Volatile Components of a Chicken Feather Hydrolysate that is Highly Attractive to the West Indian and Mexican Fruit Fly (Diptera: Tephritidae) [1, 2]

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ABSTRACT A chicken feather hydrolysate prepared by heating feathers with 6*N* hydrochloric acid was highly attractive to the West Indian fruit fly, *Anastrepha obliqua* (Macquart), and the Mexican fruit fly, *Anastrepha ludens* (Loew). In cage-top laboratory tests to determine the influence of pH on attraction, chicken feather hydrolysate, adjusted to pH 8.0, elicited the greatest response from adult *A. obliqua*; the attraction response was more than twice that observed for a 10% NuLure® (Miller Chemical and Fertilizer Corp., Hanover, PA) standard. Neutral or slightly acidic preparations of chicken feather hydrolysate were less effective. While *A. ludens* was less attractive in the laboratory tests to a 25% chicken feather hydrolysate than a 10% NuLure standard, *A. obliqua* appeared slightly more attracted to the hydrolysate than NuLure, but not significantly. In a 5-day field test using sterile released *A. ludens*, 4.5% chicken feather hydrolysate, adjusted to pH 8.0, caught almost 2.5 times more flies than 10% NuLure. Twenty-three volatile compounds that emanated from chicken feather hydrolysate were identified by headspace analysis using GC-MS and GC retention index comparison techniques. Among the volatiles were 7 ketones, 6 alcohols, 2 aldehydes, 2 chlorocarbons and 2 furans. At pH 8.0, the five most abundant compounds in decreasing order were 4-methyl-2-pentanone (76.3%), 4-methyl-2-pentanol (16.5%), 1-hexanol (3.1%), 1-heptanol (0.81%) and 2-butoxyethanol (0.64%).

KEY WORDS Lure, Mexican fruit fly, *Anastrepha ludens,* West Indian fruit fly, *Anastrepha obliqua*, volatiles, food bait, chicken feather hydrolysate

The West Indian fruit fly, *Anastrepha obliqua* (Macquart), and Mexican fruit fly, *Anastrepha ludens* (Loew), are serious pests of mangos, guavas, peaches and other commercial crops. While *A. obliqua* inhabits subtropical to tropical regions

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(i.e., Mexico to Brazil), *A. ludens* inhabits regions that are subtropical (i.e., United States to Costa Rica). Food bait attractants such as borax-buffered 10% NuLure® (Miller Chemical and Fertilizer Corp., Hanover, PA) or 2.2% torula yeast are currently used in McPhail trap (Baker et al. 1944) deployment to detect, monitor and control populations of *A. obliqua, A. ludens*, and other *Anastrepha* spp. (Anonymous 1993). Despite widespread use of these lures, research continues to develop more potent ones. Plant and animal products have long been fertile sources for new leads to insect attractants. Various degradative processes (i.e., hydrolytic, enzymatic, fermentation) of these protein-containing sources have been successful in yielding efficacious lures, NuLure and torula yeast being among them.

Intensive and large-scale production of food animals including poultry has led to waste disposal problems and potential sources of pollution (Shih 1993). As partial solution to this problem, the enzymatic conversion of chicken feathers, rich in keratins (Fraser 1969), has led to a digestible food or food supplement (Lin et al. 1992, Shih 1993).

The need to develop new uses to consume chicken feather waste and the report that a mixture of chicken feather hydrolysate alone or in combination with soybean paste is attractive to the Japanese orange fly (Mikanbai), *Bactrocera tsuneonis* (Miyake) (Matsuyama 1977), led us to investigate chicken feather hydrolysate as a candidate attractant for *A. obliqua* and *A. ludens*. This paper describes laboratory and field evaluation of chicken feather hydrolysate, factors that influence its attractancy, and the nature of the volatiles that emanate from it.

