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VOLATILE COMPONENTS OF COCOA WITH PARTICULAR REFERENCE TO GLUCOSINOLATE PRODUCTS

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Abstract—The aroma volatiles of raw, fermented and roasted cocoa beans were extracted and concentrated to valid essences using well-established techniques. Analysis by GC and GC/MS showed at least 84 components of which 13 were identified for the first time as cocoa volatiles. In total, *ca* 5, 66 and 65 μg of aroma components were obtained per g of raw, fermented and roasted cocoa beans, respectively. The most abundant groups of volatiles from fermented beans were alcohols (*ca* 40% w/w of the total volatiles) and esters (*ca* 32%), whilst those from roasted beans were pyrazines (*ca* 40%) and aldehydes (*ca* 23%). Trimethyl- and tetramethylpyrazine were also detected in fermented beans, and it is suggested that they contribute to the noticeable cocoa/chocolate aroma of fermented unroasted beans. Phenylacetonitrile, benzyl isothiocyanate and benzyl thiocyanate were all identified amongst cocoa volatiles, together showing the presence of precursor benzylglucosinolate in cocoa. Glucosinolate products were detected in roasted beans, and it seems likely that the enzyme thioglucoside glucohydrolase survived the conditions of roasting. Benzyl thiocyanate was detected only in raw beans, showing that the glucosinolate 'thiocyanate-forming factor' did not withstand conditions of fermentation.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a member of the Sterculiaceae, a family of mainly trees and shrubs. Glucosinolates are naturally-occurring thioglucosides mainly located in the Cruciferae, although they are also quite well known in the much smaller families Capparaceae and Euphorbiaceae. In addition, they occur sporadically in a number of other families, including the Resedaceae, Tovariaceae, Moringaceae, Tropaeolaceae and Caricaceae, and recently benzylglucosinolate has even been reported in mushrooms [1]. Nevertheless, glucosinolates are still normally regarded as being of cruciferous origin, and whilst a discovery in another family is no longer surprising, it is relatively unusual.

On enzymatic degradation in plant tissues, glucosinolates yield a variety of products, but the most important and most common are isothiocyanates, cyanides and, to a lesser extent, thiocyanates. Co-occurrence of such products in plant volatiles invariably indicates the presence of glucosinolate precursors. van der Wal *et al.* identified a large number of volatile components from roasted cocoa, including isobutyl cyanide, isobutyl thiocyanate and benzyl cyanide [2]. The first two, taken together, would seem to be strong evidence of the presence of isobutylglucosinolate in cocoa. However, only three of the approximately 90 known glucosinolates have so far been shown to be capable of degradation to the corresponding thiocyanate, and isobutylglucosinolate is not one of these. Thus, production of this thiocyanate in this manner would be unexpected but, if confirmed, it could provide more information concerning the possible mechanisms of thiocyanate formation. The thiocyanate apart, the identifications of isobutyl cyanide and of benzyl cyanide alone are significant, since the most likely precursors of nitriles in most plant systems would be the appropriate glucosinolates. On the other hand, the Sterculiaceae is remote from the main locus of glucosinolate occurrence, and whilst some taxonomists have related the family to the Euphorbiaceae, this is a tenuous connection, and detection of glucosinolate(s) in cocoa would be unexpected.

In these circumstances it seemed appropriate to analyse cocoa beans specifically for glucosinolates and glucosinolate products, although it should be emphasized that the previous authors [2] did not claim their products to be of glucosinolate origin. During the processing of cocoa, the raw beans, together with enclosing mucilage, are scooped out of the cocoa pods and fermented for some days. The beans are then dried, either naturally or artificially, before roasting. It is the roasted beans which are used in the manufacture of chocolate and cocoa-based beverages. In this project it was considered that the fermentation and/or roasting procedures could partially or completely inactivate the enzyme thioglucoside glucohydrolase, which is responsible for glucosinolate degradation. Therefore, to facilitate analysis of glucosinolate products, it was the raw beans which were mainly studied in this respect, but fermented and roasted beans were also assayed to evaluate any changes in glucosinolate products during processing.

