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Analytical Methods Section

Characterisation of cocoa masses: low resolution pulse NMR study of the effect of geographical origin and roasting on fluidification

C. V. Hernandez & D. N. Rutledge

Institut National Agronomique Paris-Grignon, Laboratoire de Chimie Analytique, 75231 Paris Cedex 05, France

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The evolution of solid percentage at 27·5°C for cocoa masses of various geographical origins was studied by Low Resolution Pulse NMR. Modelling of the fluidification curves revealed a bimodal system characterised by two evolution rates and two initial solid contents. A multivariate statistical analysis, based on these four parameters, separated cocoas according to process and type of roaster used. The results of the same analysis on non-roasted cocoa masses are discussed.

INTRODUCTION

The quality of the cocoa bean, its origin and the processing it has undergone all have a direct influence on the quality of the final product, chocolate. In confectionery, consumer preference is linked to the gustative and olfactory qualities of the product. These may be linked to physical and chemical properties by gas chromatographic analysis of the cocoa beans and by identification of constituents by mass spectrometry. The texture of the chocolate also depends on the hardness of the continuous fat phase and on its crystallisation state.

The crystal polymorphism of cocoa butter is of great importance in chocolate confectionery. The chocolate's quality—its crispness, fineness and gloss—depends directly on the β-crystal form (or form V). This crystal form stabilises at different temperatures depending on the triglyceride composition of the butter (Dimick & Manning, 1987; Hachiya *et al.*, 1989; Sato *et al.*, 1989).

Hardness is defined as the percentage of solid fat measured at a given temperature, following a given crystallisation cycle. All the complexity of the crystal polymorphism of cocoa butter is contained within this simple notion of Solid Fat Content (Lehrian *et al.*, 1980; Sato, 1987; Rutledge *et al.*, 1988; Arruda, 1991). For a long time, the hardness of the final product was measured by determining the chocolate bar's resistance to breaking, a coated surface's resistance to scratching or flattening, or by penetrometry. The reference method, however, is dilatometry (Wolff, 1968), but this technique is long and fastidious and therefore prone to error. Low Resolution Pulse Nuclear Magnetic Resonance may also be used for the routine determination of Solid Fat Content (SFC) melting curves (McCarten, 1974; Lambelet *et al.*, 1986; Rutledge *et al.*, 1988). This technique gives much faster results and so can be used to monitor the evolution of a sample as a function of time.

The present investigation considers the evolution of the SFC for different cocoa masses at a given temperature after a particular tempering process. Differences in the proton populations of the solid or liquid phases, as well as the rate of evolution of the solid phase, were then used to establish an index of hardness and to distinguish the different cocoas by origin.

NMR has been used by the cocoa industry to rapidly quantify the variability of the raw material as a function of its geographical origin or the year of harvest (Mohr *et al.*, 1987). Studies have shown that, during the last phase of ripening of the cacao pod, temperature plays an important role in determining the triglyceride composition of the seeds and therefore the hardness of the butter (Lehrian *et al.*, 1980).

MATERIALS AND METHODS

Sample preparation

Cocoa masses from a number of countries were studied (Table 1). In the case of drum roasters (Suppliers 1 and 2), the beans are not in direct contact with the hot air and it is possible to extract samples at different roasting stages. Supplier 3, however, uses a hot air roaster and in this case it is not possible to extract samples during the roasting process.

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Table 1. Cocoa mass samples

Geographical origin	Degree of roasting[a]	Code	Supplier number
Ivory Coast	0	IC0	1,2
Ivory Coast	1	IC1	1,2
Ivory Coast	2	IC2	1,2
Ivory Coast	3	IC3	1,2
Ghana	0	GH0	1,2
Ghana	1	GH1	1,2
Ghana	2	GH2	1,2
Ghana	3	GH3	1,2
Togo	0	TO0	4
Madagascar	0	MA0	4
Madagascar	3	MA3	3
Sumatra	0	SU0	2
Sumatra	1	SU1	2
Sumatra	2	SU2	2
Sumatra	3	SU3	2
Java	0	JA0	1
Java	1	JA1	1
Java	2	JA2	1
Java	3	JA3	1
New Guinea	0	NG3	4
Ecuador	0	EC0	2,4
Ecuador	1	EC1	2
Ecuador	2	EC2	2
Ecuador	3	EC3	2,3
Colombia	0	CO0	1
Colombia	1	CO1	1
Colombia	2	CO2	1
Colombia	3	CO3	1
Trinidad	3	TR3	3
Venezuela	3	VE3	3
Brazil	0	BRO	1,4
Brazil	1	BR1	1
Brazil	2	BR2	1
Brazil	3	BR3	1

[a] 0, Non-roasted; 1, roasted for 10 min; 2, roasted for 25 min; 3, totally roasted (35 min).

Cocoa masses were obtained from shelled beans pulverised at low temperature using a crushing roller or a helix grinder, and were stored at 25°C until used.

Masses were melted at 80°C so as to have the same continuous phase throughout the paste after cooling for 24 hours at room temperature. The glass NMR tubes (10 mm external diameter) were then filled to 30 mm, corresponding to the homogeneous region of the magnetic field of the NMR instrument's measuring head, melted once again for 20 min and left to cool. They were then ready for the tempering cycle.

Three tubes for each type of cocoa were prepared, giving 135 samples in total.

The tempering cycle must be carefully controlled to avoid irregularities during the stabilisation of the final crystal form. Variations in the properties of the samples should then only depend on the composition of butter in the masses—and therefore on their geographical origin and the changes introduced by roasting.

The samples in the NMR tubes were melted in a water bath at 80°C (± 1°C) for 30 min then placed in a second bath at 0°C (± 0·1°C) for 60 min (± 1 min). This experimental protocol is derived from the normalised IUPAC method for the tempering of fats (IUPAC, 1982).

The tubes were then introduced into the NMR apparatus maintained at 27·5°C. The progressive fluidification of the mass inside the measuring head was monitored. The temperature of 27·5°C was chosen following a preliminary survey which showed that it gave the best discrimination between masses as a function of their geographical origin.

Instrumentation and signal treatment

The Low Resolution Pulse NMR apparatus used was a Minispec pc120 (Bruker). An audio filter bandwidth of 1 MHz and a diode detector were used . The attenuation was 39 dB, the length of the relaxation delay was defined at 2 s and the value of the enhancement factor, which defines the number of scans to be executed for measurement, was 1.

