

Process for producing a seasoning

1 BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a process for producing a seasoning of excellent flavor and taste.

5 2. Description of the Prior Art

There has already been known a method of obtaining a seasoning of a high glutamic acid content which comprises, in the hydrolysis of proteinous raw materials by enzymes, subjecting the raw materials to, 10 the action of protease containing substantially no peptidase, and then subjecting the resulting product to the action of peptidase and glutaminase in the absence of sodium chloride (see, for example, Japanese Patent Publication No. 48946/82).

15 Prior art methods of preparing a seasoning by the hydrolysis of proteinous raw materials using enzyme preparations, including the above-mentioned method of preparing a seasoning disclosed in Japanese Patent Publication No. 48946/82, still have defects in that the 20 efficiency of contact and reaction between the substrate and enzyme is low even when reaction conditions such as pH and temperature are properly adjusted and further the enzyme used in the reaction cannot be employed, resulting in high production cost.

1 Accordingly, there has been eagerly awaited in
the industry the improvement of a process which gives an
enhanced contact efficiency between protein substrate
and enzyme and can thus produce a seasoning in good
5 efficiency.

SUMMARY OF THE INVENTION

 The present inventors have made extensive
studies on the reaction conditions between substrates
and enzymes in preparing a seasoning. As a result, it
10 has been found that a seasoning of high amino acid
content and markedly excellent flavor and taste can be
obtained in good efficiency by first preparing soy sauce
koji beforehand from soy sauce raw materials according
to conventional method of soy sauce production,
15 hydrolyzing the koji, and then contacting the resulting
hydrolyzate, in a liquid state at a pH of 2.5 to 8.0,
with an immobilized peptidase and/or immobilized
glutaminase in the presence of sodium chloride to react
therewith. This invention has been accomplished on the
20 basis of above finding.

 Thus, this invention relates to a process for
producing a seasoning which comprises contacting a
hydrolyzate of soy sauce koji prepared from soy sauce
raw materials, in a liquid state at a pH of 2.5 to 8.0,
25 with an immobilized peptidase and/or immobilized
glutaminase in the presence of sodium chloride to react
therewith.

1 DETAILED DESCRIPTION OF THE INVENTION

The raw materials for the soy sauce production used in this invention are those usually used in the production of soy sauce, that is to say, proteinous materials admixed with starchy materials. As examples of suitable proteinous materials, there may be listed defatted soybean, whole soybean, wheat gluten, corn gluten, purified soybean gluten, separated soluble protein, fishes and shellfishes, meats, and yeast extracts. Examples of suitable starchy materials include wheat, barley and corn.

These raw materials are subjected to customary raw material treatment such as softening of the tissue, denaturation of proteins, conversion of starch into α -form, and sterilization.

These soy sauce raw materials are inoculated with yellow koji-molds such as Aspergillus oryzae and Aspergillus soyae, which are common koji-molds for soy sauce production, and then made into koji in a customary manner for about 25 to 100 hours at 25 to 40°C by conventional methods such as koji-tray method and koji-making method under aeration to give soy sauce koji.

The hydrolysis of the soy sauce koji is carried out by adding a sodium chloride aqueous solution to the koji and hydrolyzing it at about 30° to 60°C while gently stirring the mixture just to keep the substrates from settling. The sodium chloride

1 concentration is kept at 1 to 20% (W/V), preferably 5 to
12 % (W/V), during the hydrolysis step. The hydrolysis
is carried out preferably under sterile conditions or at
a relatively elevated temperature such as 40 to 90°C,
5 preferably 45 to 65°C, for at least about 10 hours,
preferably 24 to 120 hours.

The reaction mixture obtained by the
hydrolysis of soy sauce koji as described above is
adjusted, unless its original pH lies in the range of
10 2.5 to 8.0, to a pH of 2.5 o 8.0, preferably 4.0 to 6.5,
by addition of suitable alkali or acid. When the
hydrolyzate obtained as described above is a liquid
containing no or substantially no residue of hydrolysis
(solids), it is used as such; otherwise the hydrolyzate
15 is subjected to solid-liquid separation by conventional
means such as press-filtration, filtration, and centrifu-
gation before and/or after adjusting pH to 2.5 to 8.0
with an alkali or an acid as mentioned above to obtain
a substrate in liquid form.

20 The peptidase and/or glutaminase used in this
invention is of any origin so long as they can exert
their enzymatic activity at a pH of 2.5 to 8.0.

Preferable examples of peptidase to be used
include, in the case of aminopeptidase, those of the
25 genus *Aspergillus*, *Streptomyces*, *Lactobacillus*, and
Pediococcus origin and, in the case of carboxypeptidase,
those of the genus *Aspergillus* and *Penicillium* origin.

