

Study on the Effect of *Kaempferia galanga* L. Extract on the Quality of Oyster Enzymatic Hydrolysate

Zhao Yang, Biyu Lin, Xueren Chen, Haixia Tang, Huiyi Peng, Chunlan He *

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

* Corresponding author e-mail: hchunlan2012@163.com

Abstract. To improve the quality of oyster enzymatic hydrolysate (OEH), the effect of extract of *Kaempferia galanga* L. (EKG) on improving the flavor of OEH was analyzed, and the influence of different concentrations of extract of EKG (0%, 4%, 8%) on the quality of OEH, chroma and degree of hydrolysis was further discussed, and volatile flavor components was analyzed and identified by solid-phase microextraction-gas chromatography. The results showed that the degree of hydrolysis, color, flavor, taste, and comprehensive evaluation of OEH showed an upward trend with the increase of the amount of EKG. The appearance, taste, and smell of OEH with 8% of EKG were better than those with 0% and 4%, and the color was bright and yellow-green. 17 and 25 flavor components were identified in the OEH of EKG with the addition of 0% and 4% respectively, while 28 flavor components were identified in the OEH of EKG with the addition of 8%, among which eucalyptol, 2-Propenoic acid, 3-phenyl-ethyl ester, and ethyl p-methoxycinnamate were the main volatile components in the OEH of EKG with the addition of 8%, accounting for 8.04%, 30.83% and 16% respectively. The OEH of EKG with the addition of 8% was richer in flavor substances, less fishy and halal flavor, and fresher flavor. Therefore, adding 8% of EKG to OEH was beneficial for quality improvement.

Keywords: *Kaempferia galanga* L., oyster, enzymatic hydrolysate, volatile.

1. Introduction

Oyster is a type of farmed shellfish that is quite common in the world and is one of the most resilient aquatic animals. It is particularly common in temperate tropical waters, where it adheres to other objects with its shell and feeds on tiny seaweed and organic debris in the ocean. Due to the high content of functional peptides and amino acids in oyster meat, the enzymatic hydrolysate produced from it is considered as a high-quality flavor substrate with broad application prospects [1].

1-octen-3-ol, 2-octen-1-ol, and 2, 6-nonanal are unique flavor compounds found in oysters. 2,6-nonanal has a cucumber flavor, while 1-octen-3-ol and 2-octen-1-ol are obtained through degradation of linoleic acid esters or linolenic acid ester hydroperoxides. These two substances have been identified as the main mushroom like aroma compounds unique to oysters in Jinjiang oysters [2]. However, during the enzymatic hydrolysis process, the degradation of lipooxygenase and protein can cause an increase in the fishy, bitter, and halal taste of oysters after enzymatic hydrolysis, which affects the application of flavor substrates in product processing.

Kaempferia galanga L. is a block like rhizome of plants in the ginger family. As a seasoning used in seafood, it not only has a deodorizing effect, but also has the effect of increasing the freshness of the product [3]. To understand the impact of different amounts of ginger extract on the quality of oyster enzymatic hydrolysate, this article analyzes the effects of different amounts of EKG on the quality of OEH from four aspects: sensory analysis, hydrolysis degree, chromaticity analysis, and volatile flavor components.

2. Materials and Methods

2.1. Materials

Oyster meat (frozen storage) was purchased from Raoping, Guangdong. Fresh *Kaempferia galanga* L. was purchased from Longdong Shangshe Market in Guangzhou. Trypsin (4000U/g) and

flavor protease (enzyme activity 500LAPU/g) were purchased from Henan Wanbang Industrial Co., Ltd. Sodium hydroxide and hydrochloric acid were both analytically pure.

2.2. EKG preparation

Kaempferia galanga L. was selected fresh, free from pests and diseases, pollution-free. *Kaempferia galanga* L. was washed and cut into pieces. Add 1:1 mass ratio and put them into the KS-1053 multifunctional cooking machine (Guangzhou Qihe Electric Appliance Co., Ltd., China) to beat the pulp. Filter with gauze to obtain EKG for later use [4].

