Open Access

SCHOLARENA

Journal of Food Technology and Food Chemistry

RESEARCH ARTICLE

Unraveling the Function of Recombinant Lipase and the Relationship to Pork Flavor

Xin Meng[1,2], Xiaolei Yu[1], Mo Xian[2], Yujin Cao[2], Jing Sun[1], Shuang Yang[1] and Zhen Zhang[1*]

[1]College of Food and Health, Jinzhou Medical University, Jinzhou, 121000, China
[2]CAS Key Laboratory of Bio-based Materials, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, 266101, China

[*]**Corresponding author:** Zhang Zhen, College of Food and Health, Jinzhou Medical University, Jinzhou 121000, China; Fax: 86-0416-4672179. E-mail address: woxing1981@163.com

Citation: Xin Meng, Xiaolei Yu, Mo Xian, Yujin Cao, Jing Sun, et al. (2022) Unraveling the Function of Recombinant Lipase and the Relationship to Pork Flavor. J Food Technol Food Chem 4: 103

Abstract

In order to improve pork flavor and explain the mechanism of endogenous lipase, the LPL gene was cloned and expressed in *E.coli*. The recombinant lipase and the endogenous pork lipase were added in pork respectively to determine the lipid hydrolysis and oxidation index. Meanwhile, the volatile flavor compounds were identified by electronic nose combined headspace solid-phase microextraction (HS-SPME-MS) and gas chromatography-mass spectrometry (GC-MS). Compared to the blank group without adding lipase, the other two groups had more positive effect on lipid hydrolysis and oxidation. A total of 36 kinds of volatile flavor components were detected, including hydrocarbons, heterocyclic and aromatic compounds, organic acids and esterss, especially the relative content of aldehydes increased significantly ($p<0.05$). In addition, it was further indicated that the recombinant enzyme could effectively improve the original flavor of pork and was better than endogenous enzyme.

Keywords: Meat Flavor; GC-MS; Lipase; Recombinant Strain; E-Nose

Introduction

Flavor is one of the most important sensory attributes for consumers to judge the quality and acceptability of meat and meat products together with texture, nutrition and safety, which has become a decisive factor affecting people's choice [1]. The increasing public concern about the safety of food additives has prompted the study of biological methods for the synthesis of aromatic substances. This presents new challenges for food manufacturers and technology researchers (Xu et al. 2021). With the development of food biotechnology and the improvement of consumers' demand for meat quality, meat flavor has become a research hotspot in the field of animal products processing. However, due to various influencing factors and the mechanism of meat flavor formation, the application of high quality meat products was severely restricted in the field of animal processing in China.

As a high efficiency biocatalyst, enzymes were more and more widely used in food processing than traditional chemical agents with their unique advantages[2]. Under the catalysis of enzymes, macromolecules in food can produce specific flavor substances, such as flavor precursors, proteins and fats. Enzymes that can affect the food flavor are called food flavor enzyme. As early as in 1956, Hewitt first proposed the concept of flavor enzymes [3] and studied the role of enzymes in the formation of flavor in fruit and vegetables [4]. Recently, some reports have indicated that many biochemical reactions take place in meat and meat products after adding flavor enzymes (Xu et al. 2021), which are largely responsible for the flavor characteristics of meat. Among them, endogenous lipase plays an important role in meat flavor formation during meat processing and storage [5, 6] (Waller and Feather. 1981)

On the premise of ensuring nutrition and hygiene, a food can be favored by consumers in the market depends on whether the food has certain flavor characteristics. Meat flavor is one of the most studied food for researchers because it is preferred by consumers due to its quality, as well as its delicious taste and special aroma. There are two main ways to form flavor substances in meat, one is the biosynthesis catalyzed directly or indirectly by enzymes [3], the other is produced by non-enzymatic chemical reaction through Maillard reaction [6,7]. The water-soluble components in meat, such as reducing sugars and amino acids, can induce the Maillard reaction during the thermal processing [6]. Meanwhile, the oxidation and degradation of lipids leads to the formation of meat flavor [8]. Many flavoring substances have a unique optical conformation, which is difficult to be synthesized by chemical methods. Enzyme stereoselectivity can solve this problem. Compared with chemical synthesis, enzymatic method has great advantages to synthesize flavor compounds similar to spices due to its relatively mild conditions, and it will play an increasingly important role in meat processing, meat tenderization, meat preservation, meat quality, and the utilization rate of raw materials.

