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DESCRIPTION

METHOD OF PRODUCING A SEASONING LIQUID

FIELD OF THE INVENTION

The present invention relates to an improvement of a high-temperature salt-free brewing method for soy sauce koji. In particular, the present invention relates to a method of obtaining in about 20 days a seasoning liquid with no unpleasant taste or odor, with a very high rate of nitrogen use, which involves preventing putrefaction of a soy sauce moromi mash obtainable by admixing soy sauce koji and water (or brine), and at the same time, shortening the period for lysis of the moromi mash with koji mold enzymes.

BACKGROUND OF THE INVENTION

Japanese traditional soy sauce uses protein and starch materials. As a protein material defatted soybeans or soybeans are predominantly used, and as a starch material, wheat is predominantly used. The formulation ratio of protein material and starch material that is used is approximately 1-to-1 by volume. Further, defatted soybeans are placed in a steamer and watered to 110-140% (w/w), and soybeans are placed in a steamer after selecting and washing, then after soaking in water, water is drained and then the soybeans are steamed for approximately 10 minutes with saturated water vapor, at 2.0 kg/cm² (gauge pressure). (At present, large manufacturers use continuous steamers.) The well steamed defatted soybeans or soybeans are allowed to cool (cooled) to about 35-40°C and thereafter, wheat is graded, roasted, and then wheat that is milled to about 4-tenths, and seed koji mold is added and mixed therewith. This mixture is placed in a koji-making chamber (large manufacturers use automatic koji-making devices), and while regulating the temperature koji making is performed for 3 to 4 days. Thereafter, koji is removed from the koji room. This removed koji is placed in a fermentation tank, and then salt water prepared to a suitable concentration by dissolving common salt in purified water, is mixed in (this is referred to as incorporation(fermentation)) to prepare a moromi mash. Fermentation and maturation of this moromi mash is performed for about 6 to 10 months while stirring occasionally. Matured moromi mash is wrapped in a nylon filter cloth, which is stacked, placed in a press, and liquid is pressed out. The obtained liquid (moromi mash liquid) is referred to as unrefined soy sauce. A small amount of brine is added to this unrefined soy sauce, to standardize this component. It is then pasteurized, and after adding ethanol or a biocide, clarified by removal of lees. Soy sauce that passes examination is packed into well-washed clean bottles, cans etc., and after sealing with a cap or plug, a label is applied and it is shipped as soy sauce (a product).

In the method of manufacturing the above soy sauce, the soy sauce moromi mash comprises large quantities of protein but it can nevertheless openly be allowed to stand



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without putrefaction occurring because the koji is incorporated in a strong brine and due to this immense osmotic pressure, putrefactive microorganisms (putrefactive bacteria and film forming yeasts) cannot propagate. On the other hand, it is well known that this concentrated common salt, while inhibiting putrefaction due to proliferation of putrefactive microorganisms, simultaneously imparts a strong inhibitory against the action of protease secreted from koji mold and useful bacteria, and that for this reason, the maturation period in soy sauce brewing is very long.

Therefore, if koji is digested in a salt-free or low salt (reduced salt) state, proteins can be lysed into amino acids very rapidly. (This method is called salt-free brewing.) However, if digestion is performed in a salt-free or low salt environment, while protease works well, at the same time, the phenomenon of putrefaction occurs where putrefactive bacteria (predominantly, aerobic bacilli such as *Bacillus mesentericus*, *Bacillus subtilis*, *Bacillus natto*; and anaerobic bacilli such as lactic acid bacteria, acetic acid bacteria, butyric acid bacteria, etc.) rapidly begin to propagate, the amino acids generated from proteins are broken down to form various organic acids, and these organic acids are further broken down into various foul-smelling compounds, so there is the disadvantage that the proliferation of these putrefactive bacteria must be suppressed by some method.

Here, attempts to brew soy sauce in very short periods by eliminating common salt and preventing the proliferation and activity of putrefactive bacteria by using extremely high temperatures instead of by using common salt, have been examined by many scholars since ages old. (This method is referred to as a high-temperature salt-free brewing method.) However, there are as yet no examples of industrial success.

That is to say, this method is based on the principle that if the temperature of the moromi mash is uniformly 50°C or more, even if it is completely salt-free, putrefaction is retarded, and while on the whole decomposition by microbes no longer occurs, koji mold enzymes work extremely well to lyse the moromi mash, and soy sauce flavor rapidly evolves, thereby enabling speed brewing.

This method would appear likely to work well at first glance, however, in contrast to experiments conducted in the laboratory, where this is actually performed in a factory, increasing the temperature of large quantities of moromi mash from room temperature to 50°C, or maintaining large quantities of moromi mash uniformly at 50°C is extremely difficult. Inevitably, the initial temperature of the moromi mash cools in parts to 50°C or below, decomposition occurs, and this finally proceeds to affect the entire moromi mash, leading to almost certain failure. Thus, it is common sense within this industry that it is safer not to perform this method.

As a conventional high-temperature salt-free brewing method, a method of producing soy sauce is known (Japanese Patent No. 2659105) which comprises a combination of a protein zymolysis step in which zymolysis of soy sauce koji is performed in a salt-free to low salt environment for 3 to 8 hours at 45-60°C, and a fermentation step in which common salt is added to the zymolyzed moromi mash

to a common salt concentration of 15-19% (w/v), and lactic acid bacteria and/or yeast fermentation is performed.

