Evaluation of the Representativeness of the Odor of Beer Extracts Prior to Analysis by GC Eluate Sniffing

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The importance of obtaining and proving that the odor of an extract is representative of the odor of the original product from which it was obtained, before analysis by GC-FID, GC–MS, or GC sniff, is discussed. The sensory methods, such as triangle tests, matching tests, and quantitative analysis, used to determine the representativeness of the odor of an extract are described. Beer extracts obtained by three methods were used to illustrate the interest of the sensory tests. A method using a mixture of XAD resins was proved by sensory analysis to give some extracts with sensory characteristics representative of the particular beers from which they were obtained. Such sensory evaluation of the quality of the aroma of extracts has to be systematically made when sniffing analysis is applied to new types of beer or to different types of products.

INTRODUCTION

It is generally assumed that the method used to obtain an extract for analysis by either GC–MS, GC-FID, or GC sniff produces an extract containing all compounds present in the same proportion as, and responsible for the aroma of, the actual product. The composition of the extract, however, is dependent upon the method of extraction as different classes of compounds are preferentially extracted according to the solvent and method used (Guichard and Issanchou, 1983; Etievant et al., 1986; Blanch et al., 1991).

To determine which compounds contribute significantly to the odor of a product or which are responsible for the differences between the odor of two products, it is necessary to ensure that the method of extraction yields an extract with an odor that is representative of the original product. If the aim of the analysis is to determine which compounds contribute significantly to flavor differences between two or more products, then it must be demonstrated (a) that the odor of the products themselves is significantly different, (b) that the odor of the extracts is also significantly different, and, most importantly, (c) that the sensory characteristics of the extracts are representative of the product. Van Gemert (1981) describes the difficulty of performing such evaluations due to the small quantity of extract and the poisonous or odorous nature of the solvent. He underlines that, in the literature, mention of the sensory evaluation of extracts is rare in spite of a few examples. No other authors, with the exception of Gasser and Grosch (1988) and Guichard et al. (1990), have recently published details of preliminary tests used to determine the type of odor of the extract in relation to the product being analyzed. If the representativeness of the odor of an extract has not been confirmed before analysis, the validity of the comparative data obtained must therefore be questioned.

This paper presents the sensory methods required to determine if the aroma of an extract is representative of the product from which it was obtained. Beer extracts obtained by various methods have been used as an example.

MATERIALS AND METHODS

Beer Samples. Three commercially available beer samples, two lagers (A, and B) and a lager (C) with special malt (with peat fire), were purchased from a large retail outlet and kept at 4 °C until use. The alcohol contents were 4.7, 6.1, and 6.5% and original gravities 11.4, 15, and 15.5, respectively, for beers A, B, and C. Total ester contents were 26 and 38 ppm, total alcohol contents 94 and 136 ppm, and total fatty acids (<6 carbon atoms) 5.6 and 16 ppm, respectively, for beers A and B; they were not determined for beer C.

Analytical Reagents. The XAD resins were purchased from Fluka AG, Switzerland. Each resin was washed continuously in a Soxhlet apparatus with ether and methanol for 24 h, respectively. The resins were then rinsed before use with water (10 × 50 mL).

All reagents used were of AR grade, and all water was purified by a Milli-Q system (Millipore Corp.). Smelling strips were donated by Haarmann and Reimer (Nanterre, France).

Method 1. Beer A (70 mL), CH_2Cl_2 (5 mL), and sodium chloride (18 g) were mixed in a cooled flask (250 mL) and then placed in a water bath at 30 °C for 5 min and stirred with a magnetic stirrer for 30 min. The beer/CH_2Cl_2 emulsion formed during stirring was separated from the aqueous layer and frozen in liquid nitrogen. The flask was then allowed to come to room temperature, and the CH_2Cl_2 solution, containing the aroma compounds, was progressively separated from the remaining beer.

