

Short communication

# Enhanced flavour extraction in continuous liquid–liquid extractors

Peter Apps\*, Margaux Lim Ah Tock

*Analytical Function, Bio/Chemtek, Council for Scientific and Industrial Research (CSIR), P.O. Box 395, Pretoria 0001, South Africa*

Received 15 April 2005; received in revised form 1 June 2005; accepted 6 June 2005

## Abstract

Continuous liquid–liquid extraction is a versatile, reliable and robust sample preparation technique, but there is a tendency for the solvent to make channels through the sample, causing uneven and incomplete extraction. A simple, cheap magnetic stirrer that prevents channelling improves extraction efficiency by between 2.8 and 17.6 times.

© 2005 Published by Elsevier B.V.

*Keywords:* Sample preparation; Liquid–liquid extraction; Solvent extraction; Aroma; Flavour; Fruit; Guava; Pineapple; Gas chromatography; Mass spectrometry

## 1. Introduction

“Sample preparation is one of the most critical aspects of the analysis of complex matrices for trace components and can also be the most time consuming.” [1].

Sample preparation techniques for flavour and off-flavour analysis in foods and beverages are as diverse as the analytes and matrices to which they are applied [2,3]. While solid-phase microextraction (SPME), stir bar sorptive extraction and solid phase extraction are finding increasing application in the field of flavour and aroma analysis, continuous liquid–liquid extraction (CLLE) which dates back at least to the 1960s [4], remains a rugged and reliable workhorse that provides good recoveries of analytes over a wide range of volatilities and functional groups [5]. Compared to other systems [6] the glassware is simple and the procedures straightforward. It requires no gas chromatographic inlet hardware beyond a split–splitless injector, and it can run unattended for long periods [2,7,8]. Because a small volume of solvent [5] is continuously recycled, solvent purity is less critical than with the larger volumes used in batch extractions [9]. The extract from a continuous liquid–liquid extraction can be concentrated by evaporation to a high enough concentration for the detection of trace (sub  $\mu\text{g/L}$ ) components [10], and a single

extraction yields sufficient concentrated extract for repeated analyses under different conditions, as required for example by aroma dilution analysis [11,12]. CLLE is especially valuable in exploratory flavour and fragrance research where the composition of samples in terms of viscosity, suspended solids and flavour compound concentration is variable and unpredictable.

Published applications of CLLE are limited to samples that are free-running liquids. Viscous or heterogeneous samples such as fruit or vegetable pulps can lead to incomplete and uneven extraction because the solvent repeatedly follows the same channel through the sample. Filtering or centrifuging the pulp to remove suspended solids easily solves this problem [8,12], but assumes that the flavours are all in the liquid fraction. Excessive high-speed blending in an attempt to reduce viscosity or make samples very homogenous can change flavour profiles [9]. Sufficient dilution of viscous samples to provide a free-running solution or suspension with no tendency to channelling necessarily also dilutes the analytes of interest and compromises their recovery in the extract.

If the classical design for an extractor using heavier than water solvents [4] is modified by moving the solvent return arm to the side of the sample cylinder (Fig. 1), a conventional magnetic stir bar can easily be placed in the bottom of the extractor, but for the stirring to extend up through the column of viscous sample the stirrer has to be run at high speed, which prevents separation of the solvent and sample at the

\* Corresponding author. Tel.: +27 12 841 2190; fax: +27 12 841 3594.  
E-mail address: [papps@csir.co.za](mailto:papps@csir.co.za) (P. Apps).

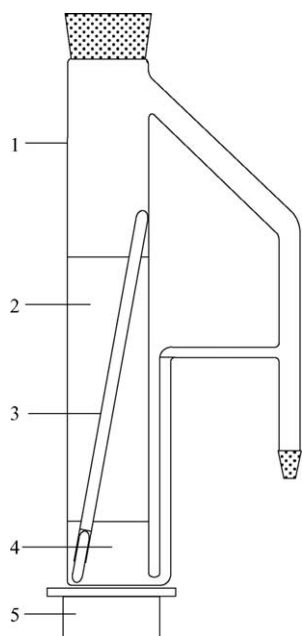


Fig. 1. Deployment of the stirring rod. (1) Extractor body (the condenser and solvent flask are not shown); (2) sample; (3) stirring rod; (4) heavier-than-water solvent; and (5) magnetic stirrer driver.



