

PRODUCTION OF HYDROLYSATE SEASONING

FIELD OF THE INVENTION

5 The present invention relates to a process for the production of a hydrolysate seasoning, more particularly to the production of hydrolysate seasoning by the biological hydrolysis of a protein containing material.

BACKGROUND OF THE INVENTION

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In our co-pending EP-A-0824873, we described a process for the production of a seasoning which comprises preparing a fermented protein koji from a protein containing material and a carbohydrate, hydrolysing the fermented protein koji at a temperature between 15°C and 60°C and a pH of from 4.5 to 10 for a period of from 6 hours to 28 days characterised in that inoculation with a culture of a lactic acid bacteria at an inoculation density of from 10^3 to 10^7 cfu/g of fermented protein koji is carried out either in the fermented protein koji stage or in the hydrolysis stage. The purpose of the lactic acid bacteria is to inhibit the growth of contaminating bacteria and protect the koji from abnormal fermentation and putrefaction caused by such contaminating bacteria.

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Some lactic acid bacteria e.g. *Lactobacillus sake* (*L.sake*) produce peptidases and fiber hydrolysing enzymes. API ZYM kit has been used to detect enzymatic activities produced by the lactic acid bacteria such as phosphatases, lipases, aminopeptidases (leucyl-, valyl-, cystyl-), protease (chymotrypsin) and carbohydrases (galactosidase, glucosidase, N-acetyl- β -glucosaminidase). However they do not produce glutaminase activity extracellularly. Among the carbohydrases that are produced, N-acetyl- β -glucosaminidase is a fiber hydrolysing enzyme. The substrate of this enzyme, N-acetyl- β -glucosamine, is commonly found in chitin. In other words, these lactic acid bacteria produce Chitinase capable of degrading the polysaccharide chains of fungal cell walls. When API 50 CHL is used in the characterisation of these lactic acid bacteria, it is shown that they are able to break down Mannose, Rhamnose, N-acetyl- β -glucosamine, Melibiose, Saccharose, Trehalose, β -Gentiobiose and Gluconate. All these are carbohydrates commonly found in plant cell wall. Among them, Gluconase is also a fungal cell wall degrading enzyme

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These properties make such lactic acid bacteria a valuable source of proteolytic and fiber hydrolysing enzymes in addition to its protective behaviour.

5 SUMMARY OF THE INVENTION

We have found that, in the production of a hydrolysate seasoning, by hydrolysing a protein containing material substrate with enzymes containing proteolytic activities and fiber hydrolysing activities, a surprising improvement in the
10 organoleptic properties of the seasoning was observed. This improvement may be achieved with or without the preparation of koji and/or in the presence or absence of lactic acid bacteria.

Accordingly, the present invention provides a process for the production of a
15 hydrolysate seasoning which comprises hydrolysing a protein containing material substrate with enzymes containing proteolytic activities and fiber hydrolysing activities.

20 DETAILED DESCRIPTION OF THE INVENTION

The protein containing material substrate may be a plant protein material such as soya, wheat germ, corn gluten, rice gluten, wheat gluten, etc. or it may be a fermented protein koji prepared from the protein containing material and a carbohydrate, for example, by fermenting one or more protein containing
25 materials together with a carbohydrate with a culture of *Aspergillus oryzae* and/or *Aspergillus sojae*. The carbohydrate may be, for instance, wheat flour, roasted wheat or wheat bran.

When the protein containing material is a gluten which will undergo a koji
30 fermentation, it is advantageously used as a substrate in the form of pellets, by forming a dough which comprises adding water to a dried gluten in an amount of from 19 to 60% by weight based on the weight of the dough, pelletising the dough and, if necessary, adding water to adjust the moisture content to from 35 to 60% by weight based on the weight of the pellets before sterilising the pellets by steam
35 treatment. The preparation of the pellets as substrate support for Koji fermentation

from the above raw materials and the conditions for koji fermentation are described in our copending SG-A-9800488-0.

5 The protein containing material is a substrate which may contain from 30 to 100% of protein, preferably from 70 to 90% of protein.

If desired, the protein containing material substrate may be inoculated with a lactic acid bacteria for protective purposes. The lactic acid bacteria may be, for instance, *L. sake*, *L. brevis*, *L. casei*, *L. curvatus*, *L. pentosus*, *L. plantarum*, *L. pentosaceus*.

