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MICROFLORA AND BRINE COMPOSITIONS FOR USE IN THE
PRODUCTION OF UNCOOKED STINKY TOFU (CHAW-TOFU) AND
METHOD FOR PRODUCING UNCOOKED STINKY TOFU (CHAW-TOFU)

Field of the Invention

The present invention provides a microflora suitable for the production of uncooked stinky tofu (Chaw-Tofu), a brine composition for producing uncooked stinky tofu suitable for the growth of the microflora and the method for producing uncooked stinky tofu by using the microflora and brine composition.

Background of the invention

Stinky tofu is a special conventional fermented food in our country. The conventional stinky tofu is produced through open fermentation which comprises curing the ingredients such as vegetables and shrimps etc. by salt; remaining in the natural environment; allowing the putrefaction of the ingredients to thus obtain the stinky brine (the fermentation liquid for stinky tofu); immersing and fermenting the tofu squares in the stinky brine for 4-6 hours; and recovering said tofu from 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).

Stinky tofu is obtained by an alkaline fermentation. For the fermentation, the microorganisms used are able to hydrolyze proteins to amino acids and peptides in accompany with release of ammonia and increase of pH value. The main microorganism used in the alkaline fermentation are *Bacillus* sp. Most of the microorganisms strains used are capable of secreting extracellular enzymes to hydrolyze proteins to amino acids

and peptides and release ammonia 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).

The strains for fermentation mainly exist in the stinky brine conventionally used. Therefore, the quality is not steady; for example, the flavor of the products is varied depending on each batch of the brines, even if comprising the same ingredients. In addition, in the production of stinky brine, the ingredients are only roughly cut and thus not easily degraded. Therefore, the growth of the microorganisms is slow. In general, it takes more than six months to complete the fermentation. Owing to the use of open fermentation, the odor produced in the fermentation cause the insects to gather together and lay eggs. The sanitation is very poor. Moreover, a variety of pathogenic and fungal infections harmful to human health could happen easily.

To develop a method for producing uncooked stinky tofu for sanitation, safety and high quality purposes is necessary

Summary of the invention

One objective of the invention is to provide a microflora suitable for the production of uncooked stinky tofu, which is obtained by screening and identification from conventional stinky brine. Therefore, a production of uncooked stinky tofu having good flavor and sanitation using the microflora is possible.

One objective of the invention is to provide a brine composition for producing the uncooked stinky tofu suitable for the growth of the microflora of the invention, and the preparation

Another objective of the invention is to provide a method for producing uncooked stinky tofu which utilizes said microflora and brine composition. The uncooked stinky tofu can be used for the production of the eatable stinky tofu by process methods such as steaming, cooking, frying and stewing with soysauce.

Brief description of the invention

The object of the invention is to provide a microflora suitable for the production of stinky tofu.

Lai, et al. has studied the production of stinky tofu wherein the screening of microorganisms for brewing stinky tofu based on the capability of hydrolyzing protein (Lai, M. N., Production of Chaw-Tofu, Food Industry 9:3 pp.25-26 (1997)). The bacteria capable of hydrolyzing protein are isolated from conventional stinky brines wherein the major of the microorganisms strains are *B. megaterium*, *B. polymyxa*, *B. pumilus* and *B. subtilis*, in which the later two strains are the major strains for fermentation (Lee, S. F., Wang, C. B., and Chang, P. P., The Isolation and Identification of Protein hydrolyzing Bacteria from Chaw-Tofu, Food Science 23:1 (1996)).

However, we found that the fermentation in the brine using a single strain is not good. According to the present invention, a microflora for fermentation was obtained by screening and identification from the conventional stinky brine.

