Eur Food Res Technol (2006) 222: 432–438
DOI 10.1007/s00217-005-0130-y

ORIGINAL PAPER

Christina Rohsius · Reinhard Matissek · Reinhard Lieberei

Free amino acid amounts in raw cocoas from different origins

Received: 3 May 2005 / Revised: 27 July 2005 / Accepted: 31 July 2005 / Published online: 26 October 2005

Abstract Raw cocoa is the processed and traded form of the cocoa seed. Fresh seeds undergo fermentation and a drying process before they are prepared for transport and shipping. Depending on the local situation in the producer region the seeds are collected from big estates and are fermented and dried in big lots or they originate from small farmer's crop trees. In the subsequent transport and trading chain raw cocoas may be mixed and stored before they finally are sold and brought to the consumer countries. Local or regional variations in cocoa plant material, fermentation procedures and drying processes finally result in a typical traded good with respect to the amino acids, which form an important part of the flavour precursors. These free amino acids and their composition result from the fermentation procedure of fresh seeds. In the course of the fermentation specific cleavage of cocoa storage proteins delivers the amino acid patterns. In this study the variation of free amino acid amount and distribution of 108 commercially fermented and traded cocoa samples and two *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum samples were determined. This examination showed clearly, that content and distribution of free amino acids in raw cocoa from different origins vary greatly (5–25 mg g^{-1} fatfree dry matter), in some cases country and even region-specific differences were apparent. It is important to notice typical, region-specific variations in the amounts and compositions of free amino acids.

Keywords Raw cocoa · Free amino acids · Fermentation · Aroma precursors · Theobroma cacao · Theobroma grandiflorum · Acrylamide

C. Rohsius (✉)
Department of Crop Science and Plant Ecology, Biocentre
Klein Flottbek and Botanical Garden,
Ohnhorststr. 18,
D-22609 Hamburg
e-mail: crohsius@iangbot.uni-hamburg.de
Tel.: +49-40-42816-388
Fax: +49-40-42816-656

R. Matissek
Food Chemistry Institute (Lebensmittelchemisches Institut LCI)
of the Association of German Confectionery Industries,
Adamsstraße 52–54,
D-51063 Köln

R. Lieberei
Department of Crop Science and Plant Ecology, Biocentre
Klein Flottbek and Botanical Garden,
Ohnhorststr. 18,
D-22609 Hamburg

Introduction

Free amino acids, oligopeptides, and reducing sugars are cocoa aroma precursors formed during the fermentation process. These precursors are converted to the typical cocoa aroma via Maillard reactions and Strecker degradation during the roasting and conching process [1–4].

The amount and composition of free amino acids changes during fermentation. Unfermented seeds contain low amounts of total free amino acids with high percentages of acidic amino acids. Fermented seeds are characterised by higher amounts of total free amino acids, the accumulation of hydrophobic free amino acids and decreased amounts of acidic free amino acids [5, 6].

The proportion of hydrophobic to acidic free amino acids changes during fermentation from 33:30 (%) in unfermented cocoa to 58:16 (%) and 46:6 (%) in fermented cocoa seeds, respectively [5, 6]. The accumulation of hydrophobic free amino acids is explained by the cleavage characteristics of two proteases of cocoa beans. The aspartic endoprotease (E.C. 3.4.23) attacks the storage proteins preferentially at sites of hydrophobic amino acids and the carboxypeptidase (E.C. 3.4.16) releases single hydrophobic amino acids [7, 8]. As a result of the different pH and temperature optima of these enzymes, proteolysis primarily depends on the fermentation conditions: duration and intensity of acidification, temperature and aeration [9]. Thus, the total amount of free amino acids and oligopeptids liberated during hydrolysis varies considerably [10–12]. The accumulation of free amino acids during the proteolysis process has mainly been analysed in experimental fermentations conducted in selected countries like Ghana, Malaysia and, in case of fermentation-like incubations, in Germany.

So far no comprehensive studies on the variation of content and composition of free amino acids in commercially fermented and dried cocoa from different origins exists. In order to display the variation of fermented, dried cocoa samples from different countries a German Foundation of the Cocoa and Chocolate Industry financed study was conducted [13]. This paper reports on the content and composition of free amino acids of 110 of these samples originating from 21 countries. Apart from *T. cacao* at least nine other species within the genus Theobroma could be used for the production of chocolate-like products [14]. From those *T. grandiflorum* (Willd. ex Spreng.) K. Schum is of growing economic importance. In contrast to cacao the used part of *T. grandiflorum (common name is* "cupuaçu") is the sweet seed pulp which is needed for the production of juices, ice creams and jams. So far the seeds were discarded. Along with the increasing production of cupuaçu seed pulp, new commercial interest arose to use the seeds as a second product. In fact, some lots have already been exported. For this reason we included two processed samples of the species *T. grandiflorum* into the study and analysed its free amino acid contents.

