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The Effect of Reaction Temperature on the Quality of Oyster Enzymatic Hydrolysate Maillard Products

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Abstract. Oyster meat was used as raw material, which was enzymatically digested under specific conditions for 3h. Then the effect of reaction temperature on the sensory, color difference, reaction rate, and volatile flavor components of the oyster enzymatic hydrolyzates and glucose meridian reaction product was investigated. The study showed that the 3h enzymatic hydrolysis of oyster produced the best flavour and sensory score of the maillard reaction product at 110℃ (110HM), which had the absorbance value of 0.788 for A294, and 0.606 for A420. 54 compounds were identified from 110HM, which mainly contained 6 alcohols (26.40%), 3 ketones (1.80%), 8 aldehydes (8.38%), 6 esters (1.71%), 16 hydrocarbons (32.84%), and 15 heterocyclic and other species (22.82%).

Keywords: Oyster; temperature; degree of hydrolysis; maillard; flavor.

1. Introduction

Oyster is the world's number one cultured shellfish and is one of the important marine biological resources available to human beings. With juicy meat and delicious flavor, the oyster is not only rich in protein, fat, zinc, calcium, phosphorus, iron and other nutrients, but also have unique healthcare functions and medicinal value, which makes it a kind of seafood treasure with high nutritional value. It has been found that oysters have various effects such as antioxidant, blood pressure reduction, blood lipid reduction and anti-tumor [1]. Oysters in China are mainly sold fresh, with a small portion used in processed foods, mainly oyster oil, dried oysters, and so on. Gao Jialong [2] and others verified that the maillard reaction could significantly improve the flavor of oyster enzymatic solution, and Zhang Jie [3] and others determined the optimal reaction conditions for deodorizing oyster enzymatic solution using maillard reaction through one-way and orthogonal experiments. In this paper, oyster meat was used as raw material, which was enzymatically digested with flavor protease (enzyme activity 500 LAPU/g) and trypsin (enzyme activity 4000u/g) under specific conditions for 3 h to obtain the enzyme hydrolyzates. Then the effects of the reaction temperature on the organoleptic, color difference, reaction rate, and volatile flavor components of the products of oyster enzyme hydrolyzates with glucose in the maillard reaction were investigated.

2. Materials and Methods

2.1. Raw materials

Oyster meat was obtained from Raoping County, Chaozhou City, Guangdong Province, China.

2.2. Reagents

Flavored protease (enzyme activity 500 LAPU/g): food grade, Novozymes China, Denmark; Trypsin (enzyme activity 4000u/g): food grade, Chongqing Xiangsheng Bioengineering Co., Ltd. Sodium hydroxide (0.05mol/L), hydrochloric acid, dextrose, and formaldehyde (36%-38%) all analytically pure, Xilong Science Co., Ltd.

2.3. Main instrumentation

DKZ-3B Electrothermal thermostatic oscillation water bath Shanghai Yiheng Scientific Instrument Co., Ltd; K9840 Kjeldahl nitrogen tester Jinan Haineng Instrument Co., Ltd; NS810 colorimeter Shenzhen SUNCH Technology Co., Ltd; UV-1800PC Spectrophotometer Shanghai Meppan Instrument Co., Ltd; GCMS-QP2010 Ultra Gas Chromatography-Mass Spectrometer Shimadzu Corporation, Japan.

2.4. Experimental methods

2.4.1. Preparation of oyster enzymatic hydrolyzates.

After thawing frozen oyster meat at room temperature, add distilled water according to the mass ratio of 1:1, and beat the pulp, the total enzyme amount was 1%, the enzyme ratio was 3:1, and the pH of enzyme digestion was 8.0, and the enzyme digestion was carried out by shaking in a water bath shaker at 55℃ for 3 h. After the end of the enzyme digestion, the enzyme was extinguished by boiling water for 10 min, and then the enzyme was cooled down at room temperature, and then centrifuged for 20 min at 4℃ under the condition of 8000 r/min, and the supernatant was taken and stored frozen at -18℃. The supernatant was frozen at -18℃ [4]. The degree of hydrolysis of oyster enzyme solution was measured as 29% by formaldehyde titration.

2.4.2. Preparation maillard reaction products.

Referring to the method of Liu Haimei [5]. The Maillard reaction products were obtained under the conditions of glucose addition of 8%, pH 7.5, reaction time of 90 min, and reaction temperatures of 100,110,120℃. They were labeled as 100HM, 110HM, and 120HM, respectively.

