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Small Yellow Ginger Juice Can be Used to Improve the Flavor and Quality of Jinjiang Oyster Meat During Enzymatic Hydrolysis Process

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Abstract. Utilizing Jinjiang oyster in Guangdong Province as the research object, the effects of small yellow ginger juice on the color, taste, hydrolysis degree, and volatile components of oyster enzymatic hydrolysate (OEH) were investigated using sensory evaluation, NS810 colorimeter measurement, formaldehyde titration, Kjeldahl nitrogen determination, and headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS/MS). The results showed that OEH exhibited sea odor, earthy odor, and rancid flavor, which decreased with the increase in the amount of small yellow ginger juice, and were replaced by pleasant smells such as fruit, flower, and lemon. The optimal improvement in the quality of OEH was achieved with the addition of 8% small yellow ginger juice.

Keywords: Jinjiang oyster; small yellow ginger; enzymatic hydrolysate; flavor components.

1. Introduction

Oysters are widespread bivalve shellfish, the most highly produced shellfish in the world, and one of the four majorly cultured shellfish in China. There are diverse oyster populations distributed along the coast of China. Jinjiang oyster is a variety with a lot of breeding and consumption in Guangdong. In recent years, the application of proteases for enzymatically digesting oysters to prepare hydrolyzed products has received widespread attention. Since oysters are rich in proteins, polysaccharides, taurine, and other nutrients, after enzymatic hydrolysis, not only are the nutrients easier for the human body to absorb, but they also exhibit good antioxidant, blood pressure-lowering, hypoglycemic, anti-tumor, and memory-enhancing effects, as well as helping to regulate the immune system and protect the liver and cardiovascular system [1-3]. The application of OEH is severely affected by the fishy odor of oysters themselves and the off-flavors produced during enzymatic hydrolysis [4]. It have been shown that the main reason for the poor flavor of OEH is the oxidation of oyster fat and protein, leading to the formation of fishy substances. After oysters are processed by pulping, the muscle tissue of the oysters is damaged, and oxygen invades in large quantities, resulting in an increase in the contact area between fats and proteins and oxygen in the air, which exacerbates oxidative cleavage. In the oxidation process, compounds such as hexanal and 2, 4-decadienal are produced due to the decomposition of alkoxy radicals, ultimately leading to the OEH developing unpleasant odors such as rancid flavor and fishy odor [5].

Small yellow ginger is a plant that can be used for both edible and medicinal purposes. Its cut surface is pure yellow, with a pungent and spicy flavor, tender meat, rich flavor, and fine fibers [6]. Small yellow ginger extract has anti-tumor, blood sugar-lowering, and cardiovascular system-improving health functions [7]. Small yellow ginger also has a notable deodorizing effect. The protease from small yellow ginger can hydrolyze proteins to enhance the palatability of food, while also facilitating digestion and absorption by the human body, thereby improving its nutritional value [8]. Furthermore, small yellow ginger contains a significant amount of ginger essential oil, which not only possesses the characteristic aromatic scent of ginger but also exhibits antioxidant and antibacterial activities [9].

Jao Y.-C. et al. [10] studied the application of ginger protease in meat tenderization and milk coagulation, providing a reference for the utilization of ginger protease in food processing. Bai Changwang [11] employed mountain ginger heads to improve the flavor of the OEH, resulting in a hydrolysate with a reduced fishy taste and a clear mountain ginger flavor. However, this method has disadvantages, such as complex operation and high cost. Nevertheless, the use of small amounts of ginger juice to improve the off-flavor of OEH has not been reported.

In this study, oysters were employed as raw materials, and different amounts of small yellow ginger juice were added during oyster enzymatic hydrolysis. After enzymatic hydrolysis, color difference, sensory evaluation, hydrolysis degree, and gas chromatography-mass spectrometry techniques were employed to explore the quality changes of oysters during the enzymatic hydrolysis process. Based on these findings, the quality improvement effects of small yellow ginger juice on the OEH were analyzed. The aim of this study is to provide a reference for the subsequent industrial application of oyster enzymatic hydrolysis products in the fields of food and healthcare products, and simultaneously, to furnish a theoretical basis for the further development of the oyster industry.

