Characterization of Cocoa Butter and Cocoa Butter Equivalents by Bulk and Molecular Carbon Isotope Analyses: Implications for Vegetable Fat Quantification in Chocolate

Jorge E. Spangenberg*

Institut de Minéralogie et Géochimie, Université de Lausanne, BFSH-2, 1015 Lausanne, Switzerland

Fabiola Dionisi

Nestlé Research Center, Vers-chez-les-Blanc, 1000 Lausanne, Switzerland

The fatty acids from cocoa butters of different origins, varieties, and suppliers and a number of cocoa butter equivalents (Illexao 30-61, Illexao 30-71, Illexao 30-96, Choclin, Coberine, Chocosine-Illipé, Chocosine-Shea, Shokao, Akomax, Akonord, and Ertina) were investigated by bulk stable carbon isotope analysis and compound specific isotope analysis. The interpretation is based on principal component analysis combining the fatty acid concentrations and the bulk and molecular isotopic data. The scatterplot of the two first principal components allowed detection of the addition of vegetable fats to cocoa butters. Enrichment in heavy carbon isotope (^{13}C) of the bulk cocoa butter and of the individual fatty acids is related to mixing with other vegetable fats and possibly to thermally or oxidatively induced degradation during processing (e.g., drying and roasting of the cocoa beans or deodorization of the pressed fat) or storage. The feasibility of the analytical approach for authenticity assessment is discussed.

Keywords: *Cocoa butter; cocoa butter equivalent; vegetable fats; carbon isotope; CSIA; authenticity*

INTRODUCTION

Chocolate is produced from fat extracted from cocoa beans. The cocoa plant is an evergreen tropical tree (*Theobroma cacao*) originating from the Amazonian rainforest and grows within 20° of the equator. Cocoa butter (CB), a yellowish fat solid at room temperature, is obtained by hydraulic pressing of the fermented, cleaned, dried, roasted, and decorticated beans. The pressed butter has a distinctive flavor and is used directly for making chocolate: a finely ground mixture of cocoa butter, sugar, some additives, and also milk solids in the case of milk chocolate. The molten chocolate mass is passed through tempering units to nucleate and partially crystallize in the fine form (β-modification) of the major glycerides. Incorrect processing and high-temperature storage can result in the CB recrystallizing in rather coarse crystals and appearing as a white bloom on the chocolate surface (*1*). The addition of crystallization germs (e.g., monounsaturated triglycerides) or another type of vegetable fat may alleviate these problems. The vegetable fats added to CB are distinguished by their functional properties (*2*, *3*), and are obtained from natural plants or produced from vegetable fats by physical separation, hydrogenation, chemical or enzymatic fractionation, and blending (*3*−*10*). Cocoa butter alternative (CBA) is the general term applying to confectionery fats used in partial or whole replacement of CB. Cocoa butter equivalents (CBEs) are individual fats or mixtures of vegetable fats, not containing lauric acid, having chemical and physical properties similar to those of CB; due to this they may be added to cocoa butter in any amount without affecting the physical behavior of the latter. Cocoa butter extenders (CBEXs) and cocoa butter improvers (CBIs) are subgroups of CBEs (*3*). CBEXs are not mixable in every ratio with CB, whereas CBIs are similar to true CBEs, but with higher contents of solid triglycerides; therefore, they are used for improving soft cocoa butters. Cocoa butter replacers (CBRs) are non-lauric plant fats with a distribution of fatty acids similar to that of CB but a completely different triglyceride structure; due to this different chemical composition they may be added to cocoa butter only in small amounts. Cocoa butter substitutes (CBSs) are plant fats containing lauric and myristic acids, with some physical similarities but chemically totally different from cocoa butter; due to this they are suitable only for whole replacement of CB. In this paper no differentiation will be made among the subgroups of CBEs (e.g., true CBEs, CBEXs, and CBIs), and we will refer to them all as cocoa butter equivalents. CBEs may be used for technological reasons (e.g., to achieve special textures and chocolate behavior) or for economical reasons, even though the prices of some CBs may be lower or comparable to those of CBEs. CBEs are produced from fats of tropical trees, including palm, shea, illipé, sal, kokum, and mango. The addition of these vegetable fats in chocolate is permitted by a recent European legislation (EU Directive 2000/36/CE of June 23, 2000, *Off. J. Eur. Community*, L197/19): they cannot exceed 5% of the final product. Therefore, cocoa butter can be replaced by ∼15% of a CBE in chocolate products containing 30% total fat. The fatty acid composition of chocolate varies with the geographical origin of the CB and type of CBEs and other additives used in its

* Corresponding author [fax +41 (0)21-692-4305; telephone +41 (0)21-692-4365; e-mail Jorge.Spangenberg@imp.unil.ch].

