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Investigation of aromatic compounds in roasted cocoa powder

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Abstract Most of the volatile compounds identified are highly significant in determining cocoa powder flavour, and this paper demonstrates that basic sensory perceptions (undesirable, bitter pungent, repulsive, fruity, nutty, floral, vegetal, and sweet chocolate) can be totally explained by aroma compounds with R^2 -adjusted values of 0.85 and greater. Samples from five geographical origins of cocoa bean were characterized by chemical compounds and sensory attributes. The aroma extracts were obtained by a two-step procedure involving (1) preliminary steam distillation under reduced pressure to evaluate the methylpyrazines generated in roasted cocoa powder by spectrophotometry (flavour index), and (2) Likens-Nickerson's simultaneous steam distillation and solvent extraction method with added NaCl. The distilled compounds were separated by adsorption chromatography in six fractions depending on the polarity. A combined total of 114 compounds were detected by gas chromatography/mass spectrometry, 110 of which were identified. About 15 components in the mean milligrams per kilogram range (1.09–4.67 mg kg⁻¹) and 95 components in the mean micrograms per kilogram range (12–980 µg kg⁻¹) were quantified. The major components of cocoa aroma were 2,3,5,6-tetramethylpyrazine, benzaldehyde, 2-phenylacetaldehyde, acetophenone, 3-methylbutyric acid, 5-methyl-2-phenyl-2-hexenal, ethyl phenylacetate, and 3-hydroxy-2-methyl-4-pyrone (mean greater than 1.30 mg kg⁻¹).

Keywords Cocoa powder · Flavour index · Aromatic compounds · Roasting

Introduction

It has been shown that cocoa-specific aroma precursors are generated by enzymatic processes which occur during fermentation of cocoa seeds. During the fermentation, fresh cocoa beans go through complex transformations: (1) the sugars from the mucilaginous pulp of the seeds are rapidly metabolized, producing volatile and nonvolatile organic acids; (2) the degradation of proteins to form peptides and free amino acids; (3) oxidation of polyphenols to form insoluble compounds, mainly *o*-quinones; and (4) hydrolysis of glycosides (mainly anthocyanins) [1–4]. A succession of microorganisms and yeasts contributes towards the development of cocoa flavour [5]. Furthermore, it has been found that the generation of these aroma precursors is dependent on the degree and the time course of the acidification of the cocoa beans and correlates with the liberation of specific hydrophobic free amino acids in the cocoa beans [6]. Roasting is a dry heat treatment which aims at the generation of certain flavours and aroma compounds. The Maillard or nonenzymatic browning reaction mainly involves the reaction of free amino groups of amino acids and reducing sugars generating the aroma compounds of food flavours [7–10]. Cocoa flavour development is influenced by the genetic constitution of the seed, postharvest processing (fermentation and drying) and manufacturing [6, 11]. Sensory quality plays an important role in the overall quality of cocoa powder, and hence in the preference of consumers. One of the major attributes of the Maillard reaction is flavour production. It was established that among these Maillard-type flavours, heterocyclic compounds with derivable aromas and low odour thresholds strongly influence the aroma, with a significant contribution to the aroma of cocoa powder [12–16]. Generally, the roasting process produces (1) the synthesis of heterocyclic aromatic compounds and the possible formation of diketopiperazines (DKPs) [17–20], (2) the effective inhibition of polyphenoloxidase activity [21] and (3) decreasing free acetic acidity, thus eliminating some undesirable volatile compounds of cocoa [22]. Roasted cocoa is one of the

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most aromatic foods, having a complex chemical constitution. Nearly 400 compounds have been isolated, fundamentally, pyrazines, thiazoles, oxazoles, pyrrole derivatives, pyridines and furans [17]. It is very difficult to assess which components are really important and which are not. Not all individual components identified should be regarded as significant aroma compounds, since the aroma impact depends on the odour concentration and odour intensity. Nevertheless, owing to the fact that the sensation intensity (I) and the stimulus intensity (S) are connected, this relationship is best evaluated by using the Stevens slope ($\ln I = n \ln S + K$) [23]. In cocoa, the pyrazines are abundantly available and can be used as tracers for the cocoa flavour. The alkalized roasted cocoa has many fewer volatile compounds than the roasted natural cocoa. Alkalization during roasting is one of several routes which may be taken by the manufacturer to modify the colour of cocoa with different susceptibilities to developing *o*-quinones and the Maillard reaction, especially nonenzymatic brown compounds. Alkalization plays a prominent role in browning, wetability or dispersability, flavour, and nutritional and biological quality of cocoa. The loss in digestibility and nutritional quality due to degradation of essential free amino acids is accompanied by destruction of potential antimutagenic, immunomodulatory and antioxidant activities of polyphenolic compounds, an important factor in the new cocoa quality [10, 24–27]. The analytical determination depends on the extraction process, the aromatic intensity of cocoa and the chromatographic procedure applied. The aromatic extract must be representative of the product, avoiding the formation of artefacts, and suitably concentrated for chromatographic sensitivity. The simultaneous extraction–distillation (SDE) techniques of Likens-Nickerson has frequently been used for cocoa, as well as subsequent extraction by CO₂ in the supercritical state [28]. Gas chromatography (GC)/mass spectrometry (MS) is the instrumental technique used most to identify the compounds of cocoa aroma. In general, there are some hundred more or less volatile individual components which are present in varying concentrations (0.02–10 mg kg⁻¹), their total concentration being only 10–200 mg kg⁻¹. For these reasons it is suitable to determine the order of the concentration of the most characteristic volatile compounds and to relate it to the aroma impact depending on the odour-activity values (concentration divided by odour threshold) and the Stevens slope. Not all individual components are identified as aroma compounds since the aroma characteristics and sensorial threshold concentrations of the components differ over a wide range. In the present work, we have optimized this method, and discovered a quantitative comparison of volatile compounds formed during roasting of cocoa beans. Very good recovery factors are measured for most chemicals when very strict conditions are maintained. With this aim the aroma fractions were isolated, and the principal aroma compounds of fermented, nonalkalized and roasted cocoa powder were identified.

Materials and methods

Samples

A total of 31 samples of fermented, nonalkalized cocoa powder of different geographical origin, unroasted and industrially roasted, were used. The import product was obtained in two different years and collected from Ghana, Cameroon, Ivory Coast, Brazil and Ecuador. The nibs were roasted in batches of 3 tons (the cocoa nib is the kernel of the bean left after removal of the shells). The roaster consisted of a rotting cylinder (5-m long and 2-m diameter) (Barth, Germany) whose envelope was heated by a combustion gas burner, in three basic steps: (1) cocoa nibs were mixed with water in natural cocoa (the term natural is often used in connection with nonalkalized cocoa powder, pH 5.60–5.90) (“natural process”) or with aqueous solution of alkali, plus incorporated air in the case of alkalized cocoa (pH 7.20–7.92) (“Dutch process”), and heated to 85 °C by steam injection into the roaster; (2) progressive heating until the selected temperature (130 °C) was reached; and (3) the selected temperature was maintained for 3 min in the roaster (total roasting process time 48 min). A thermal probe permitted measurement of the internal temperature. Samples were maintained at room temperature and analysed as soon they arrived at the laboratory.