1 Preferable example of glutaminase to be used
include those originated from such microorganisms as the
genus *Saccharomyces*, *Aspergillus*, and *Echerichia*.

 As examples of such peptidase and glutaminase
5 of microbial origin, mention may be made of a culture
liquor containing peptidase and/or glutaminase obtained
by inoculating and cultivating the cells of these
microorganisms in a medium by conventional methods,
cells separated from said culture liquor or
10 disintegrated products thereof, a crude enzyme solution
obtained from said culture liquor by filtration or
centrifugation, or purified enzyme obtained by purifying
it in a conventional manner.

 The immobilized peptidase and immobilized
15 glutaminase used in this invention can be obtained by
any conventional methods of immobilization. That is, in
the case of above-mentioned crude enzyme solution or
purified enzyme, there may be mentioned as examples of
preferred methods of immobilization, as to the ionic
20 bond method a method which comprises bonding said enzyme
to ion exchangers such as DEAE Sephadex, QAE Sephadex,
Dowex 1X1, and Amberlite IRA-45 (SEPHADEX, DOWEX and
AMBERLITE being Registered Trade Marks) and then subject-
ing it, if necessary, to crosslinking with glutaraldehyde;
as to the adsorption method one which comprises allowing
25 said enzyme to be adsorbed onto adsorbents such as active
carbon, silica gel, and alumina, and then subjecting it,
if necessary, to crosslinking with glutaraldehyde; as to

1 the covalent bond method one which comprises mixing said
enzyme with, for example, a polysaccharide activated
with cyanogen bromide or a polysaccharide to which an
epoxy group has been introduced by using a bisoxysilane
5 compound; as to the entrapping method one which
comprises mixing said enzyme with a gel base of alginic
acid salt or a liquid mixture of alginic acid salt and
silica sol and bringing the resulting mixture in contact
with a gelling agent, or mixing the enzyme with a gel
10 base of a solution of carrageenan or ^{agar}~~algae~~ which has
been formed by heating them with water, and then cooling
the resulting mixture.

In the case of above-mentioned culture liquor,
separated cells, or disintegrated cells, particularly
15 preferable methods of immobilization include those of
entrapping which comprise mixing them with a gel base of
alginic acid salt or a liquid mixture of alginic acid
salt and silica sol and bringing the resulting mixture
in contact with a gelling agent, or mixing them with a
20 gel base of a solution of carrageenan or agar, which has
been formed by heating them with water, and then cooling
the resulting mixture.

The immobilized peptidase and/or immobilized
glutaminase obtained by the procedures described above
25 are placed in an hydrolysis vessel, such as a packed
tower, stirring tank, fluidized bed contactor, bubbling
column, and film reactor, separate vessels being prefer-
ably used for the peptidase and the glutaminase when both
are present, and then the hydrolyzate of

1 soy sauce koji in liquid state at a pH of 2.5 to 8.0
mentioned above, namely a liquid substrate, is
introduced to the vessel and allowed to contact and
react, continuously or intermittently, with the
5 immobilized peptinase and/or immobilized glutaminase in
the presence of sodium chloride to give a seasoning of
excellent flavor and taste.

In the above-mentioned contact and reaction of
the liquid substrate (hydrolyzate) of a pH of 2.5 to 8.0
10 with immobilized peptidase and/or immobilized
glutaminase, the concentration of sodium chloride is
usually about 3 to 20 % (W/V), preferably about 8 to 17
% (W/V), the contact temperature is about 20 to 60°C,
preferably 25 to 50°C, and the contact time is
15 preferably about 5 minutes to 24 hours, preferably 30
minutes to 10 hours.

When both of immobilized peptidase and
immobilized glutaminase are used in the above-mentioned
reaction of the liquid substrate (hydrolyzate) with
20 immobilized peptidase and/or immobilized glutaminase, it
is preferable to bring the substrate into contact first
with immobilized peptidase and then with immobilized
glutaminase for increasing the enzyme reaction
efficiency of the substrate.

25 Although the liquid seasoning obtained after
contact with immobilized peptidase and/or immobilized
glutaminase as described above can be used as such or

1 optionally after filtration, a liquid seasoning with
more excellent flavor and taste can be prepared, if
necessary, by further ripening after usual enzyme
fermentation or suitable processing followed by usual
5 treatments such as filtration, pasteurization, and
sediment separation.

As described above, according to this
invention, it is possible to prepare efficiently a
seasoning of high amino acid content and excellent
10 flavor and taste. This invention is, therefore, is of
great significance from the industrial point of view.