Results are discussed in respect to fermentation degree and origin specificity. Results of the content and variation of the acrylamide precursor asparagine are also displayed.

Materials and methods

Samples

The following samples represent commercial arbitrage samples of fermented, dried cocoa from the port of Hamburg, number of samples in brackets: Brazil (1), Cameroon (1), Dominican Republic (1), Ecuador (9), Côte d'Ivoire (13), Ghana (7), Haiti (4), Indonesia (10: Java 4, Sulawesi 2, Sumatra 3, unspecified 1), Liberia (1), Madagascar (7), Nigeria (14), Papua New Guinea (7), São Tomé and Principe (São Tomé 1), Sierra Leone (1), Solomon Island (2), Tanzania (4), Togo (5), Trinidad and Tobago (Trinidad 1), Uganda (9) and Venezuela (4). Arbitrage samples were withdrawn from a minimum of 30% of the sound bags of one consignment; sample size was reduced by dividing until mixed samples of approximately 2 kg were achieved.

The following samples were collected elsewhere: Four monoclonal samples from Ecuador gratefully provided by Nestlé: EET 95, EET 103, EET 48 and CCN 51. Samples were fermented for 65 h in net-bags in a heap. One Ecuadorian sample provided by the company Grupo Quirollo represents a commercial, monoclonal fermentation of the clone CCN 31. One Malaysian sample collected at a fermentation centre close to Tawau, Borneo. Two Brazilian samples from the cocoa relative *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum. Fermented seeds of this species are already used for home-made chocolate or so called "cupulate" in Brazil. Samples that were not collected at the Port of Hamburg represent approximately 1 kg of randomly withdrawn material.

Analyses

Unless otherwise specified, all chemicals used were analytical grade and purchased at Merck.

Three hundred gram of deshelled raw cocoa without radiculae (only cotyledons) corresponding to approximately 300 cocoa beans were frozen for at least 24 h at –20° and slowly ground by an ultracentrifugal mill (ZU 100, Comp. Retsch, without sieve, 12er crown) at 15 000 rpm. The cocoa was sieved through a 3 mm sieve and the residue (> than 3 mm) was ground and sieved again until 98% was below 3 mm. This material was milled in a stainless steel ball homogenizer. The ground cocoa samples were defatted by a multiple cold extraction by centrifugation in a centrifugation tube with petrolether.

The water content of the defatted material was measured according to [15].

Free amino acid contents were analysed according to the method described by [5]; slightly modified: 0.500 g defatted, milled cocoa powder was stirred at $<4°C$ for 1 h with 1.4 g polyvinyl-polypyrrolidon (PVPP) and 50 ml H_2O. Immediately after adding water, the pH-value was adjusted to pH 2.5 with 50% aqueous trifluoroacetic acid solution. The solution was centrifuged for 10 min at 5000 rpm. The clear supernatant solution was filtered through a 0.45 μm filter (Multoclear, CS-Chromatographie). 30 μl of each sample were lyophilised (1 h; −20°C, 0.05 mbar) directly in the vial and kept at –20°C until analysed.

Free amino acids were separated with a reversed phase HPLC apparatus after they had been converted to *o*-phthalaldehyde (OPA) derivatives. Separating column: LicChroCART 250-4 (Merck); precolumn: Lichrospher 100 RP-18 (5 μm); column temperature: 30°C; eluants: A: 1.6 l sodium acetate solution/glacial acetic acid (50 mmol l^{-1}; pH 6.2), 50 ml MeOH (Lichrosolv®, gradient grade), 20 ml tetrahydrofuran (Lichrosolv®; gradient grade). B: 200 ml sodium acetate solution/glacial acetic acid (50 mmol l^{-1}; pH 6.2), 800 ml MeOH (Lichrosolv®, gradient grade); flow rate: 1.3 ml min^{-1}. The column was equilibrated with eluant A. Gradient ($A+B$=100% v/v): (1) 2 min 100–95% A, (2) 10 min 95–85% A, (3) 8 min 85–60% A, (4) 5 min 60–50% A, (5) 15 min 50–0% A, (6) 10 min constant 0% A, (7) 5 min 0–100% A, (8) 20 min constant 100% A. Detector: Hitachi F-1050 Fluorescence Spectrophotometer (λ_{ex}=334 nm, λ_{em}=425 nm); Autosampler:Merck-Hitachi AS-4000.

