Tracking Staling Components in Beer

THERMAL DESORPTION | Sensitive analyses are required for assessing beer ageing because beer contains only low concentrations of relevant compounds. It is possible to identify these compounds: following extraction from beer, thermal desorption-gas chromatography-mass spectrometry is used in the subsequent analysis.

BEER IS one of the most popular alcoholic beverages and its characteristic taste is widely appreciated by consumers. They expect a high-quality product with consistent properties to the end of the best before date. When beer is stored inappropriately, break-down reactions of valuable aroma substances in beer may take place at high temperatures or under the influence of strong light; as a result, beer body may be lost or off-flavours may develop. This is, among other things, attributable to carbonyl compounds. Even at low concentrations, they can be perceived as unpleasant by consumers (Table 1).

Enrichment of these volatile components on an adsorber material is a common analysis method. Substances that, due to their porous structure, can interact with the compounds to be analysed are suitable adsorbers for enriching the compounds on the material.

SPME-GC/MS (Solid Phase Microextraction) is an established method. However, the fact that only low concentrations can be extracted from the sample in view of the limited amount of useable adsorber material is a drawback. This method thus has limited sensitivity. To circumvent this drawback, an adsorption method using thermal desorption was developed for storing and extracting larger sample quantities on the adsorber.

Principle of Thermal Desorption

Thermal desorption is suitable for investigating highly volatile or semi-volatile compounds. It is a process for concentration. Components are extracted from the sample blanketed by an inert nitrogen gas flow and transferred on an adsorber material. Release (desorption) of the compounds from the adsorber follows in the thermal desorber at temperatures between 250 °C and 320 °C. The compounds are then refocused on a downstream cold trap. In a second desorption step, the cold trap is rapidly heated to temperatures between 280 °C and 310 °C and transfers the analytes into a gas chromatograph for separation of the

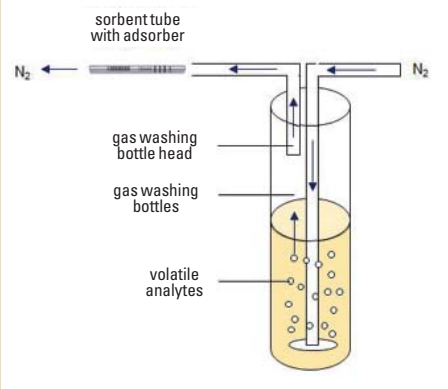

Fig. 1 Washing bottle with head for extraction of analyte and enrichment on the sorbent tube for thermal desorption

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TASTE THRESHOLD VALUES AND FLAVOUR IMPRESSION OF SOME CARBONYL COMPOUNDS [1,2,3]

Compound	Taste threshold value (µg/l)	Flavour impression
2-methylbutanal	35	bitter almond, apple, malty
3-methylbutanal	46	cherry, chocolate, malty
Benzaldehyde	515	bitter almond, cherry stone
Phenylacetaldehyde	100	hyacinth, honeyed, fruity
Methional	0,5	boiled potato, slightly spicy
(E)-2-nonenal	0,11	cardboard, cucumber, leathery
Heptanal	105	bitter, wine-like
Octanal	60	bitter, orange skin
2-furfural	15157	boiled meat, caramel-like
Nicotinic acid ethyl ester	4555	cereal-like, medicine-like
γ-nonalactone	607	coconut, vanilla, glue

Table 1

substance mix. Compounds separated are subsequently detected in a mass spectrometer.

Sample Preparation

For analysis, 100 ml of beer are placed in a 250 ml gas washing bottle and, to avoid fobbing, mixed with some droplets of a silicon-based, 10 per cent anti-foaming solution. A Drechsel pattern gas washing bottle head is inserted in the prepared washing bottle and connected to a nitrogen gas bottle via a hose. A small metal tube packed with Tenax TA/Carbograph 1TD is then attached to the second connection of the head (Fig. 1). Nitrogen is introduced in the sample solution at a rate of 900 ml/min, driving the volatile analyte out. The sorbent tube is removed after exactly 30 minutes, sealed with metal caps and placed in the thermal desorber. The enriched components are released in the desorber.