2.3. OEH preparation

Fresh frozen oyster meat was washed and added twice the mass of ultrapure water, and then add 0%, 4%, and 8% (W/W) of EKG to the KS-1053 multifunctional cooking machine (Guangzhou Qihe Electric Appliance Co., Ltd., China) for beating. Adjusted the pH of the mixture to 7.0, and added 0.2% trypsin and 0.1% flavor protease by weight to the mixture. After stirring evenly and dissolving, place it in an HH-6 digital constant temperature water bath (Changzhou Aohua Instrument Co., Ltd., China) at a temperature of 55 °C for 2 hours of enzymatic hydrolysis, and extinguish the enzyme in boiling water for 20 minutes. After cooling the enzymatic hydrolysate at room temperature, it was centrifuged at 8000 r/min for 10 minutes using an H1850R medical centrifuge (Hunan Xiangyi Centrifuge Instrument Co., Ltd., China) to obtain the supernatant, which was the OEH. The prepared OEH was stored at -18°C for later use [5].

2.4. Sensory evaluation of OEH

Five students who have undergone sensory testing course training were formed into evaluation team members to conduct a comprehensive evaluation of the appearance (0-20 score), taste (0-40 score), and odor (0-40 score) of OEH based on personal preferences in the standard sensory evaluation laboratory [6].

2.5. Determination of hydrolysis degree of OEH

The method for determination of hydrolysis degree was formaldehyde titration method [7].

2.6. Determination of chromaticity of OEH [8]

20 milliliters OEH were loaded into a transparent and smooth plastic bottle, and the measured side of the bottle was marked. The color analysis was performed using an NS810 colorimeter (Shenzhen Sanenchi Technology Co., Ltd., China).

2.7. Detection and analysis of volatile flavor components of OEH

Accurately weigh 3g OEH into a dry headspace extraction bottle, equilibrate at 55 °C for 10 minutes, and then insert 50/30um DVB/CAR/PDMS head (Supelco, USA) into a headspace bottle, adsorb for 1 hour, and then remove the extraction head. Then the flavor components were detected using GCMS-QP 2010 Ultra gas chromatography-mass spectrometry (Shimadzu Institute, Japan) [9].

The detection conditions of the gas chromatography-mass spectrometer were as follows: chromatographic conditions: Rts-5MS packed column (30m ×0.25mm, 0.25um). Using non split injection, 99.999% high-purity helium gas was used as the carrier gas, with a flow rate of 0.8 mL/min. The initial temperature of the column was 35°C, maintained for 2 minutes; 4°C/min rise to 40°C and maintain for 2 minutes; 5°C/min rise to 100°C and maintain for 10 minutes; 10°C/min rise to 230 °C and maintain for 10 minutes. The inlet temperature was 230°C. Desorb at 230°C for 5 minutes. Mass spectrometry conditions: Ionization method was EI. Electronic energy was 70eV; Ion source temperature was 200°C. Quality scanning range were 35-500m/z.

Retrieve the peaks of each color spectrum through the NIST library, remove substances with a similarity of less than 80%, and qualitatively analyze the substances based on literature reports to determine the volatile flavor components. Only compounds with relative content greater than or equal

to 0.1% were counted, and the relative content of each volatile flavor component was determined by area normalization method.

