Analysis of volatile components of Tilapia enzymolysis solution after different deodorization treatments

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Abstract. The fishy smell value was used as the detection index, and the three methods of yeast fermentation, activated carbon adsorption, and β-CD embedding were used to deodorize. The results showed that the yeast fermentation had a good deodorization effect on the tilapia enzymolysis solution (TES). The volatile components of TES after deodorization were analyzed by GC-MS, and the results showed that the smelly substances of the TES were mainly aldehydes and alcohols. The main smelly substances of TES are aldehydes and alcohols, and the main volatile compounds are heptanal, 2-heptanal, benzaldehyde, octanal, 2-octenal, (E)-nonanal, 2-octenal, (Z)-, 2,4-nonadienal, heptanol, 1-Octen-3-ol, Octenol <2-trans-> etc.

1. Introduction

Tilapia is China’s main cultured aquatic product, with the characteristics of fast growth, strong resistance, easy breeding, high yield, etc. It is a freshwater farmed fish that is the key scientific research and cultivation of the world aquaculture industry, and is known as one of the main sources of animal protein in the future[1]. Tilapia is a high-protein, low-fat, high-quality protein source with extremely high nutritional value. In order to make full use of these high-quality proteins, the fish proteolysis solution obtained by enzymolysis is rich in biologically active peptides and amino acids, and contains an appropriate proportion of essential amino acids. Its protein titer is higher than that of cow cheese protein, and it is rich in minerals and trace elements. It can be directly absorbed by human intestines and is a high-end food and nutritional supplement[2]. Although fish proteolysis solution is rich in nutrients, it usually has a heavy fishy odor, which directly affects the application prospect of aquatic protein. Therefore, how to remove fishy odor has become an urgent task for aquatic products.

At present, there are many research methods for deodorization of aquatic products, but the mechanism of deodorization is relatively little. Therefore, in this study, the fishy value was used as an indicator to compare the deodorization effects of yeast fermentation, activated carbon adsorption, and β-CD embedding treatment. Furthermore, GC-MS was used to analyze the volatile components of tilapia enzymolysis solution after different deodorization treatments, which provided a certain theoretical basis for further exploring the mechanism of tilapia enzymolysis solution deodorization.
2. Materials and Methods

2.1. Materials
*Tilapia* (fresh) purchased from Zhanjiang Dongfeng Market. Animal protein hydrolase, yeast DV-10, activated carbon, β-CD were purchased from Zhanjiang Keming Technology Co., Ltd.

2.2. Preparation of Tilapia enzymolysis solution
After cleaned, the fresh *tilapia* meat was minced by the Beater, and stored at -20°C until use. Accurately weigh about 10g of frozen *tilapia* fish meat in a 150ml Erlenmeyer flask, add ultrapure water (material-to-liquid ratio=1:3), homogenize the stirrer, and add animal protein hydrolase 0.3% (850u/g), after 3h at 50°C thermostatic oscillator, the enzyme is killed in a 100°C water bath for 10min, after rapid cooling, centrifuge in a 10000r/min centrifuge for 15min, and the supernatant is taken to obtain the *tilapia* enzymolysis solution (TES)[3].

2.3. Deodorization of TES by yeast fermentation
Take 20ml of TES and add 7g/L of yeast powder, put it in a 30°C constant temperature incubator to ferment for 1.5h, then centrifuge at 4000r/min for 10min, take the supernatant for use[4].

2.4. Deodorization of TES by activated carbon adsorption
Add 20ml of TES to a 250ml Erlenmeyer flask, add 1.5g/L of activated carbon, absorb at 40°C for 40min, then centrifuge at 4000r/min for 10min, and take the supernatant for use[5].

2.5. Deodorization treatment of TES by β-CD embedding
Take 20ml of TES in a 250ml Erlenmeyer flask, add 2.5% β-CD, act at 35°C for 30min, then centrifuge at 4000r/min for 10min, and take the supernatant for use[6].

2.6. Sensory evaluation
Sensory evaluation for fishy odour intensity was carried out according to the method of Sae-leaw and Benjakul[7] using 8 trained panelists with the ages of 25-32. Prior to the evaluation, the panelists were trained three times a week. Panelists were trained with standards for two sessions as described by Sae-leaw and Benjakul. To test the samples, all gelatin samples (7.5 g/L) were placed in a sealable plastic cup. The panelists were asked to open the sealable cup and sniff the head space above the samples in order to determine the intensity of fishy odour, with the score from 0 (none) to 5 (extremely strong fishy odour).

2.7. Gas chromatography-mass spectrometry (GC-MS) analysis
GC conditions: Column Rtx-5ms (30m×0.25mm×0.25μm), carrier gas He, flow rate 0.9mL/min, the inlet temperature is 250°C, splitless: the initial temperature is 40°C hold for 3min, increase to 90°C at 5°C/min, then increase to 230°C at 10°C/min, and maintain for 7min[8].