Materials and Methods

Test materials. NuLure, obtained from the Miller Chemical and Fertilizer Corp (Hanover, PA), was diluted to a 10% concentration with deionized water, and then buffered to a pH 8.0 with borax. NuLure was formerly PIB-7 or Staley Protein Bait No. 7 (Lopez-D. and Spishakoff 1963a,b). The chicken feather hydrolysate was prepared by the following method. A stirred slurry of 50 g of finely chopped chicken feathers in 1 liter of 6 *N* hydrochloric acid was heated at 65°C for 1.5 to 4 h. The darkened slurry was cooled and filtered through a glass-fritted funnel lined with a 1.3-cm layer of glass wool to aid the filtration of unhydrolyzed chicken feathers. Percent hydrolytic conversion at 65°C for 1.5 h and 4 h was 65 and 89%, respectively. Percent conversion = [100-(weight undissolved feathers remaining on filter ÷ initial weight of feathers) × 100]. The filtrate was then adjusted to the desired pH by addition of 50% aqueous sodium hydroxide. Solids that precipitated during the neutralization process were not removed.

Laboratory bioassay. The bioassay was a modified version of a cage-top bioassay described by Robacker and Hart (1984). Briefly, a 100-µl aliquot of the test solution was applied to a 12.7-mm diam filter paper disc attached to the bottom of a glass Petri dish. After application, the dish (inverted) was placed on top of a wire-screened cage (2 m^3) containing 1000 mixed-sex adult *A. obliqua* or *A. ludens* (3 to 7 days old) maintained only on a 6% sucrose-water diet. To eliminate a potential source of attraction, the sucrose-water diet was removed and replaced with water the night before the tests. Tests were conducted in a room held at 25°C and 70 ± 5% RH. Bioassay procedures were identical for *A. ludens*

and *A. obliqua,* except *A. obliqua* required a higher light intensity for it to respond to an attractive source. Generally, four different test materials or preparations of the same test material were bioassayed in combination with a standard (10% NuLure) and water control. Five replicates of each of these treatments were placed in the cage in a randomized complete block design. Response determinations were made as follows: counts of flies found under the area of Petri dishes were taken every 5 min for 30 min. Each test was repeated three times. Due to test schedules, one or two groups of flies were used. All flies were 3 to 7 days old. Response means and standard errors (SEM) were calculated by using a SuperAnova (Accessible General Linear Modeling) program (Abacus Concepts, Inc., Berkeley, CA).

Field test. The chicken feather hydrolysate field test was conducted with released, irradiated, Mexican fruit flies in a 'Rio Red' grapefruit orchard planted in 1990. Flies were from a culture established in 1987 and reared on artificial diet. Each batch of released flies was 3 to 4 days old. Pupae were irradiated according to the procedures established by USDA-APHIS (Moreno et al. 1991), i.e., 1 to 2 days before adult eclosion, pupae were exposed to 70-92 Gy emitted at a rate of 23.95 Gy/min from a cesium^{137} source, M-001 irradiator. After irradiation, pupae were dyed with Day-Glo fluorescent signal green color at a rate of 3 g of dye per liter of pupae. Then 150 pupae were placed in 36 × 0.5 liter cartons with screened tops. Adults in cartons were provided with 6% sugar solution and maintained at 25°C and 70% RH. Flies (approximately 5000 total) were released in trees diagonal to the trap tree the evening before trap set-up. The following morning, plastic McPhail-type traps were set out in a 6 × 6 Latin Square experimental design (6 treatments × 6 replicates). Traps were placed midway up the trees on the northeast corner and in every other tree within a row and in every other row at a distance of ≈9.1 × 14.6 m from each other. Each trap contained 300 ml of test solution. Traps were left in the field for 5 days before collection; these were taken to the laboratory where counts of flies were made. Data were analyzed using SuperAnova AGLM (Abacus Concepts, Berkeley, CA) analyses of variance (ANOVA) for Latin Square design. Data were subjected to log normal transformation. The transformation was based on an analysis of the residuals. After analysis of data, means were retransformed for presentation. Significant differences ($P < 0.05$) among means were separated by Fisher's protected least significant difference (Fisher 1949).

