Preparation and Evaluation of a Synthetic Fermented Egg Coyote Attractant and Deer Repellent

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A synthetic formulation was developed as a replacement for a fermented egg product that attracts coyotes (*Canis latrans*) and repels deer (*Odocoileus hemionus columbianus*). The development took place in three stages. First, individual volatile components (identified earlier) were mixed according to their relative concentrations in fractions of fermented egg. Then a systematic process was followed whereby one of four fractions (acids, bases, neutral headspace volatiles, and sulfur compounds) was varied while the others were held constant. An 18-member human odor panel helped in the final stage of refinement. Minor modifications in the formulation were compared with the natural fermented egg in a series of odor tests until the best match in quality was found. Behavioral tests showed that activity of the synthetic product duplicated the natural product in repelling deer and attracting coyotes. The formulation shows considerable promise as a safe means of controlling deer damage to forest and agricultural crops and as a tool for estimating coyote populations.

Most of the major volatile components in a fermented egg product (FEP) that is patented as a synanthropic fly bait (Mulla and Hwang, 1974) have been identified (Bullard et al., 1978). Hwang and co-workers (1971, 1976) have also identified several compounds in an aqueous suspension of FEP. Since fermented egg attracts coyotes and repels deer, but varies in odor quality from batch to batch, our objective was to use this analytical information in developing a synthetic mixture having biological activity similar to that of the natural FEP.

In the development of synthetic flavors for human foods the chemist seldom expects to formulate a product based solely on analytical information. Initial work indicated that our project was to be no exception. Odor evaluation would be necessary during the blending process.

Our first concern was whether or not the human nose was sensitive enough to detect subtle changes in odor quality during the blending of a synthetic mixture that would be used on animals. The use of human subjects would eliminate much of the time-consuming and expensive behavioral testing on coyotes and deer. We believed that coyotes and deer are affected by FEP because it resembles naturally attractive food (carrion) for coyotes or spoiled "rejected" food for deer. Perhaps, since canids and ungulates are exposed to a wide range of odors from decomposed proteinaceous materials in their environment, the subtle changes in odor quality were not important and the less sensitive human nose could be used.

With this in mind, we began a three-phase process. In phase I, compounds were combined in synthetic mixes according to their relative concentrations in the wire loop, volatile fatty acid, and volatile bases fractions (Bullard et al., 1978). Organosulfur compounds from the wire loop volatiles fraction were handled as a fourth fraction. The relative concentrations of these were adjusted until the odor closely resembled FEP. In phase II an 18-member human odor panel monitored further modifications until a blend was achieved that best matched a dibutyl phthalate extract of FEP. Finally, in phase III synthetic fermented egg (SFE) was compared to FEP for repellent effect in a standardized feeding test with deer and for attractant effect by a standard coyote scent-post survey technique.

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EXPERIMENTAL SECTION

Materials. When necessary, samples of individual compounds in the synthetic mixture were purified by preparative GLC so that all were at least 99% pure. All mixtures were prepared in dibutyl phthalate solution. To avoid the difficulty of comparing these liquids with a solid, we extracted FEP with dibutyl phthalate. This nonodorous solvent provided a yellow extract having an odor identical with that of FEP.

Phase I. Blending the Synthetic Mixtures. Basically, SFE was developed from the proper blending of four mixtures (components listed in Table I). Their origin is as follows.

Wire Loop Mixture. A synthetic mixture of 54 compounds was prepared according to quantitative data obtained by GLC analysis of a wire loop fraction on a 500 ft × 0.03 in. OV-101 column (Bullard et al., 1978). It included most of the compounds that were identified in FEP. Since esters predominated, both numerically and in concentrations, the mixture had a "fruity" aroma.

Acid Fraction Mixture. The volatile fatty acid content of FEP was determined by a special method (Bullard et al., 1978). Since the concentration of these compounds is higher than that of the other FEP volatiles (ca. 77% w/w) and they have low olfactory thresholds, we were concerned about the large variation in analytical values for the 1972–1975 samples (Bullard et al., 1978). Therefore, the concentration of individual fatty acids in the synthetic mixture was based on an average of their relative concentrations in the four batches. In dilute concentrations this mixture had a "cheesy" aroma.

Volatile Bases Mixture. This mixture was based on quantitative data from an early GLC procedure for the analysis of amines in the basic fraction of FEP. Circumstances forced our use of this tentative information. Much later, when the mass spectrometer was available, we were able to improve our analysis considerably. The improved method and amines found are reported elsewhere (Bullard et al., 1978).