NMR signal and its treatment

The Free Induction Decay (FID) signal is largely due to transverse relaxation of the protons and so may be

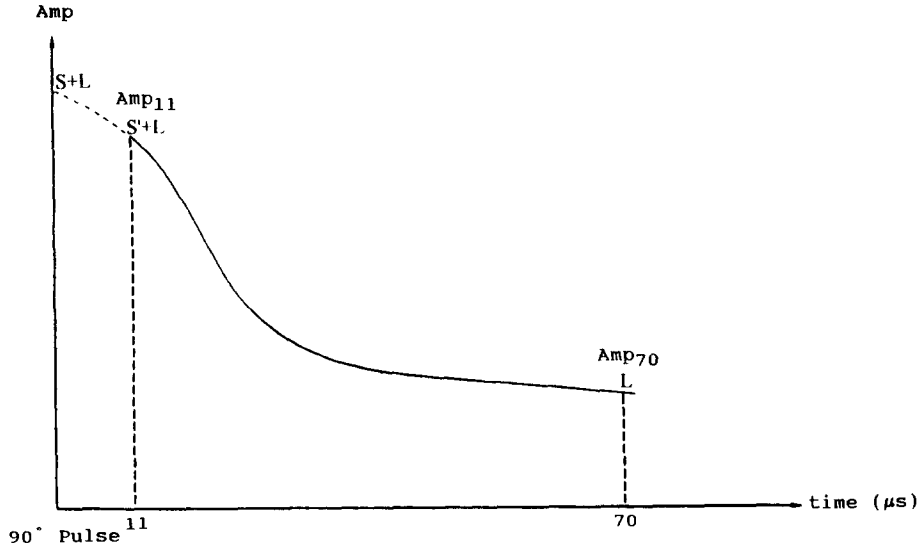

Fig. 1. Free Induction Decay.

used to determine the proportion of solid material in fat, as the transverse relaxation time (*T2*) of the solid phase protons is much shorter than that of those in the liquid phase. As shown in Fig. 1, the signal intensity following the 90° pulse is proportional to the total number of solid and liquid phase protons ($S+L$). After a delay of 70 μs, only the liquid phase protons contribute to the signal Amp_{70}. However, the signal amplitude cannot be measured just after the 90° pulse. Therefore, the quantity $S+L$ is estimated from Amp_{11}, the measurement at 11 μs (minimum delay due to instrumental constraints). To calculate the S value, a correction factor f is used.

The solid/liquid ratios were computed using the following formula

$$S/L = \frac{f \times (Amp_{11} - Amp_{70})}{f \times (Amp_{11} - Amp_{70}) + Amp_{70}} \times 100$$

Computer files were produced containing the Amp_{11} and Amp_{70} values, measured throughout the 27·5°C mass fluidification process.

Curves showing the evolution of the *S/L* ratio as a function of time were then plotted and the curves decomposed by non-linear regression, into a gaussian and an exponential component, using a program developed in the laboratory based on the Marquardt algorithm (Press *et al.*, 1988). This model was chosen as it gave a good approximation to the shape of the experimental curve.

The chi-square statistic test was used to estimate the quality of the fit of the theoretical model to the experimental data.

RESULTS AND DISCUSSION

Measurements of Amp_{11} and Amp_{70} were performed every 15 s over a period of 30 min for the first tests.

The cocoa masses were shown to take about 13 min to attain a constant *S/L* ratio at 27·5°C (Fig. 2). In preliminary studies, no significant *S/L* ratio variation was observed over a period of 3 h after the first 13 min. The total time of measurement was therefore reduced to 15 min.

The cocoa mass fluidification curve can be well approximated by the sum of a gaussian and an exponential function. The first 36 points of the fluidification curves were decomposed according to the mathematical model (I) to give empirical parameters which can then be used to discriminate the cocoa samples.

$$Y(t) = Q_S \times \exp(-t \times V_S) + Q_R \times \exp(-(t \times V_R)^2) \quad (1)$$

where $Y(t)$ is the percentage of solid at time t.

The two regions (S and R) cannot be directly assimilated to two categories of protons. They reflect differences in the rate and degree of fluidification at different stages of the process. Nevertheless, changes in the composition of the sample give rise to changes in these two regions of the curve.

The decomposition program calculates two parameters for each region of the fluidification curve: the initial solid content as a percentage (Q) and the rapidity of its evolution (V). The S index is used for the parameters of the exponential function which models mainly the region where the solid content changes slowly. The R index is used for the parameters calculated from the half gaussian representing the initial evolution where the solid ratio changes rapidly after a short, relatively stable period.

Figure 3 shows the result of a decomposition at the beginning of a curve. Calculations were done on data smoothed using a nine point Savitsky and Golay smoothing function (Savitsky & Golay, 1964; Steinier *et al.*, 1972). Figure 3 shows the two components and the smoothed experimental points.

The NMR signal is proportional to the number of protons and therefore depends on the quantity of material in the measuring head. To reduce this source

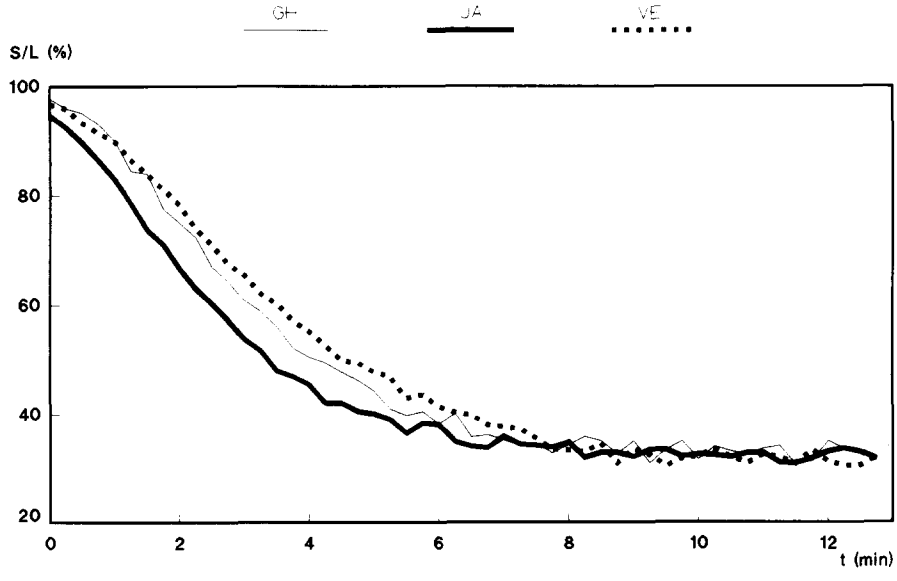

Fig. 2. Fluidification curves of three different samples: the mass of Ghana is not roasted, the masses of Java and Venezuela are roasted by, Supplier 1 and Supplier 3 respectively.

