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Archaeobotanic Evidence of the Preincaic Chiribaya Culture. Determination of Capsaicinoids in Archaeological Samples of *Capsicum frutescens* and Votive Foods

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The lack of *Capsicum* species or paprika (a basic ingredient of Peruvian foodstuff) in preincaic archaeological samples and votive foods, as evidenced by archaeobotanic studies, has stimulated the chemical analyses of these samples by HPLC methods. The results confirmed the absence of capsaicinoids in these samples whereas they were detected in more ancient fossil fruit. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: HPLC analysis; capsaicin; dihydrocapsaicin; fossil fruits; archaeological samples; *Capsicum annuum*; Solanaceae.

INTRODUCTION

The Chiribaya culture developed between the tenth and the fifteenth century AD in the valley of Rio Osmore, a torrential stream which rises in the Andean tableland and passes through the city of Moquega before flowing into the Pacific Ocean near to the present town of Ilo in the extreme south of Peru. In 1982 the Contisuyo Program began archaeological studies in the area and the results of the first excavations were published in 1990 (Watanabe *et al.*, 1990; http://members.aol.com/contisuyo/MuseoE.html). The archaeological site of Yaral, situated about 50 km from the sea and in the central section of Rio Osmore, was a rural area of about 500 ha. In its two cemeteries, the remains of cultivated products, which it was the custom to offer to the dead, were found. The major part of the items recovered corresponded to panicles of *Zea mays*, followed by tubers of *Ipomea batatas* and *Solanum tuberosum*, and of species of *Manihot* and *Pachyrrizus*. Rhizomes of *Canna edulis* and seeds of *Phaseolus* species were also present. Some specimens of food which had been cooked were found in the bottom of cups and bowls, and sometimes it was possible to determine the individual ingredients such as beans and grains of maize. However, archaeobotanic analyses revealed the absence of species of *Capsicum* or paprika, a basic ingredient of Peruvian foods in the past and continuing to this day. The total absence of this socio-economically important ingredient has stimulated many further studies amongst which has been the chemical analyses of these ancient samples with the aim of finding capsaicin, the pungent active principle of *Capsicum* species.

The analysis of capsaicinoids in fresh and dried fruits of *Capsicum*, and in their derivatives, by HPLC-UV (Jentzsch *et al.*, 1969; Jurenitsch *et al.*, 1979; Attuquayefio and Buckle, 1987; Wood, 1987; Lopez-Hernandez *et al.*, 1996; Perucka and Oleszek, 2000; Zewdie and Bosland, 2001), by methods involving spectrofluorometric detection (Woodbury, 1980), and by supercritical fluid extraction/chromatography (Peusch *et al.*, 1997; Sato *et al.*, 1999) has been reported. SFE/SFC analysis gave very low detection limits but all of the capsaicinoids were detected as only one peak. HPLC methods provide similar detection limits but permit the separation of the individual capsaicinoids.

In the present paper we report the first application of HPLC to the analysis of capsaicinoids in archaeological samples and in samples of fossil fruits dating back to the second century BC.

EXPERIMENTAL

Materials. Standard capsaicin and dihydrocapsaicin were purchased from Aldrich (Milwaukee, WI, USA). Archaeological samples were collected in cemetery number 2 at Yaral (Peru) from Chiribaya tombs where food donations were present. Samples numbered 10482 (R. 233), 10493 (R. 236) and 10523 (R. 239) from the collection (presently housed in the Contisuyo Museum, Moquegua, Peru) were studied in the present work. The fossil fruits of *Capsicum frutescens* were collected from the archeological area of Cahuachi at Nazca on the south coast of Peru. Fresh fruits of *Capsicum annuum* were used as positive controls.

