Steam Volatile Components of Roasted Barley

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Gas–liquid chromatography was used to examine the steam-volatile fraction of roasted barley. Ethanal, propanal,* 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, pentanal,* hexanal,* furfural, propanone, butanone, 2,3-butanedione,* 2,3-pentanedione, ethanol,* and pentanol* were identified in the headspace. Pyridine, pyrazine,* methylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine,* trimethylpyrazine, 2-ethyl-3-methylpyrazine,* 2-ethyl-5-methylpyrazine, 2,5-dimethyl-3-ethylpyrazine,* and 3,5-dimethyl-2-ethylpyrazine* were found in the basic fraction. Flavor aspects are discussed. The compounds with an asterisk have not previously been reported as present in roasted barley.

Roasted barley is sometimes used to provide the desired color in dark beers. In addition to this main function, flavoring effects due to the wide variety of compounds that are formed during roasting may also be encountered. In this study, the headspace volatiles of a steam distillate of roasted barley were investigated using gas–liquid chromatography (glc). Since pyrazine compounds have been reported in and are believed to make an important flavor contribution to many roasted foods such as coffee (Goldman *et al.*, 1967; Stoffelsma *et al.*, 1968) cocoa (Rizzi, 1967; van Praag *et al.*, 1968), and roasted peanuts (Mason *et al.*, 1966), the volatile basic compounds were examined.

EXPERIMENTAL

Roasted Barley. The roasted barley investigated was that used in the brewing of dark Irish beer. The maximum temperature during roasting is about 225° C.

Preparation of Roasted Barley Steam Distillate. A mixture of 1 kg of ground roasted barley and 2.5 l. of water was allowed to stand for 1 hr at 66° C. One liter of dark extract was then drawn off through a muslin filter. The residual material was further extracted with water at 77° C until a total of 7 l. of extract had been collected. This was divided into three lots of about 2.3 l., and each distilled for 2 hr at a rate of 1 ml/min.

The above procedure is modeled on the mashing process employed in brewing practice. Volatiles were also prepared by boiling 750 g of ground roasted barley with 2.5 l. of water for 2 hr at a distillation rate of 2 ml/min. Both preparations were qualitatively similar.

Headspace Analysis. Five milliliters of roasted barley steam distillate was taken in a 25 ml conical flask with 4 g of ammonium sulfate (excess to ensure saturation). The flask was sealed with a Suba-Seal rubber closure, and immersed in a water bath at 35° C for 30 min. One milliliter of headspace vapors was taken for glc analysis.

Isolation of the Basic Fraction. One liter of steam distillate was made alkaline with sodium bicarbonate and continuously extracted for 42 hr with diethyl ether. The extract was washed three times with one-third its volume of 1 *N* sulfuric acid, and the combined acid washings extracted with diethyl ether for 24 hr to remove neutral compounds. The pH of the acid layer was raised to about 7, and the basic compounds extracted into diethyl ether for 2 days. After drying (Na_2SO_4), the extract was concentrated under vacuum to 1 ml.

Classification Tests. Four classification reagents were found to be useful in the identification of the headspace volatiles. (i) Hydroxylamine hydrochloride (Bassette *et al.*, 1962). Forty milligrams of hydroxylamine hydrochloride was dissolved in 5 ml of steam distillate and allowed to stand for 30 min at room temperature. (ii) Dimedone. One hundred milligrams of dimedone and 5 ml of steam distillate were taken in a 25 ml flask and stoppered with a Suba-Seal. This was maintained at 65° C for 1 hr, and cooled to room temperature. (iii) Bromine. Sufficient bromine water to give a definite color was added to 5 ml of steam distillate. After 10 min reaction time, the excess bromine was destroyed with α-naphthol. (iv) Borohydride. Five milliliters of steam distillate was treated with 50 mg of sodium borohydride for 1 hr.

After reaction as described above the solutions were taken for headspace analysis.

Apparatus. Aerograph chromatograph model 204 equipped with dual flame ionization–electron capture detection and Philips chromatograph model PV4000 were used.

Columns and Conditions. All columns were of stainless steel.

