Supporting Information

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SI Text

Laboratory Analysis: Detailed Presentation of Methods. Quantities of burr (90–200 mg) were extracted from each sample vial using stainless steel spatulas that were cleaned after each procedure to prevent potential cross-contamination during the analytical phase. Samples were incubated with 200 to 300 μL milli-Q water at 80 °C for 30 min. After incubation, samples were vortexed and centrifuged. The resulting sediment from each sample was removed and the supernatant filtered using 5-kDa membrane filters. Filtrates were transferred to vials for UPLC/MS-MS analysis. Two unknowns supplied to the University of California Davis laboratory by T.G.P. (modern pottery samples) were prepared similarly and included in the analysis as controls.

Subsequently, a Xevo-TQ triple quadruple mass spectrometer (Waters) recorded MS and MS-MS spectra using Electro Spray Ionization in positive ion mode, capillary voltage of 3.0 kV, extractor cone voltage of 3 V, sample cone voltage of 32 V, and detector voltage of 500 V. Cone gas flow was set at 50 L/h and desolvation gas flow was maintained at 600 L/h. Source temperature and desolvation temperatures were set at 150 and 500 °C, respectively. The collision energy was varied from 16 to 26 to optimize four different daughter ions. The acquisition range was 20 to 300 Da. Pure theobromine was introduced to the source at a flow rate of 10 μL/min by using methanol:water (1:1) and 0.1% formic acid mixture as the carrier solution to develop multiple reaction monitoring method for UPLC/MS-MS operation (Fig. 2).

UPLC/MS-MS analyses of all of the samples were conducted using a Waters Acquity UPLC system connected with Xevo-TQ triple quadruple mass spectrometer. Analytical separations on the UPLC system were conducted using an Acquity UPLC HSS T3 1.7-μm column (1 × 150 mm) at a flow rate of 0.15 mL/min. The gradient started with 100% A (0.1% formic acid in H_2O) and 0% B (0.1% formic acid in CH_3CN), changed to 50% A over 3 min, followed by a 4-min linear gradient to 10% A, resulting in a total separation time of 7 min. The elutions from the UPLC column were introduced to the mass spectrometer and resulting data were analyzed and processed using MassLynx 4.1 software. Pure theobromine was used to optimize the UPLC conditions before analysis.

The UPLC/MS-MS analyses of the Olmec samples clearly showed the presence of a peak at 2.20 min (Fig. 3*D*) that matched well with the standard for theobromine (Fig. 3*B*), whereas no peaks were observed at 2.20 min in extracts from the two modern pottery control samples. We used four different multiple reaction monitoring transitions to independently confirm theobromine in the sample extracts. Indeed, all of the four multiple reaction monitoring chromatograms of theobromine-positive Olmec samples showed a peak at 2.20 min (Fig. 3*D*), which unequivocally confirmed its presence. A significant quantity of theobromine was found in eight samples (# 85, 97, 110, 119, 122, 125, 145, and 146), although theobromine also was present in trace amounts in an additional 19 samples. The remainder of the samples (n = 127) showed no detectable peaks at 2.20 min.

Summary of Sampled Pottery Types and Forms. ***Ojochi phase samples.*** Ojochi phase samples (n = 21) included bottles, incurved-rim bowls, neckless jars, and open bowls. Most were undecorated (n = 17); a minority (n = 4) exhibited fluting or punctates. The relative proportion of types for this phase corresponded to Acamaya red (43%), Caimán polished (38%), Chaya punctate (5%), Conchuda specular red (10%), and Delfín smoothed (5%).

Bajío phase samples. Bajío phase samples (n = 27) encompassed bottles, closed forms, incurved-rim bowls, necked jars, neckless jars, and open bowls. Decorative modes (n = 17) included wide incision, finger-impression, fluting, punctates, ridging, and rocker-stamping with the majority of these lacking plastic decoration. The proportion of types was Acamaya red (7%), Caamaño coarse (4%), Caimán polished (52%), Chaya punctate (7%), Cocodrilo smoothed (4%), Garza smoothed (4%), Eroded gray (4%), Pochitoca polished (11%), Tigrillo black and white (4%), and Xochiltepec white (4%).

Chicharras phase materials. Chicharras phase materials (n = 37) comprised bottles, closed forms, incurved rim bowls, necked jars, neckless jars, and open bowls. Most (n = 22) were undecorated; a minority (n = 15) showed fluting, punctates, thin or wide incision, rocker-stamp, and fingernail impressions. The proportion of types was Acamaya red (5%), Caimán polished (14%), Chipo red (5%), Conchuda specular red (24%), Garza smoothed (14%), Pochitoca polished (3%), Tejón white (3%), Reburnt Tigrillo (3%), Tigrillo black and white (19%), Tigrillo monochrome (8%), and Xochiltepec white (3%).

San Lorenzo A phase samples. San Lorenzo A phase samples (n = 21) included bottles, closed forms, incurved-rim bowls, a low annular-based open vessel, necked jars, neckless jars, and open bowls. Incising, punctates, finger impressions, ridging, rocker-stamp, modeled applications were present in the majority (n = 21), and the minority (n = 9) exhibited no decoration. The proportion of types was Caimán polished (5%), Chaya punctate (5%), Chipo red (5%), Conchuda specular red (5%), Garza smoothed (14%), Peje micaceous (5%), Pochitoca polished (10%), Tejón white (5%), Tigrillo black and white (10%), Tigrillo monochrome (24%), Tigrillo white-rimmed black (10%), and Xochiltepec white (5%).