Method 2. The volatile compounds present in three beer samples, A–C, were isolated as outlined by Hawthorne et al. (1987) except that the volatiles were desorbed from the XAD_2 resin using absolute ethanol.

Method 3. The volatiles from beers A and C were extracted using the following procedure. Three different resins, XAD_2, XAD_7, and XAD_{16}, each weighing 2 g (wet weight) were placed respectively in a wide-mouth bottle (100 mL) with solid sodium chloride (13.5 g), dilute hydrochloric acid (2 mL), and beer (45 mL). The bottles were sealed with Teflon-lined, screw-top lids and shaken for 120 min (200 rpm). The beer/resin mixture was then poured into a glass column (i.d. = 11 mm) stoppered with glass wool. Complete transfer of the resin was achieved by rinsing the bottles with saturated NaCl (4 °C, 3 × 10 mL). Residual salt water was removed from the column with nitrogen and the volatile compounds eluted stepwise with absolute ethanol (10 × 1 mL, with a 5-min wait between each milliliter) into a cooled flask. The final aliquot of ethanol was eluted under a flow of nitrogen and the flask sealed and kept at 4 °C overnight. The liquid thus obtained was decanted and stored at −20 °C until analysis. A blank sample was prepared for all batches by substituting water (45 mL) for beer.

Sensory Analysis. The panel consisted of 19 subjects (6 men, 13 women, average age of 30 years) who were recruited from the town of Dijon. The panel members were selected for their ability to memorize and recognize basic tastes and odors, as well as their ability to rank beer samples according to their acidity level and odor intensity. The number of subjects present on the panel varied from 16 to 19 due to occasional unavoidable absences.
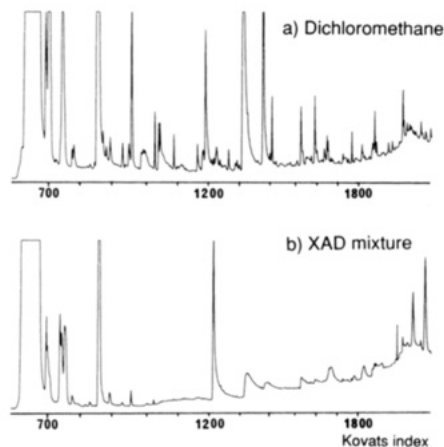
Figure 1. FID chromatograms of beer extracts obtained by two methods.

The beer samples (15 °C, 20 mL) were presented to the panel in black, coded glasses. Beer extracts were diluted with water (10 mL) to give a final ethanol concentration of 50% before being presented to the panel in clear, coded flasks (10 mL, 1 mL of extract per flask). This alcohol content was chosen as a compromise between lower values leading to extracts with very week intensity and higher values leading to extracts for which the evaluation of the odor was masked by the odor of ethanol. All samples and extracts were assessed for odor only, in isolated booths under red light, at 20 ± 1 °C. Data for the matching tests and quantitative descriptive analysis were collected directly by a PSA computer system (OP&P).

Quantitative Descriptive Analysis of Beer Samples and Extracts. A list of descriptors previously determined by the panel as being necessary to describe the odor of the beer samples was used. Beer samples and extracts were presented to the panel, one glass at a time, randomized over all subjects and all samples. Panelists were asked to assess the aroma of the samples and rate the intensity of each given descriptor on an unstructured scale of 130 mm anchored at the left end with "low" and at the right end with "high". Values were converted to 0–100 scores for data analysis.

Triangle Tests. The sensory differences among three different beers as well as their corresponding aroma extracts were assessed by triangle tests as previously described (Stone and Sidel, 1985). The panelists either smelled the flasks directly or used smelling strips (one smelling strip per flask). The subjects were instructed to use only one method of assessment for all triangle tests. When possible, all six permutations of the samples were presented with equal frequency.

