Heliyon 6 (2020) e05302

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Preparation and evaluation of mushroom (*Lentinus edodes*) and mealworm (*Tenebrio molitor*) as dog food attractant

Tao Feng [a,1], Zhongshan Hu [a,1], Yanzun Tong [a,1], Lingyun Yao [a,**], Haining Zhuang [b], Xiao Zhu [c], Shiqing Song [a], Jun Lu [a,d,*]

[a] School of Perfume and Aroma Technology, Shanghai Institute of Technology, No. 100 Hai Quan Road, Shanghai 201418, PR China
[b] Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences, Key Laboratory of Edible Fungi Resources and Utilization (South), Ministry of Agriculture, National Engineering Research Center of Edible Fungi, National R&D Center for Edible Fungi Processing, Shanghai, 201403, PR China
[c] Research Computing, Information Technology at Purdue(ITaP), Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47907 USA
[d] Faculty of Health and Environmental Sciences, Auckland University of Technology, New Zealand

ARTICLE INFO

Keywords:
Food analysis
Attractant
Palatability
Flavour analysis
Different protein sources

ABSTRACT

Chicken liver is a main protein source to prepare attractant for dog food. However, animal proteins are costly. Seeking high quality and low-cost protein sources has been a goal for the industry. Mushroom *Lentinus edodes (L. edodes)* and Mealworm *Tenebrio molitor (T. molitor)* are novel protein sources, showing high potential as raw material of attractants. In this paper, chicken liver, *L. edodes,* and *T. molitor* were used as three different protein sources to prepare attractants. Their palatability to dogs were then compared. Firstly, the enzymatic hydrolysis process of three proteins was optimized, with a degree of hydrolysis of 54.82%, 36.10% and 30.14% for chicken liver, *L. edodes*, and *T. molitor* respectively. Secondly, volatile compounds of three attractants were identified by HS-SPME/GC-MS and SDE/GC-MS. Using OAV and PLRS method, it was found that bis(2-methyl-3-furyl) disulfide, indole, methional, 2-(methyl thio) phenol, γ-butyrolacton, furfuryl alcohol, acetic acid and isovaleraldehyde were the key components. Although both *T. molitor and L. edodes* attractant showed less palatability than that of chicken liver, they could be readily improved via adding key palatable volatile compounds. The ingestion rate of dog food with attractant showed a similar trend and was higher than that of food without attractant.

1. Introduction

The palatability of pet food has been a major concern for consumers when making choice. It can be improved by adding attractants, a type of feed additives [1]. These attractants are able to stimulate pet appetite, thus increase pet food intake [2]. Fournier M. found a flavor with an added amount of less than 0.02% could effectively mask the unpleasant odor in dog food and make them more favorable to dogs without reducing palatability [3]. Catnip and its derivates have been used as attractants for cat food [4]. Arulvictor S. et al. pointed out that attractants of animal protein source can improve the palatability of pet food [5]. At present, most pet food attractants are prepared with animal proteins. Compared with animal proteins, edible fungi and insects are more novel and more prevalent sources for proteins. *Lentinus edodes (L. edodes)* is a typical mushroom containing high proteins, which has been added to pet food as supplements [6]. Insects are the most abundant and environment-friendly source of proteins in the world, and have been approved by European Union as protein sources in feed industry. *Tenebrio molitor (T. molitor)* is suitable for highly dense and large-scaled production due to its biological habits, and it is rich in specific hormones to improve immunity [7]. Also, cost of *T. molitor* is very low. Because of these advantages, *T. molitor* has been widely used in feed fields, especially in pet feed of birds and fish, which can effectively improve the immunity and reproductive rate of pets [8]. Enzymatic hydrolysis is usually the first step to prepare dog food attractants, and the quality of hydrolysates has a significant impact on the quality of attractants [9]. Therefore, it is essential to select the right enzymes and optimization of enzymatic hydrolysis.

Although chicken liver is among the most commonly used attractant ingredients today, it is expensive and has relatively high levels of heavy

* Corresponding author.
** Corresponding author.
E-mail addresses: Lyyao@sit.edu.cn (L. Yao), jun.lu@aut.ac.nz (J. Lu).
[1] These authors are equal to this work.

https://doi.org/10.1016/j.heliyon.2020.e05302
Received 25 April 2020; Received in revised form 25 July 2020; Accepted 15 October 2020

metals [10, 11]. Thus, a lot of pet nutritionists are choosing alternative food proteins as raw material of attractants. Attractants prepared from different proteins may possess various functions. And the relationships between the aroma characteristics of these three attractants and dogs' food preference remains unclear.

Therefore, this study can be summarized to: 1) optimized the enzymatic hydrolysis process of chicken liver, *L. edodes*, and *T. molitor*, 2) identification of aroma substances in three attractants, 3) palatability evaluation study with dogs. This study provided some practical guidance for application of dog food attractants from fungi and insect proteins.

2. Material and methods

2.1. Reagents and materials

Raw chicken liver and *L. edodes* were purchased from Shandong Liuhe Group. *T. molitor* were bought from Xuchang *Tenebrio molitor* breeding base in Henan Province. Dog food was obtained from Shanghai Shilin Biotechnology Co., Ltd. Annzyme Complex protease PF116 and complex protease FF104 were purchased from Angel Yeast Co., Ltd. Edible fungal hydrolase and trypsin were purchased from Nanning Pangbo Biological Engineering Co., Ltd, Guangxi province. Xylose, cysteine hydrochloride, glycine, glutamic acid, thiamine, phosphoric acid, potassium sorbate, and tert-butylhydroquinone (food grade) were purchased from Huaheng Biotechnology Co., Ltd, Anhui province. *O*-dichlorobenzene and C_5–C_{22} *n*-alkanes were purchased from Shanghai Anpu Experimental Technology Co., Ltd. Dichloromethane, anhydrous sodium sulfate, hydrochloric acid, sulfuric acid, methanol, and glacial acetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai. Tween 80 was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. All chemical reagents used were analytical grade.

2.2. Optimization of enzymatic hydrolysis process

Chicken liver, *L. edodes*, and *T. molitor* were grounded by HC-400Y grinder (Damai, China), and then single-factor experiments were conducted. The experimental design is shown in Table 1. After enzymatic hydrolysis, the enzyme hydrolysate was heated at 90 °C for 10 min to inactivate enzyme. The hydrolysate was centrifuged (8000 rpm, 5 min) and the supernatant was taken to determine the degree of hydrolysis (DH/%). The measurement formula of DH was shown in Eq. (1).

$$\text{DH}\ (\%) = (\text{free amino nitrogen content (g/mL)} / \text{total nitrogen content (g/mL)}) * 100\ \% \quad (1)$$

Free amino nitrogen was determined by formaldehyde titration and total nitrogen content was determined by Kjeldahl apparatus.

On the basis of single-factor experiments, Plackett-Burman design was applied to screen out the factors that have significant effects on DH. According to the results of Plackett-Burman experiment, the Box-Behnken design of response surface analysis was applied to optimize the conditions of enzymatic hydrolysis.

2.3. Preparation of attractants by Maillard reaction

The optimized enzymatic hydrolysate was used to prepare attractants. The Maillard reaction formula of chicken liver/*L. edodes*/*T. molitor* attractant is shown in Table 2. Reaction conditions were as the following: temperature was held at 100 °C, for 90 min. pH was adjusted to neutral with food grade phosphoric acid before reaction, and finally adjusted to 3.3 with phosphoric acid after reaction.

2.4. HS-SPME-GC-MS analysis of volatile compounds in attractants

Attractants (4.0 g) were mixed with 50 μL 1, 2-Dichlorobenzene (internal standard, 100 μL/L) (Supelco, Bellefonte, PA, USA) solution in vials. They were kept at 50 °C for 30 min. Then 50HS-SPME with stable flex fiber coated by 50/30 μm layer of DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) was added in the space of the vial for 40 min at 50 °C to absorb the volatile compounds. Volatile compounds were released by desorption for 5 min at 250 °C in GC.