For the HPLC-measurement, 800 μl borate buffer (200 mmol l^{-1} boric acid; adjusted to pH 9.5 with concentrated KOH, boiled for 5 min) were given to the lyophilised samples. Standard solutions contained 1–10 pmol μl^{-1} of each amino acid, solved in borate buffer. Subsequently 400 μl OPA-reagent was added to these solutions prior to each injection. Preparation of OPA-reagent: 100 mg *o*-phthaldialdehyde (Merck, No. 11452) previously solubilized in 2.5 ml MeOH (Lichrosolv®, gradient grade) plus

22.4 ml borate buffer (pH 9.5) plus 100 µl 2-mercapto-ethanol (Merck, No. 15433). OPA-reagent was made 24 h before being used. After injection of 20 µl of this mixture the derivatisation was stopped exactly after 2 min by passing the eluant into the column. Solution degaser: Degassex DG-4400, Phenomenex. Quantification: calculated via peak area of chromatograms from standard mixtures containing 1–10 pmol μl^{-1} of each amino acid. Standard deviation of reference substances were equal to ±2.0% except for glutamic acid, glutamine, alanine, tryptophan (±2.9–3.8%); argenine, asparagine, threonine and serine (±5.7–7.3%); glycine (±9.2%) and lysine (±9.6%).

Results

Hashim [6] and Kirchhoff [5, 16] reported total free amino acid amounts in the range of 2.4–5.1 mg g^{-1} fatfree dry matter (ffdm) for unfermented and 9.4–14.5 mg g^{-1} ffdm for freshly fermented (but not dried) cocoa beans from Malaysia and Ghana.

The results for total free amino acid contents of the 110 analysed cocoa samples varied between 5.0 and 25.2 mg g^{-1} ffdm, with a mean value of 13.0 mg g^{-1} ffdm. They were clustered into three groups containing low, medium and high amounts (Fig. 1).

Low amounts of free amino acids in the range of 5.8–8.8 mg g^{-1} ffdm were found in commercial samples from Ecuador (including five commercial Arriba type samples, graded as ASS, that contained between 6.0 and 8.8 mg g^{-1} ffdm), Haiti (5.8–8.3 mg g^{-1} ffdm), Tanzania (6.9–8.4 mg g^{-1} ffdm) and Sulawesi (6.7–6.9 mg g^{-1} ffdm) (Fig. 1).

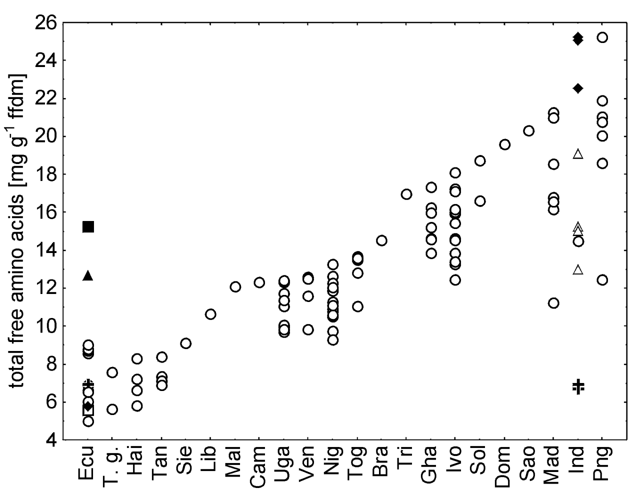

Fig. 1 Total free amino acid contents of all 110 samples. Abbreviations and symbols: Ecu: Ecuador (□ EET 95; ▲ EET 48; + EET 103; ■ CCN 31; ♦ CCN 51; ○ remaining samples from Ecuador); T.g.: *T. grandiflorum*; Hai: Haiti; Tan: Tanzania, Sie: Sierra Leone; Lib: Liberia; Mal: Malaysia; Cam: Cameroon; Uga: Uganda; Ven: Venezuela; Nig: Nigeria; Tog: Togo; Bra: Brazil; Tri: Trinidad; Gha: Ghana; Ivo: Côte d'Ivoire; Sol: Solomon Islands; Dom: Dominican Republic; Sao: São Tomé; Mad: Madagascar; Ind: Indonesia (△ Java; + Sulawesi; ♦ Sumatra; ○ not specified Indonesian sample); Png: Papua New Guinea

Controversial results were gained for monoclonal fermented cocoa from Ecuador: the clonal material "EET 95," "EET 103" and "CCN 51" contained 5.6, 6.9, and 5.7 mg g^{-1} ffdm free amino acids, whereas "EET 48," although fermented similarly, contained nearly double the amount: 12.7 mg g^{-1} ffdm. Another Ecuadorian sample also revealed higher values than ordinary Arriba samples: the monoclonal fermented material "CCN 31" contained 15.2 mg g^{-1} ffdm. "CCN 31" originates from a commercially conducted monoclonal fermentation procedure. The clonal samples "EET 48," "EET 95," "EET 103" and "CCN 51" are the result of the same scientific fermentation procedure conducted with ripe seeds in net bags.