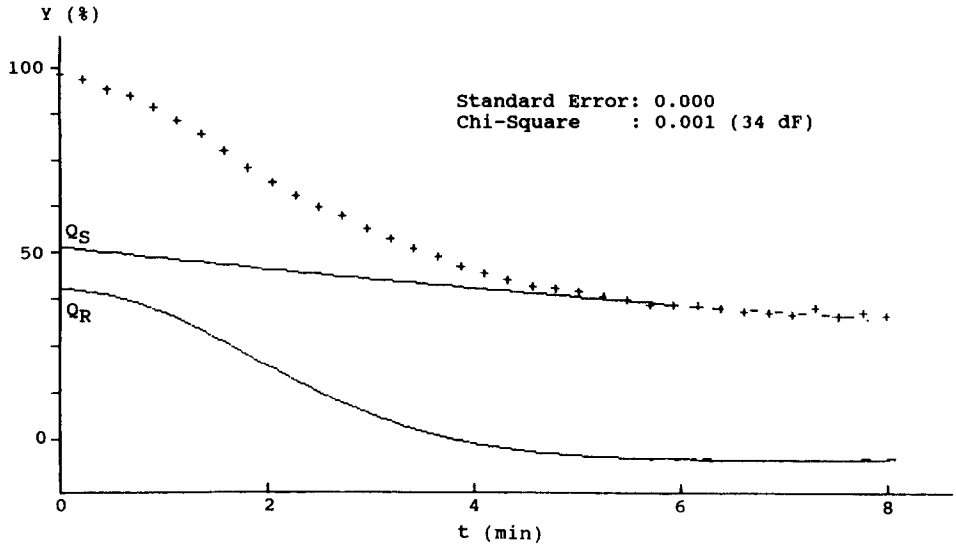

Fig. 3. Decomposition of a typical fluidification curve (mass of Ghana roasted by Supplier 2).

of variability, due to irregular filling and variations in density, the Q_S and Q_R quantities, obtained by decomposition, were divided by the mass of the sample in each tube. These new variables are named Q_{RR} and Q_{RS}.

For each cocoa mass, the averages for three tubes and the coefficients of variation are presented in Table 2. The coefficients of variation are all less than about 10%.

The scatterplot of the mean values for V_S and V_R (Table 2) does not differentiate the individuals. In the scatterplot of Q_{RS} and Q_{RR}, the samples are spread out along the Q_{RS} axis but there is no particular structure in the distribution of the individuals.

On the other hand, the plot of V_S against Q_{RS} in Fig. 4 shows that a linear trend exists between these parameters for the non-roasted samples.

The linear regression coefficient, calculated on non-roasted cocoa averages, is 0·905. The model is significant at 5%. All of the averages for Supplier 1, except for Ghana sample 1, are situated beneath the regression curve, whereas the averages for Supplier 2 are above the regression line. This supplier effect will become more evident in the following statistical analyses which were all carried out on the NMR data using the statistical package Statgraphics (STSC, Rockville Maryland, USA).

Analysis of variance

Analysis of variance (ANOVA) may be used to analyse the effect of one or more qualitative factors on a response (Lattorre, 1984). ANOVA was applied to the 135 measurements in order to analyse the effect of both the geographical origin and the degree of roasting on Q_{RR}, Q_{RS}, V_R and V_S.

The total variance is a function of the origin, the roasting and experimental error. The number of degrees of freedom is not sufficient to study the interaction between the origin and the degree of roasting.

From Table 3 it can be seen that the geographical origin of the cocoa has a significant effect on the four parameters studied, although the effect is less important for Q_{RS} The degree of roasting has a significant effect on the distribution of the number of protons

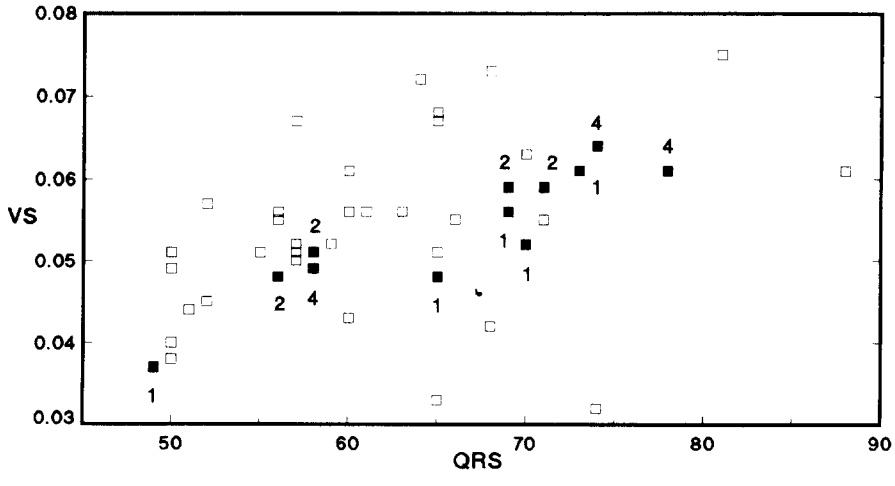

Fig. 4. Q_{RS}–V_S scatterplot of mean values for all samples. Non-roasted samples are shown in black. Beans from the three suppliers are represented by numbers 1, 2 and 4.