According to the invention, two main microflorae, A2 microbial group and S3 microbial group were obtained. The screening step comprises collecting the stinky brine samples from various places in Taiwan, diluting and plating the collected stinky brine samples with a stinky odor, selecting the desired microflora. After identification, A2 microbial

group mainly comprises *Bacillus sp.*, *Enterococcus sp.* and *Lactobacillus sp.* and S3 microbial group mainly comprises *Bacillus sp.* and *Enterococcus sp.*

The identification of the microflora was conducted by the Microbial Identification System (Miller, L. and Berger, J. (1985) Bacteria Identification by Gas Chromatography of Whole Cell Fatty Acids, HP Application Note 288:41 (1985).) According to the standard operation steps and analysis methods, the fatty acid methylated esters (FAMES) are prepared by collecting bacterial cells, saponification, methylation, extraction and washing with an alkaline solution. The resulting FAMES are analyzed with a Hewlett-Packard model HP 5890A gas chromatography which was equipped with a capillary column, a flame ionization detector, a 7673B automatic sampler, and a 3396B Integrater. Identification of the FAME fingerprints of isolates were performed using the MIDI database.

According to the identification mentioned above, the A2 microbial group mainly comprises *Bacillus sphaericus*, *Enterococcus sp.* and *Lactobacillus sp.*, which was deposited with China Center For Type Culture Collection (CCTCC) under the Budapest Treaty under accession number 98023 on 8 December 1998. The S3 group mainly comprises *Bacillus sphaericus*, *Enterococcus avium*, *Enterococcus casseliflavus* and *Enterococcus durans*, which was deposited with China Center For Type Culture Collection (CCTCC) under the Budapest Treaty under accession number 98024 on 8 December 1998

Further, the invention provides brine compositions suitable for the growth of the microflorae to produce uncooked stinky tofu, including a brine composition most

suitable for the fermentation of A2 microbial group, and a brine composition most suitable for the fermentation of S3 microbial group. The brine composition suitable for the fermentation of A2 microbial group is composed of cabbage or amaranth, bamboo, tofu, shrimp and salt. The brine composition suitable for the fermentation of S3 is composed of cabbage or amaranth, bamboo, tofu and salt, does not include shrimp.

The brine composition suitable for the fermentation of A2 microbial group preferably includes cabbage or amaranth, bamboo, 20-40 % (w/v) tofu, 2.5-10 % (w/v) shrimp and salt. The brine composition suitable for the fermentation of S3 microbial group preferably includes cabbage or amaranth, bamboo, 30-50 % (w/v) tofu and salt.

The brine composition suitable for the fermentation of A2 group more preferably includes 20-40 % (w/v) cabbage or amaranth, 10-30 % (w/v) bamboo, 30 % (w/v) tofu, 5 % (w/v) shrimp and 0.5-1.5 % (w/v) salt. The brine composition suitable for the fermentation of S3 group more preferably includes 20-40 % (w/v) cabbage or amaranth, 10-30 % (w/v) bamboo, 40% (w/v) tofu and 0.5-2 % (w/v) salt.

According to the invention, the stinky brine, which is obtained from the fermentation of A2 microbial group inoculated in the brine composition suitable for the fermentation of A2 microbial group, which can be used to produce uncooked stinky tofu having better flavor and preference. The stinky brine, which is obtained from fermentation of S3 microbial group inoculated to brine composition suitable for the fermentation of S3 microbial group, which can be used to produce uncooked stinky tofu having better appearance, flavor, texture and preference. However, in view of the poor results of the sensory preference evaluation of stinky tofu produced, the stinky brine by inoculating S3

microbial group to brine composition suitable for the fermentation of A2 microbial group. S3 microbial group is not suitable for the fermentation of brine composition containing shrimp.

The method of the invention for preparing stinky brine useful for the production of uncooked stinky tofu comprises a direct inoculation of the freeze-dried bacteria or inoculation after the incubation of the bacteria on plate, or a transformation of the stinky brine to sterilized brine composition, a fermentation in an closed-style to produce stinky brine.

The invention also provides a method for preparing uncooked stinky tofu, which uses the microflora and the brine composition of the invention. The method comprises pouring the tofu squares into the fermented stinky brine produced from the microflora and brine composition of the invention; and immersing the tofu for 0.5-12 hours, preferably 4-8 hours, at room temperature. The resulting uncooked stinky tofu should be washed with fresh water. The uncooked stinky tofu of the invention is suitable for the production of the eatable stinky tofu by such as steaming, cooking, frying and stewing steps, with soysauce.

