

Study on the Effect of Different Compound Proportion of Oyster and Mantis Shrimp on the Quality of Flavor Condiments

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Abstract. Oyster and mantis shrimp were used as raw materials to investigate the effects of their compound ratio on the degree of hydrolysis, sensory and color difference, and the volatile flavor components of the best enzymatic hydrolysis products were analyzed. The results showed that: according to the sensory evaluation score, the best compound ratio of oyster and mantis shrimp was 5:1. Under the conditions of enzymatic hydrolysis time of 2h, enzymatic hydrolysis temperature of 55°C, ratio of trypsin to flavourzyme of 3:1, addition amount of 0.7% and pH of 8.0, the umami taste score and degree of hydrolysis of enzymatic hydrolysis products were 5.71 and 28.22% respectively. In the determination of volatile flavor components, 16 volatile flavor components were detected, mainly including 2 alcohols (34.41%), 6 aldehydes (14.64%), 1 ketone (0.31%), 2 esters (0.69%) and 5 alkanes (2.53%).

Keywords: oyster; mantis shrimp; flavor condiments; volatile flavor components.

1. Introduction

With the rapid development of China's aquaculture industry, people's tastes and requirements for seasoning are becoming more and more diversified, resulting in the seasoning industry becoming more and more prosperous and developing towards advanced and natural^[1]. In particular, flavor condiments prepared from protein enzymatic hydrolysis products are widely popular because of their outstanding flavor, special flavor and high nutritional value^[2].

Oyster is a kind of seafood with low fat content and high protein content. It is popular because of its delicious taste and special flavor^[3]. In recent years, it is found that oysters and their hydrolysates have antibacterial, antioxidant, hypotensive and other physiological activities, so they have high application value in the field of food and drug development^[4]. Based on the good physiological activities of oysters and their hydrolysates, more and more scholars have focused on the preparation of flavor condiments by using protein enzymolysis technology to treat oyster^[5].

Mantis shrimp, also known as skin shrimp and pipa shrimp, is rich in nutrients. It is also an aquatic product with high protein and low fat. It tastes umami, sweet and delicious. It is easily digested and absorbed by the human body. It contains high elements such as calcium, magnesium and phosphorus. The protein content accounts for 20% and the fat content accounts for 0.7%. In addition, it also contains vitamins, astaxanthin and other nutrients^[6]. At present, mantis shrimp is mainly sold fresh or processed into shrimp processed products, and a small amount is processed into flavor sauce, which has a low utilization rate^[7]. Based on the previous research, it is found that oyster enzymatic hydrolysate has fishy smell, and mantis shrimp, as a seafood with better flavor, is mixed to prepare flavor condiments. It is expected to develop nutritional and functional components of oyster enzymatic hydrolysate and good flavor of mantis shrimp.

In this study, oyster and mantis shrimp were used as raw materials. Through single factor experiment, the effects of different compound ratios on enzymatic hydrolysis products were studied, the optimal compound ratio was selected, and the volatile flavor components of the optimal compound ratio were analyzed, so as to provide theoretical and technical support for the research of oyster flavor base.

2. Materials and methods

2.1. Materials and reagents

Fresh oyster meat (purchased from Raoping, Guangdong, frozen at -18°C in the laboratory), fresh mantis shrimp (purchased from Guangzhou Longdong farmers' market, frozen at -18°C in the laboratory), trypsin (enzyme activity: $4000\ \mu\text{g}$, Nanning Pangbo Bioengineering Co., Ltd.), flavourzyme (enzyme activity: $5000\ \mu\text{g}$, Nanning Pangbo Bioengineering Co., Ltd.), sodium hydroxide, hydrochloric acid, formaldehyde, concentrated sulfuric acid, etc. (all analytical pure reagents).

2.2. Main instruments and equipment

pH meter (Mettler-Toledo Instrument (Shanghai) Co., Ltd.); high speed centrifuge (Guangzhou Shenhua Biotechnology Co., Ltd.); constant temperature water bath pot (Changzhou Aohua Instrument Co., Ltd.); colorimeter (Shenzhen San Enchi Technology Co., Ltd.); gas chromatography-mass spectrometry (GCMS-QP2010 Ultra, Japan Shimadzu Production Institute); multifunctional machine (Guangzhou Qi he Electrical Appliances Co., Ltd.); kjeldahl nitrogen analyzer (Jinan Haineng Instrument Co., Ltd.) ; magnetic stirrer (Aika (Guangzhou) Equipment Co., Ltd.).