Whilst the aroma volatiles of roasted cocoa have been extensively studied, virtually no attention has been devoted to the volatiles of the raw and fermented beans and how these vary during processing, although it is widely accepted that fermentation produces the flavour precursors from which the flavour volatiles are then generated on roasting. However, in addition to the main objective of analysing raw, fermented and roasted cocoa beans for glucosinolate products, the opportunity was taken to carry out a more broad-based survey of the volatile constituents.

RESULTS AND DISCUSSION

Cocoa beans were obtained from a plantation at Matale, Sri Lanka. For the analysis of raw beans it was considered important that isolation of volatiles was performed within 5 days of harvesting, this being the maximum length of time for which harvested pods might be left before commencing the fermentation stage. Thus, ripe pods were freshly harvested in the plantation and transported by air to London within 2 days. Valid aroma extracts of the raw beans were then prepared immediately, using well-established procedures which have been reported previously [3]. There was no such urgency in the assay of fermented or roasted beans but these were extracted using exactly the same procedures. It was noticeable that the fermented beans had already developed slight, but significant, characteristic cocoa/chocolate aroma. All extracts were concentrated by high vacuum–low temperature distillation [3], and the resultant essences from fermented and roasted beans were found, on appropriate re-dilution, to possess characteristic cocoa aromas representative of the beans used. Similar assessment of essences from raw beans confirmed them to be a valid representation of the raw beans, but they had no typical cocoa aroma character and they were very weak. Thus, although the bulk of cocoa aroma develops on roasting, some certainly develops on fermentation alone and none is evident without these processes. Assays of raw beans were carried out in duplicate; those of fermented and roasted, in triplicate.

The concentrates were analysed by GC and GC/MS (using both electron-impact and chemical ionization mass spectrometry), and the results are given in Table 1. A number of GC columns were employed, including packed columns and fused silica capillary columns (both with PEG 20M or OV 101 stationary phases), and bonded-phase fused silica capillary columns containing either BP1 (equivalent to OV 101) or BP20 (equivalent to PEG 20M) stationary phase. The retention data given in Table 1 were obtained using a 25 m fused silica column coated with BP 20. Literature Kováts retention indices [4] of most components (on PEG 20M) are also included in the table, and they confirm the general elution sequence (although it will be noticed that phenylacetaldehyde and acetophenone show slightly different relative elution behaviour on BP 20 and PEG 20M). The qualitative data in Table 1 were mainly obtained using the two packed columns and the two bonded-phase fused silica columns; some components were best identified by GC/MS using one particular phase and/or type of column. In all instances where positive identities are given, the mass spectra obtained on GC/MS agreed with those in the literature.

The quantitative data in Table 1 show that in total about 5.4, 66.3 and 64.5 μg of aroma components were obtained per g of raw, fermented and roasted cocoa beans, respectively. Thus, the total concentration of volatiles increased considerably on fermentation, but then remained at a surprisingly similar level on roasting. However, the concentrations of many individual constituents did change significantly as a result of roasting and, of course, some components were lost entirely during the process, whilst others were produced for the first time. Considering the final product, the level of *ca* 65 ppm of total volatiles is not particularly high for a system containing both thermally-generated and enzymically-produced constituents.

Overall, 84 components were detected as cocoa volatiles, although all were not found in any one sample. Of these, 51 were positively identified and 5 were tentatively characterized; these are listed in Table 1. The numbers of identified, partially identified and unidentified components (together with quantitative data) for the three types of cocoa bean analysed are as follows: raw—17 identified (comprising 89.6% w/w of the volatiles), 1 partially identified (4.2%), 7 unidentified (6.2%); fermented—24 (90.5%), 3 (1.5%), 11 (8.0%); roasted—43 (85.6%), 2 (2.7%), 17 (11.7%). Of the fully identified components, 13 are reported here as cocoa volatiles for the first time: cyclohexane, 2-ethylfuran, methylcyclohexane, *N*-methylpyrrole, *N*-ethylpyrrole, hexan-3-ol, dimethylformamide, 2-butyl-3-methylpyrazine, benzyl isothiocyanate, ethyl hexadecanoate, benzyl thiocyanate, methyl octadecanoate and ethyl octadecanoate. The tentatively identified hexadecan-2-one would also be new for cocoa. The most common classes of compounds detected were hydrocarbons, alcohols, aldehydes, ketones, acids, esters and pyrazines. At this point it is appropriate to comment briefly on the nature of the volatile components of each particular type of cocoa bean; glucosinolate products will be considered separately.