Matching Tests. Matching tests as described by Williams et al. (1979) were applied to determine if the extracts resembled the product from which they were obtained. Two beer samples (A and B or C) were presented as control samples to each subject. A series of seven coded flasks containing 1 mL of extract from one beer sample or from the other one were presented in random order. An unbalanced set was used; for half of the panel, four of these flasks contained the aroma extract from beer A and the other three flasks the aroma extract from beer B or C. The other panel members assessed the complementary set—three flasks containing extract A and four containing the extract from either beer B or C. The panel members were instructed to evaluate and memorize the aroma of the beer samples by sniffing the glasses directly or by using the smelling strips as described for the triangle tests. Panelists were then asked to assess the odor of a randomized series of extracts in seven clear, coded flasks and determine which beer the extract resembled most. For each extract the number of times it was matched with each beer sample was established and a chi-square test was performed to test if the proportional correct matching is significant or not.

Extract Dilution Sniffing Analysis (EDSA). Serial dilutions of extracts from beer A, using methods 1 and 3 described above, were analyzed by GC sniffing until no odor-active regions were perceived. A full description of the method used has been given elsewhere (Abott et al., 1993).

PANELIST REPLICATION KOVATS IND	DICHLOROMETHANE 1 A	B	2 A	B	3 A	B	XAD MIXTURE 1 A	B	2 A	B	3 A	B
850		11	7		3	9			3			4
·			9	38		4				10		
·	138026	17527		22	413	4545	74	39	17	152	33	16
·	14			5	4							
900						4						
·	31	22	55			6	16	8				
·	3								21	37		3
950				6								
·												
·	3				4							
1000						22				5		
·	8	10	58		12	3	6		8			
·							4					
1050	97	31			7	11	31					
·	3					12			20		49	
·					34							9
1100	236	11	48		151	21	34		5			

Figure 2. Results of six Charm chromatograms made with extracts obtained by two methods. Numbers correspond to the peak areas measured on the charm aromagrams. The series of dilutions were sniffed twice (A, B) by three different subjects (1, 2, 3). The second part of the Kovats index (1100–1800) is not given because it shows similar differences. Individual boxes gather Charm values of odors detected at close retention indices.

RESULTS AND DISCUSSION

The compositions of two extracts of one beer sample (A), obtained by methods 1 and 3 (as described under Materials and Methods), were compared by FID and extract dilution sniffing analysis (EDSA). The FID trace (Figure 1) shows the major volatiles present in the two extracts that were detectable by FID, i.e., both those which are odor-active and those which are odorless but volatile. As can be seen from the two chromatograms, the CH_2Cl_2 extract contained more volatiles than the XAD_2 mixture extract. The aromagrams [Charm chromatograms as described by Acree (1984)] are presented in Figure 2. They indicate which of the regions in Figure 1 contain odor-active compounds detectable by the three panel members (1–3) during two different sessions of sniffing (A, B). The numbers corresponding to the Charm values are surface areas calculated by the program developed at INRA (Almanza A). They denote when present that an odor was perceived by one particular panel member during one

Table I. Triangle Tests for the Odor of Three Beer Samples

beer	no. of correct responses/ no. of total responses	sig[a]
A–B	11/19	*
A–B	10/17	*
A–C	15/16	***

[a] Significance: * and *** indicate significance at $p < 0.05$ and $p < 0.001$, respectively.

Table II. Triangle and Matching Tests for Beer Extracts Obtained via Method 2

beer extract	solvent	dilution of extract, % EtOH	triangle test: no. of correct responses/ no. of total responses[a]	matching tests[a]
A–B	EtOH[b]	50	9/17 ns	ns
A–C	EtOH[b]	50	11/18 *	ns

[a] Significance: ns and * indicate significant at $p < 0.05$, respectively. [b] Absolute ethanol.