2.3. Experimental method

2.3.1. Preparation of enzymatic hydrolysates from oyster and mantis shrimp with different compound ratios[8].

After the oyster and mantis shrimp frozen at -18°C were thawed at room temperature, the mass ratio of the washed oyster meat and mantis shrimp was respectively 1:1, 2:1, 3:1, 4:1, 5:1. According to the counterweight of raw materials, deionized water was added to mix and beat, and the enzymatic hydrolysate of oyster and mantis shrimp were used as the control ratio. Add 1mol/L sodium hydroxide solution or 1mol/L hydrochloric acid solution to the enzymolysis solution after stirring evenly to adjust the pH to 8.0. At the same time, trypsin and flavourzyme were added. The enzyme dosage was 0.7% of the solid of aquatic products, and the ratio was 3:1. After stirring evenly, it was put into a 55°C water bath shaking pot for enzymolysis for 2h, and the pH was adjusted every 1h. After enzymolysis, the enzyme was killed in a water bath in boiling water for 15 min, and then centrifuged for 10min under the centrifugation condition of 8000 r/min. After centrifugation, the supernatant was taken.

2.3.2. Analysis of Hydrolysis Degree of Enzymatic Hydrolysis Products with Different Compound Ratios[9].

The degree of hydrolysis requires the determination of amino acid nitrogen content and total nitrogen content. The amino acid nitrogen is determined by formaldehyde titration, and the total nitrogen content was determined by Kjeldahl method. The degree of hydrolysis (DH) was calculated according to the following formula:

$$\text{Degree of hydrolysis (DH)} = \frac{\text{amino acid nitrogen content of enzymatic hydrolysate (g)}}{\text{total nitrogen content in raw materials (g)}} \times 100\%$$

2.3.3. Sensory evaluation of enzymatic hydrolysates with different complex ratios[10].

After the training of screening and understanding five basic flavors, seven sensory assessors were selected. Umami taste (0.2% monosodium glutamate), salty taste (0.35% edible salt), bitter taste (0.02g/L quinine hydrochloride), sour taste (0.04% citric acid) and sweet taste (1% sucrose) were used as taste standards respectively, and they were diluted into a total of 5 gradients, a total of 10 points. The maximum concentration was 10 points. Each diluted concentration was reduced by 2 points, and the minimum concentration was 2 points.

2.3.4. Color difference analysis of enzymatic hydrolysis products with different complex ratios.

Measure the L^* , a^* , b^* values of enzymatic hydrolysates with different complex ratios with a color difference meter, and calculate the total color difference ΔE for 5 times for each sample as follows. The results were expressed as the mean $\pm$ standard deviation^[11].

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

2.3.5. Analysis of volatile components in enzymatic hydrolysate with optimal compound ratio[12].

Weigh 3g of each enzymatic hydrolysate with different mixture ratio, put it into a headspace extraction bottle, balance it at 55°C for 10min, insert the DVB/CAR/PDMS extraction head into the headspace bottle, pull out the extraction head after adsorption for 1h, and use it for GC-MS detection for volatile component identification.

Insert the extraction head into the sample inlet. Chromatographic conditions: Rts-5MS chromatographic column (30m $\times$ 0.25mm, 0.25 μ m). Without partial flow injection, the carrier gas is high-purity helium (99.999%), and the flow rate is 0.8mL/min. Temperature rise procedure: the initial column temperature is 35°C, maintained for 2min, increased to 40°C at 4°C/min, maintained for 2min, then increased to 100°C at 5°C/min, maintained for 10min, and then increased to 230°C at 10°C/min for 10min. The sample inlet temperature is 230°C, the desorption temperature is 230°C, and the desorption time is 5min. The mass spectrometry conditions are: ionization mode EI; The electron energy is 70eV, the ion source temperature is 200°C, and the mass scanning range is 35-500 m/z.

The detected compounds were compared by NIST11 mass spectrometry library, and the compounds with similarity greater than 80 % were retained. The relative contents of volatile flavor components were determined by area normalization method based on the qualitative analysis of the retrieved compounds in related literatures.