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The source of the enzyme containing proteolytic activities may be microorganisms such as bacteria, fungi or yeasts, etc., for instance the enzyme may be the enzyme produced during koji fermentation, the proteolytic enzymes produced by the lactic acid bacteria used to inoculate the protein material substrate, a technical

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proteolytic enzyme, or it may be a combination of two or more of these enzymes.

The technical enzyme may be, for instance, a protease, peptidase or glutaminase (e.g. Alcalase and Flavorzyme from Novo Nordisk, Glutaminase from Amano Pte Ltd). Technical enzymes are generally enzymes that have been isolated and purified to remove interfering activities which would be detrimental to processing.

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The technical enzyme containing proteolytic activities may be added to the plant protein substrate prior to koji fermentation or it may be added to the hydrolysate.

The source of the enzyme containing fiber hydrolysing activities may be microorganisms such as bacteria, fungi or yeasts, etc., for instance it may be the

25 small amount of fiber hydrolysing enzyme produced during koji fermentation, the fiber hydrolysing enzymes produced by the lactic acid bacteria used to inoculate the protein material substrate, a technical fiber hydrolysing enzyme, or it may be a combination of two or more of these sources. The technical fiber hydrolysing enzyme may be, for instance, Hemicellulase from Amano Pte Ltd, Celluclast from

30 Novo Nordisk, or Depol 40L from Biocatalyst Pte Ltd. The enzyme containing fiber hydrolysing activities may be added to the plant protein substrate prior to koji fermentation, or it may be added to the hydrolysate.

35 Among the protein substrates, corn gluten has the most significant improvement of the hydrolysis yield using an enzyme that possesses the fiber hydrolysing enzyme activity. Corn gluten contains a high percentage of carbohydrates (16.5%)

and fibers (5.7%). These complex carbohydrates form an intact membrane where corn proteins are found within. The masking effect of the carbohydrate network greatly affects the accessibility of the corn protein by proteases or peptidases. To increase the accessibility of the corn protein, it is therefore necessary to break
5 down the carbohydrate membrane by fiber hydrolysing enzymes. Although not wishing to be bound by theory, for the koji system, it is thought that these fiber hydrolysing enzymes hydrolyse the cell walls of the *Aspergillus* mycelia in koji, causing the release of intracellular koji peptidases and glutaminases into the hydrolysate. These extracellular peptidases and glutaminases would definitely be
10 more efficient, leading to an increase in formol nitrogen and glutamic acid yields.

The hydrolysis of the protein containing material substrate may be carried out at a temperature from 2° to 65°C at a pH from 4.5 to 10 for a period of from 6 hours to 28 days, e.g. as described in EP 0640294 or our co-pending patent applications
15 EP-A-0829205, EP-A-0824873 or EP-A-0913097, the entirety of which are hereby incorporated by reference. In the latter two applications, inoculation with a culture of a lactic acid bacteria at an inoculation density of from 10^3 to 10^7 cfu/g of fermented protein koji is carried out either in the fermented protein koji stage or in the hydrolysis stage. In the present invention, inoculation in the protein
20 containing material substrate with a culture of a lactic acid bacteria at an inoculation density of from 10^3 to 10^9 cfu/g of protein containing material may be carried out either in the fermented protein koji stage or in the hydrolysis stage. The hydrolysis is preferably carried out at a temperature between 15°C and 37°C for a period of from 12hr to 5 days, and more preferably at a temperature between
25 37°C and 55°C for a period of from 8hr to 2 days. Advantageously, the hydrolysis is carried out at a pH of from 4.5 to 7.5. The hydrolysis may be carried out in the absence or presence of salt, and when salt is present, the amount of salt may be up to 100% by weight based on the weight of the protein material.

30 After the hydrolysis, the hydrolysed protein material may be pressed to separate a liquid sauce from a solid residue. The liquid sauce is advantageously pasteurised e.g. at a temperature of from 90° to 140°C for a period of from 15 seconds to 30 minutes and then filtered to give a liquid seasoning. If desired, salt may be added either before or after pressing or filtering to give a product having a salt content of
35 0-60% by weight based on the weight of dried matter. If desired, the liquid sauce

may be made into a powder for instance, by concentration, then dried, e.g. vacuum dried to a low moisture content and finally milled into a powder to give a solid seasoning.