2. Materials and Methods

2.1. Material and Reagents

Oysters were purchased from RaoPing, Guangdong Province, took the meat from the shell, was stired evenly, and packed it in sealed bags, frozen at -18°C for spare. Small yellow gingers were purchased from Longdong Shangsha Market, Guangzhou. Flavor protease (4000U/g) and trypsin (50000U/g) were purchased from Nanning Pangbo Biological Engineering Co., Ltd.

2.2. Instruments and equipment

DKZ-3B electric constant temperature oscillating water tank: Shanghai Yiheng Scientific Instrument Co., Ltd., NS810 spectrophotometer: Shenzhen Sanenchi Technology Co., Ltd., GCMS-QP2010 Ultra gas chromatography-mass spectrometer (Rts-5MS chromatographic column (30m × 0.25mm, 0.25um): Shimadzu Corporation, Japan, K9840 Kjeldahl nitrogen analyzer: Jinan Haineng Instrument Co., Ltd.

2.3. Preparation of Ginger Juice

The small yellow ginger was cleaned and cut into small pieces. It was then mixed with water in a 1:1 mass ratio of ginger to water, and the mixture was mashed. The mixture was filtered, and the filtrate was retained for later use.

2.4. Preparation of OEH

To thaw the frozen oyster meat, allow it to sit at room temperature before washing it thoroughly. Then, introduce 4% and 8% (w/w) small yellow ginger juice sequentially, maintaining a feed-to-liquid ratio of 1:1, and ensure the mixture was well blended. Post-blending, adjust the pH to 7.0, add 0.2% trypsin and 0.1% flavor protease based on the slurry's total weight, stir uniformly, and incubate at 55°C for 2 hours for enzymatic digestion. Following digestion, immerse the mixture in boiling water for 20 minutes to deactivate the enzymes. Once the enzymatic hydrolysate has cooled to room temperature, centrifuge it at 8000r/min for 10 minutes to separate the supernatant, which was the OEH. The resulting OEH should be stored frozen at -18°C for subsequent use [12].

2.5. Determination of color difference

The color difference was measured at room temperature using an NS810 spectrophotometer. First, different concentrations of OEH were dispensed into separate liquid containers. Then, the brightness L, chromaticity a, and chromaticity b of the OEH samples were measured using the spectrophotometer. Before the measurements, a black and white plate calibrator was used to measure the color difference in parallel five times, and the average value was taken [13].

2.6. Sensory evaluation

The sensory assessment of OEH was conducted in a standardized sensory evaluation chamber, illuminated with white light. Following the methodology outlined by Zhang Meichao et al. [14], the sensory evaluation of OEH involved separate scoring of its appearance (20%), taste (40%), and odor (40%). A panel of five trained sensory evaluators was assembled to conduct the assessment after undergoing a training session.

2.7. Determination of hydrolysis degree

Formaldehyde titration and Kjeldahl nitrogen determination were utilized to quantify the free amino acid nitrogen content of the hydrolysate and the total nitrogen content of the raw material, respectively [15].

2.8. Determination of volatile flavor components

Headspace solid-phase microextraction conditions: weigh 3g of OEH in a headspace extraction vial, equilibrate at 55°C for 10 minutes, insert the DVB/CAR/PDMS extraction head into the headspace vial, pull out the head after 1 h of adsorption, and then insert the extraction needle into the inlet port (250°C) to be resolved for 5 minutes immediately after the end of the extraction. Chromatographic conditions: the carrier gas was high-purity helium (99.999%), and the flow rate was the temperature of the inlet was 230°C, the desorption temperature was 230℃, and the desorption time was 5 minutes. The temperature increase program: the initial temperature of the column was 35℃, kept for 2 min→4℃/min to 40℃, kept for 2min→5℃/min to 100℃, kept for 10min→10℃/min to 230℃, kept for 10min.

Mass spectrometry conditions: electron energy of 70eV, ion source temperature of 200°C, mass scan range of 35–500m/z.

The NIST11 mass spectrometry database was used to search for and analyze the chromatographic peaks. Substances with a similarity of more than 80% were selected and combined with the literature reports to characterize the substances and determine the volatile flavor components. Only compounds with a relative content of ≥0.1% were considered, and the relative content of each volatile flavor component was determined by area normalization [16]. Charts were generated using Origin 2018.

