Assessment of cocoa flavour and fat content

J.F. Clapperton

Mars Confectionery, Dundee Road, Slough SL1 4JX,UK

A fresh analytical approach to flavour assessment has revealed consistent differences between clonal cocoas grown at BAL Plantations in Malaysia. Forasteros are observed to develop more cocoa flavour and are generally less bitter and astringent than Trinitarios. In separate trials of two Forasteros, one Trinitario and one hybrid of a Trinitario and a Forastero, cocoa flavour intensity was increased and astringency reduced by post-harvest pod storage prior to separating the wet beans for fermentation. Instrumental analyses of the fermented and dried beans showed corresponding reductions in the concentrations of polyphenols which impart astringency. These effects of pod storage differed markedly between the three different types of cocoa. The two Forasteros were least affected. Cocoa flavour characteristics have been reproduced in cocoa liquors prepared from different planting materials and post-harvest, fermentation and drying treatments. Use of these liquors as flavour references has enabled producers and cocoa and chocolate manufacturers to agree on the nature and direction of flavour differences. Differences in the size and fat content of beans and in the colour and viscosity of cocoa liquors prepared from the different planting materials are reported also.

Keywords: Theobroma cacao, genetic variability, bean size, fat content, flavour.

Introduction

The mission statement of the Cocoa Research Unit on characterization includes the use of both morphological and molecular markers for purposes of identification and qualitative traits which are of direct importance. This contribution deals with butterfat content, size, colour and flavour characteristics which are valued by cocoa and chocolate manufacturers. A current project between the Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA) and BAL Plantations Sdn Bhd in Malaysia is revealing the contribution of planting materials to all of those attributes. This workshop is an opportunity to present a summary of the key results of the work to date to a select gathering of representatives of the cocoa business and related sciences. Agreed and validated information on important qualitative characteristics would be a valuable addition to the International Cocoa Germplasm Database (ICGD). In the course of presenting the results of the BAL project I shall deal with some of the main issues involved in obtaining that quality of information.

Sample preparation

As in any other analytical exercise, proper sampling and sample preparation are crucial. The results presented here are on cocoas which were fermented and dried in preparation for flavour evaluation. Every care was taken to ensure that the post harvest, fermentation and drying treatments were the same for all cocoas. Experimental designs were used for both sample preparation and taste testing to provide estimates of the differences between cocoas relative to errors between replicate preparations and tests. There have been at least three and up to six replicate preparations of each set of cocoas and between four and eight separate tests of each flavour. The three or more replicate preparations were made over different seasons to cover effects of variations in climatic conditions, just as the four or more separate taste tests were found necessary to cover the effects of differences in the sensitivity, mood and calibration of the tasters.

There are at BAL some 50 primary cocoa genotypes available in sufficient quantities to yield more than 50Kg of wet beans from a single harvest. It is possible therefore to carry out monoclonal fermentations and apply the experimental designs for sample preparation that are crucial to the success of the entire exercise.

Ripe pods from all cocoas to be tested were harvested on the same day and were held overnight prior to separation of the wet beans for fermentation. Spoiled beans were discarded. 50Kg of wet beans from the different cocoas were fermented and dried concurrently in sets of six or more so that all were exposed to the same environmental conditions throughout. One cocoa (BAL209, a progeny of PA35 and NA32) was included in every set as a check of the consistency of the processing and analytical procedures from pods at harvest through to the results of the taste tests of the cocoa liquors. In cases where there were insufficient trees to yield 50Kg of wet beans from a single harvest cocoas were tested on a 3Kg scale by inserting the wet beans in a nylon net bag in the centre of mass of a 47Kg fermentation of beans from BAL209. The beans were fermented in openweave baskets in order to maintain a constant surface to volume ratio of the fermentation heaps within and between replicates and experiments. Variable shapes and sizes of heaps resulting from differences in the flow properties of the wet beans could affect the aeration of the heaps, the course of fermentation and flavour. Under the latter conditions, effects of variations in climatic conditions could be exacerbated. The cocoas were fermented for five days with a single turn at the end of the second day. After fermentation the cocoas were sun dried. As already mentioned, the entire procedure was replicated at least three times over different seasons.

