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ARTICULO INVESTIGATIVO

CHARACTERISATION OF VOLATILE COMPOUNDS OF COCOA HUSK USING HEADSPACE SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-OLFACTOMETRY ANALYSIS

Caracterización de los compuestos volátiles de la cascarilla de cacao mediante microextracción en fase solida del espacio de cabeza y análisis por cromatografía de gases-olfatometría

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ABSTRACT

Cocoa seeds (*Theobroma cacao* L.) are known for their variety of products exhibiting pleasant sensory properties. Cocoa husk is a residue from the cocoa industry which can be used as flavouring material. A headspace solid-phase microextraction (HS-SPME) procedure followed by gas chromatography-olfactometry (GC-O) analysis is proposed to determine aroma-active compounds in cocoa husk. To determine optimal extraction conditions of the HS-SPME technique: fiber type (100 µm polydimethylsiloxane, 85 µm Carboxen/polydimethylsiloxane, 65 µm polydimethylsiloxane/divinylbenzene and 50/30 µm divinylbenzene/Carboxen/polydimethylsiloxane), extraction time (15, 20 and 25 min) and temperature (40, 50 and 60 ºC) were evaluated using surface response design. Response variables were global odour from the fibers and total chromatographic areas. GC-O in combination with HS-SPME using divinylbenzene/Carboxen/polydimethylsiloxane fiber operated at 60 ºC for 22 min could isolate most of the volatile compounds from cocoa husk. A total of 169 of them were identified, including 28 acids, 23 esters, 22 pyrazines, 16 terpenes, 12 ketones, 8 alcohols, 3 aldehydes, among others. Among them, the most odour-active compounds were acetic acid, 2,3-diethyl-5-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 3-methylbutanal, phenylacetaldehyde, 3,5-diethyl-2-methylpyrazine, 4-hidroxy-2,5-dimethyl-3(2*H*)-furanone, 3-methylbutanoic acid, 2-ethyl-5-methylpyrazine, 2,6-dimethylpyrazine, dimethyl trisulfide, 2,3,5,6-tetramethylpyrazine, 2,3,5-trimethylpyrazine, 2-methylbutanal, 2-methylpyrazine, 2-phenylacetic acid, linalool, butanoic acid, 2-methylbutanoic acid, 2,5-dimethylpyrazine and 2-phenylethyl acetate. The present method may be applied as a quality control tool for industrial laboratories.

Keywords: cocoa husk; aroma-active compounds; HS-SPME; GC-O.

RESUMEN

Las semillas del cacao (*Theobroma cacao* L.) son conocidas por su variedad de productos que exhiben propiedades sensoriales placenteras. La cascarilla de cacao es un residuo de la industria del cacao que puede ser usado como un material saborizante. En este trabajo se propone un procedimiento basado en la microextracción en fase solida del espacio de cabeza (HS-SPME) seguido del análisis gas cromatográfico-olfatometría (GC-O) para determinar los compuestos activos del aroma en la cascarilla de cacao. Para determinar las condiciones de extracción óptimas por la técnica de HS-SPME, se evaluaron: tipo de fibra (100 µm polidimetilsiloxano, 85 µm Carboxen/polidimetilsiloxano, 65 µm polidimetilsiloxano/divinilbenceno y 50/30 µm divinilbenceno/Carboxen/polidimetilsiloxano), tiempo de extracción (15, 20 y 25 min) y temperatura (40, 50 y 60 ºC), con ayuda del diseño de superficie de respuesta. Las variables de respuesta fueron el olor global desprendido de las fibras y las áreas cromatográficas totales. La GC-O combinada con la HS-SPME usando la fibra de divinilbenceno/Carboxen/polidimetilsiloxano operada a 60 ºC por 22 min pudo aislar la mayoría de los compuestos volátiles de la cascarilla de cacao. Un total de 169 de ellos fueron identificados, que incluyeron 28 ácidos, 23 ésteres, 22 pirazinas, 16 terpenos, 12 cetonas, 8 alcoholes, 3 aldehídos, entre otros. Entre ellos, los compuestos más activos del aroma fueron ácido acético, 2,3-dietil-5-metilpirazina, 2-etil-3,5-dimetilpirazina, 3-metilbutanal, fenilacetaldehído, 3,5-dietil-2-metilpirazina, 4-hidroxi-2,5-dimetil-3(2*H*)-furanona, ácido 3-metilbutanoico, 2-etil-5-metilpirazina, 2,6-dimetilpirazina, trisulfuro de dimetilo, 2,3,5,6-tetrametilpirazina, 2,3,5-trimetilpirazina, 2-metilbutanal, 2-metilpirazina, ácido 2-fenilacético, linalol, ácido butanoico, ácido 2-metilbutanoico, 2,5-dimetilpirazina y acetato de 2-feniletilo. El presente método puede ser aplicado como una herramienta para el control de calidad en los laboratorios de la industria.

