

# Microfibers for juice analysis by solid-phase microextraction

## *Microfibras para análises de sucos por microextração em fase sólida*

Renata Borchetta Fernandes FONSECA<sup>1</sup>, Lucia Maria Jaeger de CARVALHO<sup>1\*</sup>,  
Carolina Netto RANGEL<sup>1</sup>, Humberto Ribeiro BIZZO<sup>2</sup>

### Abstract

In view of the interest in analyzing volatile compounds by SPME, the following five microfibers were tested, polydimethylsiloxane; polyacrylate; polydimethylsiloxane/divinylbenzene; carboxen/polydimethylsiloxane, and carbowax/divinylbenzene, to select the one which presents the best performance for the adsorption of the volatile compounds present in the headspace of acid lime juice samples. Sample stabilization time variations (30 and 60 minutes) were assessed as well the addition of NaCl to the samples. It was verified that the chromatogram with the most adsorbed volatile compounds was obtained with PDMS/DVB microfiber at 30 minutes and the addition of 0.2 g NaCl.

**Keywords:** citrus; beverages; analysis; organic agriculture.

### Resumo

Diante do interesse de analisar substâncias voláteis pelo método de Microextração em Fase Sólida (MEFS), foram testadas cinco microfibras de polidimetilsiloxano - PDMS; poliacrilato - PA; polidimetilsiloxano/divinilbenzeno - PDMS/DVB; carboxen/polidimetilsiloxano - CAR/PMDS e carbowax/divinilbenzeno - CW/DVB no presente trabalho, a fim de selecionar a microfibra de melhor desempenho para a adsorção das substâncias voláteis presentes no headspace das amostras de suco de lima ácida (*Citrus latifolia*, *Tanaka*) cv. Tahiti, obtida por cultivo biodinâmico. As microfibras selecionadas foram PDMS/DVB e PDMS, por apresentarem cromatogramas com maior número de componentes, onde não houve adição prévia de NaCl. A seguir, as microfibras de PDMS/DVB e PDMS foram avaliadas variando-se o tempo de estabilização da amostra (30 e 60 minutos), bem como a adição de NaCl. Verificou-se que o cromatograma com mais substâncias voláteis adsorvidas foi obtido com o uso da microfibra de PDMS/DVB, no tempo de 30 minutos e com a adição de 0,2 g de NaCl.

**Palavras-chave:** frutos cítricos; bebidas; análises; agricultura orgânica.

## 1 Introduction

Brazil is well known for its conventional cultivation *citrus* production. National organic production aims at supplying the domestic market as well as presenting very good perspectives of reaching the international market.

Organic agriculture has been playing an important and gradual role as a replacement for conventional farming currently accounting for 8% of all the produce commercialized in the Brazilian market. The organic product market has been significant, being estimated around 30% in the 2000s, with fruit and edible vegetables accounting for 2% of the total amount commercialized by the local supermarket networks (AGRIANUAL, 2001). Biodiversity is the major ally of organic cultivation as it represents ecosystems of great interaction and the least intervention possible, combined with natural processes. Organic food, i.e., food cultivated without chemicals, is of high biological quality. Moreover, it is free of substances toxic to human health deriving from agricultural cultivation systems where local productive resources are handled in a harmonious and integrated way aiming at economic, environmental, social, and cultural sustainability.

Foods, fruit, juices, and fruit nectars present in their composition several substances that provide them with special flavor and smell characteristics. Volatile compounds directly affect the sensorial quality of fresh or processed fruit.

Volatile compounds may also be used to improve or standardize flavors reconstituting lost or modified aromatic characteristics besides disguising undesirable flavors and aromas of the final product when used in industry. The generally low ( $\mu\text{g}\cdot\text{L}^{-1}$ ) concentration of volatile substances may be affected by some factors, such as variety, weather, ripening stage, harvest, post-harvest treatment, and storage and processing conditions (RIU-AUMATELL et al., 2004). According to Pino (1998), it must be pointed out that the proportion of volatile compounds found may not imply sensorial importance since the most frequently identified constituents are not necessarily the main sensorial ones.

