# Identification of the Volatile Components of E802 Mazoferm Steepwater, a Condensed Fermented Corn Extractive Highly Attractive to the Mexican Fruit Fly (Diptera: Tephritidae)

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A dynamic headspace analysis was performed on the volatile components of E802 Mazoferm steepwater, a condensed fermented corn extractive, that is highly attractive in laboratory and field tests to adult Mexican fruit flies, *Anastrepha ludens* (Loew). Chemical characterization was accomplished by capillary GC and GC/MS methods. A total of 19 compounds were identified, and these were comprised of 6 alcohols, 5 esters, 3 aldehydes, 2 lactones, an alkyl furan, an acid, and a trisulfide. When E802 Mazoferm steepwater was analyzed at pH 3.9, the five most abundant compounds in the volatiles (in descending order) were 3-methyl-1-butanol (43.9%), ethyl lactate (23.7%), ethanol (15.4%), 2-methyl-1-propanol (12.8%), and ethyl acetate (0.71%). At pH 8.0, the same five compounds were again the most abundant. Except for a reversal in order for the first two compounds, the order was the same: ethyl lactate (37.3%), 3-methyl-1-butanol (32.5%), ethanol (14.2%), 2-methyl-1-propanol (10.4%), and ethyl acetate (3.09%).

**Keywords:** *Anastrepha ludens; food bait; attractant; E802 Mazoferm steepwater; volatiles; headspace analysis; lure*

## INTRODUCTION

The Mexican fruit fly, *Anastrepha ludens* (Loew), is a major pest of citrus, mangos, guava, and other subtropical fruits. McPhail traps baited with borax-buffered aqueous solutions of 10% NuLure or 2% torula yeast are currently used to detect and monitor populations of *A. ludens* (Anonymous, 1993) and to control this insect in crop-threatening situations (Lopez et al., 1969).

Certain xanthene dyes, when light activated, have been shown to be highly toxic to a variety of insects (Heitz, 1982, 1987, 1988). Interest in these dyes as potential insect control agents has steadily increased because of their relatively high margin of safety (Heitz, 1982). SureDye, one of the most intensely studied xanthene dyes, consists of phloxine B (69%) and uranine (31%). Phloxine B, an FDA-registered color additive of Rolaids and Peptobismol, was highly toxic to *A. ludens* when formulated in E802 Mazoferm steepwater, a commercially available corn-protein hydrolysate and animal food supplement (Moreno and Mangan, 1995). According to their product data bulletin, Corn Products (manufacturer of E802 Mazoferm steepwater) states, "E802 Mazoferm Condensed Fermented Corn Extractive was produced in corn wet milling when the dry corn is soaked (steeped) in warm dilute sulfurous acid solution. During the process, the grain solubles are released and undergo a mild lactic acid fermentation from naturally occurring microorganisms". Typical composition analyses of the commercial product used in this study describe a liquid high in dry solids (ca. 48%), proteins (ca. 22%), and lactic acid (10−13%). Compositions of steepwaters from various industrial processes and at different times during their steeping have been reported (Hull et al., 1996).

E802 Mazoferm steepwater is reportedly more attractive to *A. ludens* than the widely used corn-protein hydrolysate NuLure and is comparable to hydrolysates of yeast and casein (Moreno and Mangan, 1995). Moreover, this new protein hydrolysate not only attracts *A. ludens*, it also stimulates their feeding (Moreno and Mangan, 1995). In contrast, *A. ludens* appears reluctant to consume NuLure in appreciable quantities, most likely due to its high (6−13%) salt content (Buttery et al., 1983). E802 Mazoferm steepwater's ability to attract *A. ludens* in appreciable numbers coupled with its low salt content makes it an ideal bait to formulate with water soluble SureDye. In that context, we initiated the present study to identify the volatiles emanating from E802 Mazoferm steepwater that might be responsible for its long-range attraction. Compounds identified in this study could potentially be added as supplements to the commercial product and thus boost its attractancy or increase its feeding stimulancy, thereby lowering the amount of toxicant needed to achieve a specified level of control.

## MATERIALS AND METHODS

**Materials.** E802 Mazoferm steepwater (batch 1157413, 950606 production) was purchased from Corn Products (unit of CPC International Inc., Summit-Argo, IL). This solution as received had a pH of 3.9. Dilutions with water were made as needed.

