Comparison of isolation methods for papaya (*Carica papaya* L.) volatile compounds

Karina Almora Almora, Jorge A. Pino Alea and Elda Roncal Estrada.

Instituto de Investigaciones para la Industria Alimenticia, Carretera del Guatao km 3½, Ciudad de La Habana, Código Postal 19200, Cuba.

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Key words: papaya, volatile compounds, dynamic headspace, vacuum distillation, distillation-solvent extraction, gas chromatography.

RESUMEN. Para el estudio del aroma de los alimentos es necesaria la aplicación de métodos adecuados de aislamiento y concentración de los compuestos volátiles que contribuyen al aroma de las frutas. Un método adecuado permite obtener resultados más fidedignos respecto a la verdadera composición de volátiles que influyen en el aroma de un determinado producto. Para elegir un procedimiento adecuado a este fin para la fruta bomba (*Carica papaya* L) se realizó un estudio comparativo de tres métodos, que abarcan los procedimientos utilizados en otros trabajos para esta fruta. Estos son: análisis de la fase vapor (AFV), destilación a vacío y destilación-extracción con disolventes simultáneos (DES). Para la selección del método se tuvieron en cuenta criterios como: tiempo de trabajo, complejidad de la operación, volumen del extracto, factor de concentración y resultados cuantitativos mediante la utilización de la cromatografía de gases. Los resultados obtenidos para la fruta bomba (var. Maradol roja) revelaron que el método AFV posee el más corto tiempo de trabajo, poca complejidad en la operación y pequeño factor de concentración; seguido del método DES. El método DES mostró un gran número de compuestos volátiles y semivolátiles de la fruta bomba en comparación con el resto. Esto permitió concluir que el método DES es el más apropiado para el análisis de los volátiles presentes en la fruta bomba.

ABSTRACT. The first step in the study of flavor compounds of any fruit is the determination of the best extraction method for all volatile constituents that most contribute to flavor. This is extremely important to obtain reliable results concerning the exact composition of volatiles present in the fruit. The results can be utilized to produce an artificial flavor as well. Different authors have extensively investigated the aroma composition of papaya (*Carica papaya* L.) fruit. Several methods were used for the concentration of the volatile components of papaya: Dynamic Headspace (DHS), Vacuum Distillation and Simultaneous Distillation-Solvent Extraction (SDE). Each isolation procedure has its special characteristics. Consequently, the authors decided to evaluate them to determine which were the most effective for the analysis of papaya volatiles. The isolation methods were compared in terms of some experimental parameters, such as: experimental time, ease of operation, volume of extract, concentration factor and quantitative results. Concentrations of these volatiles were analyzed by GC (gas chromatography). The DHS method showed a short experimental time, ease of operation and a small concentration factor; followed by the SDE method. The SDE method produced the largest yield of both volatile and semivolatile compounds. Results lead authors to conclude that the SDE procedure is the most suitable for the analysis of volatile constituents from papaya.

INTRODUCTION

Papaya (*Carica papaya* L.) is native of tropical America, but is currently disseminated throughout the tropics. The Maradol roja variety is well known in Cuba, Mexico, Colombia and neighboring countries. The fruits have a cylindrical shape and medium weight (1,3 to 2,3 kg of pulp). The peel is yellow and the eatable pulp is intense orange with little black seeds in the, relatively small, internal cavity. This variety has a high sugar content with respect to others. Because of this, its taste is good, the pulp has a firm texture and high resistance to oxidation during ripening.[1]

The aroma composition of the papaya fruit has been extensively investigated by different authors.[2-11] But a comparison of most common concentration methods of volatiles had never been made.

The study of flavor compounds of any fruit begins by determining the best extraction method for all the volatile constituents. This is extremely important to obtain reliable results with respect to the exact composition of volatiles present in the fruit.

Several methods were used for concentration of papaya volatiles: Dynamic headspace (DHS), Vacuum distillation (VD) and simultaneous Distillation-Solvent extraction (SDE). Each isolation procedure has its characteristics. Consequently, this study was carried out in order to determine which was the most effective for the analysis of papaya var. Maradol roja.

MATERIALS AND METHODS

Material

The study was done with papaya var. Maradol roja, grown in Havana. The fruits were collected at harvest time in the "rayona" phase (appearance of the first yellow stripes). They were ripened at ambient temperature (mean value: 25,9 °C, standard deviation: 1,0 °C) and 87,5 % relatively humidity (standard deviation: 3,07 %), for a period of 7 d .

