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Triglyceride Analysis of Cocoa Beans from Different Geographical Origins

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ABSTRACT

Triglyceride analysis of cocoa beans from twenty different geographical origins was carried out by high performance liquid chromatography with an RI detector. Our results show the different compositions of the fatty fraction of cocoa beans and the discriminant analysis applied is useful for identifying samples from different origins.

INTRODUCTION

Cocoa beans of commerce are the seeds of *Theobroma cacao* L. that grows in Central and South America and in West Africa. These beans are roasted, deshelled, ground and pressed for the manufacture of cocoa butter, cocoa powder and chocolate.

The environmental temperature, climate, rainfall and sunshine, during the growth and ripening of cocoa fruit, genetics of the tree and post-harvesting and processing conditions can affect the fat composition of cocoa (Lehrian *et al.*, 1980; Chin & Zainuddin, 1984; Chalseri & Dimick, 1987).

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Considerable work has been done to elucidate the composition of cocoa butter, but the scarcity of data about the triglyceride composition of cocoa beans from different geographical origins implies that the greatest interest is in detecting and quantifying the adulteration of cocoa butter with other glyceride fats. The geographical origin of the beans, on the other hand, could affect the flavour characteristics of cocoa powder and chocolate (Padley & Timms, 1980; Fincke, 1980, 1982; Podlaha *et al.*, 1984).

Investigations reported herein were directed toward obtaining basic information about the relationship between triglyceride composition and geographical origin of samples, to contribute to the knowledge of the fat composition of cocoa beans. For this purpose we have analysed twenty samples of different geographical origins by high performance liquid chromatography.

MATERIALS AND METHODS

Instruments

The LC system used was a Perkin-Elmer Series 10 liquid chromatograph equipped with a Rheodyne Loop (10 μl) injector model 7125, coupled with a Perkin-Elmer model LC-25 refractive index detector. Quantitation was accomplished by integration with a Hewlett-Packard model 3390A. The triglycerides abbreviated SOO, PStO, LOO and LnOO indicate the family of triglycerides composed of either palmitic (P), stearic (St), oleic (O), linoleic (L) or linolenic (Ln) acids. Separation of triglycerides has been achieved on Spherisorb ODS-2 (250 × 4 mm) 5 μ.

Materials

Reference triglyceride standards were purchased from Supelco, Inc. (Bellefonte, PA). The solvents used were 'HPLC grade'. Propionitrile 'synthesis grade' (Merck) was distilled over phosphorus pentoxide.

The twenty analysed cocoa bean samples had the following geographical origins:

Ivory Coast	3 samples
Nigeria	2 samples
Brazil (Bahía, Rondonia, Amazonas, Pará)	8 samples
Ecuador (Guayaquil)	1 sample
Indonesia	4 samples
Malaysia	1 sample
Guinea	1 sample

The samples were generously supplied by Nutrexpa, SA, Spain.

The beans were dehulled manually, ground, and the oil was obtained by Soxhlet extraction with a mixture of petroleum ether (bp 40–60°C) and diethyl ether (50:50) during 4–5 h. The solvent was removed with a rotary evaporator at 40°C under nitrogen atmosphere.

The fatty extract was dissolved in a mixture of ethyl ether–propionitrile (2:3) to obtain a concentration of 100 mg/ml. Ten microlitres of the filtered solutions were injected into the liquid chromatograph. Mobile phase was propionitrile at a flow of 0·6 ml/min with the RI detector maintained at 25°C.

RESULTS AND DISCUSSION

With our chromatographic conditions all samples yielded similar chromatograms; a typical one is shown in Fig. 1. Triglyceride peaks identification was done according to Goiffon *et al.* (1981 (a) and (b)). For the reproducibility study, one sample (number 4) was injected five times and the mean percentages, the standard deviations and the coefficients of variation were calculated (Table 1). The quantification was carried out by normalisation supposing that the detector response for TG-types was the same. Each sample was analysed three times and the mean percentages for each triglyceride are shown in Table 2.