5 EXAMPLES

The following Examples further describe the present invention.

Example 1

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Water was added directly to a mixture of corn gluten (6.04kg), wheat flour (2.16kg) and wheat bran (0.43kg) to obtain a moisture content of 45%. The dough formed was fed into a mincer to form cylindrical pellets each having a diameter of 4mm and a length of 10mm. Additional moisture was introduced to the pellets
15 such that the moisture content of the pellets was 47% by weight.

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The pellets were heated to 100°C and held at the same temperature for 10 minutes and cooled to below 40°C. The cooked extrudates were mixed with a liquid suspension of 2.5g of *Aspergillus oryzae* spores inoculum and 10 g of a culture of *L. sake* at 10⁹ cfu/g. The technical hemicellulase enzyme, Hemicellulase from Amano Pte Ltd (activity= 90,000u/g), was added at a concentration of 0.43% of weight of cooked extrudate.

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During the 42 hours of Koji fermentation, the following temperature profiles were maintained for the culture bed:

0 - 25 hours	30°C
25 - 42 hours	27°C

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The Koji was mixed at 18th and 25th hours to ensure sufficient airflow through the culture bed for good ventilation. After the koji fermentation, the matured koji was harvested. Hydrolysis was carried out in a hydrolysis tank by adding water to obtain a hydrolysate with a total solid of 18%*m/m*. The hydrolysis was carried out at 35°C for 48 hours and the pH was kept constant at 6.0 with addition of a 40%
35 NaOH solution.

The corn gluten hydrolysed product has a high glutamic acid content of 1.01%*m/m*. The hydrolysed product was pressed to separate corn gluten sauce from solid residue. Salt was added to the level of 15% *m/m* before pressing. The corn gluten sauce was heat treated at 90°C for 20 minutes. The liquid sauce was concentrated by evaporation. The concentrate obtained was dried in a vacuum oven and milled into powder.

For organoleptic evaluation, 12.5g of liquid sauce or 3.5g of powder were diluted with 250 ml of boiling water. In both cases, a tasting panel of 8 experienced tasters found the product to have a better body than a product without the addition of the technical hemicellulase enzyme to the cooked extrudates.

Example 2

A similar procedure was carried out as example 1 except that the technical hemicellulase enzyme was added to the hydrolysate at a concentration of 0.25% of the weight of hydrolysate during hydrolysis instead of to the cooked extrudates before koji fermentation. The corn gluten hydrolysed product thus produced also has a high glutamic acid content of 0.97%*m/m*. For organoleptic evaluation, 12.5g of liquid sauce or 3.5g of powder were diluted with 250 ml of boiling water. In both cases, a tasting panel of 8 experienced tasters found the product to have a better body than a product without the addition of the technical hemicellulase enzyme.

Example 3

220kg of water was heated up to 35°C in a reactor. 60 kg of corn gluten powder was added into the water with stirring. 380g of technical protease enzyme, Flavourzyme 2.4L (from Novo Nordisk) were added into the hydrolysate. *L. sake* culture was also inoculated in the hydrolysate such that a count of 10⁶cfu/ml was obtained. Temperature and pH were controlled at 35°C and 6.0 respectively, for 48 hours. The hydrolysed product produced this way was found to have a higher glutamic acid (0.4%*m/m*) compared to the product without the inoculation of *L.sake* (0.2%*m/m*).

The hydrolysate was further processed as in example 1 to produce corn gluten sauce powder. A better microbiological quality during hydrolysis was also achieved compared to the product without the inoculation of *L. sake*. A tasting panel of 8 experienced tasters found the product to have a better body than a product without the addition of *L.sake*.

Example 4

A similar procedure was carried out as example 3 except that 380g of technical protease enzyme, Flavourzyme 2.4L (from Novo Nordisk) and 700g of technical hemicellulase enzyme, Hemicellulase from Amano Pte Ltd (activity= 90,000u/g), was added in the hydrolysate at the onset of hydrolysis. The corn gluten hydrolysed product thus produced has a high glutamic acid content of 0.83%*m/m*. A tasting panel of 8 experienced tasters found the product to have a better body than a product without the addition of the technical hemicellulase enzyme, i.e. an even better body to that of the product of example 3 due to the presence of further fiber hydrolysing enzyme, hemicellulase in addition to that derived from the *L.sake*.