Dynamic headspace (DHS)

Twenty grams of papaya pulp was placed in 100 mL distilled water. For the separation of volatiles, previously purified nitrogen was sparged (flow: 150 mL/min for 90 min). The volatile compounds were adsorbed in the trap-tube (60 mm X 4 mm) filled with 100 mg Tenax GC (60-80 mesh) and fitted with plugs of silanized silica wool. Trapped volatiles underwent desorption by solvent extraction with diethyl ether and then were concentrated carefully to 0,1 mL with a gentle nitrogen stream.

Vacuum distillation (VD)

Hidrodistillation was carried out with 200 g papaya pulp and 800 mL distilled water. After adjusting pH to 7 with NaOH (1 mol/L). The sample was vacuum distilled at reduce pressure (20 mm Hg) during 90 min . The condenser was kept at 0-10 °C during this operation. The aqueous distilled (200 mL) was extracted with three portions of diethyl ether (20 mL each one) and was concentrated in the Kuderna-Danish evaporator with division column Vigreux (12,5 cm X 1 cm) until 0,6 mL.

Simultaneous distillation/solvent extraction (SDE)

Two hundred grams of papaya pulp and 800 mL of distilled water was distilled and simultaneously extracted in 25 mL diethyl ether for 90 min in a Likens and Nickerson apparatus.[12] The condenser was kept at 0-10 °C during this operation.

The volatile extracts obtained by each method were dried over anhydrous sodium sulfate, concentrated to 0,1 mL and 2 mg standard (methyl undecanoate) was added before GC analysis.

Quantitative and qualitative analysis

Constituents of the volatile fraction were analyzed by GC. Measurements were performed using a Konik 4000 A GC. The GC system was fitted with a 30 m X 0,25 mm (0,25 μm film) DB-WAX type and a flame ionization detector. The sample was run on the following settings: carrier gas flow 1 mL/min; GC temperature program 60 °C for 10 min, raised to 230 °C at 3 °C/min, with 15 min hold. Sample size 1 μL .

The identification of compounds were carried out by comparison of retention indices, which were determined using a series of n-paraffins.[13]

The isolation methods were compared in terms of some experimental parameters, such as running time, concentration factor (volume of extract-volume of concentrate) and ease of operation. The chromatographic profiles obtained by the three methods were compared and the relation between compounds and the standard was taken into account.

RESULTS AND DISCUSSION

The three isolation procedures for volatiles selected for this study covered the commonly encountered procedures that can be used to isolate papaya volatiles.[2-11]

Each method was evaluated according to several experimental parameters (Table 1). The DHS method showed a shorter running time, followed by the SDE procedure. The DHS method proved to be simpler.

The concentration factor was determined from the volume ratio of initial extract to final concentrate (0,1 mL). This parameter should be as low as possible, in order to decrease the amount of impurities of the solvent. Also, if solvent volume is small, the loss of isolated volatiles is decreased. The lowest or most favorable concentration factor corresponds to the DHS method, followed by the SDE method.

Figures 1-3 show the chromatograms of papaya volatiles obtained with the three methods. The small number and area of the peaks indicate that the concentrate obtained by the DHS method is poor. On the other hand, the chromatographic profiles corresponding to the other two methods are rather similar as regards the number of compounds present, although the area is slightly larger with the SDE method.

For better comprehension, Table 2 shows the results of area determination for some of the volatiles typically present in papaya, which gives an idea of their concentrations.

Of the procedures evaluated, the SDE method allowed obtainment of the largest concentration of volatiles. The DHS method shows selectivity in the isolation of volatiles, since differences with the other two methods increase as the volatility of the compounds decrease.

The fact that the differences in areas between VD and SDE methods are not so big seems to indicate that they do not affect extraction of the volatile compounds, even if reduced pressure was not used in the SDE method. Adjusting pH to 7 protects the pulp from heat damage.

These results lead us to conclude that the SDE method is the most suitable for the analysis of volatile constituents of papaya var. Maradol roja.

CONCLUSIONS

Three well known methods were compared as regards their effectiveness in concentrating the volatiles of papaya var. Maradol roja: Dynamic Headspace (DHS), Vacuum Distillation and Simultaneous Distillation-Solvent Extraction (SDE). The DHS method has a short running time, ease of operation and small concentration factor, followed by the SDE method. But the latter shows the largest yield of volatile compounds. These results lead authors to conclude that the SDE procedure is the most suitable for analysis of the volatile constituents of papaya var. Maradol roja. This is being further studied.

Table 1. Comparative study of the extraction of the volatile constituents of papaya by different methods.

Methods	Running time[1] (h)	Ease of operation	Concentration factor[2]
DHS	2.0	+	10
VD	3.5	++	600
SDE	2.5	++	250

[1] Comprises assembly, operation and cleaning of equipment for one sample.
[2] Volume of distillate-volume of concentrate (0,1 mL).