However, there are disadvantages such that where the protein zymolysis step in which zymolysis of soy sauce koji is conducted for 3 to 8 hours in salt-free to low salt conditions at 45-60°C, is actually performed in a factory, time is required to increase the temperature of a large amount of moromi mash from room temperature to 45-60°C, and during this time contamination by, and proliferation, of putrefactive bacteria becomes a problem. Further, it is extremely difficult to maintain a large amount of moromi mash uniformly at this temperature so inevitably the moromi mash tends to cool somewhat from the initial temperature and fall to 50°C or lower, whereupon it decomposes, and putrefaction cannot be prevented.

For this reason, it is clear that there is the significant disadvantage with this method in that in order to prevent decomposition, digestion must be completed in a very short time of 3 to 8 hours, and in this protein zymolysis step, the rate of nitrogen material use is very low at 15-40%.

Therefore, in this method, there is the disadvantage that after the protein zymolysis step, in the fermentation step, a large amount of common salt, 15-19% (w/v), needs to be added to the moromi mash to inhibit putrefaction due to the proliferation of putrefactive bacteria, and at the same time further lyse the moromi mash with various enzymes such as protease secreted from the koji mold and useful microorganisms.

Further, in the fermentation step of this method, acidification by addition of a culture product of *Pediococcus halophilus* is performed to prepare (the moromi) to a pH of 5.0-5.2. Thereafter, the moromi is inoculated with culture products of *Saccharomyces rouxii* and/or *Torulopsis etchellsii*, and fermentation is performed for 3 to 6 weeks at 32-34°C while occasionally performing aeration-agitation. However, concomitant with alcohol fermentation with yeast, a odor particular to soy sauce affixes to the product. If this odor is too strong, then the product will not be suitable for use as a general seasoning and there is the disadvantage that the use thereof will be restricted.

On the other hand, in recent years, there has been a preference among consumers for soy sauce with a high nitrogen concentration. When soy sauce is produced according to a traditional method of producing soy sauce by mixing brine at a typical fermentation formulation ratio with koji obtained with a formulation ratio of protein material to starch materials of roughly 1-to-1 by volume, the product has a nitrogen concentration of more than approximately 2.0% (w/v) and it is extremely difficult to obtain a soy sauce having a good flavor.

Conventionally, it is known that to produce a soy sauce with a high nitrogen concentration, the conventional soy sauce material formulation is adjusted such that the ratio of protein material used is very high and the ratio of starch material used is low. However, in this case, as in the case of the method of producing tamari soy sauce, steamed soybean product must be granulated into balls of soybean

paste, and further because balls of soybean paste such as these have a property such that they are easily contaminated by various bacteria, the material processing and koji-making steps become very complex. Moreover, there is the problem that because the product possesses a particular tamari soy sauce odor, it is far different from the flavor of a typical strong-flavored soy sauce.

Further, to produce a soy sauce with a high nitrogen concentration, other methods are known for obtaining high-nitrogen soy sauce by adding a raw koji protein material to ordinary soy sauce koji, for example, Japanese Examined Patent Publication No. 56-8573 discloses a method of producing a reduced salt thick (strong) soy sauce which comprises performing fermentation by adding 5-20% (w/w) raw koji protein material to ordinary soy sauce koji, adding brine such that the nitrogen concentration in the moromi mash liquid is 2.2-2.5% (w/v) and the common salt concentration is 13-15% (w/v), and adding a culture product of yellow koji mold, or coarse enzymes obtained by extraction from the culture product, as required.

Further, Japanese Laid-open Patent Publication No. 55-13074 discloses a method of speed-brewing a high-nitrogen soy sauce which involves adding 21-25% (w/w) raw koji protein material to typical soy sauce koji, adding brine (to begin fermentation) such that the nitrogen concentration in the moromi mash liquid is 2.0 to 2.3% (w/v) and the common salt concentration is 13-15% (w/v), then, simultaneously with the start of fermentation, or within one week after starting fermentation, adding soy sauce lactic acid bacteria (*Pediococcus halophilus*) to 10^6 /g or more, then when the pH of the moromi mash has reached 5.0-5.3, further adding soy sauce yeast (*Saccharomyces rouxii*) to 10^5 /g or more, and either adding common salt to a common salt concentration of 16- 17.5% (w/v), as required, from 15 days to 60 days after start of fermentation, or allowing to ferment and mature without addition of common salt.

However, these methods involve the burden of lysing a raw koji protein material with a small quantity of koji and further, these methods have the disadvantage that if 25% (w/w) or more raw koji material is added, the viscosity of the moromi mash increases rapidly, reducing its ability to be press-filtered.

For this reason, the limit for the amount to be used is at most 25% (w/w), and these methods necessitate the addition, as required, of a culture product of yellow koji mold, or coarse enzymes obtained by extraction from the culture product. Further, while these inventions make it possible to obtain a soy sauce with a high nitrogen concentration, there is the disadvantage that because zymolysis of the moromi mash is performed in the presence of 13-15% (w/v) common salt, lysis by koji enzymes is inhibited, so for the components of the moromi mash to be almost totally lysed, and for the flavor of soy sauce to evolve fully takes a relatively long period of 2 to 5 months.