In the food industry, fatty acids with short chain can be released after lipase reaction, which can increase and improve the flavor and fragrance of meat [1] (Xu et al. 2021). Meat flavor is affected by many factors, including physical, chemical and biological factors. Among them, volatile flavor substances contribute most to meat flavor. Interestingly, our previous study reported that pork endogenous lipase can effectively improve meat flavor as some paper showed [4]. However, the research on the functions of enzymes and proteins related to flavor formation of meat products is still in the exploratory stage. Therefore, in order to investigate the function of enzyme and the optimum addition amount, the gene of pork endogenous lipase was cloned and expressed in *E.coli*. It was expected that the outcomes of this study would provide a comprehensive understanding of the effects of lipase on meat flavor.

Materials and Methods

Materials and chemicals

Pork was purchased from New Mart supermarket (Jinzhou, China). The competent cells of *Escherichia coli* BL21 (DE3) strain and high copy number vector pET30a (+) were stored in our laboratory. All of chemicals used in this experiment were supplied by Shenggong (Shanghai, China), which were analytically pure (95%).

Preparation of endogenous lipase from pork

The endogenous pork lipase solution was extracted from pork (fatty meat, 50g) by the two-phase aqueous method, which was simply modified by referring to the extraction method in literature [9]. The extraction condition was as follows: PEG2000 mass concentration was 32%, $(NH_4)_2SO_4$ mass concentration was 32%, and pH value was 6.5.

Bacterial strains and growth conditions

Escherichia coli BL21 (DE3) was used as a general expression strain and was propagated on LB media (1% tryptone, 0.5% yeast extract, and 1% NaCl) with adding kanamycin (50 μg/ml) as antibiotics.

RNA extraction and cDNA synthesis

Total RNA was isolated from 50 mg pork tissue using Uniq-10 column Trizol total RNA extraction kit (Shenggong, Shanghai, China) after treated with homogenizer [9]. The quantity and quality of the isolated total RNA were determined by using spectrophotometry and gel electrophoresis, respectively. First strand cDNA was synthesized from the total RNA using PrimeScript RT-PCR Kit (TaKaRa, Dalian, China) according to the manufacturer's procedures.

Construction of plasmids and recombinant strains

The LPL gene was amplified using cDNA as template with primers P1/P2, and then cloned into pET30a vector (Novagen, Madison, WI) between *EcoR* I and *Hind* III sites to create the recombinant plasmid of pET-LPL. The two pairs of primers used in RT-PCR are as follows:

P1:5'-CCGGAATTCAGGAACGCGTCCCCAGAT-3';
P2: 5'- CCCAAGCTTGCTCAGTTTCAGCCAGAC-3'.

The RT-PCR reaction was carried out as following: 94 °C for 5 min (min), followed by 35 cycles of 94 °C for 50 s (s), 60 °C for 50 s, 72 °C for 2 min, and 72 °C for 7 min. The PCR product of LPL was purified with Gel Extraction Kit (Fermentas, Hanover, MD).

The recombinant plasmid was identified by PCR reaction and enzyme digestion. Plasmids pET-LPL was transformed into *E. coli* BL21 (DE3) competent cell to construct recombinant strain of YM1. The ORF of LPL was determined by online ORF finder software (https://www.ncbi.nlm.nih.gov/orffinder/). A homology search was conducted based on a BlastX search using the NCBI Blast server. The obtained colonies were transferred into LB medium and grown at 37 °C for OD600 about 0.5, and then it was induced by IPTG (final concentration was 0.4mmol/L) for 12 hours at 28 °C. The cells were harvested, washed with distilled water three times and then dried by lyophilization for lipid analysis.

Pork sample preparation

In this experiment, three groups of pork samples were processed, one group was soaked with endogenous pork lipase (control group); the other group was soaked with recombinant pork lipase (treatment group). Meanwhile, there was another group without treating with pork lipase (blank group). Recombinant pork lipase enzyme solution and endogenous pork lipase solution (50mL) were all mixed and soaked with 50g pork at room temperature for 30min, respectively, which were as the treatment group and the control group. The enzyme solution of the blank group was replaced by double steamed water, which was separately boiled in 300 mL of boiling water for 3 min for later use.

Degree of lipid hydrolysis oxidation

The acid value and peroxide value were determined and calculated according to the method reported by Li et al [10]. The method of thiobarbituric acid reactive substances (TBARS) was applied to detect lipid peroxidation levels, which was determined with the reference to Nanjing Jiancheng Malondialdehyde (MDA) assay kit. The TBARS values were calculated as milligrams (mg) of MDA/kilogram (kg) of pork lipid. Meanwhile, the value of carbonyl and diene were analysised by the method of Jin [2].

Sensory Evaluation

All the samples were boiled for 5min without adding any salt or spices before testing and presented to 20 assessors with random codes. The sensory properties of pork were evaluated according to the method [11], especially the appearance, taste and flavor. Each assessor individually evaluated the pork on a 5-point descriptive scale.

