Constituents of Cocoa Husks

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Cocoa Husk, Mineral Elements, Vitamins, Phytic Acid, Phenolic Constituents

Mineral composition, vitamins (group B, C and D), polyphenols, purine alkaloids, sugars and phytic acid were determined in twelve samples of cocoa husk of the main varieties of cocoa beans (*Theobroma cacao* L.) cultivated in Africa and South America. Macro- (K, Mg, Ca, Na, P, and Na) and micro elements (Cu, Zn, Fe, and Cr) were quantified using an inductively coupled argon plasma-optical emission spectrometer (ICP-OES), on dry-ashed samples. Vitamins, phenolic compounds, alkaloids, sugars, and phytic acid were determined using HPLC. The mineral and vitamins intake that cocoa husk consumption could represent was evaluated with regard to the Recommended Dietary Allowances (RDA). The percentage of phytic acid is medium (0.98±0.13 g/100 g). The most abundant phenolic compounds found were caffeic acid and gentisic acid (2,5-dihydroxybenzoic acid).

Introduction

Cocoa husk has been regarded as a potential alternative to conventional sources of dietary fibre and mineral elements (Martín Cabrejas *et al.,* 1994; Serra Bonvehí and Aragay Benería, 1998; Serra Bonvehí and Ventura Coll, 1998). Depending on the cocoa variety used, the conditions of cocoa bean fermentation and cocoa butter extraction process, different composition qualities have been observed. Cocoa husk is composed mainly of polysaccharides (cellulose and hemicellulose) and lignin, along with small quantities of phenolic compounds, tannins, purine alkaloids and cocoa butter (Abbiola and Tewe, 1991). The contribution of dietary fibre to improve health is the subject of much current debate. Epidemiological studies have indicated a relationship between a diet rich in dietary fibre and a reduced incidence of colonrectal cancer. Using *in vitro* and *in vivo* assays, individual flavonoids have been shown to have a range of protective properties such as antioxidant activity (Unten *et al.,* 1991; Rice-Evans *et al.,* 1994). The mineral composition of cocoa husk is an important quality aspect (Ekpa *et al.,* 1993). One of the factors determining nutritional properties is the presence of non-nutritional components mainly phytic acid and polyphenolic compounds (Bravo *et al.,* 1994). The balance of soluble salts and vitamins plays a fundamental role that affects the nutritional properties of the cocoa husk. To evaluate the suitability of cocoa husk as a source of food for humans, we have examined the composition of its dietary fibre. Although some plant foods, especially soybeans, are potentially rich sources of iron in the diet, this iron is poorly absorbed due to the high fibre content. Another factor commonly suspected to depress the availability of iron in cereal grains and legumes is phytate (Frölich, 1984; Plat and Clydesdale, 1984; Makkar *et al.,* 1997). The aim of the present work was to determine quantitatively the mineral elements, vitamins, flavonoids, sugars, and non-nutritional compounds that are included in cocoa husk.

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Materials and Methods

Cocoa husk samples

Twelve samples of unroasted cocoa husk of the main varieties of cocoa beans (*Theobroma cacao* L.) cultivated in Cameroon, Guinea, Ivory Coast, Nigeria, Brazil, Colombia, and Ecuador were studied (Table I). The samples were obtained from processed cocoa husks. The process included a first

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Table I. Cocoa husk sampling: site of origin.

Sample n°	Country
1	Ivory Coast and Nigeria
2	Ivory Coast and Nigeria
3	Ivory Coast and Nigeria
4	Nigeria
5	Cameroon
6	Cameroon
7	Ivory Coast
8	Colombia
9	Colombia
10	Ecuador
11	Cameroon and Guinea
12	Brazil

extraction of cocoa butter from well winnowed cocoa nib (cotyledon) by expeller pressing followed by a second solvent extraction in order to remove the residual fat. Commercial-grade hexane was used in the solvent extraction. All samples came from lots destined for sale on the market, and had been collected in 1995 and 1996. The samples were stored in the dark at room temperature and analysed as soon as they arrived to the laboratory. All samples were ground to pass a 0.3-mm screen.

Physicochemical analyses

Moisture

Water content was determined using 5 g of cocoa husk in a conventional kiln at 103–105 °C for 3–4 h, until a constant weight was reached (AOAC, 1995).

Ash

Ash percentage was measured by calcination, overnight at 500°–550 °C in a furnace, to constant mass (AOAC, 1995).