3. Result analysis

3.1. Analysis of hydrolysis degree

The degree of hydrolysis is an important index to characterize the degree of protein hydrolysis. Under the conditions of enzymatic hydrolysis time of 2h, enzymatic hydrolysis temperature of 55°C and pH of 8.0, the hydrolysis degree of oyster and mantis shrimp was analyzed with the compound ratio of 1:1, 2:1, 3:1, 4:1 and 5:1 respectively. The results are shown in figure 1.

The degree of hydrolysis of oyster was 23.44% and that of mantis shrimp was 35.67%, with a difference of 12.23%. The degree of hydrolysis first increased and then decreased with the compound ratio between oyster and mantis shrimp, both between 25%-30%, and the fluctuation range was not large. When the compound ratio was 3:1, the degree of hydrolysis reached the maximum in the compound enzymatic hydrolysate, which was 28.75%. With the increase of the proportion of oyster in the compound ratio, the degree of hydrolysis began to decrease.

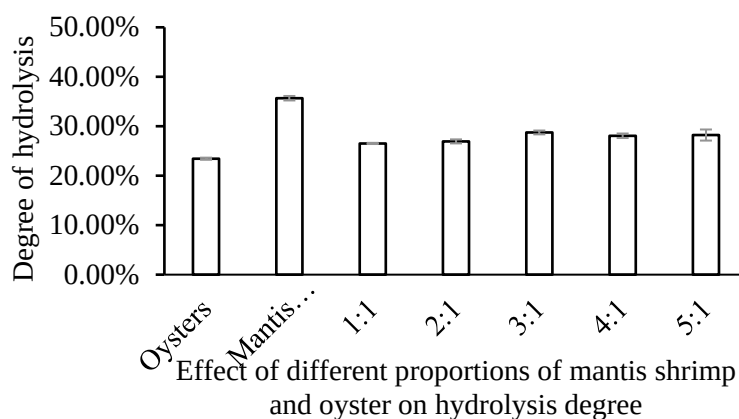


Figure 1. Analysis of hydrolytic degree of enzymatic hydrolysate from oyster and mantis shrimp with different mixed ratio

3.2. Sensory result analysis

The five basic tastes of sour, sweet, bitter, salty and umami are the basic evaluation criteria for sensory evaluation. They mainly stimulate people's taste buds to excite the brain and then form taste [13]. At present, sensory evaluation is the most commonly used method to identify the flavor condiments in food, and it is also the most direct method. The umami taste of each compound ratio was particularly obvious. The umami taste scores of oyster, mantis shrimp, 1:1, 2:1, 3:1, 4:1 and 5:1 hydrolysates were 2.57, 5.28, 4.71, 5.42, 5.28, 5.14 and 5.71 respectively. Compared with the other four basic tastes, the bitterness scores of oyster, mantis shrimp, 1:1, 2:1, 3:1, 4:1 and 5:1 hydrolysates were 1.57, 2.14, 1.57, 1.42, 1.43, 1.14 and 1.28 respectively.