Example 5

A similar procedure was carried out as in example 1 except that the koji was produced based on wheat gluten instead of corn gluten and that technical hemicellulase enzyme, Hemicellulase from Amano Pte Ltd (activity = 90,000u/g), was added to the hydrolysate at a concentration of 0.25% of the weight of hydrolysate at the onset of hydrolysis. Wheat gluten powder, at a ratio of 7:3 to the wheat gluten koji was added in the hydrolysate during the hydrolysis. The water quantity was adjusted such that the hydrolysate has a total solid of 18%*m/m*.

The hydrolysed product was found to have a higher glutamic acid (2.10%*m/m*) and formol nitrogen (0.95%*m/m*) compared to the product without the inoculation of the technical hemicellulase enzyme (1.76%*m/m* and 0.88%*m/m* respectively). A tasting panel of 8 experienced tasters found the product to have a better body than a product without the addition of the technical hemicellulase enzyme.

Example 6

5 A similar procedure was carried out as example 5 except that fiber hydrolysing enzyme was not added, corn gluten powder was added in the hydrolysate instead of wheat gluten powder and the ratio of koji to powder was 7:3 instead of 3:7. 380g of technical protease enzyme, Flavourzyme 2.4L (from Novo Nordisk) and 380g of technical protease enzyme, Alcalase 1.5L (from Novo Nordisk) were also added into the hydrolysate at the onset of hydrolysis. The hydrolysed product thus produced has a high glutamic acid content of 1.83%*m/m*.

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A tasting panel of 8 experienced tasters found the product to have a better body than a product without the addition of the *L.sake* as the source of the fiber hydrolysing enzyme.

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Example 7

A similar procedure was carried out as example 1 except that the culture of *L. sake* was omitted.

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A tasting panel of 8 experienced tasters found the product to have a better body than a product without the addition of the technical hemicellulase enzyme.

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What is claimed is:

1. A process for producing a hydrolysate seasoning comprising;
providing an aqueous solution of a protein koji prepared from a protein composition comprising one or more proteins and a carbohydrate;
inoculating the protein koji with a culture of a lactic acid bacteria at an inoculation density of from 10^3 to 10^9 cfu/g of the koji; and
hydrolyzing the protein koji with one or more enzymes that have proteolytic activity and one or more enzymes that have fiber hydrolyzing activity to produce a hydrolysate seasoning, wherein at least one of the enzymes used to hydrolyze the protein-containing material is a technical proteolytic enzyme or a technical fiber-hydrolyzing enzyme.
2. The process of claim 1, wherein the protein koji contains about 30 to 100 percent protein.
3. The process of claim 2, wherein the protein koji comprises one or more of soya, wheat germ, corn gluten, rice gluten, or wheat gluten.
4. The process of claim 1, wherein the protein koji is inoculated with material with a lactic acid bacterium selected from the group consisting of *L. sake*, *L. Brevis*, *L. casei*, *L. curvatus*, *L. pentosus*, *L. plantarum*, *L. pentosaceus*, and mixtures thereof.
5. The process of claim 1, wherein the hydrolysis is carried out at a temperature of about 15°C to 37°C for about 12 hr to 5 days.
6. The process of claim 5, wherein the hydrolysis is carried out at a temperature of about 37°C to 55°C for about 8 hr to 2 days.
7. The process of claim 1, wherein the protein koji is a fermented protein koji prepared by fermenting the protein composition with a culture comprising one or more of *Aspergillus oryzae* or *Aspergillus sojae*.

8. The process of claim 1, wherein the protein koji has been inoculated with the lactic acid bacteria culture before the protein koji is fermented or before the koji is hydrolyzed.
9. The process of claim 1, wherein the one or more enzymes that have proteolytic activity is obtained from bacteria, fungi, or yeast.
10. The process of claim 1, wherein the one or more enzymes that have proteolytic activity is one or more of a proteolytic enzyme produced during the koji fermentation or a proteolytic enzyme produced by the lactic acid bacteria that has been inoculated into the protein koji.
11. The process of claim 1, wherein the one or more enzymes containing fiber hydrolyzing activity is one or more of a fiber hydrolyzing enzyme produced during the koji fermentation, or a fiber hydrolyzing enzyme produced by the lactic acid bacteria that has been inoculated into the protein koji.
12. The process of claim 1, wherein the one or more enzymes having proteolytic activity comprise a technical proteolytic enzyme that is added to the protein koji before the protein koji is fermented.
13. The process of claim 1, wherein the one or more enzymes having fiber hydrolyzing activity comprise a technical fiber hydrolyzing enzyme that is added to the protein koji before the protein koji is fermented.
14. A process for producing a hydrolysate seasoning comprising:
providing an aqueous solution of one or more protein-containing materials;
inoculating the protein-containing material with a culture of a lactic acid bacteria at an inoculation density of from 10^3 to 10^9 cfu/g of said protein-containing material;
hydrolyzing the one or more protein-containing materials with one or more enzymes that have proteolytic activity and one or more enzymes that have fiber hydrolyzing

