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AN EMPIRICAL ASSESSMENT OF EPAZOTE (*Chenopodium ambrosioides* L.) AS A FLAVORING AGENT IN COOKED BEANS

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ABSTRACT.—A common culinary practice in Mexico and elsewhere in Mesoamerica is the use of *Chenopodium ambrosioides* L., a small herbaceous plant known most widely as epazote, to flavor black beans and other dishes. While some people find the taste and odor of this herb to be mildly disagreeable, there are good empirical reasons for its use as a flavoring agent in cooked, unrefrigerated, foods. Through a series of experimental trials we observed that beans prepared with *C. ambrosioides* remained edible, as judged by sight and smell, long after plain beans had begun to spoil. Microbiological tests revealed significant bacteriocidal activity in this species. Epazote has a large and diverse range of potential scientific and commercial applications.

Key words: epazote, *Chenopodium ambrosioides,* Mesoamerica, culinary practices, food preservative.

RESUMEN.—Una práctica culinaria frecuentemente utilizada en México y en otras partes de Mesoamérica para darles sabor a los frijoles negros y a otras comidas es el uso de *Chenopodium ambrosioides* L., una pequeña planta herbácea comúnmente llamada epazote. Aunque algunas personas encuentran el sabor y el olor de este condimento algo desagradable, existen razones empíricas para justificar el empleo de agentes para mejorar el sabor de alimentos cocinados que no son refrigerados. A través de análisis experimentales, se observó que los frijoles preparados con *C. ambrosioides* permanecían comibles, al juzgar por la apariencia y el olor de éstos, aun cuando los frijoles comunes y corrientes habían empezado a avinagrarse. Análisis microbiológicos revelaron la actividad bactericida de esta especie. El potencial de utilización con fines científicos y comerciales de esta planta es amplio y diverso.

RÉSUMÉ.—Le *Chenopodium ambrosioides* L., une petite plante herbacée plus connue sous le nom d'ambroisie, est couramment utilisé dans la cuisine du Mexique et, de façon générale, en Méso-Amérique pour aromatiser les plats de haricots noirs ainsi que d'autres plats. Quoique certaines personnes trouvent le goût et l'odeur de cette herbe légèrement désagréable, il existe d'excellentes raisons empiriques quant à l'utilisation de l'ambroisie comme aromate dans les plats cuits et non réfrigérés. À la suite d'une série d'essais expérimentaux, nous avons observé que les haricots préparés avec le *C. ambrosioides* demeuraient comestibles—

comme on peut le noter par leur aspect et leur odeur—bien après que les haricots préparés sans la plante aient commencé à se gâter. Des tests microbiologiques ont révélé une activité bactéricide significative chez cette espèce. L'ambroisie possède un grand éventail d'applications différentes ayant un potentiel à la fois scientifique et commercial.

INTRODUCTION

Many residents of Mexico and Guatemala subsist on a diet based largely on black beans and corn tortillas. These foods, of course, have served as the cornerstone in the diet of Mesoamerican cultures for thousands of years (Mangelsdorf et al. 1964). An equally ubiquitous, and possibly ancient, culinary practice is the use of epazote (*Chenopodium ambrosioides* L.) as a flavoring agent in cooked beans.[1] As one authority on Mexican cuisine remarked, "To cook black beans without it is unthinkable" (Kennedy 1978:239). Yet many individuals, especially children and adults unaccustomed to traditional Mesoamerican foods, find the taste and odor of epazote to be mildly disagreeable (Johns 1990:284). Even the plant's common name reveals much about its pungent qualities: *epazotl*, the Aztec name for this species, is based on *epatl*, their word for skunk (Coile and Artaud 1997).[2] There are, however, sound empirical reasons for cooking beans with several leaves and stems of epazote added, reasons that far overshadow any concerns one might have regarding the plant's strong taste and odor (Figure 1).

Three different experiments were conducted to better understand why this malodorous plant is so greatly valued as a flavoring agent in cooked beans. The first and second of these tests were simple in design, yet important in outcome. Moreover, each provided results that warranted a third phase of experimental investigations into the bioactivity and phytochemistry of *C. ambrosioides*. We now turn to a discussion of our methods and findings.

