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Evaluation of bitterness and astringency of polyphenolic compounds in cocoa powder

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Total polyphenols, tannins, (−)-epicatechin and colorimetric fractions were determined in six samples of three batches of cocoa beans subjected to various stages of fermentation (1–8 days), in 36 samples of non-alkalinized low fat cocoa powder and 15 samples of instant cocoa powder. The physico-chemical data obtained were related to sensory evaluation and submitted to analysis of variance, multivariate distribution (Mahalanobis distance) and Pearson Chi-squared test. From the results obtained, we can infer that optimally fermented cocoa samples have a maximum total polyphenols of 58 mg g^{-1}, a tannins of 31 mg g^{-1}, and a (−)-epicatechin of 3 mg g^{-1}. Correspondence was not found between colorimetric fraction values and sensory data. These parameters are related to the sensory properties of cocoa and can be used to confirm deficiencies.

INTRODUCTION

Among the polyphenolic and flavonoid compounds of cocoa, tannins (hydrolyzable and condensed), flavan-3-ol group and anthocyanins are responsible for the astringent taste and affect stability and digestibility of cocoa (Bate-Smith, 1973; Haslam & Lilley, 1988; Haslam *et al.*, 1992). During the fermentation process, flavan-3-ol and tannins are subject to biochemical oxidation, increased polymerization and complexation with proteins, and hence decreasing solubility and astringency. Some groups of soluble compounds, flavan-3-ol [(+)-cathechin, (−)-epicatechin and (−)-epigallocatechin] are of interest in evaluating the astringency and bitterness of cocoa (Pettipher, 1986; Villeneuve *et al.*, 1989; Thorngate & Noble, 1995). Other components, such as anthocyanins (anthocyanidin glycosides), are hydrolyzed and the resultant aglycones are oxidised to quinonic compounds, contributing to the typical brown colour of cocoa beans (Haslam, 1982; Cros *et al.*, 1982*a*, 1982*b*). The drying process reduces cocoa water content (6 g 100 g^{-1}), impedes hydrolytic rancidity of cocoa butter, inhibits polyphenoloxidase activity (PPO) and limits micro-organism development (Ribeiro & Lopez, 1983; Hor *et al.*, 1984; Villeneuve *et al.*, 1985; Reeves *et al.*, 1988; Wong *et al.*, 1990). Reactions of oxidation and polymerization of polyphenols and hydrolysis of protein continue (Biehl *et al.*, 1982). Not-hydrolyzed protein is liable to form insoluble complexes with polyphenols, reducing cocoa aromatic precursors (Zak & Keeney, 1976; McManus *et al.*, 1981; Barel *et al.*, 1985). Roasting produces Maillard (non-enzymatic) reactions and generates the aromatic compounds of cocoa. Residual polyphenolic compounds are oxidised to quinones, ending cocoa transformation (Reeves *et al.*, 1988; Heinzler & Eichner, 1991*a*, 1991*b*; Ziegleder, 1991). This phenomenon points to the need for an analysis of polyphenolic fractions related to sensory analyses in commercial processed cocoa. Complementary studies of free, fixed, volatile and non-volatile acids, amino acids, as well as alkaloids and aromatic fractions of fermented-roasted cocoa have been performed (Serra Bonvehí & Ventura Coll, 1997). In this investigation, bitterness and astringency were evaluated.

MATERIALS AND METHODS

Samples

Six samples from each of three different batches of cocoa beans subjected to various stages of fermentation (1–8 days) were analysed. The fermentation process was performed according to the recommendations of the Comissao Executiva do Plano da Lavoura Cacaueira (CEPLAC, 1980). Also analysed were 36 samples of non-alkalinized fat cocoa powder (10–12% cocoa butter) of different origins (Ghana, Ivory Coast, Nigeria,

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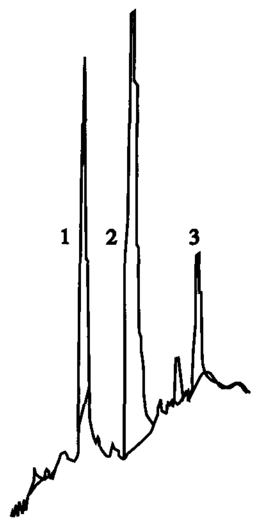

Fig. 1. HPLC chromatogram of (−)-epicatechin. 1, Gallic acid; 2, theobromine; 3, (−)-epicatechin.

