Sensory, Color, Reaction Rate, and Volatile Flavor Components of Maillard Reaction Products From Oyster Protein Hydrolysates with Different Degree of Hydrolysis

Tang Haixia, Huang Yuni, LIN Yijun, Chen Xueren, Li jiayun, Peng Huiyi, Yang Zhao*

Guangdong Food and Drug Vocational College, Guangzhou, Guangdong 510520, China

* Corresponding author e-mail: yangzhao19878@163.com

Abstract. To investigate the effects of different degree of hydrolysis on the sensory, color, reaction rate, and volatile flavor components of the Maillard reaction products in oyster enzymatic hydrolysate(OEH), oyster enzymatic hydrolysate was used as an amino acid source and reacted with 8% glucose at 110℃ for Maillard reaction. The results showed that the MRPs produced by OEH at 110℃ for 3h had the highest sensory score of 15 points and the best flavor. The color difference L* value of OHM3 was 41.26, the a* value was 3.26, and the b* value was 8.62. The absorption value of A294 was 0.785, and that of A420 was 0.486. A total of 40 components were identified from the Maillard reaction products of oyster enzymolysis solution by SPME-GC-MS. OHM3 showed shellfish flavor, meat flavor, and seafood flavor, and some pyrazines were produced, which gave it some nut-meat flavor. At a fixed temperature of 110℃, the Maillard reaction produced by OEH for 3h had good meat flavor. The Maillard reaction can effectively improve the flavor of oyster enzymatic hydrolysis products, provide a theoretical basis for the study of oyster enzymatic hydrolysis solutions to remove smell and enhance flavor, and have a good application prospect in the development of new oyster condiments.

Keywords: oyster, hydrolysis degree, Maillard reaction, volatile flavor components.

1. Introduction

Oyster is a marine shellfish, and is one of the largest cultured shellfish in the world[1]. OEH is prepared by biotechnology to explore its flavor and bioactivity, which is widely concerned by researchers. During the preparation of OEH, the oxidative degradation of oyster lipids and the degradation of proteins during enzymatic degradation resulted in a heavy fishy-bitter taste and poor flavor of OEH, which affected the consumption[2-3]. OEH have a variety of physiological activities, and its application prospect and market value are broad. The Maillard reaction is one of the important methods for deodorizing and enhancing the aroma of OEH[4]. However, the Maillard reaction is affected by many factors; pH value, temperature, time, sugar type, and amount of reaction medium in the reaction system will affect the sensory and physiological activities of MRPs[5].

At present, researchers have paid attention to the effects of reducing sugar types, reaction temperature, pH value, and other factors on the sensory, chemical composition, and biological activity of MRPs. He et al.[6] used a two-step process of enzymatic hydrolysis and Maillard reaction to produce oyster meat hydrolyzed products MRPs. Through optimized orthogonal experimental design, the flavor of oyster meat hydrolyzed MRPs was significantly improved. Wu Ruotong et al.[7] found that compared with the single addition of glycine or glutamic acid, the addition of the two amino acids effectively removed the fishy smell and enhanced the aroma of oyster peptide MRPs.

In addition, the synergistic effect of glycine and glutamic acid significantly reduced the impact of key fishy compounds, such as heptanal and octanal, on the overall odor properties, while the types of volatile flavor components increased to 21, which improved the richness of overall aroma. However, during the enzymatic digestion of oysters, different degrees of hydrolysis lead to differences in the types and contents of amino acids and small-molecule peptides in OEH.

Therefore, the quality of MRPs may be different with different hydrolysis degrees. Zhang et al.[8] found that different hydrolysis degrees had great effects on sensory properties, free amino acid content, and molecular weight distribution of MRPs from chicken protein hydrolysis. At present, few researchers have systematically studied the effects of different hydrolysis degrees on the quality of MRPs.

Temperature is an important factor affecting the rate and color difference of the Maillard reaction. Studies have shown that peptide degradation and peptide cross-linking occur simultaneously in the Maillard reaction[9]. The critical temperature of peptide degradation is 100 ℃. Above this temperature, the polypeptide rapidly degrades in the thermal degradation system. In the Maillard reaction system, the peptide chain is rapidly cross-linked at 110 ℃. During the folding and maturation of proteins, there may be a rapid cross-linking reaction between specific amino acid residues, which helps the protein form the correct three-dimensional structure to fulfill its specific biological function. Therefore, under the control of the same temperature of 110 ℃, different hydrolysis degrees were selected to investigate the sensory, color difference, light absorption value, and volatile components of MRPs as indicators, which provided a theoretical basis for the study of removing fishy smell and aroma enhancement of OEH.

