Novel Vessel for the Measurement of Dynamic Flavor Release in Real Time from Liquid Foods

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A glass vessel that will measure the dynamic flavor release of aroma volatiles from model liquid foods in real time has been built. The sample is maintained at 37 °C and stirred at constant torque. Volatiles released into the headspace are continuously swept by a constant carrier gas flow into a quadrupole mass spectrometer via a jet separator. The mass spectrometer is operated in the chemical ionization mode, and single ion monitoring of the major ion associated with each volatile allows real time detection. The apparatus was used to measure the effect of alcohol content on the dynamic flavor release of four volatile compounds from four different water/ethanol mixtures.

Keywords: *Flavor release; mass spectrometry; chemical ionization; ethanol; volatiles*

INTRODUCTION

Several attempts have been made to instrumentally measure the release of flavor compounds from foods while these foods are being eaten. The methods used can be divided into two categories: (1) the breath exhaled from the mouth is collected and analyzed by mass spectrometry (MS) or gas chromatography/MS (Soeting and Heidema, 1988; Linforth and Taylor, 1993; Delahunty et al., 1994; Taylor and Linforth, 1994); and (2) a model system is constructed, that attempts to mimic what occurs in the mouth, and the effluent from this model system is collected and analyzed by MS or GC/MS (Lee III, 1986; Roberts and Acree, 1995). Each of these method has advantages and disadvantages. There is wide inter- and intra-assessor variation when using human subjects. There are also the problems of assessor availability and the difficulties associated with connecting the assessor to the detecting device. Of course, using assessors is always more applicable to real life than using an instrumental approach, although the instrumental method can be more carefully controlled, thereby reducing variation. Also, various physical parameters can be varied by the instrumental method, allowing information on the effects of, for example, gas flow rate, temperature, and shear rate on flavor release.

In this paper, we describe an instrumental technique that we developed to measure dynamic flavor release in real time from liquid foods. Mass spectrometry was chosen as the means of detection because of its previous use as a detector for flavor release measurement and also because it was the most suitable method available to us.

For a vessel to be suitable for the measurement of dynamic flavor release, it needs to simulate the human mouth as much as possible, but in such a way as to allow successful introduction of mechanical devices, such as stirring and/or chewing apparatus. In designing the vessel, we must consider (1) inertness, (2) size, (3) shape, (4) sample introduction, (5) carrier gas flow, (6) agitation of the sample, (7) temperature, (8) gas tightness, (9) ease of modification, and (10) connection to a mass spectrometer.

MATERIALS AND METHODS

Samples. A stock solution of four volatile compounds was made up in Analar ethanol (Hayman, Witham, U.K.): maltol and vanillin both at 2000 mg L^{-1}, 2-heptanone and isoamyl acetate both at 200 mg L^{-1} (all from Aldrich Chemical Company, Gillingham, U.K.). These volatiles were chosen because they had differing molecular weights and covered a range of polarities, volatilities, and functional groups. Each compound was at a level where it contributed to the aroma of the mixture. Five milliliters of the stock solution were dissolved in either water or aqueous ethanol to give four solutions containing 5, 10, 20, and 40% ethanol. The concentration of both maltol and vanillin in each solution was 100 mg L^{-1} and the concentration of both 2-heptanone and isoamyl acetate was 10 mg L^{-1}. These concentrations are typical values found in foods (Furia and Bellanca, 1971).

Equipment. The design of the flavor-release vessel used is shown in Figure 1. The total volume of the vessel is ~125 mL, and the two taps allow the bottom part of the vessel to be removed from the system without the gas flow into the mass spectrometer being disturbed. Turning the taps on and off allows start and finish times for experiments to be monitored carefully. The bottom part of the vessel is jacketed, and water from a water bath is pumped through the jacket at a controlled temperature (for this experiment, the temperature was 37 °C). The jacket is mounted on a magnetic stirrer, which allows stirring of the sample from its center with a circular stirring bar that is driven by a constant-torque motor. Shear rates were measured with a Brookfield viscometer; a range of rates from 10 to 200 s^{-1} was achieved in the flavor-release vessel. The shear rate typically found in the human mouth during consumption of liquids is 50 s^{-1} (Shama and Sherman, 1973).

