Multidimensional Analysis of Gas Chromatographic Data, Application to the Differentiation of Clove Bud and Clove Stem Essential Oils from Madagascar

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Clove is the dried, unopened bud of *Eugenia caryophyllus* (Spreng.) Bullock and Harrison (fam. Myrtaceae), a tropical tree cultivated in Tanzania, Democratic Republic of Madagascar, Indonesia, Sri Lanka, India and Malaysia. Clove, after black pepper, is the second most popular spice used as condiment. The volatile oil of clove is obtained by water distillation from the dried flower buds but also from stems and leaves. Clove oils are used in perfumery, food industry, dentistry and in Indonesia for tobacco industry. These oils are colorless to brownish yellow liquids, having the characteristic odor and taste of clove with some differences for each type.

The chemical composition of clove oil has been recently studied by several authors (Koller, 1979 and 1981; Lawrence, 1978 and 1980; Iwamuro et al., 1983; Gopalakrishnan, 1984; Muchalal and Crouzet, 1985). Among the forty components identified to date, eugenol is the major one with 80-90% of the oil.

Lawrence (1980) has examined the differences between clove bud, stem and leaf oils using the percentage of eugenol, eugenyl acetate, total sesquiterpene hydrocarbons and the total of the non-phenolic oxygenated compounds, but the chemical composition of the three types is very similar. The discrimination used by Lawrence (1980) is based mainly on some major peaks or the sum of some major peaks.

With capillary gas chromatography, which is capable of separating a large number of components in essential oils, the choice of the peaks that contain the most information for classification can be very difficult because the large peaks are not necessarily the most informative.

Pattern recognition and multi-dimensional analysis which have been successfully applied in enological research (Kwan and Kowalski, 1980; Noble et al., 1980; Moret et al., 1984) or in lipid research (Gaydou et al., 1984 and 1985) were used to distinguish clove bud and clove stem oils from Madagascar. Because of the difference in price among these two types, clove bud oil is frequently adulterated by the stem oil. Since climatic conditions would affect the chemical compo-

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sition, samples produced during three years (1980, 1983 and 1985) were investigated to check the influence of the climatic factor on the differentiation of the two clove oil categories.

Experimental Section

Materials

The forty-four samples of clove bud and clove stem essential oils were collected in the production from lots for which genuineness and typicality were guaranteed by the "Service du Conditionnement et du Contrôle de la Qualité des Produits" of Antananarivo (Madagascar). The data set was constituted by the following samples: 22 clove bud oils (1980, 7; 1983, 8; 1985, 7) and 22 clove stem oils (1980, 7; 1983, 8; 1985, 7). All samples were stored in a refrigerator.

Gas Chromatography

Analyses by GC were done on a FID-type Girdel 30 gas chromatograph. Detector and injector temperatures were set at 230°C. The GC column used was a Carbowax 20 M WCOT glass capillary column (50 m, 0.30 mm i.d., 0.15 μm phase thickness) and the oven temperature was programmed from 70 to 210°C at 2°C min^{-1}; inlet pressure of hydrogen used as carrier gas 0.4 bar, split 40 $mL.min^{-1}$. Before the analyses of new samples were made, reproducibility of measurements over time was checked, reanalyzing some of the earlier samples. In this way differences due to possible evolution of samples and analytical conditions were prevented.

Gas Chromatography/Mass Spectrometry

Combined GC-MS was recorded on a Girdel 30 gas chromatograph linked to a Ribermag R-10-10B mass spectrometer and coupled with a Sidar data computer. The GC column was a 0.30 mm i.d.x. 50 m fused silica capillary column coated with Carbowax 20 M, 0.15 μm phase thickness. The column temperature was programmed from 70 to 210°C at 2°C.min^{-1}, carrier gas helium, ion source 220°C, ionization voltage 70 eV.

Retention Indexes and Identification

The various constituents were identified by comparison of their Kovats retention indices (I_K) with those of authentic samples purchased if available and by comparison with reported I_K values and mass spectra.