Volatile compounds in attractants were analyzed by using 7890 GC with 5975 MSD (Agilent Technologies, CA, USA). The electron ionization energy of MSD was 70 eV. The EI ion source temperature was set up at 230 °C. The chromatograms were recorded via monitoring the total ion currents from m/z = 20–350. Volatile compounds were separated by using HP-INNOWAX column (60 m × 0.25 mm × 0.25 μm) (Agilent Technologies, CA, USA). Helium was used as the carrier gas at a flow rate of 3 mL/min in a splitless mode. The quadruple mass filter, transfer line temperature and inlet temperature was running at 150 °C, 280 °C, and 250 °C, respectively. The GC oven temperature was held at 40 °C for 1 min, ramped to 230 °C at 4 °C/min and maintained for 15 min. The compounds were identified by matching the retention time of standards,

Table 1. Single-factor experiment design of enzymolysis.

Protein	Level	Factors				
		T (°C)	S: L[1]	E/S[2] (%)	t (h)	pH
Chicken liver	1	45	4:1	0.4	1	6
	2	50	3:1	0.6	2	6.5
	3	55	2:1	0.8	3	7
	4	60	1:1	1	4	7.5
	5	65	1:2	1.2	5	8
L. edodes	1	40	1:2	0.2	1	4
	2	45	1:4	0.4	2	4.5
	3	50	1:6	0.6	3	5
	4	55	1:8	0.8	4	5.5
	5	60	1:10	1	5	6
T. molitor	1	45	1:3	0.4	2	7
	2	50	1:6	0.8	3	7.5
	3	55	1:9	1.2	4	8
	4	60	1:12	1.6	5	8.5
	5	65	1:15	2	6	9

[1] Smashed protein material: Liquid (water).
[2] Enzyme/Smashed protein material * 100 %. Otherwise, enzymes used for hydrolysis of chicken liver refer to Nikolaev *et al.* [13], that of *L. edodes* refer to Lotfy *et al.* [14], that of *T. molitor* refer to Lu *et al.* [15].

Table 2. Attractants formula of three different protein sources.

Materials	Component (%)		
	Chicken liver	*L. edodes*	*T. molitor*
Enzymatic hydrolysate	80.00	91.00	48.00
Water	11.00	0.00	43.00
D-Xylose	4.70		
Cysteine hydrochloride	1.00		
Glycine	1.00		
Glutamic acid	1.00		
Thiamine	1.00		
Potassium sorbate	0.28		
TBHQ	0.02		

retention indices (RIs), and mass spectra in the NIST 11 database. The RIs of unknown compounds were determined by alkanes C_4–C_{30}.

Qualitative and semi-quantitative analyses of volatile compounds detected by GC-MS refer to Eqs. (2) and (3) [9].

$$\mathbf{RI_x} = \left(\frac{\mathbf{lg(t_{(x)})} - \mathbf{lg(t_{(z)})}}{\mathbf{lg(t_{(z+1)})} - \mathbf{lg(t_{(z)})}} + \mathbf{z} \right) \times 100, \quad (2)$$

Where t(x) is the retention time of volatile compounds. T(z) is the retention time of *n*-alkanes before and after the peak of the compound (x). z is the carbon number of n-alkanes.

$$\mathbf{w_i} = \frac{\mathbf{m_s} \times \mathbf{A_i}}{\mathbf{A_s} \times \mathbf{m_0}} \quad (3)$$

Where w_i is the concentration of volatile compounds (μg/g). m_s is the internal standard content (μg). A_i is the peak area of volatile compounds. As is the peak area of internal standard and m_0 is the weight of samples (g).

The odorous contributions of volatile compounds to attractants were evaluated by the odor activity value (OAV), which was measured as the ratio of the concentration of single compound to its detection threshold in water [12].

2.5. SDE-GC-MS analysis of volatile compounds in attractants

Attractants (25.0 g) were dissolved in water (250 mL). Dichloromethane (60 mL) was then added to the solution. They were stirred at 55 °C for 3 h in a sealed container. The extract was refrigerated for 24 h with a small amount of anhydrous sodium sulfate for dehydration. Filtrate was concentrated into 1 mL with a rotary evaporator (MLG3, Heidolph, Germany), and mixed with 20 μL 1, 2-Dichlorobenzene (100 μL/L) (internal standard). 0.2 μL mixture was then injected into GC-MS. Except for split ratio setting 10:1, other conditions were the same as used in 2.4.

2.6. Palatability, preferred food selection (PFS) and validation of key volatile compounds on attractants palatability experiment

PFS is the tendency of animals to choose a certain food first when there are two or more food choices. This experiment was performed at the School of Zoology, Shanghai Jiao Tong University. All dogs were healthy and did not receive antibiotic treatment for at least six months before the samples were collected. This experiment received ethical approval from the Animal Research Ethics Committee of Shanghai Jiao Tong University. Samples were collected according to the Animal Research Ethics Committee of Shanghai Jiao Tong University. The

Table 3. Placket-Burman design and statistical analysis of three attractants.

Protein	Source	Level		Coefficient estimate[1]	F Value	Prob > F	Significant[2]
		-1	1				
Chicken liver	Model				6.57	0.0201	**
	T (°C)	50	60	-0.80	1.23	0.31	
	S/L	3:1	1:1	1.58	4.82	0.0705	*
	E/S (%)	0.4	0.8	0.18	0.06	0.8106	
	t (h)	2	4	2.45	11.55	0.0145	**
	pH	6	7	2.81	15.19	0.008	**
L. edodes	Model				6.07	0.0242	**
	T (°C)	45	55	1.28	5.01	0.0665	*
	S/L	1:6	1:10	-0.32	0.32	0.5945	
	E/S (%)	0.2	0.6	-1.59	7.70	0.0322	**
	t (h)	2	4	2.38	17.33	0.0059	**
	pH	4.5	5.5	-0.02	0.01	0.8954	
T. molitor	Model				44.37	0.0001	**
	T (°C)	50	60	0.66	1.83	0.2249	
	S/L	1:1	1:5	-5.84	141.43	<0.0001	**
	E/S (%)	0.8	1.6	3.07	39.12	0.0008	**
	t (h)	3	5	2.26	21.07	0.0037	**
	pH	8	9	2.11	18.41	0.0051	**

[1] If the coefficient estimate was a positive number, the level of factor was positively related to DH (%). And if the coefficient estimate was a negative number, the level of factor was negatively related to DH (%).

[2] '*' stands for $P < 0.1$, and '**' stands for $P < 0.05$.

Table 4. The levels and factors of Box-Behnken design.

Protein	Code	Factor	Level		
			-1	0	1
Chicken liver [1]	A	pH	6	6.5	7
	B	S/L	3:1	2:1	1:1
	C	t (h)	2	3	4
L. edodes[2]	A	T (°C)	45	50	55
	B	E/S (%)	0.2	0.4	0.6
	C	t (h)	2	3	4
T. molitor[3]	A	t (h)	3	4	5
	B	S/L	1:1	1:3	1:5
	C	pH	8	8.5	9
	D	E/S (%)	0.8	1.2	1.6

[1] The level of other insignificant factors based on the results of single-factor experiments (Figure 5), choosing the level of highest DH, which were T = 55 °C, E/S = 0.6 %.
[2] pH = 5, E/S = 1:8.
[3] T = 55 °C.

palatability of three attractants was evaluated by two-bowl test [16]. The amounts of PFS of three attractants were evaluated by single-bowl test [16]. Finally, the key aroma substances were added to the attractants, and the two-bowl test method was used to verify the effect [1]. Food without attractants was used as a blank control. The panel of two-bowl tests consisted of 20 pet dogs of various breeds (10 Beagles, 4–5 years old, female: male = 1:1; 10 Border Collie dogs, 4–5 years old, female: male = 1:1) and similar sizes (height: 36–38cm, weight: 9–10 kg). The basic foods of dogs were used in this study. They were dry and complete foods. The basic food formula was as the following: ground whole corn, animal fat, beet pulp, ground whole wheat, meat and bone meal, vegetable oil, brewers' rice, corn, and corn gluten meal. All dogs received the same food except the three different attractants. After extrusion and sterilization of the dogs' basic food, the attractants were then sprayed onto to the food and allowed the foods to be evenly coated by attractants. The dogs received the same food without attractants before the tests for 7 days. Each two-bowl test lasted for 2 days, served 2 meals per day. The panel of single-bowl test lasted 14 days and consisted of 8 beagle dogs

Table 5. Box-Behnken design arrangement and experimental results.