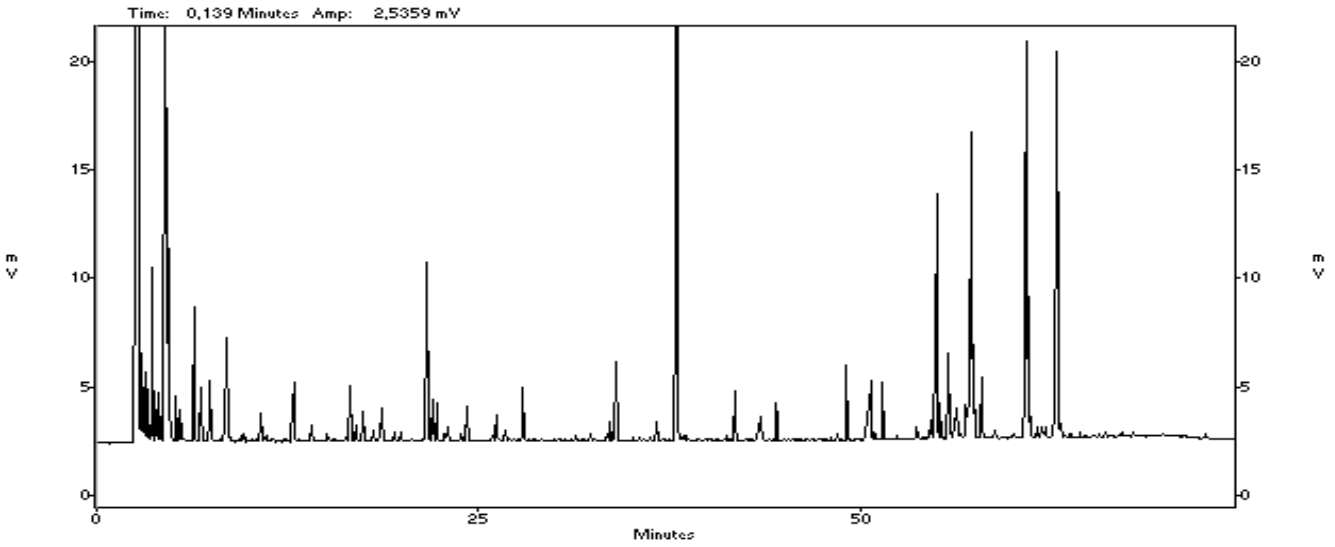

Fig. 1. *Chromatogram of papaya volatiles obtained with the Simultaneous Distillation-Solvent Extraction method (SDE).*

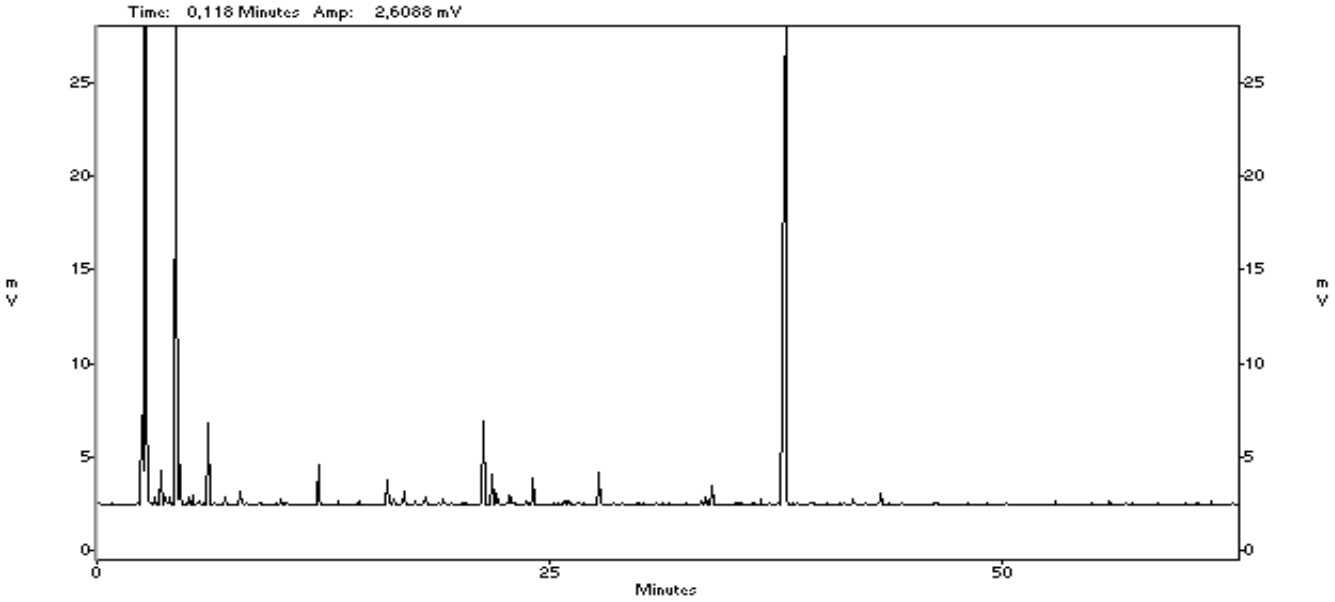

Fig. 2. *Chromatogram of papaya volatiles obtained with the Dynamic Headspace method (DHS).*