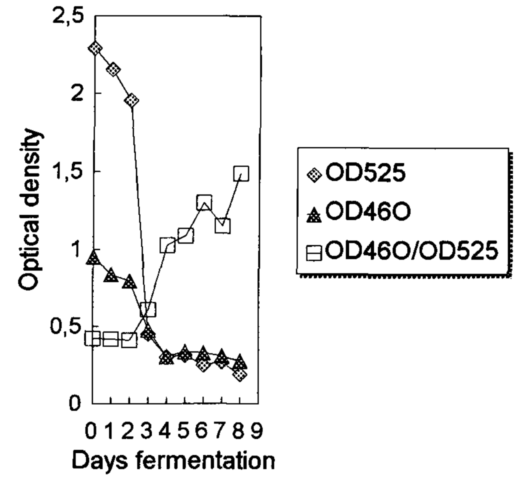

Fig. 3. Evolution of colour during fermentation of cocoa beans.

Table 1. Total polyphenols, tannins, non-tannins and (−)-epicatechin (mg/g)

Sample no.	Phenols	Non-tannins	Tannins	(−)-epicatechin
1	34.5	13.9	20.6	1.76
2	37.8	15.5	22.3	1.36
3	31.3	12.7	18.6	2.17
4	40.1	14.1	25.9	2.33
5	60.6*	29.7*	31.0	6.13*
6	62.2*	19.8	42.5*	3.11*
7	28.1	6.12	21.9	0.95
8	34.0	10.5	23.4	1.22
9	50.0	19.8	30.2	3.64*
10	19.8	11.4	8.38	1.69
11	21.5	10.5	11.0	1.31
12	34.6	14.6	19.9	1.45
13	21.7	12.2	9.5	1.93
14	27.4	13.5	14.0	1.73
15	32.4	12.7	19.7	2.21
16	35.0	17.0	18.0	2.17
17	31.7	14.9	16.8	2.02
18	34.2	16.8	17.4	1.90
19	47.9	28.5*	19.4	2.47
20	36.4	18.1	18.3	1.94
21	34.8	14.7	20.1	1.44
22	33.0	18.0	15.1	2.42
23	47.2	28.5*	18.7	1.78
24	51.1	29.5*	21.6	1.86
25	38.1	24.4	13.7	2.34
26	19.2	10.7	8.47	2.06
27	20.3	9.1	11.2	2.20
28	61.6*	27.9	33.7*	2.10
29	60.1*	28.7*	31.4*	2.30
30	50.5	22.5	27.9	1.80
31	36.9	17.8	19.1	1.23
32	34.8	14.7	20.1	1.24
33	33.0	18.0	15.1	1.97
34	36.6	21.8	14.8	0.94
35	29.0	10.8	18.2	0.97
36	33.8	21.4	12.4	1.95
X	34.3	15.7	18.3	1.79
s.d	8.64	4.8	5.66	0.45
V_{max}	51.1	27.9	31.0	2.47
V_{min}	19.2	6.12	8.38	0.94

*Outliers.

Brazil, Equador), and 15 samples of an instant cocoa powder (20–22% cocoa powder). The cocoa bean samples were milled in a coffee mill to a 2-mm particle size powder. Samples were preserved at 0–0.5°C and analyzed as soon they arrived at the laboratory. Analyses were carried out in triplicate.

Total polyphenols and tannins

The amounts of total polyphenols and tannins were determined using a modification of the method described by Marigo (1973). Cocoa (1 g) or instant cocoa powder (5 g) was extracted by agitating with 70% methanol (v/v). Phenols in the extract were determined with Folin-Ciocalteu reagent (RFC). A blank was prepared by agitating an aliquot of the extract at pH 3.5 with insoluble polyvinylpolypyrrolidone (PVP) (Polyclar AT, Carlo Erba, Milano, Italia). Absorbance was read at 760 nm, and phenols were determined using a calibration curve for 5, 25, 50, 100, 150 and 200 mg kg^{-1}

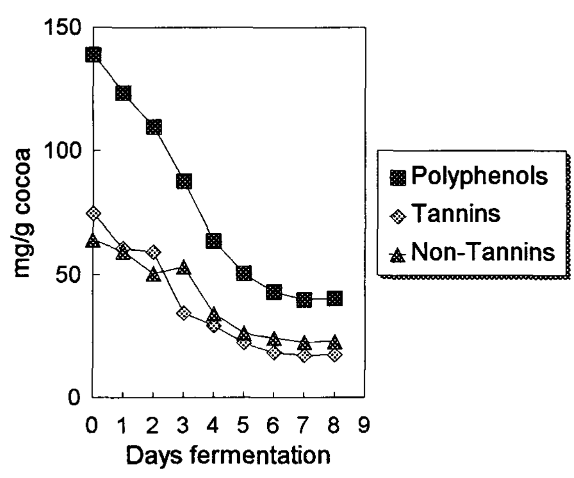

Fig. 2. Evolution of polyphenolic compounds during fermentation of cocoa beans.

of gallic acid. Tannin contents were determined by the difference of absorbances, by precipitating an aliquot of the extract at pH 4 using 1% NaCl in 10% gelatin solution.