Palabras clave: cascarilla de cacao; compuestos activos del aroma; HS-SPME; GC-O.

INTRODUCCIÓN

Chocolate is amongst the most popular favour worldwide and it is prepared by different technologies with cocoa products as main raw material mixed with other ingredients. Therefore, the flavour of cocoa products (cocoa powder, cocoa butter or cocoa liquor) is one of the most important organoleptic attribute which impacts its quality. The pleasant flavour of cocoa is mainly the result of bean genotype, geographical origin, and post-harvest treatments (fermentation and drying), roasting and storage (Afoakwa *et al.*, 2008; Kongor *et al.*, 2016).

Cocoa processing manufacturing is an important industry producing cocoa products and chocolate, but which also generate great amounts of wastes (Afoakwa, 2016). Some of these residues could provide other innovative by-products attractive to the food industry, and such is the case of cocoa husk, which can be used to yield high-value-added products as a source of valuable natural substances due the presence of polyphenols with antioxidant activity and theobromine with many stimulatory effects, and dietary fibers (Nguyen & Nguyen, 2017). Considering its intense cocoa flavour, another interesting probable use is as flavouring material.

Headspace solid-phase microextraction (HS-SPME) has been widely used to analyse the composition of food flavours (Jeleń *et al.*, 2012; Souza-Silva *et al.*, 2015). HS-SPME is a solventless technique, with simplicity and effectiveness in rapid sampling, as well as high sensitivity and reproducibility, therefore simplifying and speeding up sample preparation and analysis standardization. HS-SPME is a valuable tool for preparing samples for gas chromatography–olfactometry (GC-O) analysis, which is a procedure for the detection of aroma-active compounds in foods (Feng *et al.*, 2016).

HS-SPME has been applied to evaluate the volatile compounds from several cocoa products and chocolate, such as cocoa mass (de Brito *et al.*, 2002), cocoa powders and chocolate (Ducki *et al.*, 2008), dark chocolate (Afoakwa *et al.*, 2009), cocoa liquors (Pini *et al.*, 2004; Di Carro *et al.*, 2015), cocoa beans (Rodriguez-Campos et al., 2011, 2012; Tran et al., 2015), cocoa products and chocolate (Ascrizzi *et al.*, 2017), and microencapsulated cocoa liquor (Sanchez-Reinoso *et al.*, 2017). In some of those studies the isolation conditions were optimised because difference in the matrix of the samples generally influences the composition of the headspace, but in those reports (de Brito *et al.*, 2002; Pini *et al.*, 2004; Ducki *et al.*, 2008; Rodriguez-Campos *et al.*, 2012) the optimal conditions were selected bearing in mind only the highest number and content of volatile compounds without considering the sensory response of the HS-SPME isolated fraction.

The aim of the present research was to investigate the typical volatile compounds found in cocoa husk and their contribution to the aroma of this by-product using HS-SPME and GC-O analysis. This investigation optimised the analytical conditions, so that they can be reproduced in other researches and for quality control.

MATERIALS AND METHODS

Materials and sample

The SPME holder for manual sampling and fibers used were purchased from Supelco (Bellefonte, PA, USA). Fibers tested were 100 µm polydimethylsiloxane (PDMS), 85 µm

Carboxen/polydimethylsiloxane (CAR/PDMS), 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) and 50/30 µm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS).

A representative sample of cocoa husk (30 kg) was obtained from the major Cuban company Derivados del Cacao Baracoa (Guantánamo, Cuba). Cocoa beans were roasted in the proper industry by a progressive heating until 150 °C was reached (total roasting process time 20 min). The raw material was ground and sieved to a finely powder with a mean diameter of 550 ± 21 µm. The ground material was packed in 1-kg portions in polyethylene bags and stored at 25 °C until analysis.