For headspace analysis: (A) 150 ft × 0.02 in. i.d. capillary, coated with dinonyl sebacate, programmed from 40° C to 60° C at 0.67°/min, thereafter 2°/min to 120° C with nitrogen carrier flow 5 ml/min. (B) 150 ft × 0.02 in. i.d. capillary, coated with Polyglycol 400, operated at 50° C, with nitrogen carrier flow 8 ml/min.

For analysis of basic fraction: (C) 5 ft × 1/8 in. packed with 10% XF-1150 + 0.1% Carbowax 20M on Celite 100–120 mesh, operated at 65° C, with nitrogen carrier flow 25 ml/min. (D) 6 ft × 1/8 in. packed with 5% Carbowax 20M + 1% KOH on Gas Chrom P, 80–100 mesh, operated at 100° C, with nitrogen carrier flow 25 ml/min. (E) 170 ft × 0.02 in. i.d. capillary, coated with Polyglycol 1000, operated

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Table I. Major Headspace Volatiles of Steam Distillate from Roasted Barley

Aldehydes	Ketones
Ethanal[a]	Propanone
Propanal	Butanone
2-Methyl propanal	2,3-Butanedione[b]
2-Methyl butanal	2,3-Pentanedione[b]
3-Methyl butanal	
	Alcohols
Pentanal[a]	Ethanol[c]
Hexanal	Pentanol[c]
Furfural[b]	

[a] Although the alcohols corresponding to these aldehydes are already present in the distillate, confirmation was obtained by way of borohydride reduction as the ethanol and pentanol peaks increased significantly after this treatment. [b] The corresponding alcohols are insufficiently volatile to be detected in the headspace, but the response of the original carbonyl compounds to the highly specific electron capture detector is additional confirmatory evidence in these cases. [c] Ethanol and pentanol identifications based on glc data alone.

Table II. Retention Times of Pyrazines Relative to 2,5-Dimethylpyrazine

Pyrazine Compound	Chromatographic Column C	D	E	F
Unsubstituted	0.49	0.56	0.67	0.23
Methyl-	0.71	0.75	0.85	0.50
Ethyl-		1.06	1.17	1.02
2,5-Dimethyl-	1.00	1.00	1.00	1.00
2,3-Dimethyl-	1.14	1.11	1.13	1.09
2,6-Dimethyl-	1.00	1.02	1.11	1.07
Trimethyl-	1.57	1.51	1.53	2.12
2-Ethyl-3-methyl-		1.51	1.48	2.02
2-Ethyl-5-methyl-		1.44	1.41	2.02
2,5-Dimethyl-3-ethyl-		1.85	1.84	3.55
3,5-Dimethyl-2-ethyl-		2.02	1.98	3.80
2,5-Diethyl-		2.05	1.96	3.88
Tetramethyl-	2.18	2.18	2.18	4.26
2,5-Diethyl-3,6-dimethyl-	3.44	3.21		

Table III. Basic Compounds Identified[a] in Steam Distillate from Barley Roasted at 225° C

Pyridine[b]	Trimethylpyrazine[b]
Pyrazine	2-Ethyl-3-methylpyrazine
Methylpyrazine[b]	2-Ethyl-5-methylpyrazine[b]
2,5-Dimethylpyrazine[b]	2,5-Dimethyl-3-ethylpyrazine
2,3-Dimethylpyrazine	3,5-Dimethyl-2-ethylpyrazine

[a] Identifications based on glc data alone. [b] Wang *et al.* (1969) found these compounds and a dimethylethylpyrazine in the volatile byproducts formed during the roasting of barley at 180° C.

Table IV. Flavor Thresholds[a] of Some Pyrazines Added to Dark Irish Beer

Pyrazine Compound	Flavor Threshold (ppm)
Unsubstituted	>100
Methyl-	100
Ethyl-	10
2,5-Dimethyl-	25
2,6-Dimethyl-	3
2,3-Dimethyl-	50
Trimethyl-	1
2-Ethyl-5-methyl-	1
2-Ethyl-3-methyl-	2
2,5-Dimethyl-3-ethyl-	0.025
3,5-Dimethyl-2-ethyl-	0.005
Tetramethyl-	>100
2,5-Diethyl-3,6-dimethyl-	100

[a] The flavor threshold was taken as the minimum concentration of compound which, when added to beer, was detected consistently by one-third of the panel (for tasting procedure see Harrison, 1963).

at 100° C with nitrogen carrier flow 5 ml/min. (F) The dinonyl sebacate column, as used for headspace analysis, but operated at 65° C.