Electronic nose (e-nose, PEN3) detection

In order to evaluate the effect of recombinant lipase on meat flavor, each of the three group samples were cut into a beaker and detected immediately by e-nose (PEN3). The acquisition time of electronic nose signal was 50 s, while the cleaning time was 120 s. Each sample was repeated by 3 times. Table 1 described the performance of PEN3 portable electronic nose sensor.

Serial number	Sensor	Sensor symbol	Characteristics	Sensitivematerial and threshold value（mL/cm^{-3}）
1	W1C	R(1)	Aroma components	Methylbenzene, 10
2	W5S	R(2)	Nitrogen-oxy sensitive	Nitrogen dioxide, 1
3	W3C	R(3)	Ammonium hydroxide and Aroma components sensitive	Benzene, 10
4	W6S	R(4)	Hydrogen sensitive	Hydrogen, 100
5	W5C	R(5)	Alkane and Aroma components sensitive	Propane, 1
6	W1S	R(6)	Methane sensitive	Methane, 100
7	W1W	R(7)	Sulfide sensitive	Hydrothion, 1
8	W2S	R(8)	Ethanol sensitive	Carbon monoxide, 100
9	W2W	R(9)	Aroma components and Sulfide sensitive	Hydrothion, 1
10	W3S	R(10)	Alkane sensitive	Methane, 10

Table 1: Properties of sensor on PEN3 electronic nose

Volatile compounds analysis

Pork sample (5 g) was ground by a mincer and weighed into a 20 mL headspace vial and 3 mL saturated sodium chloride solution was added. In order to balance the sample, the product was added to the magnetic stirrer and incubated at 45 °C for 10 min. Then all the volatile compounds were analysed by solid phase micro extraction (SPME, sigma) with a 85 μm Carboxen/Polydimethylsiloxane (CAR/PDMS) fibre. The vial was incubated at 270 °C for 60 min and volatile compounds were extracted by exposing the fibre to the headspace for 30 min at 37 °C.

After that, the SPME arrows were directly transferred and desorbed in the injection port of the GC for 5 min on splitless mode for detection according to the previous method (Xu et al. 2021). Agilent 7890A GC with a mass spectrometer detector MS 5975C was used for GC-MS analysis, which was equipped with a split/splitless injector and with a HP-5MS (30 m × 0.25 mm × 0.25 μm) fused silica column from Sigma. High purith helium (99.99%) was used as carrier gas at a constant flow rate of 1 mL min^{-1}. The initial oven temperature was maintained at 57 °C and held for 3 min, then increased to 100 °C at 3 °C min^{-1} and finally programmed to rise to 260 °C at 5 °C min^{-1} for 10 min. The shunt ratio is 5:1. The mass spectrometer working conditions were as follows: the interface

temperature of chromatography-mass spectrum was 280 °C; the electron ionization (EI) was 70 eV; the ion source temperature was 230 °C and the four-stage bar temperature was 150 °C. Full scan mode was used for MS detection, at a massrange of 30-550 m/z. Three replicates were performed.

Statistical analysis

All experiments were performed in triplicate. Analyses of variance (ANOVA) were performed by SPSS software (version 19.0; SPSS). A value of p <0.05 was considered statistically significant. Principal component analysis (PCA) was done to evaluate the relationship between pork lipase and meat flavor. The PCA analysis data of electronic nose detection results of was from 47-49s data information after stability, using the software Winmuster for data analysis. GC-MS data analysis and the qualitative analysis of volatile components in the sample were all carried out according to the computer spectrum database (NIST11/Wiley7.0), and the retention time of C8-C20 n-alkanes was used to calculate the retention index of each chromatographic peak, so as to confirm the chemical composition of volatile substances. The peak area normalization method was used for the quantitative analysis of volatile components.

Results and Discussion

Isolation of the LPL gene from pork and expression in E. coli

With the aim to improve the meat flavor, the native LPL gene was isolated from pork by RT-PCR and cloned into plasmid pET30. The recombinant plasmid pET-LPL was over-expressed in *E. coli* BL21 (DE3). The bioinformatic analysis results showed that LPL shared homology with LPL from other kinds of pork and had as 99.18% identity similarity with LPL (NM_214286.1) from *Sus scrofa*.

The physicochemical properties of pET-LPL recombinant protein were predicted through the online website: the recombinant protein encodes 478 amino acids, the molecular weight was predicted to be 53.58kD, the isoelectric point was 8.72, the total number of atoms was 7,507, the atomic composition was $C_{2395}H_{3732}N_{658}O_{704}S_{18}$, and the instability index was 39.45 indicated that recombinant protein belong to stable protein. The aliphatic index was 80.13 and the Grand average-Hydropathicity (GRAVY) was -0.328 showed that recombinant protein was hydrophilic. After purification, the recombinant protein concentration was 189 μg/mL and the cloned enzyme activity was 0.932 U/mL.