2. Materials and Methods

2.1. Materials and Reagents

Oyster meat (frozen storage) was purchased from Raoping, Guangdong.Flavored protease (500 LAPU/g): Novozymes China; Trypsin (4000 U/g): Henan Wanbang Industry Co., Ltd. Sodium hydroxide, hydrochloric acid, glucose, and formaldehyde are all analytically pure.

2.2. Main instruments and equipment

GCMS-QP2010 ultra gas chromatography-mass spectrometer, Shimadzu Institute, Japan; 50μm DVB/CAR/PDMS Extractor Head Supelco, USA; NS810 Color Difference Meter, Shenzhen San enchi Technology Co., Ltd.

2.3. OEH preparation[10]

The OEH obtained were labeled as OH0, OH1, OH2, OH3, and OH4, and the hydrolysis degrees were 11.18%, 18.39%, 25.61%, 29.10%, and 32.13%, respectively, measured by formaldehyde titration.

2.4. Preparation of MRPS with different degrees of hydrolysis

Referring to the method of Liu Haimei[11], 8% glucose was added to OEH, and the reaction was carried out at 110℃ for 90 minutes under the condition of pH 7.5. After cooling, the reaction was centrifuged at 5000 r/min for 15 minutes, and the supernatant was taken and frozen at -18℃ for storage. The obtained MRPs were labeled as OHM0, OHM1, OHM2, OHM3, and OHM4, respectively.

2.5. Determination of color difference values of MRPs with different degrees of hydrolysis

Use a colorimeter to measure the brightness value (L *), redness value (a *), and yellowness value (b *) of OEH. Measure each sample 5 times and take the average of the results.

2.6. Sensory evaluation of MRPs

A sensory evaluation team consisting of 6 specially trained sensory evaluators (3 males and 3 females) was selected for sensory evaluation, and each sample to be evaluated was randomly numbered with a 3-digit number. The evaluators scored the flavor description of the Maillard products based on specific sensory evaluation criteria (Table 1). In a sensory evaluation room with a temperature of (24±1)℃, sensory evaluation was conducted, and the color and flavor were described and statistically analyzed. The evaluator takes an appropriate amount of the sample solution to be tested and puts it in the mouth. After the sample solution stays in the mouth for 20-25 seconds, it was spited out. According to the sensory evaluation criteria (see Table 1), the sensory results of this evaluation were recorded. Score the sugar aroma, fishy taste, umami taste, and bitterness of Maillard products separately, conduct sensory evaluation of their overall quality, and take the average value.

Table 1 Sensory scoring criteria for MRPs

	Sensory evaluation indicators (flavor description)			
	Candyfloss	Fishy smell	Umami	Bitterness
1	non-existent	The fishy smell is on the heavy side, obnoxious and unacceptable to the vast majority of people.	Odourless	Very pronounced bitter taste, off-putting.
2	minor, almost none	The fishy smell in general is obnoxious, and unacceptable to most people.	Slight, almost no umami	Significantly bitterness and unpleasant
3	Candyfloss is light	It's fishy, but most people can handle it.	The umami is average but noticeable.	Clearly bitterness; it is noticeable but not unpleasant.
4	Candyfloss, acceptable	Fishy smell is weak and can be felt but it is less pronounced.	Strong umami flavor	Slightly bitter, but not pronounced.
5	Strong candyfloss	No fishy smell	Strong umami	Lack of bitterness

2.7. Determination of the degree of browning of MRPs with different degrees of hydrolysis

The degree of formation of low molecular mass flavor intermediates was determined by measuring the absorbance of the samples at wavelength 294 nm. The products of the OEH Maillard reaction were pumped and filtered, the filtrate was then diluted to 200 times, and then the absorbance was measured at wavelength 294 nm with pure water as a reference, and the change in absorbance was finally analysed. The browning degree was determined by measuring the absorbance of the samples at a wavelength of 420 nm, diluting the MRPs by 100 fold, then measuring the absorbance at a wavelength of 420 nm with pure water as a reference, and finally analysing the browning degree of the Maillard reaction.