Mineral elements

To determine mineral elements, 5 g of cocoa husk was reduced to ashes at 500°–550 °C and dissolved in 2 ml acid 2 N HNO_3, made up to 50 ml with distilled water at 40 °C, and analyzed using inductively coupled argon plasma-optical emission spectrometer (ICP-OES) (Miller-Ihli, 1996). The ICP-OES used for the study was a sequential multielement analyzer (Varian Model Liberty 220). The samples were diluted to provide concentration in the proper emission range. The repeatability relative to standard deviation ranged from 1.1 to 5.3%, and the reproducibility relative to standard deviation ranged from 2 to 5%.

Inositol phosphates

Inositol tri- [IP3], tetra- [IP4], penta- [IP5], and hexaphosphate [IP6, phytic acid] were separated by HPLC, using the method of Sandberg and Ahderinne (1986) as modified by Segueilha *et al.* (1993). Inositol phosphates were extracted of 2 g of cocoa husk finely ground by agitation with 0.5 N HCl (20 ml/0.5 g) for 3 h at ambient temperature. The mixture was centrifuged at 12000×*g* for 15 min and the supernatant evaporated to dryness under vacuum at 40 °C. The dry extract obtained was recuperated in 15 ml of 0.025 N HCl and loaded on anion-exchange resin [1-X4 mesh 100–200 Cl^- form (500 mg); Bio-Rad Laboratories, Hercules, CA)] that was washed with the same volume of 0.025 N HCl (0.75 ml/min). Inositol phosphates were eluted from the resin using 25 ml of 2 N HCl (0.75 ml/min). The resulting extract was evaporated to dryness, resuspended in 2 ml of bidistilled water, and filtered at 0.45 μm before injection into the chromatographic system. HPLC-RI was carried out on a HPLC system consisting of Model 590 Waters Associate pumping units, a Model 712 Rheodyne valve loop injector fitted a 20 μl loop, and Waters Associate Model 410 Differential Refractometer detector. For phytic acid analysis, a PRP-1 (5 μm) (150 x 4.1 mm i.d.) reversed-phase analytical column was used. The mobile phase was prepared by mixing 560 ml of methanol and 410 ml of 0.035 M of formic acid. Ten milliliters of tetrabutylammonium hydroxide (TBA-OH)(40%, w/w, solution in water) were added, and the pH was adjusted to 4.3 by the addition of 72% (w/w) sulphuric acid. Solvent was pumped through a heated (40 °C) PRP-1 column at a rate of 1 ml/min (Lehrfeld, 1989). Inositol hexaphosphate (Sigma Chem. Co., St. Louis, MO) was dissolved in water and used as reference compound.

Total phenols

The sample (1.50 g) of cocoa husk finely ground was extracted by agitating with 70% methanol (v/v). Phenols in the extract were determined with Folin-Ciocalteau reagent (RFC). A blank was pre-

pared by agitating an aliquot of the extract at pH 3.5 with insoluble polyvinylpolypyrrolidone (PVP). Absorbance was read at 760 nm, and phenols were determined using a calibration curve for 5, 25, 50, 100, 150 and 200 mg/kg of gallic acid (Marigo, 1973).

Phenolic compounds

HPLC analysis of phenolic compounds was performed according to that of Serra Bonvehí and Ventura Coll (1994). The sample (3 g) of cocoa husk finely ground was dissolved in 25 ml of ethyl acetate; then 12.5 ml of 40% $(NH_4)_2SO_4$ and 2.5 ml of 20% HPO_3 were added and the flask was agitated for 20 min. The solution was poured into a separation funnel, the top phase was collected, and the extraction process was repeated. The organic phases were collected into a 100 ml flask and then concentrated to dryness under reduced pressure. The sample was redissolved in 20 ml of methanol, filtered through 0.45 μm mesh nylon non ST (Lida Manufacture Corporation, Kenosha, WI), and filled up to 25 ml. HPLC was performed following these steps: Nucleosil C_{18} column (10 μm)(4.6 mm i.d. x 250 mm); photodiode array detector at 278–282 nm and 278–350 nm; solvents: a) bidistilled water, pH 2.6 (with H_3PO_4), and b) methanol; flow rate: 2 ml/min; 0% methanol to 100% methanol in 33 min of linear gradient; loop, 20 μl. Phenolic compound quantitation was achieved by the absorbance relative to external standards.

Cocoa butter

Five grams was extracted with petroleum ether (40°–60 °C) for 6 h using a Soxhlet apparatus with previous acid hydrolysis (AOAC, 1995). Fat was determined as the difference in weight of dried samples before and after extraction.