Run	Chicken liver				*L. edodes*				*T. molitor*				
	A	B	C	DH (%)	A	B	C	DH (%)	A	B	C	D	DH (%)
1	-1	-1	0	46.614	-1	-1	0	18.070	-1	-1	0	0	16.754
2	1	-1	0	53.898	1	-1	0	17.093	-	-1	0	0	25.215
3	-1	1	0	48.310	-1	1	0	14.835	-1	1	0	0	1.461
4	1	1	0	54.103	1	1	0	27.363	1	1	0	0	10.115
5	-1	0	-1	44.878	-1	0	-1	4.384	0	0	0	-1	6.590
6	1	0	-1	53.508	1	0	-1	7.293	0	0	1	-1	11.667
7	-1	0	1	50.210	-1	0	1	12.228	0	0	-1	1	20.320
8	1	0	1	53.334	1	0	1	19.883	0	0	1	1	17.366
9	0	-1	-1	49.255	0	-1	-1	14.306	-1	0	0	-1	5.447
10	0	1	-1	51.492	0	1	-1	9.323	1	0	0	-1	12.843
11	0	-1	1	54.038	0	-1	1	18.073	-1	0	0	1	12.699
12	0	1	1	52.790	0	1	1	27.943	1	0	0	1	26.232
13	0	0	0	53.319	0	0	0	33.444	0	-1	-1	0	16.251
14	0	0	0	54.075	0	0	0	33.209	0	1	-1	0	1.861
15	0	0	0	52.642	0	0	0	35.183	0	-1	1	0	19.157
16	0	0	0	53.975	0	0	0	33.704	0	1	1	0	2.458
17	0	0	0	53.533	0	0	0	33.018	-1	0	-1	0	7.298
18									1	0	-1	0	18.376
19									-1	0	1	0	8.761
20									1	0	1	0	20.369
21									0	-1	0	-1	13.497
22									0	1	0	-1	1.462
23									0	-1	0	1	26.209
24									0	1	0	1	8.745
25									0	0	0	0	24.001
26									0	0	0	0	25.491
27									0	0	0	0	25.502
28									0	0	0	0	26.443
29									0	0	0	0	25.488

and 8 Border collie dogs, which were equally divided into 4 groups. Ingestion rate (IR) and first preference (FP) were recorded as the index of palatability and PFS during the test. It is notable that palatability and PFS of three attractants were tested through dog food with 5% addition of attractant. The measurement formula of ingestion rate (IR) was was shown in Eq. (4).

$$IR = (\text{consumption of dog feed weight (g)}) / (\text{total dog feed weight (g)}) * 100\%, \quad (4)$$

2.7. Statistical analysis

Duncan's multiple comparison tests at the level of 0.05 ($P < 0.05$) were applied to determine significant differences by SPSS Statistic 19. Each experiment was done in triplicates. Key volatile compounds (OAVs >1) data set was assigned as the X-variables and palatability index was designated as the Y-variables in PLSR analysis by Unscrambler 9.7 (CAMOAnalytics, Montclair, NJ, USA).

3. Results and discussion

3.1. Response surface mathematic model and ANOVA analysis of enzymatic hydrolysis process

Based on single-factor experiments, Plackett-Burman design (Table 3) with 5 factors and 2 levels were established and significant factors were screened out. After that, the levels and factors of Box-Behnken design (Table 4 and Table 5) were conducted to establish the response surface analysis (Figure 1), which exhibited obvious paraboloids with the highest point on the response surface. It means that the highest response value can be obtained through the regression equation of the model.

According to Table 5, ANOVA of response surface analysis designed by Box-Behnken of chicken liver, *L. edodes*, and *T. molitor* hydrolysis was shown in Table 6.

For chicken liver experiment, model p-value less than 0.0001 indicated that the model was significant. The A, C, AC, BC, A^2, B^2, C^2 p-value less than 0.05 indicated model terms were significant. P-value of the Lack of Fit just about 0.7416 implied the Lack of Fit was not significant relative to the pure error. Non-significant lack of fit was good for the model to fit.

For *L. edodes* experiment, model p-value less than 0.0001 implied the model was significant. The A, B, C, AB, AC, BC, A^2, B^2, C^2 p-value less than 0.05 indicated model terms were significant. Non-significant lack of fit was good for the model to fit. The $RPred^2$ of 0.986 was in reasonable agreement with the $RAdj^2$ of 0.994, indicating that less than 1 % variables could not be explained by this model.

For *T. molitor* experiment, model p-value less than 0.0001 implied the model was significant. The A, B, C, D, AD, BD, CD, A^2, B^2, C^2, D^2 p-value less than 0.05 indicated model terms were significant. P-value of Lack of Fit just about 0.2642 implied that the result can be raised by 26.42 % chance. The $RPred^2$ of 0.956 was in reasonable agreement with the $RAdj^2$ of 0.982, indicating that less than 2 % variables could not be explained by this model.

The above ANOVA results were similar with some literatures. Wang et al. [17] used response surface method to optimize enzymolysis of pine seed protein. They got the ANOVA results after regression and variance analysis. It was found that the regression model was significant ($p < 0.001$), lack of fit was insignificant, $R^2 = 94.85\%$, $R^2_{Adj} = 85.48\%$, which indicated that the model fits well with the experimental data and the

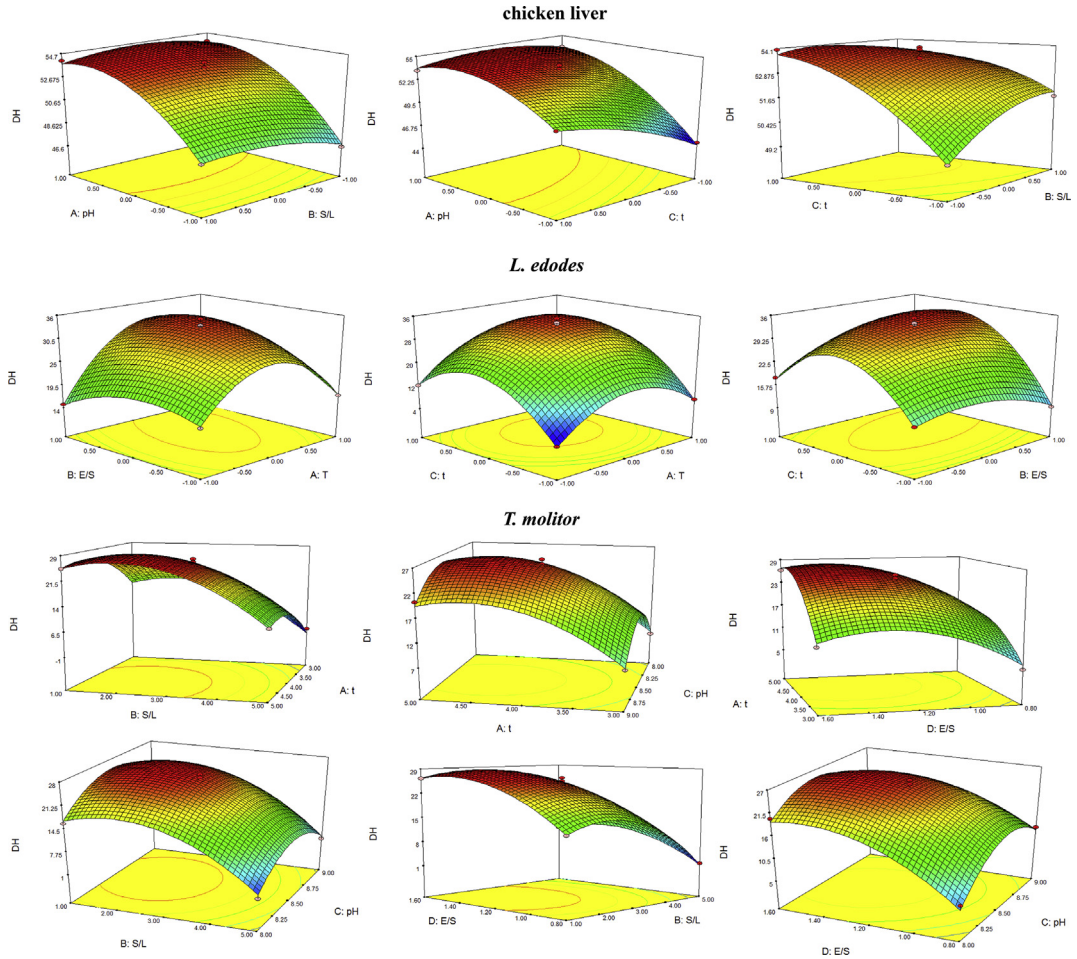

Figure 1. Response surface analysis of hydrolysis of chicken liver, *L. edodes* and *T. molitor*.

