Description

ENZYME PREPARATION FOR ADHESION-MOLDED FOODS AND METHOD FOR PRODUCING ADHESION-MOLDED FOOD

Technical Field

The present invention relates to enzyme preparations for bound and formed foods, and methods for producing a bound and formed food.

Background Art

There are many reports concerning techniques on binding food raw materials using transglutaminase. Japanese Patent No. 1927253 discloses a technique to produce a bound and formed food with the use of only transglutaminase. The invention described in this patent is groundbreaking in the sense that it developed a novel use of transglutaminase. However, because of insufficient binding strength, techniques using a variety of other components with transglutaminase have been studied, and put into practical application.

Japanese Patent Nos. 3353383 and 3353503 disclose inventions concerning bound and formed foods, in which transglutaminase and its substrate caseins are used in combination. The methods described in these patents are applicable to a wide range of food raw materials, including

not only livestock meat, but fishery products such as fish meat, squid, and crab, and fish roe such as salmon roe, herring roe, salted salmon roe, and salted cod roe. The methods described in these patents are inventions concerning highly versatile binding and forming methods that enable a food to be bound in the raw state without influencing taste and flavor.

Because of recent problems of food allergy, use of milk-derived proteins for processed foods is not always possible. In this connection, binding methods that use transglutaminase and non-casein proteins have been studied. Japanese Patent No. 3407599 discloses a binding and forming method that uses collagen and transglutaminase as the active ingredients without using casein. However, because collagen has the property to develop high viscosity when dissolved in water, the method requires the collagen to be dissolved in cold water of 10°C or less, and the bonding procedure must immediately follow the dissolving of collagen. The method is therefore problematic in terms of handling. It should also be noted that the binding strength is weak in the absence of a salt, and practical effects cannot be expected in this case.

In this connection, International Publication WO 02/080700 discloses an enzyme preparation for binding food raw materials, and a method of production of bound and formed food using the enzyme preparation. The invention described in this publication uses a specific collagen in which the total number

of hydroxyproline and proline residues (hereinafter, also referred to as "imino acids") is less than 20% of total amino acid residues in the collagen, and the specific collagen and transglutaminase are contained as the active ingredients. Patent Document 6 describes suppressing gelling of collagen at low temperatures by preferably blending, for example, salts, potassium chloride, and calcium chloride to an adhesive containing transglutaminase, collagen and salts for a bound and formed food. However, because the invention described in JP-A-2006-246716 uses calcium chloride to suppress gelling of collagen at low temperatures, the invention of this publication is completely different in the way calcium chloride is used in the present invention, as will be described later. Accordingly, the invention described in JP-A-2006-246716 does not suggest the present invention.

Further, the type of collagen that can be used as the specific collagen is essentially fishskin-derived collagen. As such, there are cases where preparations that contain fish collagen are not usable for livestock meat processed products that use pork, beef, and chicken, because the fish collagen has a different protein origin from these products. Particularly in the European market where processed foods that use raw materials originating in fish must be labeled with a listing of allergic ingredients, use of fish collagen for livestock meat processed products is often more restricted than

in other regions.

The binding techniques currently available all use specific protein materials in a blend, and no technique is available that can satisfy the need to be free of different proteins. Despite the need for a binding technique that does not use protein materials, no technique has been developed that can provide practical levels of binding strength without using protein materials.

Disclosure of the Invention

From these backgrounds, an object of the present invention is to provide an excellent enzyme preparation for binding and forming, and a method for producing a bound and formed food, with which food raw materials such as meat pieces can be sufficiently bound and formed without using protein materials.

The present inventors conducted extensive studies to solve the foregoing problems, and found that food raw materials such as livestock meat can be bound with the use of transglutaminase and calcium chloride or magnesium chloride. The present invention was completed based on this finding. Specifically, the present invention is as described below.