The dried cocoas were held in new jute sacks for three months to allow the flavours to stabilise. Coned and quartered samples were then roasted and milled into cocoa liquors using standard conditions of roasting and milling (BCCCA *loc. cit.*). The liquors were deep frozen until they were required for tasting and remained stable in that condition.

Bean size

This one is easy. The numbers of beans in 100g samples are counted. There must of course be no pre-selection, and for comparative purposes the beans should come from pods that are of a uniform state of ripeness. While the latter condition introduces a certain element of subjective judgement it is one that could easily be covered by an agreed experimental protocol. The protocol should take account also of agronomic and seasonal factors likely to affect bean size, in the same way that the procedure for sample preparation, outlined above, considered factors likely to affect flavour. For entry into the ICGD the genotype or variety should be exactly what the donor says it is. Morphological markers, isozyme analyses or genetic characterization could resolve any questions of identity, but there is no substitute for access to a well documented and maintained germplasm collection in the first place. Also there should be sufficient trees to provide substantial and representative samples. The condition of the planting materials grown at BAL Plantations certainly meets those requirements. Differences in bean counts and fat contents between genotypes from the work at BAL are listed in Table 1. The Amelonado is from a seedling population introduced originally from Ghana. The others are clonal cocoas.

Cocoa and chocolate manufacturers obtain higher yields of cocoa liquor and butter from larger beans because of the higher ratios of nib to shell. UIT1, with the best combination of bean size and fat content in the above set, would be particularly favourable as a source of cocoa butter.

Table 1 Differences in bean size and fat content.

Genotype	Bean count per 100g		Fat content of nibs (%)	
	Mean	Range	Mean	Range
Amelonado	94.5	84-110	55.6	54.7-57.1
IMC67 *	77.8	77- 83	58.2	57.2-58.9
Nanay33	103.8	102-106	54.3	53.3-55.1
Parinari7	98.5	92-103	58.2	57.9-58.4
Scavina12	123.8	122-126	54.8	53.8-55.7
UIT1 $	53.3	51- 58	58.1	57.6-58.5

* Iquitos Mixed Calabacillo. $ Unidentified Trinitario of Imperial College Selection (ICS) and Nicaraguan Criollo type. Means and ranges of four separate preparations of each cocoa.

Fat content

Estimation of fat content is also relatively straightforward. The figures in Table 1 were obtained by Soxhlet extraction of deshelled beans. In dealing with large numbers of samples Soxhlet extraction becomes tedious and errors creep in. A better option could be to use a Nuclear Magnetic Resonance (NMR) procedure (Bruker Spectrospin) calibrated with samples of known fat content. The calibration set could then be included as hidden controls in each test set in order to check the validity of the results. The NMR procedure can be adapted to estimate cocoa butter hardness also and has shown UIT1 to give a slightly harder butter than Nanay33 (Clapperton *et al.*, 1991). Chocolates made from UIT1 had a slightly harder initial bite and a slower rate of melt than those made from Nanay33 according to the same Alliance (BCCCA *loc. cit.*) recipe.

Another source of human error is frequently overlooked. When beans are deshelled by hand there is a tendency to select larger beans which are easier to deshell. Smaller and possibly immature beans tend to have a lower fat content. Wherever possible procedures should be automated. Apart from the NMR equipment this calls for a little ingenuity rather than any great expense.

Over-fermentation must be avoided. As fermentation is extended the non-fat components of the beans autolyse and the products are leached from the cotyledons thereby elevating the residual fat content. It might be preferable to avoid fermentation altogether but the cocoa and chocolate manufacturers will rightly demand that the results so obtained are made relevant by direct comparison with fermented and dried beans of the same genotype. A further phase of the work at BAL is planned to compare the fat contents of unfermented cocoas with corresponding samples of fermented and dried beans.

Flavour

Whereas fermentation might usefully be avoided in estimating fat content it is absolutely essential for flavour development. Details of the sample preparation have already been given and have stressed the care and attention neccessary to obtain valid results.

The cocoas were tasted as liquors according to experimental designs that require either three or four blind tests of each sample and allow each liquor to be compared directly with every other. Flavour terms listed in Table 2 were scored for intensity on 10cm. line scales. Data analysis was by analysis of variance. If one round of testing failed to reveal significant differences at 95% confidence on any single scale, the samples were recoded and the taste test repeated. If two such rounds failed to reveal any significant differences, based on the combined six or eight blind tests of each sample, the liquors were deemed to be similar. Where necessary, the taste testing was carried out under reduced or coloured lighting to mask any differences in colour between liquors.