Sugars

For analysis, 5 g of cocoa husk finely ground was extracted for 30 min with 40 ml of 80% ethanol (Smith *et al.,* 1986). The extract was filtered, and the filtrate was made up to 50 ml with ethanol. A 5 ml aliquot of this solution was passed through a Waters Sep-Pak C_{18} column activated with acetonitrile and water, the first 2 ml of the eluate were discarded, and the remainder of the eluate was used for determination of soluble sugars. HPLC-RI was carried out on a HPLC system consisting of Waters Associate Model 410 Refractive Index detector; column carbohydrate analysis 125 A (10 μm)(300 x 3.9 mm i.d.); flow rate 2 ml/min; mobile phase [CH_3CN/H_2O (80:20 v/v)]; column temperature 28 °C. Sugar quantitation was achieved by the absorbance relative to external standards. Retention times [RT: glucose, 3.54±0.07; fructose, 4.29±0.12; sucrose, 6.60±0.08 min].

Starch

The starch content was determined enzymatically by a Boehringer test kit method (Boehringer Mannheim GmbH, Mannheim, Germany). About 0.5 g of fat free cocoa husk was weighed. The starch was solubilized using 20 ml of dimethyl sulfoxide and 5 ml of 37% hydrochloric acid, incubated at 60 °C for 30 min. The pH was adjusted to between 4 and 5 in a 100 ml volumetric flask, made to volume with double-distilled water. We used 0.1 ml of the solution to carry out the analysis.

Purine alkaloids

HPLC analysis of purine compounds was performed according to that of Kreiser and Martin (1978). Cocoa butter was extracted by shaking twice of 0.8–0.9 g of cocoa husk into test tubes equipped with Teflon-lined screw caps with 30 ml of petroleum ether for 6 min in an ultrasonic bath. The mixture was centrifuged at 2000 x *g* for 10 min and the solvent carefully decanted. The residue free of solvent was placed in test tubes in a 60° water bath. Dried test tube and contents (to determine weight of residue) were weighed and quantitatively transferred to deionised double-distilled water (DDD). Then the flask was heated for 25 min at 100 °C. After cooling water DDD was added so that the final weight equals weight of flask plus weight of the defatted residue plus 100 g. A 25 ml aliquot of this solution was centrifuged at 2000 x *g* for 5 min, and a portion of the supernatant filtered through a 0.45 μm membrane filter. HPLC system: photodiode array detector (DAD) 278–280 nm; column Nucleosil C_{18} (10 μm)(30 cm x 4 mm i.d.); flow rate 1 ml/min; mobile phase

[methanol/acetic acid/water (20/1/79 v/v)]. Theobromine and caffeine quantitation was achieved by the absorbance relative to external standards.

Vitamins group B

Five grams of cocoa husk finely ground was weighed in duplicate into 125 ml erlenmeyer flasks. Thirty milliliters of 0.1 N HCl were added to all flasks, and the mixtures stirred. The samples were placed in to a boiling water bath for 30 min, and shaken every 5 min. After acid extraction, the samples were adjusted to pH 4–4.5 with 1 ml 2 N sodium acetate, treated with 5 ml of a 10% aqueous enzyme solution (Clara-Diastase, No. 27540 Fluka Chemikals, Buchs, Switzerland), and incubated in a 45°–50 °C water bath for 3 h according to Serra Bonvehí (1991), Chase *et al.,* (1992), and Hägg (1994). Thereafter, 1 ml of 50% trichloroacetic acid was added, and the flasks were incubated for 15 min at 90 °C. After cooling the samples to room temperature, the pH was adjusted to 3.5 with 2 N sodium acetate, the sample volume was brought to 50 ml with water, and the mixture filtered through Whatman No. 42 filter paper. Standards solutions: thiamine-HCl(B_1) (0.1 mg/ml), riboflavin (B_2)(0.1 mg/ml), nicotinamide (B_3) (1 mg/ml), and pyridoxine-HCl (B_6)(0.1 mg/ml) were prepared in 0.1 N HCl (USP Reference Standards). Stock standards were stable for several months under refrigeration. Linearity was established by running two series of 4 standard dilutions for each vitamin, ranging in concentration from 0.5 to 2 μg/ml vitamins B_1 and B_2, from 0.2 to 2 μg/ml for vitamin B_6, and 5 to 45 μg/ml for vitamin B_3. Standards were teatred as the samples. Recoveries of 4 water-soluble vitamins from spiked placebos were in the range of 86.7 to 103%. HPLC system: column Nucleosil C_{18} stainless steel (4 μm)(15 cm x 3.9 mm i.d.): mobile phase [1-hexanesulfonic acid (0.95 g) was dissolved in 1 liter water DDD containing 9.5% acetonitrile and 0.5 ml ammonium hydroxide solution was adjusted to pH 3.6 with phosphoric acid. A 950 ml portion of this solution was diluted to 1 liter with water DDD. The mobile phase was then filtered through 0.45 μm nylon filter]; injection volume 50 μl; flow rate 1 ml/min; photodiode array detector 242 nm (vitamin B_3), 254 nm (vitamin B_1) and 268 nm (vitamin B_2)(Model 996 Waters); parameters for fluorescence detection (vitamin B_6)(in series with DAD) included sensitivity 0.3, excitation wavelength 295 nm, emission wavelength 395 nm, and slit 7 nm (Model 470 Waters). Thiamine was derivatized postcolumn. Derivatization: 0.2 M KOH; 0.01% potassium ferricyanide (Gehring *et al.,* 1995). Detection; attenuation factor, 2; gain, x 100; excitation wavelength, 370 nm; emission wavelength, 430 nm. Limits of detection were 0.18 μg/ml for thiamine, 1.3 μg/ml for niacinamide and 0.11 μg/ml for riboflavin by UV detection, 0.52 ng/ml for thiamine by postcolumn derivatization and fluorescence detection, and 0.014 μg/ml for pyridoxine by fluorescence detection.