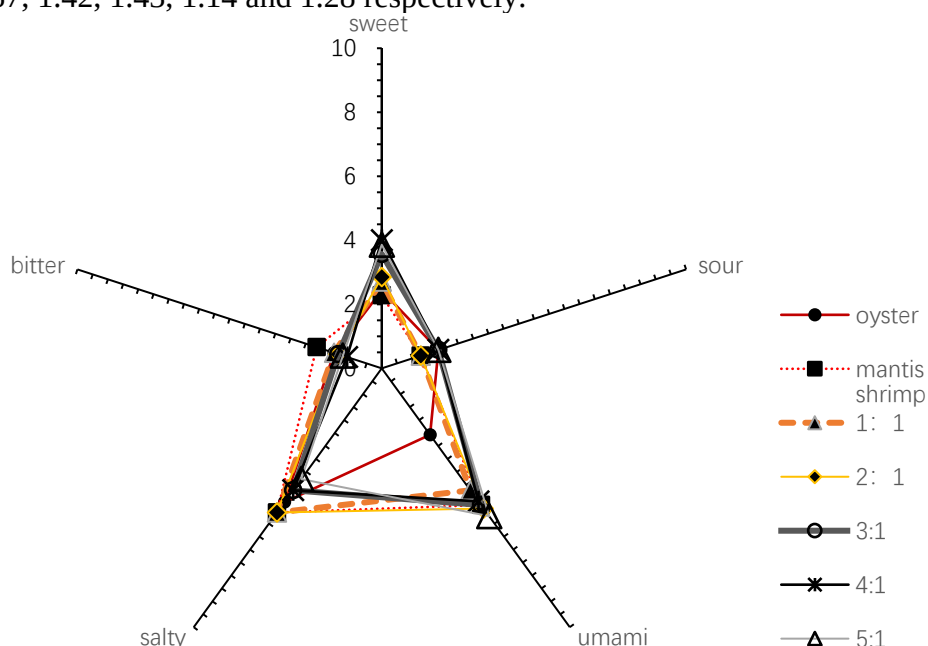


Figure 2. Radar chart of taste of enzymatic hydrolysate of oyster and mantis shrimp with different mixture ratio

3.3. Color difference analysis

The color difference of different enzymatic hydrolysates was analyzed with a color difference meter [14]. The analysis results are shown in table 3. The total color difference of enzymatic hydrolysates with different compound ratios changes with the increase of oyster quantity. With the increase of oyster quantity, the total color difference begins to increase. When the compound ratio

was 4:1, the maximum total color difference reaches 5.30 ± 0.16 , while the total color difference begins to decrease to 4.06 ± 0.17 with the compound ratio of 5:1.

Table 3. Color difference analysis of enzymatic hydrolysates of oyster and mantis shrimp with different mixed ratios

enzymatic hydrolysates	L*	a*	b*	ΔE
oyster	30.98 ± 0.46	-1.44 ± 0.13	-0.83 ± 0.09	2.19 ± 0.59
mantis shrimp	29.86 ± 0.67	-0.76 ± 0.08	-0.66 ± 0.21	1.03 ± 0.97
1:1	32.46 ± 0.10	-1.31 ± 0.32	-1.07 ± 0.05	3.64 ± 0.09
2:1	33.20 ± 0.29	-1.31 ± 0.04	-1.44 ± 0.04	4.50 ± 0.25
3:1	33.40 ± 0.09	-1.26 ± 0.02	-1.45 ± 0.08	4.56 ± 0.11
4:1	34.14 ± 0.12	-1.32 ± 0.07	-1.18 ± 0.03	5.30 ± 0.16
5:1	32.89 ± 0.14	-1.29 ± 0.07	-1.13 ± 0.08	4.06 ± 0.17

3.4. Analysis of volatile components in enzymatic hydrolysate with optimal compound ratio

3.4.1. SPME-GC-MS total ion flow chromatogram.

According to the sensory scores of different complex ratios, it is known that the highest sensory score is 5:1. The enzymatic hydrolysates of individual oyster, individual mantis shrimp and the ratio of oyster and mantis shrimp is 5:1 were selected for volatile flavor component analysis. The total ion flow chromatogram of their volatile flavor components was shown in figure 3-5.

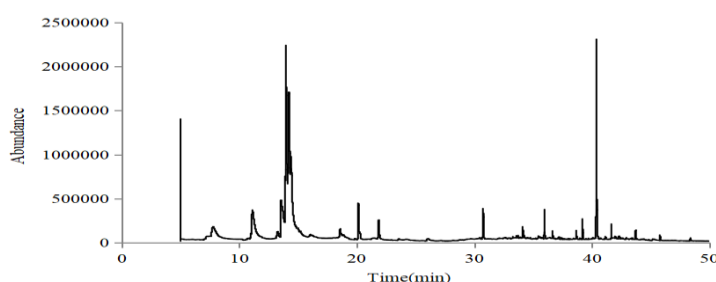


Figure 3. Total ion flow chromatogram of volatile flavor components in enzymatic hydrolysate of single oyster

