Technical Data Ⅰ

KISHIMOTO SANGYO CO.,LTD

Biotechnology Business Development Team

Nutritional Content Analysis for SESAME PEPTIDE KM-20

1.Measurement lot : 40612004

2.Result of measurement

moisture	1．9 g/100g
ash content	8．3 g/100g
protein	72．5 g/100g
fat	0．1 g/100g
carbohydrate	17．2 g/100g
sodium	33 mg/100g
energy	360 kcal/100g

Note : ‘energy’

Energy was calculated on the following basis:

protein 4kcal, fat 9kcal, carbohydrate 4kcal

Technical Data Ⅱ

KISHIMOTO SANGYO CO.,LTD

Biotechnology Business Development Team

DISTRIBUTION OF MOLECULAR WEIGHT

1.Measurement lot : 40612004

2.Outline of test

Distribution of Molecular Weight was measured on the sample by the use of Size Exclusion Chromatography (SEZ) equipped with TSKgel G2500PW$_{XL}$ column.

3.Result of the Test

Measurement result of the Molecular Weight Distribution is shown in Table 1.

Table 1 Measurement Result of Molecular Weight Distribution

range of Molecular Weight	percentage of peak area
10,000 or more	trace amount
3,000 ～ 10,000	14
1,000 ～ 3,000	38
500 ～ 1,000	22
under 500	26
total	100

Technical Data Ⅲ

KISHIMOTO SANGYO CO.,LTD

Biotechnology Business Development Team

Heat-resistance test for SESAME PEPTIDE KM-20

1.Test Method

One-hour heating treatment at 105 ℃, half-an-hour heating at 140 ℃ and half-an-hour heating at 180 ℃ was conducted respectively on SESAME PEPTIDE KM-20(Lot №. 31206001).

Measurement of ACE inhibitory activation was conducted by HPLC analysis method, IC_{50} value, the sample density in the reaction liquid in which 50% inhibition rate is shown, was sought for.

Measurement was conducted three times for each sample and the average figure was taken.

〈 sample〉

1. SESAME PEPTIDE KM-20
2. SESAME PEPTIDE KM-20 treated with one-hour heating at 105℃
3. SESAME PEPTIDE KM-20 treated with half-an-hour heating at 140℃
4. SESAME PEPTIDE KM-20 treated with half-an-hour heating at 180℃

2.Result

density(μg/ml) / sample	inhibition rate (%)				
	25	50	100	200	IC_{50}
no heating treatment	23.4	38.8	57.1	74.7	73 μg/ml
105℃, one hour heating	24.0	38.9	59.5	76.0	75 μg/ml
140℃, half an hour heating	24.9	39.9	56.3	74.2	75 μg/ml
180℃, half an hour heating	13.1	23.0	40.0	60.1	130 μg/ml

3.Consideration

By comparison of the measured ACE inhibitory activation between those heated and unheated SESAME PEPTIDE KM-20, decline of activation was not confirmed as to the‘105℃, one hour heating’ and ‘140℃, half an hour heating’ compared with the unheated one. On the other hand, activation declined about 40% in the case of ‘180℃, half an hour heating’.From this result it is concluded that SESAME PEPTIDE KM-20 has considerable heat-resistancy and can be compounded into food products manufactured at high temperature such as bread or cookie.

Technical Data Ⅳ

KISHIMOTO SANGYO CO.,LTD

Biotechnology Business Development Team

Acid-resistance and salt-resistance test for SESAME PEPTIDE KM-20

1.Test Method

Acid-resistance and salt-resistance test was conducted on SESAME PEPTIDE KM-20 (Lot №. 31206002). Citric acid buffer solution (pH 3.5) was used to test acid-resistancy and 5% salt water was used to test salt-resistancy. 1% solution of SESAME PEPTIDE KM-20 was arranged for each test.

After heating/pasteurization for 20 minutes at 85℃, each 1% solution of SESAME PEPTIDE KM-20 which was preserved for two weeks, one month and two months respectively at 5℃ were subjected to ACE inhibitory activation measurement by means of HPLC analysis, and IC_{50} value, the sample density (Dry) in the reaction liquid in which 50% inhibition rate is shown, was sought for.

Measurement was conducted three times for each sample and the average figure was taken.