3. Result and Analysis

3.1. The effect of small yellow ginger juice on color difference, sensory score, and hydrolysis degree of OEH

Table 1. The color difference, sensory score and hydrolysis degree of OEH

samples	L*	a*	b*	sensory score	hydrolysis degree
4%OEH	33.99±0.06	-1.29±0.03	-2.08±0.06	69.20±7.66	25.84%±0.00
8%OEH	34.04±0.02	-1.32±0.02	-2.11±0.07	86.00±0.00	27.26%±0.01

The color parameter serves as a quality indicator that reflects the OEH. The changes in brightness L value, chromaticity a value, and chromaticity b value of the small yellow ginger juice oyster hydrolysate with varying contents after enzymatic hydrolysis are detailed in Table 1.

As observed from Table 1, For the brightness L value, while the difference between the two groups is not pronounced, it does increase with the addition of ginger juice, with the OEH prepared with 8% small yellow ginger juice exhibiting the highest value of 34.04. Similarly, for the color a value (redness) and color b value (yellowness), the difference between the two groups is not significant, with the OEH prepared with 8% small yellow ginger juice showing the largest values of -1.32 and -2.11, respectively, indicating a light green and light blue color. The OEH prepared with 4% ginger juice has colored a and b values of -1.28 and -2.08, respectively. It can be inferred that the light blue-green color of the OEH with 4% and 8% small yellow ginger juice added was slightly attenuated. This may be due to the oxidation of fats in oysters during the enzymatic digestion process, which causes their color to gradually change from milky white to yellowish brown. As the enzymatic digestion time extends, their color deepens. The addition of small yellow ginger juice, with its antioxidant content, can slightly delay the oxidation of fats in oysters, resulting in a brighter and more acceptable color of the OEH.

As observed from Table 1, the sensory score of OEH increases with the addition of small yellow ginger juice. The total sensory evaluation score reaches its peak at 86 points when the concentration of small yellow ginger juice reaches 8%. This improvement is primarily attributed to the rising concentration of ginger juice, which not only overshadows the seafood and hydrolytic by-product odors of the oysters but also introduces the characteristic aroma of turmeric, thereby reducing unpleasant odors and elevating the sensory scores. Consequently, small yellow ginger juice enhances the flavor quality of the OEH.

According to Table 1, the degree of hydrolysis of the OEH demonstrates an increasing trend as the concentration of small yellow ginger juice increases. Among the tested samples, the OEH with 8% small yellow ginger juice exhibits the highest degree of hydrolysis, with a value of 27.26%, while the OEH with 4% small yellow ginger juice has a degree of hydrolysis of 25.84%.

3.2. The effect of small yellow ginger juice on flavor volatile components of OEH

Given that the overall flavor characteristics of the OEH could only be qualitatively assessed through sensory evaluation experiments, further quantitative analysis of the volatile flavor substances was deemed necessary to elucidate the changes in their composition and content. Consequently, the volatile flavor substances of the two OEHs were subsequently analyzed using SPME-GC-MS.

The volatile composition of the two OEHs was detailed in Table 2. 33 volatile substances in the two OEH samples were identificated. These volatile substances primarily consisted of aldehydes, alcohols, esters, ketones, alkanes, and terpenes. The predominant flavor components of the pure OEH after enzymatic hydrolysis included (Z)-4-heptenal, Hexanal, Nona-(2E, 6Z)-dienal, 1-Octen-3-ol, Octanal, Tridecanal, and Trans-2-Octen-1-ol, among others [17]. This OEH predominantly exhibited a marine odor, a rancid flavor indicative of oil oxidation, an earthy smell, and a grassy aroma. Conversely, the OEH with the addition of small yellow ginger juice featured Eucalyptol, Linalool, Bornyl acetate, 5-Hepten-2-one, 6-methyl, 1-Octen-3-ol, and Neral as its main flavor components. This OEH displayed a combination of grassy, floral, and fruity aromas, marine flavors, rancid notes, a cool pine-like aroma, and a spicy odor, which aligns with the sensory evaluation results.