EXPERIMENTAL DATA: PHASE I

The idea behind the present research originally surfaced when the senior author (ML), who has considerable fieldwork experience in Guatemala and Mexico, suspected there must be some advantageous reason for using *C. ambrosioides* to flavor cooked black beans and many other traditional foods or dishes (in addition to Kennedy 1978, see Bayless and Bayless 1987; Gerlach and Gerlach 1994; Martinez 1992; Ortiz 1967; Quintana 1986). He was also aware that many other botanicals used as spices thwarted food spoilage by curbing the growth of bacteria and fungus that rapidly invade cooked, though unrefrigerated, foods (e.g., Sherman and Hash 2001).

A simple experiment was conceived that would help resolve the enigmatic question of why it would be "unthinkable," in Kennedy's assessment, to cook black beans without epazote. With the assistance of Beth Maney, the experiment was initiated. A supply of black beans was purchased at a local grocery store. Two batches of beans (approximately 226 g [½ pound] each)—one containing fresh Mexican epazote (about a dozen whole leaves and attached stems), the other without epazote—were then cooked by vigorous boiling. This was done in the

FIGURE 1.—A *hierbera* in Mexico City's Sonora Market vends numerous fresh and dried plant products used in cooking and health care, including epazote. Photograph by Michael H. Logan.

evening. When thoroughly cooked the two batches of beans were drained, then spooned into plastic containers that were clearly labeled: "epazote" and "plain." No lids were used as the beans sat overnight at room temperature. The following morning the cooked samples were transferred out-of-doors and placed in a shaded and covered area. The month was August, with daytime highs in the lower 90s (>32°C), and evening lows in the 70s (>21°C). At 6:00 p.m. (approximately 24 hours after the beans were cooked), the senior author visually inspected the beans and then sniffed each sample. Although they looked the same, the batch without epazote had a slight odor of decay. However, the beans containing epazote smelled fresh. The samples remained out-of-doors for another evening. At 10:00 a.m. the following morning the samples were again assessed. The plain sample now exhibited signs of fungal growth. It also had a strong, disagreeable odor. Interestingly, though, the beans cooked with epazote looked and smelled fresh, save for the odor of this additive. That evening at 6:00 the plain sample was reassessed. It was thoroughly rotten, and the odor of the beans was offensive. The beans containing epazote still smelled, after approximately 48 hours, as if they remained edible. They were not eaten, however. The beans in each batch were then discarded.

This simple experiment—the "sniff test"—was repeated several times. And the results were uniformly the same. Black beans cooked with epazote remained

FIGURE 2.—Dried epazote is sold by a variety of commercial outlets in Mesoamerica and the United States. Photograph by W. Miles Wright.

fresh long after plain beans began to rot. But would use of dried epazote, rather than fresh, produce different results?

Packets of dried *C. ambrosioides* were purchased from a retail and mail order firm in Florida that specializes in herbs and spices used in preparing ethnic foods (Figure 2). The amount of epazote added to the beans followed traditional Mexican recipes (one tablespoon per half cup of beans). Interestingly, the results with dried epazote paralleled what was observed in our earlier trials. Use of fresh or dried epazote when cooking beans significantly extended the length of time during which they would remain edible, as judged by sight and, more importantly, smell.

The experiment faithfully tried to replicate the domestic environment of cooking and food storage found in the past, or among many Mesoamerican peasants today. While ceramic vessels and a wood fire were not used, the factors judged most important in these tests were to leave the cooked beans uncovered in a shaded area and at ambient daytime and evening temperatures. Reheating beans that already contained epazote would undoubtedly further extend the "shelf-life" of this staple food.

To gain better insight into the biological activity of *C. ambrosioides* as a food additive, the senior author enlisted the aid of three colleagues (the other co-authors), each having considerable laboratory experience. Additional experimentation was the logical next step.

EXPERIMENTAL DATA: PHASE II

To better control for environmental factors that could have affected the cooked samples in our earlier trials (e.g., wind-borne spores or contact by insects), we decided to place the cooked beans—those with epazote and those without—into Petri dishes, which would then be stored in an incubator at a constant temperature and light level.