Fruit juices are well defined by the COUNCIL DIRECTIVE nº 2001/112/EC of December 20, 2001, which characterizes as 100% pure any fruit juice without preservatives. On the other

Recebido para publicação em 25/5/2007

Aceito para publicação em 17/7/2008 (002547)

<sup>1</sup> Departamento de Nutrição Básica e Experimental, Instituto de Nutrição Josué de Castro, Universidade Federal do Rio de Janeiro – UFRJ, Av. Brigadeiro Trompowski, s/n, Bloco K, 1º Andar, Sala K1, 38, CEP 21941-590, Rio de Janeiro - RJ, Brasil, E-mail: [lucijaeager@gmail.com](mailto:lucijaeager@gmail.com)

<sup>2</sup> Embrapa Agroindústria de Alimentos, Av. das Américas, 29501, Guaratiba - RJ, Brasil

\*A quem a correspondência deve ser enviada

hand, organic products may be defined as products derived from cultivation systems using natural fertilizers, growth regulators, and additives produced on the farm and not chemical in nature (COUNCIL REGULATION, 1991).

According to Carvalho (2004), more advanced and sensitive equipment have been developed and improved for the detection, identification, and quantification of volatile compounds. The uses of such equipment as well as the use of extraction techniques have also been improved to minimize time and the stages that may cause these substances to lose their power or to suffer changes such as chemical artifact formation. A fast analysis of the aroma constituents may improve quality standard increasing the relation between the sensorial area and the volatile content.

Solid-phase micro extraction (SPME) is one of the new volatile extraction methods currently studied and is extremely fast and practical for the preparation of samples to be analyzed by high-resolution gas chromatography and mass spectrometry (HRGC/MS). It was reported for the first time by Berlandi and Pawliszyn (1989) when analyzing environmental contaminants in waters (effluents). SPME does not require the use of solvents, and it is based on the principle of adsorption and/or partition of the volatile compounds into polymeric microfibers followed by thermal desorption of the extract inside the chromatograph injector (CARVALHO, 2004).

The aim of this work was to select microfibers showing the best performance in the adsorption of volatile compounds in the juice of cv. Tahiti acid lime obtained by biodynamic cultivation.

## 2 Materials and methods

Eighteen kg of acid lime cultivated by biodynamic planting on *Bom Jesus* Farm – SP, June/2005 crop were used to process juice being initially separated in five lots of 10 experimental units randomly withdrawn. The juice was extracted using a multiprocessor (Wallita) and stored in 50 mL glass vials and was kept under freezing temperature at  $-10^{\circ}\text{C}$ . The juice extraction steps were controlled, so that microbiological contamination would not occur. The juice extraction was carried out at the Institute of Nutrition *Josué de Castro* laboratory at Federal University of Rio de Janeiro/UFRJ.

Analyses were carried out at *Embrapa* Food Technology, Rio de Janeiro. For the solid-phase microextraction (SPME) the following microfibers were used: polydimethylsiloxane (PDMS-100  $\mu\text{m}$ ); polyacrylate (PA-85  $\mu\text{m}$ ); polydimethylsiloxane/ divinylbenzene (PDMS/DVB-65  $\mu\text{m}$ ); carboxen/polydimethylsiloxane (CAR/PDMS-75  $\mu\text{m}$ ), and carbowax/divinylbenzene (CW/DVB-70  $\mu\text{m}$ ).