Table 2. Results of treatment of fluidification curves by the decomposition program (mean values)

Code	Q_{RR}	CV(%)	Q_{RS}	CV(%)	V_R	CV(%)	V_S	CV(%)
IC0	53	6·1	56	10·7	0·329	6·2	0·048	3·9
IC0	63	6·9	65	4·6	0·357	4·8	0·048	3·7
IC1	43	10·7	68	8·3	0·305	7·3	0·073	4·6
IC1	49	7·2	60	6·8	0·367	5·6	0·056	7·3
IC2	39	7·3	65	3·8	0·223	8·0	0·068	6·5
IC2	48	0·0	51	4·2	0·340	3·4	0·044	3·3
IC3	50	9·2	59	5·2	0·320	7·1	0·052	6·3
IC3	42	0·0	57	2·5	0·322	3·1	0·067	4·5
GH0	50	4·9	58	8·3	0·351	6·6	0·051	6·4
GH0	53	5·1	73	6·7	0·337	9·7	0·061	7·2
GH1	43	9·6	65	6·4	0·330	6·1	0·067	8·2
GH1	53	8·3	71	6·0	0·334	4·7	0·055	3·7
GH2	49	4·2	63	8·7	0·370	7·4	0·056	6·5
GH2	55	1·1	65	9·5	0·326	5·7	0·051	2·7
GH3	41	4·1	52	7·9	0·333	6·0	0·057	5·3
GH3	41	7·7	56	2·1	0·355	2·5	0·055	3·4
TO0	60	10·4	65	7·6	0·346	7·1	0·033	6·0
MA0	58	4·0	57	2·7	0·372	5·6	0·050	7·3
MA3	48	9·0	58	7·5	0·311	6·3	0·049	3·2
SU0	48	5·3	71	3·7	0·340	2·8	0·059	6·4
SU1	49	1·4	50	4·2	0·194	4·7	0·049	5·4
SU2	38	9·1	81	8·3	0·346	8·2	0·075	9·6
SU3	32	3·1	60	5·0	0·335	3·1	0·061	1·6
JA0	47	3·0	49	10·0	0·368	0·6	0·037	5·4
JA1	47	8·7	66	5·2	0·340	4·2	0·055	4·5
JA2	42	9·5	64	6·9	0·414	9·2	0·072	4·7
JA3	48	1·5	55	10·3	0·398	5·1	0·051	5·3
NG0	55	3·1	74	5·1	0·336	4·7	0·064	9·6
EC0	45	7·1	69	3·0	0·339	4·2	0·059	5·9
EC0	53	2·7	74	0·0	0·172	3·2	0·032	2·6
EC1	62	6·8	52	4·1	0·354	2·6	0·045	2·9
EC2	43	6·3	88	7·9	0·333	3·4	0·061	8·2
EC3	42	3·4	70	5·1	0·382	3·9	0·063	8·9
EC3	41	9·6	57	5·6	0·359	5·7	0·052	5·2
CO0	58	5·6	69	7·7	0·331	5·1	0·056	7·2
CO1	54	7·0	57	6·7	0·322	4·4	0·051	8·4
CO2	58	5·2	61	8·4	0·354	4·2	0·056	7·1
CO3	49	8·2	50	3·0	0·339	2·6	0·051	4·7
TR3	49	9·2	56	7·4	0·299	4·1	0·056	6·8
VE3	45	2·5	50	1·7	0·272	2·2	0·040	1·2
BR0	48	10·7	70	10·6	0·376	6·2	0·052	8·5
BR0	52	4·8	78	6·9	0·366	4·8	0·061	6·1
BR1	51	1·1	68	6·1	0·375	2·3	0·042	3·6
BR2	44	7·7	60	4·4	0·415	7·2	0·043	4·2
BR3	42	6·7	50	8·9	0·336	3·4	0·038	5·6

standard error < 0·001.
Chi-square = 0·001 (34 dF).

Table 3. Results of ANOVA of the 135 sets of data

	Source of variation	SS	dF	Mean square	F	Significance level
Q_{RR}						
	Origin	1742·36	11	158·40	3·856	0·000 1
	Roasting	1224·94	3	408·31	9·940	0·000 0
	Residual	4929·16	120	41·08		
Q_{RS}						
	Origin	1898·19	11	172·56	2·020	0·032 0
	Roasting	2378·54	3	792·85	9·282	0·000 0
	Residual	1 0250·39	120	85·42		
V_R						
	Origin	0·069 74	11	0·0061	4·206	0·000 0
	Roasting	0·015 09	3	0·0050	3·337	0·021 7
	Residual	0·180 89	120	0·0015		
V_S						
	Origin	0·003 71	11	$3·38\times10^{-4}$	2·437	0·008 9
	Roasting	0·000 58	3	$1·94\times10^{-4}$	1·402	0·245 6
	Residual	0·016 63	120	$1·38\times10^{-4}$		

between the two regions of the fluidification curves and on the rapidity of the decrease in the R region. Only the V_S parameter is not significantly influenced by roasting.

The fact that the geographical origin influences the evolution of the proton intensity in the two regions of the curve shows that the masses differ in their fluidification behaviour. The NMR technique could therefore be a simple means of differentiating the cocoa in terms of the fat hardness without requiring extraction of the butter.

The gaussian component observed in the fluidification curves reflects the initial behaviour of the masses during reheating at 27·5°C. This is influenced by the thermal inertia of the sample, in particular the crystalline lipid system created during the tempering at 0°C. Lattice modifications progressively lead to the transformation of the mass as a whole. This process accelerates about 1 min after the beginning of fluidification. V_R therefore represents the rapidity of this initial 'fusion'. According to the ANOVA, the variation of Q_{RR} depends on the origin of the mass and therefore on the chemical composition of the crystalline lipidic lattice which differs from one cocoa to another (Dimick & Manning, 1987; Chaiseri & Dimick, 1989). The possible destabilisation effect of the phospholipids on the crystalline forms may also modify the viscosity of the system. The amphiphilic nature of phospholipids has been suggested as being the basis of their importance in early crystallisation events (Arruda, 1991).

The moisture content of the cocoa masses is less than 5%, compared to the average fat content of 54% in the beans, and so its contribution to the signal intensity would not be very great. It would, however, influence the evolution of the signal. We shall see below that the moisture content does not, however, explain the discrimination between the cocoa masses.

