Influence of Chemical Composition on the Isothermal Cocoa Butter Crystallization

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ABSTRACT: The influence of chemical composition on the isothermal cocoa butter crystallization was investigated quantitatively. Apart from the fatty acid and triacylglycerol profile, the amounts of some minor components (diacylglycerols, free fatty acids, phospholipids, soap, unsaponifiable matter, iron, and primary oxidation products) were determined. With the forward model selection technique, a multiple linear regression model was established, showing the influence of chemical characteristics on the different crystallization parameters of the new model to describe the fat crystallization kinetics as developed by Foubert and others (2002). The ratios of saturated to unsaturated fatty acids and monounsaturated to diunsaturated triacylglycerols have the most important effect on the amount of crystallization, the induction time of the 2nd step of the crystallization process, and the order of the reverse reaction. The more unsaturated fatty acids and the more diunsaturated triacylglycerols, the lower the amount of crystallization; the higher the induction time for the 2nd step of crystallization, the lower the order of the reverse reaction. The amount of diacylglycerols has the most important (negative) influence on the rate constant. Other minor components with a rather pronounced influence on different crystallization parameters are the free fatty acids, phospholipids, and traces of soap.

Keywords: cocoa butter, crystallization, composition, modeling, fat

Introduction

The chemical composition of a vegetable fat, such as cocoa butter, varies depending on the growing conditions and the age of the plant (Schlichter-Aronhime and Garti 1988). In addition, the cacao variety (Chaiseri and Dimick 1989), the production process of the cocoa butter from the cacao beans (Pontillon 1998), and any refining of the butter (Hanneman 2000) can influence the composition of the cocoa butter. These differences in composition influence the physical properties of the cocoa butter, which has its importance, for example in the production process of chocolate.

Chaiseri and Dimick (1989) and Shukla (1995) observed that the induction time of crystallization as well as the solid fat content mainly depends on the triacylglycerol profile. Pontillon (1998) observed a moderate correlation between the solid fat content on the 1 hand and the amount of trisaturated and triunsaturated triacylglycerols on the other hand. However, the amount of diunsaturated triacylglycerols correlates the best with the solid fat content. The latter was also observed by Chaiseri and Dimick (1989). In a later study, the same authors (Chaiseri and Dimick 1995) discovered that samples containing a higher concentration of 1-palmitoyl-2,3-dioleolyglycerol (POO) and 1-stearoyl-2,3-dioleolylglycerol (SOO) and concomitantly a lower 1-palmitoyl-2-oleolyl-3-stearoylglycerol (POS) and 1,3-distearoyl-2-oleolylglycerol (SOS) concentration have longer induction times when they are isothermally crystallized at 26.5 °C under mild agitation (crystallization in β′ and β polymorph). Davis and Dimick (1989) statically crystallized cocoa butter isothermally at 26.5 °C. After 3 h, they observed seed crystals with a melting point of 72.4 °C and a high concentration of trisaturated triacylglycerols. Loisel and others (1998) found that during crystallization of cocoa butter in a lab-scale scraped surface heat exchanger, which leads to formation of β′ and β polymorphs, tristearoylglycerol (SSS) crystallizes 1st as a separate fraction due to its limited solubility in monounsaturated and diunsaturated triacylglycerols. Adding extra SSS consequently shortens the induction time of the 1st crystallization step but does not affect the crystallization of the remaining triacylglycerols. Hachiya and others (1989) studied the effect of seeding on the crystallization kinetics (isothermal, agitated crystallization at 30 °C) of cocoa butter and dark chocolate. They concluded that SSS in the β form does not remarkably accelerate the crystallization despite its high melting point, whereas SOS in the b form does enhance the crystallization rate.

Pontillon (1998) observed that free fatty acids increase the crystallization time of cocoa butter but only at concentrations above 2%. Shukla (1995) and Ziegleder (1988) (isothermal crystallization at temperatures between 19 °C and 23 °C leading to crystallization in the β′ polymorph) noticed that cocoa butters with a higher diacylglycerol level exhibit a slower crystallization. Gutshall-Zakis and Dimick (1993) showed that slow nucleating (when crystallizing dynamically at 26.5 °C in the β polymorph) cocoa butters contain higher amounts of phospholipids. However, Savage and Dimick (1995) and Chaiseri and Dimick (1995) (isothermal crystallization at 26.5 °C under mild agitation leading to crystallization in polymorphs β′ and β) only found a correlation between the nucleation times and the concentration of phosphatidylinositol and phosphatidylcholine.

It was the aim of this study to chemically characterize 20 different cocoa butters and to investigate quantitatively how these variables influence the isothermal, static cocoa butter crystallization, as described by the 4 parameters of the crystallization model of Foubert and others (2002). The importance of this study lies in the fact that the influence of many different chemical composition variables are studied in 1 big study. It is also important that this influence is studied using the biological variability in 20 different

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Table 1—Overview of used cocoa butters

Sample name	Sample description and supplier
CB A	Cocoa butter from West Africa (ADM Cocoa, Koog aan de Zaan, the Netherlands)
CB B	Cocoa butter from Ivory Coast (1st sample) (Barry Callebaut, Wieze, Belgium)
CB C	Cocoa butter from Nigeria (Barry Callebaut, Wieze, Belgium)
CB D	Cocoa butter from the Ivory Coast (2nd sample) (Barry Callebaut, Wieze, Belgium)
CB E	Cocoa butter from Indonesia (Barry Callebaut, Wieze, Belgium)
CB F	Cocoa butter from Malaysia (ADM Cocoa, Koog aan de Zaan, the Netherlands)
CB G	Cocoa butter from San Domingo (Barry Callebaut, Wieze, Belgium)
CB H	Cocoa butter from Ecuador (Barry Callebaut, Wieze, Belgium)
CB I	Cocoa butter from Brazil (1st sample) (Barry Callebaut, Wieze, Belgium)
CB J	Unsteamed cocoa butter of unknown origin (Barry Callebaut, Bussum, the Netherlands)
CB K	Steamed cocoa butter of unknown origin (Barry Callebaut, Bussum, the Netherlands)
CB L	Standard factory product (batch 1) (Barry Callebaut, Wieze, Belgium)
CB M	Hard cocoa butter (unknown origin) (Barry Callebaut, Wieze, Belgium)
CB N	Standard factory product (batch 2) (Barry Callebaut, Wieze, Belgium)
CB O	Cocoa butter from a mixture of beans from the Ivory Coast, Brazil, and Indonesia (ADM Cocoa, Koog aan de Zaan, the Netherlands)
CB P	Cocoa butter from Brazil (2nd sample) (Barry Callebaut, Wieze, Belgium)
CB Q	Cocoa butter causing crystallization problems in production (1st sample) (Barry Callebaut, Wieze, Belgium)
CB R	Cocoa butter causing crystallization problems in production (2nd sample) (Barry Callebaut, Wieze, Belgium)
CB S	Cocoa butter causing crystallization problems in production (3rd sample) (Barry Callebaut, Wieze, Belgium)
CB T	Cocoa butter causing crystallization problems in production (4th sample) (Barry Callebaut, Wieze, Belgium)

cocoa butters and not by adding chemical substances to cocoa butter. This is also the 1st time the influence of the chemical composition on the cocoa butter crystallization is summarized in mathematical relationships.