One gram of juice was weighed in a 4 mL flask, which was sealed with a Teflon septum and kept under magnetic stirring for 30 minutes at room temperature for sample stabilization. The SPME micro-syringe needle was inserted into the septum, and the microfiber was exposed to the atmosphere of the flask interior and the volatile compounds were extracted from the headspace for 15 minutes. The sample was kept under stirring for adsorption of the volatile compounds present in the sample headspace (YANG,

1994). The microfiber was sucked into the microsyringe, which was then withdrawn from the flask and introduced into an Agilent 6890N high resolution gas chromatograph injector (GCHR) (Agilent Technologies, Palo Alto, CA, USA), equipped with HP5 capillary column (25 m  $\times$  0.32 mm  $\times$  0.25  $\mu\text{m}$ ), under the following conditions: carrier gas ( $\text{H}_2$ ) flow  $-1.0$  mL/minutes at  $40^{\circ}\text{C}$ ; injector operated in the mode without flow division (split-less) at  $250^{\circ}\text{C}$ ; and flame ionization detector (FID) maintained at  $300^{\circ}\text{C}$ . The mass spectrometry was carried out in an Agilent 5973N using an HP5MS capillary column (25 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ) and helium as the carrier gas (1.0 mL/minutes). The electron impact technique was applied with the ion source operating at  $230^{\circ}\text{C}$  and 70eV ionization energy (ZHANG, 1994).

The volatile compounds were identified by comparing the mass spectra obtained with the mass spectrum data of Wiley 6th Ed and the calculated retention indices with the values found in the literature (ADAMS, 1995). The retention indices were calculated based on the retention times of the compounds in the samples and on the retention times of an n-alkane series injected in the same column and under the same experimental conditions described above. The experimental procedure was repeated under the same conditions for each microfiber (CEVA-ANTUNES, 2003).

After selecting the two microfibers with the best performance in the extraction/adsorption of volatile compounds, 1.0 g of juice was weighed and 0.2 g of NaCl was added at 30 and 60 minutes, stabilization time. All experiments were carried out in triplicate.

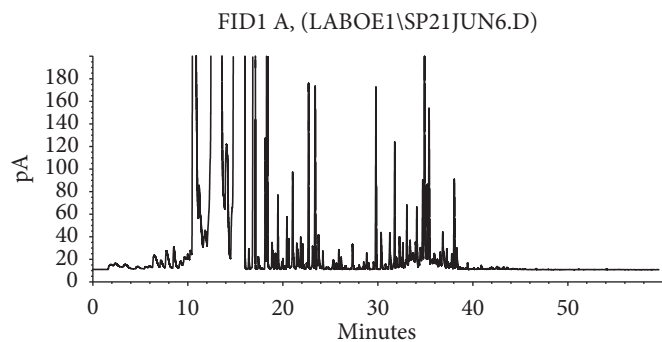
## 3 Results and discussion

SPME was found to perform with PDMS/DVB and PDMS microfibers for the extraction of the volatile compounds of acid lime juice, at the stabilization time of 30 minutes, 15 minutes of adsorption, and three of desorption without NaCl addition presenting the largest amount of adsorbed peaks, 59 and 44, respectively (Figures 1 and 2).

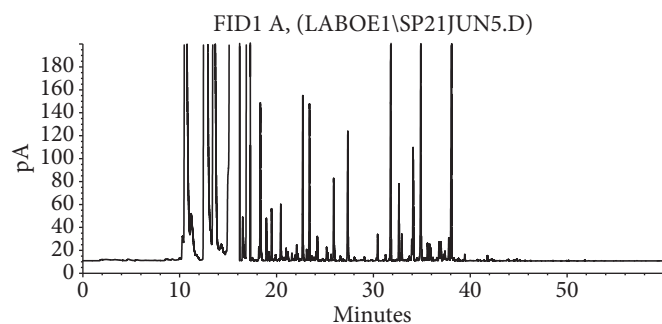
While studying some other fruits, the following authors Prudente et al. (2003) calamondin fruit; Kim et al. (2003) yuzu tea; Riu-Aumatell et al. (2004) nectarin, peach, and pear; Carvalho et al. (2000, 2002, 2004) pineapple juice, obtained good results in the adsorption of volatile compounds in several food matrices using PDMS microfiber with headspace SPME.

At optimization, 0.2 g of NaCl was added to 1 g of acid lime juice at 30 and 60 minutes. An increase in the number of volatile compounds in both microfibers at the time of 30 minutes (Figures 3 and 4) was observed. However, when the same microfibers were used within 60 minutes, with salt addition, PDMS microfiber adsorbed a smaller number of volatile compounds when compared to the samples with and without salt at 30 minutes, stabilization time.

