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Physiological Functions of Fermented Foods

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The fermented food is the food that effectively uses the microorganism as some steps of food processing. The history of fermented foods is very old same as the human race's birth. Various, traditional fermented food exists in our surroundings, and it is possible to eat readily now. Cheese, Yogurt, Bread, Wine, Beer, Sake, Vinegar, Natto, and Kimchi, etc. can be enumerated as an example of typical fermented food. Besides this, we often used Soy sauce, Cotijan, and Dried bonito, etc, as supporting player (seasoning) for the dish. We take fermented food in various shapes no relation to our consideration.

Recently, not only deliciousness of that but also the adjustment functions to the living body are paid to attention in the rise of a healthy intention of the consumer for the fermentation food.

Fermented food have possibilities that it has truly fresh regulatory function because they have lots of microorganisms that bring the functionalities into it by the resolution and conversion of food materials. Moreover, there is a history that these fermentation foods are taken as safe food for a long time, and it has an excellent characteristic on the safety of food compared with the one processed to give food the functionality newly.

1. Physiological functions SAKE and SAKE Cake

Since old times moderate SAKE drinking has been said to be the best of all medicines, and SAKE has big physiological function not only mentally but also physically. Many of the physiological functions originate in alcohol, and the research on it has mainly been made conventionally. We had searched physiologically active peptide in SAKE, it is a very-small-quantity ingredient that inhibits Proteinase and manages the metabolism of Peptide hormone, and also inquired for the purpose of the elucidation of the new living body regulation function of SAKE.

1. Angiotensin Converting Enzyme inhibitory substance

Although some systems are participating in regulation of blood pressure, the Renin-Angiotensin system is considered to have played the biggest role now. In this Renin-Angiotensin system, Angiotensin Conversion Enzyme (Angiotensin I Converting Enzyme; ACE) generates Angiotensin II that is powerful vasopressor, disassembles the Bradykinin that has a blood vessel extension action simultaneously, and raises blood pressure. Therefore, if work of ACE can be inhibited, descent of blood pressure will be

brought about. ACE inhibitory substances are found out from various foods until now, and development of the food for specified health use using these is tried. We tried to isolate ACE inhibitory substances from SAKE or its by-product.

1. ACE inhibitory substances in SAKE and its by-product

1) Isolation of the ACE inhibitory substance from SAKE

Three kinds of inhibitory peptides were isolated from *JUNMAI-SHU,* 1.6L of it was used as start materials, with performing cation exchange resin, gel filtration, and reverse phase HPLC checking for ACE inhibitory activity.

2) Isolation of the ACE inhibitory substance from SAKE Cake

A SAKE Cake was processed with the various enzymes for food processing for 24 hours, and one enzyme (Thermoase) was chosen as the index for ACE inhibitory activity. Next, it was processed with this selected enzyme for 1 to 2 hours, and comparison examination of processing time and the inhibitory activity was carried out. Consequently, also in the short-time reaction, inhibitory activity was accepted very much, and it was shown clearly that an ACE inhibitory substance exists in the form that is easy to separate in SAKE Cake. This is considered to originate in the rice protein disassembled by peptidase of molded rice in the SAKE mash. However, at the prolonged reaction, inhibitory activity became weak. This is considered that generated peptide decomposed further.

3) Structure and inhibitory activity of ACE inhibitory peptide

Six inhibitory peptides were isolated by performing gel filtration and reverse phase HPLC from the SAKE Cake hydrolyzate. IC_{50} of inhibitory peptide that isolated from SAKE and SAKE Cake are shown in Table I. Among them, longest has 5 amino acid residues and another one are short peptide of 2 to 3 amino acid residues, and also had the tendency for Tyrosine to be included in C terminus.(Table I)

Table. I ACE inhibitory peptides from SAKE and SAKE Cake

Peptide sequence	Origin	IC50(μ M)
His-Tyr	SAKE	26.1
Tyr-Gly-Gly-Tyr	SAKE	16.2
Val-Tyr	SAKE	7.1
Arg-Phe	SAKE Cake	93.0
Phe-Trp-Asn	SAKE Cake	18.3
Tyr-Trp	SAKE Cake	10.5
Val-Trp-Tyr	SAKE Cake	9.4
Ile-Tyr-Pro-Arg-Tyr	SAKE Cake	4.1
Val-Trp	SAKE Cake	1.4

