

METHOD FOR DETECTING FERMENTATION OF STINKY
BRINE

BACKGROUND OF THE INVENTION

Field of the Invention

5 The present invention provides a method of detecting the level of the fermentation of stinky brine.

Description of the Prior Art

 Stinky tofu is a special conventional fermented food in Taiwan, which is usually obtained by an alkaline fermentation. The microorganisms used for the fermentation should be able to hydrolyze proteins to amino acids and peptides. The main microorganisms used in the alkaline fermentation is *Bacillus* sp., which are capable of secreting extracellular enzymes to hydrolyze proteins to amino acids and peptides whereby ammonia is released and the pH value is increased during the fermentation (Wang, J. and Fung, D.Y.C., Alkaline-fermented foods: a review with emphasis on pidan fermentation. Crit. Rev. Microbial. 22:101, 1996).

 A conventional method of producing stinky tofu comprises curing the ingredients by salt and sustaining them in the natural environment; allowing the putrefaction of the ingredients to obtain the stinky brine; immersing and

fermenting the tofu squares in the stinky brine for 4-6 hours; and recovering said tofu from the brine (Su, Y. C. Traditional fermented foods in Taiwan, In Proceedings of the Oriental Fermented Foods, p.15, Food Industry Research and Development Institute, Taiwan, Hsinchu, 1980).

5 The evaluation of fermented stinky brine suitable for producing stinky tofu is conventionally conducted by an experienced stinky-tofu maker, who can tell the difference of the stinky odor of the fermented brines. However, due to the difference of the sensory assessment and the complex flavors of the stinky brines, the evaluation cannot be standardized. There
10 is a need to develop a standardized method for detecting the fermentation level of the stinky brine, in order to determine a fermented stinky brine suitable for producing stinky tofu.

SUMMARY OF THE INVENTION

 The objective of the invention is to provide a method of detecting
15 the fermentation level of a stinky brine.

 The other object of the invention is to provide a method of determining an optimum level of the fermentation of a stinky brine.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the change of pH value, the level of NH_3 released and the total count of the bacteria in the brine composition inoculated with A2 microflora.

Fig. 2 shows the changes of NH_3 level in stinky brine inoculated with A2 microflora and incubated at different temperature.

Fig. 3 shows the effect of the shaking rate on the changes of NH_3 level in the stinky brine inoculated with A2 microflora.

DETAILED DESCRIPTION OF THE INVENTION

The object of the invention is to provide a method of detecting the fermentation level of a stinky brine, which comprises determining the ammonia level of the brine; wherein the higher ammonia level means the higher fermentation level.

According to the invention, it is found that the ammonia will be continuously produced during the fermentation of the stinky brine. In fact, ammonia level is in a positive correlation with the stinky odor. Therefore, the ammonia level can be used to detect the fermentation level of the stinky brine. Through a control of the fermentation level by detecting the ammonia level released, we can standardize the procedures for producing stinky tofu whereby stinky tofu with sanitation, safety and identical quality can be obtained.

The other object of the invention is to provide a method for determining an optimum level of the fermentation of a stinky brine, which comprises

determining the ammonia level of the brine; wherein the optimum level of fermentation is obtained if the ammonia level reaches a predetermined values.

According to the method of the invention, the predetermined value is
5 decided by the sensory evaluation. In an embodiment, the predetermined value is 2200 to 2800mg/L, more preferably 2520mg/L.

The term "a stinky brine" refers to a brine composition which is fermented by an appropriate microorganism. Preferably, the brine composition comprises cabbage or amaranth, bamboo, 20-40 % (w/v) tofu,
10 2.5-10 % (w/v) shrimp and salt. More preferably, the brine composition comprises 20-40 % (w/v) cabbage or amaranth, 10-30 % (w/v) bamboo, 30 % (w/v) tofu, 5 % (w/v) shrimp and 0.5-1.4 % (w/v) salt. The microorganisms suitable for the fermentation of the stinky brine include more preferably microflora naturally present in the stinky brine
15 conventionally used or the purified microflora; most preferably, microflora A2 which was deposited with the China Center For Type Culture Collection (CCTCC) in Wu-han, China, under the Budapest Treaty under accession number 98023 on 8 December 1998. The microflora A2 mainly comprises the genera of *Bacillus*, *Enterococcus* and *Lactobacillus*.

20 According to the invention, we can use Nessler method to determine

the ammonia level of the stinky brine.