Statistical Analyses

Principal Component Analysis (PCA) was performed using a data set transformed into centered and reduced variables (standardized PCA). The initial data set was composed of the values taken by 14 variables and the 44 clove oil samples. Factor discriminant analysis (FDA) was performed to classify the clove oil samples either in two categories (buds and stems for each year or for all years) or three categories (the years of production for buds and stems). Further descriptions of PCA and FDA are provided by Romeder (1973), Lebart et al. (1982) and Foucart (1982). All processing was done on the computer (Hewlett Packard HP 1000) of the "Ecole Supérieure de Chimie" of Marseilles (France).

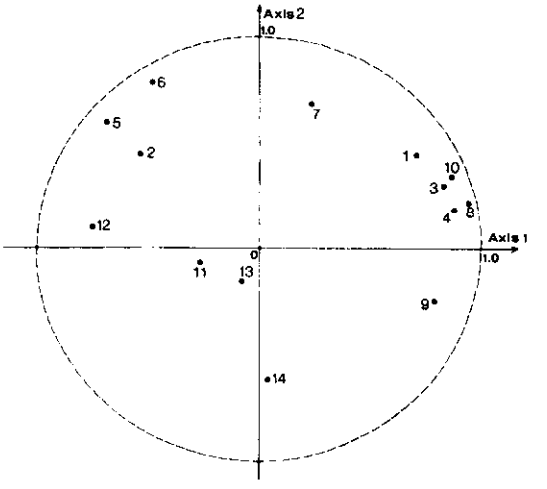

Figure 1. Graphical representation of variables of clove bud and clove stem oils on eigen vectors 1 and 2 were used in PCA for the three years investigated.

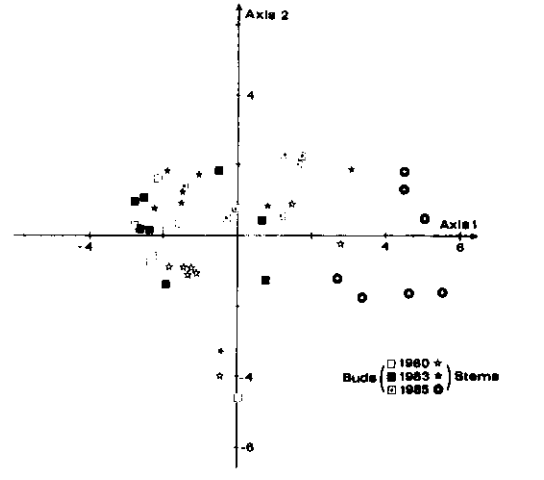

Figure 2. Graphic projection of clove bud and clove stem oil samples on axes 1 and 2 used in PCA for the three years investigated.

Results and Discussion

The essential oils of clove bud and stem oils from Madagascar obtained during the years 1980, 1983 and 1985 were examined initially by routine temperature-programmed GC using a glass capillary column coated with Carbowax 20 M. Among the complex mixture of components, 14 peaks, the area percentage of which was higher than 0.1% in, at least, one sample were retained for the differentiation of clove bud from clove stem oils.

The compounds were identified as far as possible by using GC-MS analyses and Kovats Indices determined on the Carbowax 20 M column. Results for the 22 samples of stem oils are tabulated in Table I and for the 22 samples of bud oils in Table II. Among the 14 products listed in Tables I and II, 12 were identified and only one component, (*E*)-α-bergamotene was determined for the first time in clove oil. The I_K value for this component was in agreement with those given by Maarse (1973) and the mass spectrum with those given by Vernin et al. (1984). In 1985, Muchalal and Crouzet reported that clove oil contained α-cubebene, α-copaene, β-caryophyllene and α-humulene. The oxygenated compounds such as methyl benzoate, terpinyl acetate, caryophyllene oxide, eugenol, eugenyl acetate and chavicol have been reported by Lawrence (1978). The presence of isoeugenol in clove oil was reported in 1976 by Masada.

As you can see from Tables I and II, four components have an averaged content higher than one percent: eugenol 73.5-79.7% in bud oils and 76.4-84.8% in stem oils, β-caryophyllene 7.3-12.4% in bud and stem oils, α-humulene 1.0-1.4% in bud and stem oils and eugenyl acetate 4.5-10.7% and 1.5-8.0%, respectively.