10.1021/jf001509g CCC: $20.00
Published on Web 08/17/2001

preparation (e.g., lecithins and mono- and diglycerides). The major fatty acids of CB, including palmitic (16:0, 25−30%), stearic (18:0, 31−37%), oleic (18:1, 31−38%), and linoleic (18:2, 2.2−4.8%), are accompanied by small amounts (up to 1.8%) of other saturated and polyunsaturated C_{14}−C_{20} acids (*1*). The fatty acid compositions of the vegetable fats used for production of CBEs (e.g., palm oil, 44% 16:0, 4% 18:0, 40% 18:1, and 10% 18:2; illipé butter, 18% 16:0, 46% 18:0, and 35% 18:1; shea butter, 3% 16:0, 44% 18:0, and 46% 18:1; and sal fat, 5% 16:0, 43% 18:0, and 40% 18:1) are similar to that of CB (*12*). This overlap in fatty acid composition makes the identification of the presence of CBEs and their quantification after addition to CB difficult. Different analytical approaches have been proposed for the identification and quantification of CBEs in chocolate mixtures. These include determination of fatty acid or triglyceride profiles or analysis of minor constituents as sterols, sterol degradation products, terpenes, and triterpene alcohol degradation products (*13*−*18*). Lipp and Anklam (*19*) have recently reviewed the analytical methods for the identification and quantification of CBEs in chocolate. Nevertheless, at the moment no reliable analytical method is accepted by the scientific community for the quantification of CBEs in chocolate.

Stable carbon isotope analysis is a very useful technique for assessing the authenticity of vegetable food products from plants of different photosynthetic pathways (*20*, *21*). Briefly, the carbon isotope composition of plants and their products is linked to the processes of photosynthetic atmospheric CO_2 fixation into organic compounds. The most important reactions used by plants to fix CO_2 follow the C_3 and C_4 pathways (*22*, *23*). C_3 plants use the Calvin cycle for CO_2 fixation, and their carbon isotope compositions fall into the range of −34 to −22‰. The C_4 plants comprise most plants in the tropics, including maize and sugar cane, use the Hatch−Stack cycle, and are isotopically heavier (−23 to −6‰) compared to C_3 plants. Another group of plants uses the Crassulacean acid metabolism (CAM) photosynthetic pathway, and their isotopic composition range is between those of C_3 and C_4 plants (−33 and −11‰). Factors other than the CO_2 fixation pathway, however, may also have some impact on the isotopic composition of plants. These include local atmospheric CO_2 concentration, plant variety, water availability, cultivation practices, and other factors affecting the physiology of the plant (*22*−*25*). Therefore, the carbon isotopic composition of vegetable fats and their individual lipids may provide some information on the geographical origin of C_3 and C_4 plants. Compound specific carbon isotope analysis helps to distinguish between the natural variations of $^{13}C/^{12}C$ isotope ratios ($\delta^{13}C$) of genuine C_3 or C_4 lipids and admixtures of products from different varieties of C_3 plants (*26*−*32*). We report here the chemical and isotopic composition of fatty acids of CBs from the main producer countries of the subequatorial region and compare them with single and mixtures of CBEs from different origins and suppliers. Our expectation is that the combined chemical and isotopic analyses of the fatty acids will help the identification of CBEs in chocolate mixtures and provide a further analytical tool for the quantification of mixing ratios of CBs and CBEs in chocolate.

MATERIALS AND METHODS

Materials. Twenty-one samples of pure CB were obtained from the major growing areas of Ivory Coast, Brazil, Malaysia, Ghana, Nigeria, Indonesia, Dominican Republic, Ecuador, and Sulawesi (Table 1). All of the samples were from the 1999 and 2000 growing seasons and were obtained from different commercial suppliers. To study the effects of deodorization on CB composition, deodorized and nondeodorized CBs from Ecuador, Dominican Republic, and unspecified African−Asian origins were compared. CBE samples included individual fat-based CBEs (e.g., illipé and shea), as well as 12 commercial mixtures of tropical fats such as Illexao 30-61, Illexao 30-71, Illexao 30-96, Shokao 95, Akomax R, Akomax E, Akonord XS, Akonord XT, Akonord E, Ertina 20 NUK, Ertina 20, and Ertina E3R (Table 1). Ertina 20 NUK and Ertina E3R are not permitted for use in chocolate. All analyzed samples were non-hydrogenated vegetable fats. All of the samples were stored at 4 °C in the dark prior to analysis.

Fatty Acid Analysis. The fatty acid composition was determined using a method based on the procedure described by Muuse et al. (*33*). It comprises methylation of the sample (100 mg of fat, spiked with methyl tridecanoate as internal standard) using 2 M methanolic potassium hydroxide (2 min at room temperature). After centrifugation for 5 min at 2000 rpm, the supernatant is diluted with hexane before gas chromatographic analysis.

Gas Chromatography. A Carlo Erba HRGC 5300 gas chromatograph (Brechbühler SA, Geneva, Switzerland) equipped with a flame ionization detector (FID) was used. Hydrogen was used as carrier gas (purity > 99.9997%). A CP-Sil 88 capillary column (100 m length, 0.25 mm internal diameter) coated with 100% cyanopropyl-polysiloxane (film thickness = 0.2 μm) was purchased from Chrompack-Varian (P. H. Stehelin, Basel, Switzerland). The column was held at 60 °C for 5 min, and then the temperature was increased at 15 °C/min to 165 °C, held 1 min, and then increased at 2 °C/min until the final temperature of 225 °C was reached. Each sample solution (0.5 μL) was injected. Response factors for each fatty acid relative to the internal standard (methyl tridecanoate) were calculated using a standard solution (Nu-Chek-Prep Inc., Elysian, MN). The repeatability of the fatty acid analysis is better than 5%.