Headspace solid-phase microextraction procedure

Extractions were performed in 8-mL vial PTFE/silicone septa vials (Supelco, Bellefonte, PA, USA) with 0.2 g of ground sample conditioned for 10 min at the selected temperature. The fiber was then exposed to the headspace for the selected time. To determine optimal extraction conditions of the HS-SPME technique: fiber type, extraction time and temperature were evaluated. Four fibers; extraction time (15, 20 and 25 min) and temperature (40, 50 and 60 °C) were tested. For the selection of the fiber, some initial conditions were fixed: 10 min pre-extraction and 20 min extraction at 60 °C. All analyses were made in triplicate for each sample.

Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry analysis was carried out using a QP-2010 Ultra (Shimadzu, Japan) equipped with a FID and DB-5 ms (30 m × 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) capillary column. Oven temperature was held at 50 °C for 2 min, increased at 4 °C/min up to 230 °C and held for 10 min. Carrier gas was helium at 1 mL/min. Thermal desorption was carried out at 250 °C for PDMS, PDMS/DVB and DVB/CAR/PDMS fibers, and at 280 °C for CAR/ PDMS fiber in splitless mode for 2 min, using and inlet liner of 0.75 µm i.d. The mass spectrometer was setup with the source at 250 °C, electronic ionization energy 70 eV and the mass range from 35 to 350 u.

Compounds were preliminarily identified using NIST05, Wiley 7, NBS 75k, ADAMS 2001 and in-house Flavourlib mass spectra databases, then the identities of most were confirmed by comparison of their linear retention indices, relative to C_6–C_{28} *n*-alkanes, with those of reference standards or with reported data in NISTwebbook (2020). Relative concentration was calculated based on the area of the corresponding GC peak. Each measurement was conducted in triplicate.

Direct SPME-Gas chromatography-Olfactometry

SPME-GC-O was performed following the methodology described earlier (Pino & Roncal, 2016). A HP-6890 (Hewlett-Packard Co., Palo Alto, CA, USA) was used. The injector was connected via a deactivated fused silica capillary (25 cm × 0.25 mm) to a sniffing port consisted of a cylindrically shaped aluminium device with a bevelled top and a central drill hole housing the capillary. The oven was maintained at 250 °C. Because no chromatographic separation was carried out by the short capillary, volatile compounds arrived simultaneously at the sniffing port. Three trained sniffers perceived and evaluated the resulting global odour. Sniffers were asked to smell first the ground cocoa husk (1 g) contained in a plastic cup sealed

with a pierced cap at 25°C and memorise the odour. Then they evaluated with the direct GC-O device the different SPME extracted samples, rating their similarity to the reference using a 10-cm line scale ranging from 0 (close to the reference) to 10 (far from the reference). Panelists had to smell the reference before each sample evaluation. All analyses were replicated three times.

Gas chromatography-olfactometry analysis

Gas chromatography analysis was performed in a HP-6890 (Hewlett-Packard Co., Palo Alto, CA, USA) fitted with the same capillary column and GC setup used in GC-MS. The end of the capillary column was connected to a deactivated Y-shaped glass splitter dividing the effluent into two equal parts, which were transferred via two deactivated fused silica capillaries (25 cm × 0.25 mm) to a sniffing port and an FID, respectively. Two trained sniffers (replaced at 30 min intervals to avoid fatigue) evaluated the effluents.

An approach of the analysis extract dilution analysis (AEDA) developed for GC-O analysis of roasted pistachios to estimate the odour contribution of the volatile compounds was applied in the present study (Aceña *et al.*, 2011). A series of sequentially reduced samples of cocoa husk (0.2–0.0016 g, each trial half the weight of the preceding trial) were subjected to SPME–GC–O. To these amounts were assigned FD factors of 2, 4, 8, 16, 32, 64, and 128. For each analysis, panelists recorded the detection time and gave an odour description until no odours were perceived in the GC–O effluent. The last dilution step where an odourant was detected was its flavour dilution (FD) factor. To check the linear recovery of the procedure previously described, the total peak area of the GC analyses of the initial sample and the successively reduced samples was measured and correlated with the proper sample reduction.

Statistical analysis

Results for fiber selection were processed by one-way ANOVA and Duncan's multiple range test.