We have studied the relative proportions of POP, POSt and StOSt in order to investigate the correlation between the geographical origin and these contents of triglycerides. For this purpose the amount of POP, POSt and StOSt of the different samples was normalised to 100% and the calculated values were put into a ternary diagram. The enlarged relevant

TABLE 1
Reproducibility of Triglyceride Analysis: Mean Value, Standard Deviations and Coefficients of Variation for Five Injections

Triglyceride	*Mean (%)*	*Standard deviation*	*Coefficient of variation (%)*
PLP	2·42	0·05	2·07
POO	6·72	0·07	1·04
PStL	3·35	0·07	2·09
PPO	15·02	0·14	0·93
PStO	33·65	0·23	0·68
StOSt	21·18	0·15	0·71
StOA	1·09	0·04	3·67
LOO	0·99	0·04	4·04
StStP	0·19	0·02	10·53

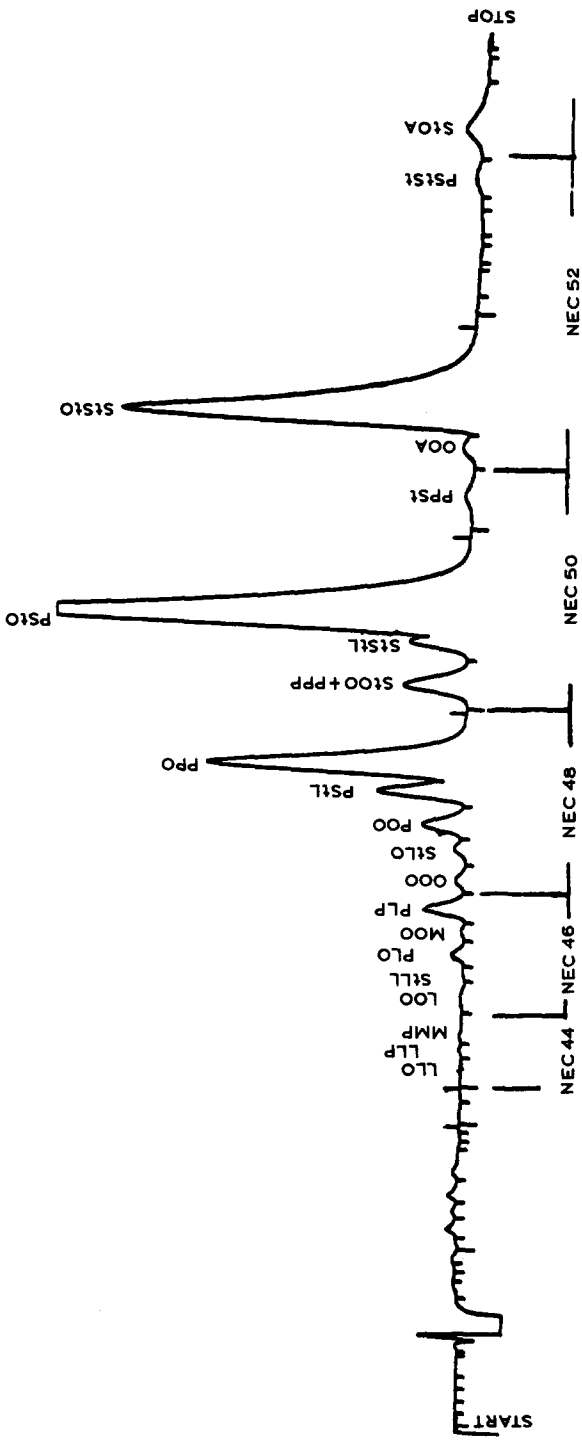

Fig. 1. Typical chromatogram of cocoa triglycerides.

TABLE 2

Quantitative Analysis

(Results are expressed in relative percentages (mean value))