The black beans were prepared in the same manner as in the previous tests. In all, three different batches of beans were cooked: two with fresh epazote (in one of these the sprigs of epazote were removed after cooking) and one without epazote. These samples were then taken to Charles Faulkner's laboratory. The beans were then placed into clearly marked, yet coded, Petri dishes. Nine dishes were used (3 with epazote left in [*ei*]; 3 with epazote removed [*er*]; and 3 plain [*p*]). Bacteria (*Micrococcus lutea*) were then added to two of the dishes in each of the three categories (*ei, er, p*), leaving one dish in each of the three groupings not spiked with bacteria. It should be noted here that only Logan knew what letters and numbers corresponded with "epazote in," "epazote removed," and "plain," as well as "bacteria yes" and "bacteria no."

All dishes were then placed inside a lighted, airtight incubator set at 26°C.[3] After 24 hours in the incubator (approximately 40 hours had elapsed since the beans had been cooked), the samples were removed and inspected (by CF) for visual signs of spoilage (e.g., any change in color or consistency). All dishes where bacteria had been added showed signs of decay, especially *p*1 and *p*2 (the plain beans with bacteria added). However, the samples prepared with epazote, but ones not containing additional bacteria (*ei*3, *er*3), showed no visible evidence of spoilage. All lids were then removed and the "sniff test" was conducted on the contents of each dish. Again, this was a blind trial. A disagreeable odor was detected (by CF) in all of the samples, save for two: the samples prepared with epazote that were not spiked with bacteria. These results warranted additional, and more rigorous, experimental trials and microbiological assessment.

EXPERIMENTAL DATA: PHASE III

Again, black beans were cooked with and without epazote in nonlaboratory conditions. El Charitto (EC) brand epazote was purchased in Mexico. One tablespoon of epazote (ca. 4 g) was added per 0.5 cup dry beans (ca. 200 g). Standard microbiological techniques were followed in order to determine bacteria counts in both samples (Harrington and McCance 1976).[4] The results were dramatic. The sample without epazote contained approximately 800 colony-forming units per ml. The sample cooked with epazote contained none. It was totally free of bacteria, yet some 42 hours had passed since the beans were cooked. After 72 hours, samples were again drawn, diluted, plated and counted. The one without epazote had more than 160 million colony-forming units per ml, while the batch with epazote had eleven thousand. These findings strongly confirmed what had been observed in our earlier trials. Epazote does extend the length of time that unrefrigerated black beans remain edible, by a magnitude of at least one full day, if not more.

Activities of EC and an epazote commercially available in the United States (Penzey's Spices [PS], Muskego, Wisconsin) were compared. The EC epazote was determined to be heavily contaminated with multiple species of bacteria that could alter bacterial counts in beans cooked with EC; therefore, for further experiments, black beans were cooked under controlled laboratory conditions using propylene oxide-sterilized epazote. Propylene oxide did not alter the chemical composition of either EC or PS epazote (data not shown). In subsequent experiments, epazote was sterilized by submersion overnight in propylene oxide that was then allowed to evaporate. To each of two sets of sterile cookware, dry black beans (200 g) and tap water (400 ml) were added. The beans were heated to boiling, then cooled to room temperature for two hours. Sterile epazote (4 g) was added to one pot and mixed well. Both batches were simmered for one hour.

The next procedure to be pursued was to assess epazote's potential, if any, to curb the growth of two common food spoilage organisms, *Escherichia coli* (O157) and *Salmonella* (8326). Again, more beans were prepared. The cooked samples were inoculated with the bacteria (10^5 per gram beans). Appropriate controls were used. Colonies were counted at 24 and 48 hours. Epazote had little or no effect on the introduced bacteria. Interestingly, the two control groups (each with epazote) had no bacteria, whereas the plain batch was laden with some type of bacteria. The naturally occurring organisms in the untreated cooked beans were classified as Gram-negative or Gram-positive by differential staining, and Gram-positive organisms were tentatively identified as a species of *Bacillus*, probably *B. cereus* (Lund et al. 2000).