Collection of volatiles. Volatiles were collected from the headspace (immediate confined area above the test material) of chicken feather hydrolysate adjusted to a pH of 4.0 or 8.0. A 100-ml aliquot of chicken feather hydrolysate, of the desired pH, was placed in a 250-ml three-neck, round-bottom flask. As the solution was stirred at ambient temperature, pre-purified nitrogen (i.e., passed through an activated charcoal bed) was swept at 300 ml/min over the chicken feather hydrolysate headspace, and volatiles were collected in a glass tube packed with 300 mg of activated charcoal (Darco®, 20-40 mesh, Aldrich Chem. 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 was pre-purified by continuous extraction (Soxhlet extractor) with methylene chloride and then benzene. After collecting volatiles for 15 h, the charcoal trap was removed and eluted with approximately 0.3 ml of methylene

chloride. The methylene chloride solution 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 chicken feather hydrolysate. GC and GC-MS techniques similar to those used to identify volatiles from bacterial supernatants (Lee et al. 1995, DeMilo et al. 1996) were used to identify the volatiles collected from the headspace of chicken feather hydrolysate.

Gas chromatograpy (GC). A Shimadzu Model GC-9A (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 µm film thickness) was used to analyze volatile components. GC peak areas were quantified using a Shimadzu CR-4A Integrator. GC operating conditions were: injector/detector temperature, 280°C; helium carrier, approximately 1 ml/min (4 kg/cm^2 head pressure), injector operated in split mode, 175:1; temperature program, 50°C 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 µm 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: ionization voltage, 70 eV; mass range, m/z 30 to 550; ion source temperature, 180°C. The mass spectra of the unknown compounds were compared with those in the Wiley/NBS spectral data base. GC-MS identifications were confirmed by comparing Kovats indices (KI) of unknowns (determined on the DB-1 column) with those determined for authentic samples (Kovats 1965).

Results and Discussion

Because pH is known to influence the composition of volatiles (Flath et al. 1989) and efficacy (Heath et al. 1994, Epsky et al. 1994) of proteinaceous baits to fruit flies, the attractivity of chicken feather hydrolysate was determined against a mixed-sex population of adult *A. obliqua* as a function of pH. Mean responses to a 30% concentration of chicken feather hydrolysate varied with pH, with a maximum mean response of 29.33 at pH 8.0 (Table 1). At pH 8.0, attraction was more than double that for the standard, 10% NuLure. The relatively high mean responses observed for chicken feather hydrolysate at pH 8.0 and 9.0, compared to those at pH 6.0 and 7.0, indicated that slightly alkaline rather than slightly acidic media are more attractive to the fruit flies.

Mean attractancy responses were determined (Table 2) for mixed-sex populations of adult *A. obliqua* exposed to varying concentrations of chicken feather hydrolysate (pH 8.0) and 10% Nulure (pH 4.0). Compared to 10% NuLure, the undiluted hydrolysate (100% chicken feather hydrolysate) was much more attractive to a mixed-sex population of *A. obliqua*. However, 25% chicken feather hydrolysate, the most dilute solution tested, elicited the greatest attraction, but like the two mid-range concentrations of chicken feather hydrolysate (50 and 75%), it was not significantly different from a mean response for 100% chicken

Table 1. Effect of pH on the attractiveness of chicken feather hydrolysate (CFH) to the West Indian fruit fly *Anastrepha obliqua* as determined in cage-top bioassays.

Treatment*	Attractancy response**	
	Mean†	SEM
Water	3.95 d	0.30
10% NuLure‡	13.57 c	2.11
30% CFH, pH 6.0	12.49 c	1.50
pH 7.0	17.85 bc	1.88
pH 8.0	29.33 a	2.39
pH 9.0	22.91 ab	3.73

*100 µl of test solution or water was applied to the filter paper disc (five replicates per treatment).
**Counts of flies under Petri dishes were made every 5 min for 30 min, then averaged.
†Analyses used means of three tests; total mean responses (n) = 734.5. Means within columns followed by the same letter are not significantly different (F = 15.6, df = 24, P = 0.0001, Fisher's protected LSD [Fisher 1949], P = 0.05).
‡Unbuffered, pH 4.0.

feather hydrolysate. Although mean responses for these mid-range concentrations were lower than that observed for concentrations at either end of the range, they still exceeded, but not significantly, the mean response for NuLure. No clear correlation could be made between attractancy and concentration. Nonetheless, the low mean response for the water control compared with observed responses for chicken feather hydrolysate and NuLure highlights the ability of the flies to move discriminatingly to different olfactory stimuli.

