Chemical Composition of Wild *Theobroma* Species and Their Comparison to the Cacao Bean†

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Theobromine, caffeine, and theophylline content were determined in different parts of the plant in two varieties of *Theobroma cacao* and two wild *Theobroma* species, *Th. bicolor* and *Th. angustifolium*. Proximate analysis was performed for the bean, pulp, and shell of the four samples, and trypsin inhibitors and hemagglutinins were also investigated in beans and leaves. Fat was the main component in the seeds of all of the samples. The highest levels of alkaloids were found in the seeds of both varieties of *Th. cacao*, theobromine being the main alkaloid found: 1.39 and 2.03 g/100 g in seeds of criollo and Costarrica varieties, respectively. In the *Th. bicolor* the highest concentration of theobromine was in the hull, 915 mg, followed by flowers, 725 mg, and leaves, 619 mg/100 g of sample. In *Th. angustifolium*, the highest concentration of theobromine was in the flowers (510 mg/100 g of sample). Caffeine was the second more important alkaloid in the cacao beans (180–920 mg/100 g of sample). In the wild theobroma species it was the alkaloid found in the lowest concentration in the seeds; in the flowers of the *Th. bicolor* it was in higher concentration (96 mg/100 g of sample). Theophylline in the *Theobroma* genus has not been previously reported. The highest concentration was found in the seeds of *Th. cacao* (357–367 mg/100 g of sample) and in the flowers and leaves of *Th. bicolor* (301 and 187 mg/100 g of sample, respectively). Trypsin inhibitor content in the seeds of *Th. cacao* was higher than in the wild species, 30–41 and 8–8.6 TUI/mg of sample, respectively. No hemagglutinins were found in any of the four samples studied.

INTRODUCTION

Many species of tropical plants belong to the *Theobroma* genus. Cuatrecasas (1964) has reported 22 species, one of which is the cacao bean (*Th. cacao*). The plant is native to America and is the only one at present with commercial significance (Hunter, 1990).

A very important cacao industry has emerged with the development of cocoa, cocoa butter, syrups, pastes, and all kinds of chocolates. For this reason, an increasing demand for cacao beans exists, and the price is steadily rising, which encourages farmers to increase production. Therefore, there is a growing demand for research on other wild species or varieties of this genus.

It is interesting to study other *Theobroma* species from several points of view including the content of theobromine, fat, and other components to determine whether they could be used as substitutes for the cacao bean and make them attractive to the food, pharmaceutical, and cosmetic industries.

Among several species of the genus *Theobroma* known in Mexico and in other countries is the cacao (*Th. cacao*) with several varieties, *Th. angustifolium*, *Th. bicolor*, *Th. biocarpum*, *Th. pentagonum*, etc. (Martínez, 1987).

In Mexico, the *Th. bicolor* and the *Th. angustifolium* species mainly grow wild in Chiapas and Tabasco and are used by the natives for different purposes.

Th. bicolor grows in widely dispersed areas, from Ecuador to Mexico, and it has different names, such as pataste in Mexico and Central America, white cacao in Ecuador, and bacao and wild cacao in Colombia. In contrast to cacao, *Th. bicolor* grows as a tree over 14 m tall; the pulp of the fruit is used by the natives to prepare a refreshing beverage, while the seeds are mixed with cacao beans as adulterant or to obtain a bitter chocolate.

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Less information exists on *Th. angustifolium*. In Brazil it is called emerald cacao and in Mexico castarrica; its growth is more limited than that of *Th. bicolor* (De la Vega and Sotelo, 1985).

In *Th. cacao* the most important component is the fat, which is widely used in the chocolate industry, in pharmaceuticals, and in cosmetics. In a previous work (Sotelo et al., 1990), the physical–chemical characteristics of the fat of *Th. cacao*, *Th. angustifolium*, and *Th. bicolor* along with other wild plants were described.

In the reviewed literature it was found that only theobromine and caffeine have been reported in the *Theobroma* genus; the same is true in the *Coffea* and *Cola* genera. However, in the genera *Ilex*, *Paullinia*, and *Thea*, theophylline was also found together with theobromine and caffeine (Raffauf, 1970; Hegnauer, 1973).