Organosulfur Mixture. The organosulfur compounds were isolated by cryogenic trapping and identified by capillary column gas chromatography–mass spectrometry of the headspace volatiles. Although one of these com-

Table I. Composition of Synthetic Fermented Egg

	Percent (wt)		Percent (wt)
Volatile fatty acids		Wire loop volatiles	
Acetic	1.35	Propyl acetate	0.006
Propionic	3.81	Propyl propionate	0.025
Isobutyric	1.32	Propyl isobutyrate	0.007
Butyric	22.40	Propyl butyrate	0.090
Isovaleric	1.46	Propyl valerate	0.005
Valeric	6.66	Propyl isocaproate	0.016
Isocaproic	1.70	Propyl caproate	0.062
Caproic	24.42	Propyl heptanoate	0.176
Heptanoic	10.16	Propyl caprylate	0.087
Caprylic	8.10	Isobutyl butyrate	0.012
		Isobutyl valerate	0.065
Amines		Isobutyl caproate	0.013
Pentyl	0.42	Isobutyl heptanoate	0.048
Hexyl	2.97	Butyl acetate	0.031
Heptyl	4.13	Butyl propionate	0.007
Trimethyl	1.90	Butyl isobutyrate	0.006
		Butyl butyrate	0.082
Organosulfurs		Butyl isovalerate	0.006
Dimethyl disulfide	0.66	Butyl isocaproate	0.020
2-Mercaptoethanol	0.17	Butyl caproate	0.060
Wire loop volatiles		Butyl heptanoate	0.095
Methyl butyrate	0.006	Isoamyl acetate	0.030
Methyl isovalerate	0.037	Isoamyl butyrate	0.013
Methyl valerate	0.044	Amyl butyrate	0.013
Methyl caproate	0.033	Amyl isocaproate	0.023
Methyl heptanoate	0.014	Amyl caproate	0.015
Methyl caprylate	0.034	2-Hexanone	0.032
Ethyl propionate	0.175	2-Heptanone	0.059
Ethyl isobutyrate	0.027	Toluene	0.015
Ethyl butyrate	0.494	Ethylbenzene	0.011
Ethyl isovalerate	0.013	*p*-Xylene	0.025
Ethyl valerate	0.468	*m*-Xylene	0.010
Ethyl isocaproate	0.061	*o*-Xylene	0.026
Ethyl caproate	1.270	Hexanal	0.041
Ethyl heptanoate	2.780	Octanal	0.011
Ethyl caprylate	1.640	α-Pinene	0.006
Ethyl nonanoate	0.048	Limonene	0.006
Isopropyl butyrate	0.006		

pounds was present in the wire loop fraction, they were treated as a special class because of their impact on odor quality.

In the blending process we subjectively compared the odor of the mixture (one or more of the four fractions in dibutyl phthalate) with an equal volume of the FEP extract. First, the concentration of the wire loop mixture having the odor intensity of the extract was established. Then the appropriate blend of fruity and cheesy notes was achieved by adding the acid fraction mixture. With the organosulfur fraction the mixture assumed an odor quality approaching that of FEP but missing an "ammonical" note. This was solved by adding an appropriate quantity of the basic amine mixture. The odor quality of the final mixture closely resembled that of the natural product.

Phase II. Odor Panel Refinement. An 18-member human odor panel assisted in the final refinement stages of SFE development. Panel members individually made odor measurements in a 8.5 ft^2 room maintained at 21 °C and swept by a stream of air purified by activated charcoal. A dye (Rit, Golden Yellow 42) was added to the synthetic mixture to give it the same color as FEP extract, thus avoiding color cues during tests. Then 0.5 mL of dibutyl phthalate solutions of either FEP extract or synthetic ingredients were tested in 30-mL Nalgene bottles. Judges were cautioned about the problems of olfactory adaptation (Stone, 1966) and were instructed to sniff the bottles briefly (10 s or less) and wait at least 15 s before sniffing a different bottle.

Selection of Odor Panel Judges. The 18 odor panel judges were selected from the Denver Wildlife Research Center staff. All candidates were volunteer men and women from a cross section of research and supporting personnel. Those selected conformed to selection criteria outlined by ASTM (Wittes and Turk, 1967) for a triangle test, an intensity rating test, and a multicomponent odor identification test.