Medium amounts of free amino acids in the range of 8–14 mg g^{-1} ffdm were observed in African samples from Sierra Leone, Liberia, Cameroon, Uganda, Nigeria and Togo. However, cocoa from the world's most important cocoa producing countries, Ghana and Côte d'Ivoire contained on average more free amino acids with mean (min-max) values of 15.4 (13.8–17.3) and 15.2 (12.4–18.1) mg g^{-1} ffdm (Fig. 1).

High amounts of total free amino acids with mean values in the range of 15.0–24.3 mg g^{-1} ffdm were found in samples from Java (Indonesia), Solomon Islands, Dominican Republic, Trinidad and Tobago, São Tomé, Madagascar, Sumatra (Indonesia) and Papua New Guinea. Of notable interest are the results of some samples from Sumatra (Indonesia) and Papua New Guinea which exceed highest reported values for total free amino acids in fermented cocoa reaching up to 25.2 mg g^{-1} ffdm [5, 6, 16–18] (Table 1, Fig. 1).

Despite the fact that many different parameters influence the final content of free amino acids in raw cocoa, country-specific variation of total free amino acids were comparatively low in Nigeria (9.2–13.3 mg g^{-1} ffdm), Uganda (9.7–12.4 mg g^{-1} ffdm) and Ghana (13.8–17.3 mg g^{-1} ffdm) (Fig. 1). Whereas high variations were found in cocoa from Indonesia (6.7–25.2 mg g^{-1} ffdm), Madagascar (11.2–21.3 mg g^{-1} ffdm) and Papua New Guinea (12.4–25.2 mg g^{-1} ffdm) (Table 1, Fig. 1). Indonesian samples could be separated according to their origins: Sulawesi samples contained 6.7–6.9 mg g^{-1} ffdm, Sumatra 22.5–25.2 mg g^{-1} ffdm and samples from Java 13.0–19.1 mg g^{-1} ffdm, another unlabelled Indonesian sample contained 14.5 mg g^{-1} ffdm free amino acids. For statistical analysis further investigations with higher sample numbers have to prove whether this observation is in fact origin-dependent.

Free amino acid distribution and total amount of free amino acids of the samples are shown as mean values per country or region in Table 1. On average the hydrophobic amino acids accounted for more than 60 mol% (15 mol% leucine, 15 mol% alanine, 10 mol% phenylalanine, 9 mol% valine, 5 mol% isoleucine and 5 mol% tyrosine), whereas acidic free amino acids and the remainders accounted for 21 mol% per group.

As previously described, the ratio of hydrophobic to acidic free amino acids changes in the course of the fermentation and thus provides information on the degree of fermentation. Samples from Sulawesi and Haiti with low

Table 1 Relative amounts of free amino acids in mol% and mean values of free amino acid concentrations in fermented cocoa beans of different origins