Fig. 2. Construction of the basic stirring rod. (1) Thin-walled glass tube and (2) Teflon-coated magnetic stir bar which is a sliding fit into the bottom of the glass tube.

bottom of the extractor, and leads to carryover of sample into the solvent flask. Rather than high speed mixing, what is required is a slow churning action extending throughout the column of sample. While a low speed shaft mixer would

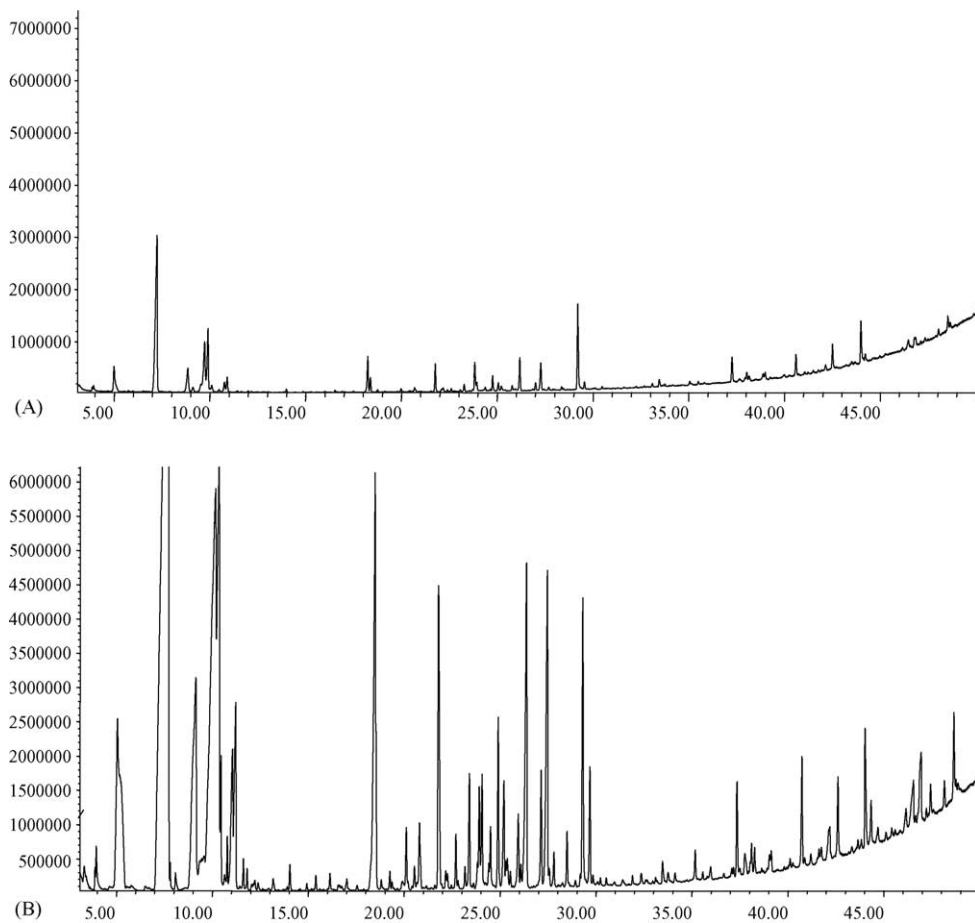


Fig. 3. TIC chromatograms from liquid–liquid extracts of guava pulp extracted without stirring (A) and with stirring (B).

prevent channelling, it would be difficult to assemble because the mixer shaft would have to run through the condenser on top of the extractor body.

## 2. Methods

During work on flavour analysis we developed a magnetic stirring rod that enhances extraction efficiency by preventing solvent channelling, and which is easy to make at low cost. The stirrer consists of a thin-walled glass tube, sealed at one end and with a pocket at the other end into which a large magnetic stir bar is a sliding fit (Fig. 2). The dimensions are not critical and can be changed to suit different sizes of extractor. For an extractor that holds approximately 500 ml of sample, a stirring rod approximately 350 mm long and 10 mm in diameter is appropriate.