(−)-Epicatechin

The amount of (−)-epicatechin was determined using a modification of the method described by Villeneuve *et al.* (1989). Cocoa (1 g) or instant cocoa powder (5 g) was extracted with 100 ml of acetone/water (75/25). The extract obtained after filtration was saturated with sodium chloride and then allowed to separate into two liquid phases. Internal standard (0.8 ml) of 0.2% gallic acid was then added to the acetone phase which was evaporated to dryness. The residue was dissolved again with 15 ml of water and washed four times with chloroform. The chloroform phase was discarded, centrifuging to 4000 rpm if necessary. The aqueous phase was filtered (0.45 μ) and filtrates were made up to 25 ml with methanol. High-performance liquid chromatography (HPLC) was performed as follows: Nucleosil C_{18} column (4.6 mm i.d.×250 mm; 10 μ); photodiode array detector at 278–282 nm; solvents: (a) bidistilled water, pH 2.6 (with H_3PO_4), and (b) methanol; flow rate, 2 ml min^{-1}; 0% methanol to 50% methanol in 16 min of linear gradient; loop, 20 μl. (−)-Epicatechin was obtained from Carl Roth GmbH (Karlsruhe, Germany). Figure 1 shows a typical chromatogram of the extract; procyanidins and theobromine were also identified.

Colour and colorimetric fractions

The colour and colorimetric fractions were determined using a modification of the method described by Cros *et al.* (1982*b*). Colour components were extracted from

Table 2. Colorimetric fractions of cocoa powder

Sample no.	OD_{525}	OD_{460}	OD_{460}/OD_{525}	OD_{525}				
				FI	FII	FIII	ϵOD	FI/FIII
1	0.189	0.376	1.99	0.027	0.035	0.105	0.167	0.26
2	0.188	0.363	1.93	0.025	0.029	0.105	0.159	0.24
3	0.225	0.389	1.73	0.020	0.020	0.105	0.145	0.19
4	0.302	0.480	1.59	0.094	0.059	0.191	0.344	0.27
5	0.766	0.897	1.17	0.257	0.083	0.317	0.657	0.81*
6	0.516	0.834	1.62	0.093	0.061	0.329	0.483	0.28
7	0.135	0.260	1.94	0.020	0.034	0.111	0.165	0.18
8	0.253	0.447	1.77	0.019	0.031	0.106	0.156	0.18
9	0.227	0.407	1.79	0.063	0.055	0.189	0.307	0.33
10	0.116	0.187	1.61	0.033	0.044	0.127	0.204	0.26
11	0.190	0.255	1.34	0.039	0.048	0.135	0.222	0.29
12	0.288	0.457	1.59	0.059	0.046	0.190	0.295	0.31
13	0.258	0.385	1.49	0.046	0.059	0.151	0.256	0.30
14	0.230	0.302	1.31	0.035	0.069	0.122	0.226	0.29
15	0.245	0.311	1.27	0.079	0.069	0.144	0.292	0.55*
16	0.224	0.291	1.30	0.042	0.060	0.137	0.239	0.31
17	0.359	0.558	1.55	0.073	0.093	0.213	0.379	0.34
18	0.264	0.368	1.89	0.046	0.075	0.181	0.302	0.25
19	0.350	0.562	1.60	0.051	0.076	0.134	0.261	0.38
20	0.164	0.241	1.47	0.036	0.031	0.125	0.192	0.29
21	0.187	0.245	1.31	0.039	0.051	0.130	0.220	0.30
22	0.214	0.282	1.32	0.048	0.062	0.165	0.275	0.29
23	0.340	0.425	1.25	0.050	0.061	0.150	0.261	0.33
24	0.480	0.510	1.27	0.080	0.118	0.208	0.406	0.38
25	0.220	0.271	1.29	0.050	0.091	0.157	0.298	0.32
26	0.216	0.278	1.29	0.042	0.062	0.155	0.259	0.27
27	0.169	0.199	1.18	0.032	0.057	0.110	0.199	0.29
28	0.459	0.629	1.37	0.088	0.096	0.240	0.424	0.37
29	0.388	0.520	1.34	0.089	0.124	0.260	0.473	0.34
30	0.472	0.614	1.30	0.094	0.103	0.251	0.448	0.37
31	0.240	0.318	1.33	0.042	0.079	0.125	0.246	0.34
32	0.372	0.585	1.57	0.061	0.085	0.241	0.387	0.25
33	0.229	0.305	1.33	0.036	0.059	0.141	0.236	0.26
34	0.312	0.460	1.47	0.073	0.065	0.187	0.325	0.39
35	0.205	0.307	1.50	0.031	0.056	0.138	0.225	0.22
36	0.307	0.470	1.53	0.064	0.077	0.179	0.320	0.36
X	0.290	0.410	1.49	0.060	0.060	0.170	0.290	0.29
s.d.	0.130	0.160	0.23	0.040	0.020	0.060	0.110	0.06
V_{max}	0.766	0.897	1.99	0.257	0.124	0.329	0.656	0.39
V_{min}	0.116	0.187	1.17	0.019	0.020	0.105	0.145	0.18