activity to produce a hydrolysate seasoning, wherein at least one of the enzymes used to hydrolyze the protein-containing material is a technical proteolytic enzyme or a technical fiber-hydrolyzing enzyme;

pressing the hydrolysate seasoning to separate a liquid hydrolysate seasoning from a solid residue;

concentrating the liquid hydrolysate seasoning to provide a concentrated hydrolysate seasoning; and

drying the concentrated hydrolysate seasoning to provide a dried hydrolysate seasoning.

15. The process of claim 14, wherein hydrolysis of the protein-containing material is carried out at a temperature from about 20° to 65°C and at a pH of about 4.5 to 10 for about 6 hours to 28 days.

16. The process of claim 14, wherein the hydrolysis of the protein-containing material is carried out with salt in an amount of up to 100% by weight based on the weight of the protein material.

17. The process of claim 14, wherein the liquid hydrolysate seasoning is pasteurized at a temperature of about 90° to 140°C for a period of about 15 seconds to 30 minutes and then filtered prior to concentrating.

18. The process of claim 14, wherein the hydrolysis is carried out in the absence of salt and further comprising adding salt either before pressing or after pressing to provide a liquid hydrolysate seasoning having a salt content of less than 60% by weight based on the weight of dry matter.

19. The process of claim 14, further comprising milling the dried hydrolysate seasoning into a powder having a water content of less than about 2 percent.

20. The process of claim 14, wherein the protein-containing material is inoculated with material with a lactic acid bacterium selected from the group consisting of L.

sake, *L. Brevis*, *L. casei*, *L. curvatus*, *L. pentosus*, *L. plantarum*, *L. pentosaceus*, and mixtures thereof.

21. The process of claim 14, wherein the protein-containing material is a protein koji prepared from a protein composition comprising one or more proteins and a carbohydrate.

22. A process for producing a hydrolysate seasoning comprising:
providing an aqueous solution of one or more protein-containing materials;
inoculating the protein-containing material with a culture of a lactic acid bacteria at an inoculation density of from 10^3 to 10^9 cfu/g of said protein-containing material; and
hydrolyzing the one or more protein-containing materials with one or more enzymes that have proteolytic activity and one or more enzymes that have fiber hydrolyzing activity to produce a hydrolysate seasoning, wherein the one or more protein-containing materials is a koji, and at least one of the enzymes used to hydrolyze the protein-containing material is a technical proteolytic enzyme or a technical fiber-hydrolyzing enzyme, and wherein the one or more enzymes that have proteolytic activity is one or more of a proteolytic enzyme produced during a koji fermentation or a proteolytic enzyme produced by the lactic acid bacteria that has been inoculated into the protein-containing material.

23. A process for producing a hydrolysate seasoning comprising:
providing an aqueous solution of one or more protein-containing materials;
inoculating the protein-containing material with a culture of a lactic acid bacteria at an inoculation density of from 10^3 to 10^9 cfu/g of said protein-containing material; and
hydrolyzing the one or more protein-containing materials with one or more enzymes that have proteolytic activity and one or more enzymes that have fiber hydrolyzing activity to produce a hydrolysate seasoning, wherein at least one of the enzymes used to hydrolyze the protein-containing material is a technical proteolytic enzyme or a technical fiber-hydrolyzing enzyme, and wherein the one or more

protein-containing materials is a koji, and the one or more enzymes containing fiber hydrolyzing activity is one or more of a fiber hydrolyzing enzyme produced during a koji fermentation, or a fiber hydrolyzing enzyme produced by the lactic acid bacteria that has been inoculated into the protein-containing material.