The optimisation of the HS-SPME procedure was evaluated by response surface methodology. The response evaluated during the experiments was the total peak area from the volatile compounds by the GC-FID analysis. The experimental factors extraction time and temperature were evaluated by a 2-factor-3-level design with four replicates at the centre point was selected using Design-Expert 8.07 (Stat-Ease Inc., Minneapolis, MN). The second-order polynomial models were assessed by the analysis of variance, determination coefficients and test for the lack of fit. Numeric optimisation was made to find a stationary point set as maximum total peak area.

RESULTS AND DISCUSSION

Selection of fiber coatings

Different types of HS-SPME fibers were compared for the extraction of the volatile compounds from cocoa husk: PDMS, CAR/PDMS, DVB/CAR/PDMS, and PDMS/DVB. In general, GC-MS analyses showed that more than 200 volatile compounds were isolated by the four fibers. The extracted components with LRI from less than 700 (LRI = 645 for acetic acid) to almost 2200 (LRI = 2197 for ethyl octadecenoate). A total of 169 of them were

identified (Table 1), including 28 acids, 23 esters, 22 pyrazines, 16 terpenes, 12 ketones, 8 alcohols, 3 aldehydes, among others. These volatile compounds have been reported in previous studies related to cocoa products (Counet *et al.*, 2002; Pini *et al.*, 2004; Serra-Bonvehí, 2005; Frauendorfer & Schieberle, 2006; Krings *et al.*, 2006; Ducki *et al.*, 2008; Rodriguez-Campos *et al.*, 2011, 2012; Tran *et al.*, 2015; Di Carro *et al.*, 2015; Sanchez-Reinoso *et al.*, 2017; Ascrizzi *et al.*, 2017).

The chromatographic areas of several constituents grouped according to their chemical nature (Table 2) clearly indicate that the apolar PDMS fiber had the lowest affinity whereas the DVB/CAR/PDMS fiber had the highest affinity for the volatile compounds from cocoa husk. The dual-coated fiber only was overcome by the CAR/PDMS fiber in the extraction of acids and terpenes; however, it had the highest extraction of pyrazines, aldehydes and ketones, which has been related to chocolate and cocoa odours (Counet *et al.*, 2002; Frauendorfer & Schieberle, 2006; Krings *et al.*, 2006). Nevertheless, considering that this study was to develop a procedure based on the use of HS-SPME-GC-O to analyse the volatile compounds from cocoa husk, it is important to evaluate the global odour of the extracts at the sniffing port. The similarity scaling resulted for the four SPME global odours with respect to the reference sample were DVB/CAR/PDMS (1.0 ± 0.3), PDMS/DVB (3.3 ± 0.2), CAR/PDMS (6.2 ± 0.3), and PDMS (8.3 ± 0.4). Therefore, the DVB/CAR/PDMS fiber was selected to characterize the volatile compounds from cocoa husk based on the use of SPME-GC-O.

Selection of temperature and extraction time

The experimental factors extraction time and temperature were evaluated by response surface methodology considering total peak area as the response, which is one of the most useful parameters for the optimisation of the HS-SPME conditions (Table 3). The regression model with coded values for the variables is given by the following equation:

$$Y = 2.42 \times 10^5 + 1.32 \times 10^5 A + 1.29 \times 10^4 B + 4.93 \times 10^3 AB + 7.86 \times 10^4 A^2 + 3.98 \times 10^3 B^2$$

where Y is the total peak area, A is the coded temperature and B is the coded extraction time.

The F-value was highly significant ($p \leq 0.001$) and the lack of fit F-values was not significant, whereas the determination coefficient (R^2) was 0.99. Therefore, the fitted model was suitable to assess the response variables as function of the selected factors.

Both factors were significant and their positive values indicate that by increasing them, the total peak area increased too. Higher extraction temperatures were not considered because Pini *et al.* (2004) found that for temperatures higher than 60 °C, Maillard reactions are likely to occur in cocoa samples. From the 15 predicted solutions, the optimal values for temperature and extraction time were 60 °C and 22 min, respectively, were selected. At this point, the desirability function hits the maximum value of 0.99 and the highest total peak area was obtained. Therefore, the selected conditions were: DVB/CAR/PDMS fiber, 0.2 g ground cocoa husk, 10 min pre-extraction, extraction at 60 °C for 22 min, and GC desorption at 250 °C in splitless mode for 2 min.