San Lorenzo B phase samples. San Lorenzo B phase samples (n = 50) included a bottle, closed forms, a collared jar, cups, a handle/lug/support, incurved-rim bowls, a low annular-based open vessel, necked jars, neckless jars, small cups, and open bowls. The variety of decoration ranged from wide and thin-line incision and fingernail impressions to effigy modeling and applications, but approximately half (n = 22) were undecorated. The proportion of types included: Caamaño coarse (4%), Chipo red (2%), Conchuda specular red (4%), Conejo orange-on-white (2%), Garza smoothed (6%), Eroded gray (10%), Mulato black (4%), Pochitoca polished (2%), Tejón white (14%), Tiburón white (2%), Reburnt Tigrillo (4%), Tigrillo black and white (10%), Tigrillo monochrome (26%), Tigrillo white-rimmed black (6%), and Xochiltepec white (4%).

Table S1. Total sampled ceramic types by phase

Type	Ojochi	Bajío	Chicharras	San Lorenzo A	San Lorenzo B	Total
Acamaya red	9	2	2			13
Caamaño coarse		1			2	3
Caimán polished	8	14	5	1		28
Chaya punctate	1	2		1		4
Chipo red			2	1	1	4
Cocodrilo smoothed		1				1
Conchuda specular red	2		9	1	2	14
Conejo orange-on-white					1	1
Delfín smoothed	1					1
Eroded gray		1			5	6
Garza smoothed		1	5	3	3	12
Mulato black					2	2
Peje Micaceous				1		1
Pochitoca polished		3	1	2	1	7
Reburnt tigrillo			1		2	3
Tejón white			1	1	7	9
Tiburón white					1	1
Tigrillo black and white		1	7	2	5	15
Tigrillo monochrome			3	5	13	21
Tigrillo white-rimmed black				2	3	5
Xochiltepec white		1	1	1	2	5
Total	21	27	37	21	50	156

Table S2. Total sampled pottery forms by phase

Pottery forms	Ojochi	Bajío	Chicharras	San Lorenzo A	San Lorenzo B	Total
Bottle	4	4	3	1	1	13
Closed form		3	9	4	4	20
Collared jar			1		1	2
Cup					2	2
Handle/lug/support					1	1
Incurved rim bowl	3	3	3	1	5	15
Ladle					1	1
Low annular based open vessel				1	1	2
Necked jar		3	1		2	6
Neckless jar	2	2	4	2	4	14
Open bowl	12	12	16	10	17	67
Small cup				2	11	13
Total	21	27	37	21	50	156

Table S3. Theobromine-positive samples with information on provenience, phase assignation, pottery type, and form

Sample ID	Site	Excavation area	Stratum	Ceramic type	Form	Chronological phase
11	San Lorenzo	SL-53	X	Caimán polished	Bottle	Ojochi
22	San Lorenzo	SL-53	IX	Xochiltepec white	Open bowl	Bajío
30	San Lorenzo	SL-53	IX	Eroded gray	Necked jar	Bajío
51	San Lorenzo	C5-6	X	Tigrillo black and white	Open bowl	Chicharras
67	San Lorenzo	C5-6	X	Tigrillo black and white	Open bowl	Chicharras
85	San Lorenzo	Grupo D: B3-17	VI/VII	Mulato black	Cup	San Lorenzo B
90	San Lorenzo	A4 Ilmenitas JZN	V	Tigrillo monochrome	Small cup	San Lorenzo B
97	San Lorenzo	Grupo D: B3-17	VII	Pochitoca polished	Bottle	San Lorenzo B
101	San Lorenzo	A4 Ilmenitas JZN	V	Xochiltepec white	Necked jar	San Lorenzo B
103	San Lorenzo	A4 Ilmenitas JZN	V	Tigrillo monochrome	Open bowl	San Lorenzo B
107	San Lorenzo	C5-6	VI	Tigrillo black and white	Open bowl	San Lorenzo B
108	San Lorenzo	C5-6	VI	Tigrillo black and white	Incurved rim bowl	San Lorenzo B
110	San Lorenzo	A4 Ilmenitas JZN	V	Caamaño coarse	Neckless jar	San Lorenzo B
111	San Lorenzo	D5-31	X	Tigrillo monochrome	Ladle	San Lorenzo B
119	San Lorenzo	C5-6	VIII	Tigrillo white-rimmed black	Incurved rim bowl	San Lorenzo A
120	San Lorenzo	C5-6	VIII	Tigrillo monochrome	Open bowl	San Lorenzo A
122	San Lorenzo	C5-6	VII	Tigrillo monochrome	Open bowl	San Lorenzo A
123	San Lorenzo	C5-6	VI	Conejo orange-on-white	Open bowl	San Lorenzo B
124	San Lorenzo	C5-6	VIII	Peje micaceous	Closed form	San Lorenzo A
125	San Lorenzo	C5-6	VIII	Garza smoothed	Neckless jar	San Lorenzo A
126	San Lorenzo	C5-6	VIII	Tejón white	Low annular based open vessel	San Lorenzo A
129	San Lorenzo	C5-6	VIII	Xochiltepec white	Open bowl	San Lorenzo A
133	San Lorenzo	C5-6	VI	Xochiltepec white	Collared jar	San Lorenzo B
145	San Lorenzo	C5-6	VI	Tejón white	Low annular based open vessel	San Lorenzo B
146	San Lorenzo	C5-6	VI	Tejón white	Open bowl	San Lorenzo B
147	San Lorenzo	C5-6	VI	Tejón white	Open bowl	San Lorenzo B
152	San Lorenzo	C5-6	VI	Tejón white	Incurved rim bowl	San Lorenzo B