Table 6. ANOVA for response surface regression model.

Protein	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Chicken liver	Model	131.195	9	14.577	58.145	<0.0001	significant
	A-pH	77.072	1	77.072	307.423	<0.0001	
	B–S/L	1.044	1	1.044	4.165	0.0806	
	C-t	15.787	1	15.787	62.969	<0.0001	
	AB	0.555	1	0.555	2.216	0.1802	
	AC	7.577	1	7.577	30.221	0.0009	
	BC	3.036	1	3.036	12.109	0.0103	
	A^2	18.472	1	18.472	73.679	<0.0001	
	B^2	1.964	1	1.964	7.833	0.0266	
	C^2	3.656	1	3.656	14.584	0.0066	
	Residual	1.755	7	0.251			
	Lack of Fit	0.430	3	0.143	0.432	0.7416	not significant
	Pure Error	1.325	4	0.331			
	Cor Total	132.950	16				
	R^2	0.987					
	R^2_{Adj}	0.970					
	R^2_{Pred}	0.933					
	Adeq Precision	24.085 > 4					
L. edodes	Model	1688.862	9	187.651	316.884	<0.0001	significant
	A-T	61.127	1	61.127	103.225	<0.0001	
	B-E/S	17.765	1	17.765	29.999	0.0009	
	C-t	229.194	1	229.194	387.036	<0.0001	
	AB	45.596	1	45.596	76.998	<0.0001	
	AC	5.632	1	5.632	9.510	0.0177	
	BC	55.158	1	55.158	93.145	<0.0001	
	A^2	456.972	1	456.972	771.680	<0.0001	
	B^2	65.809	1	65.809	111.131	<0.0001	
	C^2	641.875	1	641.875	1083.923	<0.0001	
	Residual	4.145	7	0.592			
	Lack of Fit	1.175	3	0.392	0.528	0.6868	not significant
	Pure Error	2.970	4	0.742			
	Cor Total	1693.007	16				
	R^2	0.998					
	R^2_{Adj}	0.994					
	R^2_{Pred}	0.986					
	Adeq Precision	50.313 > 4					
T. molitor	Model	2018.567	14	144.183	113.041	<0.0001	significant
	A-t	307.336	1	307.336	240.954	<0.0001	
	B–S/L	689.763	1	689.763	540.780	<0.0001	
	C-pH	6.875	1	6.875	5.390	0.0358	
	D-E/S	300.661	1	300.661	235.721	<0.0001	
	AB	0.009	1	0.009	0.007	0.9329	
	AC	0.070	1	0.070	0.055	0.8181	
	AD	9.413	1	9.413	7.380	0.0167	
	BC	1.331	1	1.331	1.044	0.3243	
	BD	7.368	1	7.368	5.777	0.0307	
	CD	16.128	1	16.128	12.645	0.0032	
	A^2	159.432	1	159.432	124.996	<0.0001	
	B^2	390.170	1	390.170	305.896	<0.0001	
	C^2	303.886	1	303.886	238.249	<0.0001	
	D^2	180.093	1	180.093	141.194	<0.0001	
	Residual	17.857	14	1.275			
	Lack of Fit	14.873	10	1.487	1.994	0.2642	not significant
	Pure Error	2.984	4	0.746			
	Cor Total	2036.424	28				
	R^2	0.991					
	R^2_{Adj}	0.982					
	R^2_{Pred}	0.956					
	Adeq Precision	32.967 > 4					

Table 7. Compositions and OAV of odorous compounds in three different attractants.

Compounds	RI[1]	KI[2]	Content (mg/kg)						Threshold[3] (mg/kg)	OAV					
			Chicken liver		*L. edodes*		*T. molitor*			Chicken liver		*L. edodes*		*T. molitor*	
			SPME	SDE	SPME	SDE	SPME	SDE		SPME	SDE	SPME	SDE	SPME	SDE
Alcohols															
methyl mercaptan	680	675	0.036	-[4]	0.040	-	-	-	0.00024	151.42	-	165.72	-	-	-
amyl alcohol	1253	1255	-	-	Tr[5]	2.710	Tr	1.656	0.14	-	-	-	19.26	-	11.82
3-octanol	1388	1394	0.034	-	0.037	-	Tr	-	0.02	1.69	-	1.85	-	-	-
furfuryl mercaptan	1419	1430	-	16.985	-	-	Tr	18.898	0.00002	-	8.49×10^{5}	-	-	-	9.45×10^{5}
1-octen-3-ol	1457	1456	0.156	-	0.355	20.948	0.016	-	0.026	6.02	-	13.66	805.71	0.63	-
2-ethyl-1-hexanol	1473	1484	0.023	-	Tr	-	Tr	-	0.26	0.09	-	-	-	-	-
Octanol	1532	1564	0.024	-	0.013	-	Tr	-	0.037	0.65	-	0.36	-	-	-
Furfuryl alcohol	1625	1660	0.144	71.027	-	-	Tr	7.993	2	0.07	35.51	-	-	-	3.99
phenethyl alcohol	1918	1921	-	-	0.022	5.671	-	-	0.07	-	-	0.32	81.01	-	-
Esters															
dextro-bornyl acetate	1576	1582	-	-	Tr	4.877	-	-	0.44	-	-	-	11.08	-	-
gamma-butyrolactone	1640	1643	0.029	6.095	Tr	-	Tr	-	0.0008	36.63	7618.69	-	-	-	-
ethyl myristate	2089	2070	Tr	4.243	Tr	-	Tr	-	0.18	-	23.57	-	-	-	-
ethyl palmitate	2221	2250	-	4.665	-	10.402	-	-	2	-	2.33	-	5.20	-	-
Acids															
acetic acid	1455	1460	0.1528	44.671	0.031	16.348	0.047	18.898	0.025	6.11	1786.83	1.24	653.93	1.89	755.93
Formic acid	1461	1470	Tr	-	Tr	-	Tr	-	14.5	-	-	-	-	-	-
Valeric acid	1721	1734	0.014	-	-	-	-	0.864	0.001	13.85	-	-	-	-	864.41
hexanoic acid	1853	1831	0.031	3.460	0.052	8.948	0.101	36.051	0.012	2.53	288.33	4.34	745.70	8.43	3004.24
octanoic acid	2020	2039	0.039	-	0.044	-	0.067	2.695	0.037	1.06	-	1.2	-	1.82	72.84
lauric acid	2458	2493	-	-	-	43.555	-	-	0.1	-	-	0.04	435.55	0.02	-
myristic acid	2715	2716	0.011	79.365	Tr	-	-	-	10	0.001	7.94	-	-	-	-
palmitic acid	2901	2890	-	107.851	-	-	-	147.947	10	-	10.78	-	-	-	14.79
Aldehydes															
isovaleraldehyde	918	924	0.082	-	0.040	-	-	-	0.003	27.31	-	13.26	-	-	-
hexanal	1077	1083	Tr	14.243	Tr	19.226	Tr	13.429	0.03	-	474.77	-	640.86	-	447.65
(E)-2-pentenal	1149	1134	-	0.914	0.012	18.213	-	1.893	1.4	-	0.65	0.01	13.01	-	1.35
heptanal	1187	1186	-	-	-	1.013	-	0.819	0.14	-	-	-	7.24	-	5.85
3-methyl-2-butenal	1191	1206	-	14.601	-	14.393	-	14.402	0.5	-	29.2	-	28.77	-	28.8
Octanal	1295	1290	-	-	Tr	2.722	Tr	2.384	0.021	-	-	-	129.58	-	113.53
nonanal	1393	1396	0.072	2.291	0.019	2.226	0.020	1.875	0.02	3.58	114.54	0.96	111.29	0.98	93.73
furfural	1468	1476	0.253	1714.472	0.114	524.964	0.181	1337.541	0.25	1.01	6857.87	0.46	2099.87	0.72	5350.17
methional	1480	1480	Tr	31.691	-	10.639	-	2.311	0.06	0.12	528.19	-	177.31	-	38.51
benzaldehyde	1519	1530	0.295	4.801	0.395	5.897	0.611	11.667	0.61	0.48	7.87	0.65	9.67	1.01	19.13
5-methyl furfural	1549	1558	-	1.045	-	-	-	1.559	1	-	1.04	-	-	-	1.56
para-tolualdehyde	1622	1638	0.020	-	Tr	-	Tr	-	0.0012	16.57	-	-	-	-	-
phenyl acetaldehyde	1660	1650	0.053	65.81	-	-	0.015	4.864	0.007	7.60	9401.44	-	-	2.12	694.92
bread thiophene	1745	1755	0.150	14.362	Tr	3.155	0.013	7.226	0.0045	33.32	3191.56	0.79	701.07	2.87	1605.78
2-undecenal	1761	1759	-	-	-	19.516	-	-	0.1	-	-	-	195.16	-	-
2,4-decadienal	1785	1797	-	-	-	9.129	-	-	0.0001	-	-	-	9.13×10^{4}	-	-
2-dodecenal	1850	1842	-	-	-	20.019	-	-	0.0014	-	-	-	1.43×10^{4}	-	-
cinnamaldehyde	2058	2043	0.032	-	Tr	-	Tr	-	0.14	0.23	-	-	-	-	-
Ketones															
3-hexanone	1040	1050	-	7.395	-	-	-	6.056	0.06	-	123.24	-	-	-	100.94
3-penten-2-one	1119	1126	-	-	-	4.916	Tr	3.463	0.07	-	-	-	70.23	-	49.47
2-heptanone	1190	1189	-	-	-	-	Tr	-	0.045	-	-	-	-	-	-
coffee furanone	1240	1242	-	3.317	-	3.052	Tr	3.271	0.25	-	13.27	-	12.21	-	13.08
1-octen-3-one	1314	1305	0.098	-	0.043	-	-	-	0.004	24.39	-	10.66	-	-	-
3-octen-2-one	1425	1408	-	-	-	-	Tr	-	0.02	-	-	-	-	-	-
3-mercapto-2-pentanone	1350	1343	0.010	-	-	-	0.010	1.689	0.0007	8.86	-	-	-	10.11	2413.24
acetophenone	1634	1645	0.054	-	0.010	-	0.014	-	0.065	0.84	-	0.16	-	0.21	-
para-methylacetophenone	1800	1797	0.012	-	Tr	-	Tr	-	0.01	1.25	-	-	-	-	-
(E)-geranyl acetone	1846	1858	0.023	-	Tr	-	-	-	0.186	0.13	-	-	-	-	-
alpha-ionone	1861	1860	-	-	0.028	-	0.010	-	0.0016	-	-	17.61	-	4.43	-