Geographical origin	*Code*	*NEC 46*			*NEC 48*					*NEC 48 NEC 50*	*NEC 50*			*NEC 52*			*NEC 54*
		LOO	*PLO (StLL)*	*PLP*	*OOO*	*StLO*	*POO*	*PLSt*	*POP*	*PPP + StOO*	*StLSt*	*StOP*	*StPP*	*OOA*	*StOSt*	*StStP*	*StOA*
Ivory Coast	1	1·03	1·46	2·57	0·80	0·77	2·49	3·23	15·97	3·40	1·53	36·84	0·47	0·46	26·69	0·62	1·67
Ivory Coast	2	0·41	0·57	2·04	0·43	0·43	2·32	3·18	15·23	3·80	1·63	37·98	0·67	0·70	28·09	0·77	1·74
Ivory Coast	3	0·74	0·78	2·37	0·60	0·54	2·59	3·29	15·83	3·92	1·47	38·31	0·50	0·46	26·67	0·52	1·40
Brazil (Bahia)	4	0·99	1·71	2·42	1·41	1·33	6·72	3·35	15·02	8·71	1·51	33·65	0·53	0·19	21·18	0·19	1·09
Brazil (Bahia)	5	0·88	1·56	2·27	1·05	0·98	5·13	3·24	14·73	7·00	1·44	35·14	0·66	0·35	24·15	0·37	1·30
Brazil (Rondonia)	6	0·20	0·45	1·84	0·36	0·33	2·04	2·89	17·63	2·95	1·43	40·21	0·40	0·52	26·48	0·50	1·77
Brazil (Rondonia)	7	0·37	0·78	2·08	0·66	0·73	3·39	3·21	15·78	4·59	1·48	37·83	0·74	0·42	25·78	0·39	1·77
Brazil (Rondonia)	8	0·44	0·53	1·61	0·81	0·45	3·17	2·76	16·85	3·88	1·08	38·87	0·70	0·46	25·71	0·46	2·20
Brazil (Amazonas)	9	0·13	0·40	1·68	0·18	0·29	1·61	3·00	17·56	2·32	1·44	40·28	0·61	0·67	27·17	0·83	1·83
Brazil (Pará)	10	0·58	0·68	1·52	1·17	0·80	5·87	3·34	15·55	8·08	1·75	35·13	0·87	0·07	22·66	0·30	1·71
Brazil (Pará)	11	0·55	0·70	1·95	0·54	0·47	3·75	3·06	16·77	5·18	1·39	37·66	0·77	0·43	24·84	0·32	1·63
Ecuador (Guayaquil)	12	0·42	0·96	1·89	0·58	0·68	4·34	3·35	15·62	6·10	1·27	36·26	0·63	0·35	25·02	0·93	1·58
Nigeria	13	0·49	0·78	2·16	0·46	0·47	2·68	3·46	15·86	3·71	1·54	38·77	0·35	0·33	27·12	0·45	1·36
Nigeria	14	0·19	0·39	1·67	0·50	0·30	2·07	2·84	16·71	2·68	1·27	40·70	0·29	0·60	27·72	0·48	1·97
Indonesia	15	0·37	0·51	2·05	0·46	0·37	2·00	3·38	15·30	3·29	1·89	40·18	0·31	0·47	29·93	0·38	2·02
Indonesia	16	0·20	0·48	1·82	0·42	0·52	3·11	3·02	15·92	4·88	1·48	37·89	0·51	0·44	26·53	0·64	2·12
Indonesia	17	0·16	0·36	1·76	0·31	0·29	2·16	3·04	15·41	3·53	1·58	39·29	0·46	0·38	28·98	0·59	1·68
Indonesia	18	0·14	0·42	1·61	0·31	0·31	2·34	2·85	16·05	3·58	1·39	39·75	0·40	0·29	28·31	0·56	1·70
Malaysia	19	0·14	0·36	1·44	0·22	0·28	1·80	2·63	14·31	3·25	1·27	40·03	0·53	0·39	31·03	0·71	1·72
Guinea	20	0·30	0·91	2·08	0·52	0·81	3·89	3·48	15·33	5·82	1·73	37·32	0·50	0·30	25·41	0·34	1·26