In order to determine if *Bacillus* species are sensitive to epazote, both treated and untreated beans were divided into two sterile flasks to which 10^5 *Bacillus* BA101/gram beans was added to one flask of each set. The flasks were incubated at 30°C, and samples were plated at 24 and 48 hours as described above to determine bacterial counts. EC and PS were evaluated in separate experiments. Approximately ten times more bacteria were isolated from untreated beans than for beans treated with EC epazote. With the addition of *Bacillus*, there was also a tenfold reduction in the number of bacteria growing in beans cooked with epazote compared to untreated beans. There was approximately twice the number of bacteria in untreated beans compared to beans cooked with PS epazote. Apparently these brands of epazote differ in their phytochemical composition, with EC epazote exhibiting greater bacteriocidal activity than PS epazote.

Dr. Duke's Phytochemical and Ethnobotanical Database was used to identify commercially-available antibacterial compounds that had previously been isolated from *Chenopodium ambrosioides*.[5] Nine compounds were tested for activity against several types of bacteria, including Gram-positive and Gram-negative organisms isolated from beans, *Salmonella* 8326, and nine strains of *Bacillus* (Table 1).[6] Geraniol and safrole were the only compounds of the nine tested that inhibited the growth of bacteria (Bard et al. 1988).

TABLE 1.—Antibacterial properties of epazote compounds.

	G (+)[1]	G (−)[1]	Sal 8326	BA 77	BA 101	E 21	E 61	E 65	E 66	E 69	E 726	E 727
Cymene	—[2]	—	—	—	—	—	—	—	—	—	—	—
Limonene	—	—	—	—	—	—	—	—	—	—	—	—
Myrcene	—	—	—	—	—	—	—	—	—	—	—	—
Geraniol	15[3]	12	11	12	14	12	11	12	15	13	12	12
Pinene	—	—	—	—	—	—	—	—	—	—	—	—
Safrole	13	10	9	8	11	10	10	10	11	10	10	10
Ferulic acid	—	—	—	—	—	—	—	—	—	—	—	—
Vanillic acid	—	—	—	—	—	—	—	—	—	—	—	—
Ascorbic acid	—	—	—	—	—	—	—	—	—	—	—	—
Control	—	—	—	—	—	—	—	—	—	—	—	—

Ten µl of undiluted cymene, limonene, myrcene, geraniol, pinene, or safrole was added to a sterile filter paper disk. Ten µl of ferulic acid (50 mg/ml in 95% ethanol), vanillic acid (50 mg/ml in 95% ethanol), or ascorbic acid (100 mg/ml) was added to a sterile filter paper disk. The treated disk was placed on a lawn of bacteria obtained by spread plating one ml 10^5 bacteria/ml suspension on TSA plates. After 24 hours the cleared zone around the filter paper disk was measured.

[1] G(+) and G(−) organisms were naturally occurring isolates from beans; Sal 8326 = *Salmonella*; BA and E = isolates of *Bacillus*.

[2] — No effect of compound on bacterial growth.

[3] Measurements are cleared diameters in mm.

DISCUSSION

The traditional use of epazote to flavor cooked beans is an empirically sound practice. Geraniol and safrole effectively retard the growth of *Bacillus* bacteria, which is a natural component in bean spoilage. This would have been particularly important in the past, as well as in contemporary settings where a sizeable number of persons lack electricity and refrigeration (Valdes-Ramos and Solomons 2002: 149). Because this species safely extends the period of time that unrefrigerated cooked beans remain edible, its potential as a preservative in other dishes, both vegetarian and meat-based, certainly warrants additional study.

Preliminary findings on a closely related species—*Chenopodium berlandieri* Moq.—suggest that it, too, retards the rate of spoilage in cooked beans due to fungal invasion. Fresh *C. berlandieri* was acquired from a local farm in Blount County, Tennessee, and three batches of black beans were prepared following the same practices done with the epazote tests described above. Beans cooked without *C. berlandieri* exhibited significant amounts of mold after 48 hours, while the control sample with *C. berlandieri* added before cooking appeared to be suitable for consumption. The third batch, prepared with dried *C. berlandieri* achenes also looked and smelled fresh some two days after they were cooked.