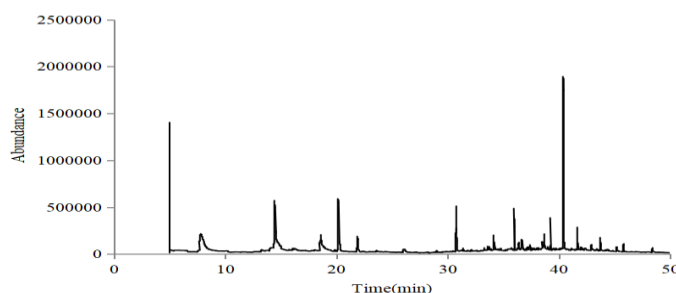


Figure 4. Total ion flow chromatogram of volatile flavor components in enzymatic hydrolysate of individual mantis shrimp

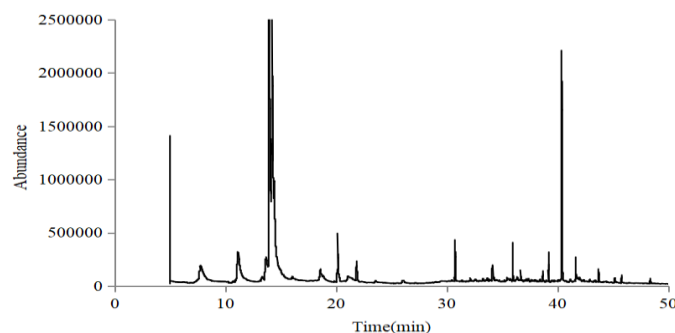


Figure 5. Total ion flow chromatogram of volatile flavor components in enzymatic hydrolysate with a compound ratio of 5:1

From Figure 3 to Figure 5, the total ion flow chromatographic separation degrees of volatile flavor components in the three enzymatic hydrolysates were mainly between 10-20 min and 30-40 min. The volatile flavor components of each compound ratio of enzymatic hydrolysate were extracted by SPME, and analyzed by GC-MS. The compounds with matching degree greater than 80 were retained. A total of 45 volatile components were identified in the three enzymatic hydrolysates. A total of 32, 20, and 16 compounds were identified from oyster alone, mantis shrimp alone, and hydrolysates with a ratio of 5 : 1.

3.4.2. Comparison of volatile flavor components in enzymatic hydrolysates with different compound ratios.

Table 4. Types and relative contents of volatile flavor components in three enzymatic hydrolysate

Category	Compound name	Relative content/%		
		Oyster	Mantis shrimp	5:1
Alcohols	3-t-Butyl-oct-6-en-1-ol	1.96	—	—
	Hexanol <2-ethyl>	0.31	—	—
	1-Octen-3-ol	23.79	—	34.11
	Eucalyptol	0.37	1.46	0.30
	2-Nonanol	—	2.41	—
	Hexadecane, 2,6,11,15-tetramethyl	—	2.11	—
	1-Pentanol, 4-methyl-2-propyl	—	4.96	—
Aldehydes	Benzaldehyde	8.24	—	4.54
	Tridecanal	0.31	—	—
	Pentanal	0.07	—	0.08
	Dodecanal	0.41	—	—
	Octanal	1.02	—	—
	Nonanal	1.15	—	1.06
	2-Hexenal	0.07	—	—
	4-Heptenal, (Z)	9.80	—	7.76
	2-Hexenal, (E)	—	—	0.11
	2,6-Nonadienal, (E,Z)	—	—	1.09
Ketones	2-Heptanone	0.35	—	0.31
	trans-Geranylacetone	0.85	—	—
	2-Heptanone, 6-methyl	0.17	—	—
Esters	Isopropyl palmitate	0.26	1.54	0.44

Alkanes	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.41	5.35	0.25
	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	0.15	3.28	—
	Dibutyl phthalate	—	2.51	—
	Dodecane	0.23	—	—
	Eicosane	0.10	1.78	—
	2-Hexene, 3,5,5-trimethyl	32.3	—	—
	5	5	—	—
	Pentadecane, 3-methyl	0.24	—	—
	2-Hexene, 2,5,5-trimethyl	6.43	—	—
	Hexadecane	0.75	4.11	0.65
	Tetradecane	1.18	2.85	0.78
	Pentadecane	0.77	3.32	0.75
	Heneicosane	0.46	—	0.22
	Hexadecane, 2,6,10,14-tetramethyl	0.10	0.84	—
	4,8,12-Tetradecatrienal, 5,9,13-trimethyl	0.62	—	—
	Hentriacontane	—	4.47	—
	Dodecane, 4,6-dimethyl	—	1.03	—
	Tricosane	—	1.78	—
	Tridecane	—	—	0.13
Heterocycles and other classes	Morpholine, 4-octadecyl	0.11	0.98	—
	Tridecanedial	0.21	—	—
	Pyrazine, 2,5-dimethyl	0.43	—	—
	Methylamine, N,N-dimethyl	—	0.98	—
	Mesitylene	—	8.86	—
	Hexadecyl octyl ether	—	1.30	—