2.8. Identification of volatile flavor components

2.8.1 Extraction of volatile flavor components

Refer to the method of Yang Zhao et al.[12]with slight adjustment. Weigh 3g of MRPs, load the sample into the headspace extraction vial using a funnel, place it in equilibrium heating at 60℃ for 10 minutes, then insert the DVB/CAR/PDMS extraction head into the headspace vial, adsorb it for 40 minutes, then pull out the head, and transfer the adsorbed extraction head to GC-MS for detection.

2.8.2 Identification of volatile flavor components

Chromatographic conditions: HP-5MS capillary column (30 m×0.25 mm, 0.25 μm). The carrier gas was high-purity helium (99.999%) at a flow rate of 1.0 mL/min, and the initial temperature of the column was50 ℃, then increased to 140 ℃ at 4 ℃/min and kept for 10 minutes, and then increased to 250 ℃ at 6 ℃/min and kept for 6 in minutes. The temperature of the injection port was 270 ℃, the sampling mode was set to'shunt', and the desorption temperature was 270 ℃.The inlet temperature was 270℃, the injection mode was set to'split flow', the desorption temperature was 270 ℃, and the desorption time was 5 minutes. The GC time was 56.83 minutes.

Mass spectrometry (MS) conditions: electron ionization source; interface temperature was 250℃;ion source temperature was 230℃;quadrupole temperature was150℃;electron energy was 70 eV; mass scanning range was 45-450 m/z.

The volatile matter components were compared and retrieved by the NIST11 mass spectrometry library for each compound detected, and the peaks of compounds with a similarity greater than 80% were selected for retention[13]. The retrieved compounds were also characterized in conjunction with relevant literature, and the relative content of each volatile flavor component was determined using area normalization, with only components with area percentages≥0.1% reported.

2.9. Data analysis

Excel 2016 and SPSS 26 software were used to analyse and process the data and produce graphs.

3. Conclusion

3.1. The color difference values of MRPs at varying degrees of hydrolysis

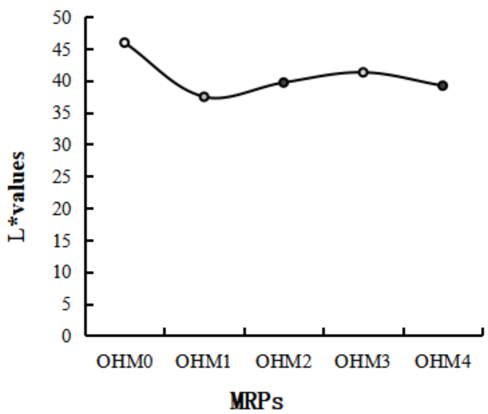

Fig. 1 L* values of MRPs with different degrees of hydrolysis

L* is a measure of the brightness of the color indicator; the greater the value, the brighter the color. As shown in Fig. 1, L* values of MRPs with different hydrolysis degrees showed an overall decreasing trend with the increase of enzymatic hydrolysis time.