Chemicals

The solvents were of analytical (Panreac, Barcelona, Spain) and GC (Merck, Darmstadt, Germany) grade. The aromatic compounds dimethyldisulfite, trithioacetone, 4-methyl-5-thiazolethanol, 2,3-pentanedione, ethyl valerate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, furfuryl propionate, ethyl decanoate, 2-phenylacetaldehyde, α -terpenyl formate, benzyl acetate, geranyl acetate, methyl phenylacetate, ethyl benzoate, ethyl phenylacetate, catechol monomethyl ether, ethyl laurate, isoamyl benzoate, 2-phenyl propionaldehyde, methyl tetradecanoate, methyl cinnamate, ethyl tetradecanoate, ethyl cinnamate, 4-hydroxybenzaldehyde, methyl palmitate, ethyl palmitate, benzenethiol, methyl stearate, ethyl stearate, furfural, pyrrole, 2-acetylfuran, 5-methylfurfural, 2-acetyl-5-methylfuran, acetophenone, 4-methylacetophenone, 2-hydroxyacetophenone, benzylideneacetone, 4-methyl-2-phenyl-2-pentenal, 5-methyl-2-phenyl-2-hexenal, 3-methylphenol, 2-acetyl-1-methylpyrrole, 4-hydroxy-3-methoxybenzaldehyde, 2-ethylpyrazine, 2,3-diethylpyrazine, 1-heptyl alcohol, 1-octyl alcohol, furfuryl alcohol, methyl salicylate, geraniol, 4-hydroxy-2,5-dimethyl-3-furanone, 2-butyl alcohol, 1-propyl alcohol, 2-pentyl alcohol, pyrazine, 3-methyl-1-butanol, 2-hexyl alcohol, 2-methyl pyrazine, 2-heptyl alcohol, 2,5-dimethylpyrazine (DMP), 2,6-DMP, 2,3-DMP, 1-hexyl alcohol, 5-ethyl-2-methylpyrazine, 2-ethyl-3-methylpyrazine, 2,3,5-trimethylpyrazine (TrMP), propionic acid, 2,3,5,6-tetramethylpyrazine (TMP), methylpropionic acid, 3-methylbutyric acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, 2-furoic acid, benzoic acid, 2-phenylacetic acid, 3-phenylpropionic acid and 3-phenylacrylic acid were obtained from Sigma-Aldrich Co. (Flavors & Fragrances) (Poole, UK), 99+ purity. 4-Ethylpyridine, 2-phenylethanol, pyrrole-2-carboxaldehyde, 3-hydroxy-2-methyl-4-pyrone, 3-hydroxy-2-methylpyridine, 1-pentyl alcohol and 2-methyl butyric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA), 99+ purity. Benzaldehyde, acetic acid, butyric acid, 5-(hydroxymethyl)furfural, benzonitrile, 2-methylphenol, 2-acetylpyrrole and 1-phenylethanol were obtained from Merck, 99+ purity. 2,3-Dihydrofuran and 2-methyl-1-propanol were purchased from Fluka Chemika (Buchs, Switzerland), 99+ purity. 2-Phenylacetamide was obtained from Acros (Geel, Belgium), 99+ purity.

Isolation procedure

The method used to determine aromatic compounds was a modified version of that proposed by Ziegler [18]. We optimized the

isolation of cocoa powder aroma compounds by preliminary steam distillation under reduced pressure in order to determine the flavour index (FI, step 1). The solution obtained was then extracted with pentane/diethyl ether (2:1 v/v) using the SDE method of Likens-Nickerson with added NaCl (step 2).

Steam distillation (step 1)

Suspension of 20 g cocoa powder in 100 ml cold ultrapure water (Milli-Q, Millipore Corp., Bedford, MA, USA) (resistance $18 \text{ m}\Omega \text{ cm}^{-2}$) was steam distilled, and 100 μl of a $1,000 \text{ mg kg}^{-1}$ solution of 4-ethylpyridine in methylene chloride was added (internal standard) [17]. The condenser was maintained at $-5 \text{ }^\circ\text{C}$ and 150 ml of the fractional distillate was collected in 40 min [29].

Flavour index

The solution obtained by steam distillation was filtered and the optical density was measured in a 10-mm quartz cell using a spectrophotometer. The extinction value obtained from the UV spectra (210–290 nm) at 278 nm (maximum absorbance) was multiplied by the collected final volume to determine the value of the FI [10].

Simultaneous SDE (step 2)

The solution obtained by steam distillation was placed in flask A of the Likens-Nickerson distillator with 10 g NaCl. In flask B, 60 ml of a mixture of pentane/diethyl ether (2:1 v/v) was introduced. Boiling chips were added to flasks A and B. Flask B was heated in an oil bath at $55 \text{ }^\circ\text{C}$ and flask A in a balloon heater. The vapours were condensed by means of a cold finger maintained at $-5 \text{ }^\circ\text{C}$ by a cryostat. After 90 min of extraction, about 55 ml of solvent, containing the aroma compounds, was collected.

Concentration

The extract was dried over 5 g anhydrous Na_2SO_4 and concentrated to about 1 ml in a Kuderna–Danish evaporator.

Adsorption chromatography on silica gel

The aroma concentrate was separated by adsorption chromatography on 3 g of silica gel 60 (activity II-III, Merck) using a column of 200×0.9 -mm inner diameter, and six fractions (40 ml each) with solvents of increasing polarity were eluted with (1) pentane, (2) pentane/methylene chloride (4:1), (3) pentane/methylene chloride (1:1); (4) pentane/methylene chloride (1:2), (5) pentane/ethyl ether (1:1), and (VI) ethyl ether. The extract was then concentrated to 0.5–1 ml in a Kuderna–Danish evaporator.

GC analytical conditions

GC was performed with a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a HP 7673 automatic sampler and a flame ionization detector (FID). The volatiles were transferred onto a 50 m fused silica column (0.32-mm inner diameter, 0.2- μm film thickness) coated with OV-351 (Supelco, Bellefonte, PA, USA). The GC conditions were as follows: 2 μl splitless injection (1.25 s valve delay); oven programmed from 60 to $220 \text{ }^\circ\text{C}$ at a rate of $5 \text{ }^\circ\text{C min}^{-1}$; $240 \text{ }^\circ\text{C}$ injector temperature; $250 \text{ }^\circ\text{C}$ detector temperature; helium carrier gas velocity $20\text{--}25 \text{ cm s}^{-1}$ [10].

Calibration factors

Solutions of the pyrazines and short-chain volatile acids (0.1% p/v) in methylene chloride were prepared: pyrazine; 2,5-DMP; 2,3,5-TrMP; 2,3,4,5-TMP; propionic acid; 2-methylpropionic acid; butyric acid; 3-methylbutyric acid; pentanoic acid; and 4-ethylpyridine (internal standard). By adding 0.50 ml of each solution in a volumetric flask of 100 ml and diluting, 5 mg kg^{-1} solutions were obtained. High-resolution GC was performed.

Calibration for quantitative analysis

Relative response coefficients for the various volatile constituents were determined by adding standard amounts (four points, in duplicates) of pure compounds to a cocoa sample prior to analysis. Retention times and areas of selected peaks, including the internal standard, were used for the statistical analysis.