Relationship between recombinant protein and attributes of pork flavor

Our previous studies had shown that pork endogenous lipase can effectively improve meat flavor. Therefore, in order to get high quality flavor meat and explain the relationship between lipase and meat flavor, pET-LPL gene was expressed in *E. coli* to obtain recombinant pork lipase, which was used for hydrolyzed lipids. The degree of lipase hydrolysis to pork fat was an important factor affecting the changes of pork flavor (Xia et al. 2021). Acid value, peroxide value, TBA value, carbonyl value and diene value are all important indexes for the degree of hydrolysis oxidation of reactive lipids. The acid value illustrated that there was little significance difference (P>0.05) between endogenous lipase and recombinant enzyme group compared with the blank group (Table 2). Since acid value was an index to reflect the content of free fatty acids (FFAs) in samples, and these FFAs were also precursors of flavor substances such as aldehydes, alcohols, methyl ketones and esters. Therefore, we speculated that this result may be related to the transformation of fatty acids. The peroxide value of the two groups added lipase was higher than that of the blank control group (P<0.05), which had a positive promoting effect on the hydrolysis and oxidation of lipids.

The level of TBA values represent the secondary oxidation products of lipids, which can be more sensitive and accurate to evaluate the degree of lipid oxidation (Sánchez-Peña et al. 2005). In comparison with the control group (667.3 nmol/ml) (P<0.05), the other two groups added lipase had a higher content of the TBA value, especially the effect of recombinant enzyme (1053.6 nmol/ml) as shown in Table 2. This result showed that recombinant lipase was important for promoting the degree of lipid oxidation and improving the flavor of meat.

The carbonyl value reflects the amount of aldehydes and ketones produced by oxidation and decomposition of fatty acids. Meanwhile, Diene value reflects the oxidation of lipids (Sánchez-Peña et al. 2005). As shown in table 2, the values of carbonyl and diene were significantly ($P<0.05$) higher in control group and the treatment group, especially the treatment group. Therefore, it could be speculated that the recombinant lipase constructed in this paper has the same ability to promote lipid oxidation and increase the flavor of meat as endogenous lipase.

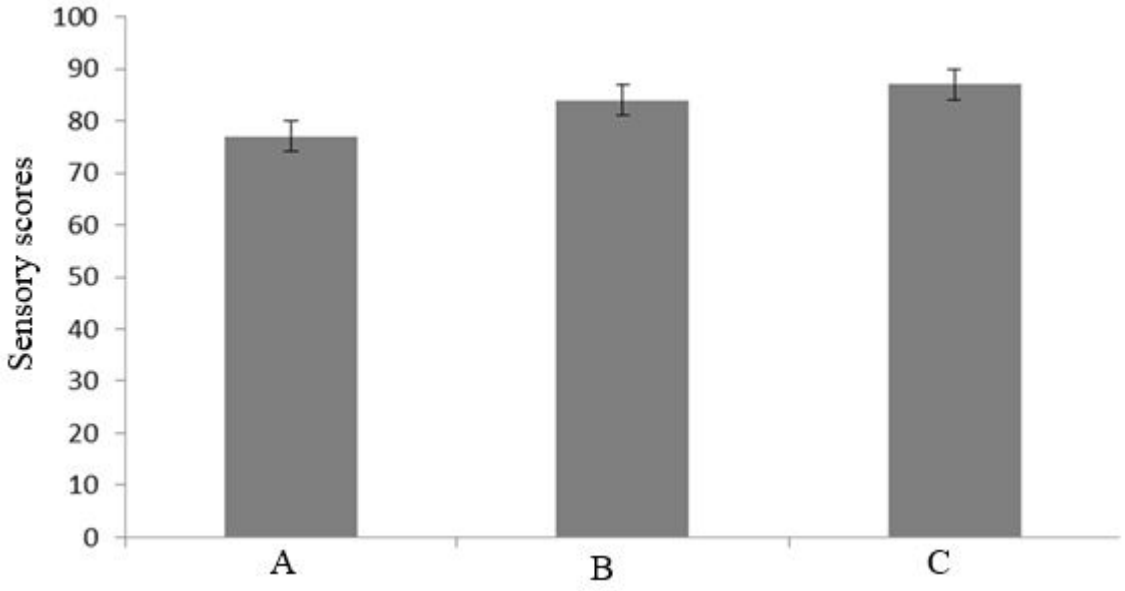
Index	Blank group	Control group	Treatment group
Acid value(mg/g)	0.754±0.01[a]	0.781±0.12 [a]	0.844±0.15 [a]
Peroxide value(mmol/kg)	0.56±0.01[a]	0.71±0.01[b]	0.78±0.02[b]
TBA value(nmol/ml)	667.3±3.18[a]	800±4.37[b]	1053.6±4.74[c]
Carbonyl value	0.222±0.01[a]	0.273±0.01[b]	0.295±0.02[b]
Diene value	0.54±0.02[a]	0.62±0.01[b]	0.69±0.01[b]