*Outliers.

cocoa (2 g) by agitating with 50 ml of a mixture 12 N HCl/CH_3OH (1 ml l^{-1}) for 45 min. The extract was filtered through 541 Whatman paper. An aliquot of filtrate (4 ml) was adjusted to 25 ml with 12 N HCl/CH_3OH. Absorbance was read at 460 and 525 nm against a blank. The colorimetric fractions were determined by introducing 2 ml of the filtered extract to a chromatographic column (1.50 cm i.d.) containing 1.5 g of previously-conditioned absorbent mixture of Silica G, Silicagel 60 and Polyclar AT (2/14/4). The fractions were eluted with 25 ml of a mixture 12 N HCl/CH_3OH (1 ml l^{-1}) (monomer anthocyanins), 25 ml of 50% formic acid (red polymers) and 25 ml formic acid (yellow and brown polymers). Absorbances were read at 460 and 525 nm against the corresponding blank and percentages representing every fraction in the total absorbance.

Sensory evaluation

Sensory evaluation was carried out by a taste panel under the conditions of the International Standard (ISO, 1985). Ten judges for the taste panel were selected to evaluate the acceptability (1) or unacceptability (0) of a sample. The sensorial attributes were smell and taste.

Statistical analyses

The results and the sensory evaluation were submitted to analysis of variance, and the multivariate distribution was made by Mahalanobis distance (Massart *et al.*, 1988), evaluated as Chi-squared, using the SPSS computer package (SPSS, 1990).

RESULTS AND DISCUSSION

In order to compare successive stages of fermentation, one sample of cocoa bean was taken daily for six days from each of the three batches fermented according to CEPLAC (1980). The results are shown in Figs 2 and 3. As can be seen in Fig. 2, polyphenols, tannins and non-tannins decreased during fermentation, and stabilized after 5–6 days. Based on six samples, each from different batches of cocoa beans, s.d. (mg g^{-1} cocoa) (at six days of fermentation) were: total polyphenols 43.1 ± 1.87; tannins 18.5 ± 1.12; non-tannins 24.6 ± 0.78.

Table 3. Statistical satisfactory/unsatisfactory results for polyphenols related to the sensory evaluation

Parameter	Sensorial accepted	Sensorial unaccepted	Totals
Correct total Polyphenols	29	3	32
Inadequate total Polyphenols	1	3	4
Totals	30	6	36

Table 4. Statistical satisfactory/unsatisfactory results for tannins related to the sensory evaluation

Parameter	Sensorial accepted	Sensorial unaccepted	Totals
Correct Tannins	29	4	33
Excessive Tannins	1	2	3
Totals	30	6	36

The OD_{460}/OD_{525} ratio increased to stabilize on the sixth day (Fig. 3). This ratio should be at least unity in well fermented cocoa (Gourieva & Tserevitinov, 1979). Table 1 shows the polyphenols, tannins, non-tannins and (−)epicatechin, and Table 2 shows colorimetric fractions of the samples of cocoa powder. An ANOVA test was performed to compare results of the polyphenol fractions obtained in relation to cocoa beans (Figs 2 and 3), and to non-alkalinised low fat cocoa powder (Tables 2 and 3); it is shown that significant differences ($P \leqslant 0.05$) exist for the values of total polyphenols and tannins contents, and OD_{460}/OD_{525} ratio. A study of outliers made by Mahalanobis distance ($P \leqslant 0.05$) established a maximum of 58 mg g^{-1} for polyphenols, 31 mg/g for tannins, 28 mg g^{-1} for non-tannins, 3 mg g^{-1} for (−)-epicatechin and a minimum of 1.1 for the OD_{460}/OD_{525} ratio in optimally-processed cocoa powder. These results coincide with the experimental values on the fifth day in optimally fermented cocoa beans. Monomeric flavan-3-ols [(+)-catechin and (−)-epicatechin] are more bitter than astringent. These compounds differ only in the absolute stereochemistry of the hydroxyl group at position C3 of the heterocycle. In contrast, higher molecular weight flavanoid polymers (polymeric flavan-3-ols) are generally more astringent than bitter (Robichaud & Noble, 1990). In recent studies, epicatechin was shown to be more bitter than catechin on an equal weight basis. Bitterness and astringency of aqueous solutions of catechin and epicatechin were evaluated by time-intensity methods (Thorngate & Noble, 1995). Epicatechin showed evidence for the astringency response above the 0.9 g $litre^{-1}$ level in aqueous solution (Thorngate & Noble, 1995). Samples 5, 6, 9, 28, 29 and 30 (Table 1) were classified as being organoleptically unacceptable.