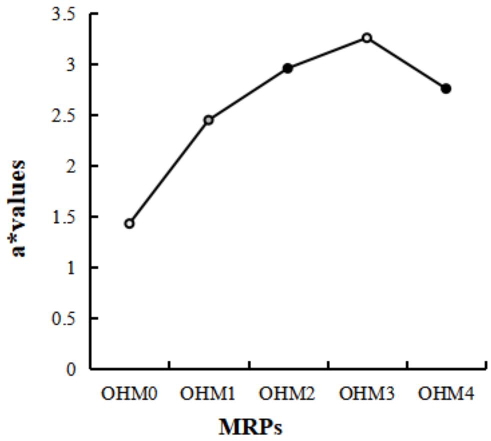

Fig. 2 a* values of MRPs with different degrees of hydrolysis

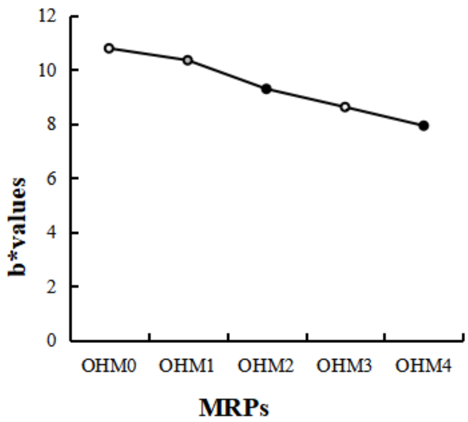

Fig. 3 b* values of MRPs with different degrees of hydrolysis

The a* value is a measure of the degree of redness or greenness, b* is a measure of the degree of yellow-blue. The larger the absolute value of a* and b*, the darker the color, and vice versa. As can be seen from Fig. 2, the a* value of MRPs gradually increased with the increase of enzymolysis time, indicating that the redness of the enzymolysis solution was deepened. However, when the enzymolysis time exceeded 3h, the a* value of the enzymolysis solution showed a slightly decreasing trend. As can be seen from Fig. 3, the change of b* value is completely opposite, and with the increase of enzymatic hydrolysis time, the b* value gradually decreases, indicating that the blueness of the enzymatic hydrolysis solution was deepened. Based on the changes of L* value, a*, and b* data, the oysters were enzymatically digested and then subjected to the Maillard reaction, which has a dramatic effect on the color. This indirectly suggests that enzymatically hydrolyzed oysters have a complex flavor. So, the color performance of OHM3 in the Maillard reaction was superior compared to others.

3.2. Sensory scores and browning of MRPs with different degrees of hydrolysis

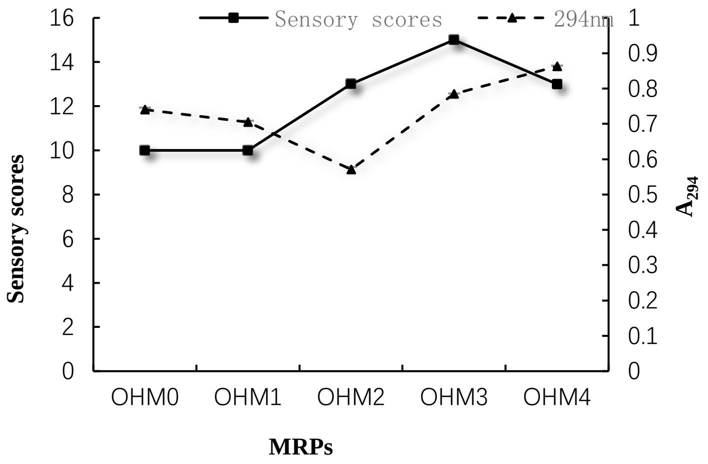

Fig. 4 Sensory score of MRPs with different hydrolysis degrees and A294nm

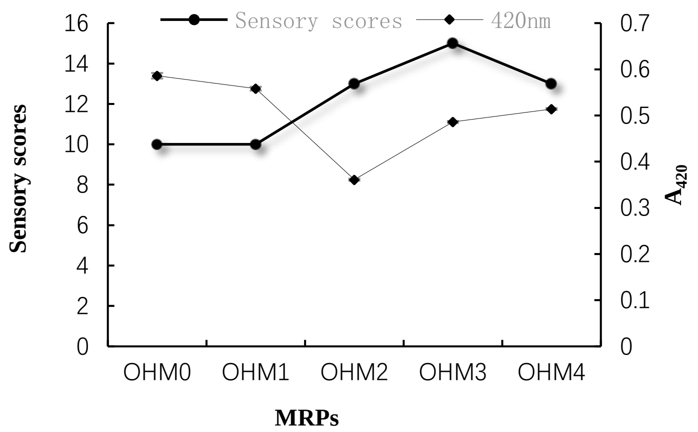

Fig. 5 Sensory scores of MRPs with different hydrolysis degrees and A294nm

According to Fig. 4 and Fig. 5, it can be seen that the sensory scores of OHM0, OHM1, OHM2, OHM3, and OHM4 were 10, 10, 13, 15, and 13, respectively.

Also according to Fig.4 and Fig. 5, it can be seen that the absorbance values at wavelength 294 nm for OHM0, OHM1, OHM2, OHM3, and OHM4 were 0.74, 0.705, 0.571, 0.785, 0.863, and at wavelength 420 nm, 0.586, 0.558, 0.361, 0.486, and 0.514, respectively. The absorbance values at both wavelengths showed a decreasing trend from OHM0 to OHM2, in which the absorbance values in OHM2 decreased to the lowest, and then they all increased gradually from OHM3 to OHM4 as well.