Gas chromatography/mass spectrometry

Identification and confirmation of aromatic compounds were achieved by GC/MS. The column was directly connected to an HP G1530 quadrupole mass spectrometer. The chromatographic conditions were the same as those used for flame ionization detection. Electron impact MS was performed at 70 eV (filament current 300 mA, electron multiplier voltage 1.7 kV, preamplifier sensitivity 10^{-7} AV^{-1}) with a source temperature of $270 \text{ }^\circ\text{C}$ and a scan range of 30–600 mass units at 13 scans s^{-1} . Spectral recording throughout elution was automatically performed with an HP 59970C MS Chemstation analytical workstation (Agilent Technologies, Waldronn, Germany). Identification was done on the basis of peak enrichment by co-injection with authentic standard compounds and comparison with the NBS/EPA/NIH mass spectra library. Identification of the volatile compounds was based on the comparison of mass spectra of unknown compounds against library data for GC/MS and the comparison of experimental and theoretical Kováts indices. The Kováts system is applied mainly in isothermal analysis. However, Van der Dool and Kratz [30] demonstrated that the Kováts system could also be applied for normal linear temperature programmed GC by simplifying the equation. Identification was considered tentative when it was based only on spectra data. The retention indices were calculated for both mixture components and reference compounds when they were available. A mixture of the aroma concentrate or reference compound and an *n*-alkane mixture (C8–C26) was injected into the gas chromatograph. An average of five replicates was taken for the calculation of retention indices, using the unadjusted retention times for both components and *n*-alkanes. The inclusion of Kováts indices in the analysis of volatiles is easily justified, because in GC/MS experiments, retention times and MS fragments are acquired simultaneously and because Kováts indices are the most widely used in expressing relative GC retention times using internal standards.

Sensory analysis

Sensory evaluations were performed in a testing room equipped with computerized booths. For the sensory analysis 12 g cocoa powder was mixed with 15 g sucrose in a 400-ml breaker. Then 300 ml water at $55 \text{ }^\circ\text{C}$ was added and the mixture was stirred into a homogeneous suspension [32]. The quantification of each attribute intensity was evaluated in comparison with the perceived intensity of the corresponding reference solution adjusted to a given concentration (Table 1). The sensory analysis was carried out under the conditions of the International Standard [33]. The samples were directly analysed by ten experts (five males and five females) to estimate the olfactory-gustatory intensity of cocoa powder in aqueous solution. Samples (20 ml) were presented in brown flasks (60 ml) closed with screw caps. Their odour intensities were evaluated after 1 h of equilibration at $21 \text{ }^\circ\text{C}$. The acceptability was

Table 1 Nature and concentration of reference solutions for each attribute

| Attribute | Reference compound | Concentration in pure water |
|------------|------------------------|-----------------------------|
| Sour | Lactic acid | 1.38 g l ⁻¹ |
| Bitter | L-Leucine | 8 g l ⁻¹ |
| Salty | Sodium chloride | 4.5 g l ⁻¹ |
| Umami | L-Monosodium glutamate | 0.6 g l ⁻¹ |
| Sweet | D-Lactose | 23.75 g l ⁻¹ |
| Astringent | Potassium alum | 0.33 g l ⁻¹ |
| Sharp | Capsaicin | 0.15 mg l ⁻¹ |

tested using an ordinal scale [scale, structured; scores, 1–5 (1 is very bad; 2 is quite bad; 3 is average; 4 is quite good; 5 is very good)].

GC sniffing

GC was performed as described earlier, the odour-active regions of the elute were evaluated, and the aroma scores of these regions were assigned by four assessors who were habitual consumers of cocoa. The assessors used a free profile to describe the odour of volatiles. Each sample was evaluated in duplicate and the results were averaged.

Odour threshold determination

Odour thresholds were determined in water following the general methods described previously [34]. The water used was distilled, and was boiled to remove about 10% of it to carry away any volatiles. Probit analysis was used to determine the threshold points (taken where 75% of the panelists gave the correct judgment) and also to determine the 95% confidence intervals.

Statistical analysis

The data were processed with the SAS system, release 6.12 (SAS Institute, Cary, NC, USA). Analysis of variance was performed at the $\alpha=0.05$ level, according to the model attribute=product+subject+product \times subject, with subject considered as a random effect. Means were compared with the Newman–Keuls multiple comparison test (Student *t* test): [$Q = (\bar{x}_{\max} - \bar{x}_{\min})/s_e$]. The CAP SAS macro was used to assess the panelists' performances [31]. To quantify the relative impact of each compound (volatile compounds) on each attribute (sensory attributes, perception), stepwise multiple linear regressions were performed with proc REG with the stepwise option to select the variables. Linear discriminant analysis was used to determine which variables discriminate between two or more naturally occurring groups. This mathematical procedure maximizes the variance between groups and minimizes the variance within each group in such a way that outsiders can be detected more easily than by principal component analysis.

Results and discussion

In this work, the combined concentrates of aroma compounds were subjected to GC/FID and GC/MS analysis to obtain the typical composition of aroma flavour components of natural roasted cocoas. The information from each sample consisting of both gas chromatographic and sensory data was checked for skewness, and the study of outliers was made by the Mahalanobis distance, evaluated

as χ^2 . Table 2 shows there were basically eight groups of sensory perception (undesirable, bitter pungent, repulsive, fruity, nutty, floral, vegetal, sweet chocolate), the sensory attribute of the volatiles, and the Kováts index. The total R^2 -adjusted values attest that 85% or more of this taste property is explained. Sweet chocolate, bitter pungent, fruity, floral, and vegetal were the most remarkable perceptions in powder cocoa. Generally, the low molecular weight aroma compounds were not detected in most samples or were present in negligible amounts. These components might have volatilized from the samples before analysis. They are also known to be affected by thermodegradation. Splitless injection can favour the formation of thermally induced artefacts. However, these compounds [e.g. methyl furoate, methyl 3-hydroxyphenylacetate and methyl 2-(4-methoxyphenylacetate)] were not detected in the chromatograms and thus did not influence the overall cocoa powder aroma. Many authors have assumed that the use of high-efficiency capillary columns reduces or avoids the possibility of coeluted components. However, the complexity of an aroma mixture makes this almost inevitable. Powder cocoa aroma was primarily composed of low molecular weight alcohols, aldehydes, ketones, heterocyclic compounds, esters, hydrocarbons, and sulfur compounds, which are typical of the flavour profile of cocoa. Different alcohols were detected in the gas chromatogram of cocoa powder volatiles. The aliphatic hydrocarbons were not important for aroma, though they may act as precursors of a number of other aroma compounds. The main compounds identified were pyrazines, aldehydes, phenols, pyrrole derivatives and furans, in agreement with Ziegler [18]. Heterocyclic aroma constituents represent the greatest amount of the cocoa aroma complex. The pyrones and furans are formed at moderate temperatures and relatively high water content; most pyrazines are typical roasting products and are generated only from well-dried cocoa. Pyrones and fura-neol are destroyed during the alkalization process. The short-chain aliphatic aldehydes and ketones cannot be quantitatively determined using SDE, because their volatility (e.g. hexanal and heptanal) does not permit the identification of all the trace aroma components. Optimization of the SDE method was carried out using different pyrazine, acid and aldehyde standards, the percentage analyte recoveries were determined by varying the parameters of interest. Different recovery results of some typical compounds are shown in Table 3, where it can be appreciated that the average was up to 85% for all the compounds with a 90-min extraction time and a temperature of the cold finger below -5 °C, and repeatability below 10%. The other aroma compounds identified were quantified with 4-ethylpyridine as an internal standard. The six aroma fractions were analysed and the components grouped for polarities: LSC I (alicyclic and aromatic hydrocarbons); LSC II (aliphatic sulfur compounds, aliphatic ketones and aromatic esters); LSC III (aliphatic and aromatic esters, aldehydes and diketones); LSC IV (furan derivatives, phenyl alkenals and aromatic ketones); LSC V (alcohols, alkylypyrazines