The attractiveness of chicken feather hydrolysate to a mixed-sex population of adult *A. ludens* was also determined from the cage-top bioassay. Test results show (Table 3) that *A. ludens* was more attracted to 10% NuLure than to 25% chicken feather hydrolysate. However, *A. obliqua* appeared slightly more attracted to the hydrolysate than NuLure, but not significantly. While the mean response difference between NuLure and chicken feather hydrolysate for *A. obliqua* was approximately 9%, the response difference between the same lures for *A. ludens* was approximately 4× greater (34%). Clearly, new studies will be required to elucidate those factors (i.e., pH, concentration, volatiles, etc.) responsible for response differences for these insects.

Five-day field catch data from a test involving trapping of released sterile adult *A. ludens* in McPhail traps baited with varying concentrations of chicken feather hydrolysate show (Table 4) that three (4.5, 9.0 and 18%) of the four concentrations tested were significantly more attractive than the 10% NuLure standard. The least effective concentration (36%) was also more attractive than NuLure, but not significantly. The relatively high attraction of *A. ludens* to

Table 2. Attractiveness of different concentrations of chicken feather hydrolysate (CFH) at pH 8.0 to the West Indian fruit fly, *Anastrepha obliqua*, as determined in laboratory cage-top bioassays.

Treatment*	Attractancy response**	
	Mean†	SEM
Water	3.16 d	0.11
10% NuLure‡	15.90 c	0.80
25% CFH	23.83 a	1.53
50% CFH	18.77 abc	2.20
75% CFH	16.83 bc	2.39
100% CFH§	21.54 ab	2.72

*100 µl of test material (prepared as aqueous solution) or water (control) was applied to the filter paper disc (five replicates per treatment).

**Counts of flies under Petri dishes were made every 5 min for 30 min, then averaged.

†Analyses used means of three tests; total mean responses (n) = 861.5; means within columns followed by the same letter are not significantly different (F = 14, df = 24, P = 0.0001; Fisher's protected LSD [Fisher 1949], P = 0.05).

‡Unbuffered, pH 4.0.

§Undiluted hydrolysate.

chicken feather hydrolysate in the field compared to NuLure was surprising in light of *A. ludens* apparent preference for NuLure in laboratory tests (Table 3). Existing quarantine restrictions prevented a field evaluation of chicken feather hydrolysate against *A. obliqua*.

No attempt was made in either field or laboratory tests to determine differential sex preference for either species to chicken feather hydrolysate. However, recent field data indicate that males and females of *A. obliqua* and *A. ludens* respond nearly equally to this lure (Moreno et al., unpubl. data).

Fig. 1 and 2 represent typical GC traces of the volatile components emanating from the chicken feather hydrolysates adjusted to pH 8.0 and 4.0, respectively. Except for the solvent peak and benzene (a contaminant unavoidably introduced onto the charcoal adsorbent through washings), arrowhead markings in the GC traces depict peaks (or areas corresponding to peaks too small to be detected at the given detector response attenuation) that were identified by GC-MS and GC retention index comparisons (Kovats 1965). Peaks lacking arrowheads remain unidentified.

Among the 23 identified volatiles were 7 ketones, 6 alcohols, 2 aldehydes, 2 chlorocarbons and 2 furans (Table 5). Only slight differences were noted in the relative peak area percentages for compounds present in the headspace volatiles at both pH 8.0 and 4.0. Without exception, differences between relative peak area percentages fell within one order of magnitude. Chlorocarbons, 1-chloro-

Table 3. Attractiveness of chicken feather hydrolysate (CFH) and NuLure to mixed sexes of adult *Anastrepha ludens* and *Anastrepha obliqua* as determined in laboratory cage-top bioassays.