Blank group treated with pure water; Control group treated with endogenous lipase; Treatment group treated with recombinant lipase

Table 2: Hydrolysis and oxidation of fat in different groups

Pork flavor detected by sensory analysis and electronic nose (e-nose)

The quality and flavor of the pork can be indicated by the sensory profile as the Figure 1 shown. The sensory analysis indicated that the pork immersed in recombinant lipase and endogenous lipase had better flavor than control, especially the effect on recombinant lipase. However, sensory evaluation is greatly influenced by subjective factors, further study should be performed on the function of recombinant lipase by Electronic nose detection.



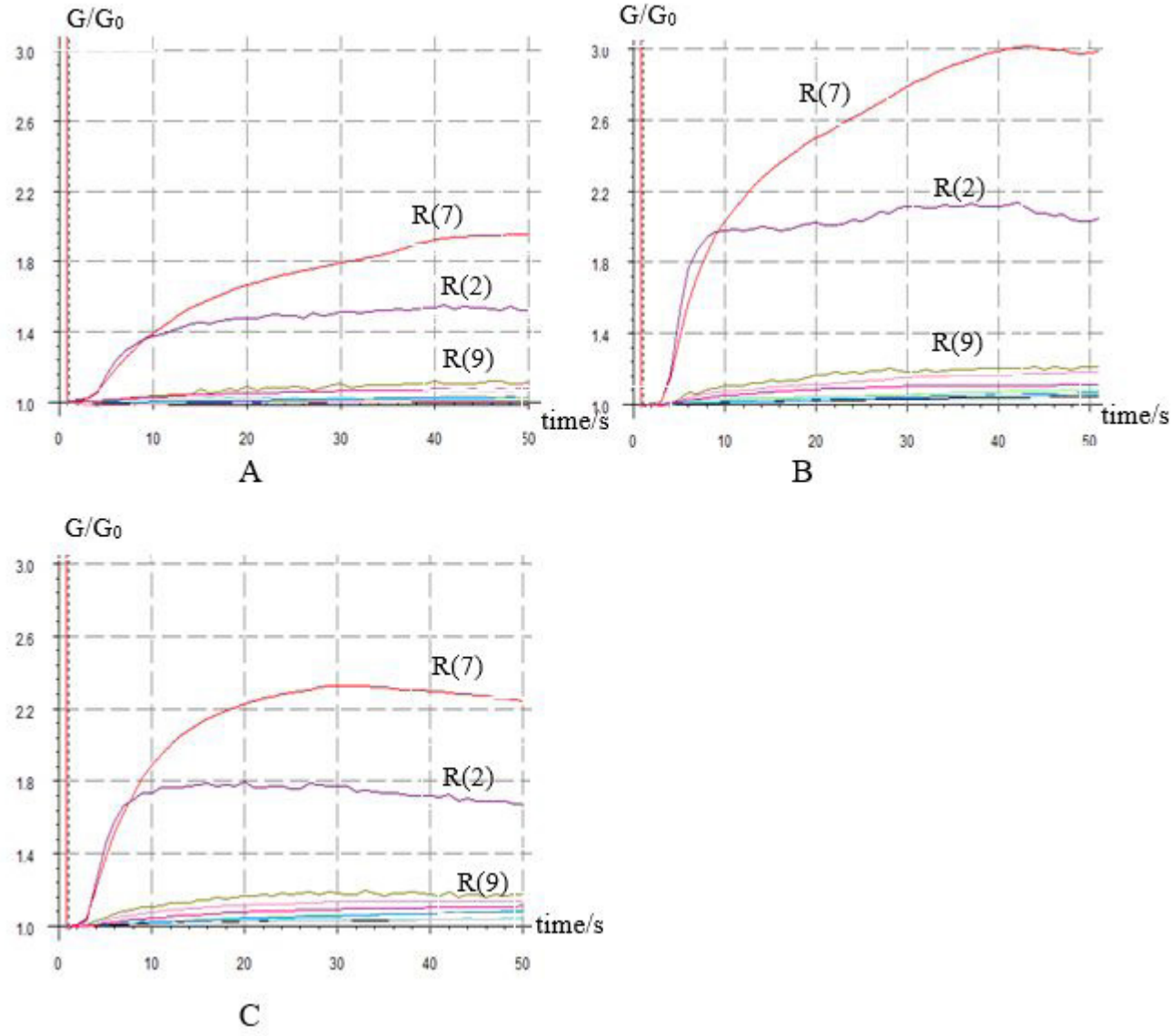
Different letters for the data points at each group point indicate significant differences ($P<0.05$)