Table 2 Sensory attributes evaluated in cocoa powder samples

| Perception | Sensory attribute | Volatile compound (IK) | |
|-------------|-------------------|--|---|
| Undesirable | Winelike | 1,056, 1,224, 1,324 | |
| | Brandy | 1,224, 1,324, 1,622 | |
| | Fatty | 1,520, 1,752, 1,990, 2,013, 2,496 | |
| | Waxy | 1,520, 2,025, 2,226, 2,425 | |
| | Oily | 1,285, 2,013, 2,386 | |
| | Coffee | 1,304, 1,438, 1,475 | |
| | Caramel | 1,318, 1,540, 1,727 | |
| | Cooked sugar | 1,631 | |
| | Malt | 1,961 | |
| | Roasted | 2,239, 2,271 | |
| | Bread | 2,070 | |
| | Medicinal | 1,000, 1,815, 2,031, 2,037 | |
| | Earthy | 1,285, 1,350 | |
| | Strong | 1,434, 1,571, 1,750 | |
| | Cheesy | 1,625, 1,638, 1,658 | |
| | Unpleasant | 2,013 | |
| | Heavy | 2,037 | |
| | Alcohol | 1,005 | |
| | Licorice | 2,070 | |
| | Creamy | 1,894 | |
| | Sulfurous | 1,036, 1,350 | |
| | Soapy | 2,025 | |
| | Camphor | 1,350 | |
| | Beefy | 2,233 | |
| | Bitter pungent | Bitter | 1,042, 1,163, 1,493 |
| | | Pungent | 1,129, 1,220, 1,434, 1,480, 1,663, 1,823, 1,894 |
| | Repulsive | Bitter almond | 1,727 |
| Wizened | | 1,350, 1,439, 2,140, 2,205 | |
| | Penetrating | 2,252 | |
| | Musty | 1,314, 1,959, 2,496 | |
| | Rancid | 1,480, 1,625, 1,638, 1,725, 1,823, 1,887 | |
| | Rancid butter | 1,539 | |
| | Faecal | 1,638, 1,725 | |
| | Putrid | 1,725 | |
| | Meaty | 1,431 | |
| | Hammy | 1,539, 1,638, 1,658 | |
| | Sweaty | 1,658, 1,725, 1,887 | |
| | Smoky | 1,815 | |
| | Sickening | 1,823 | |
| | Sour | 1,823, 1,887 | |
| | Urine-like | 2,310 | |
| | Phenolic-odour | 1,663, 1,959, 2,650 | |
| | Sharp | 1,520, 1,625 | |
| | Odourless | 2,290 | |
| | Fruity | Fruity | 1,125, 1,195, 1,224, 1,320, 1,426, 1,750, 1,752, 1,825, 1,832, 2,022, 2,125 |
| | | Apple | 1,125, 1,224 |
| | | Banana | 1,224 |
| Pineapple | | 1,426 | |
| Berry | | 1,624 | |
| Pear | | 1,622 | |
| Grape | | 1,622 | |
| Citrus | | 1,450, 1,520, 1,688 | |
| Strawberry | | 2,020, 2,022 | |
| Lemon | | 1,450 | |
| Ripe fruit | | 1,220 | |
| Nutty | | Nutty | 1,251, 1,304, 1,314, 1,373, 1,420, 1,431, 1,460, 1,571, 1,624, 2,233 |
| | | Almond | 1,445, 1,583, 1,609, 1,663 |
| | | Hazelnut | 1,431 |
| | | Roasted nuts | 1,251, 1,297, 1,377, 1,961 |
| | Burnt | 1,815 | |
| | Peanut butter | 1,314 | |
| | Peanut | 1,377 | |
| | Walnut | 2,070 | |
| | Hot sugar | 2,022 | |

Table 2 (continued)

| Perception | Sensory attribute | Volatile compound (IK) | |
|-----------------|-------------------|---|----------------------------|
| Floral | Floral | 1,426, 1,450, 1,578, 1,609, 1,688, 1,695, 1,696, 1,712, 1,750, 1,752, 1,773, 1,825, 1,832, 1,862, 1,894, 1,930, 2,254, 2,830 | |
| | Jasmine | 1,696, 1,748 | |
| | Rose | 1,743, 1,773, 1,832, 1,862, 2,382 | |
| | Lavender | 1,743 | |
| | Honey | 1,748, 1,773, 1,775, 1,862, 2,125, 2,254, 2,830 | |
| | Balsam | 1,475, 1,895, 2,020, 2,125 | |
| | Refreshing | 1,450 | |
| | Faint balsamic | 2,310 | |
| | Essences | 2,486 | |
| | Fragrant | 1,445, 1,773, 1,862 | |
| | Heavy floral | 1,762 | |
| | Wax flowers | 2,496 | |
| | Vegetal | Herbaceous | 1,195, 1,320, 1,688, 1,762 |
| | | Green | 1,195, 1,304, 1,320, 1,695 |
| | | Mild green | 1,100, 2,226 |
| Woody | | 1,445, 1,815, 2,031 | |
| Spicy | | 1,578, 1,663 | |
| Cereal | | 1,431 | |
| Raw potato | | 1,373 | |
| Sweet chocolate | Cocoa | 1,251, 1,297, 1,318, 1,377, 1,438, 1,475, 1,932, 1,968 | |
| | Chocolate | 1,163, 1,251, 1,438, 2,594 | |
| | Vanilla | 2,594 | |
| | Sweet | 1,129, 1,420, 1,445, 1,460, 1,475, 1,540, 1,609, 1,727, 1,743, 1,748, 1,762, 1,775, 1,832, 1,894, 1,895, 2,022, 2,254, 2,382, 2,594 | |
| | Sweet candy | 1,005 | |

IK Kováts retention index

Table 3 Recovery factors (mean \times 100/initial concentration, percent) and standard deviations obtained by increasing steam distillation–solvent extraction

| Compound | Recuperability | Repeatability |
|-----------------------------|----------------|---------------|
| Pyrazine | 46 | 4.6 |
| 2,5-Dimethylpyrazine | 87 | 1.1 |
| 2,3,5-Trimethylpyrazine | 92 | 1.7 |
| 2,3,5,6-Tetramethylpyrazine | 93 | 1.8 |
| Propionic acid | 89 | 1.7 |
| 2-Methylpropionic | 99 | 2.1 |
| Butyric acid | 91 | 2.7 |
| 3-Methylbutyric acid | 98 | 1.6 |
| Pentanoic acid | 98 | 2.4 |
| Benzaldehyde | 94 | 1.9 |
| Phenylacetaldehyde | 89 | 1.7 |
| Acetaldehyde | 86 | 1.4 |

and furan derivatives); and LSC VI (alkylpyrazines and short-chain volatile acids). Figure 1 shows a representative chromatogram (GC/FID) of the steam-volatile cocoa aroma of the volatile compounds in the natural roasted cocoa powder. Among the six fractions, the LSC III, LSC IV, LSC V and LSC VI fractions had the compounds that contributed to the cocoa sensorial quality to the greatest extent. Tables 4, 5, 6, 7 and 8 present the minimum, maximum, and mean concentration of the aroma compounds detected in the cocoa samples. In these tables, the chromatographic results, expressed as concentration (milligrams per kilogram) calculated by response factor, are reported as a function of retention times and Kováts indices. A combined total of 114 compounds were detected by GC, 110 of which were identified by their mass spectra, where possible in combination with their retention indices and co-chromatography with respective quality-indicative standards. The calculated standard de-