Materials and Methods

Cocoa butters

Twenty different cocoa butters were used. Table 1 gives a short description of each cocoa butter and the supplier.

Isothermal crystallization experiments by DSC

The differential scanning calorimetry (DSC) experiments were performed with a 2010 CE DSC (TA Instruments, New Castle, Del., U.S.A.) with a Refrigerated Cooling System. The DSC was calibrated with indium, azobenzene (Sigma-Aldrich, Bornem, Belgium) and undecane (Acros Organics, Geel, Belgium) before analyses. Nitrogen was used to purge the system. Cocoa butter (2.5 to 15.0 mg) was sealed in hermetic aluminum pans using sample preparation procedure B as described in Foubert and others (2003), and an empty pan was used as a reference. The applied time-temperature program was as follows: holding at 65 °C for 15 min to ensure a completely liquid state, cooling at 8 °C/min to 20 °C (± 0.05 °C) and keeping at that temperature until crystallization had finished. The crystallization peaks were integrated using a horizontal sigmoid baseline and the starting points and end points were determined using the calculation algorithm as described in Foubert and others (2003). In between the starting points and end points, the area (and thus the amount of heat released up to that moment) was calculated at 5-min intervals. The integration was performed with the Universal Analysis software version 2.5 H (TA Instruments).

Parameter estimation

The model of Foubert and others (2002) was fitted to the data. The differential equation of this model is expressed in terms of a variable h (-), which is the relative remaining crystallizable fat:

$$h = \frac{a_F - f}{a_F} \tag{1}$$

where f (J/g) is the amount of crystallization at time t (h) and a_F (J/g) is the maximum amount of crystallization. In contrast to f, which increases with time in a sigmoidal way, the variable h is related to the remaining supersaturation (that is the driving force for crystallization) and thus decreases in a sigmoidal way with time.

To obtain the model, the crystallization process was represented as if it is a combination of a 1st-order forward reaction and a reverse reaction of order n (-). The dynamics of h can then mathematically be written as follows:

$$\frac{dh}{dt} = K \times (h^n - h) \qquad h(0) = \frac{a_F - f(0)}{a_F} \tag{2}$$

in which K is the rate constant (1/h) and $f(0)$ (J/g) is related to the initially present amount of crystals. To simplify parameter estimation, the differential equation was converted to its algebraic solution for isothermal conditions (Eq. 3). Because the physical interpretation of a parameter "induction time" is more straightforward than that of the parameter $h(0)$ [or the equivalent $f(0)$] and because the induction time can be more easily extracted from a crystallization curve, it was decided to represent the equation as a function of t_ind_x instead of $h(0)$. The parameter t_ind_x is defined as the time needed to obtain x% of crystallization. x was chosen to be 1. Equation 4 mathematically shows the relation between f(0) and t_ind_x:

$$f(t) = a_F \times \left[1 - \left[1 + ((0.99^{1-n} - 1) \times e^{-(1-n)\times K \times (t - t\_ind_x)}\right]^{\frac{1}{1-n}}\right] \tag{3}$$

$$t\_ind_x = \frac{-\ln\left(\frac{(1-x)^{1-n} - 1}{(1 - \frac{f(0)}{a_F})^{1-n} - 1}\right)}{(1-n) \times K} \tag{4}$$

The model was fitted to the data series by nonlinear regression using the Sigmaplot 2000 software (SPSS Inc., Chicago, Ill., U.S.A.).

Chemical analyses

To determine the fatty acid profile, the fatty acids were esterified with methanol in the presence of the catalyst potassium hydroxide and the methyl esters were then separated by gas liquid chromatography according to the AOCS official method Ce 1-62. Cocoa butter was separated by high-resolution capillary gas chromatography into triacylglycerol fractions according to their molecular weight and degree of unsaturation using a thermostable polarizable column (Buchgraber and others 2000). The amount of diacylglycerols was determined by HPLC with evaporative light scattering detector (ELSD). A Spherisorb ODS-2 column (150 × 4.6 mm, 3-mm particles) (Varian, Sint-Katelijne Waver, Belgium) was used. Nitrogen gas was used as a carrier gas. The mobile phase consisted of 67:33 acetonitrile:dichloromethane and the flow rate was 0.6 mL/min.

The amount of free fatty acids was determined by dissolving a known quantity of fat in ethanol and by titration with aqueous sodium hydroxide according to the AOCS Official Method Ca 5a-40. The amount of phosphorus (giving an indication of the amount of phospholipids) was determined by inductively coupled plasma-atomic emission spectroscopy according to the AOCS recommended practice Ca 20-99. The amount of iron was determined by the same technique but at a wavelength of 259.94 nm. Traces of soap were determined by mixing with hydrated acetone and titration according to the AOCS Recommended Practice Cc 17-79. To determine the amount of unsaponifiable matter, the sample was saponified with ethanolic potassium hydroxide. The unsaponifiable matter was then separated from the matrix by an extraction with petroleum ether and dried until constant mass. The AOCS Official Method Ca 6a-40 was followed. The acetic acid–isooctane method (AOCS Official Method Cd 8b-90) was used to determine the peroxide value. The amounts of monoacylglycerols and secondary oxidation products (as determined by the p-anisidine value) were found to be negligible and were thus not further determined.

Principal component analysis

Principal component analysis (PCA) is a mathematical procedure that transforms a number of (possibly) correlated variables into a number of uncorrelated variables called principal components. These principal components are linear combinations of the original variables. The coefficients of the original variables in these linear combinations are chosen so that the 1st principal component accounts for as much of the variability in the data as possible and each succeeding component accounts for as much of the remaining variability as possible. Instead of working with all original variables, PCA can be performed and only the 1st 2 or 3 principal components can be used in subsequent analyses. The objective of PCA is thus to reduce the dimensionality (number of variables) of the data set while retaining most of the original variability in the data.

PCA was used in this research to reduce the dimensionality of the data set obtained from the determination of the fatty acid and triacylglycerol profile. Then the principal components explaining most of the variability were used in the multiple linear regression.

SPSS for Windows 10.0.5 (SPSS Inc.) was used to perform the PCA. Because the variables (the percentages of the different fatty acids and triacylglycerols) differed in magnitude, PCA was performed on the standardized variables. A Varimax rotation was applied to the principal components with an eigenvalue above 1. The aim of this Varimax rotation is to relate each original variable as much as possible to 1 principal component and as such facilitate the interpretation of the principal components.