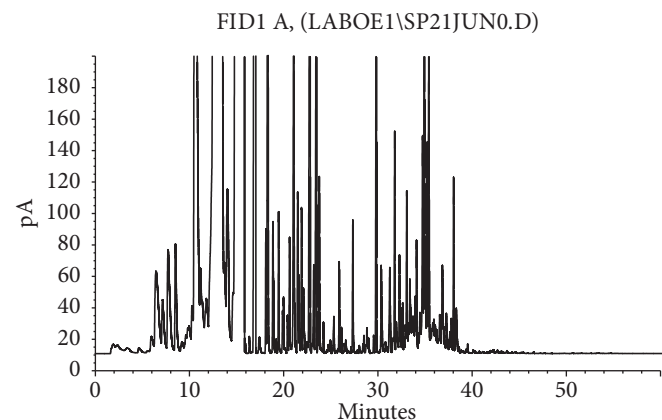
It was also observed that the addition of salt was important in the release of volatile compounds to the headspace of the samples due to its capacity of absorbing water, saturating the environment, and releasing the volatile compounds, as reported by Reto et al. (2007).



**Figure 1.** Volatile compounds of acid limejuice (biodynamic) extracted by SPME at 30 minutes without salt addition in PDMS/DVB microfiber.

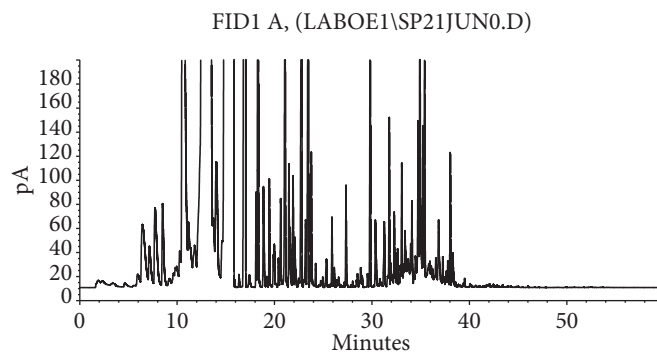


**Figure 2.** Volatile compounds of acid limejuice (biodynamic) extracted by SPME at 30 minutes without salt addition in PDMS microfiber.



**Figure 3.** Chromatogram of the volatile compounds of acid limejuice sample (biodynamic) extracted by SPME at 30 minutes with salt addition in PDMS/DVB microfiber.

PDMS/DVB microfiber adsorbed 75 volatile compounds at 30 minutes and 72 at 60 minutes of stabilization time whereas PDMS microfiber adsorbed 52 and 41 volatile substances at 30 and 60 minutes, respectively. On the other hand, in samples without NaCl addition at 30 minutes, the volatile compounds adsorbed by the same microfibers were 59 and 44, respectively.



**Figure 4.** Chromatogram of the volatile substances of acid limejuice sample (biodynamic) extracted by SPME at 60 minutes with salt addition in PDMS/DVB microfiber.

The results revealed that the microfiber PDMS/DVB presented the best performance in the adsorption of acid limejuice volatile compounds with and without salt addition. The chromatograms of the volatile compounds extracted from the acid lime juice samples using the microfiber PDMS/DVB with salt addition, at 30 and 60 minutes of stabilization time (Figures 3 and 4) are presented below.

#### 4 Conclusions

Under the studied conditions, SPME proved efficiency in extracting the volatile compounds of the juice of acid lime, cv. Tahiti, being a fast and low cost method providing process optimization. Based on the profiles of the volatile compounds in the chromatograms, PDMS/DVB and PDMS microfibers were selected as the most efficient for acid lime volatile analysis. PDMS/DVB was selected as the best microfiber under the stabilization and extraction conditions of 30 and 15 minutes, respectively.