The terminology of the test form is restricted to descriptors that can be defined using cocoa liquors as physical references to demonstrate the meanings of the terms and use of the scales. These liquors are prepared from selected planting materials and post-harvest, fermentation and drying treatments. It is necessary to be able to reproduce each specific flavour effect. Otherwise the sample does not qualify as a reference and the corresponding term is not included in the check list. These disciplines govern the development and use of the test procedure. Additional terms are included strictly on the bases of the validity and relevance of the extra information or definition they provide. Descriptors are anchored by the physical references and therefore do not lose meaning or definition on translation or use among different races, religions and cultures. That could bring about the greatest benefit of all by enabling cocoa and chocolate manufacturers to specify to producers and plant breeders the characteristics of cocoa flavour quality they require. Because the physical references relate also to selected planting materials and conditions of processing, forward control of cocoa flavour quality at source would then become a distinct possibility.

There are heavy penalties in the present system of flavour quality selection based on sampling and inspection. They reside not so much in the direct cost of the operations but in the uncertainty of the decisions and the inevitable compromise of product quality. There are no secrets relating to raw material quality. The intellectual property of cocoa and chocolate manufacturers is how to add value to the raw materials by use of production processes and product designs. The entire industry would benefit from improvements in the quality of the basic starting materials. There are effective operations in place for the selection and purchase of sugar, cereals and milk products. The same could and should be developed for cocoa beans, the principal raw material of the cocoa and chocolate industry. It is proposed to establish a small collection of reference liquors as a sub-section of the ICGD. Whether that collection grows and becomes a permanent feature of the ICGD or else is consigned to oblivion will depend on how much use is made of it for the benefit of the industry.