The flavor intermediates of MRPs with low molecular mass had specific absorption peaks at wavelength 294 nm, and the larger A294 nm, the better the formation of flavor intermediates with low molecular mass[14]. From the data, it can be seen that OHM4 has the highest absorbance value and a value of 0.863 at a wavelength of 294 nm. Therefore, OHM4 produced the best low molecular mass flavor intermediates, but at the same time the Maillard reaction was also more intense, producing more nigrosperm-like substances, which affected the sensory score.When the Maillard reaction occurred in OEH, the larger the A420 nm, the more melanoidin was generated, the darker the color of the system, and the higher the degree of browning, which was mainly due to the fact that the reaction continuously generated large molecules, such as melanoidin[15]. From the above figure, it can be seen that OHM2 was the lowest at the wavelength of 420 nm and was the turning point. OHM2, the absorbance value was increasing, the browning degree was getting stronger and stronger, and the Maillard reaction was also gradually strong.

In summary, combined with the data analysis, at 110℃, the sensory evaluation of OHM3 was as high as 15 points, the absorbance value of A294 nm was 0.785, and the absorbance value of A420 nm was 0.486, had the best flavor.

3.3. The volatile flavor components of MRPs with different degrees of hydrolysis

Forty components were identified from OHM0, OHM1, OHM2, OHM3, and OHM4. Among them, the volatile flavor component with a larger content in OHM0 was 2-Hexene, 3,5,5-trimethyl-, with the largest relative content of 15.84%, presenting a fruity aroma; the volatile flavor component with the greater content in OHM2 was octadecanal, with the largest relative content of 21.27%, presenting flavors such as a pronounced fatty odour and a waxy note; The volatile flavor components with the greater content in both OHM1 and OHM3 were eicosanal, with relative contents of 18.41% and 16.32%, respectively, with more pronounced fat notes and a slightly waxy odour with some nut-like or slightly fruity odours.

The relative content of eicosanal in OHM1 was higher than that of OHM3, but the relative content of 1-octen-3-ol in OHM1 was as high as 15.66%, presenting a strong earthy flavor, so the flavor of OHM3 was superior to that of OHM1; from the above, it can be seen that OHM0's presented a better fruity flavor, but the presence of 13.03% of eicosanal and 11.61% of 1-octen-3-ol combined to present a flavor biased towards undesirable odours such as earthy, fatty, and harry. Therefore, considering the overall factors, OHM3 was superior in terms of flavor presentation compared to the other cases.

The main volatile components, such as octanal, 1-octen-3-ol, pentanoic acid, 2-methyl, anhydride, and 2-hexene, 3,5,5-trimethyl in MRPs with different hydrolysis degrees, presented fishy, fatty, soapy, harry, and greasy flavors, and after the Maillard reaction, the main volatile components changed to decanal, nonanal, tridecanal, and pentadecanal, et cl. OHM3 showed the shellfish flavor, meaty flavor and seafood flavor of oyster. OHM3 reveals oyster shell flavor, meat flavor, and seafood flavor, and at the same time produces some pyrazines, giving it some nutty-meat flavor.

Table 2. The volatile flavor components of MRPs with different degrees of hydrolysis

Name	Relative content(%)					Odour description
	OHM 0	OHM 1	OHM 2	OHM 3	OHM 4	
Dodecanal	-	1.36	-	1.68	-	NA
Tridecanal	-	1.46	1.44	1.60	0.62	Citrus, Grapefruit, Wax, Grease
Tetradecanal	3.42	7.85	3.59	2.18	-	NA
Pentadecanal	-	2.33	1.81	2.28	0.87	Pungent, slightly bitter
Hexadecanal	-	-	3.18	6.65	1.26	Floral, Waxy
Heptadecanal	2.52	3.29	3.36	2.38	-	Pungent, slightly bitter
Octadecanal	11.08	8.97	21.27	6.15	-	Slightly fatty odor, waxy notes
Eicosanal	13.03	18.41	13.69	16.32	-	Fatty odor, waxy notes, nutty, fruity.
Lilac aldehyde C	2.12	2.37	1.59	1.52	0.82	Floral green flavor, sweet flavor
Decanal	1.71	1.42	2.08	1.44	0.77	Sweet, citrusy, waxy, floral aroma
Benzaldehyde	4.09	-	1.20	-	3.52	Almond flavor
Benzeneacetaldehyde	-	5.56	-	-	-	Hyacinth notes, fruity notes, sweet notes
Octanal	4.06	-	-	-	-	Fatty, Soapy, Lemon, Waxy
Nonanal	-	-	-	11.35	-	Spicy fatty, citrusy
2,6-Nonadienal, (E,Z)-	1.25	-	-	-	-	Violet flavor, cucumber aroma
1-Octen-3-ol	11.61	15.66	-	-	9.30	Mushroom flavor, earthy
1-Hexanol, 2-ethyl	-	1.60	-	-	-	NA
1-Heneicosanol	-	-	-	-	1.73	NA