According to the invention, we can obtain a scientific and standardized method to detect the fermentation level of the stinky brine in any fermentation condition.

5 The following examples are used for further illustrating the invention, but not for limiting the invention.

Examples

Example 1 Production of Stinky Brine

The A2 microbial group is used in a fermentation of the stinky brine
10 wherein the A2 microbial group mainly comprising the genera of *Bacillus*,
Enterococcus and *Lactobacillus*, CCTCC 98023 deposited with the China
Center For Type Culture Collection (CCTCC). The brine composition
suitable for the A2 microflora comprises 30 % (w/v) cabbage, 20%(w/v)
bamboo, 10%(w/v) tofu, 5%(w/v) shrimp and 1%(w/v) salt. The
15 ingredients were homogenized to obtain a brine medium. Eight hundred
ml of the brine medium was added to a 1000 ml bottle, and then sterilized at
121 °C for 60 minutes. Then, the A2 microflora was inoculated in the
brine composition and then the brine composition was fermented at 30 °C.

Example 2 Determination of Ammonia

20 The stinky brine as prepared in Example 1 was fermented for 10

weeks. The stinky brine was taken weekly to determine the level of ammonia. The stinky brine was centrifuged at 12000 rpm for 10 minutes. Twenty-five ml was taken from the upper part of the centrifuged and diluted 1000 times. Three drops of the mineral stabilizer (HACH) and
5 polyvinyl alcohol dispersing agent and 1 ml of Nessler reagent (HACH) to react for 1 minute. The absorption at 425nm is determined by spectrophotometer (Portable Datalogging Spectrophotometer DR/2010, HACH Co., Ltd., USA). As shown in figure 1, the level of ammonia is increased with the increase of the fermentation time.

10 **Example 3 Determination of Optimal Fermentation of Stinky Brine**

Sixty pieces of tofu squares (4.5cm×4.5cm×1.2cm) were placed in a clean plastic case. They were immersed in 1600 ml of stinky brine at room temperature for four hours. Said tofu was recovered and washed with fresh water to obtain sanitary uncooked stinky tofu.

15 The uncooked stinky tofu produced as above was refrigerated at 4 °C overnight, and then fried. The initial frying temperature was 150 °C. The evaluation of sensory preference was then carried out.

There were fifty sensory evaluators to carry out the evaluation for appearance, flavor, texture and overall liking of stinky tofu (with pickles).

20 The samples were randomly supplied and numbered. The results of the

evaluation (evaluated on a one to nine scale, 1=highly unfavorable, 9=highly favorable to conventional stinky tofu) were entered into a computer and analyzed by Statistical Analysis System (SAS). The evaluation results were shown in Table 1.

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Table 1

Results of sensory preference evaluation for stinky tofu produced by stinky brine by inoculating A2 microbial groups

Fermentation time	Appearance	Flavor	Texture	Overall liking
0.5 month	5.36 ^b	5.98 ^{ab}	6.20 ^a	6.08 ^{ab}
1 month	6.20 ^a	6.44 ^a	6.18 ^a	6.36 ^a
1.5 months	6.32 ^a	6.08 ^{ab}	5.92 ^a	6.12 ^{ab}
2 months	6.32 ^a	5.64 ^b	5.88 ^a	5.78 ^b

Note: a and b mean the mean values with the same letters in each

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column have no significant differences ($p>0.05$).

As shown in table 1, the stinky tofu fermented in one month has a better sensory performance. By comparing the data of table 1 and figure 1, the ammonia concentration of the stinky brine fermented for 1 month is 2520mg/L.

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Example 4 Production of Stinky Tofu

The stinky brine was prepared as described in Example 1 and fermented to have 2520mg/L of ammonia. Tofu squares were immersed in

the stinky brine at room temperature for four hours. Said tofu were recovered and washed with fresh water to obtain sanitary uncooked stinky tofu. After frying the tofu at 150 °C of initial temperature, we obtained stinky tofu with better appearance, flavor, texture and overall liking.

5 **Example 5 Optimum Temperature for Fermentation of Stinky Brine**

The stinky brine was prepared as described in Example 1 and respectively fermented at 20 °C, 25 °C, 30 °C, 35 °C and 40 °C. The ammonia level was determined as described in Example 2. As shown in Figure 2, after fermentation at 35 °C for 273.8 hours, the ammonia level
10 was up to 2520mg/L, which was deemed as the value of the optimum fermentation level. Therefore, the temperature of 35 °C and the fermentation time of 273.8 hours were the optimum conditions for fermentation of stinky brine.