According to table 4 and table 5, 32 compounds were identified in oyster hydrolysate alone, mainly including 4 alcohols (26.43%), 8 aldehydes (21.07%), 3 ketones (1.37%), 3 esters (0.82%), 11 alkanes (43.23%), and 3 heterocyclic and other compounds (0.75%). 20 compounds were identified from the hydrolysate of mantis shrimp, mainly including 4 alcohols (10.94%), 4 esters (12.68%), 8 alkanes (20.18%), 4 heterocycles and others (12.12%). 16 compounds were identified from the hydrolysate of oyster and mantis shrimp with a ratio of 5:1, mainly containing 2 alcohols (34.41%), 6 aldehydes (14.64%), 1 ketone (0.31%), 2 esters (0.69%) and 5 alkanes (2.53%).

Alcohols mainly have sweet plant fragrance, soft fragrance and some fishy smell^[15]. 1-octen-3-ol can produce an alcohol component similar to mushroom aroma, which mainly appears in oysters and was considered to be a unique flavor in oyster^[16]. It can be seen from table 4 that 1-octen-3-ol was detected in the enzymatic hydrolysate of oyster alone and the enzymatic hydrolysate with a compound ratio of 5:1, and the content of 1-octen-3-ol detected in the two enzymatic hydrolysates accounted for the highest content of alcohols, 23.79% and 34.11% respectively, while 1-octen-3-ol was not detected in the enzymatic hydrolysate of mantis shrimp alone.

Because the threshold value of ketones is generally high, the volatile components of ketones in the three hydrolysates are quite different, and the content is low, especially in the single mantis shrimp. Both oyster and 5:1 hydrolysate contain 2-heptanone, with the content of 0.35% and 0.31% respectively. Although esters and alkanes help to improve the overall flavor effect, the content detected in the three hydrolysates is low.

Table 5. Relative content and quantity of various compounds in volatile flavor components of three enzymatic hydrolysates

category	oyster		mantis shrimp		5:1	
	relative content(%)	number (kind)	relative content(%)	number (kind)	relative content(%)	number (kind)
alcohols	26.43	4	10.94	4	34.41	2
aldehydes	21.07	8	0	0	14.64	6
ketones	1.37	3	0	0	0.31	1
esters	0.82	3	12.68	4	0.69	2
alkanes	43.23	11	20.18	8	2.53	5
heterocycles and other classes	0.75	3	12.12	4	0	0
total	93.67	32	55.92	20	52.58	16

4. Conclusions

Through single factor experiment, the best sensory score of oyster and mantis shrimp enzymatic hydrolysate was determined with sensory score as the index under the same enzymatic hydrolysis process conditions. The obtained process conditions were as follows: the compound ratio of oyster and mantis shrimp was 5:1, the enzymatic hydrolysis time was 2h, the enzymatic hydrolysis temperature was 55°C, the ratio of trypsin enzyme to flavourzyme was 3:1, the addition amount was 0.7%, and the pH was 8.0. The umami taste scores of oyster, mantis shrimp, 1:1, 2:1, 3:1, 4:1 and 5:1 enzymatic hydrolysates were 2.57, 5.28, 4.71, 5.42, 5.28, 5.14 and 5.71 respectively. In the determination of volatile flavor components, 16 volatile flavor components were detected, mainly including 2 alcohols (34.41%), 6 aldehydes (14.64%), 1 ketone (0.31%), 2 esters (0.69%), and 5 alkanes (2.53%). In the experiment, it was found that the enzymatic hydrolysate contained some bad flavor, such as the peculiar fishy smell and rancidity taste of seafood. How to reduce these problems is the key to prepare the enzymatic hydrolysate from oyster and mantis shrimp as the flavor base.

Acknowledgments

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