This invention is further illustrated below in
detail with reference to Examples, but the invention is
not limited thereto.

15 Example 1

One liter of a liquid medium (pH 6.0)
containing 0.5 % (W/V) of defatted soybean meal and 2.0
% (W/V) of wheat bran was placed in a 5 l flask and
sterilized in a conventional manner. Aspergillus
20 awamori ATCC 20793 which had been pre-cultured
beforehand in a medium of the above-mentioned
composition was inoculated to said sterilized medium and
subjected to shaken culture at 30°C for 48 hours. The
culture liquor thus obtained was centrifuged in a
25 conventional manner to remove mycelium. The resulting
liquid was adjusted to pH 5.2 with an 1N sodium
hydroxide aqueous solution, and then 3 times its

1 amount (V/V) of cold ethanol was added thereto to cause
precipitation. After allowed to stand over-night, the
mixture obtained above was centrifuged to obtain a
precipitate. It was then dried under vacuum to give a
5 carboxypeptidase preparation (34 U/mg).

Five grams of the carboxypeptidase preparation
obtained as described above was dissolved in an acetate
buffer solution (pH 6.0) and then adsorbed onto DEAE
10 Toyopearl 650 (mfd. by Toyo Soda Mfg. Co., Ltd. - TOYOPEARL
is a Registered Trade Mark). Then, 2% (W/V) glutaralde-
hyde solution was added thereto and allowed to react at 4°C
for 16 hours to obtain an immobilized carboxypeptidase.

On the other hand, 1 ℓ of a liquid medium (pH
5.5) containing 4 % (W/V) of glucose, 6 % (W/V) of corn
15 steep liquor, 0.1 % (W/V) of potassium primary
phosphate, and 0.1 % (W/V) of magnesium sulfate was
placed in a 3 ℓ jar fermenter and sterilized in a
conventional manner. To the sterilized medium was added
50 ml of a seed culture fluid obtained by inoculating
20 beforehand Cryptococcus albidus ATCC 20293, a
glutaminase-producing yeast, to a medium of the above-
mentioned composition and subjecting it to shaken
culture at 25°C for 42 hours. It was then cultivated
under aerobic conditions at 25°C for 30 hours with an
25 air flow rate of 1 ℓ/minute and a stirring velocity of
300 r.p.m. After completion of the cultivation, the
resulting culture liquor was centrifuged and the

- 1 separated cells were washed twice with water. The cultured cells thus obtained were thoroughly mixed with 90 g of 2 % (W/V) sodium alginate, and the mixture was added dropwise by means of a syringe to a 5 % (W/V)
- 5 calcium chloride solution to obtain spherical gel granules of cells containing immobilized glutaminase.

Then, a mash for koikuchi soy sauce which had been obtained by hydrolysing normal soy sauce koji [raw material composition : defatted soybean : wheat = 50 : 50 (W/W)] at 30°C for 1 month was press-filtered in a customary manner to give a liquid [pH 5.5, NaCl 16.5 % (W/W), T.N. 1.75 % (W/V)]. The liquid was then passed continuously through a column (inner diameter: 1.5 cm) provided with a jacket, packed with 10 g of above-men-

15 tioned immobilized carboxypeptidase, and kept at a temperature of 33°C, at a rate of 0.05 ml (liquid)/minute. Then, the liquid thus obtained was passed continuously through a column (inner diameter: 1.5 cm) provided with a jacket, packed with 10 g of above-mentioned immobilized

20 glutaminase-containing cells and kept at 33°C, at a rate of 0.05 ml (liquid)/minute. Thus, a seasoning of high glutamic acid content and excellent flavor and taste as shown in Table 1 was obtained continuously.

Table 1

	before passing through column	After passing through column
T.N. * (% (W/V))	1.75	1.75
Total amino acids (mg/ml)	58.6	71.8
Glutamic acid (mg/ml)	8.8	14.5

* T.N.: Total nitrogen

1 **EXAMPLE 2**

One liter of a liquid medium (pH 6.0) containing 0.5 % (W/V) of defatted soybean meal and 2 % (W/V) of wheat bran was placed in a 5 l flask and
5 sterilized in a conventional manner. Aspergillus oryzae 460 FERM BP-983 which had been pre-cultured beforehand in a medium of the above-mentioned composition was inoculated to said sterilized medium, and subjected to shaken culture at 30°C for 48 hours. The culture liquor
10 thus obtained was centrifuged in a conventional manner to remove mycelium. The resulting liquid was subjected to ammonium sulfate fractionation and then purified by

- 1 the use of DEAE-cellulose (mfd. by Brown Co., U.S.A.) to
obtain a leucine aminopeptidase preparation.