(*continued on next page*)

Table 7 (*continued*)

Compounds	RI[1]	KI[2]	Content (mg/kg)						Threshold[3] (mg/kg)	OAV					
			Chicken liver		*L. edodes*		*T. molitor*			Chicken liver		*L. edodes*		*T. molitor*	
			SPME	SDE	SPME	SDE	SPME	SDE		SPME	SDE	SPME	SDE	SPME	SDE
beta-ionone	1931	1947	0.024	-	0.019	-	-	-	0.0012	19.81	-	16.26	-	-	-
Heterocyclics															
thiophene	1015	1021	-	-	-	14.864	-	-	0.006	-	-	-	2477.42	-	-
2-methyl thiophene	1121	1100	0.044	5.780	0.017	8.961	Tr	4.278	0.08	0.55	72.25	0.21	112.02	-	53.46
2-pentyl furan	1241	1231	0.010	0.392	Tr	-	0.018	3.017	0.27	0.04	1.45	-	-	0.07	11.17
3,4-dimethyl thiophene	1255	1250	-	-	Tr	5.903	-	-	0.008	-	-	-	737.90	-	-
fish thiol	1306	1305	-	-	-	-	0.065	-	0.00007	-	-	-	-	927.97	-
2,5-dimethyl pyrazine	1357	1348	-	-	-	-	Tr	-	0.87	-	-	-	-	-	-
2,3,5-trimethyl pyrazine	1426	1422	-	-	-	-	Tr	-	0.05	-	-	-	-	-	-
2-acetyl furan	1495	1488	-	3.472	-	-	-	2.842	10	-	0.35	-	-	-	0.28
1-furfuryl pyrrole	1829	1820	Tr	-	-	-	-	-	0.1	-	-	-	-	-	-
2-acetyl pyrrole	1989	1974	Tr	1.733	-	-	Tr	2.848	2	-	0.87	-	-	-	1.42
methyl 2-methyl-3-furyl disulfide	2105	2110	0.855	-	0.012	-	0.063	-	0.00001	8.55×10^{4}	-	1188.58	-	6319.92	-
sulfurol	2305	2302	0.474	15.157	0.094	-	0.120	37.575	0.03	15.80	505.24	3.13	-	4	1252.17
indole	2469	2453	0.017	-	-	-	-	-	0.0004	43.25	-	-	-	-	-
Phenols															
2-(methyl thio) phenol	1870	1873	-	3.911	-	-	-	-	0.8	-	4.90	-	-	-	-
phenol	1994	1989	0.037	-	Tr	-	0.013	-	0.046	0.80	-	-	-	0.29	-

[1] Retention index was calculated in agreement with literature value.
[2] Kováts indices for a HP-INNOWAX column [22].
[3] Odor thresholds were taken from Van Gemert [23].
[4] Not detected.
[5] Compositional values less than 0.01 % are noted as traces (Tr).

Table 8. Odorous compounds with an OAV >1.

Code	Compound	Code	Compound	Code	Compound
1	methyl mercaptan	17	(E)-2-pentenal	33	3-penten-2-one
2	amyl alcohol	18	heptanal	34	coffee furanone
3	3-octanol	19	3-methyl-2-butenal	35	1-octen-3-one
4	furfuryl mercaptan	20	octanal	36	3-mercapto-2-pentanone
5	1-octen-3-ol	21	nonanal	37	alpha-ionone
6	furfuryl alcohol	22	furfural	38	beta-ionone
7	phenethyl alcohol	23	methional	39	thiophene
8	dextro-bornyl acetate	24	benzaldehyde	40	2-methyl thiophene
9	gamma-butyrolactone	25	5-methyl furfural	41	2-pentyl furan
10	ethyl palmitate	26	para-tolualdehyde	42	3,4-dimethyl thiophene
11	acetic acid	27	phenyl acetaldehyde	43	fish thiol
12	hexanoic acid	28	bread thiophene	44	sulfurol
13	octanoic acid	29	2-undecenal	45	methyl 2-methyl-3-furyl disulfide
14	palmitic acid	30	2,4-decadienal	46	indole
15	isovaleraldehyde	31	2-dodecenal	47	2-(methyl thio) phenol
16	hexanal	32	3-hexanone		

model can be used to analysis and predict the results of enzymolysis of pine kernel protein. Pan et al. [18] optimized the enzymolysis of the lotus seed protein by response surface method. According to ANOVA, the model was highly significant with a p-value ($p < 0.0001$) to predict the response values. T, TE, and E^2 were significant term ($p < 0.05$). The predicated R^2 and adjusted R^2 were both close to 1, which indicated the adequacy of the model. Adequate precision value was found to be 38.878 (greater than 4), revealing that the model was sufficient for discrimination. The non-significant value of lack of fit ($p > 0.05$) also showed that the quadratic model was valid for the present study. Value of the coefficient of variation (4.61%) demonstrated a good precision and reliability of the experiments.