4) Isolation of the ACE inhibitory substance from rice bran

When the rice bran, 100-90% classification, was processed with the same enzyme as processing of SAKE Cakes for 24 hours, inhibitory activity was accepted in the reaction mixture. However, unlike SAKE Cake, inhibitory activity was not accepted at a short-time reaction. The inhibitory substance was obtained from reaction mixture by ion exchange processing and gel filtration. This inhibitory substance was heat

stable, produced precipitation with iron, calcium, cobalt, etc., but lost inhibitory activity completely by Phytase processing. Then, the inhibitory action to ACE and IR spectrum of this inhibitory substance were compared about standard Phytin, and then it showed clearly for this inhibitory substance to be Phytin.

2. Structure activity correlation of ACE inhibitory peptide

The various fragmentations of Tyr-Gly-Gly-Tyr and Ile-Tyr-Pro-Arg-Tyr were synthesized, and inhibitory activities were investigated. About Tyr-Gly-Gly-Tyr, Tyrosine in the C terminus was participating in inhibitory. On the other hand, about Ile-Tyr-Pro-Arg-Tyr, every fragmentation had strong inhibitory activity. Especially, Ile-Tyr and Pro-Arg-Tyr have strong inhibitory activities (IC_{50}), such as 2.4 and 2.5 μ M, respectively. (Table II)

Table. II Structure and activity of ACE inhibitory peptide

Fragment	IC50 (μ M)
Tyr-Gly-Gly-Tyr	3.4
Tyr-Gly-Gly	10000<
Gly-Gly-Tyr	1.3
Gly-Gly	8670
Gly-Tyr	259
Ile-Tyr-Pro-Arg-Tyr	8.5
Ile-Tyr-Pro-Arg	10
Tyr-Pro-Arg-Tyr	17.4
Tyr-Pro-Arg	16.5
Pro-Arg-Tyr	2.5
Ile-Tyr	2.4
Pro-Arg	4.1
Arg-Tyr	10.5

3. Resistance over the digestive enzyme and ACE of ACE inhibitory peptide

1) Resistance over digestive enzyme

When the resistance over Pepsin-Pancreatin digestion of Tyr-Gly-Gly-Tyr and Ile-Tyr-Pro-Arg-Tyr was investigated, it decomposed and Tyr-Gly-Gly-Tyr lost inhibitory activity. On the other hand, Ile-Tyr-Pro-Arg-Tyr held inhibitory activity as a whole, although decomposed into the fragmentation of Ile-Tyr or Arg-Tyr.

2) Resistance over ACE

Even if Tyr-Gly-Gly-Tyr and Ile-Tyr-Pro-Arg-Tyr were pre-incubated with ACE before inhibitory activity measurement, it is changeless to inhibitory activity and it did not become substrate of ACE.

4. Anti-hypertensive effects on Spontaneously Hypertensive Rats (SHR)

1) Forcible oral administration

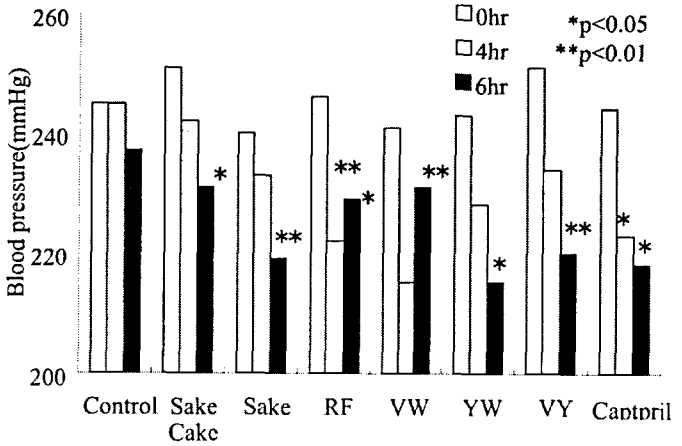

Fig. 1 Changes of blood pressure after administering ACE inhibitors

When SAKE concentration and SAKE Cake hydrolyzate and inhibitory peptides isolated from them were given in the amount of medication of 1g/kg rat and 0.1g/kg rat, respectively, blood pressure of SHR were reduced significantly for 4 to 6 hours after medication. (Fig 1)