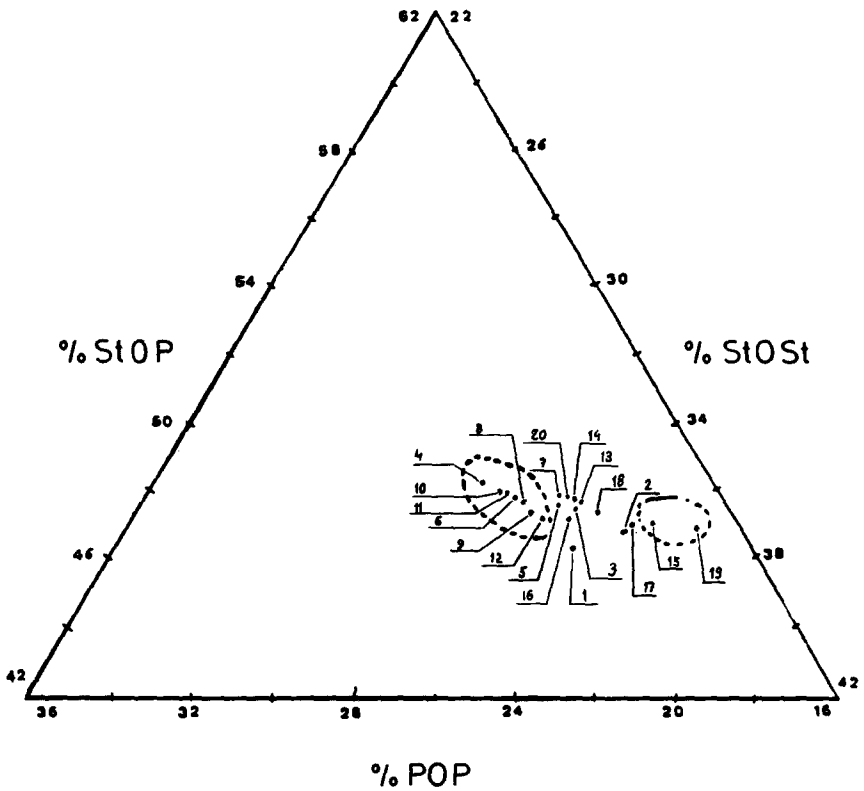

Fig. 2. Enlarged ternary diagram for StOP, POP and StOSt contents.

part of the ternary diagram is shown in Fig. 2. All cocoa samples fall within a small area but we can consider three different zones inside this area. First is the group from Malaysia and Indonesia with a low amount of POP and high amount of StOSt and, secondly, the groups from Brazil (Bahía, Pará, Rondonia and Amazonas) and Ecuador with a high amount of POP and low quantity of StOSt. Between both zones there are some samples, mainly from Africa (Ivory Coast, Guinea, Nigeria).

In order to identify the triglycerides that could be useful for the differentiation of the geographical origins of cocoa we have used stepwise discriminant analysis (SDA) as a statistical procedure, based primarily on a forward-selection process. For this purpose we have used the statistical software system from the Biomedical Computer Programs (BMDP) (1979).

Results of SDA show as triglycerides with discriminant power between LOO, StStP and PLO for the samples from Malaysia, Ecuador, Brazil (Bahía, Amazonas, Pará), Guinea and Ivory Coast. Table 3 shows the coefficients of the classification functions where variables $X1$, $X2$ and $X3$ are the LOO, PLO and StStP contents.

TABLE 3
SDA Analysis: Coefficients of the Classification Functions

(P1) *f* Ivory Coast	= 129·987 11 *X*1 − 40·522 67 *X*2 + 171·716 29 *X*3 − 85·216 13
(P2) *f* Bahía	= 45·435 57 *X*1 + 12·673 02 *X*2 + 91·848 72 *X*3 − 46·762 73
(P4) *f* Amazonas	= 86·629 73 *X*1 − 23·782 40 *X*2 + 161·056 05 *X*3 − 70·015 30
(P5) *f* Pará	= 82·025 58 *X*1 − 25·350 33 *X*2 + 98·723 04 *X*3 − 32·031 02
(P6) *f* Ecuador	= 95·929 74 *X*1 − 15·946 06 *X*2 + 189·449 48 *X*3 − 102·887 73
(P9) *f* Malaysia	= 78·382 65 *X*1 − 22·328 04 *X*2 + 140·551 51 *X*3 − 53·666 11
(P10) *f* Guinea	= −0·552 86 *X*1 + 24·629 01 *X*2 + 59·429 23 *X*3 − 23·528 82

In order to classify an unknown sample, *X*1, *X*2 and *X*3 values are substituted in the equations of Table 3 and the origin of the sample is attributed to the highest '*f*' value.

CONCLUSION

Triglyceride data obtained by reversed-phase HPLC with RI detection appears to be a rapid and powerful technique for the investigation of the geographical origin of cocoa beans, using the main triglycerides POP, StOSt, LOO, StStP for this purpose.

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