3.2. Odorous compounds in three kinds of attractants

There were 66 odorous compounds detected from attractants of chicken liver, *L. edodes*, and *T. molitor* attractant (Table 7), and 47 flavor compounds had OAV more than 1 (Table 8). The OAVs of methyl mercaptan, furfuryl mercaptan, acetic acid, hexanoic acid, hexanal, octanal, nonanal, furfural, methional, bread thiophene, methyl 2-methyl-3-furyl disulfide, and sulfurol were larger than 100, indicating they were the main aroma components of attractants. The majority of these compounds contained sulfur and nitrogen with low threshold, and they are commonly used in meat flavor. The content of sulfur compounds in chicken liver attractant was higher than those of other two. In a recent

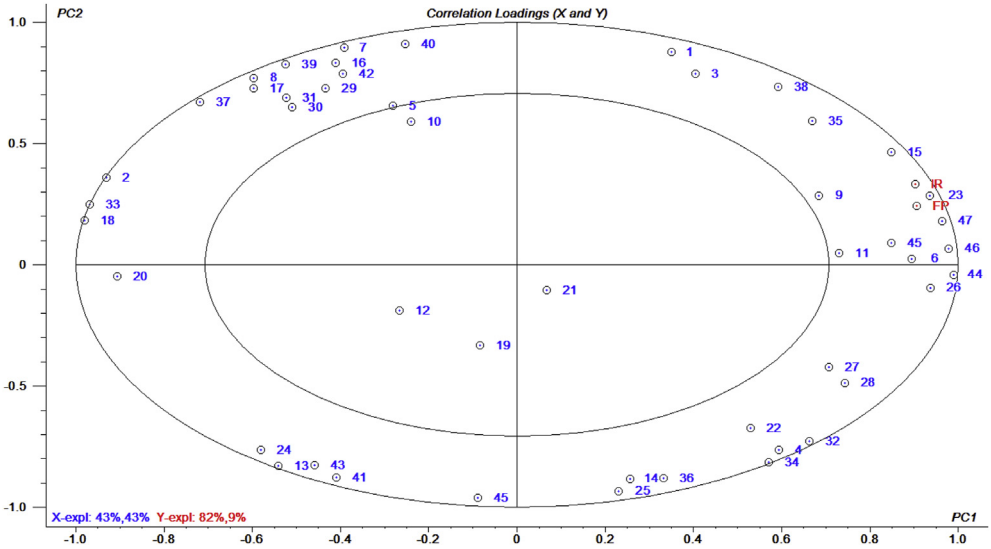

Figure 2. An overview of the variation found in the mean data from the partial least squares regression (PLSR) correlation loading plot. The model was derived from odorous compounds (OAV >1) as the X-axis and palatability index (ingestion rate and first preference) as the Y-axis. The concentric circles represent $r^2 = 0.5$ and $r^2 = 1.0$, respectively.

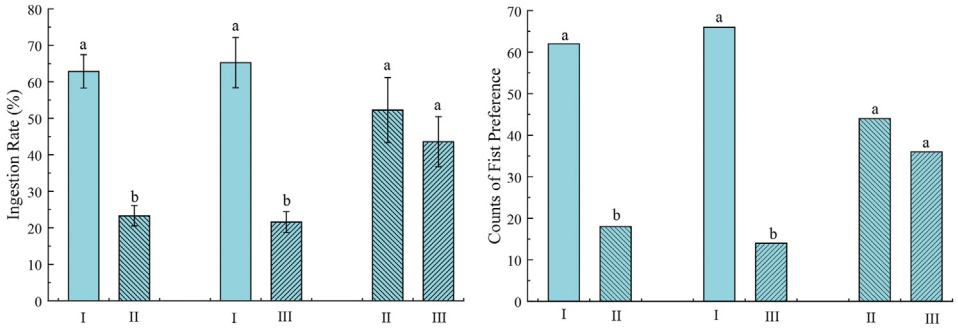

Figure 3. Significant difference in ingestion rate and first preference between three kinds of attractants ($P < 0.05$). *I means* feed added chicken liver attractant; II means feed added *L. edodes* attractant; III means feed added *T. molitor* attractant.

Table 9. Ingestion rate of four types of dog food for 14 days.

Sample	I[1] IR (%)	II	III	IV
Day 1	96.62 ± 1.97% aA[2]	93.96 ± 1.25% aB	93.50 ± 2.35% aB	90.50 ± 0.91% aB
Day 2	96.05 ± 3.19% abA	91.38 ± 1.11% abB	92.88 ± 2.29% aAB	90.38 ± 0.48% aB
Day 3	86.12 ± 12.12% abA	87.88 ± 4.39% abcA	92.63 ± 2.56% aA	88.88 ± 0.85% aA
Day 4	83.33 ± 9.54% abA	83.63 ± 5.78% abcA	87.50 ± 7.90% abcA	75.25 ± 0.65% bA
Day 5	82.62 ± 14.77% abA	82.25 ± 5.39% abcA	88.88 ± 3.01% abA	73.00 ± 1.08% bA
Day 6	82.00 ± 7.82% abA	81.63 ± 7.86% abcA	80.88 ± 10.62% bcA	72.38 ± 1.93% bA
Day 7	80.37 ± 7.69% abA	83.38 ± 5.12% abcA	78.88 ± 7.18% bcA	71.63 ± 1.38% bA
Day 8	81.96 ± 5.99% abA	83.75 ± 6.81% abcA	78.25 ± 6.84% bcA	75.25 ± 4.13% bA
Day 9	79.75 ± 3.48% abAB	84.63 ± 6.38% abcA	78.25 ± 7.10% bcAB	70.63 ± 5.15% bB
Day 10	78.63 ± 1.89% abA	80.50 ± 8.84% abcA	77.75 ± 5.45% bcA	70.88 ± 1.93% bA
Day 11	78.75 ± 1.76% abA	80.75 ± 6.81% abcA	77.38 ± 5.22% bcA	70.38 ± 3.30% bB
Day 12	78.75 ± 1.32% abA	78.63 ± 5.47% bcA	75.25 ± 4.57% cA	73.88 ± 1.44% bA
Day 13	77.96 ± 1.06% bA	77.63 ± 4.99% cA	75.38 ± 3.09% cAB	70.88 ± 1.65% bB
Day 14	78.75 ± 1.10% abA	77.00 ± 4.38% cA	75.25 ± 2.72% cAB	70.88 ± 1.55% bB

[1] I means feed added chicken liver attractant; II means feed added *L. edodes* attractant; III means feed added *T. molitor* attractant; IV means feed without attractant.
[2] Means not sharing a common lower case letter in a column are significantly different at $P < 0.05$. Means not sharing a common upper case letter in a row are significantly different at $P < 0.05$.

study, Chen et al. [19] investigated the volatile compounds of chicken liver Maillard reaction product. They found that aldehydes, especially furfural, nonanal, 3-methyl-butanal, benzaldehyde, were the main volatile compounds in the chicken liver Maillard reaction products. Meantime, they reported heterocyclic compound such as furfural contributed toasted, nutty, sweet, and caramel-like aroma. They used xylose as a reduced sugar might cause the volatile compounds difference from this study. The most characteristic volatile compound in *L. edodes* attractant was 1-octen-3-ol with a mushroom flavor. In Li et al. [20] literature, they also found that 1-octen-3-ol was a typical flavor component in *L. edodes*. The content of pyrazine in the attractant of *T. molitor* was relatively high and makes the roast note of the attractant. In Seo's study, they reported that pyrazines, pyrrolidines and carbonyls increased or appeared in roasted and fried mealworms (*Tenebrio molitor Larvae*) [21].