These results based upon 44 oil samples obtained from the production of three years show that there is some differences in the amount of eugenol and eugenyl acetate present in clove bud and clove stem oils. But the large range of variation of the two compounds show that it is difficult to perform some classification among bud and stem oils during 1980, 1983 and 1985. The use of multivariate statistical analyses is needed to try some differentiation.

Principal Component Analysis

In standardized Principal Component Analysis (PCA), the numerical values of the 14 compounds reported in Tables I and II were used to classify the 44 samples of bud and stem oils produced during the three years. The correlation coefficient matrix of compounds is given in Table III. Eugenol content showed a significant negative

correlation (r = −0.96) with eugenyl acetate. This correlation is in agreement with the fact that the longer the distillation time the higher the eugenol content and the lower the eugenyl acetate content (Lawrence, 1978). From the relationship between sesquiterpenes, there was significant positive correlation between β-caryophyllene-α-humulene (r = 0.88) and α-cubebene-(*E*)-α-bergamotene (r = 0.78). On the other hand a significant positive correlation (r = 0.90) was shown between caryophyllene oxide and one oxygenated product and these two compounds showed a significant positive correlation with α-cubebene, (*E*)-α-bergamotene and methyl benzoate (r = 0.7-0.9).

In PCA, it can be observed that the three first principal components represent 68.8% of the total variance. The factor loading between compounds and axes are given in Table IV. In figure 1, the projections of the compounds on the two first components axis 1, 41.6% and axis 2, 17.9% of the variance) is plotted. In figure 2 the projection of the 44 oil samples on to the principal components 1 and 2 is shown. According to fig. ure 1 and Table IV, one can notice that the oxygenated compound *11* and chavicol *13* have low factor loadings on axes 1 and 2 but have better factor loadings on axis 3 (−0.51 and 0.53 respectively). Caryophyllene oxide, the oxygenated compound *10*, eugenol, methyl benzoate and (*E*)-α-bergamotene are strongly positively loaded on axis 1. Eugenyl acetate, β-caryophyllene and α-copaene are negatively loaded on the first component. α-Humulene (0.77) and terpinyl acetate (0.67) are positively loaded but chavicol is negatively loaded on the second component. As shown by figure 2, if the differentiation of stem oils obtained in 1985 occurred on axis 1, because of the high content in eugenol and the low content in eugenyl acetate, the differentiation of the other samples is less evident.

A classification in three categories, taking into account the year of production, was obtained by using factorial discriminant analysis (FDA) for the 44 samples and gives 91% correct attribution. Factor loadings between compounds and axes are given in Table IV. A plot of the samples is given in figure 3. Using only bud oil samples 100% of correct attribution and with stem oil samples 95.5% of correct attribution were observed showing, therefore, the influence of climatic conditions upon the composition of clove bud and stem oils.

A classification in two categories (bud and stem oils) by using FDA for the 44 samples gives 91% of correct attribution for the three years of production together. Factor loadings between compounds and the discriminant axis is given in Table IV.

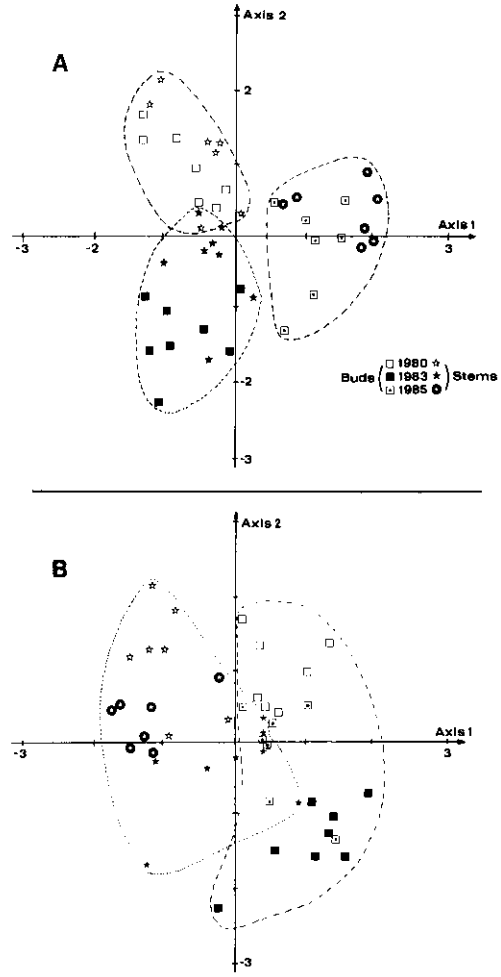

Figure 3. Graphic projection of clove bud and clove stem oil samples on axes 1 and 2 investigated in FDA. (A) Differentiation of bud and stem oils according to the three years of production. (B) Differentiation of bud oils from stem oils produced during the same years.