Amino acid [mol%]	Bra	Bra T. g	Cam	Dom	Ecu	Ecu Mon	Gha	Hai	Ind ns	Ind Java	Ind Sul	Ind Sum	Ivo	Lib	Mad	Mal	Nig	Png	Sao	Sie	Sol	Tan	Tog	Tri	Uga	Ven	Σ
N	1	2	1	1	9	5	7	4	1	4	2	3	13	1	7	1	14	7	1	1	2	4	5	1	9	4	110
																											X̄
Acidic	17.3	18.9	20.4	18.8	22.0	25.2	18.7	26.5	20.0	18.4	28.3	13.1	21.0	23.2	21.0	25.2	21.1	14.7	16.2	23.6	16.6	21.8	21.7	16.7	21.6	20.2	20.7
Asp	3.5	6.1	3.3	3.1	3.4	2.9	2.9	4.0	3.5	3.8	4.5	3.3	3.5	3.5	3.6	4.2	3.2	3.5	3.3	4.2	3.5	3.6	2.9	3.4	4.5	3.6	3.5
Asn	6.2	4.6	6.3	6.3	7.3	8.6	6.7	8.9	8.3	6.9	10.3	4.3	7.6	6.8	8.5	12.0	7.1	4.6	5.0	7.2	6.4	8.1	7.5	6.0	6.9	8.1	7.2
Glu	7.1	8.0	9.8	8.7	9.8	11.7	8.3	12.1	7.4	7.5	12.6	5.5	8.7	11.3	8.0	8.4	9.7	6.5	7.9	10.8	6.7	8.9	10.2	6.8	9.1	7.4	8.9
Gln+His[a]	0.5	0.2	1.0	0.7	1.5	2.1	0.9	1.4	0.8	0.2	0.9	0.0	1.2	1.7	0.8	0.6	1.1	0.1	0.0	1.4	0.0	1.2	1.2	0.5	1.0	1.0	1.0
Hydrohobic	60.9	60.1	61.1	57.7	59.8	57.7	60.5	57.4	60.1	59.0	60.5	60.8	59.5	56.4	58.6	55.0	59.6	60.9	58.6	59.5	60.9	61.9	59.7	59.8	60.3	60.1	59.7
Leu	16.8	12.2	16.1	17.0	15.1	14.5	16.5	12.4	15.5	16.5	13.2	14.5	15.6	13.2	15.1	13.0	15.1	16.5	16.1	14.9	16.7	15.1	14.8	16.8	15.7	15.2	15.2
Ala	14.2	16.8	14.4	14.8	15.5	15.6	14.6	15.3	14.7	14.4	14.7	20.0	14.5	13.2	16.5	15.4	14.7	16.1	16.3	12.6	15.7	15.5	15.5	14.1	15.0	14.7	15.3
Phe	11.1	8.8	10.9	9.6	9.4	9.2	10.7	7.7	10.6	10.4	9.1	8.9	10.2	9.6	10.0	9.7	9.9	10.6	9.7	10.1	10.9	9.4	10.4	10.9	9.2	10.2	9.9
Tyr	5.4	4.2	5.7	4.8	4.8	4.8	5.1	5.2	5.3	5.1	5.5	3.9	4.9	5.7	4.4	4.3	5.0	5.0	4.7	5.2	5.3	4.9	5.4	5.0	4.7	5.4	4.9
Val	8.2	10.9	8.7	7.1	9.3	8.4	8.7	10.0	8.8	7.9	10.8	8.4	9.0	8.8	8.6	7.9	9.3	8.2	7.5	10.5	7.7	10.2	8.5	8.3	9.8	9.1	9.0
Ile	5.2	7.2	5.2	4.4	5.8	5.2	5.0	6.9	5.2	4.7	7.1	5.0	5.2	5.8	5.3	4.7	5.6	4.7	4.2	6.3	4.4	6.7	5.1	4.8	5.9	5.6	5.5
Other	21.8	20.9	18.5	23.4	18.2	17.1	20.8	16.1	19.9	22.6	11.2	26.1	19.5	20.4	20.4	19.8	18.4	24.4	25.2	16.9	22.5	16.3	17.7	23.5	18.1	19.7	19.5
Trp[b]	0.0	0.4	0.5	0.0	0.4	0.1	0.2	0.5	0.6	0.5	0.9	0.0	0.5	0.2	0.2	0.0	0.5	0.1	0.0	0.8	0.3	0.3	0.4	0.0	0.3	0.1	0.3
Lys[c]	6.2	5.0	5.5	7.6	4.5	4.3	5.6	3.7	4.3	5.9	1.8	10.1	5.1	5.0	5.7	4.9	4.4	7.2	8.2	3.9	6.0	3.9	4.4	7.0	4.1	4.8	5.1
Ser	3.9	3.6	2.8	2.9	3.4	3.2	3.8	2.8	4.4	3.8	3.2	3.4	3.9	4.0	3.4	3.6	3.9	4.3	4.0	4.2	4.1	3.4	3.3	4.2	3.8	3.9	3.7
Gly	2.2	2.4	1.9	2.1	1.9	2.3	2.1	2.0	1.8	2.8	0.0	3.5	1.6	2.5	2.7	3.2	1.6	2.9	2.7	0.9	2.4	1.8	2.0	2.2	1.8	2.2	2.1
Arg	5.8	5.5	4.2	7.6	4.9	4.4	5.5	4.0	5.4	5.9	2.8	5.5	5.2	4.9	5.0	4.3	4.5	6.0	6.3	4.3	6.5	4.0	4.4	6.4	4.7	4.9	5.0
Thr	3.7	4.0	3.6	3.2	3.4	3.4	3.6	3.7	3.3	3.7	2.4	3.6	3.4	3.8	3.4	3.8	3.4	3.9	4.0	2.8	3.2	3.3	3.3	3.7	3.3	3.7	3.5
Met[b]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Total faa mg g^{-1} ffdm	14.5	6.6	12.3	19.6	7.4	9.2	15.4	7.0	14.5	15.6	6.8	24.3	15.2	10.6	17.4	12.1	11.3	20.0	20.3	9.1	17.7	7.4	12.9	17.0	10.9	11.6	13.0
SD	–	1.4	–	–	1.5	4.4	1.2	1.1	–	2.5	0.2	1.5	1.7	–	3.4	–	1.1	3.9	–	–	1.5	0.7	1.1	–	1.1	1.3	4.7