Higher Extraction Yields

Though concentration takes place on the sorbent tube, concentrations of some compounds extracted are too low for unequivocal determination. Sample preparation had thus to be optimised. In order to increase extraction yield, tests using various pH values were run, also with sample solution enriched with salt (addition of 6 M NaCl solution).

A neutral aqueous standard solution was measured as control. The resulting intensities of the various compounds were defined as basis and related to the intensities of all optimisation tests. By way of illustration, a starting intensity of 100 is assumed. When intensity is 80 in the follow-on test, this would indicate a drop in extraction of 20 per cent. Fig. 2 shows the percentage changes of pH value and salt tests. Drops in extracts were interpreted as a negative number. In order to simulate beer pH, an acidic buffer solution of pH = 4 was prepared. Compared to the neutral solution, yield of extraction of all compounds without exception was lower for acidic extraction, intensity of γ-nonalactone tasting of glue registering the sharpest drop by 69 per cent.

For comparison, an alkaline buffer solution (pH = 10) was prepared. Its extraction behaviour clearly deviated from that in the acidic solution. Some compounds also reacted negatively to extraction at pH = 10, however, the yield of aromatic and linear aldehydes extracted e.g. phenylacetaldehyde increased by 68 per cent.

In polar compounds, a water envelope might also form, this will cause fixation of components in the sample solution. Addition of salt will rupture this envelope in that the compounds are deprived of available water. In order to check this salting-out effect, a 6 M sodium chloride solution was added to the sample. An increase in extraction was noted for most compounds. This was most obvious for nicotinic acid ethyl ester tasting of medicine as well as for γ-nonalactone (increases of more than 438 per cent and 530 per cent).

Both parameters were combined for use in the beer matrix. However, extraction yield was lower for phenylacetaldehyde and benzaldehyde as, in combination, the negative effect of the salt solution overwhelmed the positive one of the alkaline pH value. Using a salt-containing sample solution in the weakly alkaline range (pH = 8), yield of the two compounds rose again. As a conse-

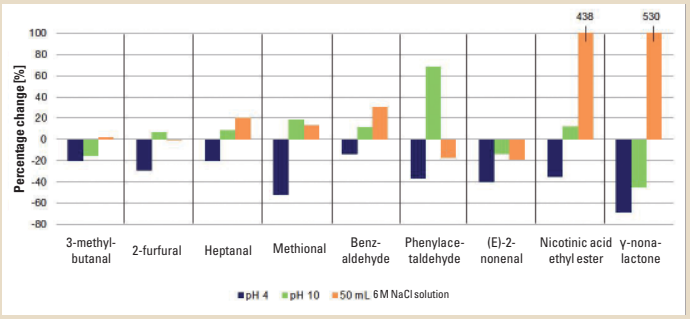

Fig. 2 Extraction as a function of pH value and salt content (percentage change based on aqueous neutral standard solution)

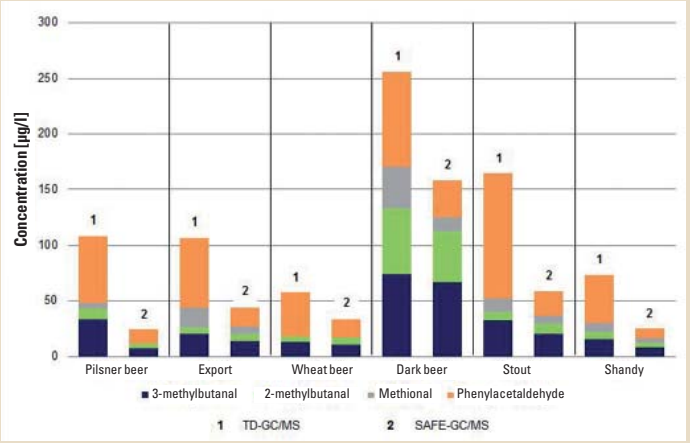

Fig. 3 Concentrations of some staling compounds in different beer types measured using TD-GC/MS and SAFE GC/MS

quence, these parameters were integrated in sample preparation.