A classification in two categories for each year gives 100% of correct attribution, showing therefore that it is possible to realize the differentiation of clove bud from clove stem oils each year of production.

Stepwise discriminant analysis (SDA) was used for the determination of the compounds giving the best pattern recognition. The results obtained are given in Table V. The compounds having the better discriminant power in the differentiation of bud from stem oils are eugenol, α-cubebene, the oxygenated product *11*, terpinyl acetate, (*E*)-α-bergamotene and caryophyllene

Table I. Composition and Range of Variation of the Main Compounds in Clove Stem Oils Production and Their Kovats Indexes During Three Years

Peak n° on CW20M	I_K[b]	Compound[a]	Years of Production													
			1980[c]				1983[d]				1985[c]				1980-1985[e]	
			min.	max.	mean	s.d.	min.	max.	mean	s.d.	min.	max.	mean	s.d.	mean	s.d.
1	1415	α-cubebene	0.10	0.30	0.16	0.07	0.11	0.25	0.17	0.04	0.17	0.26	0.22	0.03	0.18	0.06
2	1477	α-copaene	0.12	0.16	0.14	0.01	0.12	0.16	0.14	0.01	0.11	0.14	0.12	0.10	0.14	0.01
3	1558	(E)-α-bergamotene	0.02	0.09	0.03	0.02	0.03	0.07	0.04	0.02	0.05	0.07	0.06	0.09	0.04	0.02
4	1565	methyl benzoate	0.04	0.13	0.06	0.03	0.02	0.24	0.08	0.06	0.16	0.24	0.21	0.03	0.12	0.08
5	1582	β-caryophyllene	7.32	11.77	10.49	1.39	8.57	12.41	11.12	1.21	8.03	9.95	9.27	0.61	10.5	1.20
6	1650	α-humulene	0.96	1.29	1.21	0.11	1.06	1.43	1.33	0.11	1.06	1.36	1.19	0.09	1.26	0.10
7	1732	terpinyl acetate	0.13	0.18	0.15	0.02	0.15	0.20	0.18	0.01	0.07	0.20	0.16	0.04	0.17	0.03
8	2016	caryophyllene oxide	0.13	0.35	0.21	0.08	0.17	0.47	0.25	0.09	0.29	0.48	0.43	0.07	0.28	0.12
9	2140	eugenol	79.62	81.25	80.60	0.58	76.42	80.51	77.97	1.36	82.71	84.82	83.98	0.72	80.80	2.65
10	2195	oxyg.compd[f]	0.01	0.07	0.04	0.02	0.03	0.09	0.05	0.02	0.05	0.09	0.08	0.01	0.05	0.02
11	2198	oxyg.compd[f]	0.01	0.05	0.02	0.01	0.02	0.04	0.02	0.01	0.01	0.02	0.01	0.00	0.02	0.01
12	2230	eugenyl acetate	4.17	6.57	4.81	0.77	4.40	8.04	6.58	1.23	1.54	1.95	1.80	0.15	4.40	2.12
13	2303	isoeugenol	0.14	0.18	0.16	0.01	0.15	0.20	0.17	0.02	0.15	0.19	0.17	0.01	0.17	0.02
14	2347	chavicol	0.04	0.65	0.23	0.22	0.06	0.18	0.09	0.04	0.05	0.11	0.07	0.02	0.11	0.11

[a]Only compounds having a content higher than 0.1% have been retained.
[b]Experimentally determined Kovats indexes
[c]Determined upon 7 samples
[d]Determined upon 8 samples
[e]Determined upon 22 samples
[f]Oxygenated compounds not identified

Table II. Composition and Range of Variation of the Main Compounds in Clove Bud Oils During Three Years Production