The second region, S, modelled by the exponential function, corresponds to the evolution of the more stable crystalline form, that is the lipid part which gives a more or less 'hard' characteristic to the cocoa butter. We may therefore assume that the greater the S fraction, the harder the fat. If the values Q_{RR} and Q_{RS} only depended on the quantity of rapidly evolving and slowly evolving fats in the sample, the ratio Q_{RS}/Q_{RR} would be directly related to the relative hardness of the mass. This is not the case.

The average composition of the shelled beans is 11·5% proteins and 16·5% carbohydrate, cellulose, starch and pentosans; these proportions are far from negligible. Q_{RS} could therefore be influenced by variations in the relative proportions of these constituents,

from one mass to another. Therefore, the Q_{RS} parameter may be overestimated as it would also take into account the constituent molecules. These molecules may also play a role in the fluidification of the mass. In the first part of the curves, their influence is masked by the changes due to fat transformation which is a much more rapid process. On the other hand, in the second part, the modification of these chemical species may become apparent. Therefore, Q_{RS} would represent the behaviour of both the protons linked to the constituent molecules and to fusion of the more stable crystalline forms of the triglycerides.

As the sample is heated at 27·5°C, changes in the three-dimensional protein configuration may occur, creating a more fluid environment.

The tail part of the 27·5°C stabilisation is also characterised by V_S. As shown in Table 3, the phase S kinetic parameter is not altered by roasting, while a more detailed ANOVA study (not shown) demonstrated that although it does not decrease regularly with degree of roasting, Q_{RS} is significantly lower at degree 3 than at degree 0. The thermal treatment to which the beans are subjected is brutal, therefore modifying the entire cellular structure so that lipids which were initially separated in the cellular structure of the beans progressively form a uniform medium. As this would facilitate the fusion, it is normal to see the number of less mobile protons decrease during roasting.

If the region S, the 'hard' part of the mass, reflects the behaviour of the saturated triglycerides in the lipid fraction, the appearance of even small quantities of unsaturated molecules, which are known to be formed as a result of the thermal treatment, could explain the variation in Q_{RS} (Chaiseri & Dimick, 1989). Hachiya *et al.* (1989) demonstrated the influence of composition on the time and temperature treatments required during the preparation of chocolate, by adding different pure triglycerides, the amount ranging from 0·01 to 0·50 Wt% with respect to the total fat content. They showed that SOS accelerated the crystallisation whereas SSS had the opposite effect.

The same ANOVA demonstrated a significant and regular decrease in the Q_{RR} value during roasting. This decrease in the proportion of the rapidly evolving constituents could be due to the progressive elimination of moisture from the masses during roasting.

The geographical origin of the beans has an impact on the V_R and V_S parameters (Table 3) and is more significant than the degree of roasting. This is in agreement with the fact that the triglyceride composition certainly varies more with origin than with roasting. The hardness of cocoa butter and masses is influenced by the geographical origin as a result of changes in lipidic composition caused by climatic conditions (Lehrian *et al.*, 1980). The cocoa butter composition is dominated by monounsaturated triglycerides: 2-oleodistearin (SOS), 2-oleodipalmitin (POP) and 1-palmito-2-oleo-3-stearin (POS), which represent 65–78% of the lipidic fraction. Variability of the ratio of trisaturated and triunsaturated (triolein) triglycerides between different cocoas is less important (Minifie, 1989). On the other hand, the proportion of diunsaturated triglycerides, such as the stearodiolein (SOO), may vary by a factor of four, while palmitodiolein (POO) may vary by a factor of eight (Lehrian *et al.*, 1980; Arruda, 1991).

These studies clearly show the relationship which exists between the composition of the cocoa butter and the fat crystallisation rates and crystalline form.

Discriminant analysis

A discriminant analysis may be applied to data in order to find discriminant functions which maximise the distance between known groups within the data set (Forina *et al.*, 1986). The purpose of this analysis is to create a predictive pattern to classify new individuals into known groups.

In this type of analysis, it is assumed that the different groups have similar covariance matrices and that the differences between the groups come from the position of their centroids in the multidimensional space defined by the variables (Forina *et al.*, 1986).

All the following multivariate analyses were performed on the complete set of standardised individual results rather than on the mean values.

Constitution of groups

A Principal Component Analysis was first performed to detect similarities and differences between the individuals (Forina *et al.*, 1986).

In order to find groups, it is better to use data for individuals taken at the same degree of roasting. In the following studies, only the non-roasted samples and the completely roasted beans are taken into account.

The results of the analysis performed on the raw Q_{RR} Q_{RS}, V_R and V_S data for these two roasting levels are presented in Tables 4 and 5. Table 4 shows that 96% of the total variability of the non-roasted cocoas can be preserved with just three Principal Components. We can therefore visualise the disposition of these cocoas in a three dimensional space. Similarly for roasted cocoas, 91·6% of the total variability can be represented using three Principal Components.

Non-roasted samples

The first Principal Component (PC1), which accounts for 50% of the total variability, is negatively influenced by Q_{RS}, V_R and V_S while V_R contributes significantly to PC2. Indeed, if the slow part of the fluidification curve resulted from changes in behaviour of the constituent molecules, then little of the total variability would be explained by V_S and Q_{RS}. It is reasonable to suppose that the differences in the constituent molecules of cocoa beans from different geographical origins are less important than the variability in their lipid composition. Therefore, the fact that V_S and Q_{RS} both contribute significantly to the total variability implies that this part of the curve largely reflects changes in the lipid mobility.

Table 4. Results of principal component analysis

Roasting step	Principal component	Percent of variance	Cumulative percentage
0	1	50·134 93	50·134 93
	2	28·349 85	78·484 77
	3	17·503 37	95·988 15
	4	4·011 85	100·000 00
3	1	50·794 11	50·794 11
	2	21·346 04	72·140 14
	3	19·458 44	91·598 59
	4	8·401 40	100·000 00

Table 5. Principal components loadings

Roasting step	Var.	PC1	PC2	PC3	PC4
0	Q_{RR}	0·507 66	−0·185 74	0·782 54	0·308 89
	Q_{RS}	−0·512 68	−0·514 36	0·424 07	−0·541 07
	V_R	−0·178 47	0·836 68	0·428 89	−0·290 13
	V_S	−0·669 01	0·030 02	0·154 42	0·726 40
3	Q_{RR}	0·434 69	0·593 25	0·603 91	0·307 24
	Q_{RS}	−0·537 87	0·562 96	0·144 77	−0·610 58
	V_R	−0·403 40	−0·509 28	0·757 39	0·065 37
	V_S	0·599 18	0·267 90	−0·201 74	0·726 99

In Fig. 5a the different cocoas are plotted on the plane of the first two Principal Components. Cocoa masses from the three suppliers are clearly separated into groups along the first Principal Component.