Advantages Compared to Solvent Extraction

Solvent-controlled extractions are often used for determining staling components. Compared to this method, enrichment on adsorber material has considerable advantages because solvent extractions are very cost and labour intensive e.g. due to high solvent consumption and a complicated arrangement. In order to compare thermal desorption with solvent-controlled extractions, different beers were analysed using both TD-GC/MS and SAFE-GC/MS (Solvent Assisted Flavour Evaporation) [4]. In the SAFE analysis, staling components are extracted from beer with diethylether and distilled in a high vacuum.

For comparison, different beer types were selected having varying compositions of components depending on the brewing process. The readings showed that concentrations of the various staling components differed (see Fig. 3). Moreover, concentrations measured with the two methods varied widely as significantly lower amounts of compounds were extracted using SAFE-GC/MS. This is an important factor for assessing the methods as solvent extraction is inferior to thermal desorption in terms of sensitivity, not to speak of commercial aspects. Compounds with concentrations below 1 µg/l are not detectable with SAFE extraction. This is particularly relevant for analysing (E)-2-nonenal that, anyway, has low concentrations and has a cardboard or cucumber flavour and is being as an important indicator for staling. When comparing both methods under the test conditions selected, this compound having low concentrations can be clearly detected using only thermal desorption.

As already mentioned, when using a sample solution rich in salt for thermal desorption, higher concentrations of nicotinic acid ethyl ester and γ-nonalactone can be extracted and detected. Very considerable increases in phenylacetaldehyde tasting of honey and methional having a slightly spicy flavour are also noted and can be attributed to the selected alkaline pH value.

Method Validation

Based on the good results compared to other methods, the thermal desorption GC/MS method was validated for routine use. A five per cent ethanolic standard solution and a beer sample were used. In each case, ten parallel samples were analysed. Data collected made it possible to calculate the recovery rate of staling components, being one validation parameter. Recoveries of between 80 and 120 per cent are regarded as appropri-

ate for volatile compounds [5]. 73 per cent of compounds fell within that range including linear, saturated and unsaturated aldehydes, Strecker aldehydes and Maillard products.

The limits of determination as of which compounds could also be quantitatively detected ranged between 0.14 µg/l for 2-methylbutanol smelling of malt and 6.75 µg/l for γ-nonalactone. In most compounds, the limits of determination were below concentrations of those in fresh beer, meaning that staling components are analysable using TD-GC/MS.

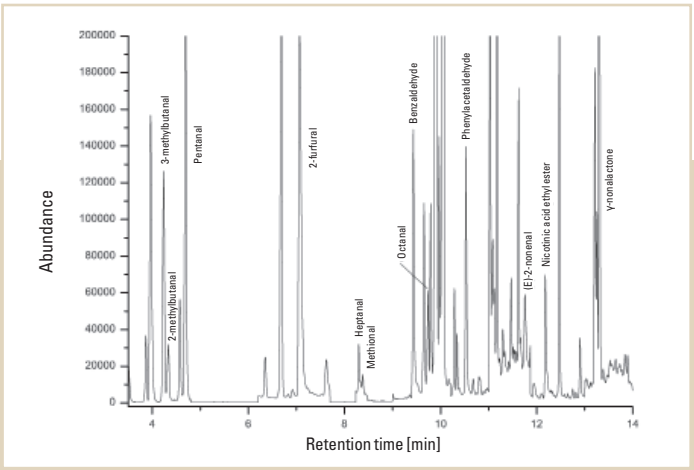

Fig. 4 Chromatogram of a freshly dispensed Pilsner beer

Conclusion

Using the method developed, it is possible to determine important staling-relevant compounds in fresh beer as the limits of determination of these compounds are low. Chromatographically, many volatile components having a role in beer ageing can be found (Fig. 4). Optimisation of extraction during sample preparation, achieved by addition of sodium chloride for salting out, and caustic solution for setting an alkaline pH value was a very important step for effective quantification of analytes. TD-GC/MS is a valuable alternative to more labour and cost intensive methods in view of shorter sample preparation and a reduction in the amounts of chemicals used.

Extensive measurements with different beer types have also been performed. Analyses showed that concentrations in beer vary widely as concentrations depended on the respective technological brewing process for specific beer types and associated diversity of components. Further analyses made it possible to predict the degree to which a particular beer type might age during storage and the concentrations to be expected after storage. “ ■

Literature

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