Five grams of the leucine aminopeptidase
preparation obtained above was dissolved in a phosphate
5 buffer solution (pH 7.0), and then adsorbed onto 100 g
of moistened DEAE-Sephadex A-25 (mfd. by Pharmacia Co.,
Sweden). Then, 2 % (W/V) glutaraldehyde solution was
added thereto and allowed to react at 4°C for 16 hours
to obtain an immobilized leucine aminopeptidase.

- 10 On the other hand, 1 ℓ of a liquid medium (pH
5.5) containing 4 % (W/V) of glucose, 6 % (W/V) of corn
steep liquor, 0.1 % (W/V) of potassium primary
phosphate, and 0.1 % (W/V) of magnesium sulfate was
placed in a 3 ℓ jar fermenter and sterilized in a
15 conventional manner. To the sterilized medium was added
50 ml of a seed culture fluid obtained by inoculating
beforehand Cryptococcus albidus ATCC 20293, a
glutaminase-producing yeast, to a medium of the above-
mentioned composition and subjecting it to shaken
20 culture at 25°C for 42 hours. It was then cultivated
under aerobic conditions at 25°C for 30 hours with an
air flow rate of 1 ℓ/minute and a stirring velocity of
300 r.p.m. After completion of the cultivation, the
resulting culture liquor was centrifuged and the
25 separated cells were washed twice with water. The cells
thus obtained was suspended in an acetate buffer
solution (pH 6.0) with ice cooling, then disintegrated

1 by means of an ultrasonic disintegrator (mfd. by Nippon
Seiki Seisakusho K.K.) at 20 KC, and centrifuged to
obtain a solution containing glutaminase.

5 The solution containing glutaminase obtained
above was adsorbed onto QAE-Sephadex (mfd. by Pharmacia
Co., Sweden). Then, 2 % (W/V) glutaraldehyde solution
was added thereto and allowed to react at 4°C for 16
hours to obtain an immobilized glutaminase.

Then, a mash for koiguchi soy sauce which had been
10 obtained by hydrolysing normal soy sauce [raw material
composition : defatted soybean : wheat = 50 : 50 (W/W)]
at 30°C for 1 month was press-filtered in a customary
manner to give a liquid [pH 6.0, NaCl 16.5 % (W/V),
T.N. 1.75 % (W/V)]. The liquid was then passed contin-
15 uously through a column (inner diameter: 1.5 cm) provided
with a jacket, packed with 10 g of above-mentioned immo-
bilized leucine aminopeptidase and kept at a temperature
of 35°C, at a rate of 0.05 ml (liquid)/minute. Then,
the liquid thus obtained was continuously passed through
20 a column provided with a jacket, packed with 10 g of the
above-mentioned immobilized glutaminase and kept at 35°C,
at a rate of 0.05 ml (liquid)/minute. Thus, a seasoning
of high glutamic acid content and excellent flavor and
taste as shown in Table 2 was obtained continuously.

Table 2

	before passed through column	After passed through column
T.N. (% (W/V))	1.75	1.75
Total amino acids (mg/ml)	58.6	70.5
Glutamic acid (mg/ml)	8.8	10.8



CLAIMS

1. A process for producing a seasoning which comprises contacting a hydrolyzate of soy sauce koji prepared from soy sauce raw materials, in a liquid state at a pH of 2.5 to 8.0, with an immobilized peptidase and/or
5 immobilized glutaminase in the presence of sodium chloride to react therewith.
 2. A process according to claim 1, wherein the concentration of sodium chloride is 3 to 20 % (w/v).
 3. A process according to claim 1 or 2, wherein
10 the contact temperature is 20 to 60°C.
 4. A process according to any of claims 1 to 3, wherein the contact time is 5 minutes to 24 hours.
 5. A process according to any of claims 1 to 4, wherein the contact of the hydrolyzate and the immobilized
15 enzyme is carried out by contacting the hydrolyzate first with the immobilized peptidase and then with the immobilized glutaminase.
 6. A process according to any of claims 1 to 5, wherein the immobilized peptidase and immobilized glutamin-
20 ase are each placed in a separate packed tower, stirring tank, fluidized bed contactor, bubbling column or film reactor.
 7. A process for producing a seasoning carried out substantially as hereinbefore specifically described or as illustrated in either of the foregoing Examples.
 - 25 8. A foodstuff which has been seasoned using a seasoning produced by a process according to any of claims 1 to 7.
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