In use the stirring rod runs with the magnetic follower resting on the bottom of the extractor (Fig. 1), which has a powerful stir driver beneath it, running at low speed. As the magnetic follower moves around the base of the extractor the glass tube churns the whole of the sample column.

To demonstrate the performance of the stirrer we prepared samples from guavas by blending 603 g of fruit with 100 g sodium chloride and 400 ml water. In duplicate, 200 ml of the slurry was diluted with 100 ml of water and extracted with dichloromethane for 5.5 h; one replicate with a stirring rod and the other without. A similar test was run with pineapple pulp prepared from 750 g of fruit, 140 g of sodium chloride and 100 ml of water, diluted 1:1 with water. The dilute extracts were concentrated to 2 ml by evaporation under vacuum and analysed by capillary GC–MS on a 30 m × 0.32 mm × 0.25 μm polyethylene glycol column in a Hewlett Packard 6890 GC coupled to a Hewlett Packard 5973 MS. TICs (70 eV) were integrated with Chemstation software and spectra were searched against the Wiley 275 library.

## 3. Results

With uniformly viscous pulps, such as that from guava, the use of the stir bar increases the concentration of the extract by more than an order of magnitude (Fig. 3; Table 1). With less viscous samples containing suspended solids, such as

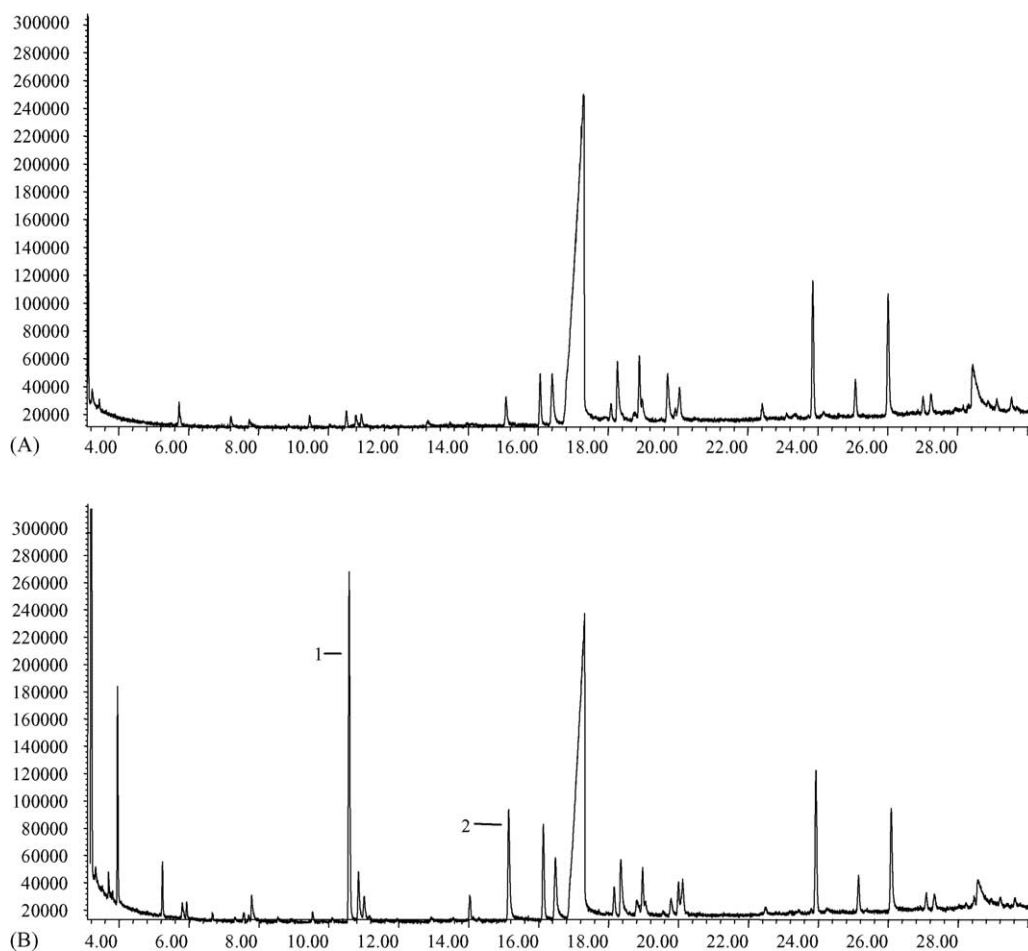


Fig. 4. TIC chromatograms from liquid–liquid extracts of pineapple juice extracted without stirring (A) and with stirring (B). Peak 1: methyl hexanoate and peak 2: methyl ester of thiomethylpropanoic acid.

Table 1  
TIC peak areas from GC–MS analysis of guava pulp extracts prepared by continuous liquid–liquid extraction with and without stirring

Retention time (Fig. 3)	Area without stirring (Fig. 3A)	Area with stirring (Fig. 3B)	Enhancement of extraction efficiency
5.000	30,238,398	418,193,760	13.8
7.24	207,698,646	2,481,368,378	11.9
8.84	28,299,362	409,486,446	14.5
9.73	65,727,836	1,107,360,384	16.8
9.90	53,614,942	496,937,718	9.3
18.24	26,474,322	464,963,965	17.6
21.77	17,082,175	228,674,427	13.4
23.83	15,752,444	61,778,526	3.9
26.18	23,679,444	288,680,362	12.2
27.28	21,472,465	255,641,188	11.9