**Collection of Volatiles.** Volatiles were collected from the E802 Mazoferm steepwater concentrate (pH 3.9) or from a portion of concentrate adjusted to a pH of 8.0 by addition of 10% aqueous sodium hydroxide. A 100 mL aliquot of E802 Mazoferm steepwater, of the desired pH, was placed in a three-neck, round-bottom flask. As the solution was stirred at ambient temperature, prepurified nitrogen (i.e., passed through an activated charcoal bed) was swept at 300 mL/min over the headspace, and volatiles were collected in a glass tube packed

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**Table 1. Attractancy of Aqueous Preparations of Selected Food Baits to Adult Mixed-Sex *A. ludens* As Determined in Laboratory and Field Tests**

| food bait[b] | concn (%) | attractancy[a] laboratory test (mean responses[c,d] ± SEM) | attractancy[a] field test (mean flies trapped[d,e] ± SEM) |
|---|---|---|---|
| torula yeast | 2.22 | 21.7 ± 0.94 c | 72.7 ± 7.1 a |
| NuLure | 10.0 | 43.5 ± 1.8 a | 23.7 ± 2.7 b |
| E802 Mazoferm | 10.0 | 37.7 ± 2.4 ab | 94.0 ± 15.1 a |
| water (control) | | 6.74 ± 0.29 d | 5.67 ± 0.92 c |

[a] For information on insects, test methods, and data on other baits evaluated within same test, see Moreno and Mangan (1995). [b] All baits were tested at pH 8.0. [c] $N = 880.5$. [d] Means in the same column followed by the same letter are not significantly different [$p = 0.05$; Fisher's protected least significance (Fisher, 1949)]. [e] $N = 2183$.

with 300 mg of activated charcoal (Darco, 20−40 mesh, Aldrich Chemical Co., Milwaukee, WI). Activated charcoal was used as the trapping agent because of its high efficiency to adsorb a variety of organic compounds (Heinz et al., 1966). The charcoal used in the collection trap was prepurified by continuous extraction (Soxhlet extractor) with methylene chloride and then benzene. (Caution: benzene and methylene chloride, cancer suspect agents, should be handled with care and adequate ventilation.) After collecting volatiles for 15 h, the charcoal trap was removed and eluted with ca. 0.3 mL of methylene chloride. The methylene chloride was analyzed without concentration. The efficiency of the charcoal trap was determined by inserting a second charcoal trap (equal size and load) in the purge stream after the first trap. GC analysis of the eluate from the second trap showed complete absence of any volatiles associated with the Mazoferm substrate.

**Gas Chromatography (GC).** A Shimadzu Model GC-9A gas chromatograph (Shimadzu, Columbia, MD) equipped with a flame ionization detector (FID) and a bonded DB-1 (J&W Scientific, Folsom, CA) fused-silica capillary column (60 m × 0.248 mm i.d., 0.25 $\mu$m film thickness) was used to analyze volatile components. GC peak areas were quantified using a Shimadzu CR-4A integrator. GC operating conditions: injector/detector temperature, 280 °C; helium carrier, ca. 1 mL/min (4 kg/cm$^2$ head pressure); injector operated in split mode, 175:1; temperature program, 50 to 250 °C at 5 °C/min.

**Gas Chromatography/Mass Spectrometry (GC/MS).** GC/MS was performed on a Hewlett-Packard 5890A GC/MS equipped with a 5971A MSD and a HP5 (Hewlett-Packard, Avondale, PA) bonded fused-silica capillary column (25 m × 0.2 mm i.d., 0.11 $\mu$m film thickness). GC conditions used were the same as those described for GC analysis on the Shimadzu instrument except that the injection port was operated in the splitless mode. MS conditions (EI mode) used were as follows: ionization voltage, 70 eV; mass range, $m/z$ 30−550; ion source temperature, 180 °C. The mass spectra of the unknown compounds were compared with those in the Wiley/NBS spectral data base. Identifications were also made by comparing Kovats indices (KI) of unknowns with those determined for authentic samples (Kovats, 1966).