The following examples are used for further illustrating the microflora, brine composition used for fermentation and method for preparing uncooked stinky tofu of the invention, but not for limiting the invention.

Examples

Example 1

Screening and Preservation of the Microflora of the Invention

Ten samples of the stinky brines were collected from various locations in Taiwan two samples were produced by the inventors, as shown in Table 1. Ten mL of stinky brine was transferred to the sterilized brine medium. After the submerged fermentation at 30 °C for one month, three operators smelt the fermented brines to determine which brine has a stinky odor. The above step was repeated to make sure the production of the stinky odors. The fermented brines transferred from samples B, G and J did not produce the stinky odor. The transferred brines medium with a stinky odor after the fermentation were diluted and plated into TSA medium. After the aerobic incubation at 30 °C for 24 hours, the bacteria in each of the dilutions were respectively scraped by loop and inoculated to another sterilized brine medium. After one month of the close style of fermentation at 30 °C, the same operators smelt the fermented brines and determine which dilutions still have the stinky odor. The types of the microflora were decreased and the essential microflora thereof were determined. The results were shown in Table 2. The fermented brine inoculated with microflora at 10^3 x dilution with a stinky odor were E, S and W. The fermented brine inoculated with microflora at 10^2 x dilution with a stinky order were A and F. In addition, the fermented brines inoculated with microflora through dilution and plating of the samples C, D, H and I did not produce stinky orders. The desired microbial groups were preserved by freezing and re-designated as A2, E3, F2, S3 and W3. The microbial groups were activated from the frozen culture and inoculated in the brine composition to carry out the stinky odor-production test. The

results were shown in Table 2. The microbial groups A2 and S3 produced a stinky odor after the activation.

For identifying the species contained in A2 microbial group and S3 microbial group, the microflora were diluted and plated on TSA medium. Isolates were randomly selected after the incubation aerobically at 30 °C for 2 days and were identified by the Microbial Identification System. We found that A2 microbial group was mainly composed of *Bacillus sphaericus*, *Enterococcus* and *Lactobacillus* sp. S3 microbial group was mainly composed of *Bacillus sphaericus*, *Enterococcus avium*, *Enterococcus casseliflavus* and *Enterococcus durans*.

Table 1

The pH values, NaCl percentages and total counts of microorganisms of stinky brine samples collected in Taiwan

Sample No.	Sampling Place	pH	NaCl %	Total Counts of Microorganisms (cfu/mL)
A	Wang Chi Stinky Tofu, Kuang Fu Road, Hsinchu City	5.72	1.8	9.0×10^8 (aerobic) 8.5×10^8 (anaerobic)
B	Stinky tofu vender on Chung Cheng road, Hsinchu City	5.34	1.3	1.3×10^8 (aerobic) 1.3×10^8 (anaerobic)
C	Stinky tofu vender on Tung Hwa street Taipei City	5.54	1.9	5.6×10^7 (aerobic) 1.1×10^8 (anaerobic)
D	Du Chaw Chih Chia, Kuang Chou Street, Taipei	7.72	1.4	9.0×10^7 (aerobic) 2.7×10^8 (anaerobic)

E	Stinky tofu store on Lin Yi street, Taipei	5.64	2.9	1.0×10^9 (aerobic) 1.0×10^9 (anaerobic)
F	Ku Ting Market, Taipei	5.20	1.8	1.3×10^9 (aerobic) 1.4×10^9 (anaerobic)
G	Wu Miao Market, Kaohsiung	6.12	1.5	1.4×10^7 (aerobic) 2.4×10^7 (anaerobic)
S	South Men Market, Taipei	6.08	2.2	1.8×10^9 (aerobic) 1.5×10^9 (anaerobic)
W	West Men Market, Taipei	6.85	7.3	3.1×10^8 (aerobic) 6.0×10^8 (anaerobic)
H	Yi Yin Co., Da Pi, Yunlin	—	—	—
I	Conventional stinky brine made by ourselves (1)	—	—	—
J	Conventional stinky brine made by ourselves (2)	—	—	—

Table 2

Results of stinky odor production at different screening steps in the process of screening microflora from stinky brine samples