Table 5. Statistical satisfactory/unsatisfactory results for (−)-epicatechin related to the sensory evaluation

Parameter	Sensorial-accepted	Sensorial-unaccepted	Totals
Correct (−)Epicatechin	29	4	33
Excessive (−)Epicatechin	1	2	3
Totals	30	6	36

Table 6. Polyphenols, tannins and non-tannins (mg g^{-1} cocoa) of instant cocoa powder

Sample no.	Phenols	Non-tannins	Tannins	(−)Epicatechin
1	7.89	5.08	2.81	0.26
2	11.7	7.21	4.46	0.31
3	6.04	4.09	1.95	0.27
4	10.3	6.43	3.87	0.26
5	2.86	1.11	1.75	0.30
6	3.14	1.41	1.73	0.31
7	3.83	1.84	1.99	0.29
8	3.99	1.56	2.43	0.32
9	7.16	4.57	2.59	0.18
10	7.13	4.13	3.00	0.20
11	7.02	5.65	1.37	0.24
12	7.17	3.93	3.20	0.18
13	6.75	3.29	3.46	0.32
14	6.27	4.50	1.77	0.25
15	5.73	3.79	1.94	0.24
X	6.46	3.91	2.55	0.26
s.d.	2.44	1.83	0.90	0.048
V_{max}	11.7	7.21	4.46	0.32
V_{min}	2.86	1.11	1.37	0.18

We evaluated the correspondence between the statistical satisfactory/unsatisfactory results and sensory evaluation (Tables 3–5). Samples classified as having ‘excessive polyphenols content’, ‘excessive tannins content’ and ‘excessive (−)-epicatechin content’ have at least one parameter over the maximum values established for optimally fermented samples. The Pearson Chi-squared test performed using results from Table 3–5 showed the existence of significant differences ($P \leqslant 0.05$) between accepted and unaccepted samples in terms of analytical and sensory evaluation. On the basis of these results, polyphenol, tannins and (−)-epicatechin contents have been related to the sensory score and the maximum values of these parameters could be used as an objective specification of the acceptance of product. The application of this specification to instant cocoa powder confirms the acceptance of the cocoa powder samples to be used as ingredients (Table 6). One can observe that the samples which are incorrectly fermented according to the percentages of polyphenols, tannins and (−)-epicatechin contents, are acceptable for OD_{460}/OD_{525} ratio (Gourieva & Tserevitinov, 1979). This contrast demands a study of different colorimetric fractions in low fat cocoa powder.

In Table 2, the FI fraction represents the anthocyanins and hydroxycinnamic compounds, the FII red polymers, and the FIII fraction yellow and brown polymers. These fractions undergo different chemical evolution, during the fermentation and processing of cocoa beans, and it was hypothesized that their sensory properties would also differ. In the FI fraction the anthocyanins decrease quickly, whereas in the FIII fraction the yellow and brown polymers slowly increase. The FII fraction (red polymers) does not undergo significant modifications (Bourzeaix *et al.*, 1979, 1982; Cros *et al.*, 1982*b*). Consequently, the FI/FIII ratio goes through complex transformations during the fermentation and processing of cocoa beans. According to Cros *et al.* (1982*b*) the FI/FIII ratio of optimally fermented cocoa beans has to be lower than 0.30. Thus, the samples numbered 12, 15, 16, 17, 19, 23, 24, 25, 31, 34 and 36 can be classified as deficiently fermented cocoa beans, in keeping with this ratio, not corresponding with high polyphenol, tannin, and (−)-epicatechin contents (Table 2). The Pearson Chi-squared test ($P \leqslant 0.05$) confirms the non-correspondence between colorimetric fractions and sensory evaluation. Polyphenols, tannins and (−)-epicatechin were critical factors in defining the quality of cocoa used in chocolate manufacture.

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