Table 4 Compounds identified in fraction LSC II

| Sensorial attribute | Odour threshold ($\mu\text{g l}^{-1}$) | IK | Compound | RRT | Mean concentration (mg kg^{-1}) | Odour value |
|-------------------------------------|--|-------|-------------------------------|------------------|--|-------------|
| Sulfurous | 0.30 | 1,036 | 3. Dimethyldisulfite | 0.59 \pm 0.024 | 0–0.096 (0.044) | 146.7 |
| | | 1,386 | 4-Ethylpyridine (IS) | 1 | | |
| | | 1,350 | 21. Trithioacetone | 1.02 \pm 0.027 | | |
| Sulfurous, earthy, camphor, wizened | | 1,558 | 40. 2,3-Dihydrofuran | 1.53 \pm 0.024 | 0–0.075 (0.032) | |
| | | 2,233 | 92. 4-Methyl-5-thiazolethanol | 2.75 \pm 0.032 | | |
| Beefy, nutty | 10,800 | | | | | 0.0035 |

The odour value is the concentration divided by the odour threshold.
RRT retention time

Table 5 Compounds identified in fraction LSC III

| Sensorial attribute | Odour threshold ($\mu\text{g l}^{-1}$) | IK | Compound | RRT | Mean concentration (mg kg^{-1}) | Odour value |
|---|--|-------|--|------------|--|-------------|
| Bitter | 20 | 1,042 | 4. 2,3-Pentanedione | 0.61±0.010 | 0–0.096 (0.057) | 2.85 |
| Fruity, apple | 1.5 | 1,125 | 7. Ethyl valerate | 0.69±0.013 | 0–0.093 (0.067) | 44.67 |
| Fruity, apple, banana, winelike, brandy | 1.9 | 1,224 | 12. Ethyl hexanoate | 0.76±0.017 | 0–0.084 (0.062) | 32.63 |
| Winelike, brandy | 2.2 | 1,324 | 20. Ethyl heptanoate | 0.95±0.015 | 0–0.088 (0.059) | 26.82 |
| Fruity, floral, pineapple | | 1,386 | 4-Ethylpyridine (IS) | 1 | | |
| Bitter | 350 | 1,426 | 26. Ethyl octanoate | 1.19±0.016 | 0–0.079 (0.053) | |
| Spicy, floral | | 1,493 | 36. Benzaldehyde | 1.38±0.022 | 0.5–1.89 (1.38) | 3.94 |
| Almond | | 1,578 | 42. Furfuryl propionate | 1.60±0.027 | 0–0.082 (0.027) | |
| Pear, grape, brandy | 19 | 1,583 | 43. Benzointrile | 1.61±0.013 | 0–0.079 (0.028) | |
| Berry, nutty | 4 | 1,622 | 45. Ethyl decanoate | 1.71±0.014 | 0–0.45 (0.031) | 1.63 |
| Pungent, phenolic-odor, spicy, almond | | 1,624 | 46. 2-Phenylacetaldehyde | 1.72±0.025 | 2–8.90 (4.67) | 116.75 |
| Herbaceous, citrus | | 1,663 | 51. 2-Hydroxybenzaldehyde | 1.80±0.018 | 0–0.097 (0.060) | |
| Floral, jasmine | | 1,688 | 52. α -Terpenyl formate | 1.82±0.010 | 0–0.38 (0.16) | |
| Rose, lavender, sweet | 9 | 1,696 | 54. Benzyl acetate | 1.84±0.013 | 0–0.033 (0.029) | |
| Sweet, honey, jasmine | 60 | 1,743 | 58. Geranyl acetate | 1.91±0.011 | 0–0.041 (0.030) | 3.33 |
| Fatty, floral, fruity | | 1,748 | 59. Methyl phenylacetate | 1.92±0.032 | 0–0.047 (0.038) | 0.35 |
| Sweet, honey | 650 | 1,752 | 61. Ethyl benzoate | 1.92±0.016 | 0–0.029 (0.021) | |
| Smoky, medicinal, woody, burnt | 10 | 1,775 | 64. Ethyl phenylacetate | 1.97±0.024 | 0.35–1.87 (1.33) | 2.05 |
| Fruity, floral | 19 | 1,815 | 65. Catechol monomethyl ether | 2.07±0.021 | 0–0.084 (0.047) | 4.70 |
| Balsam, sweet | | 1,825 | 67. Ethyl laurate | 2.10±0.020 | 0.16–0.38 (0.24) | 12.63 |
| Floral | | 1,895 | 72. Isoamyl benzoate | 2.18±0.019 | 0.12–0.45 (0.34) | |
| Fatty | | 1,930 | 73. 2-Phenyl propionaldehyde | 2.22±0.018 | 0–0.088 (0.031) | |
| Balsamic, strawberry | | 1,990 | 79. Methyl tetradecanoate | 2.30±0.020 | 0.56–1.78 (1.22) | |
| Waxy, soapy | | 2,020 | 81. Methyl cinnamate | 2.34±0.021 | 0–0.096 (0.067) | |
| Fruity, balsam, honey | 67 | 2,025 | 83. Ethyl tetradecanoate | 2.35±0.010 | 0.12–0.45 (0.28) | |
| Waxy, mild green | 2,000 | 2,125 | 87. Ethyl cinnamate | 2.54±0.013 | 0–0.088 (0.071) | 1.06 |
| Penetrating | 13,500 | 2,190 | 89. Methyl palmitate | 2.67±0.010 | 0–0.058 (0.045) | |
| Oily | 200 | 2,226 | 91. Ethyl palmitate | 2.73±0.012 | 0–0.039 (0.026) | 0.013 |
| Waxy | | 2,252 | 95. Benzenethiol | 2.79±0.020 | 0.17–0.34 (0.25) | 0.0185 |
| | | 2,386 | 101. Methyl stearate | 3.09±0.023 | 0–0.021 (0.014) | 0.027 |
| | | 2,425 | 102. Ethyl stearate | 3.18±0.026 | 0–0.045 (0.033) | |
| | | 2,445 | 103. Ethyl <i>cis</i> -9-octadecenoate | 3.20±0.024 | 0.15–1.76 (0.98) | |
| | | 2,560 | 106. Ethyl 9,12-octadecadienoate | 3.44±0.034 | 0.08–0.35 (0.18) | |

Detection thresholds in aqueous solution (micrograms per litre)

viation for the retention indices was less than 0.5%. The retention times for the aroma components agreed with the retention indices of available reference compounds. Where reference compounds were not available, the retention indices were compared with values reported in the literature for polar stationary phases. Some retention indices were not found in the literature and comparisons could not be performed. A large increase in methylpyrazines was detected and quantified as the FI in the preliminary steam distillation (step 1) by spectrophotometry at 278 nm. The concentration of all methylpyrazines varied with the temperature and the time of roasting. In the majority of samples the FI values generated were acceptable (FI>45). The optimal FI was detected for roasting at 130 °C for 16 min (heating-up period to reach 130 °C, 48 min) and at 140 °C for 10 min (heating-up period to reach 140 °C, 58 min) with addition of reducing sugars [20]. During cocoa roasting, the contents of some compounds related to DKPs (or cyclic dipeptides) can be increased, thus having an influence on the sensorial quality of cocoa. Our results show a minor DKP content when cocoa was roasted at 130 °C.