Group 1 contains most of the masses from Supplier 1 whereas Group 2 is essentially made up of samples from Supplier 2. During the transformation process used by Supplier 2, the beans pass through an infrared beam for a short time to facilitate their shelling before roasting. This is not the case for the beans of Supplier 1 which are, in most cases, shelled after roasting, except for three origins—Brazil, Ghana and the Ivory Coast—which are shelled and ground before roasting. Group 1' contains three individuals from Supplier 1.

Individuals from Supplier 4 are rather widely spread. Figure 5a shows that they form two major subdivisions

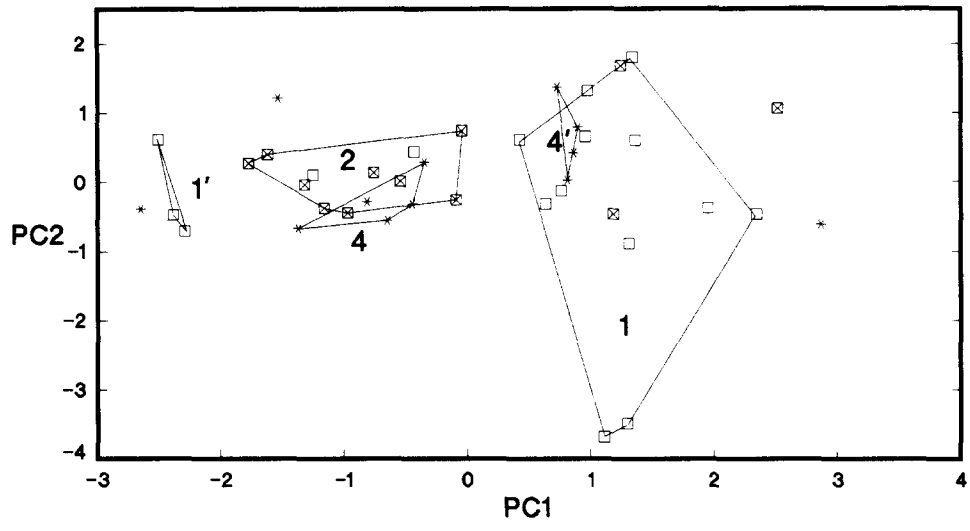

Fig. 5a. Eigenvector projection of non-roasted cocoa samples. 1: Supplier 1; 2: Supplier 2; 4: Supplier 4.

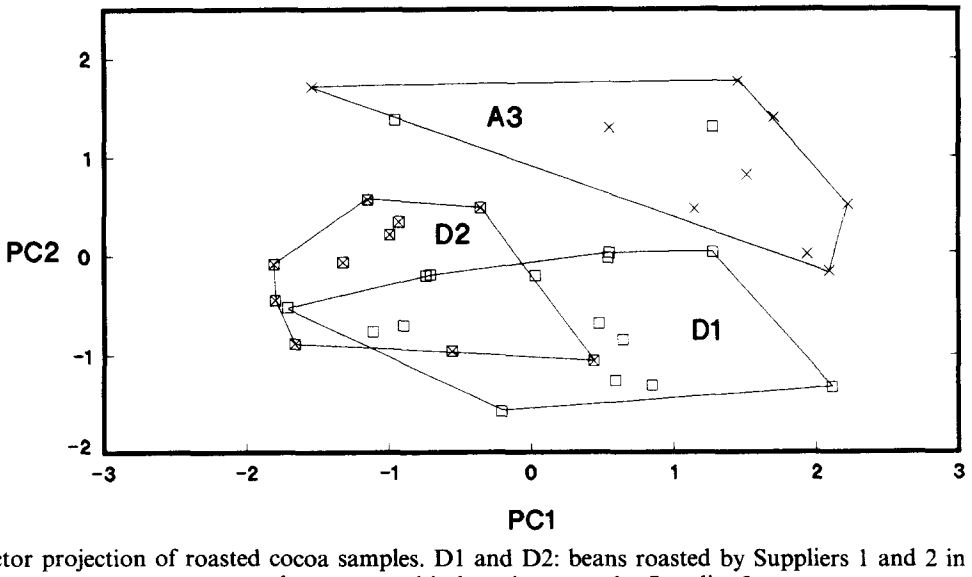

Fig. 5b. Eigenvector projection of roasted cocoa samples. D1 and D2: beans roasted by Suppliers 1 and 2 in drum roasters; A3: beans roasted in hot air roaster by Supplier 3.

Table 6. Results of discriminant analysis of non-roasted masses

Discriminant function	Eigenvalue	Relative percentage	Canonical correlation	Wilks statistic	Chi-squared	dF	Significance level
1	4·671 86	95·02	0·907 57	0·139 93	61·948 00	16	0·000 00
2	0·157 96	3·21	0·369 34	0·793 67	7·279 20	9	0·608 08
3	0·069 38	1·41	0·254 72	0·919 04	2·659 39	4	0·616 34
4	0·017 50	0·36	0·131 13	0·982 81	0·546 33	1	0·459 82

(4 and 4'), overlapping groups composed of individuals from 2 and 1, respectively, although PC3 clearly separates groups 2 and 4. Three outliers from Supplier 1 are distributed over the length of PC1 and one outlier from Supplier 2 is close to the group of Supplier 1.

Roasted samples

All four variables contribute significantly to PC1, which explains more than 50% of the variability.

The projection of the individuals on to the PC1–PC2 plane clearly distinguishes cocoas that have been hot air roasted (A3) from those roasted in drums (D1 and D2). The two groups of drum roasted cocoas are also separated to a large extent (Fig. 5b). Two D1 individuals are isolated from the rest of their group. Those individuals in D2 which overlap the Group D1 in the PC1–PC2 plane are separated along PC3.

Factorial discriminant analysis

Non-roasted samples

The Factorial Discriminant Analysis on the non-roasted samples was performed using the five groups (1, 1', 2, 4, 4') shown in Fig. 5a, without the outliers. Although there are two samples from Supplier 1 in Group 2 and two samples from Supplier 2 in Group 1, they will be considered as belonging to the group corresponding to their supplier.