n-Caproic acid vinyl ester	1.72	-	-	-	-	Fruity, estery
2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	-	-	-	-	0.70	Floral
1-Norbornanemethanol, acetate	1.21	1.25	3.54	2.32	1.73	Fruity, floral
Ethanone, 1-(2-aminophenyl)	-	-	-	-	1.35	Stimulating odor
4-Penten-2-one, 4-methyl-	1.52	-	-	-	-	Fruity, green
2,3,5-Trimethyl-6-ethylpyrazine	-	-	-	-	0.65	Meaty
Pyrazine, trimethyl	-	-	8.18	-	9.21	Roasted, nutty, coffee, cocoa aromas
Pyrazine, 3-ethyl-2,5-dimethyl	9.36	7.50	10.38	14.99	10.40	Roasted, caramelized
Pyrazine, 2-methyl-6-(1-propenyl)-, (E)	-	-	2.36	1.67	1.68	Nutty, Coffee
Pyrazine, 2,5-dimethyl-3-(3-methylbutyl)	-	3.65	6.52	4.22	2.53	Roasted, Nutty
2,5-Dimethyl-3-(2-methylbutyl)pyrazine	-	-	1.12	2.21	-	Roasted, Nutty
Pyrazine, 2,5-dimethyl-3-(2-methylpropyl)	-	-	-	1.11	-	Fatty, caramelized
trans-2-(2-Pentenyl)furan	1.87	-	-	-	-	Vegetable, grassy
Acetic acid, diethyl-	1.41	-	-	-	-	Faint ammonia odor
Pentanoic acid, 2-methyl-, anhydride	2.92	-	-	-	-	Pungent odor characteristic of acid anhydride
2-Hexene, 3,5,5-trimethyl-	15.84	16.05	13.64	-	8.59	Fruity
3-Dodecyne	1.80	1.26	-	1.40	0.96	Fatty odor
Cyclohexene, 4-(1,1-dimethylethyl)	-	-	2.03	-	-	NA
Pentadecane	-	-	1.90	2.44	0.75	NA
Hexadecane, 1,1-bis(dodecyloxy)	-	3.63	-	-	0.53	Petroleum odor, solvent-like odor
Heptadecane	2.12	2.67	5.47	3.07	-	NA
Cyclohexane, 1-butenylidene	-	1.23	-	-	-	NA

Note: "-" indicates none, "NA" indeterminate.

4. Discussion

At 110℃, OHM3 had a pleasant and good meat flavor with an organoleptic score as high as 15 points. The absorbance values also showed a regular change with the digestion time, and the absorbance values of OHM3 were 0.785 at A294 nm and 0.486 at A420 nm, which were in the best condition for the flavor, and OHM3 had better color performance compared with the other cases.

The MRPs identified 40 components in the volatile gas analysis. The major volatile components in OEH, such as octanal, 1-octene-3 alcohol, eicosanal, and 2-hexene 3,5,5-trimethyl gave the OEH a fishy, fatty, soapy, harry, and greasy flavor. However, after enzymatic hydrolysis and the Maillard reaction, the main volatile components were changed to decanal, nonanal, tridecanal and pentadecanal, etc. OHM3 focused on the shellfish, meaty, and seafood flavors of oysters, and some pyrazines were also produced, which conferred a unique nutty-meaty flavor to the oysters.

The Maillard reaction can effectively improve the flavor of oyster enzymatic products and provide a theoretical basis for the study of OEH deodorisation and flavor enhancement, which have a good application prospect in the development of new oyster flavoring products.

Acknowledgments

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