The main objective of the present work was to study the chemical composition, especially to detect and determine the three methyl xanthines content in the two wild species of *Theobroma* and its comparison with the cacao plant (*Th. cacao*).

No previous reports were found in the literature with regard to theophylline in *Theobroma* species.

The particular objectives of this work were as follows: (1) to determine the theobromine, caffeine, and theophylline content in different parts of the plant and beans of two varieties of *Th. cacao* and in the two wild species *Th. bicolor* and *Th. angustifolium*; (2) to carry out the proximate analysis of the beans of the four samples and compare the results to those obtained in a previous study; and (3) to investigate the presence of trypsin inhibitors and hemagglutinins in the material.

MATERIALS AND METHODS

The samples were collected in the suburbs of Tapachula, Chiapas, Mexico, and classified at the Biology Institute of the Universidad Nacional Autónoma de México.

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Table I. Proximate Composition of the Two Varieties of *Th. cacao* and the Two Wild *Theobroma* Fruits

sample	g/100 g of sample					
	moisture	protein	fat	fiber	ash	carbohydrate
Th. cacao						
Criollo variety						
seed	52.50	7.88	23.92	3.13	2.07	10.50
shell	94.69	0.42	0.06	1.53	0.53	2.75
Costarrica variety						
seed	61.01	6.34	19.56	3.17	1.41	3.62
shell	90.08	1.00	0.10	3.44	0.98	4.39
Th. bicolor						
seed	52.31	11.36	17.00	8.80	1.74	8.79
shell	50.59	3.25	0.37	31.98	1.11	12.59
pulp	92.14	1.25	0.29	0.83	0.70	4.79
Th. angustifolium						
seed	44.17	5.55	17.48	11.30	1.82	19.68
shell	44.68	3.51	0.17	28.56	1.89	10.19

Table II. Theobromine, Theophylline, and Caffeine Content in the Different Parts of the Fruit of *Th. cacao* Varieties and Wild *Theobroma* Species and the Effect of Fermentation on It

sample	mg/100 g of dry sample					
	theobromine		theophylline		caffeine	
	fermented	not fermented	fermented	not fermented	fermented	not fermented
Th. cacao						
Costarrica variety						
seed	2021.0	2034.0	357.9	325.4	180.0	182.0
hull	108.1	107.0	15.6	19.7	23.2	36.3
shell	22.3	17.4	7.6	5.8	5.6	5.1
Criollo variety						
seed	1474.8	1387.7	367.0	473.9	919.7	769.6
hull	143.4	93.6	25.7	13.0	13.0	72.3
shell	19.6	17.6	15.3	18.8	25.2	22.7
Th. bicolor						
seed	113.9	171.0	19.6	21.6	11.0	14.7
hull	878.7	915.8	21.0	21.1	19.2	23.5
shell	12.9	11.7	5.3	27.3	7.2	13.8
pulp	143.5	146.8	45.3	52.2	16.4	15.8
Th. angustifolium						
seed	33.0	36.8	8.4	9.6	3.5	5.7
hull	29.4	33.2	13.6	11.3	3.8	5.2
shell	3.2	3.0	5.2	4.7	8.2	8.0

Samples of two varieties of cacao (*Th. cacao*), Criollo and Costarrica, and of two wild species, pataste (*Th. bicolor*) and castarrica (*Th. angustifolium*), were collected.

From each sample, duplicate analyses of different parts of the plant were performed: The seeds (beans), pulp, shell, and skin of the beans (hull) were separated from the fruit, as well as the leaves and flowers.

The pulp was obtained only from the *Th. bicolor* since this part of the fruit in the other *Theobroma* species is tightly adhered to the shell, which makes it very difficult to remove.

The beans, shell, and hull were divided into two batches (1 and 2). The samples from batch 1 were dried in the oven at 50 °C and then milled in a Thomas Willey mill to pass a 1-mm sieve. The samples of batch 2 were wrapped in paper for 4 days to simulate the process of fermentation used by the natives to improve the concentration of theobromine in the external parts of the seeds. Then the samples were dried and milled as batch 1. The flowers and leaves were only dried at 50 °C and milled.