Reference Standards. The compounds listed in Table I were obtained from commercial sources. Of those listed in Tables II, III, and IV, pyridine, pyrazine, methylpyrazine, and 2,5-dimethylpyrazine were available commercially; the remainder were synthesized according to known procedures (Behun and Levine, 1961; Ishiguro and Matsumura, 1958; Kamal and Levine, 1962; Kipping, 1929; Klein and Spoerri, 1951).

RESULTS AND DISCUSSION

Headspace analysis showed more than 40 peaks. Of these, 13 were greater than 1 in. high at maximum obtainable sensitivity and were arbitrarily defined as major volatiles. The classification reagents were of considerable use in the identification of these peaks. Thus, hydroxylamine hydrochloride distinguished carbonyl compounds and noncarbonyl compounds by removing the former from the headspace (Bassette *et al.*, 1962). Similarly, dimedone was of value in distinguishing aldehydes from simple ketones, while bromine detected unsaturated linkages. With borohydride volatile derivatives, *i.e.*, the corresponding alcohols, were formed and the identification of these provided strong confirmatory evidence for the identification of the original carbonyl compounds.

When tested on a solution containing a range of carbonyl compounds, 3-methylbutanal (simple aldehyde), butanone (simple ketone), crotonaldehyde (α,β-unsaturated aldehyde), methyl vinyl ketone (α,β-unsaturated ketone), and 2,3-butanedione (α-diketone), it was found that hydroxylamine hydrochloride and borohydride reacted as expected. Dimedone, in addition to removing aldehydes, also removed 2,3-butanedione and a considerable amount (40%) of methyl vinyl ketone, due presumably to the activated keto grouping in these molecules. This anomalous reaction did not, fortunately, lead to confusion of aldehyde and α-diketone peaks, since the latter responded strongly to the electron capture detector when the sample was run with dual flame ionization–electron capture detection. The reaction of the unsaturated carbonyl compounds with bromine was extremely time-dependent. If the reaction was allowed to proceed long enough for complete removal of this class (30 to 60 min), considerable attack on 3-methylbutanal also occurred. Ten minutes reaction time was adopted as optimal, as reaction with the unsaturated carbonyl compounds had proceeded far enough to be recognized, while attack on the other carbonyls was still minimal.

Table I lists the identification of the major headspace volatiles. These identifications are based on the classification tests, retention times on the dinonyl sebacate and Polyglycol 400 capillary columns, retention times of the corresponding alcohols obtained by borohydride reduction on the same columns, and response to the electron capture detector. Some of these compounds have been previously identified in the volatile material formed during the roasting process using a lower roasting temperature (Shimizu *et al.*, 1969; Wang *et al.*, 1968). The minor headspace volatiles as defined above have not been investigated in detail.

Table II shows the relative retention times obtained for authentic samples of pyrazines when run on the four columns (C, D, E, and F) as previously described. The compounds identified in the basic fraction are listed in Table III. Pyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 2,5-dimethyl-3-ethylpyrazine, and 3,5-dimethyl-2-ethylpyrazine have not previously been reported as present in roasted barley.

Although 2,6-dimethylpyrazine and/or ethylpyrazine may be present, they were insufficiently resolved from other peaks on any of the columns used to allow a positive statement regarding their presence.

The possible contribution made by the compounds identified in this work to the flavor of dark beer was investigated. The carbonyl compounds listed in Table I, despite the flavor potency of some (Harrison and Collins, 1968), are likely to be extensively reduced during the fermentation process to relatively bland alcohols.

Pyrazines were found to vary considerably in flavor potency (Table IV). The dimethylethylpyrazines in particular are extremely potent. In normal brewing practice a considerable amount of these compounds is removed from the dark worts by the hop boil, at which stage a 5% boil-off takes place. Laboratory experiments show that if the hop boil is carried out under reflux conditions so that the volatiles normally lost in the boil-off are retained, the resulting beer has a very strong pyrazine-type flavor which is generally disliked by tasters. Methods for quantitation of pyrazines in beer are being developed.

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