Abbreviations: Bra: Brazil; Bra T.g.: *T. grandiflorum*; Cam: Cameroon; Dom: Dominican Republic; Ecu: Ecuador; Ecu Mon: EET 95, EET 48, EET 103, CCN 31, CCN 51; Gha: Ghana; Hai: Haiti; Ind ns: non specified sample of Indonesia; Ind Java: Java; Ind Sul.: Sulawesi; Ind Sum.: Sumatra; Ivo: Côte d'Ivoire; Lib: Liberia; Mad: Madagascar; Mal: Malaysia; Nig: Nigeria; Png: Papua New Guinea; Sao: São Tomé; Sie: Sierra Leone; Sol: Solomon Islands; Tan: Tanzania, Tog: Togo; Tri: Trinidad; Uga: Uganda; Ven: Venezuela

[a]glutamine and histidine were not separated by HPLC and are both included in the sum of acidic amino acids

[b]in 25 samples the amount of methionine and threonine was not analysed, accordingly the N value differed for the respective countries as following: Gha: 2; Hai: 3; Java: 3; Sum: 1; Ivo: 11; Mad: 4; Nig: 11; Png: 6; Tan: 3; Tog: 4; Uga: 6; Ven: 2

[c]in 13 samples the amount of lysine was not analysed, accordingly the N value differed for the respective countries as following: Ivo: 9, Mad: 5, Nig: 11, Png: 6, Tan: 3, Uga: 8, Ven: 3

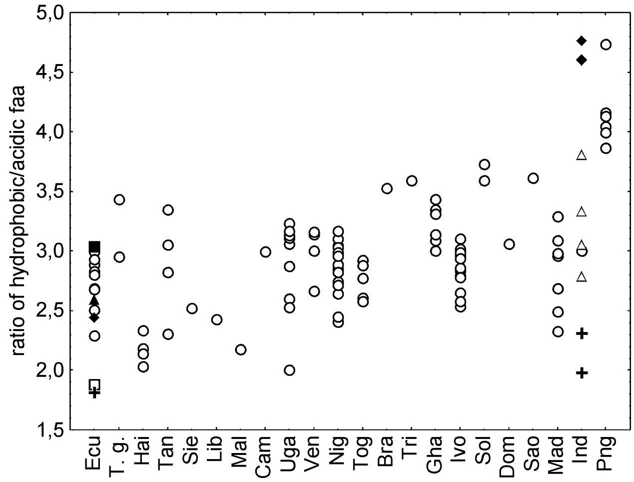

Fig. 2 Ratio of hydrophobic/acidic free amino acids of all 110 samples. Abbreviations: faa: free amino acid; symbols: as in Fig. 1

amounts of total free amino acids concurrently revealed a low ratio between 2.0 and 2.3 of hydrophobic to acidic free amino acids (Figs. 2 and 3). Similar low values were calculated for the Ecuadorian monoclonal samples "EET 95" (1.9), "EET 103" (1.8) and "CCN 51" (2.4). Ratios of 2.6 and 3.0 were found for the monoclonal fermented samples "EET 48" and "CCN 31." Ratios as high as 4.6–4.8 were observed in samples with higher total free amino acid amounts e.g. the Sumatra samples and some Papua New Guinea samples (Figs. 2 and 3). As illustrated in Fig. 3, a positive correlation (r=0.7; p<0.01) between total amount of free amino acids and the ratio of hydrophobic to acidic free amino acids was observed.