Multiple linear regression

To investigate the influence of chemical composition variables on the crystallization parameters, multiple linear regression with a forward model selection was used. Multiple regression fits a linear model between a dependent variable (the different crystallization parameters) and different independent variables (chemical composition variables). Each independent variable has a partial regression coefficient indicating its influence on the dependent variable, while controlling for the other independent variables in the model. A multiple regression model can be built in 2 ways. With standard methods all independent variables are entered into the model at the same time, whereas with stepwise methods the independent variables are entered step by step, based on an F test. Such an F test allows deciding whether a more complex model is significantly better than the simpler model. In this research, a stepwise method, more specifically forward model selection was used. This means that the independent variable with the highest F value (lowest significance) and thus the highest correlation with the dependent variable is added to the model 1st. In a 2nd step, the variable, which then has the highest F value, is added. The model is complete when all variables with an F value above a minimum value (F-to-enter [FIN]) or a significance value below a maximum value (PIN = maximal value of P for the variable to be entered in the model), are added. A PIN value of 0.05 was used in this research.

The regression analysis was performed with SPSS for Windows 10.0.5 (SPSS Inc.). The means of the chemical composition variables were used. For the crystallization kinetics parameters, the individual values of the 5 repetitions were used.

Results and Discussion

Chemical composition of cocoa butters

Table 2 presents the relative amounts of each fatty acid and Table 3 presents the relative amounts of each triacylglycerol present. To reduce the dimensionality of the data from the fatty acid and triacylglycerol profile PCA was applied. The 1st and 2nd principal component explain 36.5% and 19.2% of the total variance of the data set, respectively. The 3rd principal component explains another 15.2% so that the 1st 3 principal components together explain 70.9% of the total variance in the data set. Figure 1 shows the loading plot of the 1st 2 principal components. The loadings are the coefficients that are used to compute the principal components from the original variables. A loading plot thus shows which of the original variables determine the values of the principal components the most. Figure 2 shows the values of these 1st 2 principal components for each of the cocoa butters. The 1st principal component is mainly determined by the ratios of saturated to unsaturated fatty acids and monounsaturated to diunsaturated triacylglycerols. Cocoa butters containing a high percentage of unsaturated fatty acids and diunsaturated triacylglycerols have a high negative value for the 1st principal component and vice versa. This means that the Brazilian cocoa butters (CB P and I), the cocoa butter partly from Brazilian beans (CB O), and the other South American cocoa butters (CB G and H) contain relatively more unsaturated fatty acids and diunsaturated triacylglycerols. This agrees with the results of Chaiseri and Dimick (1989), Klagge and Sen Gupta (1990), Schlichter-Aronhime and Garti (1988), and Shukla (1995).

The 2nd principal component is mainly influenced by the percentage of tripalmitoylglycerol (PPP), 1-myristoyl-2-oleolyl-3-palmitoylglycerol (MOP), and palmitic acid (positive influence) and the percentage of 1,3-dipalmitoyl-2-inoleylglycerol (PLP), 1-palmitoyl-2-linoleyl-3-stearoylglycerol (PLS), and 1,3-distearoyl-2-linoleylglycerol (SLS) + trioleolylglycerol (OOO) (negative influence). As shown in Figure 2, the 2nd principal component does not lead to a strong grouping of the cocoa butters. The high values for cocoa

Table 2—Fatty acid composition of 20 cocoa butters. Mean and standard deviation of 2 repetitions are reported.

Sample	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Arachidic acid (%)
A	26.2 ± 0.2	36.6 ± 0.1	33.6 ± 0.2	2.74 ± 0.18	0.895 ± 0.049
B	25.6 ± 0.1	36.5 ± 0.1	34.1 ± 0.0	2.77 ± 0.12	1.04 ± 0.040
C	26.5 ± 0.0	37.1 ± 0.2	33.1 ± 0.1	2.25 ± 0.05	1.03 ± 0.060
D	26.4 ± 0.4	36.5 ± 0.3	33.5 ± 0.1	2.70 ± 0.01	0.985 ± 0.035
E	26.1 ± 0.6	37.3 ± 0.3	33.3 ± 0.2	2.40 ± 0.11	0.990 ± 0.028
F	25.7 ± 0.1	37.1 ± 0.0	33.7 ± 0.1	2.41 ± 0.05	1.08 ± 0.010
G	26.9 ± 0.1	34.4 ± 0.1	34.8 ± 0.1	2.93 ± 0.04	1.00 ± 0.080
H	27.1 ± 0.0	35.4 ± 0.1	33.7 ± 0.2	2.62 ± 0.01	1.08 ± 0.030
I	25.1 ± 0.8	34.3 ± 0.4	36.4 ± 0.3	3.37 ± 0.13	0.910 ± 0.014
J	27.1 ± 0.3	36.3 ± 0.1	33.1 ± 0.2	2.67 ± 0.14	0.825 ± 0.205
K	25.8 ± 0.0	36.9 ± 0.2	33.5 ± 0.1	2.81 ± 0.14	0.995 ± 0.120
L	26.1 ± 0.1	36.4 ± 0.1	33.9 ± 0.0	2.61 ± 0.07	1.01 ± 0.000
M	27.6 ± 1.2	36.3 ± 0.9	32.7 ± 0.6	2.26 ± 0.08	1.07 ± 0.010
N	25.0 ± 0.2	37.7 ± 0.2	33.6 ± 0.0	2.72 ± 0.01	1.01 ± 0.060
O	26.0 ± 0.5	36.0 ± 0.1	34.2 ± 0.1	2.87 ± 0.11	0.960 ± 0.127
P	24.9 ± 0.3	32.9 ± 0.1	37.6 ± 0.1	3.71 ± 0.01	0.930 ± 0.028
Q	26.2 ± 0.2	37.0 ± 0.1	33.3 ± 0.2	2.61 ± 0.08	0.990 ± 0.014
R	26.0 ± 0.1	37.1 ± 0.0	33.5 ± 0.2	2.41 ± 0.06	0.980 ± 0.057
S	26.7 ± 0.8	36.5 ± 0.4	33.4 ± 0.1	2.37 ± 0.13	0.995 ± 0.205
T	26.4 ± 0.1	36.3 ± 0.1	33.8 ± 0.1	2.55 ± 0.05	0.980± 0.042