	Attractancy response**			
	A. ludens		*A. obiqua*	
Treatment*	Mean†	SEM	Mean†	SEM
Water	9.60 c	0.28	9.8 b	0.23
10% NuLure (pH 4.0)	51.81 a	2.47	43.11 a	2.57
25% CFH (pH 8.0)	38.56 b	1.53	47.19 a	3.14

*Dose applied for NuLure and chicken feather hydrolysate was 100 µl/disc.
**Counts of flies under Petri dishes were made every 5 min for 30 min, then averaged. Means for *A. ludens* and *A. obliqua* were determined from independent tests.
†Calculated from 3 tests, 10 replicates/test. Means within columns followed by the same number are not significantly different (for A. ludens $F = 164.7$, df = 27, $P = <0.001$ and for *A. obliqua* $F = 76.3$, df = 27, $P = <0.001$, Fisher's protected LSD [Fisher 1949], $P = 0.05$); mean flies counted were 646 and 583 for *A. ludens* and *A. obliqua*, respectively.

hexane and 1,4-dichlorobutane could be rationalized as secondary products formed by acid reaction with 1-hexanol and tetrahydrofuran, respectively. The five most abundant volatiles collected from the headspace of chicken feather hydrolysate at pH 8.0 in decreasing order were 4-methyl-2-pentanone (76.3%), 4-methyl-2-pentanol (16.5%), 1-hexanol (3.1%), 1-heptanol (0.81%) and 2-butoxyethanol (0.64%). It is notable that four out of the five most abundant compounds are low molecular weight primary or secondary alcohols. Low molecular weight alcohols also were reported among the most abundant components in the volatiles of two bacteria-produced supernatants that were highly attractive to *A. ludens* (Lee et al. 1995, DeMilo et al. 1996). However, none of those alcohols corresponded to alcohols identified in the chicken feather hydrolysate volatiles.

We have demonstrated that a hydrolysis product of chicken feathers elicits high attraction to two *Anastrepha* spp. This attraction was comparable to that of an often used standard, NuLure. We have also identified 23 compounds in the headspace volatiles of chicken feather hydrolysate at two different pH values. Differences noted between relative peak area percentages for volatile components found in the headspace of chicken feather hydrolysate at both pHs were small. Highest attraction was observed for a chicken feather hydrolysate that was adjusted to pH 8.0. Chicken feather hydrolysates with neutral (pH 7.0) or slightly acidic (pH 6.0) were less attractive. Laboratory and field studies are in

chloride. The methylene chloride solution 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 chicken feather hydrolysate. GC and GC-MS techniques similar to those used to identify volatiles from bacterial supernatants (Lee et al. 1995, DeMilo et al. 1996) were used to identify the volatiles collected from the headspace of chicken feather hydrolysate.