A: Blank(cheating with water); B: Control group(cheating with endogenous lipase);

C: Treatment group; cheating with recombinant lipase;

Figure 1: Results of sensory evaluation of samples

Meanwhile, an electronic nose (PEN3) was used to characterize and classify the flavor changes of three kinds of pork cheated with different lipase. Among all the three different groups of pork, the location of each curve was similar after treating with pure water, pork lipase and recombinant lipase respectively. However, the level of G/G_0 was changed clearly. This results showed that when certain proportion of lipase was added to pork, the types of volatile flavor substances in pork did not change, but the contents of some substances changed in different degrees, especially the sensor of R(7), R(2), R(9). The content of sulfide, nitrogen oxides and aromatic components increased significantly ($P<0.05$), which indicated that the addition of recombinant lipase can effectively improve the original flavor and quality of pork. Moreover, in order to examine the relationship of the chemicals and the meat flavor with the aroma compounds after dealing with lipase, a principal component analysis (PCA) was performed (Figure 2).



A: Blank(cheating with water); B: Control group(cheating with endogenous lipase);

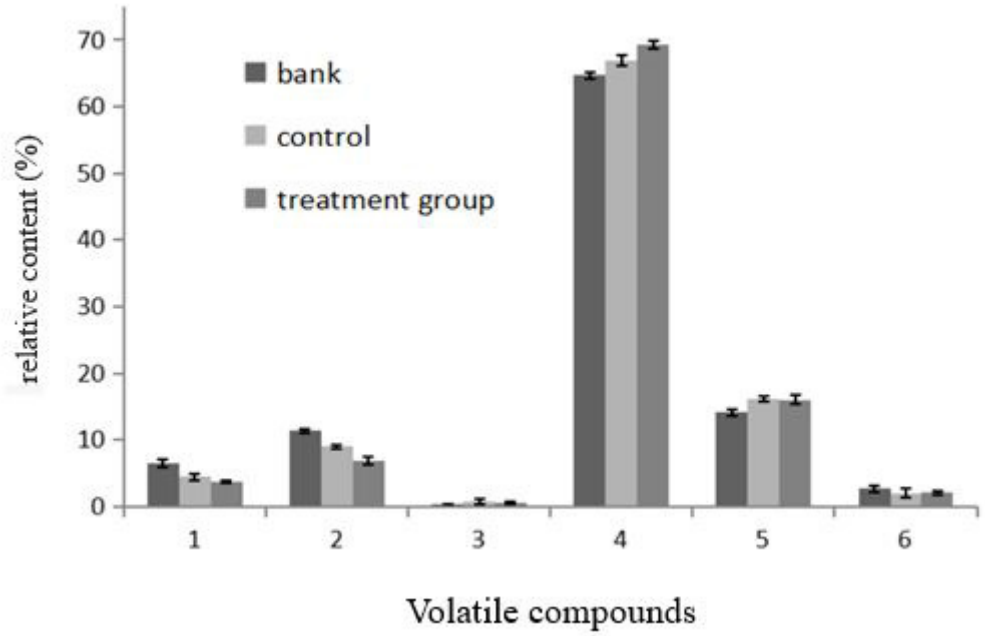
C: Treatment group (cheating with recombinant lipase)

Figure 2: Curve of sensor responses of pork samples before and after lipase treatment

Each ellipse in the figure represents the data collection point of pork flavor of the same batch. Two principal components were able to explain the total variability. The variance contribution rate of the first principal component was 92.85%, while the variance contribution rate of the second principal component (PC2) was 6.97%. The total contribution rate was 99.82%, which was greater than 95%, indicating that the information contained in PC1 and PC2 could represent the main characteristics of volatile flavor of pork samples. This result showed that the three groups of pork samples were different in flavor and the electronic nose could effectively distinguish the flavor characteristics of pork samples before and after lipase treatment. In order to further clarify the specific components of lipase promoting the increase of pork volatile substances, GC-MS was used for analysis and detection.

Volatile compounds analysis by GC-MS

Volatile compounds were analysed in the headspace of pork by SPME-GC-MS. A total number of 36 compounds were identified and the results were shown in Figure 3. In the non-lipase treated pork (blank group), 19 volatile substances were detected. Meanwhile, there were 27 volatile substances and 26 in the endogene-treated pork (control group) and in the recombinant enzyme treated pork (treatment group) respectively. According to the functional groups, these volatile compounds were mainly includes hydrocarbons, heterocycles and aromatic families, organic acids and esters, aldehydes, ketones, alcohols, etc [8]. Compared with the pork samples without lipase treatment, the types and contents of volatile compounds in the pork samples treated with endogenous enzyme and recombinant enzyme were changed, and all these volatile components were closely related to the formation of meat flavor.