Vitamin D compound

Vitamin D_2 and D_3 were determined by reversed-phase liquid chromatography with UV detection. The sample (5 g) finely ground was saponified 30 min at 60 °C and extracted into 60 ml hexane according to Sliva *et al.,* (1992) with solid-phase extraction silica column (500 mg/2.8 ml). HPLC system: column Nucleosil C_{18} (5 μm)(25 cm x 4.6 mm i.d.); mobile phase gradient combination of (A) acetonitrile, (B) methanol and (C) ethyl acetate; photodiode array detector, 265 nm; volume, 250 μl; standard solution (Vit. D_2 and Vit. D_3 3 μg/ml)(USP reference standard).

Vitamin C

Vitamin C was determined by HPLC with UV detection. The sample (20 g) finely ground was extracted by agitation with 70 ml of 0.05% disodium ethylenediaminetetraacetate (EDTA) for 10 min in an ultrasonic bath, centrifuged at 15000x*g* for 10 min, quantitatively transfer supernatant to 100 ml volumetric flask, and dilute to volume with solution EDTA according to Ashor *et al.,* (1984). HPLC system: column Aminex HPX-87 (30 cm x 7.8 mm); mobile phase 0.009 N H_2SO_4; photodiode array detector, 245 nm; standard solution L-ascorbic acid (20 μg/ml).

Phenolic, vitamins B and purine alkaloids identification

Flavonoids, vitamins B and purine alkaloids were identified by their UV spectra which had been recorded with a photodiode array detector

coupled to HPLC. The different phenolic compounds were identified by their bathochromic movement of band I (320–380 nm) and band II (240–270 nm) using hydroxylation, methylation and metal complexes in accordance with Markham (1982), as well as co-chromatography with pertinent markers.

Statistical analyses

Analysis of variance (ANOVA) and multiple range LSD test were conducted considering the factors mineral elements, vitamins, phytic acid, sugars, phenols and origen of samples. Statistical package software Statgraphics (SAS, 1990) was used to made the calculations.

Results and Discussion

The moisture content was in the range 3.6 to 7.8 g/100 g, mean 6.72±1.2 g/100 g. The moisture content was lower than the critical moisture content (8 g/100 g) for mould growth in cocoa (Schwan *et al.,* 1995), depending of the relative equilibrium moisture contents in different environments and lenghts of time of sun drying. The variation of ash and mineral elements contents could be a result of factors such as soil pH, soil nutrients, and/or level of fertilizer applied. With regard to human nutritional aspects, cocoa husk has a significant mineral content. Table II shows ash, mineral elements, sugars, polyphenolic compounds, and phytic acid. The total amount of mineral elements was high (x=10.7±0.88 g/100 g) this is 1.7 times higher than the mineral elements content in all bran cereals (6 g/100 g)(Schweizer *et al.,* 1984). The ash content of the cocoa bean varies between 2 and 3.8 g/100 g (Olaofe *et al.,* 1987). The mean content of all mineral elements presents at much higher levels than the other cocoa products. The difference was significant ($P \leq 0.05$). All samples were good sources of potassium, magnesium, calcium, phosphorus, copper and zinc. These results therefore suggest that cocoa husk can provide appreciable amounts of these minerals in cocoa-

Table II. Constituents of cocoa husks (g/100 g).