2.4.3. Sensory evaluation.

Seven students (3 males and 4 females, aged 18-21 years old) with professional sensory training were selected for the sensory evaluation and scored the flavor descriptions of the maillard products. Sugar aroma, fishy taste, fresh taste, and bitterness of the maillard product were scored respectively, and the comprehensive quality was sensory evaluated and averaged. 7 students were scored according to their taste and flavor according to the unified scoring standard under the same external environmental temperature conditions and mouthwash water.

2.4.4. Measurement of color difference value.

The color difference meter was used to determine the color of the oyster enzymatic solution. Each sample was determined five times and the results were averaged [6].

2.4.5. Determination of extent of reaction.

The enzyme solution of oyster after the maillard reaction was filtered, the filtrate was diluted 200 times, and the absorbance was measured at 294nm with ultrapure water as the reference, and the change of absorbance was observed. The final stage of the maillard reaction produced a black essence-like substance, which was absorbed at the wavelength of A420nm. The browning degree of the maillard reaction was detected by diluting the product of the maillard reaction 100 times and measuring its absorbance at the wavelength A420nm with ultrapure water as a reference [7].

2.4.6. Determination of volatile flavor components.

The method of Yuan Lin [8] and other references, headspace SPME conditions: weigh 0.5g of oyster enzyme solution maillard reaction product, with deionized water to 50mL, 4mL in a 20mL SPME bottle, 60℃ extraction for 30 min. Extraction immediately after the end of the extraction into the injection port (250℃) desorbed for 4 min.The extraction head in the first time needs to be used in 250℃ aged 1 h. The extractor head should be aged at 250℃ for 1 h when used for the first time.

GC conditions: HP-5 capillary column (5% phenyl, 95% polymethylsiloxane) specifications: 30 mx0.32mm, 0.25μm; carrier gas for high-purity helium (99.999%), helium flow rate of 1.0 mL/min;

non-split-flow injection, inlet temperature: 250℃; column temperature: initial temperature of 40℃ constant temperature for 3 min to 6℃/min to 200℃, then 10℃/min to 10℃ to 200℃. 10℃/min to 250℃, keep 10 min.

MS conditions: the ionization source was electrospray ionization, the ion temperature was 150℃, the GC-MS transmission line temperature was 250℃, the mass scanning range was 33~300u, and the scanning rate was 0.220 s/scan. The electron energy of electrospray ionization was 70eV.

The NIST11 mass spectrometry database was used to search and analyze the peaks, take the substances with similarity greater than 80%, and characterize the substances in combination with literature reports to determine the volatile flavor components. Only compounds with relative content ≥0.1% were counted, and the relative content of each volatile flavor component was determined by area normalization.

3. Result

3.1. Sensory analysis of Maillard products

The final data showed that the overall sensory evaluation of the 110HM was presented a strong and distinct umami flavor, weak fishy taste, sugar aroma, and bitterness. Hou Qing'e [9] also found that after enzymatic hydrolysis of oysters, the freshness of the enzymatic hydrolysis products was better, with the highest sensory score for 110 HM and the lowest comprehensive sensory score for 120 HM.