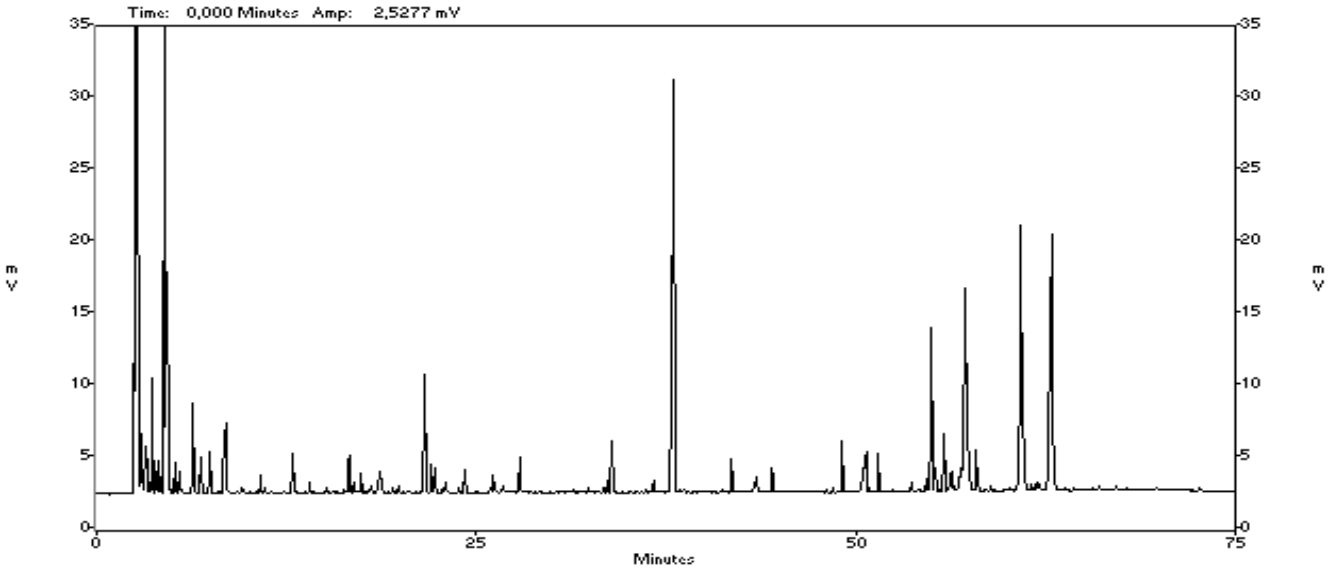

Fig. 3. *Chromatogram of papaya volatiles obtained with Vacuum Distillation method (VD).*

Table 2 Results of area determination for volatiles typically present in papaya by the three methods.

Retention time (min)	Compounds	Area ratios (compound/standard) · 100		
		DHS	VD	SDE
4.32	methyl butanoate	0.4	2.7	3.0
4.69	3-methylbutanol	109.0	128.0	134.0
17.15	ethyl hexanoate	0.9	1.6	2.0
18.23	benzyl alcohol	1.6	1.8	2.0
21.76	(Z)-linalool oxide	10.5	20.0	20.0
22.45	(E)-linalool oxide	0.4	2.9	3.0
23.14	linalool	1.0	7.0	7.1
26.29	terpinen-4-ol	0.5	3.1	3.0
34.12	benzyl isothiocyanate	2.0	7.0	7.1
44.62	ethyl dodecanoate	0.4	3.8	4.0
	hexadecanoic acid	0.0	35.0	39.0
—	TOTAL	$17 \cdot 10^6$	$69 \cdot 10^6$	$78 \cdot 10^6$

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Convocatoria

SIMPOSIO ESTUDIANTIL INTERNACIONAL DE INGENIERIA QUIMICA

8 al 13 de marzo de 2004.
Hotel Villa Panamericana, Ciudad de La Habana.

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Para mayor información:

Akaena Serrano Jaime, Presidenta Comité Organizador
Teléfono: 202 2253. Fax: (537) 267 1574
Correos electrónicos: seiq@quimica.cujae.edu.cu akaena@quimica.cujae.edu.cu