Matching Odor Intensity. Since odor intensity is an olfactory component that can influence odor judgements, the first step was to match the intensities of the synthetic mixture from phase I and the FEP extract. A series of five dilutions of the synthetic were arranged from left to right in order of increasing intensity. Judges were instructed to begin at the left (to alleviate adaptation) and select the bottle most like the FEP extract in intensity. The concentration selected more than 50% of the time then occupied the central position in another series of five dilutions having a narrower concentration range. This process continued until the responses were such that a plot of selections vs. concentrations was essentially Gaussian in shape. The final concentration was determined by the equation

$$\frac{n_a[A] + n_b[B] + \cdots n_e[E]}{N} = [S]$$

where $[A]$ through $[E]$ is the concentration in μL/mL of synthetic in the candidate test mixture and $[S]$ the final calculated concentration. The total number of selections is N and the number of selections of each candidate concentration is n_a through n_e with the lower case subscript denoting the upper case candidate mixture.

Matching Odor Quality. The triangle test (Byer and Abrams, 1953) was used to achieve the final SFE blend. Each judge received five sets of odor samples consisting of two samples of FEP extract and one of synthetic or vice versa and asked to select the "odd" sample.

The relative concentrations of the four synthetic mixtures were established early in the blending process. Adjustments were then made in the concentrations of certain components within the mixtures. After testing had been completed, judges were frequently asked to describe how the odd sample generally differed from the other two and what "note" should be enhanced or suppressed to obtain a better match. If general agreement among the judges was good, the mixture was modified accordingly. Always, one of the four mixtures (i.e., wire loop, organosulfur, base, and acid) was modified while the other three remained unchanged. This process continued until a blend was found that produced a 40% error rate in triangle test selections. That formulation (Table I) was designated synthetic fermented egg (SFE).

Results and Discussion. The ingredients and their relative concentrations are found in Table I. The correct selection on triangle tests was only changed from 70% at the beginning to 60% at the end. This reflected the fact that the synthetic mixture did not require much of a change during phase II. As testing proceeded some of the judges became more adept at odor discrimination and were making perfect scores on every test while others responded at about the chance level of 33.3%. Since there is considerable variation in fermentation and putrefaction products of plant and animal matter even under controlled conditions (Bullard et al., 1978) the 60% selection level became the established criterion. The question was not one of discrimination but whether or not SFE smelled highly similar to FEP.

Phase III. Animal Behavioral Tests. Odor intensity tests were conducted as described above to adjust the intensity of SFE odor in a test formulation to that of a given amount of FEP. The appropriate SFE formulation was selected after human odor panel testing determined it to be equal in odor intensity to FEP.

Deer Repellency Tests. Details of the apparatus and procedure for evaluating deer repellents are described elsewhere (Campbell and Bullard, 1972). Briefly, deer (*Odocoileus hemionus columbianus*) were given a choice between a standard food and a test food. A 2- to 3-s eating period on a particular food is considered a choice. The method incorporates the brief exposure, foods-together principle (Young, 1968) where post-ingestional factors do not influence preference.

The repellent tetramethylthiuram disulfide (TMTD) has been used as the standard (Campbell and Bullard, 1972) for comparison with experimental materials. A 0.01% TMTD formulation provides the marginal acceptance necessary for comparison. If either the standard or candidate concentrations are too high or low, the preparation would tend to be 100% rejected or accepted.

The food base for both standard and candidate formulations was a pelleted mixture of ground deer feed containing 2% ground Douglas fir needles. Either 0.01% TMTD, 0.004% FEP, or the SFE dibutyl phthalate equivalent of 0.004% FEP (0.00009% SFE) were mixed in fresh corn oil and coated on 250 g of pelleted food base. The treatments were packaged in polyethylene bags and placed in the test pans. The bags served as liners so that treatments could be changed with each deer thereby minimizing contamination from animal sources.

Six deer were individually tested in two replications of 20 choices each. Preferences were calculated by the following formula:

$$\text{percent acceptance} = \frac{\text{number of choices of test food} \times 100}{\text{total choices for standard and test foods}}$$

Results and Discussion. Mean acceptance was 24.5% for the SFE formulation and 48% for FEP. A two-factor analysis of variance of the data (Winer, 1971) revealed that although the acceptance of the SFE formulation was numerically lower they were equal at $P = 0.14$. Since the carousel test is limited to comparison of candidates at low concentrations the maximum repellency must be determined by other means. Subsequent semifield tests indicated that the repellency can be increased considerably simply by increasing the SFE concentration. However, weathering problems must be overcome before a long-term test of field efficacy can be conducted. SFE must be formulated in a weather-resistant delayed release matrix that can be applied to plants.

Coyote Attractancy Tests. The test method is a slight modification of one reported earlier by Linhart et al. (1977). It is similar to the method used by the U.S. Fish and Wildlife Service to obtain annual indices of relative coyote (*Canis latrans*) abundance throughtout the western United States (Linhart and Knowlton, 1975). The test was conducted February 6 through 15 (during the coyote breeding season) at Zapata, Texas.