Table 3—Triacylglycerol composition of 20 cocoa butters

Sample	PPP (%)	MOP (%)	PPS (%)	POP (%)	PLP (%)	PSS (%)	POS (%)	POO (%)	PLS (%)	PLO (%)	SSS (%)	SOS (%)	SOO (%)	SLS+OOO (%)	SOA (%)
A	0.222	0.232	0.889	17.7	1.61	0.646	41.3	2.29	2.86	0.434	0.777	25.5	3.01	1.33	1.21
B	0.385	0.253	0.688	18.3	1.79	0.334	41.7	2.40	2.88	0.364	0.223	25.2	2.86	1.40	1.21
C	0.514	0.262	0.836	18.3	1.36	0.464	43.0	1.80	2.51	0.322	0.282	25.7	2.09	1.27	1.33
D	0.383	0.252	0.726	18.1	1.69	0.564	41.9	2.35	2.90	0.433	0.252	25.1	2.73	1.28	1.24
E	0.333	0.232	0.717	17.5	1.79	0.485	41.8	2.37	2.81	0.465	0.293	25.8	2.75	1.28	1.31
F	0.221	0.201	0.844	17.8	1.84	1.03	40.7	2.38	2.67	0.493	0.563	25.9	2.82	1.29	1.32
G	0.364	0.233	0.577	19.4	1.91	0.263	41.4	3.08	3.00	0.435	0.182	23.2	3.54	1.35	1.13
H	0.383	0.302	0.948	18.9	1.55	0.696	41.0	2.36	2.46	0.383	0.615	25.2	2.86	1.04	1.27
I	0.256	0.205	0.583	17.0	2.18	0.317	38.7	5.02	2.97	0.337	0.235	23.8	5.96	1.38	1.10
J	0.231	0.211	0.934	17.8	1.70	1.46	40.4	2.47	2.79	0.512	0.824	25.4	2.86	1.23	1.15
K	0.222	0.212	0.829	17.8	1.75	0.566	40.7	2.28	2.87	0.222	0.617	26.1	3.06	1.59	1.22
L	0.201	0.211	0.715	18.3	1.61	0.866	41.4	2.54	2.91	0.362	0.362	25.0	3.02	1.36	1.22
M	0.344	0.253	0.740	17.6	1.54	0.40	41.1	2.44	2.76	0.456	0.588	25.9	3.23	1.37	1.34
N	0.192	0.222	0.656	18.4	1.82	0.525	41.1	2.53	3.01	0.424	0.252	25.0	3.17	1.51	1.21
O	0.213	0.192	0.729	17.6	1.86	0.435	40.9	3.18	2.98	0.405	0.324	24.9	3.69	1.35	1.20
P	0.307	0.184	0.368	16.6	1.74	0.194	38.6	5.96	3.10	0.317	0.205	23.1	7.00	1.33	1.05
Q	0.404	0.272	0.768	17.4	1.51	0.839	41.4	2.53	2.72	0.404	0.717	25.2	3.14	1.39	1.25
R	0.423	0.282	0.907	17.8	1.62	0.474	41.5	2.36	2.75	0.454	0.262	25.9	2.76	1.23	1.32
S	0.415	0.273	0.961	17.4	1.69	0.648	40.1	2.45	2.72	0.466	0.668	26.3	3.13	1.47	1.31
T	0.354	0.273	0.830	18.3	1.71	0.486	40.7	2.46	2.71	0.445	0.668	25.3	3.07	1.43	1.25

butters H and C reflect their higher PPP, MOP, and palmitic acid contents. The high negative values for cocoa butters N and K reflect their higher PLS and SLS + OOO contents. The 3rd principal component (not depicted in Figure 1) is mainly influenced by the percentage of the trisaturated triacylglycerols 1-palmitoyl-2,3-distearoylglycerol (PSS), SSS, and 1,2-dipalmitoyl-3-stearoylglycerol (PPS) and by the percentage of 1-palmitoyl-2-linoleyl-3-oleolylglycerol (PLO). All 4 triacylglycerols have a positive influence on the 3rd principal component. Cocoa butter J has a high value for the 3rd principal component because of its high percentage of the trisaturated triacylglycerols PPS, PSS, and SSS.

Table 4 shows the amounts of the different minor components (free fatty acids [FFAs]), diacylglycerols [DGs], phospholipids as determined by the amount of phosphorus [P], peroxide value [PV], unsaponifiable matter [UM], soap [S], and iron [Fe]) in the 20 cocoa butters. The amount of free fatty acids varies from 1.16% to 2.77%. No link between the country of origin and the amount of free fatty acids could be detected. This is probably because not so much the country of origin but the production process and possible refining determines the amount of free fatty acids. High free fatty acid values can be due to the use of beans from diseased pods, hydrolysis by lipase from mould contamination caused by insufficient drying, extended fermentation, or too quick drying of the beans, which does not allow for an adequate loss of the volatile acids (Chaiseri and Dimick 1989; Hancock and Fowler 1994; Pontillon 1998).

The percentage of diacylglycerols varies from 0.59% to 2.22%. All cocoa butters causing crystallization problems in production contain rather low amounts of diacylglycerols, which may be surprising, considering their rather high free fatty acid levels. It is, however, possible that the diacylglycerols hydrolyze further. This would not be surprising, bearing in mind that the kinetics of attack of a diacylglycerol is faster than that of a triacylglycerol due to the lower amounts present (Pontillon 1998).

Table 4—Minor components of 20 cocoa butters

Sample	Free fatty acids (% oleic acid)	Diacylglycerols (%)	Phosphorus (ppm)	Peroxide value (meq/1000 g)	Unsaponifiable matter (%)	Soap (ppm sodium stearate)	Fe (ppm)
A	1.62 ± 0.02	0.59	22.6 ±0.1	0.05 ± 0.07	0.34 ± 0.04	69.4 ± 29.0	0.836 ± 0.074
B	2.18 ± 0.02	0.70	16.9 ± 0.4	0.20 ± 0.14	0.31 ± 0.02	124.0 ± 7.0	0.565 ± 0.099
C	2.77 ± 0.02	1.1	11.5 ± 0.2	1.8 ± 0.1	0.53 ± 0.00	3.05 ± 0.01	1.00 ± 0.03
D	1.91 ± 0.06	1.1	15.9 ± 0.7	0.35 ± 0.07	0.40 ± 0.05	0.0 ± 0.0	1.66 ± 0.61
E	1.58 ± 0.02	0.81	18.7 ± 0.1	0.54 ± 0.06	0.38 ± 0.01	172.0 ± 19.0	0.938 ± 0.174
F	1.20 ± 0.04	1.2	63.0 ± 1.0	0.64 ± 0.07	0.35 ± 0.05	329.0 ± 0.0	1.17 ± 0.04
G	1.32 ± 0.04	0.69	2.3 ± 0.20	0.44 ± 0.07	0.42 ± 0.01	27.4 ± 4.2	0.140 ± 0.037
H	1.18 ± 0.02	1.1	2.71 ± 0.05	0.83 ± 0.19	0.43 ± 0.01	7.62 ± 2.16	1.17 ± 0.23
I	1.66 ± 0.02	0.72	41.6 ± 0.3	0.20 ± 0.14	0.43 ± 0.00	157.0 ± 2.0	1.77 ± 0.04
J	1.52 ± 0.01	0.62	39.8 ± 0.5	0.55 ± 0.07	0.37 ± 0.05	220.0 ± 5.0	2.55 ± 0.08
K	1.37 ± 0.04	1.3	35.3 ± 0.6	0.64 ± 0.08	0.37 ± 0.01	165.0 ± 4.0	2.86 ± 0.04
L	2.25 ± 0.07	0.72	35.6 ± 0.4	3.1 ± 0.4	0.35 ± 0.04	149.0 ± 0.0	1.66 ± 0.08
M	1.16 ± 0.01	0.81	49.8 ± 0.2	0.30 ± 0.01	0.33 ± 0.00	82.4 ± 4.2	1.75 ± 0.07
N	1.38 ± 0.01	0.72	41.6 ± 0.2	1.1 ± 0.1	0.39 ± 0.05	195.0 ± 53.0	2.71 ± 0.04
O	1.79 ± 0.02	2.2	7.7 ± 0.20	0.0 ± 0.0	0.36 ± 0.04	0.0 ± 0.0	0.573 ± 0.150
P	1.60 ± 0.02	0.86	26.5 ± 0.3	0.15 ± 0.07	0.44 ± 0.03	51.9 ± 4.4	0.302 ± 0.047
Q	1.72 ± 0.01	0.88	37.1 ± 0.4	4.4 ± 0.2	0.45 ± 0.07	188.0 ± 26.0	5.71 ± 0.09
R	1.93 ± 0.04	0.81	39.4 ± 0.4	7.9 ± 0.8	0.34 ± 0.01	248.0 ± 4.0	5.56 ± 0.08
S	1.91 ± 0.02	0.78	48.4 ± 0.5	4.7 ± 0.9	0.41 ± 0.00	207.0 ± 16.0	5.18 ± 0.07
T	2.08 ± 0.02	0.64	38.9 ± 0.4	2.8 ± 0.5	0.38 ± 0.02	165.0 ± 5.0	4.27 ± 0.06