Not unexpectedly, the composition of volatiles from raw beans was very simple and the overall concentration was relatively low. The major constituent was styrene (3.74 μg/g, 68.8% of the sample), but an appreciable amount of dimethylformamide (0.46 μg/g, 8.5%) was also produced. Otherwise, the volatiles comprised a few simple, common alcohols, aldehydes and ketones; no pyrazines were detected.

As already indicated, fermented cocoa beans produced a larger number of volatiles in much larger total concentration. The major groups of compounds were alcohols (39.8% of the sample) and esters (31.6%), which is not surprising taking into account the microbiological basis of the fermentation process. 2-Phenylethanol (20.0%) and 3-methylbutan-1-ol (17.0%) were the most abundant constituents, followed by their two acetates (10.7 and 8.9%, respectively). Of particular interest was the detection of relatively small amounts of two alkylpyrazines, the trimethyl (0.26 μg/g, 0.4%) and tetramethyl (0.15 μg/g, 0.2%) derivatives, and presumably they contributed to the noticeable cocoa/chocolate aroma of the fermented beans. This is the first detection of trimethylpyrazine in fermented, unroasted cocoa beans, but tetramethylpyrazine has been previously identified, and at a surprisingly similar level (0.2 μg/g) [5].

The formation of such alkylpyrazines is usually associated with heat-treated food systems, but tetramethylpyrazine has been shown to be the most readily formed pyrazine during cocoa roasting and accounted for almost all of the alkylpyrazine content of beans which had been subjected to only the very mild roasting conditions of 70° for 30 min [5] (normally roasting is carried out at 100–140° for 45–90 min). Since natural cocoa fermentation temperatures can rise as high as 50° [6], and this level is likely to be maintained for *ca* 50 hr [7], clearly a thermally-induced origin of tetramethylpyrazine is possible. Furthermore, the compound has only been found in well-fermented beans and it was absent from lightly or non-fermented varieties [5]. However, microbial formation of tetramethylpyrazine in fermented beans is also possible, and it has been detected in several fermented foods including natto, vinegar, wine and beer [8]. Kosuge