Sample preparation. All samples (0.5–1.0 *g*; see Table 1) were accurately weighed, pulverised in a blender after immersion in liquid nitrogen, and extracted with 80% ethanol (10 mL) at room temperature for 24 h with constant stirring. The solutions were filtered through Whatman (Maidstone, UK) number 42 filter paper, and the solvent evaporated at 40°C under vacuum. The

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Table 1. Quantitative analyses of capsaicinoids in fresh fruits and archaeological samples

Sample	Sample weight (mg)	Capsaicin (mg)[a] (percentage content)	Dihydrocapsaichin (mg)[a] (percentage content)
Capsicum annuum, fresh fruits	499	0.429 ± 0.004 (0.086%)	0.154 ± 0.003 (0.031%)
Capsicum frutescens, fossil fruits	966	0.036 ± 0.005 (0.0037%)	0.049 ± 0.005 (0.0051%)
10482 (R.233)[b]	1072	nd[c]	nd
10493 (R.236)[b]	984	nd	nd
10523 (R.239)[b]	1146	nd	nd

[a] Mean ± standard deviation ($n = 3$).
[b] Archaeological sample from cemetery number 2 at Yaral (Peru).
[c] nd = none detected.

residues were redissolved in 80% methanol in a volumetric flask for analysis (Jentzsch *et al.*, 1969).

Analytical methods. TLC analyses were performed using Merck (Darmstadt, Germany) Kieselgel 60 F_{254} pre-coated plates. After development in hexane:ethyl acetate:methanol (70:25:5), layers were sprayed with a 1% methanolic solution of 2,6-dichloroquinonechloroimide and then exposed to ammonia vapour: a blue spot evidenced a positive reaction for capsaicinoids.

HPLC analyses were performed on a Waters (Milford, MA, USA) model 600E chromatograph equipped with a UV-photodiode array detector set to monitor at 280 nm. A Merck RP-18 LichroCart column (250 × 4 mm i.d.) was subjected to gradient elution with a mobile phase consisting of acetonitrile:water containing 0.1% trifluoroacetic acid, from (100:0) to (0:100) in 50 min at a flow rate of 1 mL/min: 20 µL samples (in 80% methanol) were analysed. Peaks were identified by comparison of their retention times with those of pure standards and by mean of their UV spectra (capsaicin, λ_{max} at 225 and 279 nm; dihydrocapsaicin, λ_{max} at 228 and 281 nm). Qualitative and quantitative analyses were performed at 280 nm using a standard solution containing capsaicin (65.69%) and dihydrocapsaicin (32.46%). Different concentrations were analysed by HPLC and a calibration curve constructed by plotting peak area against concentration. All samples were measured in triplicate and the results expressed as mean (± standard deviation).

RESULTS AND DISCUSSION

Whilst HPLC has already been employed in the analysis of archeological materials, the technique has been mainly used to identify and quantify amino acids and proteins in samples of animal origin (Ulrich *et al.*, 1987; Gurley *et al.*, 1991; Kaufman and Manley, 1998; Fitznar *et al.*, 1999). This is the first time that HPLC-UV analysis has been applied to fossil material of plant origin. The use of ethanol for the extraction of the capsaicinoids from both fossil and fresh fruit samples of *Capsicum* was appropriate (Gbolade *et al.*, 1997; Santamaria *et al.*, 2000). The HPLC method was found to be suitable for the detection and the quantitative analysis of these compounds in the extracts: the response was linear within the range 10–1000 µg and the limit of detection was 0.5 µg.

The results of the quantitative analyses are reported in Table 1. Capsaicin and dihydrocapsaicin could be readily detected in fresh fruits of *C. annuum* and also in the fossil fruits of *C. frutescens*. On the other hand, the votive foods either did not contain these substances, or their levels were below the level of detection, supporting the results of the archaeobotanic analyses which had revealed the absence of *Capsicum* species. It is clear that the lack of detection of capsaicinoids could not be attributed to their decomposition over time since the compounds could be identified and quantitatively determined in fossil fruits that dated back to a period early than that of the Chiribaya culture.

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