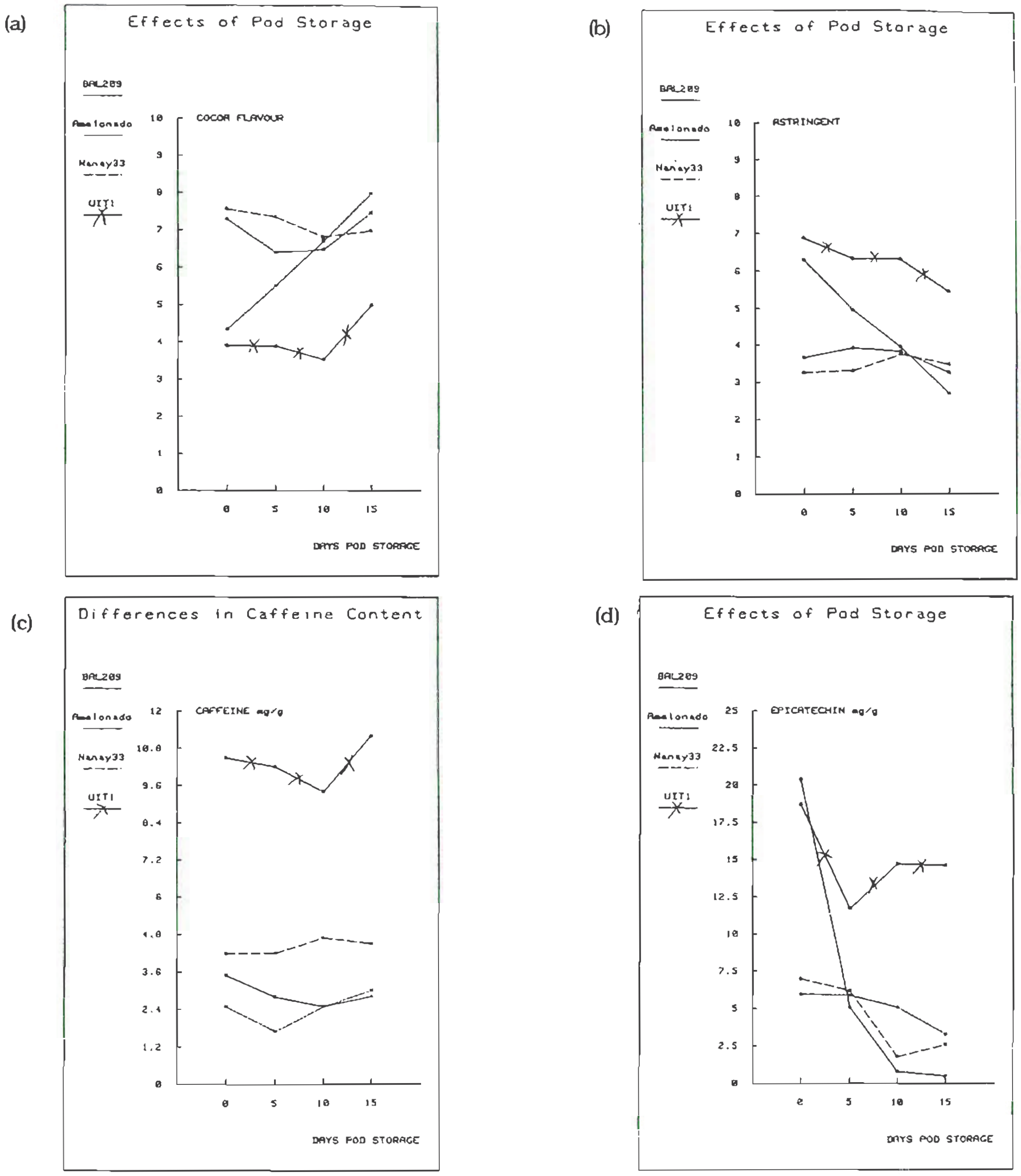
Frequent reference is made in Table 2 to cocoas that are featured in Figure 1, below, which shows the results of a series of pod storage trials. The trials were replicated three times over a period of 15 months. Cocoa liquors from all three replicates were tasted "blind" by an individual assessor. All three replicates showed the same pattern of flavour differences. The flavour effects are therefore reproducible thereby meeting the first criterion for the use of the cocoa liquors as flavour references. The second criterion is that the use of the liquors as training standards must be effective. The sensory results for cocoa flavour intensity and astringency shown in Figure 1 are the combined results of the individual assessor and a panel of 15 trained assessors from BAL Plantations who tasted the samples from the second replicate. Differences recorded by all individual panellists were consistent and statistically significant at or above 95% confidence. The training and taste testing followed the procedures and disciplines outlined above, except that flavour references other than those marked * were used for the panel training. The highlighted samples have been used subsequently to illustrate the flavour differences which have been widely recognised and agreed by representatives of different cocoa and chocolate manufacturers. The indications are that the cocoas from the pod storage trials should provide an even better set of flavour references than those used originally to train the BAL panel. Beans from the trials are available. The corresponding cocoa liquors could be the first flavour references that are forwarded to the ICGD.

Table 2 Flavour terms, definitions and references.

Term	Meaning, references and use of scale
Cocoa flavour intensity	Affected by both genotype and process. References:(High scores) *Amelonado; *Nanay33; *BAL209 with 15 days pod storage. (Low scores) *UIT1; *BAL209 with zero days pod storage.
Acid/sharp	Sharp,pungent smell and immediate sharp taste. Vinegar. Affected by conditions of drying (Duncan *et al.*, 1989). High scores:- Cocoa beans dried at >60C. Low scores:- Sun dried cocoas; unfermented cocoas.
Astringent	Mouth drying/puckering. Affected by both genotype and extent of fermentation. High scores:- *UIT1; *BAL209 without pod storage; *Epicatechins; unfermented cocoas. Low score:- *BAL209 with 15 days pod storage.
Bitter	Quinine like. Taste develops slowly at back of tongue. Reference:- *Caffeine or theobromine diluted to taste.
Raw/green	Characteristic (beany) flavour of unfermented cocoa beans, also astringent and bitter. High scores:- Cocoa liquors made from slaty beans. Mid-scale scores:- Underfermented cocoas. Zero scores:- Fully fermented cocoas.
Fragrant/floral	Sweet aromatic top note of cocoa flavour. Increased by post harvest pod storage. High scores:- *Amelonado, *Nanay33 and *BAL209 all with 15 days pod storage. Low scores:-*Same genotypes and other cocoas without pod storage.
Brown fruit	Dried fruit, raisins, currants, dates, prunes; liquorice. High scores:- Scavina6, Scavina12.
Late sour	The gradually developing astringency or sourness of under-ripe fruit. The time-scale effect distinguishes it from acid sourness. High score:- *UIT1
Viscous/thick	A physical viscosity and mouthfeel effect. High scores:- *BAL209 with 15 days pod storage; *Amelonado; *Nanay33. Low score:-*BAL209 without pod storage.
Smoky	An effect of careless drying or storage of finished cocoa. Wood smoke, pungent, phenolic. High score:- Wet cocoa dried over a smoky wood fire. Can be diluted with uncontaminated cocoa to illustrate use of the scale. Zero score:- Sun dried cocoas not exposed to wood smoke during processing and subsequent storage.