Table 6 indicates that only function 1 (DF1) discriminates significantly between the five groups. This function correctly describes the group structure as the correlation coefficient is high and it explains 95% of the total variability. The Wilks statistic for this function is low, which means that the probability of the null hypothesis of no significant difference between the groups is low. DF1 is positively influenced by both Q_{RS} and V_S and therefore by the behaviour of the S portion of the fluidification curves.

Figure 6a shows the projection of the cocoas on to the plane of the two first Discriminant Functions for the groups observed in the PCA on the non-roasted samples.

Table 7. Classification ability of discriminant analysis on non-roasted samples

Predicted group	1	1'	2	4	4'
Actual group					
1	42·86	0	7·14	7·14	42·86
1'	0	100	0	0	0
2	0	9·09	72·73	18·18	0
4	0	0	20·00	80·00	0
4'	0	0	0	0	100

The validity of the discriminant model is tested by calculating the percentage of individuals correctly classed into each group. More than 70% of the Group 2 individuals are correctly classed, while almost 20% are classed into Group 4 (Table 7). These incorrectly classed individuals are already apparent in the PCA plot (Fig. 5a). The calculation of the discriminant functions improved the separation of Group 4. On the other hand, the discriminant analysis could not extract Group 4' from Group 1 (Fig. 6a). Therefore, nearly 43% of the masses of Supplier 1 are included in this subgroup (Table 7).

As can be seen from the position of the groups on the DF1 axis and the loadings of Q_{RS} and V_S (Fig. 6*a*), the cocoas from Supplier 2 contain more constituents which contribute to the slowly evolving region of the fluidification curves.

Masses from Supplier 4 are separated into two groups, and Group 4' remains within the region of Group 1. Therefore, masses from Madagascar and Togo (Group 4') have behaviour during fluidification similar to that of samples from Brazil and Java in Group 1.

Roasted samples

The groups D1, D2 and A3, without the three isolated individuals shown in Fig. 5b, were used to calculate the Factorial Discriminant Functions.

Table 8 shows the results of the discriminant analysis on the masses after roasting. Table 9 shows the loadings of the critical variables. As can be seen from the

Table 8. Results of Discriminant Analysis on roasted masses

Discriminant function	Eigenvalue	Relative percentage	Canonical correlation	Wilks statistic	Chi-square	dF	Significance level
1	2·778 59	80·38	0·857 53	0·157 70	54·487 96	8	0·000 00
2	0·678 16	19·62	0·635 70	0·595 89	15·272 11	3	0·001 60

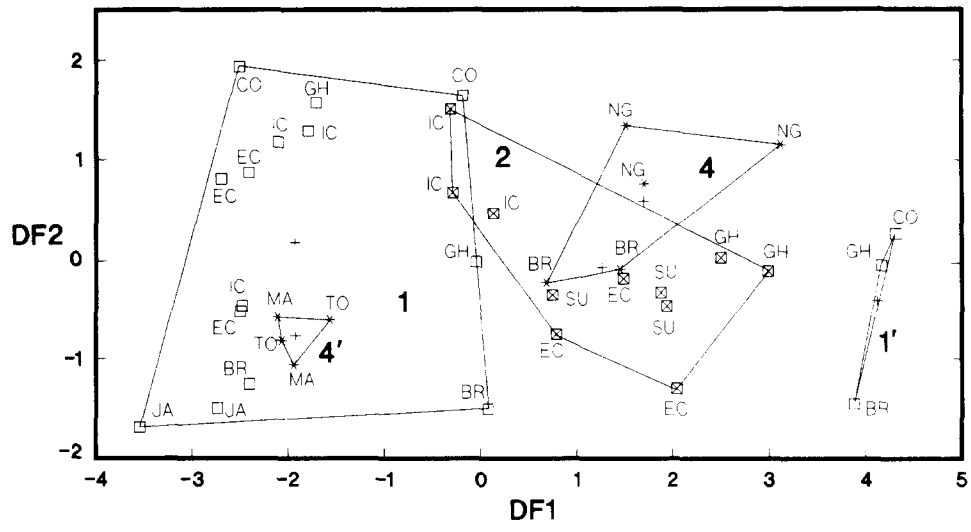

Fig. 6a. Plot of Discriminant Function 1 vs Discriminant Function 2 with non-roasted samples. Crosses indicate centroid position. 1: Supplier 1; 2: Supplier 2; 4: Supplier 4. Meanings of abbreviations given in text.

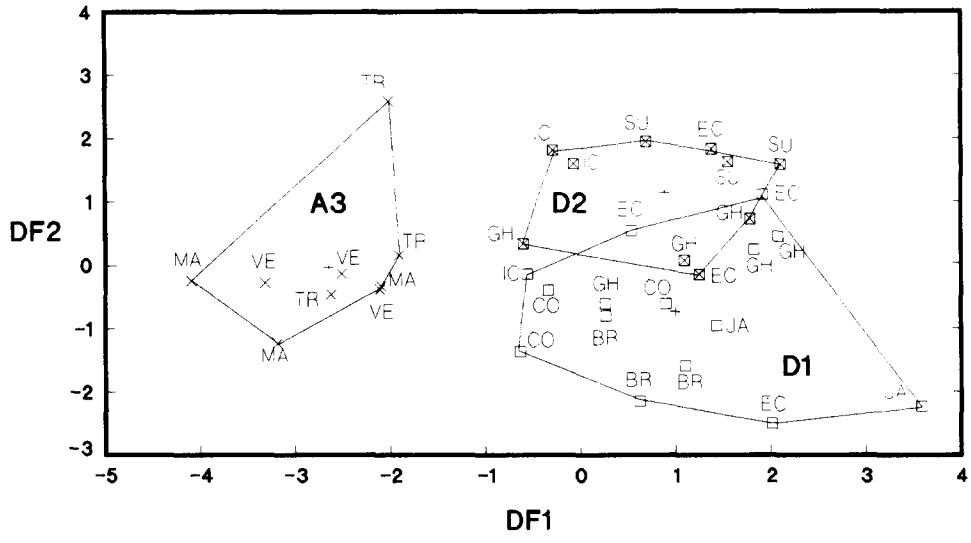

Fig. 6b. Plot of Discriminant Function 1 vs Discriminant Function 2 with roasted samples. Crosses indicate centroid position. D1 and D2: beans roasted by Suppliers 1 and 2 in drums roasters; A3: beans roasted in hot air roaster by Supplier 3. Meanings of abbreviations given in text.

chi-square test of the Wilks statistic (Table 8), both Discriminant Functions are significant. The values of the canonical correlation coefficients show that the groups are rather scattered. The variables V_R and Q_{RR} contribute significantly to DF1, which means that the initial part of the fluidification curve discriminates the different groups most (Table 9).