The 10 considerations for the design of the vessel were addressed as follows: (1) inertness—glass is ideal and stirring can be monitored visually; (2) size—the vessel is too large at present, but a vessel of ~50 mL would be ideal; (3) shape—a regular cylinder was chosen to simplify calculations; (4) sample introduction—a wide inlet allows quick sample introduction, and taps divert flow and maintain mass spectrometer vacuum; (5) carrier gas flow—maximized through use of a jet separator; (6) agitation of the sample—achieved with a stirring bar; (7) temperature—maintained by water jacket and circulating water bath; (8) gastightness—the Quickfit inlet is not ideal, and SVL fittings (Bibby Sterilin, Stone, U.K.) would be more gastight; (9) ease of modification—there is space available for two side arms, allowing addition of, for example, saliva; and (10) connection to a mass spectrometer—a jet separator was used.

High purity helium (99.995%) is passed through the vessel from a cylinder at a flow rate that is controlled with a manually operated flow controller. The upper limit of the flow controller is 100 mL min^{-1}.

The flow rate of gas into the mass spectrometer is limited by the ability of the pumps to maintain an operational vacuum.

S0021-8561(95)00687-X CCC: $12.00

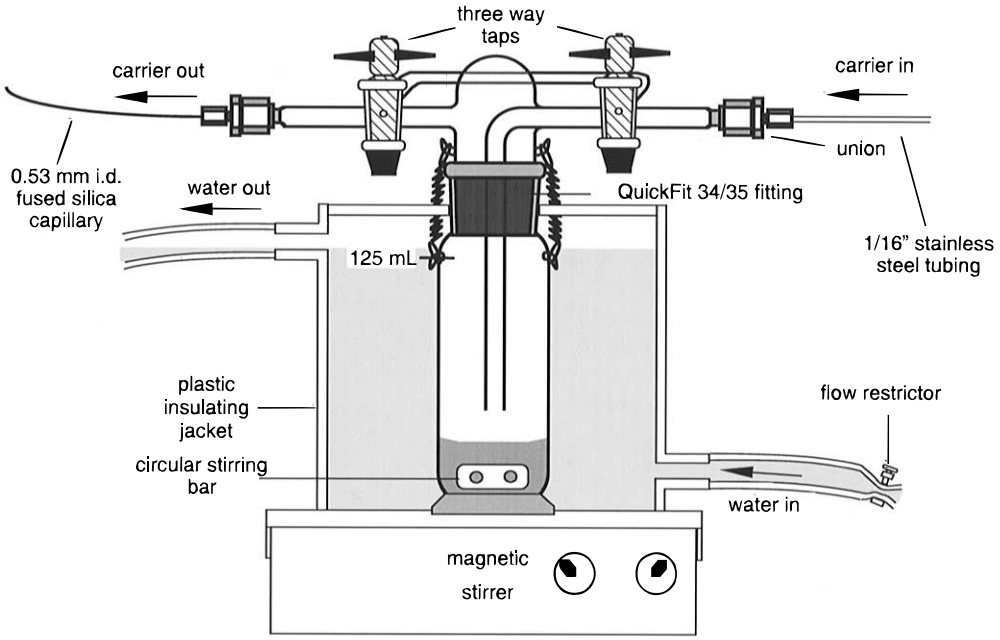

Figure 1. Vessel for the measurement of dynamic flavor release.

For the mass spectrometer used, a Hewlett-Packard 5988A with a Pascal workstation (Hewlett-Packard, Palo Alto, CA), this flow rate, when measured at atmospheric pressure, was ~5 mL min^{-1}. At least 30 mL min^{-1} needs to flow over the sample to give a reasonable flow through the flavor-release vessel. Even at this flow rate, the vessel will only be fully evacuated every 4 min. To allow such high flow rates through the flavor-release vessel, a jet separator (SGE Ltd., Ringwood, Australia) was installed in the roof of the Hewlett-Packard 5890 gas chromatograph between the vessel and the mass spectrometer (Throck Watson, 1969). The jet separator was heated at 100 °C with one of the detector heaters for the gas chromatograph. The maximum flow rate possible with the jet separator was 30 mL min^{-1}, which was the flow rate used for the experiment. A 1-m length of 0.53-mm i.d. fused silica (J&W, Folsom, CA) connected the flavor-release vessel to the jet separator. The fused silica was maintained at 37 °C by the oven of the gas chromatograph. A piece of $^1/_{16}$ in. i.d. glass-lined steel tubing connected the outlet of the jet separator to the mass spectrometer.

