Figures 2A and 2B represent a small proportion of the total number of carbonyl compounds detected. Nevertheless, it can be seen that levels of methional, 4-methoxybenzaldehyde, heptagonal and (E)-2-nonenal tend to increase. Phenylacetaldehyde appears to increase and then decrease and octanal appears to decrease. Although full quantitative analysis of these aldehydes has not been carried out, it can be seen that the area

TABLE III. Aldehydic components of lager as detected by GC-olfactometry

Compound	RRI[a]	Odour description	Presence in lager			
			F[a]	NA11w[a]	NAly[a]	FA[a]
Methional	904	Potato	√	√	√	√
Octanal	1001	Aldehydic	√	ND[a]	ND	ND
Phenylacetaldehyde	1048	Floral, honey	√	√	√	√
Aldehyde 1	1067	Aldehydic	ND	√	√	√
Aldehyde 2	1073	Aldehydic, mushroom	√	√	√	√
Aldehyde 3	1095	Aldehydic	√	√	√	√
Nonanal	1099	Aldehydic	√	√	√	√
(E,Z)-2,6-Nonadienal	1151	Aldehydic, papery	√	√	√	√
(E)-2-Nonenal	1163	Aldehydic, papery	√	√	√	√
Aldehyde 4	1220	Aldehydic	√	√	√	√
4-Methoxybenzaldehyde	1267	Aubepine, aniseed	ND	√	√	√
(E)-2-Decenal	1270	Soapy, aldehydic	√	√	√	√
Aldehyde 5	1285	Aldehydic	√	√	√	√
Aldehyde 6	1385	Aldehydic	√	√	√	√

a   see Table I

counts of phenylacetaldehyde and methional are present at relatively higher levels than (E)-2-nonenal. Also the pattern of change seen with these aldehydes is similar to that seen with GCO for octanal and 4-methoxybenzaldehyde. Increases in the levels of (E)-2-nonenal[9], methional[30] and phenylacetaldehyde[23,27] during ageing of beer have been reported. The presence, however, of 4-methoxybenzaldehyde, although previously reported in beer[22], has not been associated with time

dependent increase during storage. It is possible, however, that the unknown compound having "sweet, aniseed" flavoured, identified as increasing during forced ageing of lager by Schieberle[27], could be 4-methoxybenzaldehyde. This tentative identification is based on similarities in Relative Retention indices, since this and Schieberle's study used similar chromatographic columns (i.e. stationary phases).

TABLE IV. Sulphur containing compounds of lager as detected by GC-olfactometry

Compound	RRI[a]	Odour description	Presence in lager			
			F[a]	NA11w[a]	NAly[a]	FA[a]
3-Methyl-2-butenethiol	823	Sunstruck	√	√	√	√
2-Methyl-3-furanthiol	870	Meaty	√	√	√	√
Furfuryl thios	912	Roasted, coffee	ND[a]	√	√	√
Sulphur 1	983	Sulphury	√	√	√	√
Sulphur 2	1024	Metallic, sulphury	√	√	√	√
Sulphur 3	1192	Metallic, sulphury	√	√	√	√

a   see Table I

Table IV shows some sulphur containing compounds identified from the aromagrams of the four lagers. Most of these components were common to all samples. The compound named as furfuryl thiol, not previously reported in beer, however, appears in the naturally aged and forced aged samples only. Furfuryl mercaptan is a known flavour impact component of coffee[28]. The component, 3-methyl-2-butenethiol, responsible for sunstruck flavour, was common to all the beer samples studied, although none of the samples would be described as being sunstruck. Again, with reference to Schieberle's earlier work[27], the unknown sulphury compound described as decreasing with forced ageing conditions is probably 3-methyl-2-butenethiol, based again on the similar Relative Retention indices and odour descriptions found in the two studies.

TABLE V. Phenols of lager as detected by GC-olfactometry

Compound	RRI[a]	Odour description	Presence in lager			
			F[a]	NA11w[a]	NAly[a]	FA[a]
Guaiacol	1094	Phenolic	√	√	√	√
3,5-Dimethylphenol	1170	Phenolic, leather	√	√	√	ND[a]
Phenol 1	1201	Musky, Geosmin-like	√	√	√	√
4-Ethylguaiacol	1288	Spicy, phenolic	√	√	√	√
Phenol 2	1304	Smoky, phenolic	√	ND	ND	√
4-Vinylguaiacol	1323	Spicy, phenolic	√	√	√	√
Phenol 3	1331	Medicinal, phenolic	√	√	√	ND
Phenol 4	1366	Phenolic	√	√	√	√
Vanillin	1413	Vanillin	√	√	√	√
Phenol 5	1437	Musky, phenolic	√	√	√	√
Phenol 6	1456	Sweet, phenolic	√	√	√	√
Phenol 7	1575	Smoky, phenolic	ND	√	ND	√

a   see Table I

Table V shows some phenolic compounds as detected by GCO. Here it can be seen that the flavour component, 4-vinylguaiacol is common to all samples tested. Conditions of forced ageing, however, have been shown to result in reduced levels of 4-vinylguaiacol[27]. It is also important to note that this beer would not have been expected to carry a phenolic flavour impression, nor did it carry one according to sensory evaluation.