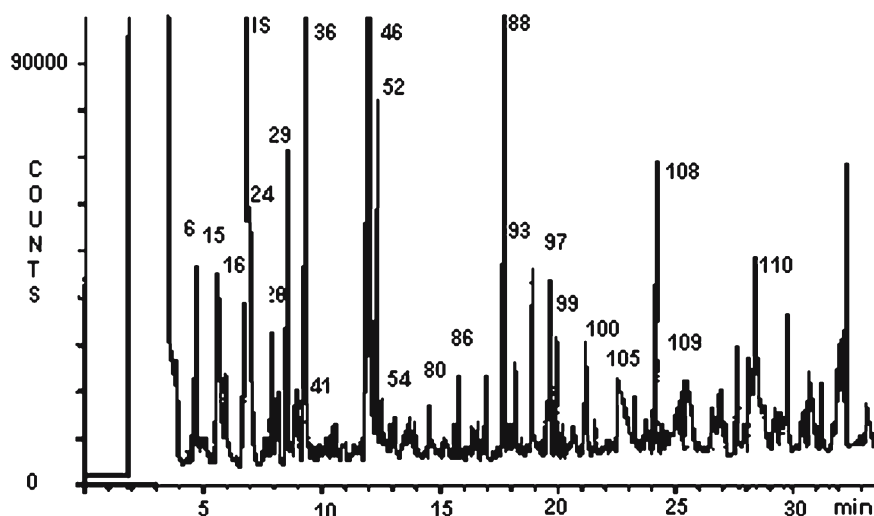
In the LSC I fraction a minimum concentration (less than 0.1 mg kg⁻¹) of alkanes (C10–C20) was identified in accordance with Hoskin and Dimick [35]. In the LSC II fraction dimethyldisulfite, trithioacetone, and 4-methyl-5-thiazolethanol predominated. A total of 19 esters were identified in large quantities in the LSC III fraction. The compounds benzaldehyde, 2-phenylacetaldehyde, α -terpenyl formate, ethyl phenylacetate, ethyl *cis*-9-octadecenoate and ethyl 9,12-octadecadienoate were identified in different concentration ranges (Table 5). Aldehydes and esters represented 20–25% of the aroma compounds, phenylacetaldehyde, benzaldehyde, ethyl phenylacetate and methyl tetradecanoate among them, distributed in the LSC II and LSC III fractions (Tables 4, 5). In the LSC IV fraction, some compounds were identified that give a intense bitter taste to cocoa, such as 4-methyl-2-phenyl-2-pentenal and 5-methyl-2-phenyl-2-hexenal. Most of the heterocyclic compounds identified have high volatility, and were detected in amounts between 0 and 0.5 mg kg⁻¹, and only for some compounds between 0.5 and 2.5 mg kg⁻¹ (Table 6). Among them, acetophenone and the benzylineacetone predominated. LSC V fraction re-

Table 6 Compounds identified in fraction LSC IV

| Sensorial attribute | Odour threshold ($\mu\text{g l}^{-1}$) | IK | Compound | RRT | Mean concentration (mg kg^{-1}) | Odour value |
|---|--|-------|----------------------------------|------------|--|-------------|
| Sweet, woody, fragrant, almond | 3,000 | 1,386 | 4-Ethylpyridine (IS) | 1 | 0.12–1.45 (0.87) | 0.29 |
| | | 1,445 | 31. Furfural | 1.27±0.027 | | |
| Nutty, sweet | 20,000 | 1,460 | 33. Pyrrole | 1.39±0.025 | 0–0.078 (0.025) | 0.0013 |
| Sweet, balsamic, cocoa, slightly coffee | 10,000 | 1,475 | 34. 2-Acetylfuran | 1.35±0.028 | 0–0.071 (0.021) | 0.002 |
| Sweet, caramel | | 1,540 | 39. 5-Methylfurfural | 1.49±0.030 | 0.016–0.32 (0.16) | |
| Strong, nutty | | 1,571 | 41. 2-Acetyl-5-methylfuran | 1.59±0.011 | 0–0.035 (0.016) | |
| Floral, almond, sweet | 65 | 1,609 | 44. Acetophenone | 1.67±0.024 | 0.64–3.82 (2.25) | 34.62 |
| Fruity, floral, strong | 0.027 | 1,750 | 60. 4-Methyl acetophenone | 1.92±0.027 | 0–0.078 (0.044) | 629.6 |
| Heavy floral, herbaceous, sweet | | 1,762 | 62. 2-Hydroxy acetophenone | 1.94±0.024 | 0.045–0.41 (0.29) | |
| Sweet, floral, pungent, creamy | | 1,894 | 71. Benzylideneacetone | 2.18±0.027 | 0.22–1.68 (1.12) | |
| Cocoa | | 1,932 | 74. 4-Methyl-2-phenyl-2-pentenal | 2.22±0.021 | 0.025–0.31 (0.18) | |
| Musty, phenolic-odor | | 1,959 | 75. 2-Methylphenol | 2.25±0.022 | 0.022–0.18(0.092) | |
| Cocoa | | 1,968 | 78. 5-Methyl-2-phenyl-2-hexenal | 2.28±0.028 | 0.52–1.94 (1.38) | |
| Medicinal, woody | | 2,031 | 84. 3-Methylphenol | 2.36±0.024 | 0.031–0.21 (0.12) | |
| Medicinal, heavy | | 2,037 | 85. 4-Methylphenol | 2.37±0.022 | 0.026–0.23 (0.089) | |
| Bread, walnut, licorice | 170,000 | 2,070 | 86. 2-Acetylpyrrole | 2.44±0.028 | 0.021–0.38 (0.16) | 0.0009 |
| | | 2,248 | 94. 2-Acetyl-1-methylpyrrole | 2.78±0.025 | 0.019–0.31 (0.12) | |
| Fatty, musty, wax flowers | | 2,496 | 105. 5-Hydroxymethylfurfural | 3.30±0.025 | 0.17–0.47 (0.29) | |
| Chocolate, sweet, vanilla | 20 | 2,594 | 108. Vanillin ^a | 3.51±0.030 | 0.23–0.44 (0.31) | 15.5 |

^a 3-Methoxy-4-hydroxybenzaldehyde

Fig. 1 Gas chromatography/flame ionization detection chromatogram of natural cocoa powder aroma



vealed the presence of 32 components, of which one could be identified as pyrazines (Table 7). The pyrazines are generally considered very important compounds of roasted cocoa; some are specific (e.g. 2,5-dimethylpyrazine). Furthermore, the compounds identified in this fraction, presented the main sensorial attributes of roasted cocoa, including linalool, which is responsible for the typical floral smell [36]. In addition to 3-hydroxy-2-methyl-4-pyrone (maltol), relatively high amounts of 1-phenylethanol and pyrazine were also quantified. The pyrazine-related compound comprised just over 40% of the cocoa powder essence, and also taking into account their aroma properties. These compounds could therefore

be considered to be particularly important contributors to characteristic roasted cocoa powder, especially DMP and TrMP (Table 8). Excluding TMP, which is biosynthesized during cocoa fermentation, the rest of the pyrazines originated from Maillard reactions during roasting. The concentrations of all of them varied with the temperature and the time of roasting treatment. These specific components were measured with the FI. The values obtained in the different samples analysed are conceivable with the range that best defines the roasting process [20]. Furthermore, the concentrations of certain pyrazines present in the cocoa beans, in particular, DMP and TMP, were found to be largely proportional to the pod storage period.