Proximate analysis was performed on the beans, pulp, and shell. The reducing and nonreducing sugars were determined in the shell and pulp. These determinations were done according to the techniques described by the AOAC (1984).

Alkaloids Determination. The theobromine, theophylline, and caffeine contents were determined according to the high-performance liquid chromatography (HPLC) technique described by the AOAC (1984) with minor modifications.

Alkaloids Extraction Method. Samples (0.5–1.0 g) were placed in a 125-mL Erlenmeyer flask with boiling chips; 50 mL of boiling demineralized water was added and then boiled for 20 min. All of the contents were placed in a 100-mL volumetric flask along with the washing water of the original flask; the solution was then cooled to room temperature (25 °C), and an internal standard of coumarine was added (100 μg/mL). The solution was then brought to a final volume of 100 mL, well mixed, and filtered. From this solution 20 mL was taken and the fat extracted with 10 mL of petroleum ether. The water phase was filtered through a 0.45-μm Millipore membrane filter, and from this filtered solution quadruplicate determinations were done by HPLC. Standard curves of theobromine, theophylline, and caffeine were prepared (Sigma, St. Louis, MO) by dissolving the solid alkaloid in boiling demineralized water to have a working solution of 100 μg/mL. From this solution the standard curve was prepared at concentrations from 1.0 to 10.0 μg/mL. For the standard curves of the three alkaloids the correlation coefficients of area vs concentration were greater than 0.997.

Liquid Chromatography. HPLC was performed on a Varian 5000 chromatograph equipped with a Vista 401 and variable UV detector set at λ = 280 nm, with a recorder paper speed of 0.5 cm/min, and a Micro-Pak MCH-10 column, 30 cm long × 4 mm i.d. The flow rate of the mobile phase, water/acetic acid/methanol (69:1:30), was 0.8 mL/min.

Trypsin inhibitor determinations were done on the beans and leaves according to the technique described by Kakade et al. (1974) using the synthetic substrate benzoyl-DL-arginine *p*-nitroanilide (BAPNA, Sigma).

Hemagglutinins Determination. The technique described by Jaffé et al. (1974) was employed using rabbit red blood cells.

Table III. Trypsin Inhibitor Content in Seeds and Leaves and Sugar Content in the Shell and Pulp of Cacao and Wild *Theobroma* Species

sample	trypsin inhibitors, TUI[a]/mg of dry sample	sugar content		
		reducing sugar	nonreducing sugar	total sugar
Th. cacao				
Costarrica variety				
seeds	39.06			
leaves	13.75			
shell		3.79	ND[b]	3.79
Criollo variety				
seeeds	41.43			
leaves	7.83			
shell		13.82	5.48	19.30
Th. bicolor				
seeds	8.00			
leaves	23.65			
shell		ND	ND	ND
pulp		13.56	32.65	46.21
Th. angustifolium				
seeds	8.57			
leaves	15.27			
shell		3.15	ND	3.15

[a] TUI, trypsin units inhibited (Kakade et al., 1974). [b] ND, not detected.