The relative preference of candidate attractants (scents) was determined by assessing the number of scent stations that were visited during the test period. A station was a cleared 3-ft-diameter circle covered with sifted earth or sand upon which tracks and other marks can be imprinted. A 7 mm high × 29 mm diameter round plastic capsule (manufactured by Lab-Tek Products of Westmont, Ill., for biological tissue processing) containing the attractant is located in the center and supported 1.3 cm above the ground by a 10-penny nail. The quantity of attractant is either 1.2 g of a powder or 2 mL of a liquid absorbed in a cotton ball. The stations are checked daily and recorded as visited if any coyote tracks are found. Other behavioral information is gained through observation of any signs of urination, defecation, scratching, digging, biting, pulling, and rolling or carrying the scent capsule. These signs are then cleared for the next day's observations. The attractants were renewed at each station every 5 days during the test.

A polyamide resin was used to delay the release of liquid SFE so that its odor intensity and longevity would be comparable to solid FEP. A 33.3% solution of SFE in propylene glycol was absorbed into 12–16 mesh particles of Polyamide Resin 1351 (General Mills Chemicals, Inc., Minneapolis, Minn.) yielding a powdered formulation that contained 4% attractant (w/w). SFE resin has been compared with liquid SFE coated on 100/120 mesh glass beads in triangle tests of odor quality. We found no differences in odor quality ($P < 0.05$) in the formulations. Therefore, the resin apparently does not modify odor quality, at least for humans. It was tested against FEP, concentrated SFE (undiluted liquid), a volatile fatty acid (VFA) mix (10% acetic, 7% propionic, 3% isobutyric, 40% butyric, 30% isovaleric, and 10% isocaproic), and a blank capsule.

Results and Discussion. The results are given in Table II. A three-factor analysis of variance showed that concentrated SFE received significantly more coyote visits

Table II. Coyote Responses to Odor Attractant Stations

		Other elicited behavior								
Attractant	Visits	Urination	Defecation	Scratch	Dig	Biting	Pulling	Carrying	Rolling	Total
SFE resin	86	16		4	10	2	22	17	1	72
Conc. SFE	180	40	4	9	20	2	83	73	18	249
FEP	117	13	2	4	12	8	44	39	1	123
VFA	136	17	2	5	22	1	54	51	11	163
Control	34	5			2		2	2		11

than other treatments ($P < 0.01$). Mean separation by Duncan's multiple range test showed VFA responses to be equal to FEP but greater than for the SFE resin. The SFE resin and FEP were equal. All treatments were better than the blank control. Subsequent tests have yielded similar results.

Responses to the blank capsule indicate that some of the coyote responses cannot be attributed entirely to the scents. However, odor intensity and quality of the scents appear to be the dominant factors in eliciting coyote responses. The greater response for concentrated SFE compared with SFE resin (42.7 times higher per capsule) indicates the importance of intensity. On the other hand, the VFA mixture (Linhart et al., 1977), which had been the primary attractant did not attract as many visits or behavioral responses as did the concentrated SFE. Yet all VFA ingredients are present in SFE and both are undiluted. Although the relative detection thresholds are unknown, we must assume that since both were undiluted liquid mixtures, the differences in responses were primarily related to odor quality. On the other hand, the VFA treatment received more visits and elicited nearly twice the total behavioral response of SFE-resin, probably because there were 42.7 times more of the attractant in the VFA capsule. Therefore, it appears that when intensities are equivalent odor quality is important, but when they are not, the stronger scent will elicit greater response.

Response to odor quality may be partially associated with the pheromonal effects of the breeding season. Trimethylamine and all of the C_2 to C_6 volatile fatty acids have been positively or tentatively identified in fox (Albone and Fox, 1971) and positively identified in coyote and dog anal gland secretions (Preti et al., 1976). However, there is no consistent difference in the pattern of volatiles that is indicative of estrus state or gender in coyotes and dogs. SFE has become a standard for comparison in this test during the entire year and has not been surpassed by a wide variety of commercial and special trapper lures.

CONCLUSION

Our utilization of analytical information and a stepwise blending process has resulted in a synthetic product that elicits behavioral responses in coyotes and deer similar to those elicited by natural FEP. Furthermore, the deer repellency and coyote attractancy responses can be increased simply by increasing the intensity of exposed SFE.

An important contribution of this work is the development of processes whereby humans can be used to evaluate odors in formulations of foods for animals. A synthetic mixture that smelled like the natural fermented egg to humans elicited appropriate responses in deer and coyotes. Although the task may be easier for a familiar odor having a normal variation in quality, a developmental process such as this can be an asset in developing flavors for animals.

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