TABLE VI. Maillard reaction products of lager as detected by GC-olfactometry

Compound	RRI[a]	Odour description	F[a]	NA11w[a]	NA1y[a]	FA[a]
				Presence in lager		
2-Acetyl-1-pyrroline	920	Roasted, Basmati-like	√	√	√	√
Trimethylpyrazine	1003	Cocoa, roasted	√	√	√	√
3,5-Dimethyl-2-ethylpyrazine	1086	Cocoa, pyrazine	√	√	√	√
Maillard 1	1105	Pyrazine-like, roasted	ND[a]	ND	ND	√
Maillard 2	1160	Cocoa, pyrazine	√	√	√	√
Maillard 3	1173	Hay-like, pyrazine	√	√	√	√
Maillard 4	1187	Earthy, hay-like	ND	√	√	ND
Maillard 5	1350	Hay-like	√	ND	ND	ND

a   see Table I

Table VI shows some of the Maillard reaction products from the four beer samples. The named Maillard reaction products were common to all of the samples. The unnamed components, listed because the odour descriptions were typical of Maillard products, indicated that their levels may be related to storage conditions. 2-Acetyl-1-pyrroline, a known flavour impact compact of Basmati Rice[15], and trimethylpyrazine, have both been reported previously in beer[22,27]. The compound, 3,5-dimethyl-2-ethylpyrazine, named on the basis of its Relative Retention index, has not been reported previously in beer but is a known flavour component of other food systems[25]. Previous work[24] has indicated relatively little change in Maillard products during prolonged storage, most of the change in levels of Maillard products taking place within a few days after packaging. It is thought that the fresh sample used in this study is probably at least 1 week old from the date of packaging, hence any change as described by Qureshi *et al*[24] would probably already have taken place.

Table VII shows some lactone compounds found in the four beer samples by GCO. All of the three named

TABLE VII. Lactones of lager as detected by GC-olfactometry

Compound	RRI[a]	Odour description	F[a]	NA11w[a]	NA1y[a]	FA[a]
				Presence in lager		
γ-Octalactone	1267	Coconut	ND[a]	√	√	√
γ-Nonalactone	1372	Coconut, lactone-like	√	√	√	√
γ-Decalactone	1480	Peaches, lactone-like	√	ND	ND	√

a   see Table I

compounds have been previously found in beer[20]. There is some evidence that the γ-octalactone is present at lower levels in the fresh sample than in aged samples. Similarly, γ-decalactone is present at lower levels in naturally aged samples. The possible role of lactones in the development of stale flavour formation has been suggested by Hashimoto[17].

TABLE VIII. Hop-derived compounds of lager as detected by GC-olfactometry

Compound	RRI[a]	Odour description	F[a]	NA11w[a]	NA1y[a]	FA[a]
				Presence in lager		
Hop 1	1067	Citronellol-like, terpene	√	ND[a]	ND	√
Geraniol	1258	Floral, geraniums	√	√	ND	ND
(E)-β-Damascenone	1397	Cooked fruit	√	√	√	√

a   see Table I

Table VIII shows some compounds originating from hop that have been found in the four samples of lager. The compound, (E)-β-damascenone was common to all four samples. It has been found in our laboratories to be one of the flavour impact components of a number of hop oil fractions. Furthermore, it has been reported previously as increasing during conditions of forced ageing[27] and is believed to be a carotenoid degredation product[31]. Geraniol and the unknown hop compound (hop1, RRI:1067) appeared to decrease in level during conditions of ageing. Hop flavours are known to be susceptible to oxidation and hop aroma of beer has been observed to change during the ageing process[26].