The phosphorus content varies between 2.30 ppm and 63 ppm, equaling 0.006% to 0.16% phospholipids. It can be remarked that all problem-causing cocoa butters (CB Q to T) contain above-average amounts of phosphorus and thus phospholipids.

The peroxide value ranges from 0 to 7.9 meq/1000 g. It is obvious that all problem-causing cocoa butters have a high peroxide value. No real trend as function of country of origin could be detected.

The amount of unsaponifiable matter varies from 0.31% to 0.53%. Included in the unsaponifiable matter are compounds such as higher aliphatic alcohols, sterols, pigments, and hydrocarbons.

The soap content varies from 0 ppm to 329 ppm. High soap contents in cocoa butter may be due to alkalizing of the cocoa nib or mass before pressing. If this alkalizing is not carefully performed, saponification can take place (Cros and Bianchi 1998; Meursing 1994).

The amount of iron varies between 0.140 ppm and 5.71 ppm. All problem-causing cocoa butters contain very high amounts of iron compared with the average. These high iron contents may be due to old machines or press devices used in the production and refining of cocoa butter.

Two rather high Pearson correlation coefficients between different chemical composition variables could be detected. The phosphorus content is positively correlated with the soap content, and the iron content is positively correlated with the peroxide value. A possible explanation for the 1st observation is that some refining treatments remove phospholipids as well as traces of soap (Gutshall-Zakis and Dimick 1993). The 2nd observation can most probably be explained by the pro-oxidant activity of iron. Hashim and others (1997) showed that the presence of a pro-oxidant such as iron accelerates the oxidation of refined cocoa butter.

Isothermal crystallization at 20 °C measured by DSC

The isothermal crystallization at 20 °C of the different cocoa butters was measured by DSC. An example of an obtained DSC ther-

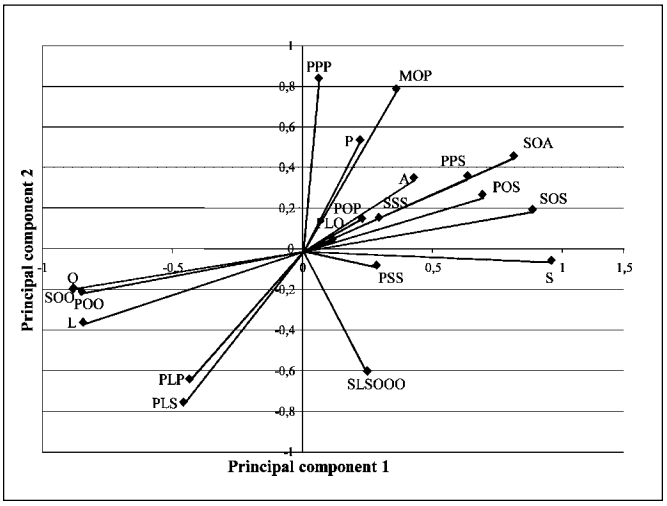

Figure 1—Loading plot of the 1st 2 principal components

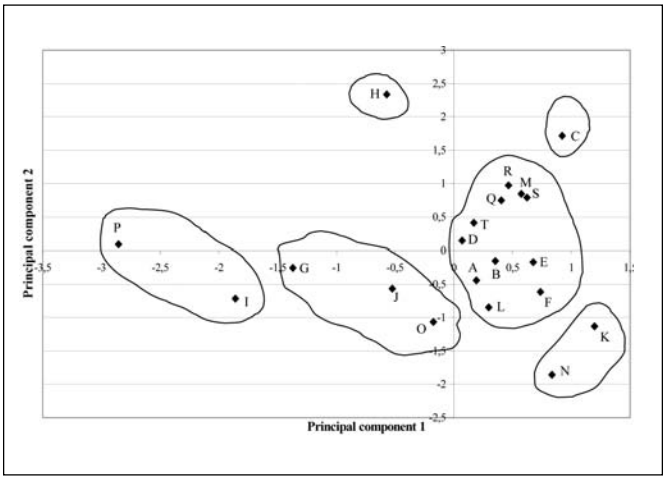

Figure 2—Separation of cocoa butters based on the 1st 2 principal components

Table 5—Parameters of the crystallization model for 20 different cocoa butters[a]