(1) An enzyme preparation for bound and formed foods, comprising transglutaminase, and calcium chloride or magnesium chloride as active ingredients, wherein the amount

of calcium chloride is 0.007 to 0.03 g per 1 U of the transglutaminase in said enzyme preparation, or wherein the amount of magnesium chloride is 0.022 to 0.025 g per 1 U of the transglutaminase in said enzyme preparation.

(2) A method for producing a bound and formed food, comprising adding 0.6 g of calcium chloride and 75 to 100 units of transglutaminase per 100 g of a food raw material.

(3) A method for producing a bound and formed food, comprising adding 0.7 to 0.9 g of calcium chloride and 25 to 100 units of transglutaminase per 100 g of a food raw material.

(4) A method for producing a bound and formed food, comprising adding 1.4 to 1.6 g of magnesium chloride and 60 to 100 units of transglutaminase per 100 g of a food raw material.

(5) The method according to any of (2) to (4), wherein the food raw material is meat, and wherein the bound and formed food is bound and formed meat.

The transglutaminase to be used in this invention is an enzyme used for catalyzing an acyl-transfer reaction in a γ-carboxyamide group in a glutamine residue in a protein or peptide chain. An ε-(γ-Glu)-Lys bond is formed in and between protein molecules by the action of the transglutaminase as an acyl receptor exerted on an ε-amino group of a lysine residue in the protein. An enzyme of any origin can be used as the transglutaminase to be used in the present invention, as long

as it has transglutaminase activity. For example, enzymes that originate in microorganisms, such as those from actinomycetes (see Japanese Patent No. 2572716), and from *Bacillus subtilis* (see Japanese Patent No. 3873408) can be used. Further, the enzyme may be of guinea pig liver origin (see Japanese Patent No. 1689614), microorganism origin (see WO96/06931), animal origin including cow blood and pig blood, fish origin including salmon and red sea bream (see Seki *et al.*, *Nippon Suisan Gakkaishi*, 1990, 56, 125-132), or oyster origin (see U.S. Patent No. 5,736,356). Further, for example, the enzyme may be those produced by genetic recombination (see, for example, Japanese Patent No. 3010589, JP-A-11-75876, WO01/23591, WO02/081694, and WO2004/078973). That is, any transglutaminase can be used in the present invention, and the origin and method of production are not limited. However, considering functionality for food applications and ease of handling, it is preferable to use transglutaminases of microorganism origin (Japanese Patent No. 2572716), which can be commercially mass-produced, and are available at low cost.

The activity unit of the transglutaminase used in the present invention is measured and defined as follows. Specifically, a reaction is performed by using benzyloxycarbonyl-L-glutaminylglycine and hydroxylamine as a substrate, and, after converting thus-generated hydroxamic acid into an iron complex in the presence of trichloroacetic

acid, an amount of the iron complex is measured at an absorbance of 525 nm. An enzyme amount that enables generation of 1 micromol of hydroxamic acid in one minute is defined as 1 unit which is an activity unit of transglutaminase. Details of this measurement method which is called hydroxamate method have already been reported (for example, see Registered No. 2572716).

The essential components calcium chloride and magnesium chloride in the present invention may be of any grade, provided that they are usable for food. Calcium chloride or magnesium chloride may be used alone, or calcium chloride and magnesium chloride may be used in combination. The use of calcium chloride and/or magnesium chloride considerably increases the binding effect.

In the enzyme preparation of the present invention, the mixture ratio of the constituent components transglutaminase and calcium chloride or magnesium chloride is such that the calcium chloride content is preferably 0.007 to 0.03 g per 1 U of transglutaminase in the preparation, or the magnesium chloride content is preferably 0.022 to 0.025 g per 1 U of transglutaminase in the enzyme preparation. The transglutaminase is mixed in 1 to 200 units per gram of the enzyme preparation.