Figure 3 shows the first two dimensions from PCA of four flavour attribute of lager samples A, B, C and D used in this GCO study. These results illustrate the difference in overall sensory perception of the four beer samples. The fresh sample being associated with a more bitter character, the moderately, naturally aged sample (B) with a more papery character, and the forced aged sample with a sherry-type flavour.
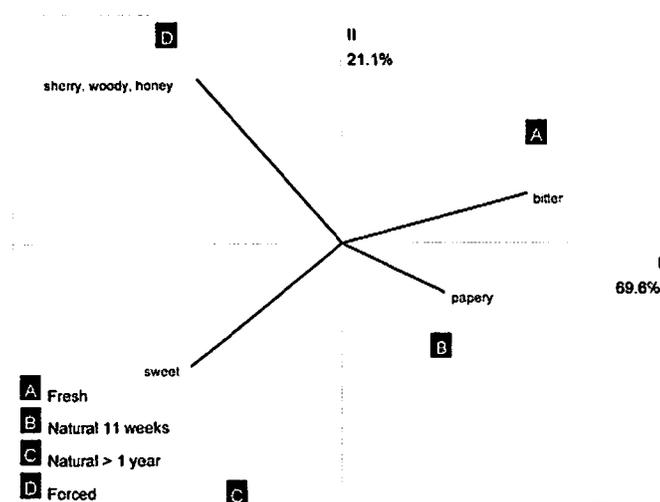

FIG. 3. The first two dimensions from PCA of four flavour attributes of fresh, naturally aged and forced aged lager.

DISCUSSION

This study indicates that the combined approach of GCO, GC-ECD after derivatisation and sensory analysis can provide additional insight into stale flavour formation in beer. Demonstrating time dependent change, for individual flavour impact components can, however, be difficult particularly for those flavour impact components present at levels for which conventional chromatographic analysis is difficult. Furthermore, the situation is complicated, because it is known that extraction conditions used to isolate volatiles for analysis by GCO can influence the profile of flavour impact components that are observed[2]. Not surprisingly, therefore, the relatively few flavour impact components that are known to be dependent upon storage conditions, are present in beer at levels which can be measured by robust quantitative analytical procedures.

Esters, and higher alcohols are measured routinely in brewing, however, it is not clear if these components change in a predictable direction during the ageing process, although, the decrease in ester character of beer has been observed during ageing[19]. The formation of aldehydes during ageing of beer has led to the development of increasingly sensitive methods for their measurement[11]. Methods for the specific measurement of (E)-2-nonenal[9] have been developed because the flavour impact of this compound so closely correlates with one of the perceived flavour changes in aged beer, namely cardboard flavour[18].

The focused activity on the formation of aldehydes in beer has led to the proposals of several plausible mechanisms for their formation[12] although it is still not clear which of these mechanisms results in the greatest proportion of flavour change during storage. There does, however, appear to be two distinct groups of flavour active aldehydes. Aldehydes that appear to be derived from the oxidative degradation of unsaturated fatty acids, e.g. (E)-2-nonenal, and aldehydes that are derived from the non-oxidative Strecker degradation of amino acids e.g. phenylacetaldehyde. Whichever of these and other related mechanisms predominate, however, the role of aldehyde formation is clearly implicated as a contributor towards the formation of stale flavour in beer.

The reliable measurement of other groups of flavour impact components of beer in relation to how they change with time has not been the subject of such intense investigation. The use of quantitative GCO by charm analysis or aroma extract dilution analysis may conveniently give useful information as to the quantitative levels of volatile flavour impact components of beer as they change with time and storage conditions. Importantly, GCO helps to identify only those volatile components which have a high flavour activity[29]. It avoids compounds which despite being both readily detectable by other chemical analysis and subject to changes in level during storage, contribute little to overall flavour impact.

Apart from the aldehydes and esters, this study has indicated the involvement in beer ageing of some well known flavour impact components of other food systems. Furfuryl thiol, for example, is an important and potent flavour impact component of coffee[19], whereas 2-methyl-3-furanthiol is a flavour impact component of roasted meat[13]. If further work demonstrates either of these to be more widely associated with beer flavour staling, this may indicate the benefit of investigating the time dependent change of sulphur containing compounds in beer on a more general level.

The contribution of hop derived volatile flavours towards the appearance of stale flavour formation in beer is interesting, as here the impact upon stale flavour formation may be more related to the disappearance of these components rather than their formation. Although the composition of hop aroma in beer has been the subject of much study[7], very little work, if one excludes the development of sunstruck flavour, has been carried out on the changing levels of hop derived volatile compounds during storage of packaged beer. Late hop aroma is a desirable quality of some lagers, it follows therefore that the deterioration in the levels of flavour impact components that give rise to late hop aroma e.g. geraniol may be associated with the onset of stale flavour perception.

On a more general note, this study has indicated that a large proportion of the flavour impact components of beer, including some of those thought to be involved in the development of stale flavour are present in examples of both fresh and aged beers. Relatively few compounds have been either newly formed or have completely disappeared during ageing of beer from the point of packaging. This suggests and supports the view which is increasingly presented, that conditions exist in the brewing process which allow for the formation of many of the flavour impact components that contribute to the perception of stale flavour formation[21]. Clearly, however, after packaging, conditions exist which allow for the continued formation and degradation of these flavours, thereby altering the overall flavour balance of the product. Stale flavour perception in beer, therefore, would appear to be, at least in part, a function of the concentration and interaction of a wide range of flavour impact components that are common to both fresh and aged beers alike.