3. Conclusion

3.1. The effect of different concentrations of EKG on the sensory of OEH

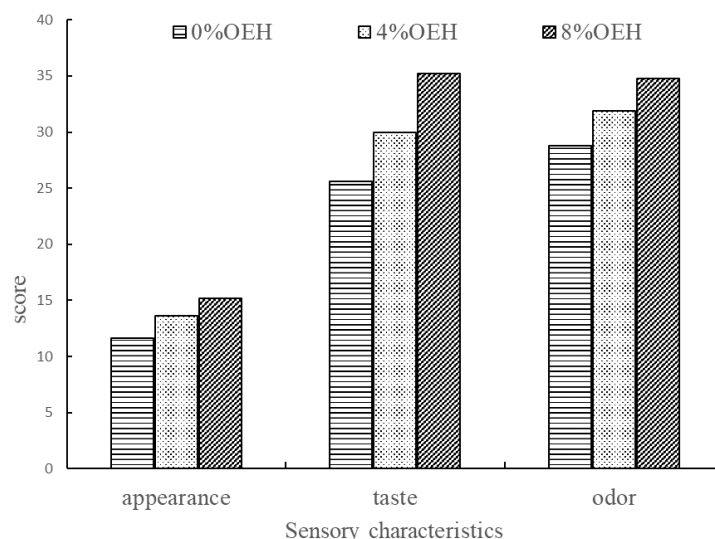


Figure 1. Sensory evaluation of different concentrations of EKG of OEH

As shown in Fig. 1, when the concentrations of EKG were 0%, the total score of sensory analysis was 66. When the addition amount was 4%, the total score was 75.5. When the addition amount was 8%, the total score was the highest, at 85.2. The scores of appearances, taste, and odor all increased with the increase of the concentration of EKG, and the appearance, taste, and odor scores of OEH with EKG added at 8% were significantly different from those of OEH with EKG added at 0% and 4%. The flavor of OEH gradually improves with the increase of EKG concentration, which may be due to the pleasant volatile flavor components of *Kaempferia galanga L.* itself, which not only masks the taste of oyster itself, but also releases the pleasant herbal flavor of *Kaempferia galanga L.* itself.

3.2. The effect of different concentrations of EKG on the hydrolysis degree of OEH

Oyster enzymatic hydrolysis is a complex degradation process, in which the structure and cleavage sites of the raw protein undergo changes. During the enzymatic hydrolysis of oysters, protein macromolecules are degraded into small peptides and free amino acids. From Fig. 2, it can be seen that as the amount of EKG increases, the degree of hydrolysis also increases. When the amount of EKG added was 0%, the degree of hydrolysis of OEH was 25.20%. When the amount of EKG added was 4%, the degree of hydrolysis of OEH was 27.23%. When the amount of EKG added was 8%, the degree of hydrolysis of OEH was 31.02%.

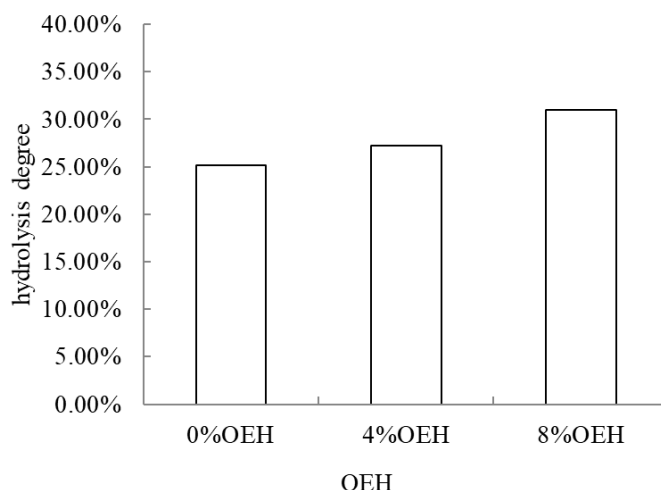


Figure 2. Hydrolysis degree of different concentrations of EKG of OEH

3.3. The effect of different concentrations of EKG on the chromaticity of OEH

Color is an important parameter for studying the quality of the enzymatic hydrolysate. To study the color difference of the OEH after adding different concentrations of EKG, the L * value, a * value, and b * value was measured using a colorimeter, and the color change of the OEH with different concentrations of EKG was studied.