Table 9. Discriminant function loadings

Roasting step	Var.	DF1	DF2
0	Q_{RR}	−0·191 05	0·912 50
	Q_{RS}	0·538 61	−0·325 17
	V_R	0·157 44	−0·763 50
	V_S	0·796 17	0·479 08
3	Q_{RR}	−0·743 95	−0·319 15
	Q_{RS}	−0·545 16	0·300 95
	V_R	1·056 83	−0·448 53
	V_S	0·114 73	0·709 00

It is obvious that one of the first effects of the roasting process is the elimination of moisture from the masses. Because the thermal conductivity of water is greater than that of lipids, one would expect to observe a decrease in V_R during roasting. However, the discrimination of the three groups is not simply a consequence of the decrease in the water content of the samples. If this were the case, Supplier 1, who roasts at the highest temperature, should give the lowest V_R values. As can be seen in Fig. 7, V_R is lower for Suppliers 2 and 3 than for either the non-roasted masses or those of Supplier

Table 10. Classification ability of discriminant analysis on roasted samples

Predicted group	D1	D2	A3
Actual group			
D1	80·00	20·00	0
D2	20·00	80·00	0
A3	0	0	100

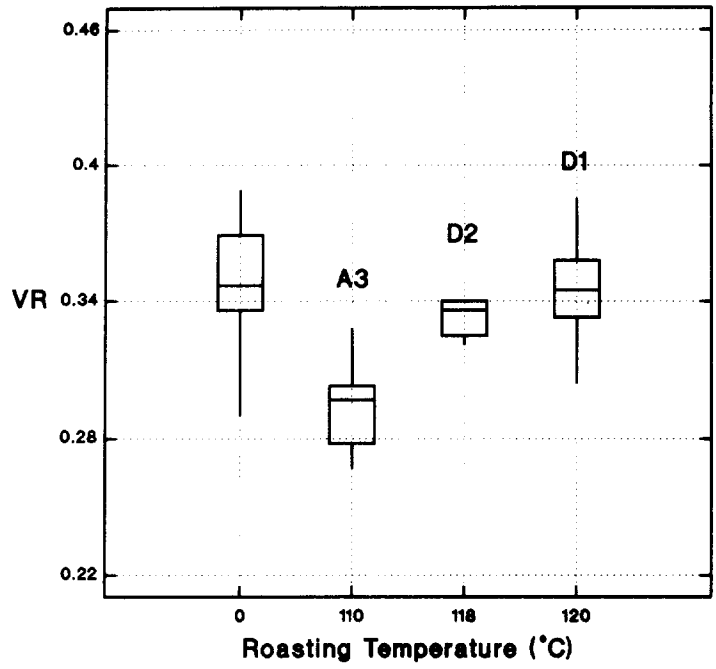

Fig. 7. Multiple box-and-whisker plot for VR subdivided into four groups: 0 for the non-roasted masses; A3 for the masses roasted at 110°C by Supplier 3; D2 for the masses roasted at 118°C by Supplier 2; and D1 for the masses roasted at 120°C by Supplier 1.

3. The modifications of fluidification parameters must therefore be the result of another phenomenon such as variation in the level of unsaturated triglycerides. If this level is low, the initial rate of fluidification of cocoa masses is also low. Oxidation of fat is probably more important at 120°C than at 110°C, and this would explain why Supplier 1 produces much more fluid masses with a higher V_R. It therefore appears that the composition of the rapidly changing part of the lipid fraction varies with the type of roaster used.

Within each group, however, replicates are not close enough to be able to distinguish samples of different origins (Fig. 6*b*).

The D1 group is the most scattered and 20% of D1 samples are classified as belonging to D2. Although the roasting parameters (time, temperature) used by each supplier are apparently similar, masses of the same geographical origin treated by these two suppliers are not in proximity in the DF1–DF2 plane (Fig. 6*b*). The technological parameters therefore appear to have as great an impact on the fluidification properties as the geographical origin.

The other two groups, A3 and D2, are conserved at respectively 100 and 80% (Table 10).

CONCLUSION

The study by Low Resolution Pulse NMR of the fluidification of cocoa masses at 27·5°C after tempering at 0°C may be used to characterise samples according to the evolution of their solid content. The analysis of variance on the quantitative parameters Q_{RR}, Q_{RS}, and on the kinetic parameters V_R, V_S, calculated from the decomposition of the fluidification curves, has shown that the geographical origin has a significant influence on the fluidification process.

Principal Components Analysis and Factorial Discriminant Analysis may be used to group together non-roasted bean masses with similar fluidification behaviour. In this way, New Guinea cocoa, which is similar to that of Sumatra, is separated from the Madagascar and Togo cocoas, these being close to Java cocoa. It is obvious that we cannot obtain simplified separations for continents because of the large variety of cocoas. Also the statistical analysis suggests that the quality of the discrimination between masses depends on the technological history of the beans and on their storage conditions. When only considering the beans from one supplier, the differentiation as a function of the geographical origin is more apparent.

Roasting would appear to have an effect on both the constituent molecules (protein, carbohydrate, etc.) and the lipids. The signal measured by NMR is therefore the result of two phenomena, the crystalline transitions and fusion of the lipids, and changes in the configuration of non-lipid molecules. Although these phenomena have distinct kinetics, the data are not sufficient to differentiate between them.

Discriminant analysis on roasted cocoas clearly shows the repercussions of the choice of the processing technique on the fluidification of the mass at 27·5°C. This will be reflected in the rheological properties of the cocoa butter. This study therefore shows the importance of the choice of the roaster for industries which produce cocoa butter.

Similar studies should help industrialists to improve the control of production processes by helping them find better roasting conditions for different beans.

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