REFERENCES

1. Abbott, N., Etiévant, P.X., Issanchou, S. & Langlois, D., *Journal of Agricultural and Food Chemistry*, 1993, **41**, 1698.

2. Abbott, N., Etiévant, P.X., Langlois, D., Lesschaev, I. & Issanchou, S., *Journal of Agricultural and Food Chemistry*, 1993, **41**, 777.

3. Acree, T.E., *Analytical Chemistry News and Features*, March 1, 1997, 170.

4. Acree, T.E. & Barnard, J., *Trends in Flavour Research*, Maarse, H. & van der Heij, D.G. (Eds.) Elsevier, Amsterdam, 1994, 211.

5. Ahrenst-Larsen, B. & Hansen, H.L., *Brauwissenschaft*, 1963, **16**, 1393.

6. Bennett, S.J.E., *Food Taints and Off Flavour*, Saxby, M.J. (Ed), Chapman & Hall, 1996, 290.

7. Deinzer, M. & Yang, X., *Monograph-European Brewing Convention*, 1994, **22**, 181.

8. van den Dool, H. & Kratz, P.D., *Journal of Chromatography*, 1963, **11**, 463.

9. Drost, B.W., van den Berg, R., Freijee, F.J.M., van der Velde, E.G. & Hollemans, M., *Journal of the American Society of Brewing Chemists*, 1990, **48**, 124.

10. Etiévant, P.X., Moio, L., Guichard, E., Langlois, D., Leschaeve, I., Schlich, P. & Chambellant, E., *Trends in Flavour Research*, Maarse, H. & van der Heij, D.G. (Eds) Elseview, Amsterdam, 1994, 179.

11. Grönqvist, A., Siirilä, J., Virtanen, H., Home, S. & Pajunen, E., *Proceedings of the 24th Congress of the European Brewery Convention*, Oslo, 1993, 421.

12. Hasimoto, N., *Brewing Science*, Pollock, J.R.A. (Ed) Academic Press, London, 1981, 347.

13. Hofmann, T. & Shieberle, P., *Journal of Agricultural and Food Chemistry*, 1995, **43**, 2187.

14. Kaneda, H., Kano, Y., Osawa, T., Ramarathnam, N., Kawakishi, S. & Kamada, K., *Journal of Food Science*, 1988, **53**, 885.

15. Kerler, J., van der Ven, J.G.M. & Weenen, H., *Food Reviews International*, 1997, **13(4)**, 553.

16. King, B.M., *Proceedings of the 5th Convention of the Institute of Brewing (C&SA Section)*, Victoria Falls, 1995, 9.

17. Kobayashi, N., Kaneda, H., Kano Y and Koshino, S., *Journal of the Institute of Brewing*, 1993, **99**, 143.

18. Meilgard, M.C., *The Brewers Digest*, 1972, **72**, 48.

19. Meidaner, H., Narziss, L. & Eichhorn, P., *Proceedings of the 23rd Congress of the European Brewing Convention*, Lisbon, 1991, 401.

20. Moll, M., *Beers and Coolers*, Intercept, Andover, 1991, 276.

21. Narziss, L., *Journal of the Institute of Brewing*, 1986, **92**, 346.

22. Nijssen, L.M., Visscher, C.A.& Maarse, H., *Volatile Compounds in Food. Qualitative and Quantitative Data*, TNO, Zeist, The Netherlands, 1996.

23. Pollock, J R A., *Proceedings of the 12 Convention of the Institute of Brewing (Aust. & NZ Section)*, 1971, 135.

24. Qureshi, A.A., Burger, W.C. & Prentice, N., *Journal of the American Society of Brewing Chemists*, 1979, **37**, 161.

25. Rychlik, M. & Grosch, W., *Lebensmittel-Wissenschaft und-Technologie*, 1996, **29**, 1.

26. Ryder, D.S., Power, J. & Siebel, R.E., *Proceedings of the 2nd Convention of the Institute of Brewing (C&SA Section)*, Johannesburg, 1989, 266.

27. Scchieberle, P., *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, 1991, **193**, 558.

28. Semmelrock, P. & Grosch, W., *Journal of Agricultural and Food Chemistry*, 1996, **44**, 537.

29. Siebert, K.J. & Acree, T.E., *Cerevisia Biotechnology*, 1993, **18**, 29.

30. Visser, M.K. & Lindsay, R.C., *Proceedings of the American Society of Brewing Chemists*, 1971, 230.

31. Winterhalter, P., *Biotechnology for Improved Foods and Flavours*, American Chemical Society, 1996, 295.