SUMMARY OF THE INVENTION

The present invention is directed to providing a method of obtaining, in about 20

days, a seasoning liquid with no unpleasant taste or odor, and moreover, almost no odor particular to soy sauce, with a very high rate of nitrogen use which involves preventing putrefaction of a soy sauce moromi mash, and at the same time shortening the period for lysis of the moromi mash with koji mold enzymes.

The present inventors, as a result of focused research conducted to solve the above problem, found that when soy sauce koji was admixed with 70-80°C hot water, a moromi mash with an initial temperature of 50-57 °C could be instantly prepared and contamination with putrefactive bacteria could be avoided during preparation, and further that by introducing the prepared moromi mash into a fermentation tank fitted with a thermal jacket having warm water flowing it, the initial temperature could be easily maintained at a constant, and a seasoning liquid with a nitrogen use rate of 80% or more could be obtained in a extremely short period of 15 to 30 hours.

Further, conventionally, the production of a seasoning liquid that is rich in flavor by preparing a soy sauce moromi mash by admixing a typical soy sauce koji with water or brine, then admixing a raw koji protein material and allowing to ferment and mature, is known. However, this method has the disadvantage that where the added amount of the material is 25% (w/w) or more, viscosity of the moromi mash rapidly increases and the press-filterability becomes poor.

The present inventors found that as a soy sauce koji for the above described method of production, a high quality soy sauce koji could be obtained by using a soy sauce koji using wheat bran, obtained by admixing steam-denatured protein material with heat-denatured wheat bran, inoculating with seed koji mold, and thereafter performing typical koji-making.

Further, while in principle culture (koji-making) of soy sauce koji is performed by inoculating all of the material for production of soy sauce with koji mold, a method is known in which koji mold is not cultured on a part of the total material for soy sauce production (hereinafter, referred to as raw koji material) and this is added at the start of fermentation (hereinafter, sometimes referred to as reduced koji fermentation). However, the present inventors found that when the above-described soy sauce koji using wheat bran is used, there is provided the effect that even if the ratio of a raw koji material relative to total material is 50%, this material is rapidly hydrolyzed, and a seasoning liquid with a high nitrogen concentration, that has almost no odor particular to soy sauce, and which is salt-free or essentially almost totally salt-free, can be obtained.

Further, in a method of producing soy sauce using typical soy sauce koji, using 25% (w/w) or more raw koji material invites a rapid increase in the viscosity of the moromi mash. However, where the soy sauce koji using wheat bran is used in place of soy sauce koji, there is provided the effect that the increase in viscosity of the moromi mash can be controlled to a relatively low level and the press-filterability of the moromi mash can be improved.

Further, the present inventors found that when a moromi mash having an initial

temperature of 50-57°C is prepared by admixing soy sauce koji with 70-80°C hot water by means of a rotary displacement-type pump, and the moromi mash is introduced into a fermentation tank fitted with a thermal jacket by means of the pump while maintaining the initial temperature, then there is provided the effect that a moromi mash having a desired initial temperature mechanically can be easily obtained, and the initial temperature of the moromi mash can be maintained at a constant in the fermentation tank. That is, the present inventors found that instant preparation of a large amount of moromi mash having a temperature of 50-57°C and maintenance of the moromi mash uniformly and continuously at a desired temperature without putrefaction of the moromi mash can certainly be performed at a factory level.

Further, the present inventors found that after zymolysis of the moromi mash, a moromi mash liquid with a pH of 5.3 or lower could be obtained by adding soy sauce lactic acid bacteria to the lysed moromi mash cooled to room temperature after mixing with common salt, and performing lactic acid fermentation at a common salt concentration of 17-20% (w/v) and an initial temperature of 20-35°C, and that when after pasteurization of this moromi mash liquid, it is allowed to stand in a static tank, the pasteurized lees rapidly precipitate and the mash separates into clear seasoning liquid and a lees fraction allowing a clear seasoning liquid to be easily obtained. Thus, it is possible to obtain a seasoning liquid with good flavor in an extremely short period of time while preventing putrefaction of the moromi mash.

The present invention was completed on the basis of these findings, i.e. the present invention is (1) a method of producing a seasoning liquid, which comprises admixing soy sauce koji and 70-80°C hot water to prepare a moromi mash having an initial temperature of 50-57°C; and performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously and maintaining the initial temperature.

Further, the present invention is (2) a method of producing a seasoning liquid, which comprises admixing a soy sauce koji, a raw koji protein material, and 70-80°C hot water to prepare a moromi mash having an initial temperature of 50-57°C, performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously and maintaining the initial temperature.

Further, the present invention is (3) a method of producing a seasoning liquid, which comprises admixing heat-denatured wheat bran with steam-denatured protein material; inoculating with seed koji mold; admixing soy sauce koji obtained by koji-making according to ordinary methods, with 70-80°C hot water to prepare a moromi mash having an initial temperature of 50-57°C; and performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously and maintaining the initial temperature.

Further, the present invention is (4) a method of producing a seasoning liquid, which comprises admixing soy sauce koji and 70-80°C hot water by means of a rotary

displacement-type pump to prepare a moromi mash having an initial temperature of 50-57°C; introducing the moromi mash into a fermentation tank fitted with a thermal jacket while maintaining the initial temperature; and performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously and maintaining the initial temperature.