## RESULTS AND DISCUSSION

Results from laboratory tests (Moreno and Mangan, 1995) showed that a 10% solution of E802 Mazoferm steepwater, adjusted to pH 8.0, was comparable to a 10% solution of NuLure (pH 8.0) and exceeded (i.e., ca. 2×) a 2.22% solution of torula yeast (pH 8.0) in its attractiveness to adult mixed-sex *A. ludens* (Table 1). In contrast, field test data of identical concentrations of the hydrolysates showed that while E802 Mazoferm steepwater was comparable to torula yeast, it was considerably more attractive (i.e., ca. 4×) than NuLure (Table 1). Since moderate (i.e., 40%) to high (i.e., 70%) concentrations of E802 Mazoferm steepwater were reportedly (Moreno and Mangan, 1995) more attractive than torula yeast or NuLure at comparable concentrations, we selected the full-strength concentrate of E802 Mazoferm steepwater to conduct a dynamic headspace analysis to identify the volatiles emanating from that substrate (mixture of suspended solids in a dark amber liquid).

Since E802 Mazoferm steepwater was highly effective at a pH 8.0 in laboratory and field tests (Moreno and Mangan, 1995; Table 1), we conducted the headspace analysis of the concentrate at pH 8.0 as well as at pH 3.9, the pH of the supplied commercial product. Typical gas chromatographic profiles of the volatiles obtained from the hydrolysate at both pH values are shown in Figure 1[A (pH 8.0) and B (pH 3.9)]. Compounds eluting from the GC columns were identified by comparing their GC/mass spectra and Kovats indices to those obtained for commercially obtained standards. Chemical identities, Kovats indices (KI), and relative concentrations for unknowns and standards are listed in Table 2. Exclud-

**Table 2. Volatiles Identified in E802 Mazoferm Steepwater at Indicated pH**

| GC peak no.[a] | compound | ref KI[b] | pH 3.9 KI[c] | pH 3.9 relative area %[g] | pH 8.0 KI[c] | pH 8.0 relative area %[g] |
|---|---|---|---|---|---|---|
| 1 | ethanol | *d* | *d* | 15.4 | *d* | 14.2 |
| 2 | acetic acid | *d* | *d* | 0.560 | *d* | 0.178 |
| 3 | ethyl acetate | 600 | 600 | 0.712 | 600 | 3.09 |
| 4 | 2-methyl-1-propanol | 612 | 611 | 12.8 | 611 | 10.4 |
| 5 | 3-methylbutanal | 632 | 631 | 0.659 | 631 | 0.105 |
| 6 | 3-methyl-1-butanol | 719 | 719 | 43.9 | 718 | 32.5 |
| 7 | 2-methyl-1-propyl acetate | 755 | 755 | 0.158 | 755 | 0.056 |
| 8 | ethyl lactate | 797 | 798 | 23.7 | 799 | 37.3 |
| 9 | furfuryl alcohol | 835 | 833 | 0.366 | 832 | 0.960 |
| 10 | 1-hexanol | 853 | 854 | 0.023 | 854 | 0.030 |
| 11 | 3-methylbutyl acetate | 858 | 858 | 0.414 | 859 | 0.057 |
| 12 | $\gamma$-butyrolactone | 864 | 861 | 0.130 | 862 | 0.014 |
| 13 | $\beta$-methyl-$\gamma$-butyrolactone | 916 | 913 | 0.020 | 914 | *f* |
| 14 | dimethyl trisulfide | 949 | 950 | 0.120 | 950 | 0.183 |
| 15 | furfuryl acetate | 966 | 966 | 0.038 | 966 | 0.013 |
| 16 | 2-pentylfuran | 980 | 980 | 0.360 | 980 | 0.016 |
| 17 | phenylacetaldehyde | 1013 | 1012 | 0.019 | 1011 | 0.041 |
| 18 | phenethyl alcohol | 1090 | 1091 | 0.055 | 1090 | 0.175 |
| 19 | safranal | 1181 | 1181 | 0.008 | ND[e] | ND |

[a] Peak numbers correspond to those in Figure 1. [b] Reference Kovats indices; calculated from retention time data of authentic sample obtained on a DB-1 capillary column. [c] Kovats indices of individual compounds in volatiles; calculated from retention time data on a DB-1 capillary column. [d] Not determined. [e] ND, not detected. [f] Trace amount (compound's presence was detected by GC/MS). [g] Percentage of the total peak area calculated for all identified peaks in the GC trace except for the solvent and benzene peaks.