29.21	64,108,755	180,539,444	2.8

The degree to which stirring enhances extraction efficiency is expressed as the ratio of the peak areas.

pineapple pulp, the quantitative effect is less striking, but there are clear changes in extract profile (Fig. 4).

#### 4. Discussion

Use of the stir bar extends the application of CLLE to samples that are not free-running liquids, thereby reducing sample manipulations with their associated artefacts [9]. The stir bar can be sealed inside the glass tube, but this has the disadvantage that the end of the glass tube then tends to scratch the bottom of the extractor. Adding fins or other projections to the sides of the bar increases its stirring action, at the cost of making it slightly more difficult to make; we found that a polyethylene disc encircling the stir bar about half way up was a fast and simple modification that helped if samples contain suspended solids.

#### Acknowledgement

This work was supported financially by CSIR Parliamentary Grant.

#### References

- [1] C.F. Poole, I.D. Wilson, *J. Chromatogr. A* (2000) 1.
- [2] T.H. Parliment, in: R. Masili (Ed.), *Techniques for Analyzing Food Aroma*, Marcel Dekker, New York, 1997, p. 1.
- [3] J.G. Wilkes, E.D. Conte, Y. Kim, M. Holcomb, J.B. Sutherland, D.W. Miller, *J. Chromatogr. A* 880 (2000) 3.
- [4] H. Stage, L. Gemmeker, *Chem. Z.* 88 (1964) 517.
- [5] M.H. Leahy, G.A. Reineccius, *Analysis of Volatiles*, Walter de Gruyter & Co., Berlin, 1984, p 19.
- [6] P.X. Etievant, *Crit. Rev. Food Sci.* 36 (1996) 733.
- [7] C. Weurman, *J. Agric. Food Chem.* 17 (1969) 370.
- [8] L. Zea, L. Moyano, J. Moreno, B. Cortes, M. Medina, *Food Chem.* 75 (2001) 79.
- [9] S. Elss, C. Preston, C. Hertzog, F. Heckel, E. Richling, P. Schreier, *LWT Food Sci. Technol.* 38 (2005) 263.
- [10] H.S. Okamoto, J. Dhoot, S.K. Perera, *Determination of 1,2,3-Trichloropropane in Drinking Water by Continuous Liquid–Liquid Extraction and Gas Chromatography/Mass Spectrometry*, California Department of Health Services, Berkeley, 2002, p. 1.
- [11] A. Buettner, P. Schieberle, *J. Agric. Food Chem.* 47 (1999) 5189.
- [12] S. Wu, U. Krings, H. Zorn, R.G. Berger, *Food Chem.* 92 (2005) 221.