Further, the present invention is (5) a method of producing a seasoning liquid, which comprises admixing soy sauce koji and 70-80°C hot water to prepare a moromi mash having an initial temperature of 50-57°C; performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously, and maintaining the initial temperature; adding common salt and soy sauce lactic acid bacteria to the zymolysis moromi mash; and performing lactic acid fermentation at an initial temperature of 20-35°C with a common salt concentration of 17-20% (w/v) to obtain a moromi mash liquid having a pH of 5.3 or lower.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is explained in detail below.

To carry out the present invention, first, a soy sauce moromi mash having an initial temperature of 50-57°C is prepared by admixing soy sauce koji with 70-80°C hot water.

The soy sauce koji used here includes a solid state koji obtainable by performing ordinary methods, material processing of koji-making materials such as a mixture of protein materials and starch materials and further, where necessary other materials; performing solid state culture using soy sauce koji mold used in the production of ordinary soy sauce, and making koji according to ordinary methods.

Examples of protein materials include round soybeans, defatted soybeans, wheat gluten, corn gluten, etc. and examples of starch materials include, wheat, barley, wheat bran, kaoliang, starch cake, etc.

These can be used independently or in combination.

Where starch materials are to be formulated with protein materials, the formulation ratio of both will differ depending on the nitrogen concentration of the seasoning liquid to be obtained. However, for example, in the case where a typical soy sauce is to be obtained, it is preferable to adopt a formula for starting materials (formula for raw materials prior to watering or heat processing) where the protein material/starch material ratio (V/V) is from 80/20 to 20/80.

Using a soy sauce koji obtained by admixing heat-denatured wheat bran with steam-denatured protein material, inoculating this with seed koji mold, and thereafter performing koji making according to ordinary methods (hereinafter, referred to as a soy sauce koji using wheat bran) is preferable to soy sauce which uses typical roasted and milled wheat because it has a high enzyme titer and a high quality soy sauce koji can be obtained.

Further, while in principle koji-making of soy sauce koji is performed by inoculating all of the material for production of soy sauce with koji mold (hereinafter, sometimes referred to as total koji fermentation), there is known a method wherein koji mold is not cultured on a part of the total material for soy sauce production (i.e. raw koji material) and this is added at the start of fermentation (hereinafter, sometimes referred to as reduced koji fermentation). However, when the above-described soy sauce koji using wheat bran is used, it is preferable that the ratio of added raw koji material used relative to total material is about 50%. The added amount is preferably from 25 to 50%, more preferably from 25 to 40% and most preferably from 30 to 35%.

Protein material is steam-denatured by steam processing under ordinary pressure, pressure-steamed in a sealed vessel, or swelling-denaturing by placing the material in a pressure-heating steamer, and pressure-heating for a short term by saturated water vapor or super-heated water vapor then rapidly releasing into atmospheric pressure.

Further, starch material is heat denatured by steam processing under ordinary pressure, or by roasting and milling, etc. After the steam-denatured protein material is admixed with heat-denatured starch materials, and water content regulated, it is inoculated with seed koji mold, and koji-making is performed at for example 20-40°C, for 30 to 80 hours.

The seed koji mold to be used here can be any koji mold used for soy sauce and suitable examples include *Aspergillus soyae* and *Aspergillus oryzae*.

Next, the temperature of the hot water to be admixed with this soy sauce koji is required to be 70-80°C. When hot water having a temperature of 70°C or lower is used, it is difficult, after admixing, to obtain a soy sauce moromi mash having an initial temperature of 50 °C or more. In contrast, a temperature exceeding 80 °C make it possible to obtain a soy sauce moromi mash with an initial temperature of 50°C or more, however, it is not preferable because useful enzymes such as protease and cellulase accumulated in the soy sauce koji are deactivated and inhibited by contact with hot water and zymolysis cannot be performed at high efficiency.

The amount of hot water to be used should be enough to sufficiently immerse the koji in hot water, and is generally, relative to the weight of materials prior to material processing, preferably between 1.5 to 4 parts by volume (v/w).

Zymolysis of protein needs to be performed at a common salt concentration of 0-5% (w/w), and a high rate of dissolved nitrogen use cannot be achieved in a short time with a common salt concentration in excess thereof.

At this time, addition as appropriate raw koji protein material to the koji as required is preferable because a stronger flavored seasoning liquid can be obtained.

The amount of a raw koji protein material, relative to the total weight of a soy sauce koji (in terms of untreated materials) and a raw koji material (in terms of untreated material), is preferably from 25 to 50%, more preferably from 25 to 40% and most preferably from 30 to 35%.

Further, as a raw koji protein material to be used in the present invention, defatted soybeans, round soybeans, peeled soybeans, soybean meal, wheat gluten, corn gluten, etc., that are steamed by ordinary methods or steam-denatured by swelling-denaturing etc., are preferable.

Next, 70-80°C hot water (or brine having the same temperature) is admixed with the above-described soy sauce koji to a common salt concentration of 0-5% (w/w).

Alternatively, 70-80°C hot water (or brine having the same temperature) is admixed with a mixture of a soy sauce koji and a raw koji protein material to a common salt concentration of 0-5% (w/w).