The enzyme preparation for binding of the present invention comprises transglutaminase and calcium chloride

and/or magnesium chloride as the active ingredients, and may additionally comprise other components, including, for example, known food excipients such as lactose, sucrose, maltitol, sorbitol, dextrin, branched dextrin, cyclodextrin, silicon dioxide, cellulose, starches, polysaccharides, gums, and pectin. The enzyme preparation may also comprise sodium bicarbonate, sodium citrate, sodium phosphate, calcined calcium, calcium phosphate, calcium carbonate, magnesium carbonate, sodium chloride, potassium chloride or various polyphosphates such as sodium pyrophosphate, sodium tripolyphosphate or sodium metaphosphate. It is also possible to appropriately mix components such as flavor enhancers, sugars, spices, artificial colors, coloring agents, ascorbic acids and salts thereof, emulsifiers, or fats and oils.

The enzyme preparation for binding and forming of the present invention is not necessarily required to be provided as a mixture of transglutaminase and calcium chloride or magnesium chloride contained in the same container, and may be provided in the form of a kit that contains these components in separate containers. The enzyme preparation may be a powder or a liquid.

In the present invention, the bound and formed food refers to food products obtained by binding and forming food raw material pieces measuring 3 mm or more, preferably 1 cm or more in the smallest side, and excludes paste products, such

as sausage and *kamaboko*, produced from minced or paste raw materials. Any food raw material may be used as long as the material is a protein-based food. Examples of food raw material include meat such as beef, pork, horse, mutton, goat, rabbit, chicken, duck, and domestic duck; various types of fish meat; shellfish; crustaceans such as shrimp and crab; mollusks such as squid and octopus; and fish roe such as salmon roe, and salted salmon roe. These are merely examples, and the food raw material is not limited to these.

Binding of food pieces or small food materials requires a tensile strength of 80 g/cm^2 or more as measured with a rheometer (Fudo Kougyou). A tensile strength less than 80 g/cm^2 is not suited for practical use, because it causes the materials to detach during the production or preparation of a bound and formed food.

As described above, the present invention is applicable to all kinds of protein-based food raw materials, enabling production of bound and formed products without addition of protein materials (purified protein or extracted protein) such as casein and collagen. The present invention can therefore solve the problem of the meat processing industry looking for ways to be free of different proteins, and to be free of the need to put a labeling of allergic ingredients.

The following methods can be used to produce a bound and formed food by binding food raw materials.

- A method using an enzyme preparation that comprises transglutaminase and calcium chloride or magnesium chloride as the active ingredients

- A method using transglutaminase and calcium chloride or magnesium chloride that are separately purchased

Either method is usable.

In either case, the calcium chloride is used in an amount of preferably from 0.6 to 0.9 g (inclusive), more preferably from 0.6 to 0.8 g, in terms of a weight of dihydrate crystals, per 100 g of the food raw material. Above 0.9 g, the taste of calcium chloride becomes strong, and the bound and formed food cannot have good taste. Below 0.6 g, sufficient binding cannot be obtained even with the use of transglutaminase, and binding becomes insufficient in the bound and formed food.

The transglutaminase is used in an amount of preferably 75 to 100 units, more preferably 90 to 100 units per 100 g of the food raw material when the calcium chloride content is 0.6 g per 100 g of the food raw material. When the calcium chloride content is 0.7 to 0.9 g per 100 g of the food raw material, the transglutaminase is used in an amount of preferably 20 to 100 units, more preferably 30 to 90 units per 100 g of the food raw material. When the transglutaminase is used in amounts below these ranges, binding does not develop sufficiently. Above these ranges, the cost of the transglutaminase becomes too much of a factor.