	Sample n°													
Parameter	1	2	3	4	5	6	7	8	9	10	11	12	X	SD
Moisture	6.30	7.44	6.90	7.00	7.10	7.60	6.60	7.30	7.80	7.70	5.30	3.60	6.72	1.20
Ash	11.6	10.5	11.9	9.80	9.30	10.2	10.8	10.9	11.4	12.0	10.7	9.70	10.7	0.88
Cocoa butter	2.50	2.20	2.30	2.30	2.40	2.20	2.30	2.30	2.20	1.80	2.30	3.00	2.32	0.27
Glucose	1.01	0.88	1.06	1.32	1.19	1.18	1.26	1.11	0.97	0.87	1.12	1.30	1.11	0.15
Fructose	0.60	0.46	0.62	0.42	0.98	0.46	0.90	0.38	0.36	0.39	0.41	0.70	0.56	0.21
Sucrose	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
Sugar total	1.61	1.34	1.68	1.74	2.17	1.64	2.16	1.49	1.33	1.26	1.53	2.00	1.66	0.31
Starch	1.10	1.30	1.07	0.95	1.40	1.20	0.85	0.97	1.10	1.20	1.30	1.18	1.13	0.17
Polyphenols 1	0.44	0.58	0.52	0.65	0.81	0.75	0.54	0.93	0.76	0.64	0.84	0.78	0.69	0.15
Polyphenols2	0.31	0.25	0.33	0.45	1.15	0.71	0.65	0.87	0.87	1.00	1.01	0.53	0.68	0.31
Tannins	0.05	0.12	0.07	0.10	0.17	0.11	0.15	0.24	0.23	0.19	0.45	0.10	0.17	0.11
Non-Tannins	0.26	0.13	0.26	0.35	0.98	0.60	0.50	0.63	0.64	0.81	0.56	0.43	0.51	0.24
Gentisic acid	0.35	0.49	0.41	0.54	0.66	0.60	0.44	0.70	0.56	0.48	0.66	0.67	0.55	0.11
Caffeic acid	0.09	0.09	0.11	0.11	0.15	0.15	0.10	0.23	0.22	0.16	0.18	0.11	0.14	0.05
Phytic acid	0.87	0.98	1.20	0.93	1.11	1.15	0.88	1.07	0.97	0.89	0.78	0.95	0.98	0.13
P	0.66	0.61	0.66	0.60	0.67	0.64	0.58	1.00	0.76	0.50	0.94	0.75	0.69	0.15
Ca	0.26	0.40	0.51	0.32	0.26	0.25	0.36	0.31	0.24	0.44	0.27	0.33	0.33	0.09
Mg	0.67	0.83	1.29	0.77	0.86	0.79	0.87	1.27	0.99	0.74	1.07	1.00	0.93	0.20
K	1.25	1.27	1.80	1.79	1.62	1.40	1.72	1.69	1.64	1.75	1.82	1.80	1.63	0.21
Cr mg/kg	6.67	7.74	9.65	7.74	8.16	7.84	9.93	8.96	7.27	8.02	6.72	11.3	8.33	1.38
Cu mg/kg	23.5	58.2	66.2	37.5	41.0	42.1	37.2	45.3	47.1	31.4	54.7	33.1	43.1	12.1
Zn mg/kg	27.5	65.8	69.3	34.8	33.1	37.3	30.0	75.3	69.8	55.2	46.1	49.3	49.5	17.3
Fe mg/kg	310	730	670	643	687	805	562	332	276	638	528	605	566	173
Na mg/kg	396	1427	1922	810	1451	1005	439	1396	469	624	278	616	903	530
Vit. B1 mg/100 g	0.16	0.08	0.08	0.10	0.30	0.31	0.10	0.12	0.19	0.11	0.17	0.07	0.15	0.08
Vit. B2 mg/100 g	0.12	0.18	0.18	0.13	0.10	0.27	0.10	0.19	0.28	0.11	0.31	0.09	0.17	0.08
Vit. B6 mg/100 g	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
Vit. D µg/100 g	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
Theobromine	0.93	0.81	0.95	0.76	1.45	1.39	1.00	1.22	1.12	0.65	1.52	0.96	1.06	0.28
Caffeine	0.09	0.07	0.09	0.07	0.13	0.11	0.09	0.20	0.16	0.10	0.13	0.09	0.11	0.04