Next, a moromi mash having an initial temperature of 50-57°C is introduced directly and immediately into a tank fitted with a thermal jacket without allowing the initial temperature to fall. Then zymolysis is performed in the tank at a common salt concentration of 0-5% (w/v) while stirring intermittently or continuously and maintaining the initial temperature.

According to the present inventor's experiments, where a moromi mash having an initial temperature less than 50°C is introduced into a large fermentation tank having a structure such that the entire surface of circumference wall is tightly covered with a thermal jacket (outer tube) through which hot water flows, heating of the moromi mash to 50°C or more (increasing the temperature by even 1°C), requires an enormous amount of energy, and is extremely difficult.

In contrast to this, when moromi mash that has a temperature of 50-57°C is introduced into a fermentation tank fitted with a thermal jacket, it is relatively easy to maintain the initial temperature of the moromi mash, by operating the thermal device of the tank fitted with a thermal jacket such that the initial temperature does not fall.

It is necessary to perform hydrolysis for 15 to 30 hours. If the period is too short, the rate of nitrogen use becomes poor, and a exceeding 30 hours is not preferable because, warm-brewing odor affixes strongly, or a burnt odor affixes, due to the high temperature.

Further, examples of rotary displacement-type pump include a pump comprising a cylinder having a material input opening at one end and a material discharge opening at the other end, a rotating screw within the cylinder, a driving means for the screw, and an opening for a hot water supply nozzle at the material input opening or within the cylinder or mid-way thereof (screw conveyor, screw feeder, uniaxial extruder, biaxial extruder) and a uniaxial eccentric screw pump (mono-pump) (Japanese Laid-open Patent Publication No. 60-142078, Japanese Laid-open Patent Publication No. 62-29781, Japanese Laid-open Utility Model Publication No. 2-54391). Of these, a uniaxial eccentric screw pump is preferable.

Moromi mash that has completed hydrolysis may be press-filtered while hot and converted into a product. However, it is preferable to produce a moromi mash liquid by adding common salt to a concentration of 17-20% (w/v) and after mixing uniformly, adding lactic acid bacteria (*Pediococcus halophilus*) to give a 10^3 - 10^5 /g moromi

mash, particularly a 10^4 microbe/g moromi mash, fermenting at 25-30°C for 10 to 14 days, obtaining a lysed moromi mash having a target pH of 5.3 or lower, and then press-filtering according to a typical method of soy sauce production.

It is important to have the common salt concentration at 17% or more. That is, a common salt concentration that is lower than this is not preferable because there is a danger that damaging microbes such as putrefactive bacteria and film-forming yeast will proliferate during the lactic acid fermentation that is performed thereafter.

Further, it is important to keep the pH of the moromi mash liquid at 5.3 or lower by lactic acid fermentation. By this processing, when the moromi mash liquid is allowed to stand in a static tank after pasteurization, the pasteurized lees rapidly precipitate, and the mash separates into a clear seasoning liquid and a lees fraction, allowing a clear seasoning liquid to be easily obtained. Further, this is preferable because it provides the effect that a flavorsome seasoning liquid can be obtained in a very short period of time while preventing putrefaction of the moromi mash.

In contrast, a pH greater than 5.3 is not preferable because post-pasteurization precipitation-separation of cloudy substances (less etc.) takes time and moreover separation becomes difficult.

Examples

Below the present invention is explained in more detail by way of Examples.

Example 1

(A salt-free protein zymolysis method of producing a seasoning liquid)

In cylindrical tanks having a volume of 40L, moromi mash was prepared having each of the temperatures indicated in Table 1, by quickly mixing by hand, 10kg of soy sauce koji obtained by admixing steamed defatted soybeans with roasted and milled wheat, inoculating with seed koji mold and making koji according to typical methods of producing soy sauce koji, together with 15L hot water having each of the temperatures indicated in Table 1. These were placed in a temperature controlled room, and stirred intermittently once per hour, in salt-free conditions, while maintaining the initial temperature, and zymolysis was performed for a total of 18 hours. Thereafter, the mash was press-filtered to obtain a salt-free seasoning liquid having a very good flavor, and having almost no odor particular to soy sauce.

The total microbe count of each of the fractions was measured at the start of the moromi mash (immediately after preparation) and after 18 hours of zymolysis. Further, rate of nitrogen use of the zymolyzed moromi mash liquid was measured.

These results are shown in Table 1.

The total microbe count was measured according to the following method:
(Measurement of total microbe count)

A plate culture using a sugar-added bouillon medium was performed (culture temperature: 32°C; 72 hours culture), and colonies counted.

Table 1

Fraction	Total microbe count (microbes/g)		Rate of nitrogen use	Remarks
	Start	After 18 hours		
43°C	2×10^6	5×10^{10}	83	unpleasant taste/odor
48°C	1×10^6	3×10^{10}	84	"
50°C	5×10^5	2.5×10^2	85	no unpleasant taste or flavor
57°C	5×10^5	1.4×10^2	85	"
60°C	5×10^4	1.0×10^2	56	"
65°C	1×10^4	8×10^1	37	"

From the results in Table 1, it is clear that in fractions where the initial temperature of the moromi mash was lower than 50°C, there was substantial proliferation of miscellaneous microbes found in soy sauce koji during zymolysis of moromi mash and the salt-free moromi mash putrefied.