#### Acknowledgments

The authors are grateful for the financial support provided by the foundation CNPq (The National Council for Scientific and Technological Development) and to Embrapa – Food Technology for allowing the use of the GCHR/MS equipment.

#### References

- ADAMS, R. P. **Identification of Essential Oil Components by CG/MS**. Carol Stream, Illinois, USA: Allured Publishing Corporation, 1995.
- AGRIANUAL. **Anuário Estatístico da Agricultura Brasileira**. São Paulo: FNP, Consultoria e Comercio, 2001.
- BERLARDI, R.; PAWLISZYN, J. The application of chemically modified fused silica fibers in the extraction of organic water matrix samples and their rapid transfer to capillary columns. **Water Pollution Research Journal of Canadá**, v. 24, p. 179, 1989.
- CARVALHO, L. M. J. et al. Clarification of pineapple juice (*Ananas comosus*, L. Merrill) by UF. **Alimentaria - Revista de Tecnologia e Higiene de los Alimentos**, v. 316, p. 129-132, 2000.
- CARVALHO, L. M. J. et al. Volatile compounds in hydrolyzed pineapple (*Ananas comosus*, L. Merrill), Perola variety and, clarified

- pasteurized juices obtained by solid-phase microextraction. **Alimentaria - Revista de Tecnologia e Higiene de los Alimentos**, v. 33, p. 127-132, 2002.
- CARVALHO, L. M. J. **Abacaxi (*Ananas comosus*, L. Merrill), cv. Perola: obtenção e avaliação de suco clarificado por membranas**. Seropédica, 2004. 209 p. Tese – (Doutorado em Ciências), Departamento de Fitotecnia, Universidade Federal Rural do Rio de Janeiro.
- CEVA-ANTUNES, P. M. N. et al. Analysis of volatile compounds of taperebá (*Spondias mombin* L.) and cajá (*Spondias mombin* L.) by simultaneous distillation and extraction (SDE) and solid phase microextraction (SPME). **Journal of Agricultural and Food Chemistry**, v. 51, p. 1387-1392, 2003.
- COUNCIL DIRECTIVE n° 2001/112/EC of 20 December 2001.** Relating to fruit juices and certain similar products intended for human consumption.
- COUNCIL REGULATION (EEC) n° 2092/91 of 24 June 1991.** On organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs.
- KIM, T. H. et al. Aroma dilution method using GC injector split ratio for volatile compounds extracted by headspace solid-phase microextraction. **Food Chemistry**, v. 83, p. 151-158, 2003.
- PINO, J. A. Los constituyentes volátiles del tamarindo (*tamarindus indica* L.). **Alimentaria – Revista de Tecnologia e Higiene de los Alimentos**, n. 292, p. 85-87, 1998.
- PRUDENTE, A. D.; XU, Z.; KING, J. M. Comparison of volatile components between ripe and unripe calamondin (*Citrofortunella mitis*, J. Igram and H.E. Moore). In: **IFT Annual Meeting**, Chicago, USA, 2003. (Session 45C – Food Chemistry: flavor and aroma activity). Disponível em: <[http://ift.confex.com/ift/2003/techprogram/paper\\_19558.htm](http://ift.confex.com/ift/2003/techprogram/paper_19558.htm)>. Acesso em: 30 nov. 2005.
- RETO, M. et al. Analyses of vitamin K in green tea leaves and infusions by SPME-GC-FID. **Food Chemistry**, v. 100, p. 405-411, 2007.
- RIU-AUMATELL, M. R. et al. Characterization of volatile compounds of fruit juices and nectars by HS/SPME and GC/MS. **Food Chemistry**, v. 87, n. 4, p. 627-637, 2004.
- YANG, X.; PEPPARD, T. Solid-phase microextraction for flavor analysis. **Journal of Agricultural and Food Chemistry**, v. 42, p. 1925-1930, 1994.
- ZHANG, Z.; YANG, Z.; PAWLISZYN, J. Solid-phase microextraction. **Analytical Chemistry**, v. 66, p. 845A-853A, 1994.