Table 1. Volatile constituents of cocoa beans

Component	R_t (min)	Kováts index (lit)*	Raw		Fermented		Roasted	
			% rel abund.	μg/g	% rel. abund.	μg/g	% rel abund	μg/g
Cyclohexane	0 4	765	—	—	—	—	0.3	0 19
2-Methylfuran	0 5	866	—	—	—	—	0.5	0 32
2-Ethylfuran	0.8	951	—	—	—	—	tr	tr
Pentanal	1 0	1002	—	—	—	—	0 6	0.39
Methylcyclohexane	1 3	—	—	—	—	—	0.4	0.26
Toluene	1 7	1055	—	—	2 3	1.49	0.4	0 26
2-Methylpropan-1-ol	2.3	—	—	—	1 5	0 98	—	—
Dimethyl disulphide	2.4	1081	—	—	tr	tr	0 8	0 52
Pentan-2-ol	2.6	1091	—	—	—	—	1.8	1 16
3-Methylbutyl acetate	2.9	1110	—	—	8.9	5 91	—	—
N-Methylpyrrole	3.4	1139	—	—	—	—	0 8	0 52
N-Ethylpyrrole	3.7	—	—	—	—	—	0 5	0 32
Hexan-3-ol	4.0	—	—	—	—	—	0.6	0.39
Pyridine	4.2	1180	—	—	—	—	0 8	0.52
3-Methylbutan-1-ol	4.3	1184	—	—	17 0	11 29	1.0	0.64
Pentan-1-ol	5.0	1213	—	—	0 4	0 27	1.3	0.84
Methylpyrazine	5.2	1251	—	—	—	—	2.7	1.74
Styrene	5.6	—	68.8	3 74	—	—	tr	tr
A trimethylbenzene	6 2	—	—	—	0 3	0 19	—	—
Heptan-2-ol	7.2	1284	—	—	0 9	0 58	—	—
Dimethylformamide	7.9	—	8.5	0 46	1 0	0 69	—	—
A C_4-alkylbenzene	8 0	—	—	—	0.3	0 19	—	—
2,5-Dimethylpyrazine	8 0	1306	—	—	—	—	4 8	3.09
2,6-Dimethylpyrazine	8 3	1325	—	—	—	—	5 5	3.54
2-Ethyl-3-methylpyrazine	9.2	1381	—	—	—	—	2 6	1 67
Trimethylpyrazine	9.4	1387	—	—	0.4	0 26	6 3	4.06
2,5-Dimethyl-3-ethylpyrazine	9.9	—	—	—	—	—	2 6	1.68
Ethyl octanoate	10.2	1423	—	—	1 7	1 10	—	—
2,6-Dimethyl-3-ethylpyrazine	10.2	—	—	—	—	—	1 5	0 97
Tetramethylpyrazine	10.5	1458	—	—	0.2	0.15	5.5	3.54
2,5-Diethyl-3-methylpyrazine	10.8	—	—	—	—	—	0 7	0 45
2,6-Diethyl-3-methylpyrazine	11.0	—	—	—	—	—	3.2	2.06
Benzaldehyde	11 6	1502	3 7	0.20	5.3	3.48	4 2	2 71
5-Methyl-2-furfural	13.1	1563	—	—	—	—	0.6	0.39
Phenylacetaldehyde	13.3	1646	2 0	0.11	7.6	5.05	15.9	10.25
Acetophenone	14.0	1627	0 9	0.05	0.8	0.55	4 0	2.57
A C_5-alkylpyrazine	14.6	—	—	—	—	—	2 1	1.35
A C_5-alkylpyrazine	15.0	—	—	—	—	—	0.6	0.39
2-Butyl-3-methylpyrazine	15 2	—	—	—	—	—	1 6	1.03
Methyl phenylacetate	15 4	1747	—	—	—	—	0 3	0.19
2-Phenylethyl acetate	15.7	1785	—	—	10.7	7.11	0 7	0 45
2-Butyl-3,5-dimethylpyrazine	16 0	—	—	—	—	—	0 4	0 26
Ethyl dodecanoate	16 2	1826	—	—	4.3	2.82	0.5	0.32
2-Phenylethanol	17 3	1859	3.5	0.19	20.0	13.18	3.6	2.32
Phenylacetonitrile	17 7	—	tr	tr	1.2	0.79	0 7	0 45
5-Methyl-2-phenylhex-2-enal	20.4	—	—	—	—	—	1 5	0 97
Ethyl tetradecanoate	21 1	2027	—	—	2.0	1.34	—	—
Benzyl isothiocyanate	21 8	—	2.2	0.12	0.3	0.19	0.3	0 19
Ethyl cinnamate	22.0	—	—	—	0 1	0 08	—	—
? Hexadecan-2-one	22 8	—	4.2	0 23	0.9	0 60	—	—
Ethyl hexadecanoate	23.0	2245	—	—	1 0	0.69	0.4	0.26
Benzyl thiocyanate	23.8	—	tr	tr	—	—	—	—
Methyl octadecanoate	24.1	—	—	—	tr	tr	3.1	2.00
Ethyl octadecanoate	26.3	2470	—	—	2.9	1.95	0 6	0.39
Tetradecanoic acid	29.4	—	—	—	—	—	1 2	0.78
Hexadecanoic acid	37 1	—	—	—	—	—	0 8	0 52

*Literature = [4]; tr = trace (< 0.1 %).

and Kamiya found it to be a metabolic product of *Bacillus subtilis* grown on various media [9] and this organism has been reported to dominate the microbial population during the later stages of cocoa fermentation [10]. Zak *et al.* have shown tetramethylpyrazine to be produced in unroasted Trinidad and Brazilian beans at levels of *ca* 0.15 μg/g (cf. Table 1) after 6 days of fermentation (the normal period) and associated its formation with the presence of *B. subtilis* [10].

The fact that trimethylpyrazine has now been detected (and at roughly similar levels) in unroasted beans, but has not previously been reported as a fermentation product, tends to support the thermal origin for such pyrazines in fermented cocoa beans.