Further, it is clear that a temperature of 60°C or more is not preferable because the rate of nitrogen use falls dramatically. In contrast to this, in fractions where the initial temperature of the moromi mash was 50°C-57°C, even though they were totally salt-free, decomposition did not occur and moreover, an enzyme hydrolyzed fluid having an extremely high rate of dissolved nitrogen use was obtained.

Example 2

(A salt-free protein zymolysis method of producing a seasoning liquid using a soy sauce koji using wheat bran: all koji fermentation)

Four parts by weight of steamed defatted soybeans prepared according to a typical method of producing soy sauce koji was admixed with 1 part by weight of separately steamed wheat bran and soy sauce koji mold was uniformly inoculated thereto.

Next, this was aerated with 25-30°C warm air for 45 hours and koji-making performed to prepare a soy sauce koji using wheat bran.

Four thousand kilograms of the obtained soy sauce koji using wheat bran having an initial temperature of 28°C was admixed with 7000L of 75°C hot water by means of a rotary displacement-type uniaxial eccentric screw pump (NE type eccentric screw pump, manufactured by Heishin Ltd.), to prepare a moromi mash having an initial temperature of 55°C. While maintaining the initial temperature, moromi mash was introduced into a fermentation tank fitted with a thermal jacket by means of the pump over a period of 1.5 hours, and while maintaining the initial temperature, zymolysis was performed stirring by aerating intermittently at a rate of once every hours under salt-free conditions to obtain a zymolyzed moromi mash.

Next, this moromi mash was press-filtered to obtain a salt-free protein zymolyzed seasoning liquid.

Analysis of the components of the seasoning liquid obtained in the above described Example 2, are shown in Table 2.

Analysis of components was performed according to *Shoyu Jikken-hou*, Editorial Committee for Shoyu Jikken-hou, Japan Soy Sauce Laboratory, Soy Sauce Brewer's Association News Agency, Issued March 1, 1985. (Analysis of components was performed in each of the below Examples in a similar manner.)

Table 2

NaCl	T.N.	pH	Col.	Alc.	Total microbe count	L.A.
% (w/v)	% (w/v)			% (v/v)	microbes/g moromi mash	% (w/v)
0	1.78	5.8	26	0	350/g	0

Note: NaCl: common salt, T.N.: total nitrogen, Col.: color, Alc.: ethanol, L.A.: lactic acid

Comparative Example

Further, for the purpose of comparison, in the salt-free protein zymolysis method of producing a seasoning liquid of the above Example 2, "7000L of 20°C tap water" was used in place of "7000L of 75°C hot water" and otherwise a similar process was followed, and the mash introduced into the fermentation tank over a period of 1.5 hours, and thereafter the thermal jacket was heated to raise the initial temperature of the moromi mash to reach 55°C. This took approximately 5 hours.

Thereafter, while maintaining the initial temperature of 55°C, zymolysis was performed while stirring by aerating intermittently at a rate of once every hours under salt-free conditions. Thereafter, the mash was press-filtered to obtain a salt-free protein zymolyzed seasoning liquid.

Above, in the present invention and the comparative example, total microbe count was taken from moromi mash (start) collected from the discharge opening of the rotary displacement-type uniaxial eccentric screw pump (NE-type eccentric screw pump, manufactured by Heishin Ltd.). Further, an organoleptic test was conducted on the seasoning liquid. These results are shown in Table 3.

Table 3

	Initial temperature of moromi mash at preparation (°C)	Initial temperature of moromi mash at the time of zymolysis (start)	Total microbe count (microbes/g) (After 6.5 hours)	Organoleptic test
Present invention	55°C	Initial temperature: 55°C microbe count : 1×10^7	initial temperature : 55 microbe count : 3×10^5	good

Comparative example	25°C	Initial temperature : 25°C microbe count : 2×10^7	initial temperature : 55 microbe count : 3×10^{10}	poor
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Note 1: (Organoleptic test) good: no unpleasant taste or odor; poor: unpleasant taste/odor.

It can be understood from Table 3 that in the comparative example it took about 6.5 hours to uniformly increase the temperature of the moromi mash from an initial temperature in the vicinity of room temperature to 55°C. Thus there is the significant disadvantage that during this time microbes present in the soy sauce koji propagated voraciously and the moromi mash putrefied.

This may appear to be uncomplicated in a small-scale experiment in a laboratory, but in contrast to laboratory work, where this is actually performed in a factory with a large filled fermentation tank, it is extremely difficult to rapidly raise the temperature of a large amount of moromi mash from around room temperature to a uniform 50-57°C and during this period decomposition of the moromi mash inevitably occurs and it is clear that such an approach is certain to fail.

Example 3

(A salt-free protein zymolysis method of producing a seasoning liquid which uses a soy sauce koji using wheat bran: reduced koji fermentation)

Two parts by weight of steamed defatted soybeans prepared according to a typical method of producing soy sauce koji was admixed with 1 part by weight of separately steamed wheat bran and soy sauce koji mold was uniformly inoculated thereto.

Next, this was aerated with 25-30°C warm air for 45 hours and koji-making performed to prepare a soy sauce koji.

This koji in comparison to the koji of Example 2, had a relatively large ratio of steamed wheat bran to steam defatted soybeans. The wheat bran covered the surface of the defatted soybeans so that the defatted soybean surface moisture was reduced thereby inhibiting the propagation of miscellaneous microbes. As a result, the total microbe count was extremely small, being approximately one hundredth or less than in Example 2, thereby allowing a sanitary soy sauce koji to be obtained.