Other reported optimum HS-SPME conditions were extraction at 60 °C and 45 min for cocoa mass (de Brito *et al.*, 2002) and cocoa liquors (Pini *et al.*, 2004), 60 °C and 15 min for cocoa and chocolate powders (Ducki *et al.*, 2008), and 60 °C and 30 min for cocoa beans

(Rodriguez-Campos *et al.*, 2012). These differences in extraction time should be explained considering the different type of matrix analysed in each study. Thus, the high fat content in samples like cocoa mass and liquor causes a low release of volatile compounds affecting their extraction (Ducki *et al.*, 2008).

GC-O analysis

With the SPME technique, no physical extract is obtained contrary to other conventional techniques and therefore, the usual AEDA cannot be practical. However, the AEDA approach based of the analysis of sequentially reduced samples from the initial sample could be a valuable tool for ranking aroma-active compounds according to their relative odour potency. The linear recovery of the volatile compounds was confirmed via correlation of the total peak area of the GC analyses of the initial sample and the successively reduced samples with the ratio 1/FD factor. The determination coefficient (R^2) was 0.99, significant at $p \leq 0.001$. No bias was found because the confidence limits of the independent variable included the zero. Likewise, the slope of the line resulted significantly different from zero, and hence there is proportionality of the chromatographic response. Because there were no deviations from the linear trend in total peak areas, it was assumed that this procedure can be applied to assess FD factors of the aroma-active compounds considering the last sample reduction.

Under the HS-SPME optimum conditions the cocoa husk was analysed by the described AEDA (Table 4). The results yielded 21 aroma-active compounds in the FD factor range of 4–128, so they are probably very important to the aroma of cocoa husk. According to their chemical group, the most aroma-active compounds in cocoa husk were 9 pyrazines, 5 acids, 3 Strecker aldehydes (3-methylbutanal, 2-methylbutanal, and phenylacetaldehyde), and one furanone (4-hidroxy-2,5-dimethyl-3(2*H*)-furanone), sulphur-compound (dimethyl trisulfide), terpene (linalool) and ester (2-phenylethyl acetate). Compounds with the highest FD factors (128–64) were acetic acid, 2,3-diethyl-5-methylpyrazine, and 2-ethyl-3,5-dimethylpyrazine followed by 3-methylbutanal, phenylacetaldehyde, 3,5-diethyl-2-methylpyrazine, and 4-hidroxy-2,5-dimethyl-3(2*H*)-furanone. A somewhat lower FD factors of 32–16 were found for 3-methylbutanoic acid, 2-ethyl-5-methylpyrazine, 2,6-dimethylpyrazine, dimethyl trisulfide, and 2,3,5,6-tetramethylpyrazine. A last group, with FD factors of 8–4, was constituted by 2,3,5-trimethylpyrazine, 2-methylbutanal, 2-methylpyrazine, 2-phenylacetic acid, linalool, butanoic acid, 2-methylbutanoic acid, 2,5-dimethylpyrazine, and 2-phenylethyl acetate.

These results confirmed the important role of acids, pyrazines, and Strecker aldehydes in the aroma of cocoa husk. Excepting linalool and acids, the rest of volatile compounds were related to the thermal degradation during roasting of cocoa beans where the Maillard reactions occur (Belitz *et al.*, 2009).

In good agreement with most of the earlier studies in cocoa products (Serra-Bonvehí, 2005; Krings *et al.*, 2006; Frauendorfer & Schieberle, 2006, 2008; Liu *et al.*, 2017) these volatile compounds were found as potent odourants.

Table 1. Volatile compounds identified in cocoa husk by SPME-GC-MS.

Compound	LRI[a]
Acetic acid	645
3-Methylbutanal	654
2-Methylbutanal	658
2,3-Pentanedione	702
Propanoic acid	711
3-Hydroxy-2-butanone	718
3-Methylbutan-1-ol	741
Dimethyl disulfide	744
Ethyl 2-methylpropanoate	751
Pyridine	753
2-Methylpropanoic acid	785
2,3-Butanediol	789
Ethyl 2-hydroxypropanoate	815
Butanoic acid	821
2-Methylpyrazine	826
2-Furfural	836
Ethyl 2-methylbutanoate	851
2-Furfuryl alcohol	854
Ethyl 3-methylbutanoate	859
3-Methylbutyl acetate	881
3-Methylbutanoic acid	883
2-Heptanone	890
2-Methylbutanoic acid	895
n-Nonane	900
Heptanal	905
Pentanoic acid	907
2-Acetylfuran	910
2-Ethylpyridine	913
γ-Butyrolactone	918
2,5-Dimethylpyrazine	919
2,6-Dimethylpyrazine	920
Methyl hexanoate	927
2,3-Dimethylpyrazine	935
α-Pinene	939
2-Methylbutylpyridine	no
Benzaldehyde	960
4-Methylpentanoic acid	965
Dimethyl trisulfide	976
2-Methylbutylpyrazine	977
β-Pinene	979
Phenol	981