15 **Example 6 Determination of Optimum Shaking Rate for Fermentation of Stinky Brine**

The stinky brine was prepared as described in Example 1. The stinky brine was fermented in a stationary condition and agitated at 75 rpm and 150 rpm. The ammonia level was determined as described in Example 2. As shown in Figure 3, the fermentation of stinky brine in a
20 stationary condition was up to 2520mg/L. The fermentation at stationary

condition is a better condition for fermentation.

Example 7 Determination of the Preferred Capping Method

The stinky brine was prepared as described in Example 1 and fermented in a tightly capped bottle and a loosely capped bottle. The ammonia level was determined as described in Example 2. As shown in Table 2, the ammonia level of the fermentation in the tightly capped bottle was not up to 2520mg/L. Therefore, the optimum fermentation in the tightly capped bottle could not be obtained. Therefore, the fermentation in the loosely capped bottle was preferred.

10 Table 2 The Comparison of Capping Method for the Fermentation with A2 Microflora

Capping method	Ammonia concentration before incubation (mg/L)	Ammonia concentration 4 weeks after incubation (mg/L)
Tightly capped	180	456
Loosely capped	180	3430



We Claim:

1. A method for detecting the level of fermentation of stinky brine, which comprises determining the ammonia level of the brine; wherein a higher ammonia level means a higher level of fermentation.
2. A method for monitoring the level of fermentation of stinky brine for optimal fermentation, which comprises determining the ammonia level of the brine; wherein a optimum level of fermentation is obtained if the ammonia level of the brine reaches a predetermined value.
3. A method of Claim 2, wherein the predetermined level of ammonia is from 2200 to 2800 mg/L.
4. A method of Claim 3, wherein the predetermined level of ammonia is at least about 2520 mg/L.
5. A method of Claim 1, wherein the brine composition comprises cabbage or amaranth, bamboo, about 20-40% (w/v) tofu, about 2.5-10% (w/v) shrimp and salt.
6. A method of Claim 2, wherein the brine composition comprises cabbage or amaranth, bamboo, about 20-40% (w/v) tofu, about 2.5-10% (w/v) shrimp and salt.



7. A method of claim 5, wherein the composition comprises about 20-40% (w/v) cabbage or amaranth, about 10-30% (w/v) bamboo, about 30% (w/v) tofu, about 5% (w/v) shrimp and about 0.5-1.4% (w/v) salt.
8. A method of claim 6, wherein the composition comprises about 20-40% (w/v) cabbage or amaranth, about 10-30% (w/v) bamboo, about 30% (w/v) tofu, about 5% (w/v) shrimp and about 0.5-1.4% (w/v) salt.
9. A method of Claim 1 wherein the brine composition is fermented by a microflora naturally present in the stinky brine.
10. A method of Claim 2 wherein the brine composition is fermented by a microflora naturally present in the stinky brine.
11. A method of Claim 1 wherein the brine composition is fermented by an isolated microflora.
12. A method of Claim 2 wherein the brine composition is fermented by an isolated microflora.
13. A method of claim 9, wherein the microflora mainly comprises the microorganisms of the genera *Bacillus*, *Enterococcus*, and *Lactobacillus*.



14. A method of claim 10, wherein the microflora mainly comprises the microorganisms of the genera *Bacillus*, *Enterococcus*, and *Lactobacillus*.
15. A method of claim 11, wherein the microflora mainly comprises the microorganisms of the genera *Bacillus*, *Enterococcus*, and *Lactobacillus*.
16. A method of claim 12, wherein the microflora mainly comprises the microorganisms of the genera *Bacillus*, *Enterococcus*, and *Lactobacillus*.
17. A method of claim 15, wherein the microflora is substantially the same as the microflora identified as A2 and deposited with the China Center for Type Culture Collection (CCTCC) as accession number 98023.
18. A method of claim 16, wherein the microflora is substantially the same as the microflora identified as A2 and deposited with the China Center for Type Culture Collection (CCTCC) as accession number 98023.
19. A method of claim 17 wherein the microflora is the microflora identified as A2 and deposited with the China Center for Type



Culture Collection (CCTCC) as accession number 98023.

20. A method of claim 18, wherein the microflora is the microflora identified as A2 and deposited with the China Center for Type Culture Collection (CCTCC) as accession number 98023.