Table 7 Identification compounds fraction LSC V

| Sensorial attribute | Odour threshold ($\mu\text{g l}^{-1}$) | IK | Compound | RRT | Mean concentration (mg kg^{-1}) | Odour value |
|--------------------------------------|--|-------|---|------------|--|-------------|
| Medicinal | 500 | 1,000 | 1. 2-Butyl alcohol | 0.56±0.008 | 0–0.087 (0.021) | 0.042 |
| Sweet candy, alcohol | 9,000 | 1,005 | 2. 1-Propyl alcohol | 0.57±0.010 | 0–0.078 (0.032) | 0.0036 |
| Winelike | 1,800 | 1,056 | 5. 2-Methyl 1-propanol | 0.62±0.009 | 0–0.069 (0.045) | 0.025 |
| Mild green | 4,000 | 1,100 | 6. 2-Pentyl alcohol | 0.67±0.010 | 0–0.089 (0.065) | 0.016 |
| Bitter, chocolate | 300 | 1,163 | 9. 3-Methyl-1- butanol | 0.72±0.012 | 0.02–0.45 (0.27) | 0.9 |
| Fruity, green, herbaceous | 10 | 1,195 | 10. 2-Hexyl alcohol | 0.74±0.009 | 0–0.089 (0.054) | 5.4 |
| Pungent, ripe fruit | 4,000 | 1,220 | 11. 1-Pentyl alcohol | 0.76±0.010 | 0–0.076 (0.045) | 0.011 |
| Earthy, oily | 3 | 1,285 | 14. 2-Heptyl alcohol | 0.84±0.012 | 0.12–0.38 (0.23) | 76.67 |
| Fruty, green, herbaceous | 2,500 | 1,320 | 19.1-Hexyl alcohol | 0.93±0.009 | 0–0.091 (0.041) | 0.016 |
| | | 1,386 | 4-Ethylpyridine (IS) | 1 | | |
| Sweet, nutty | 6 | 1,420 | 25. Linalool oxide (<i>cis</i> -furanoid) | 1.18±0.020 | 0.01–0.36 (0.21) | 35 |
| Nutty, hazelnut, cereal, meaty | | 1,431 | 27. 2,3-Diethylpyrazine | 1.21±0.025 | 0.11–0.27 (0.16) | |
| Wizened | 3 | 1,439 | 30. 1-Heptyl alcohol | 1.25±0.012 | 0–0.065 (0.021) | 7 |
| Floral, citrus, lemon, refreshing | | 1,450 | 32. Linalool oxide (<i>trans</i> furanoid) | 1.28±0.018 | 0–0.054 (0.012) | |
| Sharp, fatty, waxy, citrus | 110 | 1,520 | 37. 1-Octyl alcohol | 1.44±0.010 | 0–0.044 (0.019) | 0.17 |
| Cooked-sugar | 2000 | 1,631 | 48. Furfuryl alcohol | 1.73±0.020 | 0–0.069 (0.021) | 0.011 |
| Floral, green | 4.7 | 1,695 | 53. Linalool (<i>cis</i> -pyranoid) | 1.84±0.025 | 0–0.045 (0.018) | 3.83 |
| Floral | | 1,712 | 55. Linalool (<i>trans</i> -pyranoid) | 1.86±0.026 | 0–0.078 (0.032) | |
| Caramel, sweet, bitter-almond | 40 | 1,727 | 57. Methyl salicylate | 1.89±0.025 | 0–0.083 (0.044) | 1.10 |
| Rose, honey, fragrant, floral | 750 | 1,773 | 63. 1-Phenylethanol | 1.96±0.018 | 0.18–1.57 (1.12) | 1.49 |
| Floral, sweet, rose, fruity | 40 | 1,832 | 68. Geraniol | 2.11±0.017 | 0–0.062 (0.031) | 0.78 |
| Rose, honey, fragrant, floral | 1,100 | 1,862 | 69. 2-Phenylethanol | 2.15±0.020 | 0.12–1.26 (0.76) | 0.69 |
| | | | | 2.18±0.020 | 0.13–0.47 (0.27) | |
| | | | | 2.22±0.028 | 0.18–0.41 (0.32) | |
| Malt, roasted-nuts | 35,000 | 1,961 | 76. 3-Hydroxy-2-methyl-4-pyrone | 2.26±0.027 | 0.28–1.69 (1.37) | 0.039 |
| | | 1,965 | 77. Pyrrole-2-carboxaldehyde | 2.27±0.029 | 0.12–0.38 (0.21) | |
| | | | | 2.30±0.027 | 0.13–0.41 (0.28) | |
| | | | | 2.32±0.025 | 0.11–0.39 (0.21) | |
| Sweet, fruity, strawberry, hot sugar | 0.03 | 2,022 | 82. 4-Hydroxy-2,5-dimethyl-3-furanone | 2.34±0.030 | 0.21–1.78 (0.76) | 25,333.3 |
| Wizened | | 2,140 | 88. 3-Hydroxy-2-methylpyridine | 2.57±0.030 | 0.14–0.38 (0.23) | |
| Wizened | | 2,205 | 90. 3-Hydroxy-6-methylpyridine | 2.70±0.023 | 0.12–0.41 (0.27) | |
| Roasted | | 2,239 | 93. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4-pyrone | 2.76±0.030 | 0.28–1.87 (0.89) | |
| Roasted | | 2,271 | 97. 3,5-Dihydroxy-6-methyl-4-pyrone | 2.83±0.026 | 0.02–0.37 (0.12) | |

Also the relationship between the alkylpyrazines DMP, TMP and TrMP are used for assess the degree of roasting applied to cocoa. Ziegler [1] evaluated roasted cocoa flavour from DMP/TMP and DMP/TrMP indexes; in agreement with these values and with the FI values obtained, the majority of the samples were correctly roasted. The alkylpyrazines and sulfurated compounds detected in the LSC II, LSC V and LSC VI fractions can easily modify the cocoa aroma quality. In general, their presence varied with the patterns and number of free amino acids present in cocoa bean. The formation of pyrazines and other Maillard products in the reaction of free amino acids and sugars has generally been studied in model systems. Also the short-chain volatile acids were identified in the LSC VI fraction, propionic acid, butyric acid, 2-methyl-

propionic acid and 3-methylbutyric acid among them. All of them in large quantities would have a negative effect on cocoa aromatic quality (Table 8). Different proportions of the alcohols and acids were detected. Generally, these values were highly variable. Alcohols C3–C8 are produced mainly by the reduction of their corresponding aldehydes and/or ketones. The majority were found only in small amounts. The opposite effect was observed for 3-methyl-1-butanol and 2-heptyl alcohol (mean concentration greater than 0.20 mg kg^{-1}) (Table 7). Generally, the acids were detected in higher concentration depending on the fermentation process (Table 8). For example, 3-methylbutyric acid had extremely variable values ($0.59\text{--}4.18 \text{ mg kg}^{-1}$). The various combinations of volatile compounds present impart the flavour attributes of cocoa