MS conditions: ionization mode EI, electron energy 70eV, emission current 80μA, interface temperature 250°C, ion source temperature 200°C, detection voltage 1000V, mass scanning range 33~450 m/z.

3. Results and Discussion

3.1. Sensory evaluation after different deodorization treatments
According to the results of sensory evaluation analysis as table1, the fishy taste value of TES is up to 4.1±0.12, indicating a heavier smell. But after yeast fermentation, activated carbon adsorption and β-CD embedding treatments, the fishy smell value is lower than that of TES, respectively 1.5±0.51, 2.8±0.33, 3.0±0.27. It means that after the deodorization treatments, the fishy smell value decreases, which shows that all three treatment methods have a certain deodorization effect, but the best treatment is the yeast fermentation with lowest value.
Table 1. Fishy taste value after different deodorization treatments

| different methods | TES    | Yeast Fermentation | Activated Carbon Adsorption | β-CD Embedding |
|-------------------|--------|--------------------|-----------------------------|---------------|
| the fishy taste value | 4.10±0.12 | 1.5±0.51 | 2.8±0.33 | 3.0±0.27 |

3.2. GC-MS analysis

Pure MS was obtained by analyzing the components in different retention time periods. Then compare the MS database to perform a qualitative search on the identified components. TES and three deodorization methods were able to detect 68, 73, 64, 68, substances, including aldehydes, alcohols, alkanes, ketones and other substances. Further using the peak area normalization method [9] for quantitative analysis, the results are shown in Table 2. According to Table 2, there is minimal change before and after deodorization of types and contents of ethers, acids, esters and ketones. The content of nitrogen-containing compounds also decreases, but after activated carbon adsorption treatment, the content increases. After the deodorization treatment, the contents and types of alkanes and olefins increase obviously, but the threshold is higher. The contents and types of aldehydes show higher, and the difference varies greatly before and after deodorization. Finally, the main aldehydes and alcohols were selected for comparative analysis. The results are shown in Table 3.

3.2.1. Aldehydes

According to Table 2, there were 23 types of aldehydes in TES with a content of 49.17%. After deodorization treatment, the aldehydes are significantly reduced in the yeast fermentation, activated carbon adsorption and β-cd embedding. The types of aldehydes were reduced to 14, 14, 17 with contents of 16.43%, 28.93%, and 39.88%, respectively. According to Table 3, the main aldehydes of TES are heptanal, 2-heptanal, benzaldehyde, octanal, 2-octenal, (E)-nonanal, 2-oecenal, (Z)-, 2,4-nonadienal, 2-undecenal. After the yeast fermentation treatment, the aldehydes were reduced in it and heptanal, 2-heptanal, and 2-undecenal were not detected. By the activated carbon adsorption treatment, (E)-nonanal, 2-oecenal, (Z)- and 2-undecenal were not detected, the content of octenal is increased, and the content of other aldehydes is reduced. After β-CD embedding treatment, the aldehydes were reduced except (E)-nonanal. There were no 2-octenal, 2-oecenal, (Z)-, 2,4-nonadienal, 2-undecenal in it. Because of the type and content of aldehydes are relatively high, and the threshold is low, it has a large contribution to the flavor of the TES. The result is similar to YO-SHIWA et al. [10] they used distillation extraction to collect volatile compounds from fresh sardine minced meat and sardine minced meat stored at 10 °C for 24 hours and analyzed by GC-MS. The results show that 2, 4-heptadienal, 3, 5-octadienal, 2,4-decadienal and 2, 4, 7-decadienal are the main components that form the characteristic odor of sardines. Therefore, in this experiment, heptanal, 2-heptanal, benzaldehyde, octanal, 2-octenal, (E)-nonanal, 2-oecenal, (Z)-, 2, 4-nonadienal, 2-undecenal were detected as the main flavor.

3.2.2. Alcohols

There were 15 types of alcohols accounting for about 26.63% in TES according to Table 2. After yeast fermentation treatment, the types of alcohols increases by one, and the content increases to 55.91%. After activated carbon and β-CD embedding treatment, the types and contents of alcohols were reduced, their types were 7, 8 respectively, and their contents were 11.29% and 18.30%, respectively. According to Table 3, the main alcohols of TES are ethanol, heptanol, 1-octen-3-ol, octenol <2-trans>, linalool. After the yeast fermentation treatment, the alcohols increased significantly, and the content of ethanol increased significantly, which was as high as 16.1%. The possible reason is that the yeast used is saccharomyces cerevisiae. During the deodorization process, aldehydes are used as substrates to produce alcohols. The detected saturated alcohols such as hexanol, heptanol. can produce a softer odor. And because their threshold is relatively high, and the contribution to the flavor is very small, thereby
it may reduce the sensory taste value of the TES[11]. After activated carbon and β-CD embedding treatment, its alcohol content is basically reduced, which may be due to its special molecular structure to adsorb or embed the small molecules of the liquid[12]. So, their smell is reduced to a certain extent.