Sample	a_F (J/g)	t_ind_x (h)	K (/h)	n (-)
A	71.9 ± 2.0	0.445 ± 0.004	6.64 ± 0.15	4.02 ± 0.15
B	70.8 ± 0.9	0.520 ± 0.011	4.45 ± 0.10	4.22 ± 0.17
C	65.6 ± 1.7	0.563 ± 0.011	2.61 ± 0.03	6.46 ± 0.23
D	75.1 ± 3.3	0.500 ± 0.021	4.41 ± 0.30	4.34 ± 0.20
E	76.0 ± 1.9	0.506 ± 0.017	4.39 ± 0.11	4.58 ± 0.27
F	75.7 ± 2.1	0.421 ± 0.013	4.59 ± 0.23	5.34 ± 0.24
G	71.2 ± 1.5	0.547 ± 0.012	6.44 ± 0.37	3.86 ± 0.26
H	69.0 ± 3.2	0.498 ± 0.006	4.12 ± 0.22	4.00 ± 0.13
I	58.6 ± 0.5	1.16 ± 0.05	4.55 ± 0.40	3.00 ± 0.17
J	76.2 ± 2.2	0.532 ± 0.012	4.17 ± 0.19	3.82 ± 0.10
K	74.6 ± 1.0	0.578 ± 0.020	4.61 ± 0.31	4.14 ± 0.27
L	65.5 ± 2.1	0.523 ± 0.021	5.01 ± 0.47	3.92 ± 0.33
M	78.9 ± 2.3	0.412 ± 0.005	5.67 ± 0.34	4.90 ± 0.42
N	77.7 ± 2.3	0.414 ± 0.018	5.48 ± 0.27	5.35 ± 0.32
O	60.5 ± 1.3	0.707 ± 0.044	2.87 ± 0.26	3.39 ± 0.27
P	49.1 ± 2.6	1.45 ± 0.17	3.86 ± 0.91	2.11 ± 0.43
Q	75.6 ± 1.9	0.478 ± 0.028	3.80 ± 0.51	3.93 ± 0.23
R	76.3 ± 2.2	0.655 ± 0.012	3.15 ± 0.37	4.71 ± 0.31
S	65.3 ± 2.1	0.758 ± 0.031	2.72 ± 0.37	3.85 ± 0.36
T	72.4 ± 0.2	0.513 ± 0.018	5.06 ± 0.25	3.69 ± 0.10
Mean	70.3	0.61	4.4	4.2
Standard deviation[b]	7.6	0.26	1.1	0.9

[a]Mean and standard deviations from 5 repetitions are reported. [b]Standard deviation only taking into account the mean values for the different cocoa butters. a_F = maximum amount of crystallization; K = rate constant; n = order of reverse reaction; t_ind_x = induction time.

mogram is shown in Figure 3. Foubert (2003), on the basis of pulsed nuclear magnetic resonance (pNMR), DSC (stop and return experiments) and real-time XRD experiments, proposed that the isothermal cocoa butter crystallization at 20 °C is a 2-step process with formation of α crystals in the 1st step and formation of β′ crystals in the 2nd step. In the 1st step, part of the melt crystallizes in the α polymorph, whereas the 2nd step consists of α-mediated β′ crystallization. The latter means that the already formed α crystals transform into β′, leading to the formation of extra α crystals, which in turn transform into β′. No β′ crystals are formed directly from the melt, and no transformation from β′ to β took place in the time interval of the analysis. In the isothermal DSC experiments, the 1st crystallization step was visible as a small peak at the start of the isothermal period. However, due to overlap with the temperature equilibration, it is impossible to integrate this peak. Therefore, only the main crystallization peak was integrated, and the model of Foubert and others (2002) was fitted to the data series. Taking into account the previously proposed mechanism, this main peak represents the polymorphic transition of already formed 1:α crystals into β′ and the α-mediated β′ crystallization. The amount of α crystallization in the 1st step could be estimated by DSC by holding the sample at the crystallization temperature up to the starting point of the β′ crystallization, heating up the sample and integrating the melting peak. An example of a thus obtained DSC melting profile is shown in Figure 4. Considering that the enthalpy of fusion of the α polymorph of cocoa butter is about 80 J/g (Riiner 1970; Chapman and others 1971), the mass fraction of α crystals can be calculated from the area of the melting peak. For a standard factory product, this mass fraction of α crystals was approximately 8%. Using this mass fraction of α crystals formed in the 1st crystallization step, the contribution to the main DSC crystallization peak of their transformation to β′ can be calculated, taking into account the heat of polymorphic transition of 20 J/g (Riiner 1970; Chapman and others 1971). The contribution appears to be about 2 J/g and is thus negligible compared with the total area of the main peak, which is around 70 J/g. Parameter a_F thus represents the total amount of heat released in the 2nd step of crystallization, equaling the mass fraction β′ crystals formed in this 2nd step (taking account the latent heat of the β′ polymorph of about 100 J/g (Riiner 1970; Chapman and others 1971). Parameter t_ind_x represents the induction time of the polymorphic transition from a to β′. K is the rate constant, and n is the order of the reverse reaction. The latter reflects how long the reverse reaction affects the crystallization process: the higher n, the shorter its influence. Table 5 shows the mean values and standard deviations of the 4 crystallization parameters for each of the cocoa butters. Clearly the Brazilian cocoa butters have comparable characteristics (low a_F values, high t_ind_x values, low n values).

Influence of chemical composition on isothermal crystallization kinetics

Multiple linear regression with forward model selection was used to investigate the influence of chemical composition variables on the different crystallization kinetics parameters (a_F, t_ind_x, K, and n). To reduce the dimensionality of the data from the fatty acid and triacylglycerol profile, the 3 principal components obtained in the previous section were used as independent variables in the regression instead of the percentages of all fatty acids and triacylglycerols separately.

Influence of chemical composition on a_F

The value of the crystallization parameter a_F corresponds to the amount of crystallization in the 2nd step of the crystallization process and is thus related to the equilibrium amount of solid fat. The amount of α crystallization (not determined in this research) should, however, be added to a_F to obtain the total equilibrium amount of solid fat.

The results of the multiple linear regression with forward model selection with a_F as the dependent variable are presented in Table 6. Table 6A gives the order in which the different independent variables are added to the regression model and the corresponding R^2 values. This R^2 value corresponds to the amount of variance of the dependent variable that is explained by the regression model, thus by the independent variables entered in the regression model.

It could be deduced that the 1st principal component explains most of the variance of a_F (38.2%). Other chemical composition variables having a significant ($\alpha = 0.05$) influence on a_F, include the amounts of free fatty acids and diacylglycerols. These 3 independent variables together explain nearly 60% of the variance of a_F.

Table 6B reports the regression equation for the model with these 3 independent variables entered. The regression coefficients indicate the change in the dependent variable (in this case a_F) when the value of that independent variable increases with 1 unit, whereas the values of the other independent variables is kept constant. The standardized regression coefficients are also reported in Table 6B. These are dimensionless and give an indication of the relative importance of each independent variable: the variable with the highest absolute value has the most important influence on the dependent variable. The sign of the standardized regression coefficient indicates whether an increase in the value of the independent variable leads to an increase or a decrease in the value of the dependent variable.

Table 6B shows that the value of the 1st principal component has the most important influence on a_F: when the percentage of unsaturated fatty acids and diunsaturated triacylglycerols increases, a_F decreases. This result matches the findings of Chaiseri and Dimick (1989), Pontillon (1998), and Shukla (1995). The 1st authors explained the lower equilibrium amount of solid fat for cocoa butters with a high concentration of diunsaturated triacylglycerols by the double bond in the sn-3 position. This causes extra kinking in the structure and so interrupts the molecular packing of the monounsaturated triacylglycerols. Another possible explanation for the lower equilibrium amount of solid fat is that the diunsaturated triacylglycerols do not crystallize at 20 °C because of their β′ melting point below the crystallization temperature (2.5 °C for POO and 8.6 °C for SOO [Hagemann 1988]). Consequently, a lower percentage of triacylglycerols is able to crystallize under the given conditions.