Three thousand five hundred kilograms of the obtained soy sauce koji having an initial temperature of 28°C was admixed, using a rotary displacement-type uniaxial eccentric screw pump (NE type eccentric screw pump, manufactured by Heishin Ltd.), with 700 kg of steamed defatted soybeans prepared in accordance with a typical method of producing soy sauce, 7000L of 78°C hot water and 200 kg of common salt to prepare a moromi mash having an initial temperature of 55°C. The moromi mash was introduced into a fermentation tank fitted with a thermal jacket by means of the pump over a period of 1.5 hours while maintaining the initial temperature. Zymolysis was performed in the presence of approximately 3% (w/w) common salt while stirring by aerating intermittently at a rate of

once every hours and maintaining the initial temperature. Next the moromi mash was press-filtered to obtain a protein zymolyzed seasoning liquid which was rich in flavor, had a common salt concentration of approximately 3% (w/w) and nitrogen concentration similar to that of Example 2.

It is clear from this result that according to the present invention, if the ratio of wheat bran used relative to steamed defatted soybeans is increased, a sanitary koji having a low miscellaneous microbe count can be obtained.

Further, it is clear that by preparing a soy sauce koji using a less than typical amount of protein material, and using the remainder of protein material not in the koji-making but adding it directly to the moromi mash, a seasoning liquid of equal quality can be obtained.

Example 4

(method of producing a seasoning liquid in which lactic acid fermentation is performed after zymolysis of proteins)

Three thousand five hundred kilograms of the soy sauce koji having an initial temperature of 28°C that was obtained in Example 3 was admixed, using a rotary displacement-type uniaxial eccentric screw pump (NE type eccentric screw pump, manufactured by Heishin Ltd.), with 700 kg of steamed defatted soybeans prepared in accordance with a typical method of producing soy sauce, 7000L of 78°C hot water and 200 kg of common salt to prepare a moromi mash having an initial temperature of 55°C. The moromi mash was introduced into a fermentation tank fitted with a thermal jacket by means of the pump over a period of 1.5 hours while maintaining the initial temperature. Zymolysis was performed under salt-free conditions while stirring by aerating intermittently at a rate of once every hours and maintaining the initial temperature to obtain a zymolyzed moromi mash.

Next, 2500kg of common salt was introduced into the above described zymolyzed moromi mash, and while homogenized by air-stirring, the mash was allowed to stand for 2 days (48hours).

After allowing to stand, the mash was cooled to around 30°C, lactic acid bacteria (*Pediococcus halophilus*) was added to give a 10^4 microbes/ g moromi mash, which was allow to ferment at 28°C for 10 days. A lysed moromi mash having a target pH of 5.3 or lower was obtained. This was subjected to press-filtration according to typical soy sauce production methods to provide a moromi mash liquid.

After pasteurizing this moromi mash liquid according to ordinary methods, it was allowed to stand in a static tank, whereupon the pasteurized lees rapidly precipitated and the mash separated into clear seasoning liquid and a lees fraction allowing a clear seasoning liquid to be easily obtained. Further, the obtained seasoning liquid had almost no odor particular to soy sauce, was completely free of alcohol, and had an extremely good, rich flavor. Further, the seasoning liquid could be obtained in the very short period

of approximately 2 weeks.

An analysis of the components of the obtained seasoning liquid was performed. The results are shown in Table 4.

Table 4

NaCl	T.N.	pH	Col.	Alc.	Total microbe count	L.A.
% (w/v)	% (w/v)			% (v/v)	microbes/g moromi mash	% (w/v)
19.75	1.730	5.25	22	0	215/g	0.74

Example 5

(protein hydrolysis method of producing a seasoning liquid having a different period of hydrolysis with enzymes.)

A seasoning liquid was obtained by a method identical to the method of the above Example 1, except that the period of lysis was as described in Table 5 below.

In this Example, results of the relationship between rate of nitrogen use and nitrogen concentration of the lysis liquid at the start and over the course of time, and results of an organoleptic test are indicated.

Table 5

	Start	5	12	16	18	22	30	35
Rate of nitrogen use	-	43	60	80.5	85.3	86.6	86.7	87.4
Nitrogen concentration	-	1.11	1.58	1.69	1.80	1.83	1.83	1.85
Organoleptic evaluation	-	Flavor good	Flavor good	Flavor good	Flavor good	Flavor good	Flavor somewhat good	Flavor poor (Note 1)

Note 1: warm brewing odor and burnt odor present.

It is clear from the results in Table 5 that when the period of lysis was less than 15 hours, i.e. at 12 hours, rate of dissolved nitrogen use was extremely poor at 60%. On the other hand, it is clear that a period of lysis exceeding 30 hours was not preferable because a warm-brew odor strongly affixed and a burnt odor affixed and there was deterioration of flavor. In contrast, it is clear that when the period of lysis was from 15 hours to 30 hours, values for the rate of nitrogen use of 80% or more were obtained and moreover there was no danger of deterioration of flavor.