Table 1 shows that although roasted cocoa beans gave much the same total concentration of volatiles as fermented beans, the pattern of volatiles was entirely different. Thus, the major classes of volatiles on roasting became pyrazines (14, 25.88 μg/g, 40.1% of the sample) and aldehydes (5, 14.71 μg/g, 22.8%). However, alcohols (8.3%) and esters (5.6%) were still produced in reasonable concentration. The major volatile constituent was phenylacetaldehyde (10.25 μg/g, 15.9%), followed by trimethylpyrazine (6.3%), tetramethylpyrazine (5.5%) and 2,6-dimethylpyrazine (5.5%). It would appear from these results that in our system, trimethylpyrazine was the most readily formed pyrazine on roasting, in contrast to previously mentioned earlier work [5], and this would also fit in with our results for fermented beans (i.e. trimethyl > tetramethyl). Reineccius *et al.* have investigated pyrazine formation during the roasting of cocoa beans, and they found that between 0.8 and 7.1 μg/g of pyrazines were produced, depending on the vigour of the roasting conditions [5]. Clearly, the amount detected in this work (25.88 μg/g) is much greater. This may be due to the use of more sensitive analytical methodology or, more likely, to the use of more strongly roasted beans. In addition, apparently the potential for generating pyrazines is greater in well-fermented beans, in which fructose becomes the dominant sugar [5] and it has already been observed that the beans used in this study were particularly well-fermented.

Most of the changes which occurred in the overall pattern of volatiles during the processing of raw cocoa beans to the roasted product are fairly readily understandable. The generation of large quantities of pyrazines on roasting is a good example. Similarly, the fact that alcohols and esters reached a peak in fermented beans (alcohols, 0.19 → 26.38 → 5.36 μg/g; esters, 0 → 20.95 → 3.61 μg/g) is reasonable on the basis of the expected chemical changes during fermentation and roasting. Aldehydes, on the other hand, increased fairly regularly throughout (0.31 → 8.53 → 14.71 μg/g). They are important contributors to good cocoa flavour and most are formed via Strecker degradations. Thus, the abundant phenylacetaldehyde in the volatiles of roasted beans originated from phenylalanine.

With regard to the specific investigation of possible glucosinolate occurrence in cocoa beans, it can be seen from Table 1 that of the three previously reported [2] possible glucosinolate products only one, benzyl cyanide (phenylacetonitrile), was detected in this work. It should be emphasized that very careful, high sensitivity searches were made by selected ion monitoring (SIM) GC/MS specifically for isobutyl cyanide (on ions *m/z* 68, 83) and isobutyl thiocyanate (*m/z* 72, 73, 115), and also isobutyl isothiocyanate (*m/z* 72, 73, 115). All cocoa samples were investigated in this manner but all results were negative. However, in addition to confirming the previous identification of benzyl cyanide in roasted cocoa [2], both of the other major degradation products of benzylglucosinolate, the isothiocyanate and thiocyanate, were also positively identified in this work (Table 1). This is the first time that these three glucosinolate products have all been identified together in cocoa volatiles, and provides virtually positive proof of the occurrence of the precursor glucosinolate in cocoa.

However, assay of simple glucosinolates as such (as TMSi-derivatives) in plant tissue is now a relatively straightforward procedure, and using this method it was possible to obtain supporting evidence for the presence of benzylglucosinolate in cocoa. Because of the relatively poor overall sensitivity of the procedure (in comparison with high sensitivity GC assay of autolysis products) and the very low concentration of benzylglucosinolate in cocoa, it was not possible to obtain a full mass spectrum of the TMSi-derivative on GC/MS. However, by means of high sensitivity SIM-GC/MS (on ions *m/z* 73, 75, 103, 129, 133 and 147—for TMSi-glucoside—and on *m/z* 89, 91 and 116—specifically for TMSi-benzylglucosinolate), confirmation of the presence of benzylglucosinolate in cocoa was obtained. There would thus appear to be little doubt that benzylglucosinolate does occur in cocoa, and this is the first report of the occurrence of any glucosinolate in cocoa or in any member of the Sterculiaceae. However, the amount of the compound in cocoa (*ca* 3.5 μg/g in fermented beans) is very small.