L * represents the brightness and darkness of the color. The smaller the measured value, the darker the sample. The larger the measured value, the brighter the sample. A * represents the redness and greenness of the color. A positive measurement indicates that the sample is closer to red, while a negative measurement indicates that it is closer to green. B * represents the yellow blue hue of the color. A positive measurement indicates that the sample is closer to yellow, while a negative measurement indicates that the sample is closer to blue. From Table 1, the brightness of different amounts of EKG added was higher than that of ultrapure water, and the brightness increases with the increase of EKG added. When the EKG added was 8%, the brightness was the highest. The color of EKG with different amounts added tends to be yellow green, which was more visually acceptable to people.

Table 1. The chromaticity of different concentrations of EKG of OEH

samples	L*	a*	b*
ultrapure water	29.12±0.21	-0.95±0.01	-0.51±0.02
0%OEH	34.03±0.11	-1.4±0.09	-2.27±0.03
4%OEH	35.40±0.75	-1.29±0.03	-2.05±0.05
8%OEH	36.23±0.10	-1.34±0.03	-2.13±0.03

3.4. The effect of different concentrations of EKG on the volatile flavor components of OEH

The volatile components of the OEH were extracted and analyzed by solid-phase microextraction-gas chromatography, retaining compounds with a similarity of $\geq 80\%$. A total of 42 compounds were identified in OEH. 17, 25, and 28 compounds were identified in the OEH with 0%, 4%, and 8% EKG added.

1-octen-3-ol and eucalyptol in alcoholic substances exhibit mushroom like, greasy, earthy, and pungent odors, which can produce unpleasant odors. The above substances contribute significantly to the odor of OEH. Although 1-octen-3-ol was still detected in the OEH after adding EKG, however, its content decreases from 29.75% to 4.23%, and the content of eucalyptol also decreases from 8.61% to 8.04%. Research has found that peppermint p-Mentha-1,5-dien-8-ol and linalool present mint, fruity, and floral aromas, producing a pleasant odor. The highest content of p-Mentha-1,5-dien-8-ol in the OEH with an 8% addition of EKG was 1.9%. Linalool was not detected in the OEH with 0%

and 4% addition of EKG, but Linalool was detected in the OEH with an 8% addition of EKG, with a content of 0.6%.

Aldehydes and esters generally have lower thresholds and significant flavor characteristics for OEH, with very low concentrations producing a strong flavor. Hexanal presents a fishy odor and produces a bad flavor. After adding EKG, this flavor component was not detected. 2-Propenoic acid, 3-phenyl-ethyl ester, and ethyl p-methoxycinnamate exhibit fruity and floral aromas, but were not detected in the addition of 0% EKG of OEH. After adding EKG, these two substances were detected, and the detection concentration increased with the increase of the addition amount. The highest detection content was found in the 8% EKG of OEH, increasing from 25.75% and 10.57% to 30.83% and 16%, respectively.

Ketone substances generally have a sweet floral and fruity aroma. 2-undecanone contributes to the pleasant flavor of the OEH of EKG. It was not detected in the OEH with 0% and 4% addition of EKG, but was detected in the 8% EKG of OEH with a content of 0.18%.

After analysis and comparison, the unpleasant flavor produced by the lipid oxidation and cleavage of OEH, such as hexanal, was not detected. The relative content of 1-octen-3-ol and eucalyptol decreased, and the content in the OEH with an 8% addition of EKG was 4.23% and 8.04%, respectively. In addition, many new and pleasant volatile substances were detected in the OEH of EKG, and the highest content was found in the OEH with an 8% addition of EKG, such as linalool, 2-Propenoic acid, 3-phenyl-ethyl ester, ethyl p-methoxycinnamate and 2-undecanone. 1-octen-3-ol, eucalyptol, 2-Propenoic acid, 3-phenyl-ethyl ester, and ethyl p-methoxycinnamate were the main volatile components in the OEH of EKG. Although unpleasant odors such as 1-octen-3-ol and eucalyptol were still the main flavor components, their relative contents have decreased from 29.75% and 8.61% to 4.23% and 8.04%, respectively. It is indicated that the antioxidant components in EKG effectively inhibit lipid oxidation during oyster enzymatic hydrolysis. In addition, pleasant flavor components such as 2-Propenoic acid, 3-phenyl-ethyl ester, ethyl p-methoxycinnamate, and 2-undecanone have become new volatile components, contributing significant fruit and ginger aromas to the OEH, indicating that EKG has a good effect on improving the flavor of the OEH.