In contrast, the volatile substances in OEH with the addition of small yellow ginger juice, which predominantly contributes to unpleasant fishy and fatty flavors, were either reduced or entirely eliminated as the concentration of ginger juice increased. For instance, the content of 1-Octen-3-ol in the 4% OEH decreased to 4.22%, whereas in the 8% OEH, it decreased to 2.39%. Similarly, the content of (Z)-4-heptenal in the 4% OEH decreased to 5.41%, and in the 8% OEH, it decreased to 3.21%. Moreover, Nona-(2E, 6Z)-dienal completely disappeared in the 8% OEH. The volatile components of small yellow ginger, such as Linalool, Eucalyptol, Citronellol, 5-Hepten-2-one, 6-methyl, and trans-Citral (trans-3, 7-Dimethyl-octa-2, 6-dien-1-al), emerged as the new primary flavor components of the OEH, imparting pleasant odors with notes of fruitiness, floral aromas, and a citrusy character, effectively masking the fishy and off-flavors of the OEH. Consequently, it is evident that small yellow ginger juice not only mitigates the undesirable odors resulting from OEH but also significantly enhances the flavor quality of the OEH.

Table 2. The volatile components of OEH

Serial Number	Name	Relative content (%)		Odor characterization
		4%OEH	8%OEH	
1	1-Octen-3-ol	4.22	2.39	Earthy, mushroomy odor
2	Eucalyptol	16.58	17.41	Has a pungent odor similar to camphor
3	Linalool	4.39	4.58	Floral, Woody
4	Fenchyl alcohol	0.1	0.12	—
5	L-.alpha.-Terpineol	3.31	3.77	—
6	Citronellol	0.14	0.55	Rose Scent
7	(-)-Globulol	—	0.28	—
8	1-(1-Butyny) cyclopentanol	0.27	—	—
9	2-Naphthalenemethanol, decahydro-.alpha., .alpha., 4a-trimethyl-8-methylene-, [2R-(2.alpha., 4a.alpha., 8a.beta.)]	0.48	0.53	—
10	Nerolidol	0.38	0.46	Grassy, rose and lily scent
11	(R)-(+)-Beta-citronellol	0.35	—	—
12	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)	1.78	1.55	—
13	(Z)-4-heptenal	5.41	3.21	Grassy, fatty flavor
14	Nona-(2E, 6Z)-dienal	3.79	—	Strong violet and cucumber-like aroma
15	Decanal	—	0.12	Cucumber flavor
16	Neral	1.22	1.07	Lemon Scent
17	trans-3, 7-Dimethyl-octa-2, 6-dien-1-al	1.96	1.65	Lemon Scent
18	5-Hepten-2-one, 6-methyl	19.69	18.13	Fruity
19	2-Heptanone	0.15	0.16	Fruity
20	2-Undecanone	0.16	0.2	Fruity, tallowy
21	2-Hexene, 3, 5, 5-trimethyl	5.62	3.74	—
22	Funebrene <alpha->	0.25	0.29	—
23	Hexadecane <n->	0.2	0.2	—
24	Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis (1-methylethenyl)-, [1S-(1.alpha., 2.beta., 4.beta.)]	0.27	0.32	—
25	Copaene	0.16	0.17	—
26	(1R)-2, 6, 6-Trimethylbicyclo [3.1.1] hept-2-ene	—	0.15	—
27	2, 2, 4-Trimethyl-1, 3-pentanediol diisobutyrate	0.19	0.13	—
28	1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	0.14	—	Slightly aromatic odor
29	Tetradecanoate <isopropyl->	—	0.11	—
30	Bornyl acetate	0.24	0.28	Strong refreshing pine-like aroma and spicy flavor
31	(+)-2-Bornanone	0.53	0.33	Characteristic scent of camphor and a light minty aroma.
32	Isoborneol	0.59	0.22	Has a smell similar to camphor
33	Bicyclo [2.2.1] heptan-2-ol, 1, 7, 7-trimethyl-, (1S-endo)	7.52	6.49	—

Note: "-" indicates none.