* See Figure 1.

Figure 1 Results of sensory and instrumental analyses.



On completion of the sensory analyses of the sixteen cocoas depicted in Figure 1, coned and quartered samples were submitted under code for analyses of bittering agents and polyphenols. The results shown in Figure 1 (sections c and d) confirm the differences in bitterness and astringency between the different cocoas (Clapperton *et al.* 1992). A purified fraction of polyphenols comprising epicatechin and higher oligomers from UIT1 displayed the same intensity and time scale effects of bitterness and astringency experienced in tasting cocoa liquors. Fractions containing higher oligomers were less bitter and more astringent. On the bases of these results caffeine and epicatechin are included in Table 2 as flavour references in addition to the use of entire cocoa liquors.

In order to keep the taste testing on schedule with the production of samples and the project plan it was agreed that the primary tasting should be carried out by a single expert taster and key results confirmed independently by panels at BAL and in the UK. The UK panel comprises trained and experienced panellists from Cadbury, Mars, Nestle and United Biscuits. So far, differences identified by the individual expert have been confirmed. That is, in addition to the above instrumental-analytical confirmation of the differences in bitterness and astringency.

The following are the two main flavour classes that have emerged from the work so far on the characterization of a wider range of cocoas grown at BAL Plantations. Scale refers to the 50Kg and 3Kg heap fermentations and also to a number of 250Kg box fermentations which were used to check that the flavour effects being observed were not merely an artefact of the small scale fermentation procedures. In all cases the flavours were consistent between replicates and the different scales of fermentation.

KA5-201 displayed a mild smoky flavour. When first observed it was thought that the conditions of drying or storage were at fault and that some smoke contamination had occurred. But in keeping with the experimental protocol, all six cocoas included in the set had been exposed to the same environmental conditions throughout and the smoky note was not observed in any other of the five co-samples. The same occurred in the other two replicate preparations of the test set. Confirmation of the flavour is being sought in other Keravat clones and by the independent taste panels.

The two Scavina cocoas had a distinct fruity flavour which was observed also but at lower intensity in the Nanays and Parinaris included in Group 1 (Table 3). De Witt (1954), reporting the results of a study of clonal cocoas in Trinidad, described Scavina12 as having a heavy auxilliary flavour typical of prunes or liquorice although there were considerable variations between both individual tasters and replicate preparations. Glendinning (1965) reporting the results of plant breeding trials in Ghana noted the frequency of comments on a fruity/floral note in a progeny of Scavina12 and suggested that Scavina and the Ecuador Nacional cocoa representing Arriba flavour might be related. In the latter study the test cocoas were scored for overall acceptance by different cocoa and chocolate manufacturers. The presence of specific flavour effects were not recorded systematically and the occasional comments were confused with personal likes and dislikes. The advantage of the present taste test procedure is that it is directed at identifying the flavour characteristics that can be produced from different planting materials and processing conditions quite separate from the particular requirements of any individual manufacturer.

Table 3 Group 1. Moderate to well developed cocoa flavour, low astringency and bitterness, mostly dark coloured beans and liquors and viscous/thick liquors.

Cocoa	Scale (Kg)	Details
Amelonado	50,250	Lower Amazon Forastero.
IMC53	3	Upper Amazon Forastero. Iquitos Mixed Calabacillo.
IMC105	3	
KA5-201	50	Keravat Trinitario.
NA31	3	Upper Amazon Forastero, Nanay.
NA33	3,50,250	
PA7	50,250	Upper Amazon Foastero, Parinari.
PA137	3	
SCA6	50	Upper Amazon Forastero, Scavina.
SCA12	50,250	
SPA9	50	Forastero, Seleccion Palmira
SPA10	3	
(UIT1 x NA32) x SCA12		Hybrid, tested on 3Kg scale.