Isotopic Analysis of Bulk Oil by Elemental Analysis/Isotope Ratio Mass Spectrometry (EA/IRMS). The bulk fats were analyzed for carbon isotope composition using an on-line Carlo Erba 1108 elemental analyzer (EA) connected to a Finnigan MAT Delta S isotope ratio mass spectrometer (IRMS) via a Conflo II split interface (EA/IRMS). The EA oxidized all of the organic compounds under a stream of helium and oxygen by flash combustion in a quartz tube packed with oxidizing catalyst (chromium oxide, silver-coated cobalt oxide) at 1020 °C. The oxidation products passed through a reduction reactor packed with elemental copper and copper oxide at 640 °C to remove excess oxygen and to reduce the nitrous products (NO_x) to elemental nitrogen. Water was removed using anhydrous magnesium perchlorate, and the gases entered a chromatographic column for separation of N_2 from CO_2, which were then analyzed for their isotopic composition on the IRMS. The stable carbon isotope ratios are reported in the delta (δ) notation as the per mil (‰) deviations relative to the Pee Dee Belemnite limestone (PDB) standard

$$\delta^{13}C = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3$$

where $R = {}^{13}C/{}^{12}C$. The reproducibility of the EA/IRMS, assessed by replicate analyses of a laboratory standard material (glycine, working value = −26.1‰ $\delta^{13}C$), was better than 0.1‰ (1 SD).

Isotopic Analysis of Individual Fatty Acids by GC/C/IRMS. Separation and methylation of the fatty acids from the cocoa butter samples were performed acording to a modified procedure previously described (*31*). Compound specific carbon isotope analyses of the fatty acid methyl esters (FAMEs) were obtained by the use of a Hewlett-Packard 6890 GC coupled to a Finnigan MAT Delta S IRMS by a combustion (C) interface III (GC/C/IRMS) under a continuous helium flow (*34*, *35*). The combustion interface is composed of a ceramic furnace with

Table 1. Fatty Acid Composition of Cocoa Butters (CBs) and Cocoa Butter Equivalents (CBEs)