Table 8 Identification compounds fraction LSC VI

| Sensorial attribute | Odour threshold ($\mu\text{g l}^{-1}$) | IK | Compound | RRT | Mean concentration (mg kg^{-1}) | Odour value |
|---------------------------------------|--|-------|---------------------------------------|------------|--|-------------|
| Pungent, sweet | 180,000 | 1,129 | 8. Pyrazine | 0.69±0.020 | 0–0.067 (0.043) | 0.00024 |
| Nutty, cocoa, chocolate, roasted-nuts | 60 | 1,251 | 13. 2-Methylpyrazine | 0.78±0.021 | 0.11–0.37 (0.29) | 4.83 |
| Cocoa, roasted nuts | 1,700 | 1,297 | 15. 2,5-Dimethylpyrazine | 0.86±0.030 | 0.23–1.69 (1.10) | 0.65 |
| Nutty, coffee, green | 9,000 | 1,304 | 16. 2,6-Dimethylpyrazine | 0.88±0.020 | 0.11–0.39 (0.24) | 0.027 |
| Peanut-butter, musty, nutty | 6,000 | 1,314 | 17. 2-Ethylpyrazine | 0.91±0.020 | 0.15–0.41 (0.30) | 0.05 |
| Caramel, cocoa | 2,500 | 1,318 | 18. 2,3-Dimethylpyrazine | 0.92±0.027 | 0.01–0.44 (0.21) | 0.084 |
| | 100 | 1,363 | 22. 5-Ethyl-2-methylpyrazine | 1.04±0.028 | 0.12–0.47 (0.28) | 2.8 |
| Nutty, raw potato | 130 | 1,373 | 23. 2-Ethyl-3-methylpyrazine | 1.06±0.030 | 0.13–0.42 (0.21) | 1.62 |
| Cocoa, roasted nuts, peanut | 1,800 | 1,377 | 24. 2,3,5-Trimethylpyrazine | 1.07±0.020 | 0.21–1.71 (0.82) | 0.46 |
| | | 1,386 | 4-Ethylpyridine (IS) | 1 | | |
| Strong, pungent | 32,948 | 1,434 | 28. Acetic acid | 1.22±0.030 | 0–0.11 (0.056) | 0.0017 |
| Chocolate, cocoa, coffee | 10,000 | 1,438 | 29. 2,3,5,6-Tetramethylpyrazine | 1.24±0.040 | 0.52–8.28 (4.65) | 0.47 |
| Pungent, rancid | 20,000 | 1,480 | 35. Propionic acid | 1.37±0.030 | 0.12–0.46 (0.31) | 0.016 |
| Rancid butter, hammy | 130 | 1,539 | 38. 2-Methylpropionic acid | 1.48±0.020 | 0.21–1.92 (0.79) | 6.08 |
| Sharp, cheesy, rancid | 240 | 1,625 | 47. Butyric acid | 1.72±0.030 | 0.28–1.87 (0.86) | 3.58 |
| Rancid, cheesy, faecal, hammy | 700 | 1,638 | 49. 3-Methylbutyric acid | 1.74±0.030 | 0.59–4.18 (2.45) | 3.5 |
| Cheesy, sweaty, hammy | 100 | 1,658 | 50. 2-Methylbutyric acid | 1.77±0.030 | 0.52–1.14 (0.77) | 7.8 |
| Putrid, faecal, sweaty, rancid | 3,000 | 1,725 | 56. Pentanoic acid | 1.88±0.030 | 0.12–0.48 (0.34) | 0.11 |
| Pungent, sickening, rancid, sour | 3,000 | 1,823 | 66. Hexanoic acid | 2.09±0.020 | 0.25–1.91 (1.16) | 0.39 |
| Rancid, sour, sweaty | 3,000 | 1,887 | 70. Heptanoic acid | 2.17±0.030 | 0–12–0.49 (0.31) | 0.10 |
| Unpleasant, oily, fatty | 3,000 | 2,013 | 80. Octanoic acid | 2.32±0.030 | 0.021–0.37 (0.19) | 0.063 |
| Sweet, floral, honey | 10,000 | 2,254 | 96. 2-Phenylacetic acid | 2.79±0.038 | 0.12–1.91 (1.09) | 0.11 |
| Odourless | | 2,290 | 98. 2-Furoic acid | 2.87±0.027 | 0.12–0.46 (0.29) | |
| Urinelike, faint balsamic | | 2,310 | 99. Benzoic acid | 2.93±0.030 | 0.13–1.82 (0.79) | |
| Sweet, rose | | 2,382 | 100. 3-Phenylpropionic acid | 3.08±0.030 | 0.11–1.88 (0.81) | |
| Essencies | | 2,486 | 104. <i>N</i> -(2-Phenethyl)formamide | 3.28±0.030 | 0.13–0.46 (0.23) | |
| | | 2,590 | 107. <i>N</i> -(2-Phenethyl)acetamide | 3.50±0.027 | 0.14–0.49 (0.31) | |
| Phenolic odour | | 2,650 | 109. 2-Phenylacetamide | 3.63±0.028 | 0.12–0.48 (0.26) | |
| Honey, floral | | 2,830 | 110. 3-Phenylacrylic acid | 4.07±0.040 | 0.21–1.90 (1.27) | |

powder. For these reasons all of the aroma components of cocoa can be subdivided in four groups: (1) a group of sulfurated compounds originating from the hydrolysis of the glucosylonates, fundamentally identified in the LSC II fraction; (2) a group of compounds that give a fruity smell to cocoa, identified in the LSC III fraction (esters, aliphatic aldehydes and ketones), all having retention times inferior to benzaldehyde; (3) a group of terpenic compounds that give a floral smell to cocoa, identified in the LSC V fraction; and (4) a group of compounds that give vegetal smell to cocoa, identified in the LSC III fraction, all having retention times superior to benzaldehyde. The Pearson χ^2 test, performed using the results in Tables 4, 5, 6, 7 and 8, showed significant differences ($p \leq 0.05$) between the aromatic compounds. The discriminant flavour compounds were benzaldehyde, 2-phenylacetaldehyde, ethyl phenylacetate, isoamyl benzoate, ethyl laurate, methyl tetradecanoate, ethyl tetradecanoate, benzenethiol, 2-methylpyrazine, 2,3-DMP, 2,5-DMP, 2,6-DMP, 2-ethylpyrazine, 2,3,5-TrMP, 2,3,5,6-TMP, ethyl *cis*-9-octadecenoate, furfural, acetophenone, 2-hydroxyacetophenone, benzylideneacetone, 3-methoxy-4-hydroxybenzaldehyde, 5-methyl-2-phenyl-2-hexenal, butyric acid,

propionic acid, 2-methylpropionic acid, 2-methylbutyric acid, 3-methylbutyric acid, pentanoic acid, hexanoic acid, heptanoic acid, benzoic acid, 2-phenylacetic acid, 1-phenylethanol, 2-phenylethanol, 3-methyl-1-butanol, 5-ethyl-2-methylpyrazine, 3-phenyl propionate, 4-hydroxy-2,5-dimethyl-3-furanone, 2,3-dihydro-3,5-dihydroxy-6-methyl-4-pyrone, 3-hydroxy-2-methyl-4-pyrone and 3-phenylacrylic acid. The results indicated that benzaldehyde, 2-phenylacetaldehyde, ethyl phenylacetate, acetophenone, 5-methyl-2-phenyl-2-hexenal, TMP, 3-hydroxy-2-methyl-4-pyrone and 3-methylbutyric acid (mean greater than 1.30 mg kg^{-1}), were the major odour compounds of cocoa powder, irrespective of its geographical origin. The results indicate that quantifying single compounds was not sufficiently accurate; instead, using total weight percentages of chemical structures was preferable. Finally, the probable contribution of the different compounds identified to the aroma generated was also evaluated. The second column in Tables 4, 5, 6, 7 and 8 lists the odour thresholds for most of the compounds identified, measured in water solution. Also listed in the sixth and seventh columns are the concentration and concentration to threshold ratios (odour values) for most of the

compounds identified. For such foods, this ratio would indicate which compounds are present well above their threshold (concentration-to-threshold ratio greater than 1), and are capable of contributing to the odour, and those which are well below their threshold (concentration-to-threshold ratio less than 1), and not capable of contributing to the odour. This ratio could be considered a measure of the probability that a compound contributes to the odour (aroma) of the cocoa powder.

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