3.3. Correlation between odorous compounds and palatability

The correlation of PLSR was analyzed by taking OAV of odorous compounds (Table 8) as X variable and palatability index (IR and FP) as Y variable (Figure 2). The derived PLSR model showed that PC1 and PC2 explained 86 % and 91 % respectively, which demonstrated that optimal number of components in model was determined by two principal components (PC). The variance contribution rates of two ellipses in the model were $r^2 = 0.5$ (medial ellipse) and $r^2 = 1$ (lateral ellipse). The variables dispersed between two ellipses indicated that they contributed more to flavor, while the variables in medial ellipse indicated that contribution of flavor was less than 50%. The two Y variables were very close, indicating that the effects of IR and FP are similar, which accurately reflected the results of palatability experiments. Chen et al. [24] also used such a method to find the correlation between volatile compounds and palatability value of seven dog food attractants.

The X variables around the palatability index indicated that volatile compounds were highly correlated with palatability. Among them, methyl 2-methyl-3-furyl disulfide (45) and 2-(methyl thio) phenol (47) has strong meat and garlic flavor, which commonly exists in chicken flavor. Methional (23) has the typical flavor of baked potatoes, which can contribute attractant the sense of roasted. Although indole (46) has an unpleasant odor of fecal, its combination with some sulfur-containing substances at low concentrations can enhance the authenticity of chicken flavor. Both ϒ-butyrolactone (9) and isovaleraldehyde (15) have favorable fat flavor, which could be combined with other aldehydes and esters to form a round fat flavor. Acetic acid (11) has a pungent acidity, which satisfies dog's preference for sour taste, and its aroma is more penetrating than other short chain fatty acids. In Chen's study, they reported that 23 aroma compounds such as hexanoic acid, acetaldehyde, heptanone, butyl hexanoate, heptyl formate, methyl pyrazine, 2,5-dimethyl pyrazine, 2-heptanone, pentanal, ethyl decanoate, heptanal, octanal, pentanol, acetone, ethyl caprylate, 3-methyl butanal, anisole, 2-ethyl hexanol, 2-pentyl furan, 2,3-butanediol, benzaldehyde, ethyl vanillin, and vanillin were related to the palatability of dry dog foods [24]. Three aroma compounds (benzaldehyde, vanillin, and 2,5-dimethyl pyrazine) were selected and added to dry dog food to validate the PLSR results [24]. These results are mostly inconsistent with our findings. The reason might be related to the great difference of the technical conditions of Maillard product reaction. Furfuryl alcohol (6) has a typical caramel odor, which exists in Maillard reaction products. This caramel-flavored compound combined with sulfur compounds can bring roasted meat flavor. In Chen's finding, they also reported that furfuryl alcohol was detected in all samples and was known to play an important role in the flavor of dry dog foods [24]. Chicken liver attractant is better than both *L. edodes* attractant and *T. molitor* attractant in terms of palatability. It is proved that attractant with combined flavor of meat, roast, fat, caramel, and sour taste can effectively improve the palatability of dog food, which meets dogs' feeding preference [25].

3.4. Palatability and PFS of three attractants

Two-bowl tests were used to evaluate palatability through recording ingestion rate (IR) and first preference (FP). As shown in Figure 3, the IR and FP of food with chicken liver attractant (I) were significantly higher than those of food with *L. edodes* attractant (II) or *T. molitor* attractant (III) ($P < 0.05$). Compared with II or III, the IR of I reached 62.85 %. While corresponding IR of II or III was less than 25 %. Chen et al. reported that the intake ratios (IR) of the seven basal dry dog foods ranged from 43.8 to 74.6, which indicated that dogs consumed most of samples and had a good acceptance towards these attractants [24]. These findings were similar with ours results. Moreover, the FP of I accounted for more than 75 % in 80 cases, indicating that the experimental dogs had better acceptance and preference for the flavor of chicken liver attractant. Although IR and FP of II were slightly higher than those of III, no significant difference was observed. It highlighted that the least preference of the experimental dogs to *T. molitor* attractant. The palatability test verified that chicken liver was the preferred protein source to prepare dog food attractant.

The IR of preferred food as index of three attractants was evaluated by a 14-day single-bowl test (Table 9). The results showed that IR declined for all four foods. As shown in Figure 4, the IR of I, II, and III fluctuated greatly in some specific days, but without significant difference. In summary, the IR of dog food added with attractant showed a similar trend, and was higher than that of food without attractant.

3.5. Validation of key volatile compounds on attractants palatability

It's widely known that the overall flavor of attractant is hard to be changed greatly via a single compound. The key odorous compounds should be formulated into flavor accordingly based on GC-MS determination ratio. Then the compensated flavor was added to *L. edodes* attractant and *T. molitor* attractant at 0.1 % concentration respectively. A two-bowl test was implemented to compare the palatability of non-flavored chicken liver attractant (A) with that of flavored *L. edodes* attractant (B/f) and flavored *T. molitor* attractant (C/f) (Table 10).

There was no significant difference ($p > 0.05$) in IR and FP between B/f and C/f and A. The IR of B/f or C/f was not distinguished from that of A, which was about 49 %. Compared with the results in Figure 5, IR of B/f and C/f increased by 26 and 27% respectively, whereas that of A decreased by 14 %. In addition, FP of B/f and C/f accounted for 47 % of the total experimental times, which increased by 22 % compared with results shown in Figure 3. In Chen's study, they added the key aroma

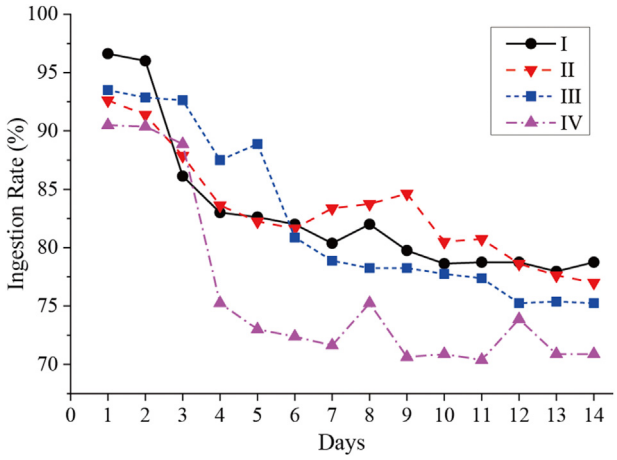

Figure 4. Variation trend of ingestion rate of four kinds of dog feed. *I means* feed added chicken liver attractant; II means feed added *L. edodes* attractant; III means feed added *T. molitor* attractant; IV means feed without attractant.

Table 10. Validation of key volatile compounds on attractants palatability.

Attractant		IR (%)	FP
A[1]		49.23 ± 4.32 a	42 a
B/f[2]		48.67 ± 5.22 a	38 a
A		50.89 ± 5.67 a	43 a
C/f[3]		49.25 ± 3.33 a	37 a
Code	**Compound**	**Content (%)**	
		Bb applied	**Cc applied**
6	furfuryl alcohol	2.30	2.30
9	gamma-butyrolactone	0.34	0.33
11	acetic acid	1.98	1.81
15	isovaleraldehyde	0.67	1.30
23	methional	0.15	0.15
45	methyl 2-methyl-3-furyl disulfide 10 %	1.38	1.33
46	indole	0.26	0.27
47	2-(methyl thio) phenol	0.55	0.57
	Salad oil	99.45	99.43

[1] represented non-flavored chicken liver attractant.
[2] represented flavored *L. edodes* attractant.
[3] represented flavored *T. molitor* attractant.