It has been proven that glycerides and phospholipids in oysters are degraded by lipase and phospholipase to produce a large amount of free fatty acids. On the one hand, free fatty acids continue to decompose to yield small molecules such as aldehydes and alcohols, on the other hand, free fatty acids are more easily oxidized than glycerides and phospholipids, resulting in a rancid odor [18]. Aldehydes are the substances that have the greatest influence on the flavor of oysters before and after enzymatic hydrolysis. The threshold value of aldehydes was very low, meaning that even a very low concentration can produce a very strong flavor, which has a significant effect on the flavor of oyster liquid [19]. The relative content of aldehydes in the 4% and 8% OEH was 12.38% and 6.05%, respectively. It can be seen that the relative content of aldehydes decreased as the increase of the addition of small yellow ginger juice, and the lowest relative content of aldehydes was found in the enzyme digest with the addition of 8% small yellow ginger juice, which indicates that small yellow ginger juice can significantly inhibit the oxidation of lipids in the OEH and protect the quality of the OEH.

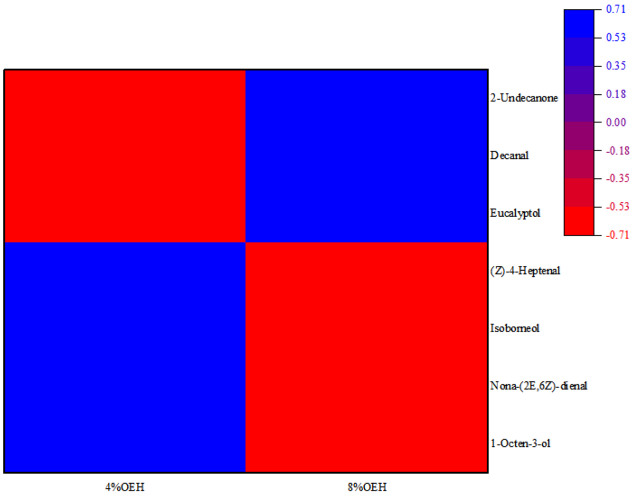

Figure 1. Thermographic analysis of key fishy substances of OEH

In accordance with the literature [20], seven compounds responsible for the fishy odor of oyster meat were identified in this study, encompassing two alcohols, three aldehydes, one ketone, and one terpene. As depicted in Fig. 1, Eucalyptol, a by-product of adding ginger enzyme solution, exhibited a stimulating odor similar to camphor, so its relative content appeared to increase as the reaction progressed. Except for Eucalyptol, Decanal, and 2-Undecanone, the relative contents of the other five substances decreased compared to the reaction with the addition of 4% small yellow ginger juice. Among these, the most obvious decrease was observed for Nona-(2E, 6Z)-dienal, which is a degradation product of fat desaturase acting on linoleic acid, and it disappeared in the 8% OEH. This may be due to the fact that the antioxidant components in ginger juice can effectively inhibit the degradation of linoleic acid, leading to the decrease or even disappearance of its relative content. Overall, the relative contents of fishy odor compounds such as 1-Octen-3-ol and (Z)-4-heptenal decreased to a certain extent, with a more significant effect observed in the 8% ginger juice compared to the 4% ginger juice, indicating that the 8% ginger juice had a better impact on improving the flavor of the OEH.

4. Discussion and Conclusions

The color difference, sensory, hydrolysis degree, and volatile components were analyzed throughout the enzymatic process. The addition of small yellow ginger juice eliminates the fishy and rancid flavors in the OEH and decelerates its spoilage. Combined with other studies, it was found that increasing the addition of ginger juice, on the one hand, could increase the generation of amino nitrogen and the hydrolysis degree, on the other hand, it would make the enzymatic hydrolysate spicy, deepen the color of enzymatic hydrolysate, and aggravate the undesirable odor. Furthermore, due to the low juice yield of small yellow ginger, increasing the amount of small yellow ginger juice would lead to higher costs. Therefore, considering the color difference, sensory evaluation, and volatile components analysis of the OEH, the optimal amount of small yellow ginger juice was selected as 8%.

In conclusion, the incorporation of small yellow ginger juice not only does not negatively impact the taste and appearance of the OEH but also reduces the relative content of unwanted flavor components. Additionally, the antioxidant components in ginger juice could effectively inhibit the lipid oxidation of oysters during oyster enzymatic hydrolysis, which had a beneficial effect on the quality of OEH. Finally, according to the analysis of sensory, color difference, hydrolysis degree, and volatile components, the optimal addition amount of small yellow ginger juice was selected as 8%.

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