Table 1. (continued)

Compound	LRI[a]
Benzonitrile	984
6-Methyl-5-hepten-2-one	986
Methyl 2-hidroxy-4-methylpentanoate	988
Myrcene	990
2-Octanone	991
Methyl 2-hidroxy-3-methylpentanoate	993
1,3,5-Trimethylbenzene	996
2-Ethyl-5-methylpyrazine	999
Hexanoic acid	1002
2,3,5-Trimethylpyrazine	1005
2-Methyl-2-pentenoic acid	1012
1,4-Cineole	1015
2-Pyrrolaldehyde	1018
2-Acetylpyrazine	1023
p-Cymene	1025
Limonene	1028
2-Ethyl-1-hexanol	1030
1,8-Cineole	1031
2-Acetylpyridine	1034
Benzyl alcohol	1032
3-Hidroxy-4,4-dimethyl-dihydrofuran-2-one	1037
Phenylacetaldehyde	1041
3-Ethyl-2-methylpyrazine	1044
1-Ethyl-2-formylpyrrole	1046
4-Hidroxy-2,5-dimethyl-3(2*H*)-furanone	1055
γ-Hexalactone	1059
γ-Terpinene	1060
1-Phenylethanol	1063
Acetophenone	1065
2-Acetylpyrrole	1070
cis-Linalool oxide	1073
2-Acetyl-5-methylpyrrole	1075
2-Ethyl-3,5-dimethylpyrazine	1078
Heptanoic acid	1080
2,3,5,6-Tetramethylpyrazine	1086
p-Cymenene	1091
2-Nonanone	1090
Methyl benzoate	1101
Linalool	1097
2-(2-Methylpropyl)-5-methylhex-2-enal	1103
Nonanal	1105

Table 1. (continued)

Compound	**LRI**[a]
Maltol	1111
2-Phenylethanol	1107
2-Ethylhexanoic acid	1120
Isophorone	1122
1-Phenyl-2-propanone	1124
2-Formyl-5-methylpyrrole	1127
2,3-Dimethyl-5-propylpyrazine	1130
Terpinen-1-ol	1134
Phenylacetonitrile	1142
6,7-Dihydro-5-methyl-5*H*-cyclopentapyrazine	1145
Veratrole	1145
4-Ketoisophorone	1148
cis-β-Terpineol	1150
3,5-Diethyl-2-methylpyrazine	1159
5-Methylundecane	1156
2,3-Diethyl-5-methylpyrazine	1163
Benzoic acid	1170
Methyl phenylacetate	1179
2-(3-Methylbutyl)pyrazine	1180
Octanoic acid	1185
Terpinen-4-ol	1183
Methyl salicylate	1195
α-Terpineol	1189
2-Decanone	1192
Ethyl octanoate	1197
Decanal	1202
2,5-Dimethyl-3-(2-methylpropyl)pyrazine	1208
hexylcyclohexane	1237
Ethyl 2-phenylacetate	1247
Carvone	1243
2-Phenylacetic acid	1252
2-(3-Methylbutyl)-6-methylpyrazine	1262
2-Phenylethy acetate	1268
2-Phenyl-2-butenal	1291
Nonanoic acid	1295
2-Undecanone	1293
2-Methylnaphthalene	1297
2-(2-Methylpropyl)-3,5,6-trimethylpyrazine	1298
Carvacrol	1299
n-Tridecane	1300
Undecanal	1307

Table 1. (continued)