Table 2. The types and contents of volatile components in different deodorization treatment of TES

| Types               | Yeast Fermentation | Activated Carbon Adsorption | β-CD Embedding |
|---------------------|--------------------|-------------------------------|---------------|
|                     | Types Relative Contents (%) | Types Relative Contents (%) | Types Relative Contents (%) | Types Relative Contents (%) |
| Alcohols            | 15 26.63           | 16 55.91                      | 7 11.29       | 8 18.30          |
| Nitrogen-containing compound | 3 9.13 | 2 6.30 | 3 15.52 | 2 2.48 |
| Ethers              | 2 1.25             | 4 2.86                        | 0 0.00        | 0 0.00           |
| Aldehydes           | 23 49.17           | 14 16.43                      | 14 28.93      | 17 39.88        |
| Acids               | 1 1.00             | 0 0.00                        | 0 0.00        | 1 3.86           |
| Ketones             | 8 6.87             | 4 2.14                        | 2 0.58        | 3 1.66           |
| Alkanes             | 7 2.45             | 24 12.81                      | 28 26.03      | 27 25.51        |
| Olefins             | 6 2.59             | 2 1.04                        | 6 12.00       | 7 5.31           |
| Esters              | 3 0.92             | 7 2.51                        | 4 5.66        | 3 3.02           |
| Add up              | 68 100             | 73 100                        | 64 100        | 68 100           |

Table 3. Selected volatile compounds in different treatment of TES

| Compounds          | TES Retention time | Relative Contents (%) | Yeast Retention time | Relative Contents (%) | Activated Carbon Retention time | Relative Contents (%) | β-CD Embedding Retention time | Relative Contents (%) |
|--------------------|--------------------|-----------------------|----------------------|-----------------------|---------------------------------|-----------------------|-------------------------------|-----------------------|
| Ethanol            | 5.316              | 1.97                  | 5.324                | 16.1                  | 5.337                           | 1.17                  | 5.295                         | 0.97                  |
| Heptanol           | 7.897              | 3                     | 7.884                | 9.47                  | 7.918                           | 0.76                  | 7.82                          | 1.51                  |
| 1-Octen-3-ol       | 8.015              | 9.15                  | 8.175                | 4.92                  | ND                              | ND                    | 8.13                          | 4.34                  |
| Octenol <2-trans-> | 10.77              | 1.57                  | 10.136              | 1.52                  | 10.784                          | 1.7                   | 10.73                         | 0.8                   |
| Linalool           | ND                  | ND                    | 11.752              | 1.31                  | 11.743                          | 4.42                  | 11.635                        | 1.95                  |
| Heptanal           | 6.075              | 3.87                  | ND                   | ND                    | 6.082                           | 2.21                  | 6.115                         | 2.77                  |
| 2-Heptanal         | 7.517              | 3.02                  | ND                   | ND                    | 7.529                           | 1.63                  | 7.548                         | 2.15                  |
| Benzaldehyde       | 7.662              | 1.8                   | 7.676                | 0.36                  | 7.671                           | 1.25                  | 7.685                         | 2.44                  |
| Octanal            | 8.83               | 4.11                  | 8.838                | 1.57                  | 8.834                           | 2.64                  | 8.850                         | 3.74                  |
| 2-Octenal, (E)-    | 10.46              | 4.36                  | 10.468               | 2.47                  | 11.857                          | 7.69                  | ND                            | ND                    |
| Nonanal            | 11.858             | 8.93                  | 11.863               | 4.86                  | ND                              | ND                    | 11.864                        | 11.23                 |
| 2-Decenal, (Z)-    | 16.495             | 2.2                   | 16.566               | 1.68                  | ND                              | ND                    | ND                            | ND                    |
| 2,4-Nonadienial    | 18.07              | 4.55                  | 18.156               | 0.6                   | 18.159                          | 0.24                  | ND                            | ND                    |
| 2-Undecenal        | 19.38              | 1.14                  | ND                   | ND                    | ND                              | ND                    | ND                            | ND                    |

4. Conclusion
The main smelly substances of TES are aldehydes and alcohols, and the main volatile compounds are heptanal, 2-heptanal, benzaldehyde, octanal, 2-octenal, (E)-nonanal, 2-oecenal, (Z)-, 2, 4-nonadienal, heptanol, 1-Octen-3-ol, Octenol <2-trans-> etc. Yeast fermentation is better than activated carbon adsorption and β-CD embedding in both deodorization treatment. Fermentation deodorization can better remove odorous substances. The possible reason is that yeast uses the main odorous substance aldehydes to react it as a substrate to produce alcohol. It can remove fishy smell better, and the special aroma produced by fermentation can also mask fishy smell to a certain extent.
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