sample	fat[a]	origin	myristic (14:0), wt %	palmitic (16:0), wt %	palmitoleic (16:1), wt %	stearic (18:0), wt %	oleic (18:1), wt %	linoleic (18:2), wt %	arachidic (20:0), wt %	behenic (22:0), wt %	other fatty acids[b]
CB-8	CB, crude, pressed	Ivory Coast	0.05	25.37	0.27	36.85	32.84	2.68	1.11	0.20	0.26 (17:0), 0.17 (18:3), tr
CB-12	CB MP 1699	Brazil	0.08	23.75	0.25	32.79	37.52	3.89	0.91	0.22	0.21 (17:0), 0.20 (18:3), tr
CB-13	CB MP 1999	Ghana/Ivory Coast	0.10	25.52	0.26	36.24	33.21	2.78	1.04	0.19	0.23 (17:0), 0.18 (18:3), tr
CB-14	CB MP 1799	Malaysia	0.12	25.22	0.24	37.01	32.98	2.37	1.14	0.20	0.24 (17:0), 0.19 (18:3), tr
CB-19	CB RDM, deo	Africa/Asia	0.10	25.48	0.25	36.43	33.18	2.76	1.04	0.18	0.26 (17:0), 0.18 (18:3), tr
CB-23	CB AA, deo	Africa/Asia	0.11	25.48	0.25	36.69	33.01	2.69	1.04	0.19	0.23 (17:0), 0.13 (18:3), tr
CB-27	CB, deo	Ghana	0.12	25.46	0.23	36.69	32.99	2.51	1.18	0.18	0.27 (17:0), 0.18 (18:3), tr
CB-28	CB, deo	Malaysia	0.09	25.05	0.23	37.35	32.96	2.39	1.11	0.19	0.24 (17:0), 0.18 (18:3), tr
CB-29	CB, deo	Nigeria	0.16	25.22	0.26	35.92	33.47	3.02	1.03	0.18	0.20 (17:0), 0.19 (18:3), tr
CB-30	CB, ppp, deo	West Africa	0.11	25.72	0.26	35.96	33.23	2.79	1.05	0.20	0.25 (17:0), 0.18 (18:3), tr
CB-31	CB, ppp, deo	70% West Africa/ 30% Far East	0.11	25.51	0.24	36.51	33.06	2.67	1.05	0.21	0.26 (17:0), 0.18 (18:3), tr
CB-32	CB, ppp, deo	50% W Africa/ 50% Far East	0.09	25.37	0.25	36.56	33.20	2.65	1.05	0.21	0.26 (17:0), 0.17 (18:3), tr
CB-33	CB, ppp	Peru	0.07	27.86	0.29	31.66	35.14	3.25	0.96	0.20	0.24 (17:0), 0.17 (18:3), tr
CB-34	CB, ppp	Nigeria	0.13	25.58	0.27	35.29	33.74	3.21	0.99	0.16	0.23 (17:0), 0.19 (18:3), tr
CB-35	CB, ppp	Indonesia	0.11	25.13	0.27	36.88	33.06	2.50	1.12	0.22	0.24 (17:0), 0.17 (18:3), tr
CB-50	CB, bio, deo	Dominican Republic	0.11	25.38	0.28	36.18	33.34	2.97	1.03	0.15	0.21 (17:0), 0.19 (18:3), tr
CB-53	CB, bio, nondeo	Dominican Republic	0.09	25.91	0.26	35.93	33.22	2.67	1.11	0.18	0.26 (17:0), 0.19 (18:3), tr
CB-58	CB, nondeo	Ecuador	0.09	24.71	0.30	33.89	36.00	3.29	0.92	0.20	0.22 (17:0), 0.22 (18:3), tr
CB-59	CB, deo	Ecuador	0.09	25.2	0.33	34.62	34.91	3.04	0.96	0.20	0.24 (17:0), 0.20 (18:3), tr
CB-56	CB, ppp	Malaysia/Papua New Guinea	–[c]	–	–	–	–	–	–	–	
CB-57	CB, deo	Sulawesi	–	–	–	–	–	–	–	–	
CBE-1	CBE, Choclin 135557		0.55	38.88	nd[d]	21.27	32.55	3.44	0.84	0.11	0.14 (12:0), 0.15 (17:0), 1.81 (t18:1), tr
CBE-3	CBE, Coberine 154655		0.42	33.29	nd	27.69	31.76	2.94	0.96	0.12	0.12 (17:0), 2.32 (t18:1), tr
CBE-4	CBE Illexao 30-71		0.63	40.70	0.04	22.06	32.01	3.04	0.89	0.08	0.12 (17:0), tr
CBE-5	CBE, Illexao 30-96		0.33	22.18	nd	39.77	32.02	2.97	1.33	0.13	0.10 (17:0), 0.78 (t18:1), tr
CBE-7	CBE, Illexao 30-61		0.43	30.46	0.02	31.70	32.38	2.92	1.15	0.13	0.11 (17:0), 0.28 (t18:1), tr
CBE-9	CBE, Shokao 95		0.87	55.29	0.06	6.82	32.09	3.22	0.52	0.08	0.16 (17:0), 0.34 (t18:1), tr
CBE-11	CBE, Akomax R		0.44	33.78	0.03	26.05	34.11	3.22	1.01	0.14	0.13 (17:0), 0.43 (t18:1), 0.11 (18:3), tr
CBE-20	CBE, Akomax E		0.39	27.04	0.04	33.22	33.80	3.37	1.13	0.11	0.11 (17:0), 0.10 (24:0), 0.26 (t18:1), tr
CBE-21	CBE, Akonord XS		0.81	54.26	0.07	5.88	33.28	4.05	0.44	0.07	0.13 (12:0), 0.14 (17:0), 0.20 (t18:1), 0.25 (18:3), tr
CBE-22	CBE, Akonord XT		0.79	55.43	0.08	5.97	32.58	3.61	0.44	0.07	0.10 (12:0), 0.14 (17:0), 0.11 (t18:1), 0.24 (18:3), tr
CBE-23	CBE, Akonord E		0.51	37.15	0.06	22.52	34.35	3.39	0.90	0.12	0.14 (17:0), 0.39 (t18:1), 0.11 (24:0), tr
CBE-24	CBE, Ertina 20 NUK		0.57	38.57	0.07	22.65	33.09	2.67	0.50	0.45	0.12 (12:0), 0.13 (17:0), 0.61 (t18:1), 0.16 (24:0), tr
CBE-26	CBE, Ertina 20		0.55	38.69	0.05	22.30	33.52	2.89	0.57	0.43	0.10 (12:0), 0.12 (17:0), 0.27 (t18:1), 0.17 (24:0), tr
CBE-27	CBE, Ertina E3R		0.43	31.28	0.05	29.95	33.47	2.64	0.56	0.57	0.12 (17:0), 0.19 (24:0), 0.31 (t18:1), tr
CBE-31	CBE, Chocosine, Illipe		0.37	32.52	0.06	29.09	33.79	1.81	1.44	0.08	0.12 (12:0), 0.13 (17:0), 0.24 (t18:1), tr
CBE-32	CBE, Chocosine, Shea		0.49	30.79	0.02	31.27	32.18	2.94	1.05	0.13	0.22 (12:0), 0.13 (17:0), 0.39 (t18:1), 0.10 (24:0), tr

[a] ppp, pure prime pressed; deo, deodorized. [b] Lauric (12:0), margaric (17:0), α-linolenic (18:3), lignoceric (24:0), traces (tr) mainly of lauric (12:0), pentadecanoic (15:0), heptadecenoic (17:1), and eicosenoic (20:1). [c] –, not analyzed. [d] nd, not detected.