The magnesium chloride is used in an amount of preferably 1.4 to 1.6 g, in terms of a weight of hexahydrate crystals, per 100 g of the food raw material. Above 1.6 g, the taste of the magnesium chloride becomes strong, and the bound and formed food cannot have good taste. Below 1.4 g, sufficient binding cannot be obtained even with the use of the transglutaminase, and binding becomes insufficient in the bound and formed food. The transglutaminase is used in an amount of preferably 60 to 100 units per 100 g of the food raw material when the magnesium chloride content is 1.4 to 1.6 g per 100 g of the food raw material. When the transglutaminase is used in amounts below this range, binding does not develop sufficiently. Above this range, the cost of the transglutaminase becomes too much of a factor.

Note that sufficient binding is the state in which the tensile strength as measured with a rheometer is 80 g/cm^2 or more.

Production of a bound and formed food by the binding of food raw materials can use either a method in which the enzyme preparation for binding of the present invention is used by being dissolved in a solvent, or a method in which the enzyme preparation is mixed with food raw materials in powdery form. Specifically, the essential components transglutaminase, calcium chloride, and magnesium chloride are mixed with the food raw materials either separately or at the same time in

the form of a solution or a powder. Both of these methods are confined within the method for producing bound and formed foods of the present invention.

Brief Description of the Drawings

Fig. 1 is a diagram representing the relationship between the binding strength and the amounts of calcium chloride and transglutaminase added (Example 1).

Fig. 2 is a diagram representing the relationship between the binding strength and the amounts of magnesium chloride and transglutaminase added (Example 1).

Best Mode for Carrying Out the Invention

The present invention is described below in detail based on Example and Comparative Example. It should be noted, however, that the technical scope of the present invention is not limited by the following Example.

Example 1

A commercially available transglutaminase (Activa TG; Ajinomoto Co., Inc.; specific activity, 1,000 units/g) of *Streptomyces mobaraensis* origin was used as the transglutaminase. Note that *Streptomyces mobaraensis* had been called *Streptoverticillium mobaraense* before 1990. Magnesium chloride hexahydrate (from Tomita Pharmaceutical

Co., Ltd.) and calcium chloride dehydrate (from Tomita Pharmaceutical Co., Ltd.) were used. As shown in Table 1, the transglutaminase, calcium chloride, and magnesium chloride were weighed, and dissolved in 12 ml of water. The resulting mixed solutions were then used as enzyme preparations of the present invention.

Table 1. Formulations of Enzyme Preparations

Test group	1	2	3	4	5	6	7	8
Transglutaminase (U)	90	90	90	90	90	180	180	180
Calcium chloride (g)	1.2	1.5	1.8	2.1	2.4	1.2	1.5	1.8
Test group	9	10	11	12	13	14	15	
Transglutaminase (U)	180	180	270	270	270	270	270	
Calcium chloride (g)	2.1	2.4	1.2	1.5	1.8	2.1	2.4	
Test group	16	17	18	19	20	21		
Transglutaminase (U)	180	180	180	180	180	180		
Magnesium chloride (g)	2.7	3.0	3.3	3.6	3.9	4.2		

Each enzyme preparation so prepared was thoroughly mixed with small pieces of pork ham (about 2 cm cube, a total of 300 g), and the mixture was packed into a casing tube that had a folding width of 75 mm. The tube was left at 5°C for 18 hours to allow the transglutaminase reaction to proceed. Thereafter, the tube was placed in a -40°C freezer, and preserved therein for later evaluation. The frozen bound pork was then sliced into a thickness of 9 mm and a width of 25 mm.

Figs. 1 and 2 show the results of tensile strength measurements performed in the raw state with a rheometer (Fudo Kougyou) after thawing. In contrast to the tensile strength

of 80 g/cm^2 as the conventional index of a practical binding strength measured in the same manner, the values of binding strength measured in this evaluation were above this reference value (80 g/cm^2) in the test groups that had the calcium chloride content of 2.1 g (0.7 g per 100 g of the food raw material) or more from among the test groups 1 to 5, and 6 to 10 in which the transglutaminase was added in 90 U and 180 U (30 U and 60 U per 100 g of the food raw material). the values of binding strength also exceeded the reference value in the test groups that had the calcium chloride content of 1.8 g (0.6 g per 100 g of the food raw material) or more from among the test groups 11 to 15 in which the transglutaminase was added in 270 U (90 U per 100 g of the food raw material). Further, the value of binding strength was above the reference value in the test group that had the magnesium chloride content of 4.2 g (1.4 g per 100 g of the food raw material) or more from among the test groups 16 to 21 in which magnesium chloride was added. As these results demonstrate, practical binding strength can be obtained by using calcium chloride, magnesium chloride, and transglutaminase in the appropriate ranges.