1, Hydrocarbon; 2, Heterocyclic and aromatic families; 3, Organic acids and lipids; 4, Aldehyde; 5, Ketones; 6, Alcohols

Figure 3: Relative amounts of volatile compounds in pork samples

Among the volatile substances present in control group and treatment group pork, 5 of them were identified as potential flavor contributors by GC-MS, including Hydrocarbons, heterocyclic, aromatic family compounds, aldehyde and ketones (Table 3).

Compounds	RT (min)	Total%		
		Blank	Control	Treatment
Hydrocarbon				
Hexane	3.82	2.46	2.61	1.75
Eicosane	48.78	---	0.24	---
Tetracosane	11.30	0.95	---	---
3-Methylpentane	3.62	---	---	0.24
2-Methyl-4-methylene hexane	16.96	---	0.21	---
Subtotal		3.41	3.05	1.99
Heterocyclic and aromatic families				
Trichloromethane	4.11	2.18	0.56	0.16
Chlorooctadecane	48.75	0.49	---	---
Dexterpendiene	19.36	0.41	0.80	0.56
Benzocyclobutene	12.72	---	---	0.99
Benzene	4.82	1.40	0.10	0.48
Methylbenzene	7.52	---	1.39	0.92
Ethylbenzene	11.28	---	0.87	---
O-xylene	11.29	---	---	0.88
M-xylene	11.64	---	---	1.11
Paraxylene	11.68	3.69	2.09	---
Styrene	12.74	4.41	2.33	---
2 - Pinene	14.597	0.19	---	0.20
4-Bromobenzyl amino-1-bromobenzene	27.45	---	0.35	0.24
2-N-pentyl furan	17.55	1.70	1.85	2.23
N-[dimethylaminomethyl] azacyclic propane	5.50	---	---	0.79
Subtotal		11.39	8.98	6.85
Organic acids and lipids				
Levotartaric acid	3.44	---	0.29	---
Carbamic acid	25.07	---	0.21	---
Ethyl phenylpropionate	23.77	---	0.15	0.43
Monoisopropyl diisothiocyanate carbonate	21.01	0.11	0.10	0.16
Subtotal		0.11	0.75	0.59
Aldehyde				
Hexanal	8.73	60.20	62.34	63.92
Heptanal	13.19	1.59	1.92	2.47
Octanal	18.14	---	---	0.39
Nonanal	23.20	1.81	2.07	2.35
2-methyl-heptanaldehyde	5.76	---	0.07	0.16
2-Methyl-hexadecanal	3.07	---	0.37	---
Methyl nonacetaldehyde	3.08	1.09	---	---
(E)-2-Heptene aldehyde	15.99	---	0.21	---
Subtotal		64.69	66.98	69.29
Ketones				
3-Octanone	19.87	8.24	10.21	9.26
2,3-Acetyl caproyl	17.26	5.88	5.95	6.76
Subtotal		14.12	16.16	16.02

Alcohols				
N-amyl alcohol		1.07	0.43	0.63
2-Ethylhexanol		1.65	0.97	0.77
1-Octene-3-ol	17.11	----	0.62	0.67
Subtotal		2.72	2.02	2.07

Table 3: Volatile compound compositions for pork samples

Aromatic compounds are hydrocarbons, which have been referred to as a class of aromatic substances obtained from plant gums in history. In the process of steaming the meat, aromatic compounds are considered to be the main aroma components and help to improve the overall flavor quality as some aromatic hydrocarbons have strong aromatic flavor (Zhao et al. 2017). Some studies have shown that these aromatic hydrocarbons are possible to be important intermediates in the formation of heterocyclic compounds. As what had been reported on meat flavor [5], the Heterocyclic compounds, especially those containing sulfur or nitrogen, had a low threshold and were mainly derived from Maillard reaction. It was mean that the flavor could be formed from the pyrolysis of amino acids and the degradation of thiamine. They have a sulfur-like aroma, and most of them have meat aroma, which is an important flavor of meat flavor. In our experiment, the relative concentrations of 2-pinene, 4-bromobenzylidene amino-1-bromobenzene and 2-n-pentyl furan detected in the control and treatment groups were higher than those in the blank group. These compounds played an important role in the formation of pork flavor and were considered to play a significant role in the formation of meat flavor in flavor studies.

As an important carbonyl compound in the formation of meat flavor, aldehydes are precursors of other aromatic compounds, including heterocyclic compounds [5]. Aldehydes have fatty and fresh fragrance and are commonly used as flavoring spices, such as hexanal, n-heptanaldehyde, valeraldehyde and octanal [8] expecially hexanal, which has grass and leaf fragrance and is a landmark product of fat oxidation. Previous studies have shown that straight-chain aldehydes of C6-C10 have a low threshold value and are the most important volatile flavor compounds in all cooked meat products, and play an important role in improving the flavor of meat [12]. As shown in Table 3, the relative contents of aldehydes in control group (66.98%) and treatment group (69.29%) were significantly higher than the blank one (64.69%). These aldehydes inclueded N-hexanal, n-heptanaldehyde and n-octanal, especially N-hexanal. The relative content of n-hexaldehyde in the pork treated with recombinant enzyme had reached 70%, which indicated that recombinant enzyme could promote the increase of pork flavor compounds and enhance aroma.