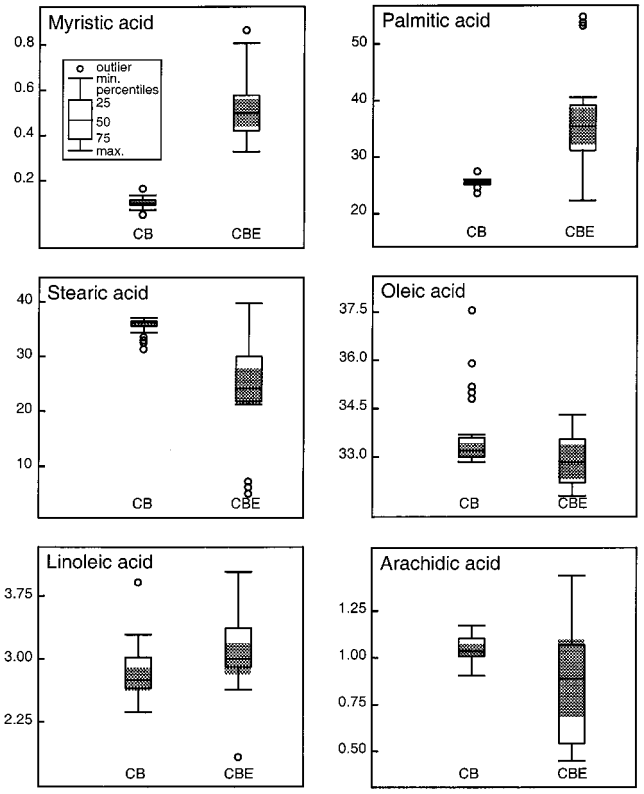

Figure 1. Ranges and medians of composition of main fatty acids in CB and CBE samples. The gray boxes display the 95% CI values for comparing medians.

copper oxide and platinum catalyst at a temperature of 940 °C. An He-flushed Nafion membrane prevented water from reaching the ion source of the IRMS. The GC was equipped with an HP-FFAP fused silica capillary column (50 m × 0.20 mm i.d.) coated with polyethylene glycol−TPA modified as stationary phase (film thickness = 0.33 μm). Helium was used as carrier gas (1 mL/min flow rate), and the injection was manual and splitless. Injector temperature was 200 °C, to prevent potential isomerization of the unsaturated fatty acids. After an initial period of 2 min at 100 °C, the column was heated to 250 °C at 5 °C/min followed by an isothermal period of 10 min. The performance of the GC/C/IRMS system, including the GC and combustion furnace, was evaluated every 10 analyses by injection of a mixture of FAMEs of known δ^{13}C values. The background subtraction and δ^{13}C values were calculated by using ISODAT 7.4 software. The reproducibility assessed from six replicate analyses of the samples ranged between ±0.1 and ±0.4‰ (1 SD). The accuracy of the GC/C/IRMS analyses was monitored by co-injection of a FAME laboratory standard of known isotopic composition. The isotopic shift due to the carbon introduced in the fatty acid methylation was corrected by a mass balance equation (*31*).

Statistical Analysis. Principal component analysis (PCA) by the software package Data Desk 3.0 was used to reduce the GC, GC/C/IRMS, and bulk butter δ^{13}C data set to a limited number of independent variables (principal components).

RESULTS AND DISCUSSION

Fatty Acid Contents. The composition of the main fatty acids in the analyzed CBs and CBEs is given in Table 1. CBs differ from CBEs by their high content in stearic acid and relatively low content in myristic and palmitic acids (Figure 1). The significant scatter of the content in saturated fatty acids of the CBEs (22.2−55.4% 16:0, 5.9−39.8% 18:0, and 0.4−1.4% 20:0) reflects mainly the variation in the source of the vegetable fats and the different mixing ratios used for their preparation. The concentrations of oleic acid are similar in both CBs (32.8−37.5%) and CBEs (31.7−34.5%). The scatter of the compositions of fatty acids for the CBs probably reflects the variation in CB variety and other factors affecting plant physiology, such as climatic conditions of the area, water-use efficiency of the cultivars, salinity, temperature, and pH of the irrigation water. Furthermore, the maturation stage of the cocoa bean and the conditions of bean fermentation may affect the fatty acid composition of the extracted butter. In CBE samples oleic acid (9*cis*-octadecenoic) is often accompanied by low levels of 18:1 *trans* isomers. In fact, high-temperature treatments (>200 °C) may produce isomerization of the *cis* monounsaturated acids by cleavage of the double carbon bond in the natural *cis* isomer and rearrangement of the atoms in a *trans* configuration (*36*, *37*). Furthermore, inadequate storage of the fats may induce oxidative degradation of the triglycerides. An increase in peroxidase activity in the cocoa seeds is observed during fermentation and drying of the beans (*38*).

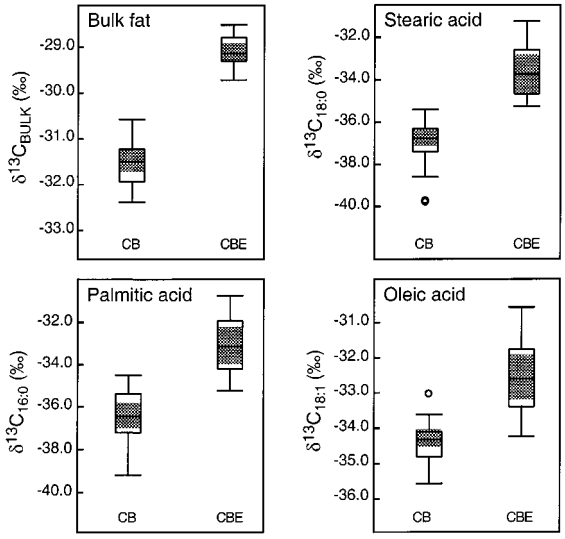

Figure 2. Ranges and medians of carbon isotope ratios of bulk fat and individual fatty acids of CB and CBE samples. The gray boxes display the 95% CI values for comparing medians.