Table 2. The volatile flavor components of different concentrations of EKG of OEH

Name	Relative content (%)		
	0% OEH	4%OEH	8%OEH
1-Octen-3-ol	29.75	6.9	4.23
Cyclodecanol	1.8	—	—
1-(1-Butyny)cyclopentanol	0.78	—	—
4-Heptenal, (Z)	—	6.01	1.93
Eucalyptol	—	8.61	8.04
2-Nonanol	—	0.73	0.68
p-Mentha-1,5-dien-8-ol	—	1.64	1.9
4-methyl	—	0.64	0.91
Terpineol	—	0.21	0.29
Linalool	—	—	0.6
Hexanal	1.45	—	—
Benzaldehyde	0.89	—	—
Nona-(2E,6Z)-dienal	4.1	0.17	1.38
Decanal <n->	0.26	—	—
Tetradecanal	0.46	—	—
Hexadecanal	—	0.17	—
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	0.33	0.2	—
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.58	0.3	0.14
Dibutyl phthalate	0.34	—	—
2-Propenoic acid, 3-phenyl-ethyl ester	—	25.75	30.83
Ethyl p-methoxycinnamate	—	10.57	16
2-Butanone, 4-(2,2-dimethyl-6-methylenecyclohexyl)	0.24	—	—
Car-3-en-2-one	—	—	0.32
2-Undecanone	—	—	0.18
2-Hexene, 3,5,5-trimethyl	21.75	10.55	6.11
Dodecane	0.11	—	—
Tetradecane	0.34	—	—
Pentadecane	0.46	3.14	—
Myrcene	—	0.94	0.62
3-Carene	—	6.3	5.12
Cyclohexane	—	0.16	0.18
Humulene	—	0.15	0.17
Cyperene	—	0.45	0.5
3-t-Butyl-oct-6-en-1-ol	0.63	—	—
Cymene <para->	—	0.6	0.51
Bicyclo	—	5.75	6.15
Cubebene	—	0.19	0.24
2,4-Di-tert-butylphenol	—	0.35	0.33
Cadinene	—	0.52	0.66
Car-3-en-2-one	—	—	0.32
1H-Cycloprop	—	—	0.2
1-Isopropyl-4,7-dimethyl	—	—	0.1

Note: "-" indicates none.

4. Discussion

With the increase of the amount of EKG added, the hydrolysis degree, color, flavor, taste, and comprehensive evaluation of OEH show an upward trend. The appearance, taste, and odor of the OEH with an 8% addition of EKG were better than those with 0% and 4% addition of EKG. The color was bright and yellow green. 17 and 25 flavor components were identified in the OEH of EKG with an addition of 0% and 4%, respectively, while 28 flavor components were identified in the OEH of EKG with an addition of 8%. Among them, eucalyptol, 2-Propenoic acid, 3-phenyl-ethyl ester, and ethyl p-methoxycinnamate were the main volatile components in the OEH of EKG with an addition of 8%, accounting for 8.04%, 30.83%, and 16%, respectively. The addition of 8% EKG of OEH resulted in richer flavor compounds, reduced fishy and halal flavors, and increased freshness. In summary, the OEH with an 8% addition of EKG exhibits the best color, flavor, taste, and comprehensive evaluation.

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