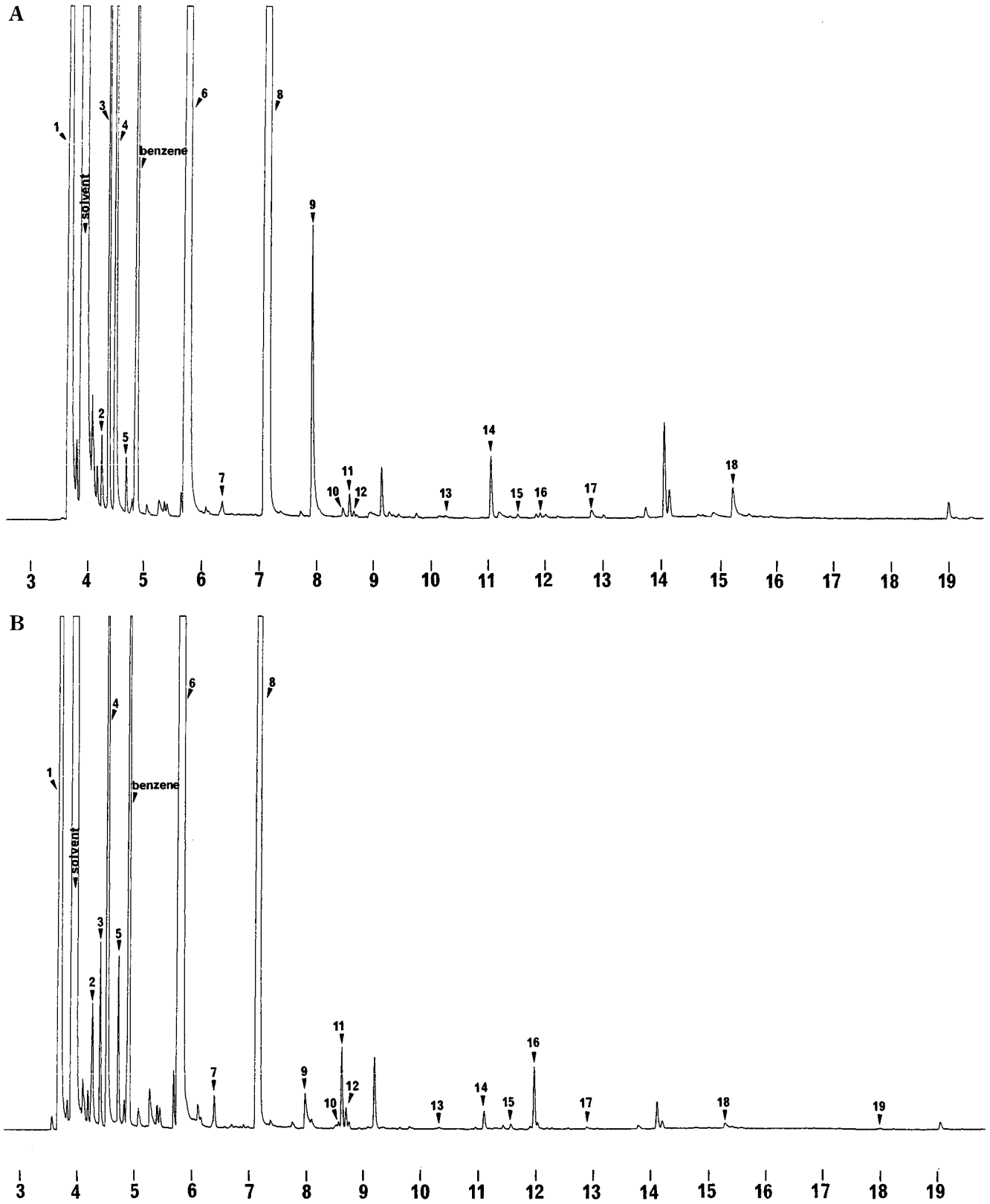


**Figure 1.** Gas chromatograms of volatile components observed from E802 Mazoferm steepwater: (A) at pH 8.0; (B) at pH 3.9.

ing benzene (a contaminant introduced onto the activated charcoal as a result of a prerinse step), a total of 19 compounds were identified. Of the 19 compounds (GC peaks 1−19), 18 were common to both of the chromatograms representing analyses at pH 3.9 and 8.0. Despite this commonality, relative concentrations for these volatiles varied with pH, but the differences were almost exclusively within 1 order of magnitude. Safranal, while detected as a trace component at pH 3.9, was undetected at pH 8.0; no attempt was made to determine whether or not safranal was still present in the liquid phase. Among the 19 volatile constituents identified in volatiles of E802 Mazoferm steepwater, 6 were alcohols, 5 were esters, 3 were aldehydes, and 2 were lactones. The remaining compounds consisted of an alkylfuran, an acid, and a trisulfide.