These trials, though simplistic, are especially interesting because this species of *Chenopodium* held an important place in the diet of prehistoric Indians in the eastern woodlands of North America as early as three to four thousand years ago (Fritz 1999; Gremillion 1993; Smith 1984, 1985a, 1985b). Perhaps these peoples, like their counterparts in Mesoamerica, had discovered the food preservation qualities of *Chenopodium*. Our research on *C. berlandieri* continues, yet it should be noted here that both *C. berlandieri* and *C. ambrosioides* were eventually domesticated by aboriginal peoples in the past. Domestication confirms how culturally important these species once were.

In Mesoamerica, though, the cultural importance of *C. ambrosioides* has persisted to the present day (e.g., Heiser 1985:82–99). Aside from its role as a flavoring agent, and by extension a food preservative, it is eaten fresh as a potherb by Indians in northern Mexico (Bye 1981:116). Leaves and stems of this plant contain important amounts of calcium, phosphorous, and vitamin C (Ortiz de Montellano 1990:240). The fruits or achenes of epazote, which are rich in protein, also hold value as a food (Minnis 2000:223). While most residents of the United States would view chenopods to be lowly weeds, these plants have considerable worth as an alternative crop (Coile and Artaud 1997).

Epazote is also culturally valued for its medicinal properties (e.g., Moerman 1998; Morton 1981). It is widely used in the Americas and beyond to combat a large array of health-related problems. Frequently it is the remedy of choice for controlling intestinal worms (Berlin et al. 1996:413–417), yet its efficacy as a vermifuge in humans is, to some degree, uncertain (Kliks 1985; but also see Kightlinger et al. 1996).[7] While epazote's role as a curative must be of considerable antiquity, this cannot be determined archaeologically. However, it is described as a useful medicinal herb in early post-Conquest ethnohistorical sources (e.g., Orellana 1987). The volume of literature on *C. ambrosioides* as a medicinal plant far overshadows what has been published on this species as a dietary item.

In addition to its bacteriocidal properties, *C. ambrosioides* has been shown to possess other realms of biological activity. It is a strong allelopathic species, in that its leaves exude compounds that prohibit or delay seed germination of other nearby species (Jimenez-Osornio et al. 1996). Researchers have also learned that *C. ambrosioides* has insecticidal and repellent properties (Morsy et al. 1998; Su 1991; Tapondjou et al. 2001). Antiviral (Verma and Baranwal 1983), antifungal (Kishore et al. 1989; Montes-Belmont and Carvajal 1998), trypanocidal (Kiuchi et al. 2002), and molluscicidal (Hmamouchi et al. 2000) activities have also been reported for this species. Other studies have shown that *C. ambrosioides* has hypotensive (Gohar and Elmazar 1997) and antidermatophytic (Kishore et al. 1996) activities as well. It becomes obvious that this species has a vast number of potential uses, and in a variety of industries, ones ranging from agriculture and pest control to human (Lall and Meyer 1999) and veterinary medicine (Perezgrovas et al. 1994).[8]

CONCLUSIONS

Precisely when and where prehistoric peoples of Mesoamerica discovered the food-preservation qualities of epazote is not known. How this discovery was made, though, is easier to reconstruct. Olfaction was undoubtedly the critical element lying behind this acquired knowledge concerning *C. ambrosioides.*

The Archaic Period of Mesoamerican prehistory (ca. 8000 B.C. to 2000 B.C.) witnessed a huge number of cultural innovations. None was equal in importance to the domestication of plants. Dozens of different species were transformed from their wild states into useful foods (West and Augelli 1989:220). By 2000 B.C., maize and beans held an important place in the diet of these early peoples. They, and no doubt their predecessors, also consumed *C. ambrosioides* (Manglesdorf et al. 1964:434). There is clear evidence that the seeds of this plant were roasted and eaten. It is also highly probable that fresh leaves and stems were eaten as well. Placing sprigs of this edible potherb into a stone, and later ceramic, vessel that contained beans and water brought to a boil would be a logical method of adding volume, if not variety, to a meal soon to be eaten. Leftover beans would remain in the vessel or a gourd bowl until reheated for the next meal. And humans invariably employ smell, coupled with sight, as a proximate gauge of a food's edibility.