Bulk Isotopic Composition. The δ^{13}C values of the bulk CBs (−32.4 to −30.6‰) and CBEs (−29.7 to −28.5‰) show isotopic compositions typical for plants using the C_3 photosynthetic pathway (Table 2). The scatter patterns of the δ^{13}C values of the CBs (1.9‰) and CBEs (1.2‰) are statistically different (Figure 2). These variations may be attributed to differences in the origin and variety of the fat and to mixing of different vegetable fats in the factories. Some isotopic differences may be partially explained by natural variations affecting the isotope effect during photosynthetic fixation of carbon dioxide (e.g., climate and water availability). Additionally, chemical changes (e.g., isomerization and oxidation of oleic and linoleic acids) during processing of the vegetable fats induce an isotopic enrichment in ^{13}C in the residual fat. In fact, the ^{12}C−^{12}C single or double bonds are more labile than the ^{12}C−^{13}C bonds. Therefore, loss of ^{13}C-depleted moieties occurs during thermal or oxidative breakdown of C−C bonds in the vegetable fats. However, the δ^{13}C values of the deodorized cocoa butter samples from Ecuador (CB-59) and Dominican Republic (CB-50) are similar to those of nondeodorized samples (CB-53 and CB-58). Deodorization of cocoa butter seems not to affect its isotopic composition. Addition of oxidable compounds to CB,

Table 2. Carbon Isotope Ratios of Bulk Fat and Individual Fatty Acids of Cocoa Butters and Cocoa Butter Equivalents

			δ^{13}C, ‰, PDB				
sample	fat[a]	origin	bulk fat	palmitic	stearic	oleic	linoleic
CB-8	CB, crude, pressed	Ivory Coast	−31.3	−37.5	−37.0	−34.4	−32.6
CB-12	CB MP 1699	Brazil	−31.2	−37.3	−36.2	−33.9	−33.7
CB-13	CB MP 1999	Ghana/Ivory Coast	−31.5	−36.6	−36.4	−34.6	−32.8
CB-14	CB MP 1799	Malaysia	−32.0	−37.9	−39.8	−35.1	−35.4
CB-19	CB RDM, deo	Africa/Asia	−31.6	−36.8	−37.7	−34.0	−34.1
CB-23	CB AA, deo	Africa/Asia	−31.6	−36.4	−36.9	−34.8	−32.7
CB-27	CB, deo	Ghana	−31.0	−35.8	−35.5	−33.0	−31.8
CB-28	CB, deo	Malaysia	−32.0	−37.1	−36.1	−34.1	−33.4
CB-29	CB, deo	Nigeria	−32.4	−35.3	−36.6	−35.3	−35.1
CB-30	CB, ppp, deo	100% West Africa	−31.2	−36.3	−36.7	−34.6	−32.4
CB-31	CB, ppp, deo	70% West Africa/30% Far East	−31.1	−35.5	−37.6	−35.2	−32.3
CB-32	CB, ppp, deo	50% West Africa/50% Far East	−31.8	−34.5	−35.5	−35.6	−34.9
CB-33	CB, ppp	Peru	−30.6	−37.0	−36.1	−34.2	−32.9
CB-34	CB, ppp	Nigeria	−32.2	−37.8	−37.1	−34.5	−34.0
CB-35	CB, ppp	Indonesia	−31.9	−39.3	−38.6	−34.3	−33.1
CB-50	CB, bio, deo	Dominican Republic	−31.3	−36.4	−37.4	−35.1	−33.9
CB-53	CB, bio, nondeo	Dominican Republic	−31.2	−36.8	−37.3	−34.3	−33.7
CB-58	CB, nondeo	Ecuador	−30.6	−35.3	−36.5	−34.2	−33.1
CB-59	CB, deo	Ecuador	−30.8	−35.1	−36.5	−33.9	−33.8
CB-56	CB, ppp	Malaysia/Papua New Guinea	−31.7	−34.8	−35.4	−33.6	−31.4
CB-57	CB, deo	Sulawesi	−31.9	−35.2	−37.1	−34.2	−33.9
CBE-1	Choclin 135557		−29.2	−30.7	−31.2	−30.5	−32.6
CBE-3	Coberine 154655		−29.0	−32.1	−32.5	−31.7	−31.5
CBE-4	Illexao 30-71		−29.0	−31.7	−33.5	−33.2	−32.8
CBE-5	Illexao 30-96		−28.5	−32.7	−31.7	−32.5	−31.7
CBE-7	Illexao 30-61		−28.9	−33.5	−33.0	−31.9	−32.2
CBE-9	Shokao 95		−29.4	−34.2	−34.5	−33.8	−33.9
CBE-11	Akomax R		−28.7	−34.2	−33.8	−32.6	−32.7
CBE-20	Akomax E		−28.5	−33.8	−34.8	−31.7	−32.1
CBE-21	Akonord XS		−29.6	−34.1	−34.2	−34.2	−32.3
CBE-22	Akonord XT		−29.4	−32.4	−35.2	−33.1	−33.9
CBE-23	Akonord E		−28.7	−31.0	−31.3	−30.9	−32.5
CBE-24	Ertina 20 NUK		−29.2	−31.3	−32.1	−31.8	−32.2
CBE-26	Ertina 20		−29.3	−34.5	−34.7	−33.8	−[b]
CBE-27	Ertina E3R		−29.2	−31.9	−33.6	−34.2	−32.8
CBE-31	Chocosine, Illipe based		−29.7	−34.0	−34.7	−33.0	−33.2
CBE-32	Chocosine, Shea based		−29.1	−35.2	−34.8	−31.4	−30.4