Compound[a]	Years of Production													
	1980[c]				1983[d]				1985[c]				1980-1985[e]	
	min.	max.	mean	s.d.	min.	max.	mean	s.d.	min.	max.	mean	s.d.	mean	s.d.
α-cubebene	0.11	0.17	0.14	0.02	0.01	0.25	0.16	0.08	0.18	0.29	0.23	0.04	0.18	0.07
α-copaene	0.14	0.17	0.15	0.01	0.12	0.16	0.14	0.01	0.14	0.15	0.14	0.00	0.14	0.01
(E)-α-bergamotene	0.02	0.03	0.02	0.00	0.02	0.07	0.04	0.01	0.04	0.06	0.05	0.01	0.04	0.01
methyl benzoate	0.05	0.08	0.06	0.01	0.04	0.13	0.07	0.03	0.04	0.15	0.11	0.04	0.08	0.03
β-caryophyllene	7.32	12.40	11.21	1.61	9.20	12.12	10.99	0.93	10.59	11.77	11.38	0.35	11.20	1.10
α-humulene	0.96	1.44	1.31	0.15	1.17	1.38	1.30	0.06	1.26	1.42	1.33	0.04	1.31	0.10
terpinyl acetate	0.13	0.18	0.15	0.02	0.14	0.17	0.16	0.01	0.16	0.20	0.18	0.01	0.16	0.02
caryophyllene oxide	0.16	0.22	0.19	0.02	0.17	0.29	0.22	0.05	0.23	0.34	0.29	0.04	0.22	0.06
eugenol	76.35	79.62	77.98	0.98	73.50	77.02	75.29	1.06	74.22	79.73	78.40	1.77	77.10	1.91
oxygenated compd.[f]	0.03	0.05	0.03	0.01	0.03	0.07	0.05	0.01	0.04	0.07	0.06	0.01	0.04	0.01
oxygenated compd.[f]	0.01	0.13	0.04	0.04	0.01	0.02	0.01	0.00	0.01	0.06	0.02	0.02	0.03	0.03
eugenyl acetate	6.09	7.29	6.63	0.36	8.11	10.72	9.67	0.84	4.54	9.22	5.50	1.58	7.38	2.08
isoeugenol	0.15	0.22	0.18	0.02	0.14	0.23	0.17	0.02	0.15	0.21	0.16	0.02	0.17	0.02
chavicol	0.04	0.47	0.14	0.14	0.05	0.18	0.09	0.04	0.05	0.10	0.07	0.02	0.10	0.09

[a]Only compounds having a content higher than 0.1% have been retained.
[b]Experimentally determined Kovats indexes
[c]Determined upon 7 samples
[d]Determined upon 8 samples
[e]Determined upon 22 samples
[f]Oxygenated compounds not identified

Table III. Correlation Coefficient Matrix Compounds used for the Differentiation of Clove Bud and Clove Stem Oils[a]

	α-copaene	(E)-α-bergamotene	methyl benzoate	β-caryophyllene	α-humulene	terpinyl acetate	caryophyllene oxide	eugenol	oxyg. compd[b]	oxyg. compd[c]	eugenyl acetate	iso-eugenol	chavicol
α-cubebene	- 0.03	0.78**	0.58**	- 0.27	- 0.06	0.35*	0.71**	0.32*	0.73**	- 0.21	- 0.40**	- 0.05	- 0.05
α-copaene		- 0.23	- 0.47**	0.53**	0.49**	0.10	- 0.37*	- 0.56**	- 0.35*	- 0.01	0.46**	0.13	- 0.11
(E)-α-bergamotene			0.61**	- 0.48**	- 0.21	0.32*	0.82**	0.45**	0.80**	- 0.20	- 0.48**	- 0.04	- 0.05
methyl benzoate				- 0.51**	- 0.24	0.21	0.86**	0.59**	0.84**	- 0.27	- 0.59**	- 0.11	- 0.15
β-caryophyllene					0.88**	0.18	- 0.53**	- 0.55**	- 0.43**	0.27	0.38*	- 0.16	- 0.38*
α-humulene						0.28	- 0.30*	- 0.52**	- 0.15	0.17	0.34*	- 0.11	- 0.49**
terpinyl acetate							0.25	0.04	0.36*	- 0.08	- 0.15	- 0.09	- 0.31*
caryophyllene oxide								0.61**	0.90**	- 0.24	- 0.62**	- 0.11	- 0.08
eugenol									0.42**	- 0.12	- 0.96**	- 0.06	0.03
oxyg. compound[b]										- 0.17	- 0.46**	- 0.16	- 0.03
oxyg. compound[c]											0.06	- 0.05	0.17
eugenyl acetate												0.06	0.02
isoeugenol													- 0.03