Table 6B also shows that higher amounts of free fatty acids and diacylglycerols have a negative influence on a_F. Jacobsberg and Oh Chuan (1976) obtained similar results for palm oil. They even established a regression equation between the solid fat content and the free fatty acid content. For cocoa butter, Gutshall-Zakis and Dimick (1993) observed that refining, which involves elimination of minor components such as free fatty acids and diacylglycerols, increases the amount of solid fat.

Table 6—Multiple linear regression with forward model selection with a_F as dependent variable: A = variables entered; B = resulting regression model.

A

Variables entered	R^2
Principal component 1	0.382
Principal component 1 + free fatty acids	0.503
Principal component 1 + free fatty acids + diacylglycerols	0.596

B

Independent variable	Regression coefficient ± standard error	Standardized regression coefficient
Principal component 1	6.2 ± 0.6	0.72
Free fatty acids	–8.2 ± 1.4	–0.39
Diacylglycerols	–6.8 ± 1.6	–0.29
Intercept	91.0 ± 3.0	

Influence of chemical composition on t_ind$_x$

Table 7 represents the results of the multiple linear regression with forward model selection with t_ind_x as the dependent variable. This parameter represents the induction time of the 2nd step of crystallization, that is, the α-mediated β′ crystallization.

The value of the 1st principal component has by far the most important influence on t_ind_x. This variable alone already explains 55% of the variance of t_ind_x. The higher the percentage of the saturated fatty acids and the monounsaturated triacylglycerols, the lower the value of t_ind_x. Four other independent variables have a significant ($\alpha = 0.05$) influence on t_ind_x: the amount of diacylglycerols, phosphorus, and free fatty acids have a positive influence, while the value of hte 3rd principal component has a negative influence on t_ind_x. The latter means that a high percentage of trisaturated triacylglycerols leads to a shorter induction time for the 2nd step of crystallization. Table 7B shows the resulting regression model with t_ind_x as dependent variable.

The parameter t_ind_x represents the induction time of the 2nd step of crystallization and thus depends on the induction time for

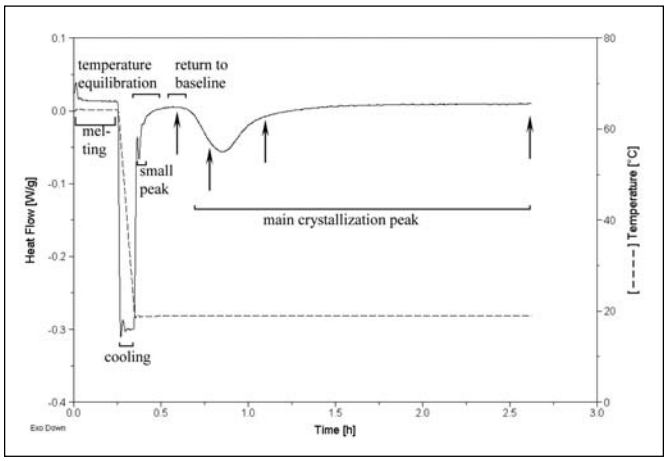

Figure 3—Example of isothermal cocoa butter crystallization at 20 °C as measured by DSC

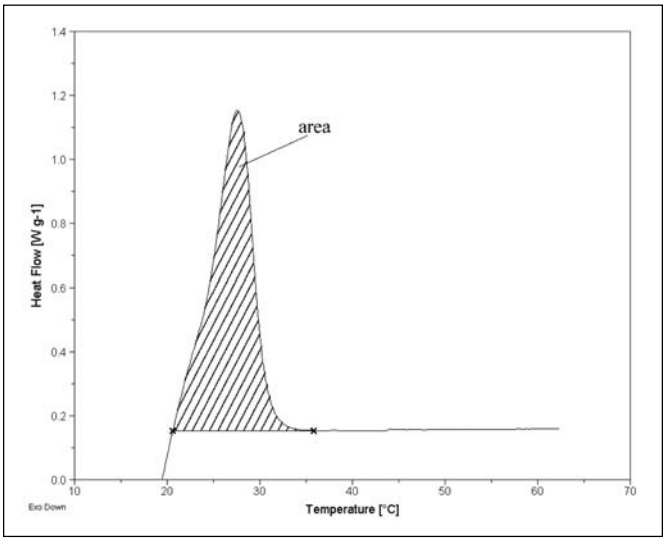

Figure 4—Integration of melting peaks obtained by stop and return experiments

the polymorphic transition from α to β′. Cebula and Smith (1992) and Smith and others (1994) also observed that diacylglycerols retard polymorphic transitions in cocoa butter equivalents and trilaurin, respectively. The structural irregularities caused by free fatty acids, diacylglycerols, and phospholipids may explain the stabilization of the metastable a phase.

Influence of chemical composition on *K*

Table 8 presents the results of the multiple linear regression with forward model selection with the rate constant *K* of the crystallization process as the dependent variable. Only 53.7% of the variance of *K* is explained by the chemical composition variables determined in this research.

K is the only crystallization parameter on which the ratios of saturated to unsaturated fatty acids and monounsaturated to diunsaturated triacylglycerols (reflected by the value of the 1st principal component) do not have the most important influence. The 1st and 2nd principal component have an impact on the rate constant but to a lower extent than the amount of free fatty acids, diacylglycerols, and soap. The 1st principal component has a positive influence, whereas the 2nd one has a negative influence. This means that the higher the percentage of unsaturated fatty acids and diunsaturated triacylglycerols, the lower the rate constant. Furthermore, an increase in the percentage of PPP and MOP or a decrease in the percentage of PLP, PLS, and SLS + OOO leads to a lower rate constant. Chaiseri and Dimick (1995) also observed that higher percentages of diunsaturated triacylglycerols retard the crystallization of cocoa butter. They attributed this phenomenon to the interference of their extra oleate chain with the molecular packing of the monounsaturated triacylglycerols. The influence of the triacylglycerols with the most important influence in the 2nd principal component has never been studied before. Table 8B summarizes the regression model with *K* as dependent variable.

The variables with the most important influence on the rate constant are the amount of diacylglycerols and the amount of free fatty acids. Both have a negative influence. Pontillon (1998) also observed that free fatty acids decrease the crystallization rate of cocoa butter. Shukla (1995) and Ziegleder (1988) also noticed that cocoa butters with a higher diacylglycerol level display a slower crystallization. The negative effect of diacylglycerols on the crystallization rate of milk fat was explained by Wright and others (2000): as the diacylglycerols become incorporated in the solids, they create irregularities in the growing crystal because of the hydroxyl group they contain instead of a fatty acid chain. This polar region, or the structural vacancy created in the lattice, may hinder the incorporation of triacylglycerol molecules into the crystal and thus the subsequent crystallization. Wähnelt and others noticed that 1,2 as well as 1,3 diacylglycerols (added to cocoa butter) increased the crystallization (isothermal crystallization at 18 °C in α and β′ polymorph) time of cocoa butter, which can be related to both an increased induction time and a decreased rate constant. These results are in accordance with the results obtained in this research.