The final entry in Group 1 is an F2 hybrid from a cross of Scavina12 with a progeny of UIT1 and NA33. There have been more direct comparisons of UIT1 and NA33 than of any other cocoas in the course of the present study and these have emerged as the anchor points of two sets of contrasting flavours listed under Group 1 and Group 2 (Table 4). The hybrid cocoa displayed more of the flavour characteristics of NA33 and also the distinctive fruity flavour of SCA12.

All of the cocoas listed in Groups 1 and 2 were tested without pod storage. As already indicated in Figure 1, BAL209 with 10 or 15 days pod storage would have been included in Group 1. There are indications from other trials in progress as part of the project that other cocoas respond differently to pod storage, although so far none have responded as dramatically as BAL209.

Cocoas in the two contrasting groups tended to differ also in bean size. Most of the cocoas in Group 1 were between 0.8 and 1.0g per bean (bean counts in the range of 95 to 130). Most in Group 2 were between 1.2 and 1.8g (counts of between 55 and 90 beans per 100g).

A further phase of the project is planned to examine more genotypes, particularly ICS and other Keravat clones and more progenies. Further work will be directed also at the refinement of the small scale fermentation procedure to allow the study to be extended laterally to other cocoa germplasm collections and environments. A study of the inheritance of flavour characteristics is already in progress.

Table 4 Group 2. Low cocoa flavour intensity, high astringency/bitterness, mostly pale coloured beans and liquors and less viscous liquors.

Cocoa	Scale (Kg)	Details
Amaz2/1	50	Upper Amazon Forastero
BAL209	3,50	A hybrid from "PA35" x NA32
CC10	3	Trinitario. Cacau Center, Costa Rica. (CC10 is a Matina derivative)
CC11	3	
EET399	3	Hybrid, Silecia Oriente.
NA32	50	Upper Amazon Forastero, Nanay.
NA34	50	
10P	50	Trinitario, Soconusco Mexico.
PA13	50	Upper Amazon Forastero, Parinari.
"PA35"	50	Unidentified Trinitario.
PA138	50	Upper Amazon Forastero, Parinari.
PA156	50	
SCA9	50	Upper Amazon Forastero, Scavina.
UF163	3	Trinitario. United Fruit, Costa Rica.
UF676	3	
UIT1	3,50,250	Unidentified Trinitario. ICS and Nicaraguan Criollo type.
UIT2	50	

Acknowledgements

The studies described here are funded by the Biscuit, Cake, Chocolate and Confectionery Alliance. The co-operation of staff and management at BAL Plantations is gratefully acknowledged. Members of the various taste panels are thanked for their honest effort and continued support.

References

Brucker Spectrospin Ltd, Banner Lane, Coventry CV4 9GH, UK.

Clapperton, J., Yow, S.T.K., Chan, J., Lim, D.H.K., Lockwood, G., Romanczyk, L. and Hammerstone, J. (1991) Effects of cocoa genotype on flavour, *Proc. Malaysian Cocoa Conf.*, Kuala Lumpur, in press

Clapperton, J., Romanczyk, L., Hammerstone, J., Yow, S.T.K., Lim, D.H.K. and Lockwood, G. (1992) Polyphenols and cocoa flavour, *Proc. of Meeting of Groupe Polyphenol*, JIEP 92, Lisbon

De Witt, K.W. (1954) The flavour assessment of cocoa, *Reports on Cacao Res.*, Imperial College of Tropical Agriculture, Trinidad, pp. 77-81

Duncan, R.J.E., Godfrey, G., Yap, T.N., Pettipher, G.L. and Tharumarajah, T. (1989) Improvement of Malaysian cocoa bean flavour by modification of harvesting, fermentation and drying methods - the Sime/Cadbury process, *The Planter* **65** 157-173

Glendinning, D.R. (1965) Experiences in flavour evaluation of cocoa varieties, *Trop. Agric. (Trinidad)* **42**(4) 297-304

The Biscuit, Cake, Chocolate and Confectionery Alliance, 11 Green Street, London W1Y 3RF, UK, Small scale preparation of samples of chocolate