Several peaks [i.e., retention times 9.20 (0.13%), 13.80 (0.05%), 14.12 (0.28%), and 14.21 (0.11%) min] in the

GC trace for volatiles emanating from steepwater at pH 8.0 remain unidentified due to poor library matches or lack of authentic standards from which comparisons could be made. The same peaks were observed at pH 3.9.

Data from the analysis at pH 3.9 showed that the five most abundant compounds were (in descending order) 3-methyl-1-butanol (43.9%), ethyl lactate (23.7%), ethanol (15.4%), 2-methyl-1-propanol (12.8%), and ethyl acetate (0.71%). Of particular note is that of the five most abundant compounds, four were hydroxlyated, i.e., three alcohols and one hydroxy ester.

Strikingly similar results were observed in the volatiles collected from the slightly basic (pH 8.0) concentrate; the same five compounds were the most abundant in the volatiles. These were, in decreasing order of abundance, ethyl lactate (37.3%), 3-methyl-1-butanol (32.5%), ethanol (14.2%), 2-methyl-1-propanol (10.4%), and ethyl acetate (3.09%). The order of abundance in the volatiles at pH 8.0 was essentially the same as the order observed for volatiles at pH 3.9 except for a reversal of the first two compounds (ethyl lactate and 3-methyl-1-butanol). Ten lipophilic components of corn steepwater have also been identified (Hull et al., 1996). One of these (phenethyl alcohol) was identified as a constituent of E802 Mazoferm steepwater volatiles (Table 2). Although lactic acid is the most prominent organic constituent of corn steepwater (Wright, 1987; Hull et al., 1996), it was not detected in the E802 Mazoferm steepwater volatiles. However, appreciable amounts of ethyl lactate and ethanol present in the volatiles derived from E802 Mazoferm steepwater (Table 2) strongly suggested the parent acid's (lactic) presence in the liquid concentrate.

Interestingly, 3-methyl-1-butanol, a predominant constituent in E802 Mazoferm steepwater, was also a predominant constituent in volatiles from supernatants derived from *Klebsiella pneumoniae* (Lee et al., 1995) and *Citrobacter freundii* (DeMilo et al., 1996). It is of further interest that although 3-methyl-1-butanol was not a reported volatile constituent of NuLure (formerly PIB-7), its corresponding aldehyde, 3-methylbutanal, was (Matsumoto et al., 1985; Buttery et al., 1983; Flath et al., 1989) and was present in relatively large amounts (Matsumoto et al., 1985). Although E802 Mazoferm steepwater and NuLure are corn protein hydrolysates, only four of the volatile compounds identified in E802 Mazoferm steepwater were reported constituents of NuLure steam distillates (Flath et al., 1989). These were 3-methylbutanal, furfuryl alcohol, dimethyl trisulfide, and phenethyl alcohol.

The importance of nitrogen-containing compounds in enhancing the attractiveness of protein baits to fruit flies has been the focus of considerable research. Bateman and Morton (1981) reported that increasing the pH of a yeast hydrolysate increases its attractiveness, a property that could be correlated with an increase in ammonia. Morton and Bateman (1981) suggested that high attraction of a tryptic hydrolysate of bovine albumin to the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), was due to the presence of feeding stimulants (amino acids and peptides) and olfactory stimulant, ammonia. Mazor et al. (1987) reported a positive correlation between the catch of female Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), and the rate of release of ammonia from various protein baits. Moreover, nitrogen-containing compounds such as pyrazines (Buttery et al., 1983; Matsumoto et al., 1985), and 2-acetylpyrrole (Buttery et al., 1983) were reported in the volatiles of NuLure. Despite these reports, we were unable to detect the presence of ammonia, alkyl-substituted amines, or pyrazines in the volatiles of E802 Mazoferm steepwater.

In summary, we have identified a total of 19 compounds present in the volatiles of E802 Mazoferm steepwater. To our knowledge, this is the first report on the chemical characterization of the volatiles of this protein hydrolysate. Laboratory and field studies are currently in progress to evaluate these materials, individually or as mixtures, as candidate attractants for the Mexican fruit fly or related tephritids. We hope that information derived from these and subsequent studies uncover supplements that when added to E802 Mazoferm steepwater will enhance its effectiveness as an attractant and feeding stimulant for fruit flies.

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