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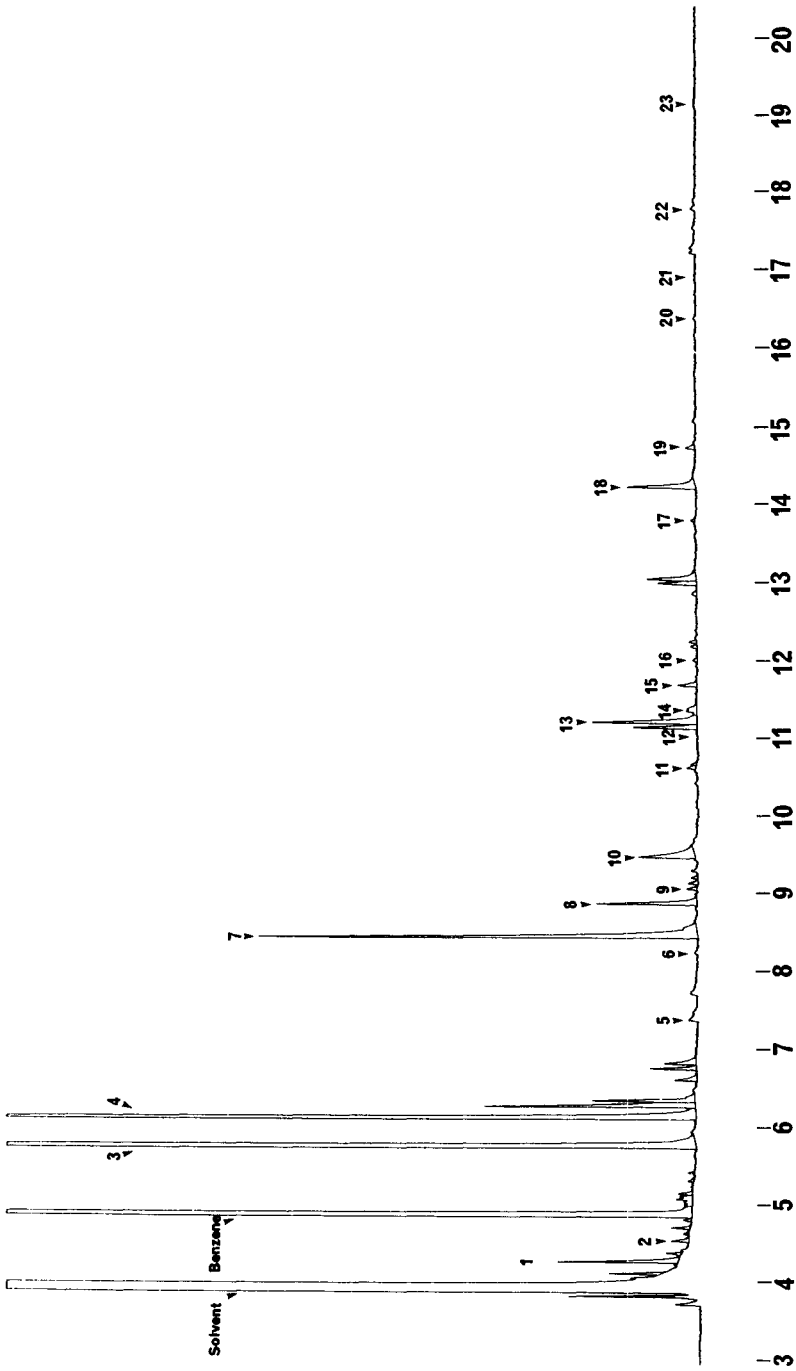

Fig. 1. Gas chromatogram of volatile components observed from chicken feather hydrolysate at pH 8.0. Vertical axis = detector response; horizontal axis = retention time in min.