The results of our original experimental trials—the "sniff" test—revealed, in no uncertain terms, that beans prepared with epazote seemed safe to eat long after the beans lacking epazote smelled foul. The same observation undoubtedly occurred, and repeatedly so, among women in the past. A culinary tradition, one seen today, emerged from this simple, though important, discovery about epazote and its value as a food additive. And the microbiological data identified in the third phase of our experimental trials confirmed the merits of this widely diffused and ancient custom. Kennedy (1978) was indeed correct—there are strong empirical reasons why it would be unthinkable to prepare black beans without epazote.

Hopefully the findings of this study will stimulate additional research on this small herbaceous plant originally domesticated in Mesoamerica thousands of years ago. The potential scientific and commercial applications of *C. ambrosioides* are considerable. This species certainly warrants further cross-disciplinary inquiry.

NOTES

[1] *C. ambrosioides* is a small (up to 1 m) herbaceous plant native to tropical and subtropical zones in the Americas, although it has naturalized to many other locales beyond its original range. It has been exported abroad and is now found in several countries in Africa, Asia, and Europe. The strongly scented leaves are alternate, lanceolate or oblanceolate in shape (ca. 4–12 cm long) with toothed, lobed, wavy, or smooth margins. It is known by at least fifty different common names, depending on the region and languages spoken. In English it is frequently called goosefoot or lamb's-quarters due to the shape of its leaves. The genus *Chenopodium* contains, worldwide, approximately 150 species. Some of these are of considerable cultural importance, especially in the Americas.

[2] These authors use a variant spelling, one common in Guatemala: "apazote."

[3] This setting on the incubator was chosen because it would simulate ambient daytime (noon) temperatures in much of Mesoamerica.

[4] Following an incubation period, three beans were placed in 10 ml sterile 0.75% NaCl, vortexed, serially diluted and plated in duplicate on tryptic soy agar (TSA). Plates were incubated at 30°C. Bacterial colonies were counted after 24 hours.

[5] Duke, Jim. n.d. Dr. Duke's Phytochemical and Ethnobotanical Databases. Agricultural Research Service, U.S. Department of Agriculture. [http://www.ars-grin.gov/duke/] (verified 9 December 2003)

[6] Ten μl of each compound was added to a sterile filter paper disk. The treated disk was placed on a lawn of bacteria obtained by spread plating one ml 10^5 bacteria/ml suspension on TSA plates. Bacteria tested included Gram-positive and Gram-negative organisms isolated from beans, *Salmonella* 8326, and *Bacillus* sp. isolates BA77, BA101, E21, E61, E65, E66, E69, E726, and E727. Cultures of *Bacillus* were obtained from the collection of Bonnie Ownley, University of Tennessee. After incubating 24 hours at 30°C, the diameter of cleared bacterial growth was measured. Cymene, limonene, and pinene were obtained from Aldrich Chemical, Milwaukee, WI. Myrcene, geraniol, safrole, ferulic acid, vanillic acid and ascorbic acid were obtained from Sigma Chemical, St. Louis, MO. Cymene, limonene, myrcene, geraniol, pinene, and safrole were used as concentrated oils. Ferulic acid and vanillic acid were suspended at 50 mg/ml in 95% ethanol and ascorbic acid was suspended at 100 mg/ml in distilled water.

[7] The anthelmintic activity of *C. ambrosioides* is due to the presence of ascaridole, which is known to be toxic to round worms (*Ascaris lumbricoides*), as well as a number of other parasitic organisms. Oil of Chenopodium was once widely used to expel intestinal worms, yet the dosage required to be totally effective approached the lethal limit in humans.

[8] Co-author Beth Maney, who is an avid horse breeder, found during our epazote tests that a water-based solution of *C. ambrosioides*, when applied daily as a wash to the soles of her horses' hooves, effectively eliminated hoof (thrush) infection from her animals. It proved superior to some common, over-the-counter, remedies designed to control this equine hoof infection.

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