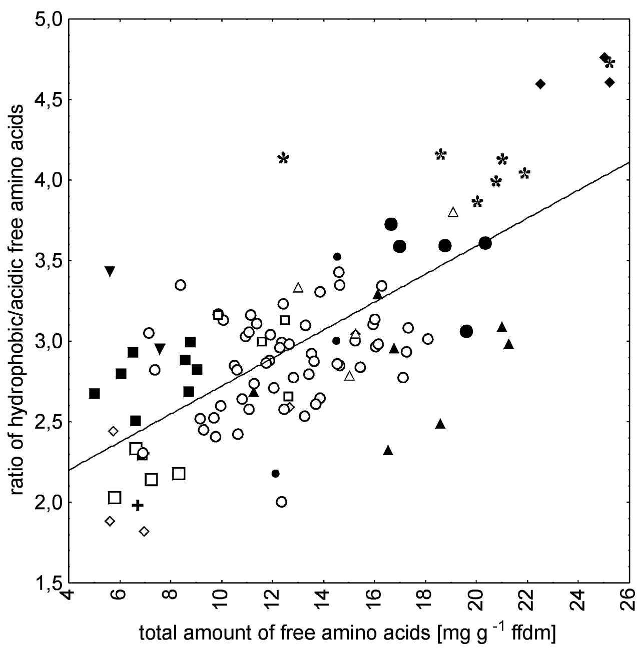

Fig. 3 Ratio of hydrophobic/acidic free amino acids versus total free amino acid content. Symbols: (○) African cocoa samples: Côte d'Ivoire, Cameroon, Nigeria, Uganda, Tanzania, Togo, Ghana, Sierra Leone, Liberia; (□) Haiti; (◇) Ecuador (EET 48, EET 103, EET 95, CCN 31, CCN 51); (■) Ecuador; (▲) Madagascar; (•) Solomon Islands, Trinidad, São Tomé, Dominican Republic; (□) Venezuela; + Sulawesi; (△) Java; (⧫) Sumatra; *Papua New Guinea; (▼) *Theobroma grandiflorum*; (●) Malaysia, Brasil, Indonesia (unspecified sample). Correlation coefficient: r=0.7; p < 0.01

Theobroma grandiflorum

Data on aroma precursors of fermented *T. grandiflorum* seeds is still scarce. The two *T. grandiflorum* seed samples from Brazil contained 5.6 and 7.6 mg g^{-1} ffdm free amino acids, the ratio of hydrophobic to acidic amino acids were 3.4 and 3.0, respectively (Figs. 1 and 2).

Acrylamide

In the current discussion, free amino acids in foodstuffs are linked to the potential formation of acrylamide. Acrylamide is formed via Maillard reactions. Asparagine in combination with reducing sugars is generally seen as the main precursor for the formation of acrylamide [19–23]. The cocoa samples contained between 397 and 1 947 mg kg^{-1} ffdm asparagine, on average 924 mg kg^{-1} ffdm. For comparison: in potatoes 5 435-8 120 mg kg^{-1} dm asparagine (fresh-stored) is detectable; reducing sugar contents of potatoes are as high as 1 970-4 614 mg kg^{-1} dm (fresh-stored) [24], whereas unfermented cocoa contains 271 mg kg^{-1} and fully fermented contains 80 mg kg^{-1} reducing sugars [9].

The asparagine content was positively correlated (r=0.7; p<0.01) with the total amount of free amino acids (Fig. 4). Accordingly, highest amounts were present in samples

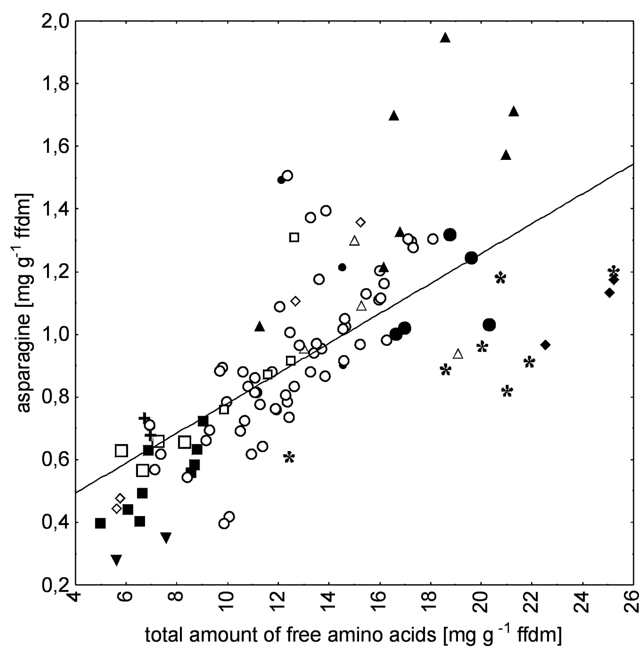

Fig. 4 Asparagine contents versus total free amino acid content. Symbols as in Fig 3. Correlation coefficient: r=0.7; p < 0.01

from Sumatra (Indonesia), Papua New Guinea and Madagascar, lowest amounts were recorded in *Theobroma grandiflorum* samples and samples from Uganda, Nigeria, Ecuador, Tanzania and Haiti.

Discussion

Formerly many studies about the changes of free amino acids in the course of the fermentation have been undertaken in the context of aroma precursor formation. But so far no comprehensive study focussing on the variation of the content and distribution of free amino acids of the commodity raw cocoa has been made.

Many parameters effect the composition of the commodity raw cocoa: climate in the course of ripening, degree of ripeness, nutritional state of the mother plant, genetic material and the processing steps fermentation, drying, transport and storage.

This examination showed clearly that content and distribution of free amino acids in raw cocoa from different origins varies greatly and although many factors influence the content of free amino acids, in some cases country and even region-specific differences were apparent. Causes of such differences remain unclear, since commercial samples usually lack information on fermentation and drying practices as well as planting material used. But, even though sample-specific information is lacking, overall information on the common fermentation practices and knowledge of the planting materials in use is available for many countries and regions.