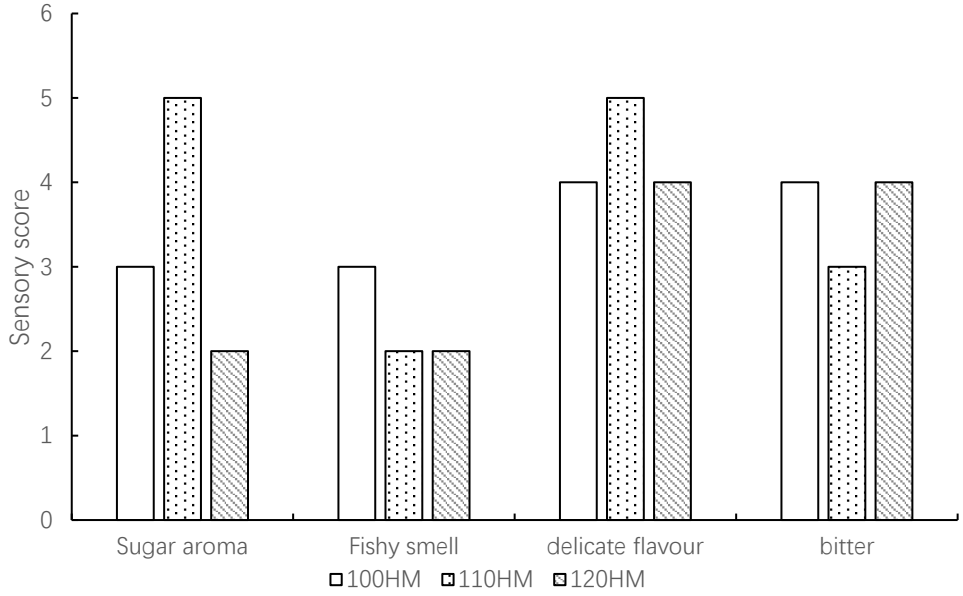

Figure 1. Sensory evaluation chart of Maillard products

3.2. Color difference value of Maillard products

As can be seen from Table 1, the L* value of 100HM was the largest, with a value of 46.12, and that of 120HM was the smallest, with a value of 35.34. The a* value of 120 HM was the largest with a value of 4.46, and the value of 100 HM was the smallest with a value of 0.37. The b* value of 100HM was the largest, with a value of 10.45, and the b* value of 120HM was the smallest, with a value of 6.55.

Table 1. Color difference value of Maillard products

samples	100HM	110HM	120HM

L *	46.12	40.34	35.34
a *	0.37	3.56	4.46
b *	10.45	8.26	6.55

3.3. Absorbance values of Maillard products

The Maillard reaction mainly occurs between reducing sugars and amino acids. Different reducing sugars have varying reactivities and flavor change impacts. The reactivity order of common sugars was D-xylose > L-arabinose > hexose > disaccharide [10]. For the Maillard reaction study, the most commonly available glucose was selected. The higher the absorbance value, the more significant the color change, indicating a stronger degree of the Maillard reaction. Temperature has a significant impact on the Maillard reaction, with a 10℃ temperature difference leading to a 3- to 5-fold variation in the browning rate. As the enzymatic reaction time extends, the absorbance and flavor of the Maillard reaction solution at wavelengths of 294 nm and 420 nm will vary, and the degree of the Maillard reaction will generally follow a certain trend. With the rise in enzymatic reaction temperature, the absorbance of oyster Maillard products at 294 nm and 420 nm gradually increases, meaning the degree of the Maillard reaction gradually rises, which is consistent with the results of Dong Zhijian [11].

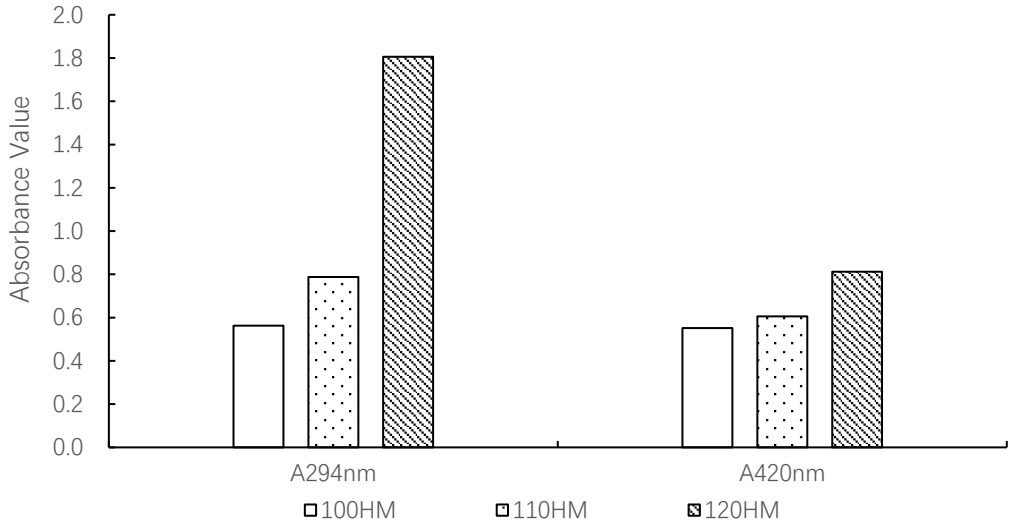

Figure 2. Absorbance values of Maillard products

3.4. Comparison of volatile flavor components in Maillard products

Table 2. The types and relative content of volatile flavor components of Maillard products