SIM-GC/MS searches were also made for other glucosinolates (e.g. methyl, 2-phenethyl) and their degradation products in all cocoa samples, but none was found. It may well be that benzylglucosinolate is the only glucosinolate in cocoa. In fact, although there are now quite a few reports of glucosinolates outside the Cruciferae and the order Capparales, these include only a very small number of the approximately 90 known naturally-occurring glucosinolates, which are always glucosinolates biosynthesized directly from α-amino acids without any chain-elongation stages. Thus, benzylglucosinolate (from phenylalanine) has been found in a number of widespread sources (e.g. papaya [11] and mushroom [1]). On this basis it would not be surprising if the one glucosinolate to occur in cocoa were the benzyl derivative.

It is interesting to consider how the amounts of benzylglucosinolate products varied during cocoa processing. The relevant data, extracted from Table 1, are summarized in Table 2. That the products were formed at all, shows that not only does cocoa contain the glucosinolate substrate but it also contains the necessary associated enzyme, thioglucoside glucohydrolase. In early work, exogeneous enzyme (from mustard) was added to appropriate extracts of raw cocoa beans to ensure, as far as possible, complete degradation of any glucosinolates present (and hence to facilitate analysis). In fact, this was unnecessary, in that the results obtained both with and without added enzyme were the same. Thus, cocoa contains native thioglucosidase.

It is especially interesting that added enzyme was even unnecessary with roasted beans (Table 2). In this case, either the thioglucosidase survived the conditions of roasting (as well as fermentation) or the products were formed non-enzymically. The latter is certainly possible, and glucosinolates have been shown to degrade thermally,

Table 2. Glucosinolate products from cocoa beans

Glucosinolate product	Raw		Fermented		Roasted	
	% rel. abund.	μg/g	% rel. abund.	μg/g	% rel. abund.	μg/g
Phenylacetonitrile	tr	tr	1 2	0 79	0.7	0.45
Benzyl isothiocyanate	2.2	0.12	0 3	0 19	0.3	0.19
Benzyl thiocyanate	tr	tr	—	—	—	—

tr = trace (< 0.1 %).

in the absence of any enzyme, to yield the corresponding nitriles and isothiocyanates [12], but the conditions necessary for benzylglucosinolate (a temperature of 200°) are rather more extreme than those normally used in roasting cocoa beans (145° in this case). Thus the former possibility is the more likely, and there is no doubt that the thioglucosidase is a very robust enzyme. Although it has been claimed, on limited evidence, that it does not survive in cooking (boiling) of Brussels sprouts [13], from a previous detailed study of its thermal stability in *Lepidium sativum* seeds, it was still active even after heating at 135° for 30 min [14] (more severe conditions were not assessed). It is possible that thioglucosidases of different origins may have different thermal stabilities, but in any case the findings for seeds are more directly related to cocoa beans, and on that basis it is quite possible that the conditions of roasting (*ca* 145° for *ca* 40 min) were insufficient to inactivate the enzyme completely. It can be seen from Table 2 that overall a lesser amount of benzylglucosinolate products was given by roasted beans compared with fermented beans, and this may well have been due to partial inactivation of the thioglucosidase on roasting.

The data in Table 2 also show that benzyl thiocyanate was only detected in extracts from raw beans. Particular efforts were made to detect it in other samples, but even when high sensitivity SIM-GC/MS was used this proved impossible. As mentioned previously, only three glucosinolates appear capable of degradation to thiocyanate, but as benzylglucosinolate is one of the three, this identification is not unreasonable. However, the detailed mechanism of formation of thiocyanate from glucosinolate is not known, although the involvement of a heat-labile 'thiocyanate-forming factor' has been shown [14]. In *L. sativum* seeds this 'factor' was inactivated by heating at 125° for just over 4 hr or by heating at 135° for 30 min [14]. The fact that benzyl thiocyanate could not be detected in roasted cocoa beans therefore agrees well with these earlier findings, in that the conditions of roasting would be expected to inactivate the 'thiocyanate-forming factor'. However, obviously the conditions of fermentation were also sufficient to have inactivated the factor prior to roasting (Table 2) and this is, perhaps, surprising. The cause of inactivation at this stage is unknown, but the effect of an elevated temperature (*ca* 45–50°) for an extended period of time (*ca* 4 days) during fermentation might be significant.