Another chemical composition variable with a significant ($\alpha = 0.05$) influence on the rate constant is the amount of soap. The higher the amount of soap, the lower the rate constant. The influence of soap on the crystallization kinetics of fats has never been studied in detail, but it is known by chocolate manufacturers that cocoa butters containing high soap contents show crystallization problems.

It has to be remarked that β′ crystals have to grow before sufficient heat is released for the DSC to detect it, and as such the induction time as measured by DSC is also associated with crystal growth (Toro-Vazquez and others 2002). That is why t_ind_x may not only depend on the induction time for the polymorphic transition from α to β′ but also on the growth rate of the β′ crystals. It may be expected then that the chemical composition variables influencing the rate constant *K*, also influence t_ind_x but in the opposite way because a higher rate constant leads to a shorter induction time. Three of the 5 chemical composition variables influencing the rate constant *K* have an opposite influence on t_ind_x.

Table 7—Multiple linear regression with forward model selection with t_ind_x as dependent variable: A = variables entered; B = resulting regression model.

A

Variables entered	R^2
Principal component 1	0.549
Principal component 1 + principal component 3	0.605
Principal component 1 + principal component 3 + phosphorus	0.671
Principal component 1 + principal component 3 + phosphorus + free fatty acids	0.709
Principal component 1 + principal component 3 + phosphorus + free fatty acids + diacylglycerols	0.752

B

Independent variable	Regression coefficient ± standard error	Standardized regression coefficient
Principal component 1	–0.25 ± 0.02	–0.91
Principal component 3	–0.06 ± 0.02	–0.24
Phosphorus	0.006 ± 0.001	0.39
Free fatty acids	0.17 ± 0.04	0.25
Diacylglycerols	0.17 ± 0.04	0.22
Intercept	–0.02 ± 0.1	

Table 8—Multiple linear regression with forward model selection with *K* as dependent variable: A = variables entered; B = resulting regression model.

A

Variables entered	R^2
Free fatty acids	0.145
Free fatty acids + diacylglycerols	0.302
Free fatty acids + diacylglycerols + peroxide value	0.388
Free fatty acids + diacylglycerols + peroxide value + unsaponifiable matter	0.433
Free fatty acids + diacylglycerols + peroxide value + unsaponifiable matter + soap	0.460
Free fatty acids + diacylglycerols + peroxide value + unsaponifiable matter + soap + principal component 2	0.508
Free fatty acids + diacylglycerols + peroxide value + unsaponifiable matter + soap + principal component 2 + principal component 1	0.537

B

Independent variable	Regression coefficient ± standard error	Standardized regression coefficient
Free fatty acids	–1.3 ± 0.3	–0.41
Diacylglycerols	–2.2 ± 0.3	–0.61
Peroxide value	–0.02 ± 0.07	–0.04
Unsaponifiable matter	–3.8 ± 2.2	–0.15
Soap	–0.007 ± 0.002	–0.49
Principal component 2	–0.47 ± 0.13	–0.36
Principal component 1	0.28 ± 0.11	0.21
Intercept	11.0 ± 1	

Table 9—Multiple linear regression with forward model selection with *n* as dependent variable: A = variables entered; B = resulting regression model.

A

Variables entered	R^2
Principal component 1	0.442
Principal component 1 + unsaponifiable matter	0.506
Principal component 1 + unsaponifiable matter + iron	0.558
Principal component 1 + unsaponifiable matter + iron + diacylglycerols	0.586
Principal component 1 + unsaponifiable matter + iron + diacylglycerols + peroxide value	0.605
Principal component 1 + unsaponifiable matter + iron + diacylglycerols + peroxide value + phosphorus	0.627

B

Independent variable	Regression coefficient ± standard error	Standardized regression coefficient
Principal component 1	0.90 ± 0.08	0.87
Unsaponifiable matter	7.3 ± 1.5	0.36
Iron	–0.40 ± 0.09	–0.65
Diacylglycerols	–0.39 ± 0.21	–0.14
Peroxide value	0.17 ± 0.08	0.32
Phosphorus	0.02 ± 0.008	0.20
Intercept	1.9 ± 0.7	

Influence of chemical composition on *n*

Table 9 presents the results of the multiple linear regression with forward model selection with *n* as the dependent variable. This crystallization parameter represents the order of the reverse reaction of the crystallization process. Some hypotheses about the detailed nature of this reverse reaction can be given: local remelting due to a local temperature rise caused by the release of latent heat of crystallization, redissolving of crystals as suggested by Smith and others (2001), or a combination of both could be the reason for the existence of a reverse reaction. The order of the reverse reaction reflects how long it affects the crystallization process: the higher *n* is, the shorter is its influence. It can be deduced that the 6 chemical composition variables having a significant ($\alpha = 0.05$) influence on *n*, together explain 62.7% of the variance of *n*. The value of the 1st principal component has the most important (positive) influence, meaning that higher percentages of saturated fatty acids and monounsaturated triacylglycerols increase the value of *n*. A higher percentage of saturated fatty acids and monounsaturated triacylglycerols increases the melting point of the cocoa butter. At the same crystallization temperature, the necessary temperature increase for local remelting increases and so the reverse reaction may lose its influence faster, leading to an increase in the value of *n*.

Other chemical composition variables with a positive influence include the amount of unsaponifiable matter, the peroxide value, and the amount of phosphorus. The amount of iron and the diacylglycerol content have a negative influence on *n*. The influence of the diacylglycerols may be explained as follows: partial glycerides such as diacylglycerols may act as a template and thus induce a larger number of nuclei and crystals (Vanhoutte 2002) which, consequently, remain smaller. Smaller crystals have a higher surface-to-volume ratio and thus promote redissolving of molecules. Then the importance of the reverse reaction decreases slower, which is reflected in a lower value for *n*. It is possible that iron molecules act as heterogeneous nuclei and also lead to a higher amount of smaller crystals and a lower value of *n*.

Conclusions

Differences in chemical composition of cocoa butter cause differences in the crystallization kinetics. The ratios of saturated to unsaturated fatty acids and monounsaturated to diunsaturated triacylglycerols have the most important influence on all crystallization parameters except on the rate constant *K*. The diacylglycerols and free fatty acids have a similar influence on crystallization: they have a negative influence on the equilibrium amount of solid fat, the growth rate, and the polymorphic transition. Other minor components with an important influence on the crystallization kinetics are the phospholipids and traces of soap.

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