Mass Spectrometry. The mass spectrometer was operated in the chemical ionization mode (Rose, 1990), with 99.95% pure isobutane from a lecture bottle as the ionizing gas (Argo International Ltd., Basildon, U.K.). The pressure at the center of the ion source was ~10^{-4} Torr. The isobutane cylinder was closed when the system was not in use and opened 1 h before sample analysis commenced. The mass spectrometer was manually tuned with perfluorotributylamine (mass spectrometer grade, Fluorochem Ltd., Old Glossop, U.K.) immediately before data acquisition.

The stock solution was analyzed by chemical-ionization GC/MS, and the spectrum of each compound in the solution gave a strong protonated molecular ion and little fragmentation under these conditions. Ion chromatograms for m/z 115 (2-heptanone), 127 (maltol), 131 (isoamyl acetate), and 153 (vanillin) all showed only one peak, which meant that the protonated molecular ion (**M**+**1** ion) was discriminating for each of the four volatiles. Hence, single-ion monitoring with the four ions listed could be used to discriminate each compound. Single ion monitoring increases sensitivity, relative to scanning across the whole mass range, by up to a factor of 1000 (Rose, 1990).

The flavor-release measurements were made with single-ion monitoring. The dwell time for each ion was 100 ms and the cycle time for each set of ions was 500 ms. Hence, two data points were obtained for each volatile compound per second.

Measurement of Flavor Release. Samples were maintained at 4 °C until just prior to analysis. An aliquot of solution (20 mL) was poured into the flask, and a stirring bar was added. The flask was then connected to the top of the vessel with metal springs to prevent separation of the vessel due to pressure buildup. Data acquisition commenced and, after a sample incubation time of 1 min, stirring began at 300 rpm. At the same time, the taps were opened, allowing carrier gas to sweep the headspace into the mass spectrometer. Data acquisition continued until the flavor-release curves had maximized and then began to fall. Sample incubation time was minimized so that the sample would be as similar as possible to that normally consumed.

The samples were each run in triplicate. A blank, which was an empty flavor-release vessel under the same conditions as for the samples, was run between each set of samples. The baseline for each ion in the blank was subtracted from the corresponding ion in each sample.

Absolute values for the flavor release for each volatile were not measured. In addition to the partition of volatiles between the solution and the headspace, there is a partition caused by the volatiles passing through the jet separator. The heavier a molecule is, the greater is its momentum and so the number of the heavier molecules entering the mass spectrometer will be relatively higher than that of the lighter molecules. Additionally, the degree of fragmentation of the **M**+**1** ion generated in the mass spectrometer will vary for the different volatiles, making quantification difficult and prone to error. Instead, the release of volatiles is measured relative to their amounts in the 5% alcohol solution, which served as a standard.

RESULTS AND DISCUSSION

A dynamic flavor-release curve was simultaneously obtained for each volatile compound in the solution in real time. The curves obtained were similar in shape to those observed in sensory time-intensity experiments (Lee and Pangborn, 1986) but were obtained over ~10 min rather than ~30 s for the equivalent sensory assessment.

Although the aromas of vanillin and maltol could both be perceived at 100 ppm, their concentrations in the headspace were too low to be detected by the mass spectrometer in all of the samples. Isoamyl acetate and 2-heptanone, although present in solution at concentrations 10 times lower than vanillin and maltol, were easily detected, with a minimum signal-to-noise ratio of 1000. Hence, only data for isoamyl acetate and 2-heptanone were analyzed.