Category	Name	Relative content (%)		
		100HM	110HM	120HM
Alcohols	1-Octen-3-ol	20.75	16.45	6.88
	1-Hexanol, 2-ethyl	1.43	1.33	1.29
	1-(1-Butyny) cyclopentanol	—	0.48	0.33
	3,6-Nonadien-1-ol, (E, Z)	0.43	—	0.53
	1-Norbornanemethanol, acetate	2.08	1.86	1.71
	1-Octyn-3-ol, 4-ethyl	0.60	—	—
	Cyclohexanol, 5-methyl-2-(1-methylethyl)	0.51	—	—
	1-Heptanol, 2-propyl	—	0.26	0.98
	trans-2-Dodecen-1-ol	10.04	6.02	3.14

	Cyclohexanol, 2,4-bis (1, 1-dimethylethyl)	0.47	—	—
Subtotal		36.31	26.40	14.85
Ketones	Ethanone, 1-(1H-pyrrol-2-yl)	—	—	1.09
	Ethanone, 1-(2-aminophenyl)	1.11	—	—
	1H-Inden-1-one, 2,3,3a,4,7,7a-hexahydro-, trans	—	—	0.57
	2-Nonen-4-one	—	—	0.64
	2-Nonanone	0.56	—	—
	2-Nonanone, 3-(hydroxymethyl)	—	0.69	0.69
	Bicyclo [2.2.1] heptane-2,5-dione, 1,7,7-trimethyl	0.51	—	0.34
	7-Decen-2-one	0.42	0.42	0.37
	5,9-Undecadien-2-one, 6,10-dimethyl	1.13	0.70	1.16
Subtotal		3.72	1.80	4.86
Aldehyde	Benzaldehyde	2.19	2.81	—
	Benzeneacetaldehyde	—	3.02	6.55
	2,6-Nonadienal, (E, Z)	—	0.28	0.57
	Nonanal	—		2.72
	Lilac aldehyde C	1.20	0.74	1.36
	Decanal	0.58	0.57	1.43
	Furane-2-carboxaldehyde, 5-(4-nitrophenoxymethyl)	—	—	0.68
	2-Dodecenal, (E)	—	0.28	—
	5-Methyl-2-phenyl-2-hexenal	—	—	0.51
	Tridecanal	0.49	0.47	0.58
	7-Tetradecenal, (Z)			0.27
	Tetradecanal	0.20	0.22	3.02
	Pentadecanal-	0.23	—	0.68
	7-Hexadecenal, (Z)	—	—	0.26
	Heptadecanal	0.42	—	1.81
	9-Octadecenal, (Z)	—	—	0.21
	13-Octadecenal, (Z)	—	—	0.18
	Eicosanal-	—	—	0.32
	Tetracosanal	—	—	0.45
	Pentanal, 5-(methylenecyclopropyl)	0.30	—	—
Subtotal		5.59	8.38	21.59
Esters	Methyl salicylate	1.21	—	0.70
	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	—	0.39	0.17
	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	0.21	0.19	0.51
	Sulfurous acid, butyl tridecyl ester	0.20	0.12	—
	Acetic acid, trifluoro-, undecyl ester	—	0.20	—
	Carbonic acid, decyl undecyl ester	—	—	0.28
	Sulfurous acid, cyclohexylmethyl hexadecyl ester	—	0.50	—
	Sulfurous acid, hexyl undecyl ester	0.23	0.30	—
Subtotal		1.85	1.71	1.65