EXPERIMENTAL

Cocoa beans were obtained from a plantation and associated factory at Matale, Sri Lanka. Freshly harvested cocoa pods were transported by air to London for assay within 3 days. Samples of roasted beans were also obtained from Gill and Duffus Ltd., 201 Borough High Street, London.

Sample preparation. (a) *Raw cocoa beans.* Fresh pods were cut open and the beans removed from the fibrous white mucilage. One sample (100 g) was ground, mixed with distilled H_2O (350 ml) and extracted using a modified [15] Likens and Nickerson apparatus [16], as described previously [3]. 2-Methylbutane (40 ml) was used as solvent and extraction was carried out for 2 hr. A second sample of ground raw beans (120 g) was defatted by means of a Soxhlet apparatus using Et_2O. The defatted beans were mixed with distilled H_2O (350 ml) containing 50 mg of a crude thioglucosidase preparation (from mustard—prepared by the method of Schwimmer [17]) and 20 g Na ascorbate (enzyme co-factor). The mixture was allowed to stand for 1 hr and extracted as above. (b) *Fermented cocoa beans* Ground fermented beans (120 g), without defatting or exogeneous enzyme, were submitted to Likens and Nickerson extraction as for raw beans. (c) *Roasted cocoa beans.* Ground roasted beans (50 g) were extracted as above. However, extraction was facilitated, without affecting the result, by prior defatting. All extractions were carried out at least in duplicate, and all extracts were subsequently concd to 1.0 ml as described previously [15].

GC. FID-GC: 25 m × 0.2 mm i.d. fused silica capillary containing PEG 20M or OV 101 or BP 20 bonded-phase (equivalent to PEG 20M) or BP1 bonded-phase (equivalent to OV 101); H_2 0.8 ml/min; temp. programme 70° for 3 min, followed by an increase at 8°/min to 180°; detector and injection point heaters, 275° and 250°, respectively; typically 0.1 μl or 1.0 μl injected at 25 : 1 split. Other columns used were 1.5 m (and 5.5 m) × 4 mm i.d glass packed with either 10 % PEG 20M or 3 % OV 101 coated on 100–120 BSS mesh acid-washed Diatomite C, using N_2 (30 ml/min) and the same temp. programme.

GC/MS. A Kratos MS 25 instrument was used, linked on-line to a Kratos DS 50S data processing system and equipped with a computer-controlled multi-peak monitoring (MPM) unit. The same GC conditions as above were used, with He as carrier gas. Packed columns and bonded-phase fused silica columns were mainly used. The single-stage, all-glass jet separator was at 250° Significant operating parameters of the MS during EIMS were. ionization voltage, 70 eV; ionization current, 100 μA; source temp., 225°; accelerating voltage, 1.33 kV, resolution, 1500; scan speed, 1 sec/decade (repetitive throughout run). Identical conditions were employed during CIMS except for: reagent gas, isobutane; ionization potential, 100–110 eV; emission current, 5 mA. The MPM unit was extensively employed in high-sensitivity SIM-GC/MS, using the same GC columns and conditions as described previously.

Quantitative assessment. Samples were prepared in such a manner that a known aliquot of the cocoa bean sample was

analysed. Quantitative data were then derived mainly from the TIC trace during GC/MS, but also from the GC–FID traces. Known amounts of a selection of identified compounds (toluene, pyridine, pentan-1-ol, methylpyrazine, styrene, trimethylpyrazine, benzaldehyde, 2-phenylethanol, phenylacetonitrile and benzyl isothiocyanate) were injected under the same analytical conditions to enable assessment of absolute amounts of components in the samples.

TMSi-glucosinolate assay. Bjorkman's method [18] was used to prepare TMSi-derivatives of glucosinolates in suitable extracts of dried, ground fermented cocoa beans (200 g); a standard TMSi-benzylglucosinolate was also prepared. Products were analysed by GC, GC/MS and SIM-GC/MS using a 1.5 m glass column packed with 3% OV 101, a temp. programme of a 2°/min increase from 200 to 280°, and a flow rate (N_2 or He) of 40 ml/min. Under these conditions, TMSi-benzylglucosinolate had an R_t of 21 min.

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