[a] ppp, pure prime pressed; deo, deodorized. [b] −, not analyzed.

Table 3. Principal Component Analysis Performed on the Fatty Acid Composition and Carbon Isotope Ratios of Bulk Oil and Main Individual Fatty Acids of Cocoa Butters and Cocoa Butter Equivalents

	principal components				
	1	2	3	4	5
variance proportion	59.9	15.2	11.8	6.9	3.7
unrotated factor loadings					
16:0	0.80	−0.19	0.56	−0.056	−0.04
18:0	−0.82	0.06	−0.56	0.08	0.04
18:1	−0.26	0.84	0.12	−0.45	−0.04
18:2	0.47	0.68	0.11	0.55	0.07
$\delta^{13}C_{bulk}$	0.91	0.05	−0.21	−0.10	−0.12
$\delta^{13}C_{16:0}$	0.89	0.01	−0.21	−0.16	0.33
$\delta^{13}C_{18:0}$	0.92	0.01	−0.28	−0.07	0.16
$\delta^{13}C_{18:1}$	0.840	0.06	−0.35	0.03	−0.37

possibly added from foreign fats, may induce ^{13}C enrichment due to oxidative rancidity.

Isotopic Composition of Individual Fatty Acids. The δ^{13}C values of the CB fatty acids vary between −39.8 and −31.4‰, and those of the CBEs range between −35.2 and −30.4‰ (Table 2). The individual fatty acids of the CBs are isotopically lighter than those of the CBEs (Figure 2). The difference in the δ^{13}C values of the bulk CBs and CBEs (up to −4‰, median $\Delta^{13}C_{CB-CBE} = -2.4$‰) and of their individual fatty acids (up to −8.6‰, median $\Delta^{13}C_{CB-CBE} = -3.3$‰ for 16:0; up to −8.6‰, median $\Delta^{13}C_{CB-CBE} = -3.0$‰ for 18:0; up to −5.1‰, median $\Delta^{13}C_{CB-CBE} = -1.8$‰ for 18:1; and up to −5.0‰, median $\Delta^{13}C_{CB-CBE} = -0.9$‰ for 18:2) are too small to be used for the quantification at low level of vegetable fats added to cocoa butters. To test the aptitude of the analytical approach to distinguish mixtures of CB and CBE, the composition of a mixture of 85% CB and 15% CBE was calculated using a two-component mass balance equation and the median values of the fatty acid concentrations and isotopic ratios

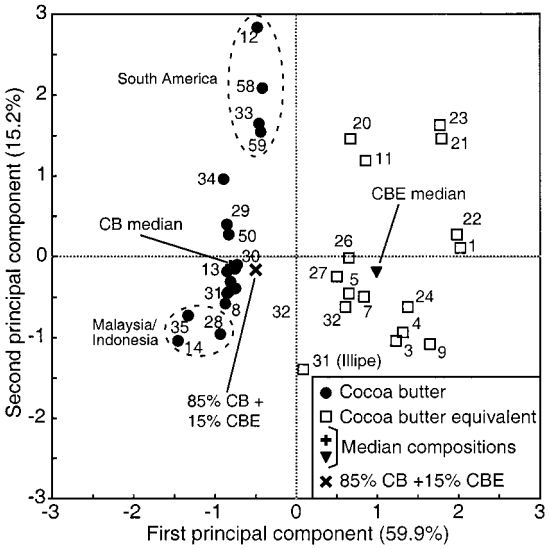

Figure 3. Scatterplot of the scores from the first two principal components for the CB and CBE samples. Loadings of the principal components are given in Table 3.