Sample No.	Transfer	Inoculation with microflora diluted and inoculated on plate (dilution folds)	Inoculation with microflora activated from frozen culture
A	+ *	$+(10^2)$	+
B			
C	+		

D	+		
E	+	$+(10^3)$	
F	+	$+(10^2)$	
G			
S	+	$+(10^3)$	+
W	+	$+(10^3)$	
H	+		
I	+		
J			

* : + represents the production of stinky odor

Example 2

Brine Composition and Production of Stinky Brine

The brine composition suitable for the fermentation of A2 microbial group comprised 30 % (w/v) cabbage, 20%(w/v) bamboo, 30%(w/v) tofu, 5%(w/v) shrimp and 1%(w/v) salt.

The brine composition suitable for the fermentation of S3 microbial group comprised 30%(w/v) cabbage, 20%(w/v) bamboo, 40%(w/v) tofu and 1%(w/v) salt. After the ingredients were homogenized, 200ml brine medium was poured into 250ml serum bottle and then sterilized at 121 °C for 45 minutes. Eight hundred ml of the brine medium in a 1000 ml serum bottle was sterilized at 121 °C for 60 minutes.

The microflora for fermenting the brine was activated from frozen culture Seventy-five

μl bacterial liquid was even plated on TSA (Trypticase Soy Agar (BBL, Maryland)) medium and was incubated aerobically at 30 °C for 24 hours. The bacteria were scraped from plate by loop and inoculated to the sterilized brine medium. The close style of fermentation was carried out at 30 °C for one month.

Example 3

Production of Uncooked Stinky Tofu

Sixty pieces of tofu squares were placed in a clean plastic case. They were immersed in 1600 ml of stinky brine at room temperature for four hours. The uncooked stinky tofu were recovered and washed with fresh water to obtain sanitary uncooked stinky tofu.

Example 4

Sensory Preference Evaluation

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

There were fifty sensory evaluators to carry out the sensory preference evaluation for appearance, flavor, texture and preference of stinky tofu (with pickles). The samples were randomly supplied and numbered. The results of sensory preference evaluation (evaluated on the basis of nine score, 1=highly unfavorable, 9=highly favorable to conventional stinky tofu) were treated with a computer and analyzed by Statistical Analysis System (SAS). The evaluation results were shown in Tables 3 and 4

Table 3

Results of sensory preference evaluation for stinky tofu produced by inoculating the A2 and S3 microbial groups to brine composition containing shrimp

Microflora No.	Appearance	Flavor	Texture	Preference
A2	6.28 ^a	5.96 ^a	6.14 ^a	6.34 ^a
S3	5.56 ^b	5.20 ^b	5.62 ^b	5.42 ^b

a and b: The mean values with the same letters in each column have no significant differences.

Table 4

Results of sensory preference evaluation for stinky tofu produced by inoculating A2 and S3 microbial groups to brine composition not containing shrimp.

Microflora No.	Appearance	Flavor	Texture	Preference
A2	6.30 ^a	5.86 ^{ab}	6.04 ^a	6.08 ^b
S3	6.36 ^a	6.50 ^a	6.28 ^a	6.78 ^a

We claims:

1. A microflora suitable for the production of uncooked stinky tofu designated A2, mainly comprising the genus of *Bacillus*, *Enterococcus* and *Lactobacillus*, which was deposited with China Center For Type Culture Collection (CCTCC) under the Budapest Treaty under accession number 98023 on 8 December 1998.
2. A microflora according to Claim 1, wherein the microflora mainly comprises *Bacillus sphaericus*, *Enterococcus sp.* and *Lactobacillus sp.*
3. A microflora suitable for the production of uncooked stinky tofu designated S3, mainly comprising the genus of *Bacillus*, *Enterococcus* and *Enterococcus*, which was deposited with China Center For Type Culture Collection (CCTCC) under the Budapest Treaty under accession number 98024 on 8 December 1998
4. A microflora according to Claim 2, wherein the microflora mainly comprises *Bacillus sphaericus*, *Enterococcus avium*, *Enterococcus casseliflavus* and *Enterococcus durans*.