Hydrocarbons	1,3-Heptadiene, 2,3-dimethyl	—	—	0.64
	1-Octene, 6-methyl	0.71	—	0.50
	2-Hexene, 3,5,5-trimethyl	26.02	21.95	5.82
	Decane, 1-iodo	1.17	0.44	0.61
	Undecane	1.09	0.73	—
	3-Dodecyne	1.20	0.89	—
	Cyclopropane, 1-methyl-2-octyl-	—	0.42	—
	Dodecane	1.77	0.96	0.59
	Cyclohexane, 1,2,4,5-tetraethyl	—	0.35	—
	Dodecane, 4,6-dimethyl	0.14	—	—
	3,5-Dimethyldodecane	—	—	0.34
	Tetradecane	4.01	2.48	1.72
	1,7-Dimethyl-4-(1-methylethyl)cyclodecane	0.37	—	—
	Dodecane, 2,6,10-trimethyl	0.27	—	—
	Dodecane, 2,6,11-trimethyl	0.25	—	0.14
	Pentadecane	2.78	1.17	2.24
	1,1'-Biphenyl, 2,2',5,5'-tetramethyl	0.24	0.18	—
	Hexadecane	0.75	0.51	0.48
	Nonane, 2,2,4,4,6,8,8-heptamethyl	0.40	0.23	—
	Pentadecane, 3-methyl	0.20	—	—
	Heptadecane	1.19	0.75	1.05
	Dodecane, 5-cyclohexyl	0.22	—	—
	Dodecane, 6-cyclohexyl	—	0.13	—
	Octadecanal	2.08	1.47	10.80
	10-Methylnonadecane	0.34	—	—
	Hexadecane, 1,1-bis(dodecyloxy)	0.70	—	0.15
	Tritetracontane	0.32	0.18	—
Subtotal		45.48	32.84	23.94
Heterocycles and other categories	Pyrazine, 2,5-dimethyl	—	0.52	—
	Benzothiazole	4.19	2.07	2.90
	Pyrazine, trimethyl	—	5.81	6.54
	Pyrazine, 2-methyl-6-(1-propenyl)-, (E)	—	1.67	—
	2-Methyl-3-propylpyrazine	—	0.43	0.62
	Pyrazine, 3-ethyl-2,5-dimethyl	1.76	6.58	3.25
	Hexanoic acid, 2-ethyl	—	—	0.53
	2-Isoamylpyrazine	—	—	1.08
	2-Isoamyl-6-methylpyrazine	—	—	0.51
	Pyrazine, 2,5-dimethyl-3-(2-methylpropyl)	—	0.38	0.27
	Pyrazine, 2-butyl-3,5-dimethyl	—	0.17	—
	2,5-Dimethyl-3-(2-methylbutyl) pyrazine	—	0.76	—
	Pyrazine, 2,5-dimethyl-3-(3-methylbutyl)	—	2.05	1.45
	Pentanoic acid, 2-methyl-, anhydride	—	—	0.28
	2-Bromo dodecane	—	0.49	—

	4-t-Butyl-2-(1-methyl-2-nitroethyl) cyclohexanone	—	0.34	0.32
	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)	0.46	0.38	0.34
	2-Dodecen-1-yl(-)succinic anhydride	—	0.64	—
	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)	0.16	—	—
	Heptyl tetradecyl ether	—	0.54	—
Subtotal		6.57	22.82	18.08

Note "-" indicates not detected.

Table 3. The quantity and relative content of volatile flavor components of Maillard products

Category	100HM		110HM		120HM	
	Relative content (%)	Quantity (piece)	Relative content (%)	Quantity (piece)	Relative content (%)	Quantity (piece)
Alcohols	36.31	8	26.40	6	14.85	7
Ketones	3.72	5	1.80	3	4.86	6
Aldehyde	5.59	8	8.38	8	21.59	15
Esters	1.85	4	1.71	6	1.65	4
Hydrocarbons	45.48	21	32.84	16	23.94	13
Heterocycles and other categories	6.57	4	22.82	15	18.08	12
Total	99.52	50	93.95	54	84.97	57

As shown in Tables 2 and 3, 50 compounds were identified from 100HM, which mainly containing 8 alcohols (36.31%), 5 ketones (3.72%), 8 aldehydes (5.59%), 4 esters (1.85%), 21 hydrocarbons (46.08%), and 4 heterocyclic and others (6.57%). 54 compounds were identified from 110HM, which mainly containing 6 alcohols (26.40%), 3 ketones (1.80%), 8 aldehydes (8.38%), 6 esters (1.71%), 16 hydrocarbons (32.84%), and 15 heterocyclic and others (22.82%). 57 compounds were identified from 120HM, which mainly containing 7 alcohols (14.85%), 6 ketones (4.86%), 15 aldehydes (21.59%), 4 esters (1.65%), 13 hydrocarbons (23.94%) and 12 heterocycles and others species (18.08%).

4. Conclusion

In sensory evaluation, the highest sensory score was obtained at 110HM, and the lowest overall sensory score was obtained at 120HM. The L* value of 100HM was the largest with a value of 46.12; the L* value of 120HM was the smallest with a value of 35.34. The a* value was the largest under 120HM with a value of 4.46, the a* value of 100HM was the smallest with a value of 0.37. The b* value of 100HM was the largest with a value of 10.45. The absorbance values also showed a regular change with the change of temperature. With the rise in enzymatic reaction temperature, the absorbance of oyster Maillard products at 294nm and 420nm gradually increases. 50,54,57 compounds were identified from 100HM, 110HM, 120HM respectively. The results of this study can provide new ideas for the high-value application of oysters, and the Maillard product of oyster enzymatic hydrolysis solution may have the potential to be a high-quality flavor base material.

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