Compound	**LRI**[a]
2,5-Dimethyl-3-(3-methylbutyl)pyrazine	1323
Phenylpropionic acid	1333
Methyl anthranilate	1337
Eugenol	1357
5-Methyltridecane	1360
γ-Nonalactone	1363
Decanoic acid	1386
2,3,5-Trimethyl-6-(3-methylbutyl)-pyrazine	1390
Vanillin	1394
n-Tetradecane	1400
cis-α-Bergamotene	1413
Coumarin	1434
Geranyl acetone	1455
5-Methyl-2-phenyl-2-hexenal	1486
n-Pentadecane	1500
β-Bisabolene	1506
Tridecanal	1510
5-Methylpentadecane	1551
Dodecanoic acid	1568
Ethyl dodecanoate	1595
n-Hexadecane	1600
Benzophenone	1628
Tridecanoic acid	1677
n-Heptadecane	1700
(*Z*)-9-Tetradecenoic acid	1780
Tetradecanoic acid	1784
Ethyl tetradecanoate	1796
n-Octadecane	1800
(*Z,E*)-Farnesyl acetate	1822
Pentadecanoic acid	1868
1-Hexadecanol	1876
Ethyl pentadecanoate	1895
n-Nonadecane	1900
Methyl hexadecanoate	1922
(*Z*)-9-Hexadecenoic acid	1953
Hexadecanoic acid	1960
Ethyl hexadecanoate	1993
n-Eicosane	2000
2-Methylpropyl hexadecanoatoe	2025
Heptadecanoic acid	2053
Manool	2057

Table 1. (continued)

Compound	LRI[a]
Methyl octadecenoate	2125
Oleic acid	2141
Octadecanoic acid	2170
Ethyl oleate	2179
Ethyl octadecanoate	2197

[a] *Linear retention indices in DB-5ms capillary column*.

Table 2. Effect of fiber type on the chromatographic area (×107 area units) of volatile compounds from cocoa husk[a]

Compound	PDMS	PDMS/DVB	CAR/PDMS	DVB/CAR/PDMS
Acids	605 d	13,498 c	116,643 a	106,425 b
Esters	692 b	733 a	324 c	876 a
Pyrazines	146 c	4069 b	12,380 a	13,112 a
Aldehydes & ketones	778 d	5964 c	52,948 b	68,952 a
Alcohols	315 d	23,804 c	66,475 b	119,885 a
Terpenes	22 c	2006 b	5428 a	1870 b
Total	2557 d	50,074 c	254,199 b	311,120 a

[a]*Different letters in the same row indicate significant difference at* $p \leq 0.05$.

Table 3. Matrix of the experimental design for SPME optimization

Run	Temperature (min)	Extraction time (º C)	Total peak area (x 10^7)
1	50	25	253,483
2	60	25	465,232
3	50	20	241,631
4	40	20	189,467
5	50	20	237,345
6	40	25	190,438
7	50	20	242,426
8	60	20	451,340
9	60	15	432,421
10	50	15	222,187
11	50	20	245,576
12	40	15	177,342

Table 4. Odour-active compounds found in coca husk.

Compound	Odour quality	FD[a]
Acetic acid	sour	128
2,3-Diethyl-5-methylpyrazine	nutty	128
2-Ethyl-3,5-dimethylpyrazine	coffee	128
3-Methylbutanal	malty, chocolate	64
Phenylacetaldehyde	honey	64
3,5-Diethyl-2-methylpyrazine	cocoa, chocolate	64
4-Hidroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	64
3-Methylbutanoic acid	rancid, cheese-like	32
2-Ethyl-5-methylpyrazine	cocoa	32
2,6-Dimethylpyrazine	cocoa	16
Dimethyl trisulfide	onion	16
2,3,5,6-Tetramethylpyrazine	coffee	16
2,3,5-Trimethylpyrazine	toasted, cacao	8
2-Methylbutanal	malty, chocolate	8
2-Methylpyrazine	toasted, chocolate	8
2-Phenylacetic acid	honey	8
Linalool	flowery	8
Butanoic acid	butter	4
2-Methylbutanoic acid	sweaty	4
2,5-Dimethylpyrazine	popcorn	4
2-Phenylethyl acetate	honey	4

[a] *Flavour dilution factor*.

CONCLUSIONS

Gas chromatography-olfactometry in combination with headspace solid-phase microextraction using divinylbenzene/Carboxen/polydimethylsiloxane fiber operated at 60 °C for 22 min could isolate most of the volatile compounds from cocoa husk. The identified 21 odourants were originated from wide range of acids, pyrazines, Strecker aldehydes, and one furanone, sulphur compound, terpene, and ester. The combined procedure is a simple, sensitive, reproducible, rapid and low-cost method which may be applied as a quality control tool for industrial processing of cocoa husk.

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