[a]Determined for the 44 samples investigated
[b]Oxygenated compound, peak n°10 on the CW20M column.
[c]peak n°11
*significant at the 5% level
**significant at the 1% level

Table IV. Factor Loadings Between Compounds and Axes by Using PCA or FDA of Clove Oil Samples

Compound[a]	PCA			FDA		
	axis			Versus year of production		bud vs stem
	1	2	3	1	2	
1	0.60	0.43	0.26	0.98	- 0.17	0.38
2	- 0.55	0.43	0.40	- 0.99	- 0.03	0.01
3	0.82	0.28	0.21	0.92	- 0.38	- 0.29
4	0.87	0.16	- 0.05	0.99	- 0.13	0.17
5	- 0.69	0.58	- 0.31	- 0.99	- 0.06	0.02
6	- 0.49	0.77	- 0.19	- 0.75	- 0.66	- 0.40
7	0.22	0.67	- 0.05	0.76	- 0.65	- 0.12
8	0.93	0.20	0.05	0.99	- 0.12	0.39
9	0.78	- 0.27	- 0.37	0.78	0.62	- 1.48
10	0.85	0.32	0.08	0.92	- 0.38	- 0.33
11	- 0.28	- 0.08	- 0.51	- 0.58	0.81	0.24
12	- 0.76	0.09	0.42	- 0.81	- 0.59	- 0.53
13	- 0.08	- 0.17	0.53	- 0.88	0.47	- 0.00
14	0.03	- 0.63	0.16	- 0.66	0.75	- 0.21

[a]For the name of compounds see Table I

Table V. Discriminant Power of Components used in the Differentiation of Clove Bud and Clove Stem Oils

Number of variable	Differentiation of Buds from Stems			Differentiation by Year of Production		
	Component	D.P.[a]	Correct attribution[b]	Component	D.P.[a]	Correct attribution[b]
1	eugenol	0.384	-	eugenyl acetate	0.522	-
2	α-cubebene	0.414	35	caryophyllene oxide	0.931	35
3	oxyg. compound[c]	0.442	36	chavicol	1.00	35
4	terpinyl acetate	0.465	35	(E)-α-bergamotene	1.06	38
5	(E)-α-bergamotene	0.481	34	α-copaene	1.12	37
6	caryophyllene oxide	0.503	33	eugenol	1.21	39
7	oxyg. compound[d]	0.520	36	α-humulene	1.28	39
8	β-caryophyllene	0.531	37	oxygenated compound[d]	1.32	38
9	chavicol	0.547	37	isoeugenol	1.33	39
10	α-humulene	0.554	35	oxygenated compound[c]	1.35	39
11	eugenyl acetate	0.562	38	α-cubebene	1.36	40
12	methyl benzoate	0.565	39	β-caryophyllene	1.37	40
13	α-copaene	0.565	39	methyl benzoate	1.38	41
14	isoeugenol	0.565	39	terpinyl acetate	1.39	40

[a]Discriminant power
[b]44 samples were investigated
[c]Oxygenated compound, peak n°11 on the CW20M column
[d]Peak n°10

oxide. Some compounds, such as β-caryophyllene, α-humulene, eugenyl acetate and methyl benzoate, have a lower discriminant power since they are highly correlated with some compounds cited above. The differentiation by year of production is easier and only six compounds (eugenyl acetate, caryophyllene oxide, chavicol, (E)-α-bergamotene, α-copaene and eugenol) permitted 88.6% of correct classification.

Conclusion

The differentiation of clove bud from clove stem oils produced in Madagascar seems to be possible using the content of less than 15 components, for each year of production. The influence of climatic conditions plays an important role on the chemical composition of these oils and more data are needed to realize a differentiation independent of the year of production or to detect an adulteration of clove bud oil with clove stem oil.

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