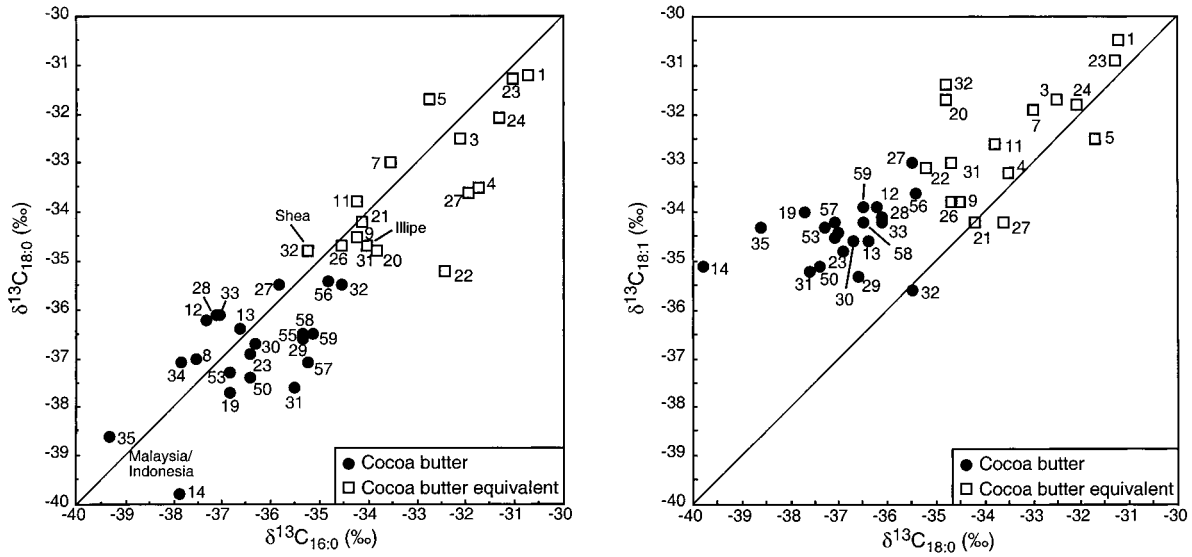

Figure 4. Carbon isotope composition of oleic acid ($\delta^{13}C_{18:1}$) versus stearic ($\delta^{13}C_{18:0}$) and palmitic acid ($\delta^{13}C_{16:0}$).

of the CBs and CBEs. This model mixture is seen as an approximate procedure for the mixture of CB and CBE and as an alternative to laboratory-prepared mixtures, which represent only a limited number of the possible combinations of CBs and CBEs. CBs are readily separated from the CBEs by PCA combining the fatty acid composition and the carbon isotope data of the bulk oil and the major fatty acids (Table 3; Figure 3). The model mixture (85% CB and 15% CBE) plots between the clusters of CBs and CBEs and can be separated from the CB median in the scatterplot of the scores of the first two principal components. Similar calculations of mixtures of other CBs and CBEs show this approach works in mixtures of CB with 15% Choclin (CBE-1), CBE Akonord XS (CBE-21), Akonord XT (CBE-22), or Akonord E (CBE-23). Mixtures with illipé fat (outlier in Figure 3) plot in the cluster of CBs. In addition, the first two principal components give information on the geographical origin of pure CBs. The CBs from Brazil (CB-12), Ecuador (CB-58), and Peru (CB-33), known to be soft CBs, are clearly separated from the CBs from Indonesia (CB-35) and Malaysia (CB-14 and CB-28), known as hard CBs (Figure 3). The distribution of the CBs and CBEs in the $\delta^{13}C_{18:0}$ versus $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:1}$ versus $\delta^{13}C_{18:0}$ diagrams reflects their different vegetable origins (Figure 4). Loss of isotopically light moieties during thermal or oxidative alteration of unsaturated acids explains the distinctive shift toward less negative $\delta^{13}C_{18:1}$ of the CBs seen in the $\delta^{13}C$ values of 18:0 and 18:1 of CBs and CBEs. Spangenberg et al. (*31*, *32*) demonstrated that a substantial separation of vegetable oils from the 1:1 line in the $\delta^{13}C_{18:0}$ versus $\delta^{13}C_{16:0}$ diagram may be used as an indication of mixing of oils and oxidative and/or thermal alteration of the unsaturated acids. Furthermore, mixing of fats of slightly different 18:1/16:0 and 18:0/16:0 concentration ratios will also induce a significant deviation from the 1:1 line in the $\delta^{13}C$ scatterplots of the major fatty acids.

Conclusions. Bulk stable carbon isotope composition and compound specific $\delta^{13}C$ measurements of the major fatty acids allow the fats from CBs and CBEs to be distinguished. PCA combining the fatty acid concentrations and the bulk and molecular isotopic ratios serve to detect low-level mixture (15%) of various CBEs in CB with slightly different fatty acid compositions. This approach seems to be unable to detect illipé fat in CB. We believe that combining the bulk and individual fatty acid stable carbon isotope composition with established qualitative methods (e.g., determination of sterol and triterpene degradation products) and quantitative methods based on the analysis of triacylglycerol subfractions will improve the precision of quantification of CBE added to CB. To validate the analytical approach, a systematic study methodologically similar to the present study with use of a blind design of randomized mixture combinations of CB and CBE of different sources and geographical origins is in progress.

ACKNOWLEDGMENT

We are grateful to C. Blancpain-Gostely, E. Prior, and B. Hug for technical assistance.

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Received for review December 21, 2000. Revised manuscript received June 26, 2001. Accepted July 5, 2001. The organic geochemical laboratory including the GC/MS and GC/C/IRMS facilities at the Department of Mineralogy and Geochemistry, University of Lausanne, was funded by the Swiss National Science Foundation (Grant 21-59198.99) and the University of Lausanne. This is a contribution to the IGCP 429.

JF001509G