Table III. Triangle and Matching Tests for Beer Extracts Obtained via Method 3

beer extract	solvent	dilution of extract, % EtOH[b]	triangle test: no. of correct responses/ no. of total responses[a]	matching test[a]
A–C	EtOH[b]	50	14/19 ***	*

[a] Significance: * and *** indicate significance at $p < 0.05$ and $p < 0.001$, respectively. [b] Absolute ethanol.

particular series of sniffing. The absence of a number indicates that no odor could be detected at such retention index by this particular panelist. These aromagrams illustrate that, although the difference in the number of odor-active compounds extracted by the two methods and detected by sniffing was less than that observed by FID, there were several important quantitative and qualitative differences in the individual odors of the extracts. These results highlight the need to determine the representativeness of the odor of the extracts by sensory analysis before any valid comparisons of the extracts from different products or processes can be made.

To determine the significance of the differences in the odor of extracts from two different products, the odor of the products must be significantly different. Triangle tests confirmed that the odor of beer A was significantly different from that of beers B and C at $p < 0.05$ and 0.001, respectively (Table I).

The second method of extraction was one which has been commonly used for the analysis of beer samples (Hawthorne et al., 1987) and has the advantage over the former method in that it gives an extract which is easier to test by sensory analysis as the solvent used is both safe and naturally present in alcoholic beverages. Triangle tests demonstrated that only the odor of the extracts from beers A and C obtained by this method were significantly different when presented to the panel in 50% EtOH (Table II). Analysis of these two extracts by matching tests, however, demonstrated that the odor of these extracts did not resemble that of the respective beers from which they were obtained.

As the difference in odor between beers A and C was more significant than that between beers A and B (Table I), beers A and C were used for all further work.

Extraction of beers A and C using a mixture of three XAD resins gave extracts with an odor perceived by the panel as being significantly different (Table III). Matching tests conducted on the beers and their extracts were also significant. The panel members were able to match the extract from beer A with beer A 30 of 48 times. The extract

Table IV. Quantitative Descriptive Analysis of Beers A and C and Their Corresponding Extracts (Method 3)

descriptor	beer A[a]	beer C[a]	p[c]	extract A[b]	extract C[b]	p[c]
banana	14	37	0.0001	15	27	0.0183
coffee	12	19	0.0306	15	12	0.6139
caramel	9	16	0.0329	10	11	0.8841
butter	6	13	0.0129	7	5	0.5697
smokey	13	20	0.0937	4	11	0.1015
burnt	3	8	0.0686	2	3	0.7611
yeast	13	19	0.0993	15	22	0.0760
honey	15	19	0.3690	21	11	0.0556

[a] Mean of 19 subjects over 2 replicates. [b] Mean of 18 subjects over 1 replicate. [c] p value determined by a two-related samples t-test.

from beer C was matched with beer C 34 of 50 times. Furthermore, quantitative descriptive analysis demonstrated that three common descriptors, banana, smokey, and yeast, were used by the panel to significantly differentiate between the two beer samples and between their two extracts (Table IV).

From the data presented in this table and the results from the matching tests, it can therefore be concluded that this method of extraction provides an extract which was more representative of the original sample. Most importantly, these results allow further comparative analyses to be performed on the extracts with the knowledge that the results obtained can be more directly related to the aroma of the beer.

CONCLUSION

A method using a mixture of XAD resins was developed to give a beer extract which was subsequently shown to have sensory characteristics more representative of the beer from which it was obtained than other extracts tested. This method has many advantages over commonly used extraction methods in that (a) the entire procedure can be conducted at low temperatures, therefore removing any possibility of thermally catalyzed rearrangement of the compounds in the extract; (b) the method is efficient and inexpensive and could easily be automated; (c) only small volumes of beer are required to give an extract of a suitable concentration for analysis by EDSA; and (d) the final extract does not need to be concentrated before analysis.

It must be stressed, however, that although the method of extraction was demonstrated, by sensory analysis, to give extracts with an odor representative of the beers from which they were obtained, any other product being treated by this method must undergo strict sensory analysis, triangle tests, matching tests and quantitative descriptive analysis to ensure that the extract obtained is representative of the product in question.

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