Ketones and aldehydes are all belong to carbonyl compounds. However, the contribution of ketones to flavor was less than aldehydes. Volatiles anaylsis showed that the level of ketones were only 16%, which was consistent with the previous results. In fact, although ketones in pork were produced by thermal oxidation of unsaturated fatty acids and degradation of amino acids, they were not closely related to the aroma of meat and played a subtle role in the overall aroma of meat [10], which generally have a creamy and fruity aroma.

The production of meat flavor is caused by various organic compounds produced by complex biochemical changes of the inherent ingredients in meat. The flavor of pork mainly comes from the decomposition, oxidation, reduction and other chemical reactions of flavor precursors, such as various volatile flavor substances, aldehydes, ketones, and some heterocyclic compounds. Therefore, the volatile compounds analysis is an important approach to explore the changes of meat flavor and to evaluate compounds related on the aroma of pork after cheating with lipase [13]. In our reasearch, Electronic nose combined with GC-MS was used to detect the effect of recombinant enzyme on pork flavor, which providing theoretical basis for the application of recombinant enzyme in meat production.

Conclusion

In this study, in order to investigate the function of lipase on meat flavor, lipoprotein lipase gene (LPL) was cloned from pork and expressed in *E.coli*. The results of lipid hydrolytic oxidation index showed that the recombinant enzyme had a similar effect with endogenous lipase and had a positive effect on lipid hydrolysis and oxidation, both of them were higher than the blank group. It can

positively regulate the hydrolysis and oxidation of fat as well as the endogenous lipase, which promoted the formation of pork flavor up to a point. A total of 36 kinds of volatile flavor components were revealed through a combination of electronic nose and GC-MS, which mainly for the hydrocarbons, aromatic and heterocyclic compounds, organic acids and esters, aldehydes, ketones, alcohols, etc. In addition, the effect of recombinant enzyme was better than that of endogenous enzyme. It was further indicated that the recombinant enzyme could effectively improve the original flavor of pork.

References

1. Wei Jia, Qingyun Shi, Rong Zhang (2020) Unraveling proteome changes of irradiated goat meat and its relationship to off-flavor analyzed by high-throughput proteomics analysis. Food Chemistry 127806.

2. Jin GF, Zhang JH, Yu X (2010) Lipolysis and lipid oxidation in bacon during curing and drying-ripening. Food Chemistry 123: 465-71.

3. Sigmund Schwimmer (1963) Alteration of the Flavor of Processed Vegetables by Enzyme Preparations. Food Science 28: 460-6.

4. Re Campbel, Drake MA (2013) Invited review: The effect of native and nonnative enzymes on the flavor of dried dairy ingredients. Campbell and Drake 96: 4773-83.

5. Huan Y, Zhou GH, Zhao GM (2005) Changes in flavor compounds of dry-cured Chinese Jinhua ham during processing. Meat Science 71: 291-9.

6. Song Shiqing, Tang Qi, Li Fan (2017) Identification of pork flavour precursors from enzyme-treated lard using Maillard model system assessed by GC-MS and partial least squares regression. Meat Science 124:15-24.

7. Gao Pei, Xia Wenshui, Li Xinzhi (2020) Optimization of the Maillard reaction of xylose with cysteine for modulating aroma compound formation in fermented tilapia fish head hydrolysate using response surface methodology. Food Chemistry 331:1-10.

8. Mottram DS (1998) Flavor formation in meat and meat products. Food Chemistry 62: 415-24.

9. Tian JW, Lei ZC, Qiu P (2014) Purification and Characterization of a Cold-Adapted Lipase from Oceahobacillus Strain PT-11. Plos One 9: 1-7.

10. Liu Y, Su H, Song HL (2018) Comparison of four extraction methods, SPME, DHS, SPME, DHS,SAFE, versus SDE, for the anaylsis of flavor compouds in Natto. Food Analytical Methods 11: 343-54.

11. Al-Bachir M, Zeinou R (2014) Effect of gamma irradiation on the microbial load, chemical and sensory properties of goat meat. Acta Alimentaria 43: 264-72.

12. Flores M (2018) Understanding the implications of current health trends on the aroma of wet and dry cured meat products. Meat Science 144: 53-61.

13. Waller GR, Feather MS (1983) The maillard reaction in foods and nutrition. American Chemical Society 585-614.