

## METHOD FOR PRODUCING A HYPOALLERGENIC WHEAT FLOUR

### FIELD OF THE INVENTION

The present invention relates to a method for producing a hypoallergenic wheat flour and a hypoallergenic wheat flour produced by the method. More particularly, the present invention relates to a hypoallergenic wheat flour useful as a food material for cereal-allergy patients and a wheat-flour processed foods available from such a wheat flour.

### DESCRIPTION OF THE RELATED ART

The number of patients suffering from food allergy is increasing throughout the world. Cereal-associated allergy is considered to be particularly a serious problem, because cereals such as rice and wheat are consumed as the staple food in most countries.

To solve this problem, the present inventors developed a low-allergen rice in 1990 (J. Food Sci., 55, p. 781, 1990; J. Food Sci., 55, p. 1105, 1990; J. Nutrition, 44, p. 51, 1991; Trends Food Sci., 4, 1993). The developed product was approved in 1993 as the first physiologically functional food for specified health uses by the Japanese Ministry of Health and Welfare, and is now being commercially supplied widely to patients with rice-associated allergy.

On the other hand, although wheat flour is used in quantities as a raw material for bread, noodles and pasta serving as the stable food for many people in the world, achievement of a hypoallergenic wheat flour applicable to food preparation has not as yet been successful to date. In this respect, the present inventors isolated a wheat-antigenic peptide having the amino acid sequence of SEQ ID NO. 1 from a chymotrypsin hydrolysate of gluten which is an antigen for most of wheat-allergy patients (Biosci. Biotech. Biochem., 59, p. 1596-1607, 1995), and found out that the epitope of this peptide is the portion of the amino acid sequence shown in SEQ ID NO. 2, and that the amino acid residues essential for the epitope function are glutamine residue (Gln) first from the N-terminal and the fourth and fifth proline residues (Pro) (Proceedings of annual meeting of Jap. Agr. Chem., 1995). With a view to inactivating epitope of this wheat allergen, the present inventors successfully produced a hypoallergenic wheat flour by treating the flour with collagenase which recognized proline residue, and processed the resultant wheat flour into bread and pasta (Biosci. Biotech. Biochem., 58, p. 388-390, 1994).

However, since this collagenase is an enzyme produced from an anaerobic bacillus, Clostridium, it is not approved as a food additive, and has a definitive drawback in that hypoallergenic wheat flour treated with collagenase is not applicable to food preparation as a raw material.

### SUMMARY OF THE INVENTION

The present invention has an object to provide a method for producing a hypoallergenic wheat flour applicable to food preparation and a resultant hypoallergenic wheat flour, as well as wheat flour processed food products produced from this hypoallergenic wheat flour.

The present invention provides a method for producing a hypoallergenic wheat flour, which comprises the steps of mixing water or an aqueous solution of ethanol to wheat flour, and then mixing a protease having a high collagenase-like activity and being applicable to food preparation with the mixture.

In an embodiment of this method, the protease should preferably be an enzyme having a low amylase activity, in

addition to a high collagenase-like activity. More specifically, bromelain is the most suitable as the protease used in the method of the present invention because of a high collagenase-like activity and a low amylase activity.

In another embodiment of the foregoing method, furthermore, water in an amount of from 0.05 to 100 times, or more preferably, from 0.1 to 10 times, or an aqueous ethanol solution having a concentration of up to 20% in an amount of from 0.05 to 100 times, or more preferably, from 0.1 to 10 times, should be mixed with the flour to prepare a mixture, and the protease in an amount of from 0.01 to 10 wt. %, or more preferably, from 0.1 to 5 wt. % relative to wheat flour should be mixed with the mixture.

The foregoing method is applicable to any of various types of wheat flour such as hard flour, quasi-hard flour, medium flour and soft flour.

The present invention provides a hypoallergenic wheat flour, of which the epitope of IgE-binding site having the amino acid sequence of SEQ ID NO. 2 or 3 is inactivated.

The present invention further provides a wheat flour processed food products produced from the hypoallergenic wheat flour as a raw material. These wheat flour processed food products include all products of wheat flour such as bread, noodles, coatings of shao-mais and the like, pasta, pizza dough and cakes.

The present invention more further provides a manufacturing method of a hypoallergenic bread, which comprises the steps of mixing a carbonate and an acid with the hypoallergenic wheat flour thereby generating carbon dioxide from the mixture, and then baking the mixture.

In addition, the present invention provides also a peptide and a peptide derivative having the amino acid sequence of SEQ ID NO. 2 or 3, the derivative having acetylates glutamine residue at the N-terminal. This peptide and peptide derivative serve as a haptene molecule because they have a binding ability with IgE against wheat allergen but not having antigenicity.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a gel filtration pattern of chymotryptic hydrolysate of gluten;

FIG. 2(A) illustrates a chromatogram of chymotryptic hydrolysate of gluten and FIG. 2(B) a chromatogram of an antigenicity peak;

FIG. 3 illustrates concentration-dependent inhibition values of a peptide derivative;

FIG. 4 illustrates changes in the amount of histamine released by the peptide derivative;

FIG. 5 is a histogram illustrating the pH-dependency of the collagenase-like activity of bromelain;

FIG. 6(A) illustrates results of SDS-PAGE of a hypoallergenic wheat flour treated with bromelain and FIG. 6(B) illustrates the same with a non-treated wheat flour;

FIG. 7(A) is a resistgram of a hypoallergenic wheat flour treated with bromelain and FIG. 7(B) is a resistgram of a non-treated wheat flour; and

FIG. 8 is a farmogram of the hypoallergenic wheat flour treated with bromelain.

### DETAILED DESCRIPTION OF THE INVENTION

The epitope of wheat flour, which is the basis of the method for producing a hypoallergenic wheat flour of the present invention was determined as follows.