For example, countries and regions like Ecuador, Haiti, Tanzania and Sulawesi are known for traditionally short or, in the case of Sulawesi, often omitted fermentation processes [25]. With the exception of the monoclonal fermented samples from Ecuador, all samples from respective countries contained low amounts of free amino acids.

In contrast, higher amounts of free amino acids were found in West African countries like Ghana and Côte d'Ivoire. Both countries are considered to produce bulk cocoa with a reliable average quality produced from 3 to 6 days heap fermentations [25].

In the case of Indonesia, samples could easily be separated according to their amounts of free amino acids. These differences are likely to be origin specific: the proveniences Sumatra, Sulawesi and Java are characterised by differing clonal material planted and/or fermentation practices applied [25, 26].

It is known, that well fermented cocoa contains about 8 to 14 mg g^{-1} ffdm and unfermented cocoa contains as low as 2 to 4 mg g^{-1}ffdm of free amino acid, however, it was unknown so far, that contents as high as 25 mg occur in fermented cocoas. Interestingly these high amounts of free amino acids were limited to samples from Madagascar, Papua New Guinea and Java, Indonesia. The high variation of the results from Madagascar and Papua New Guinea remains unexplained.

Controversial results of the monoclonal fermented samples from Ecuador indicate that other factors apart from the applied fermentation procedure may influence the final amount of free amino acids of raw cocoa, possibly the genetic background of the crop trees. However, even for unfermented cocoa seeds, significant differences have been reported: according to Kirchhoff [5] and Hansen [6] fresh cocoa seeds contain between 2 and 4 mg g^{-1} ffdm. Cros [17] describes a comparative study on unfermented cocoas comprising nine Forasteros from Côte d'Ivoire, one Sanchez from the Dominican Republic and one Trinitario from Madagascar. The author stated that in contrast to the rest of the samples the Trinitario contained double the amount of total free amino acids. In addition to the fermentation process, therefore, the initial amount of free amino acids contributes to the total amount of free amino acids in fermented cocoa seeds. The longer the fermentation process the lesser this influence. It remains an open question whether these differences are caused by the degree of ripeness, abiotic factors or clonal material. It does show, however, that in the case of insufficient or short fermentation procedures the initial content of free amino acids in unfermented beans can be decisive.

Theobroma grandiflorum

Data on fermented *T. grandiflorum* seeds is so far lacking. According to Reisdorff et al. [27] the seeds of *T. grandiflorum* could be used as a source for chocolate-like aroma if fermentation procedures were adapted to the seed characteristics. In respect to the formation of aroma precursors the main differing seed characteristics are seed size, morphology of seed testa and cotyledon [28], amount of vicilin class globulin and the activity of the endoprotease [27]. Compared to *T. cacao* the values for the amount of free amino acids in the processed seeds of *T. grandiflorum* indicate a low- to normal fermentation; the ratio of hydrophobic to acidic free amino acids suggests that the seeds of *T. grandiflorum* underwent sufficient fermentation. Thus, in terms of amount and composition of free amino acids both samples provide the prerequisites for the formation of chocolate-like aroma, if cocoa seeds were set as a standard.

However, further research on the formation of aroma precursors in *T. grandiflorum* seeds in the course of the fermentation needs to be conducted in order to appraise these values accordingly.

Acrylamide

Free amino acids, esp. asparagine in combination with reducing sugars build the basis for acrylamide formation within the Maillard reaction in the roasting process. Raw cocoa contains in general considerably low amounts of asparagine and reducing sugars.

Conclusions

Generally, fermented cocoas can be differentiated from unfermented cocoas by the amount of free amino acids and by the ratio of hydrophobic to acidic free amino acids. In the case of genetically identical material, the authors of this study propose using this ratio as a suitable indicator for the fermentation procedure. In cocoa lots composed of seeds derived of various clones sufficient fermentation in general results in the same factors i.e. high content of free amino acids and high ratio of hydrophobic/acidic free amino acids. In the case of selected cocoa varieties or subspecies like Arriba or Criollo it may be possible that due to testa differences the acidification and, as a result, the induction of proteolysis occurs earlier and thus the high amounts of free amino acids and ratios are reached after a shorter period of time.

In terms of free amino-acids-related aroma precursors, the seeds of *Theobroma grandiflorum* are suitable for the production of chocolate-like products.

Acknowledgements We would like to thank the members of the "Hamburger Rohkakaoverein" that provided cocoa material from the Hamburg Harbour. For providing monoclonal fermented cocoa from Ecuador we especially thank Dr. Bucheli (Nestlé). The work was part of a project financed by the Foundation of the German Cocoa and Chocolate Industry.

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