Table 1. Mean Values of I_{max}, T_{max}, m_1, and m_2 for 2-Heptanone and Isoamyl Acetate in Ethanol Solutions

		ethanol concentration[a]			
flavor compound	parameter	5%	10%	20%	40%
2-heptanone	I_{max}	1 370 000 (348 000)	879 000 (134 000)	471 000 (75 300)	90 700 (13 400)
	T_{max}	7.0 (0.9)	8.3 (1.4)	9.2 (0.6)	12.7 (0.7)
	m_1	416 000 (202 000)	343 000 (64 800)	262 000 (21 100)	79 000 (18 900)
	m_2	202 000 (76 700)	108 000 (24 200)	50 800 (5 300)	7 120 (1 020)
isoamyl acetate	I_{max}	1 661 000 (463 000)	1 163 000 (202 000)	716 000 (117 000)	158 000 (30 900)
	T_{max}	6.9 (0.6)	8.1 (0.9)	8.9 (1.1)	12.2 (0.5)
	m_1	422 000 (224 000)	413 000 (106 000)	390 000 (40 100)	115 000 (31 100)
	m_2	245 000 (85 600)	145 000 (28 500)	79 900 (4 220)	13 000 (3 100)

[a] Values on parentheses are standard deviations.

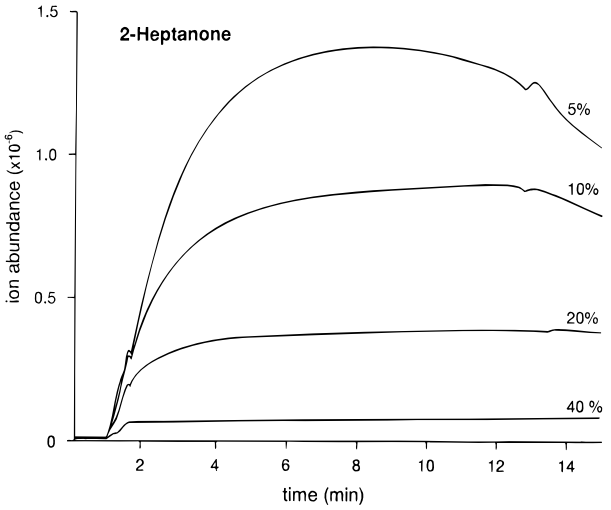

Figure 2. Effect of alcohol concentration on the dynamic flavor release of 2-heptanone and isoamyl acetate.

To compare the flavor-release curves, parts of the curve needed to be defined and measured. The parts of the curve of interest are I_{max}, the maximum intensity of the curve corresponding to the greatest amount of flavor in the headspace; T_{max}, the time after stirring commences when I_{max} is reached; and m, the initial gradient of the curve (m), which represents the initial increase in release and is approximately linear in this experiment.

Both the intensity and the shape of the curves were affected by the concentration of ethanol present, although at each ethanol concentration, the release curves of 2-heptanone and isoamyl acetate were similar. As the alcohol concentration increases the maximum intensity for each flavor release curve decreases (Figure 2). The curve becomes more angular, with a flatter apex.

Two competing effects influence the shape of the flavor release curve: (1) An exponential increase in release of flavor, with time, into the headspace from the food matrix. The exponential coefficient will depend on such factors as the rate of diffusion of the flavor molecule into the headspace and the surface area of the liquid/gas interface. (2) Transport of the flavor molecules from the headspace to the mass spectrometer. As carrier gas flow rate increases, the number of flavor molecules removed from the headspace per second will increase.

As ethanol concentration of the solution increases, the vapor pressure of the flavor molecules decreases, which suppresses release into the headspace. Equilibrium is reached more slowly and the headspace concentration of the flavor molecules is reduced. Transport of the volatiles to the mass spectrometer is constant. This combined effect will lead to a reduction in I_{max} and an increase in T_{max} as ethanol concentration increases, as seen for both isoamyl acetate and 2-heptanone (Table 1).

Table 2. Analysis of Variance of the Dynamic Flavor-Release Curves of Isoamyl Acetate and 2-Heptanone from Ethanol-in-Water Solutions

parameter	I_{max}	T_{max}	m_{11}	m_2
concentration of ethanol	★★★	★★★	★★★	★★★
volatile	★★★	NS	★★★	★★★
interaction	★★	NS	NS	★★★

[a] (★★★) $p < 0.001$; (★★) $p < 0.01$, (★) $p < 0.05$, (NS) not significant).