Comparative Example 1

Calcium oxide (calcined calcium),an inorganic salt of calcium as is calcium chloride, is known to bind food materials when added in appropriate amounts. Thus, it was investigated

whether use of calcium oxide would provide the same effect obtained with the use of calcium chloride. Comparisons using various other salts were also made. Each salt presented in Table 2 was dissolved in 12 ml of water with 180 units of transglutaminase, and, after the solution was thoroughly mixed with small pieces of pork ham (about 2 cm cube, a total of 300 g), the mixture was packed into a casing tube that had a folding width of 75 mm. The tube was left at 5°C for 17 hours to allow the transglutaminase reaction to proceed. Thereafter, the tube was placed in a -40°C freezer, and preserved therein for later evaluation. Thereafter, tensile strength was measured in the same manner as in Example 1, and comparison was made. The results are presented in Table 2.

As shown in Table 2, of all groups in which 2.1 g of salt (0.7 g per 100 g of raw food) was added, biding was sufficient only in the calcium chloride-added group, and sufficient binding strength could not be obtained in groups that had other salts. The calcium oxide-added group had binding strength close to a sufficient value; however, the meat turned white in color, and was not desirable in terms of appearance. The discoloration is considered to be caused because calcium oxide is alkali. The results therefore demonstrated that the binding technique using calcium oxide being a calcium salt as well was completely different from the binding technique using calcium chloride and transglutaminase, and that calcium oxide

could not be used as a replacement of calcium chloride.

Table 2

Salts	Amount added (for 300 g of meat)	Binding strength	Note
Calcium chloride	2.1 g	104.9 g/cm^2	Good appearance
Calcium oxide	2.1 g	75.7 g/cm^2	Turned white; undesirable
Calcium lactate	2.1 g	- (Not bound)	-
Calcium gluconate	2.1 g	- (Not bound)	-
Magnesium sulfate	2.1 g	- (Not bound)	-
Potassium chloride	2.1 g	- (Not bound)	-
Sodium chloride	2.1 g	- (Not bound)	-

Industrial Applicability

According to the enzyme preparation and the method for producing bound and formed food of the present invention, food raw materials such as meat pieces can be bound and formed sufficiently, without using casein, collagen, or other protein materials as auxiliary agents, and therefore it is highly useful in the field of food.

Claims

1. An enzyme preparation for bound and formed foods, comprising transglutaminase, and calcium chloride or magnesium chloride as active ingredients, wherein the amount of calcium chloride is 0.007 to 0.03 g per 1 U of the transglutaminase in said enzyme preparation, or wherein the amount of magnesium chloride is 0.022 to 0.025 g per 1 U of the transglutaminase in said enzyme preparation.

2. A method for producing a bound and formed food, comprising adding 0.6 g of calcium chloride and 75 to 100 units of transglutaminase per 100 g of a food raw material.

3. A method for producing a bound and formed food, comprising adding 0.7 to 0.9 g of calcium chloride and 25 to 100 units of transglutaminase per 100 g of a food raw material.

4. A method for producing a bound and formed food, comprising adding 1.4 to 1.6 g of magnesium chloride and 60 to 100 units of transglutaminase per 100 g of a food raw material.

5. The method according to any of claim 2 to 4, wherein the food raw material is meat, and wherein the bound and formed food is bound and formed meat.