2.Result

density(μg/ml) / sample	preservation days	inhibition rate (%)				IC_{50}
		25	50	100	200	
untreated	–	20.3	39.7	52.8	72.0	88 μg/ml
treated with citric acid buffer solution (pH3.5)	2 weeks	22.9	36.2	56.5	73.1	86 μg/ml
	1 month	23.4	35.5	59.6	72.1	87 μg/ml
	2 months	20.3	39.0	53.5	70.9	89 μg/ml
treated with 5% salt water	2 weeks	23.9	37.8	56.9	74.0	87 μg/ml
	1 month	19.0	32.9	53.8	73.9	90 μg/ml
	2 months	20.5	34.7	54.4	78.1	88 μg/ml

3.Consideration

As the result of acid-resistance and salt-resistance test on SESAME PEPTIDE KM-20, decline of ACE inhibitory activation was confirmed after preservation of 2 weeks, 1 month and 2 months respectively both in the citric-acid buffer solution (pH3.5) as acid-resistance test and in the 5% salt water as salt-resistance test following the heating/pasteurization for 20 minutes at 85℃.

From the above results, it has been presumed that SESAME PEPTIDE KM-20 remains stable without being resolved.

Technical Data Ⅴ

KISHIMOTO SANGYO CO.,LTD
Biotechnology Business Development Team

Artificial digestion test for SESAME PEPTIDE KM-20

1.Test Method

Artificial digestion test was conducted on SESAME PEPTIDE KM-20 using pepsin, trypsin,chymotrypsin and small-intestine fluid, 320g of SESAME PEPTIDE KM-20 was dissolved into 10mg of 0.075 N HCL which includes 20g of pepsin, then after the reaction process for 4 hours at 37 ℃ it was neutralized 0.2N NaOH. Next, 10ml of 0.1M phosphoric acid buffering solution was added to 1g of rat's small-intestine acetone powder and was dissolved, then 200μl of the supernatant fluid obtained by centrifuging (3500rpm 15minutes) of the dissolved fluid was added to the abovementioned neutralized pepsin solution, and was again made to react for 4 hours at 37℃ and pH 7.0. After the reaction, the solution was heated in boiling water for 5 minutes so that its digestive enzyme would get inactivated.

After the digestion of pepsin,in the same manner as above, each 10ml from 20mg of trypsin (4hours at 37℃,0.1M phosphoric acid buffering solution of pH 8.0), 20mg of chymotrypsin (4 hours at 37℃, 0.1M phosphoric acid buffering solution of pH 8.0), 20mg of trypsin +20mg of chymotrypsin (4 hours at 37℃, 0.1M phosphoric acid buffering solution of pH 8.0) were added for reaction, thereby gaining each digestive fluid. As a control, enzyme was likewise inactivated as to the solution taken by sampling in the course of processing. Measurement of ACE inhibitory activation was conducted by means of HPLC analysis regarding artificial digestive solution of each SESAME PEPTIDE KM-20 gained through the above process, and IC_{50} value, the sample density(Dry) in the reaction fluid in which 50% inhibition rate is shown, was sought for. Measurement was conducted three times for each sample and the average figure was taken.

2.Result

Digestion	IC_{50} (μg/ml)	Remaining ACE inhibitory activity(%)
None	80	100
Pepsin (pH 2.0, 4 hours)	70	114
Pepsin ⇒ Trypsin (pH 8.0, 4 hours)	75	107
Pepsin ⇒ Chymotrypsin (pH 8.0, 4 hours)	78	103
Pepsin ⇒ Chymotrypsin + Trypsin (pH 8.0, 4 hours)	78	103
Pepsin ⇒ Intestinal fluid (pH 7.0, 4 hours)	80	100

3.Consideration

As the result of artificial digestion regarding SESAME PEPTIDE KM-20, decline of ACE inhibitory sctivation was not confirmed in any processing using pepsin, trypsin, chymotrypsin or small-intestine fluid.

Rather, slight rise of activation was confirmed. It is considered that the advances toward low molecule and high activation occurred due to the work of various enzymes, and because the real ACE inhibitory sctivation peptide was caused, it led to the rise of activation consequently.

From the above result, it is presumed that although SESAME PEPTIDE KM-20 is affected by the action of the above three kinds of enzyme (pepsin,trypsin and chymotrypsin) and various proteinase in intestines, it can keep retaining ACE inhibitory sctivation capability.