The curves were compared by analysis of variance. Two values of m were examined: m_1, a measured value, equivalent to the intensity after 1 min; and m_2, a calculated value equal to I_{max}/T_{max}. The calculated value was more reproducible than the measured value, which was affected more by the buildup of flavor compounds in the headspace before the gas flow was diverted through the vessel. Natural logarithms rather than the real values of I_{max} were compared, because the variation in I_{max} across the replicates was skew, with greater variation at lower ethanol concentrations.

The results of the analysis of variance are shown in Table 2. Ethanol concentration was shown to significantly affect all of the values used to define the curves ($p < 0.001$); I_{max}, m_1, and m_2 all decreased with increasing ethanol concentration, whereas T_{max} increased. The T_{max} values for both isoamyl acetate and 2-heptanone were similar, although I_{max}, m_1, and m_2 were all significantly less for 2-heptanone. The interaction between flavor compound type and alcohol concentration showed that the differences between the two compounds for I_{max} and m_2 significantly increased as ethanol concentration increased, with the effect of ethanol being greater for 2-heptanone, which is the less polar of the two compounds. However, an F test showed that this interaction is very small, relative to the main effects.

Williams and Rosser (1981) injected the headspace from a series of fruit drinks containing different concentrations of ethanol and found that the ethanol concentration affected the partition coefficient of four volatile compounds, which included isoamyl acetate. Their experiment was carried out at 21 °C; at this temperature, maximum flavor release occurred at an

ethanol concentration of 0.75% for all of the volatiles. A maximum ethanol concentration of 15% was examined. Thus, a direct comparison of the data in our experiment with that of Williams and Rosser is not possible. In a paper by de Roos and Wolswinkel (1994), release of volatiles from a 30% ethanol-in-water solution was significantly less than from water. The change in release was greater for some volatiles than others, although no obvious trend was observed. A combined effect of, for example, polarity and boiling point may exist. Both papers used partition coefficient measurements to determine flavor release, although de Roos and Wolswinkel state this technique is a suitable indicator of flavor release in a dynamic system.

The use of the mass spectrometer as a detector for dynamic flavor release is accompanied by several problems. Isobutane is one of the 'softest' of the commonly available ionizing gases, which means that it produces a strong molecular ion with little fragmentation of the compounds analyzed. However, it contains the most impurities of all the ionizing gases and, even with high purity gas, the ion source of the mass spectrometer needs regular cleaning. Chemical ionization also reduces the sensitivity of the mass spectrometer, resulting in the need to run standards regularly. High flow rates of air into the mass spectrometer caused by evacuating the flavor release vessel reduce the lifetime of the electron multiplier of the mass spectrometer from 2 years to as little as 3 months, making the technique quite expensive in consumables.

The current vessel is only suitable for liquid foods, and more sophisticated shearing methods will be needed to simulate the action of the tongue and teeth working together. Also the internal volume of the mouth varies during eating and the gas flow in the mouth is pulsed, rather than regular. Moreover, the top of the vessel is not jacketed, so condensation may occur there.

The mass spectrometer used is not the most suitable one available. A small benchtop mass spectrometer with a simple ion source and a turbomolecular pump would be much less troublesome. The simple ion source would result in less down time and less training for users, and the high specification pump would allow more flexibility in the selection of flow rate.

The technique is not robust and requires a large amount of training to obtain good quality results. However, there has been no other system published in the literature or marketed that allows the measurement of flavor release in real time.

Future work will involve the design of smaller vessels, incorporating all of the features of the current vessel. The smaller vessel will evacuate more quickly, allowing curves to be more like sensory flavor-release curves. Some of these vessels will incorporate a cutting blade for more solid foods. Other ports can be added to the vessel, so that saliva can be added during shearing and food samples may be put in the vessel without introducing air to the mass spectrometer.

Comparison of the data obtained from the flavor-release vessel with that obtained from sensory time-intensity measurement of these samples is essential to validate the use of this device. The shape of the release curves produced by the flavor-release vessel are very similar to those produced by human subjects (Lee and Pangborn, 1986), albeit on a different time scale. The instrumental method will enable us to assess the effect of the composition and rheology of liquid foods on the release of volatile flavor compounds under a variety of mixing conditions.

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Received for review October 19, 1995. Revised manuscript received January 18, 1996. Accepted August 16, 1996.[⊗] We thank the Office of Science and Technology for funding this work.

JF950687K

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1996.