Effect of the Invention

According to the present invention it is possible to instantly prepare a moromi mash with an initial temperature of 50-57°C by admixing soy sauce koji with 70-80°C hot

water. Further it was possible to prevent contamination of the moromi mash by putrefactive bacteria during preparation and because the prepared moromi mash was introduced into a fermentation tank fitted with a thermal jacket through which warm water flowed, the initial temperature could easily be maintained at a constant. Thus a seasoning liquid having a rate of nitrogen use of 80% or more could be obtained in the very short time of 15 to 30 hours.

Further, conventionally, when producing seasoning liquid that is rich in flavor by preparing a soy sauce moromi mash by admixing typical soy sauce koji with water or brine, admixing a raw koji protein material and allowing to ferment and mature, if the added amount of the material is 25% (w/w) or more, the viscosity of the moromi mash increases and its ability to be press-filtered is degraded. However, the present invention in the above described method of producing the seasoning liquid that is rich in flavor uses soy sauce koji using wheat bran as a soy sauce koji. This allows the increase in viscosity of the moromi mash to be controlled to a relatively low level. Thus there is provided a effect of improving the press-filterability of the moromi mash.

Further, because the present invention uses a soy sauce koji using wheat bran as a soy sauce koji, even if the ratio of a raw koji material to total material is 50%, there is provided the effect that this material is rapidly hydrolyzed to obtain a seasoning liquid with high nitrogen concentration, that has almost no odor particular to soy sauce, and is salt-free or essentially almost totally free of salt.

Further, because a moromi mash having an initial temperature of 50-57°C is prepared by admixing soy sauce koji with 70-80°C hot water by means of a rotary displacement-type pump, and the moromi mash is introduced into a fermentation tank fitted with a thermal jacket by means of the pump while maintaining the initial temperature, there are provided the effects that it is possible to mechanically and easily obtain a moromi mash having a desired initial temperature, and to maintain the initial temperature of the moromi mash at a constant within the fermentation tank. Thus, there is provided the effect that instant regulation of a large amount of moromi mash to 50-57°C, and moreover, uniform and continuous maintenance of the moromi mash at a desired temperature such that there is no putrefaction of moromi mash is certainly possible on an industrial scale.

Further, in the present invention, after zymolysis of the moromi mash, the lysed moromi mash is admixed with common salt and cooled to room temperature, soy sauce lactic acid bacteria are added, and lactic acid fermentation is performed at a common salt concentration of 17-20% (w/v) and an initial temperature of 20-35°C to provide a moromi mash liquid having a pH of 5.3 or lower. Whereby there is provided the effect that, after pasteurization of this moromi mash liquid, it is allowed to stand in a static tank, whereupon the pasteurized lees rapidly precipitate and the mash separates into clear seasoning liquid and a lees fraction allowing a clear seasoning liquid to be easily obtained.

Further, in the present invention, lactic acid bacteria are added to hydrolyzed moromi mash but yeast is not added, so a seasoning liquid having 0% alcohol can be

obtained. Further, a seasoning liquid with very good flavor which has no odor particular to soy sauce, and which is salt-free or essentially almost free of salt can be obtained in the very short time of 30 hours to 20 days.

What is claimed is:

1. A method of producing a seasoning liquid, which comprises:
 - (a) admixing a soy sauce koji and 70-80°C hot water to prepare a moromi mash having an initial temperature of 50-57°C; and
 - (b) performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously and maintaining the initial temperature.
2. A method of producing a seasoning liquid, which comprises:
 - (a) admixing a soy sauce koji, a raw koji protein material, and 70-80°C hot water to prepare a moromi mash having an initial temperature of 50-57°C; and
 - (b) performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously and maintaining the initial temperature.
3. The method of producing a seasoning liquid according to claim 2, wherein relative to total materials, 25-50% (w/w) raw koji protein material is used.
4. A method of producing a seasoning liquid, which comprises:
 - (a) admixing a heat-denatured wheat bran with a steam-denatured protein material;
 - (b) inoculating with seed koji mold;
 - (c) admixing a soy sauce koji obtained by koji-making according to ordinary methods with 70-80°C hot water to prepare a moromi mash having an initial temperature of 50-57°C; and
 - (d) performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously and maintaining the initial temperature.
5. The method of producing a seasoning liquid according to claim 4, wherein relative to protein material, 20-50% (w/w) wheat bran is used.
6. A method of producing a seasoning liquid, which comprises:
 - (a) Admixing a soy sauce koji and 70-80°C hot water by means of a rotary displacement-type pump to prepare a moromi mash having an initial temperature of 50-57°C, and introducing into a fermentation tank fitted with a thermal jacket while maintaining the initial temperature; and
 - (b) performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously and maintaining the initial temperature.

7. A method of producing a seasoning liquid, which comprises:
 - (a) admixing a soy sauce koji and 70-80°C hot water to prepare a moromi mash having an initial temperature of 50-57°C;
 - (b) performing zymolysis for 15 to 30 hours in the presence of a common salt concentration of 0-5% (w/v), while stirring intermittently or continuously, and maintaining the initial temperature;
 - (c) adding common salt and soy sauce lactic acid bacteria to the zymolysis moromi mash; and
 - (d) performing lactic acid fermentation at an initial temperature of 20-35 °C with a common salt concentration of 17-20 % (w/v) to obtain a moromi mash liquid having a pH of 5.3 or lower.
8. The method of producing a seasoning liquid according to claim 7, wherein lactic acid fermentation is performed for 10 to 14 days.