1: spectrophotometric method; 2: chromatographic method.

based products. The presence of antinutritional compounds, mainly phytic acid and condensed tannins, reduces the bioavailability of such elements generating a major retention by the insoluble fibre. But in products with a high amount of dietary fibre, like cocoa husk, the retention of mineral elements is low. Fe, Ca and Zn are the most important (Plat and Clydesdale, 1984; Indouraine *et al.,* 1995). The correlation between minerals and soluble dietary fibre was calculated (Serra Bonvehí and Aragay Benería, 1998) and did not exceed the Pearson-Lee limit ($P \leq 0.05$). However, Frölich (1984) detected high correlation in cereals. Phytates have been shown to reduce the biovailability of minerals and to inhibit proteolytic and amyolytic enzymes (Nolan and Duffin, 1987). Tri-, tetra-, and pentaphosphate inositol were not detected. In mature plant seeds, the inositol phosphates occur mainly as hexaphosphate, but during food processes including prolonged heat treatment, it is likely that inositol phosphates and others are formed (Serra Bonvehí and Ventura Coll, 1997a). Phytic acid content was determined by HPLC as 0.98±0.13 g/100 g (Table II). A series of samples of different foods containing 0.008 to 10.7 g/100 g phytic acid were analysed (Lehrfeld, 1994). According to these results we conclude that the percentage of phytic acid is not substantial. In contrast to K, Na is present in low average in cocoa husk (x= 90.3 mg/100 g)(Table II). It is admitted that product supplies a mineral element in the diet when its content is higher than 15% of the Recommend Dietary Allowances (RDA) of the National Research Council (NCR, 1989). Among the mineral elements identified, Ca, P, Fe, Mg and Zn surpassed its RDA [Ca, 800 mg; P, 800 mg; Fe, 14 mg; Mg, 300 mg; Zn, 15 mg]. Cr is present in low average. The polyphenolic and flavonoid compounds are responsible for the astringent taste and affect stability and digestibility of cocoa husk (Serra Bonvehí and Ventura Coll, 1997b). Table II shows the soluble polyphenols (x= 0.69±0.15 g/100 g), tannins (x= 0.17±0.11 g/100 g), and non-tannins (x= 0.51± 0.24 g/100 g). Polyphenols were also quantified by spectrophotometry and chromatography. No significant differences ($P \leq 0.05$ were found between the two results. As a result of its low content, cocoa husk is less astringent than cocoa bean. Among the compounds of the phenolic fraction, caffeic acid and gentisic acid (2,5-dihydroxybenzoic acid) were identified as the most important in the cocoa husk (x= 0.69±0.15 g/100 g). Since the lipid content is low (≤ 3 g/100 g) (Table II) only vitamin D was identified. Vitamins B_1 (x=0.15±0.083 mg/100 g) and B_2 (x = 0.17± 0.079 mg/100 g) were the most important among the water-soluble vitamins, and their average is near to the 15% of the RDA needed. Vitamin B_6 was found in a very small amount and vitamin C was not identified. During each fermentation, chemical and physical changes were monitored (Tomlins *et al.,* 1993). Theobromine is the predominant alkaloid and its presence depends on the origin, ripeness and fermentation process (De Vries *et al.,* 1981). During the fermentation approximately a 40% of the theobromine content is lost by diffussion and migrator to the cocoa husk (Schwan *et al.,* 1995). As a result cocoa mass tasts less bitter than cocoa husk. Among the samples analyzed, 73% of them, showed a theobromine content higher than 0.91 g/100 g (x= 1.06±0.28 g/100 g). Caffeine only represents 10% of the total purine alkaloids (Table II). During the roasting process of the cocoa husk theobromine was bound to various diketopiperazines (DKPs) generating an accentuated bitter taste (Pickenhagen *et al.,* 1975; Van Der Greef *et al.,* 1987). The free sugar content is low (x= 1.66± 0.31 g/100 g) among them, glucose (x= 1.11±0.15 g/100 g) and fructose (x= 0.56±0.21 g/100 g) are the most important. The amount of sucrose is very low. Starch was measured in a low quantity (x= 1.13±0.17 g/100 g) (Table II). The analysis of variance of the chemical constituents values between samples and origen of samples showed no significantly differences ($P \leq 0.05$).

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