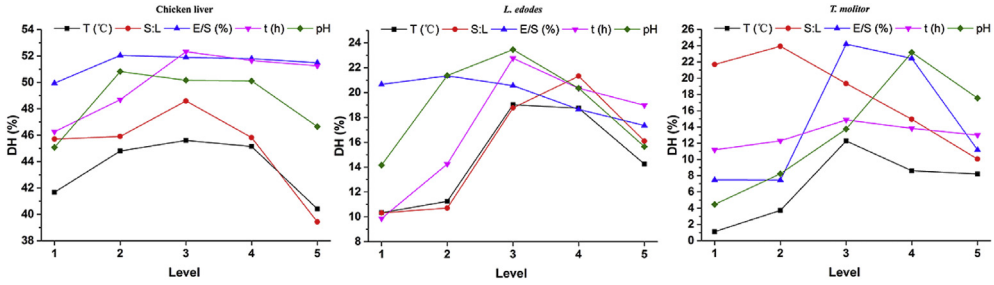

Figure 5. Effect of different factors on enzymatic hydrolysis of chicken liver, *L. edodes* and *T. molitor* (level setting refers to Table 1).

compounds to dry dog food to validate the PLSR results [24]. The addition of those compounds positively impacted on the flavour of the dry dog foods and their presence significantly increased the palatability of the all the samples [24]. This methodology was also used in our study. Therefore, animal experiments can be used to verify the practical effect of 8 key volatile compounds on attractant palatability. The characteristic flavor of chicken liver attractant with significant palatability was imitated by compensating with the addition of key odorous compounds, which effectively compensated for the insufficient key flavor of non-meat protein source, such as *L. edodes* and *T. molitor*, in the preparation of meat-flavor attractant.

4. Conclusion

In summary, the disadvantage of *L. edodes* or *T. molitor* attractant palatability compared to chicken liver attractant could gradually be overcome by addition of key odorous compounds.

The key flavor of attractant were methyl mercaptan, furfuryl mercaptan, acetic acid, hexanoic acid, hexanal, octanal, nonanal, furfural, methional, bread thiophene, methyl 2-methyl-3-furyl disulfide, and sulfurol. The key volatile compounds affecting palatability were 2-methyl-3-furyl disulfide, indole, methional, 2-(methyl thio) phenol, gamma-butyrolactone, furfuryl alcohol, acetic acid, and isovaleraldehyde, which could improve palatability of *L. edodes* and *T. molitor* attractant.

Declarations

Author contribution statement

Tao Feng: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zhongshan Hu, Yanzun Tong: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Lingyun Yao, Jun Lu: Conceived and designed the experiments; Wrote the paper.

Haining Zhuang, Xiao Zhu: Performed the experiments.

Shiqing Song: Performed the experiments; Analyzed and interpreted the data.

Funding statement

This work was supported by the National Natural Science Foundation of China (31771942), Natural Science Foundation of Shanghai (17ZR1429600), and Shanghai Local Capacity Building Projects (16090503800).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] G. Aldrich, K. Koppel, Pet food palatability evaluation: a review of standard assay techniques and interpretation of results with a primary focus on limitations, Animals 5 (1) (2015) 43–55.

[2] C. Tobie, F. Péron, C. Larose, Assessing food preferences in dogs and cats: a review of the current methods, Animals 5 (1) (2015) 126–137.

[3] M. Fournier, Method for Preparing Wet Pet Food Products Having an Improved Appeal to Pet Owners and at Least a Maintained Palatability to Pets, 2016. WO/2016/001323.

[4] T.K. Chen, N.B. Trivedi, Animal Food Palatability Enhancer and Method of Use and Manufacture Thereof: US, 2005. US 20050276881 A1.

[5] A.V. Suresh, K.P. Vasagam, S. Nates, et al., Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, Litopenaeusstylirostris fed diets formulated with high levels of poultry byproduct meal, Aquaculture 319 (1) (2011) 132–140.

[6] V. Nair, Fermented Soy Nutritional Supplements Including Mushroom Components: US, 2011. US20150216918A1.

[7] X. Tan, H. Chen, R. Huang, E. Hao, N. Zhang, S. Jia, Research progress of the insect protein feed development, Feed Rev. 3 (2015) 32–34.

[8] K. Martin, S.J.M. Krammer-Lukas, Feed Composition for Companion Animals, 2011. US Patent, US20110052751 A1, https://patents.google.com/patent/US20110052751A1/en.

[9] J. Yi, X. Huang, F. Yang, Q. Nie, J. Dai, B. Li, J. Hu, A review on the application and research progress of feed attractant in pet, Feed Industry 37 (2016) 61–64.

[10] R.T. Hussain, M.K. Ebraheem, H.M. Moker, Assessment of heavy metals (Cd, Pb and Zn) contentsin livers of chicken available in the local marketsof Basrah city, Iraq, Basrah J. Vet. Res. 1 (11) (2012) 43–51.

[11] P. Zhuang, H. Zou, W. Shu, Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: field study, J. Environ. Sci. 21 (6) (2009) 849–853.

[12] M. Shui, T. Feng, Y. Tong, H. Zhuang, C. Lo, H. Sun, L. Chen, S. Song, Characterization of key aroma compounds and construction of flavor base module of Chinese sweet oranges, Molecules 24 (13) (2019) 2384–2396.

[13] I.V. Nikolaev, S. Sforza, F.D. Lambertini, I. Yu, V.P. Khotchenkov, V.G. Volik, A. Dossena, V.O. Popov, O.V. Koroleva, Biocatalytic conversion of poultry processing leftovers: optimization of hydrolytic conditions and peptide hydrolysate characterization, Food Chem. 197 (2016) 611–621.

[14] S.N. Lotfy, H.H.M. Fadel, A.H. El-Ghorab, M.S. Shaheen, Stability of encapsulated beef-like flavourings prepared from enzymatically hydrolysed mushroom proteins with other precursors under conventional and microwave heating, Food Chem. 187 (2015) 7–13.

[15] W.J. Lu, T. Han, H.Y. Zhang, H.W. Li, Y. Lv, On enzymatic hydrolysis of protein in *Tenebrio molitor* with trypsin, J. Beijing Univ. Agri. 27 (2012) 77–80.

[16] J. Ramírez, G. Gilardoni, M. Jácome, J. Montesinos, M. Rodolfi, M.L. Guglielminetti, C. Cagliero, C. Bicchi, G. Vidari, Chemical composition, enantiomeric analysis, AEDA sensorial evaluation and antifungal activity of the essential oil from the Ecuadorian plant LepechiniamuticaBenth (Lamiaceae), Chem. Biodivers. 14 (12) (2017) 1–11.

[17] S.N. Wang, L.Z. Jiang, Y. Li, D.D. Li, X.N. Sui, Optimization on aqueous enzymatic extraction conditions of pine seed protein by response surface method, Proc. Eng. 15 (2011) 4956–4966.

[18] A.D. Pan, H.Y. Zeng, G.B.F.C. Alain, B. Feng, Heat-pretreatment and enzymolysis behavior of the lotus seed protein, Food Chem. 201 (2016) 230–236.

[19] X. Chen, Y. Zou, D.Y. Wang, G.Y. Xiong, W.M. Xu, Effects of ultrasound pretreatment on the extent of Maillard reaction and the structure, taste and volatile compounds of chicken liver protein, Food Chem. 331 (2020) 127369.

[20] B. Li, C.Y. Liu, D.L. Fang, B. Yuan, Q.H. Hu, L.Y. Zhao, Effect of boiling time on the contents of flavor and taste in Lentinus edodes, Flavour Fragrance J. 34 (2019) 506–513.

[21] H. Seo, H.R. Kim, I.H. Cho, Aroma characteristics of raw and cooked Tenebrio molitor larvae (mealworms), Food Sci. Animal Res. 40 (4) (2020) 649–658.

[22] T. Tsukahara, N. Matsukawa, S. Tomonaga, R. Inoue, K. Ushida, K. Ochiai, High-sensitivity detection of short-chain fatty acids in porcine ileal, cecal, portal and abdominal blood by gas chromatography-mass spectrometry, Anim. Sci. J. 85 (2014) 494–498.

[23] E.S. Kovats, Gas chromatographic characterization of organic substances in the retention index system, Adv. Chromatogr. 1 (1965) 229–247.

[24] M.S. Chen, X.M. Chen, J. Nsor-Atindana, K.G. Masamba, J.G. Ma, F. Zhong, Optimization of key aroma compounds for dog food attractants, Anim. Feed Sci. Technol. 225 (2017) 173–181.

[25] M.J. Hopkins, G.T. Macfarlane, Changes in predominant bacterial populations in human faeces with age and with Clostridium difficile infection, J. Med. Microbiol. 51 (2002) 448–454.