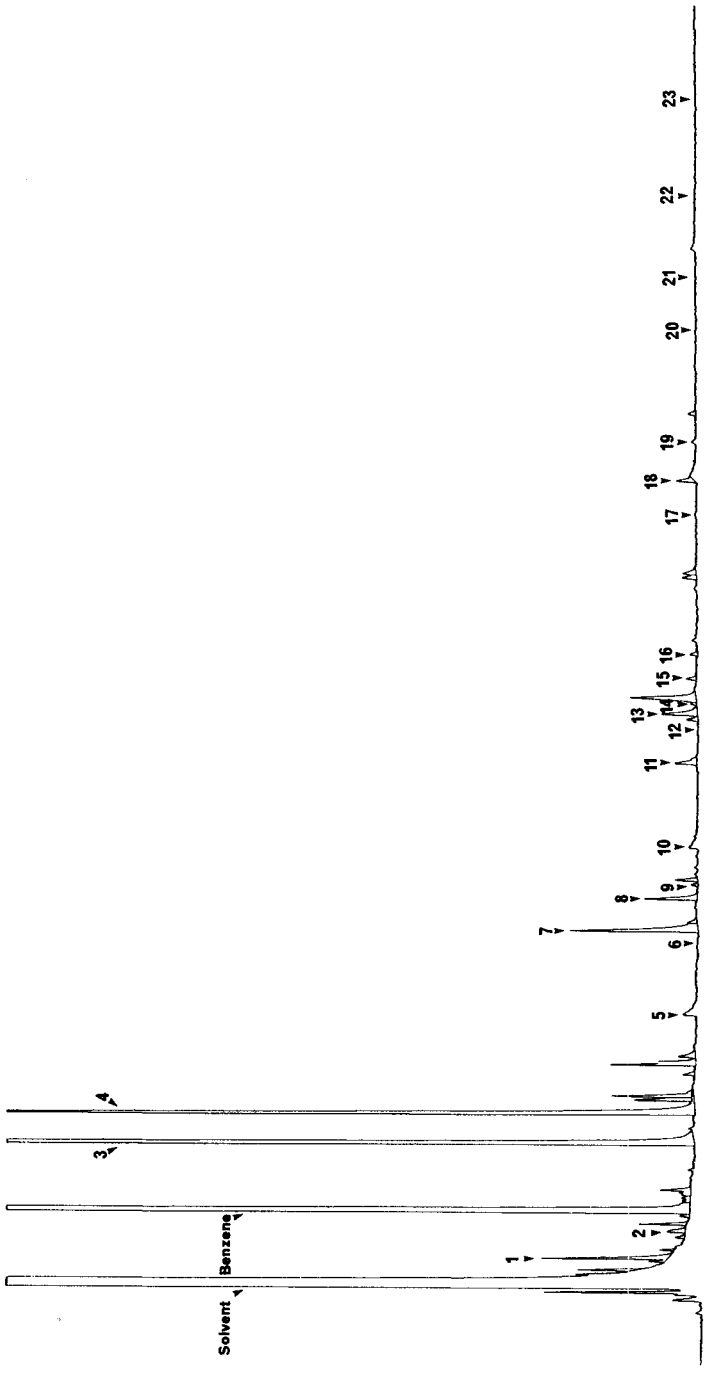

Fig. 2. Gas chromatogram of volatile components observed from chicken feather hydrolysate at pH 4.0. Vertical axis = detector response; horizontal axis = retention time in min.

Table 5. Volatiles identified from chicken feather hydrolysate at pH 4.0 and pH 8.0.

GC peak no.*	Compound	Ref. KI**	pH = 4.0		pH = 8.0		mass spectral ions, *m/z*,	
			KI†	Rel. Area%	KI†	Rel. Area%	M+(intensity)	base
1	2-butanone	‡	‡	1.30	‡	0.624	72 (19)	43
2	tetrahydrofuran	615	615	0.096	614	0.103	72 (32)	42
3	4-methyl-2-pentanone	721	720	79.7	720	76.3	100 (16)	43
4	4-methyl-2-pentanol	743	742	14.4	741	16.5	ND§	45
5	2-furaldehyde	805	804	0.262	803	0.111	96 (100)	96
6	1-chlorohexane	842	842	¶	842	0.029	ND	91
7	1-hexanol	854	854	1.88	852	3.07	ND	56
8	2-heptanone	870	869	0.636	869	0.639	114 (5)	43
9	1,4-dichlorobutane	877	877	0.077	877	0.059	ND	55
10	2-butoxyethanol	893	891	0.174	892	0.641	ND	57
11	benzaldehyde	934	934	0.374	935	0.063	106 (95)	77
12	dimethyltrisulfide	949	949	¶	949	¶	126 (100)	126
13	1-heptanol	956	955	0.571	955	0.807	ND	70
14	phenol	960	959	0.058	960	0.128	94 (100)	94
15	2-octanone	971	971	0.123	971	0.122	128 (4)	43
16	2-pentylfuran	980	980	0.100	980	0.032	138 (18)	81
17	acetophenone	1040	1041	¶	1042	0.027	120 (34)	105
18	1-octanol	1057	1056	0.243	1056	0.582	ND	41
19	2-nonanone	1073	1073	0.077	1073	0.069	142 (7)	43
20	camphor	1128	1128	¶	1128	0.021	152 (28)	95
21	2-pentylthiophene	1149	1149	¶	1148	¶	154 (21)	97
22	2-decanone	1175	1175	¶	1175	¶	156 (6)	43
23	γ-octanoic lactone	1218	1219	¶	1219	0.025	142 (1)	85

*Peak numbers correspond to those in Fig. 1 and 2.

**Kovats indices calculated from retention time data of authentic sample obtained on a DB-1 capillary column.

†Kovats indices calculated from retention time data on a DB-1 capillary column.

‡not determined.

§ND = not detected.

¶trace amount (compound's presence was detected by GC-MS).

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