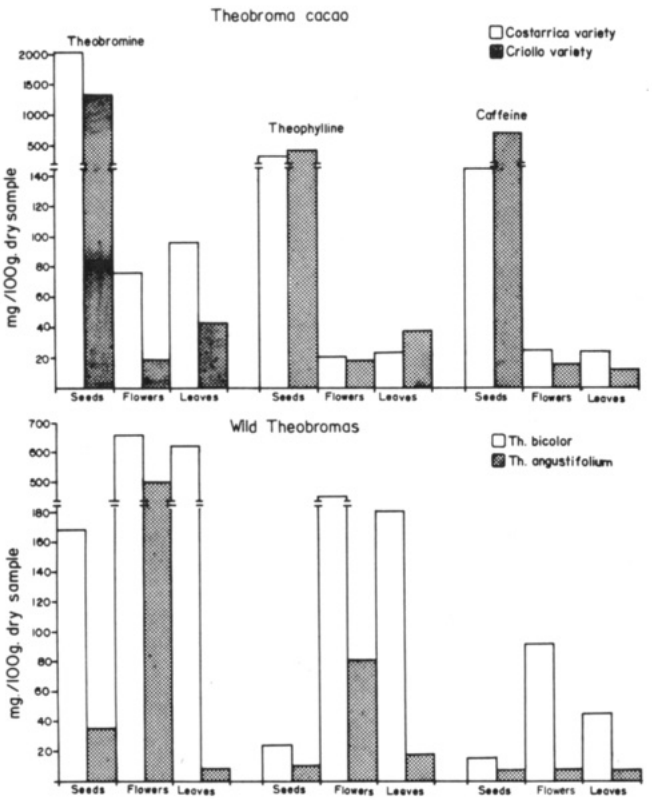

Figure 1. Alkaloid concentration in seeds, flowers, and leaves of the two varieties of *Th. cacao* and two wild species.

RESULTS AND DISCUSSION

The proximate composition in the *Theobroma* studied is shown in Table I. Fat was the main component of the beans, with the highest content in the two varieties of *Th. cacao*: 19.6 and 23.9%. The crude fiber was very high in both wild species. *Th. bicolor* showed the highest protein concentration (14.4%), which, expressed on a dry basis, increased to 23.83%; the lowest concentration was found in *Th. angustifolium* (5.5%). These results agree with those previously reported (Sotelo et al., 1990); however, the fiber content in the wild seeds was higher in the samples studied in the present work.

The three alkaloids were found in the four *Theobroma* species studied and in the different selected parts of the plants. The alkaloid content in the seed, hull, and shell of the two varieties of *Th. cacao* and the two wild species is shown in Table II. The highest content of the three alkaloids in the cacao varieties was found in the seeds and the lowest in the shell.

According to the findings of other authors (Roelofsen, 1958), in the fermented cacao fruit the theobromine of the seeds migrates to the shell; however, no significant differences were found in the alkaloid content between the fermented and nonfermented samples.

In the wild *Th. bicolor* the hull showed a higher concentration of theobromine than in the seed. Also, the pulp showed important concentration of this alkaloid.

Th. angustifolium showed the lowest concentration of the three alkaloids in the seed, hull, and shell.

Figure 1 illustrates the distribution of the theobromine, caffeine, and theophylline in the three different parts of the plant of *Th. cacao* and wild *Theobroma* species (seeds, flowers, and leaves). In the two varieties of *Th. cacao* the seeds were the most important reservoir of alkaloids, while in *Th. bicolor* the flowers and leaves have higher concentrations of the three alkaloids than the seeds. On the other hand, in *Th. angustifolium* the flower had the main content of theobromine and theophylline, while caffeine content was low and quite similar in the seeds, flowers, and leaves.

As was mentioned before, no information was found in the literature on the theophylline content in *Th. cacao*.

Table III shows the trypsin inhibitor content. The highest concentration was found in the seeds of the two cacao varieties, but in the wild species, the leaves had the highest concentration of this antiphysiological component; these values were lower relative to those observed in the seeds of *Th. cacao*. No hemagglutinins were found in any of the different parts of the *Theobroma* species studied.

Also shown in Table III is the sugar content in shells of the *Theobroma* species and in the pulp of *Th. bicolor*, which was the only one that could be removed from the shell. In all of the samples, except *Th. bicolor* pulp, the total sugar content was low, and probably most of it was glucose or fructose or both, since the direct reducing sugar value was higher or it was the only component. The pulp of *Th. bicolor* had a high total sugar content; the nonre-

ducing sugar had the highest value observed, and possibly it was sucrose. The sweet flavor and probably other components are the reasons the natives use it to prepare refreshing beverages.

Although the content of the three alkaloids and their distribution and concentration in all parts of the two wild *Theobroma* species were different from that in cacao, the present study confirmed the high fat concentration in the wild *Theobroma*, which makes them useful as a possible substitute for cacao.

Our work describes for the first time the theobromine, caffeine, and theophylline content measured in the seeds, hull, shell, flowers, and leaves of the same plant of two varieties of *Th. cacao* and in two wild species. Using HPLC, the three alkaloids were well separated in a few minutes. Theobromine, caffeine, and theophylline were always present in the different parts of the plant and in all of the species studied.

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Registry No. Theobromine, 83-67-0; caffeine, 58-08-2; theophylline, 58-55-9; trypsin inhibitor, 9035-81-8.