2) Long-term feeding examinations

In order to examine whether a SAKE-lees hydrolyzate fully functions as a source of protein of feed, when the feeding examination was performed by the Wister rat, even if it substituted for the SAKE-lees hydrolyzate to 50% of casein, for growth, it became clear that a difference is not seen. Subsequently, when SHR was bred for three weeks with the feed, which substituted the SAKE Cake hydrolyzate for 50% of the casein of refining feed, a significant blood-pressure descent was shown after the 10 days of feeding. (Fig. 2)

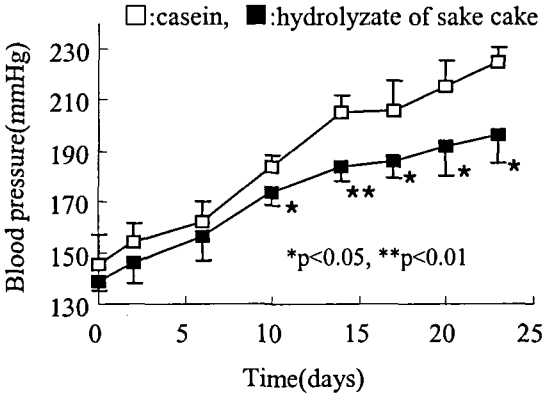

Fig. 2 Changes of blood pressure of SHR

II. Prolyl Endpeptidase inhibitory peptide

Vasopressin participates in the process of study and memory within a brain, and if the decomposition progresses, it might be said that amnesia advances. Moreover, it turns out that an Alzheimer's disease patient's amount of Vasopressin is fewer than that of normal people. Vasopressin is decomposed by Prolyl Endopeptidase (PEP). Although PEP in a brain is working normally in healthy people, if a regulation mechanism separates in a certain reason, Vasopressin will be decomposed beyond necessity and injury will appear in memory maintenance. It is reported that various PEP inhibitory substances show an anti-amnesia action to a Scoporamine guidance amnesia rat. Then, since it was accepted when PEP inhibitory activity was searched about SAKE or the by-product, isolation of an inhibitory substance was tried.

1. PEP inhibitory substance in SAKE and SAKE Cake

1) PEP inhibitory substance from SAKE Cake

SAKE Cake Pepsin hydrolyzate dissolved in 20% acetonitrile, containing 0.1% TFA, and was adsorb to ODS, and then eluted by 60% acetonitrile. By repeating reverse phase HPLC, three kinds of inhibitory peptides were isolated, and structure was determined.

2) Isolation of the PEP inhibitory substance from SAKE

By repeating reverse phase HPLC and gel permeation HPLC, three kinds of inhibitory peptides were isolated from 2L of *JUNMAI-SHU*. N terminus of these peptides were blocked and these peptides had

determined structure for the first time by carrying out Pyroglutamate Aminopeptidase processing. (TableIII) As mentioned above, inhibitory peptides obtained from SAKE and SAKE Cake consisted of an 8 to 14 amino acids residue, and were the gruterine origin of the rice protein.

Table. III PEP inhibitory peptide from sake Cake and sake

	Sequence	Origin	IC50(μ M)
A:	Ser-Pro-Phe-Trp-Asn-Ile-Asn-Ala	Sake Cake	42.8
B:	Leu-Ser-Pro-Phe-Trp-Asn-Ile-Asn-Ala	Sake Cake	29.0
C:	Leu-Leu-Ser-Pro-Phe-Trp-Asn-Ile-Asn-Ala	Sake Cake	24.3
D:	pGlu-Leu-Phe-Asn-Pro-Ser-Thr-Asn-Pro-Trp-His-Ser-Pro	Sake	24.3
E:	pGlu-Leu-Phe-Asn-Pro-Ser-Thr-Asn-Pro-Trp-His-Ser-Pro-Arg	Sake	14.1
F:	pGlu-Leu-Phe-Gly-Pro-Asn-Val-Asn-Pro-Trp-His-Asn-Pro-Arg	Sake	11.8

2. Physiological functions of AMAZAKE

Do you know AMAZAKE, the sweet drink made from fermented rice? The origin of the AMAZAKE are in the age of ancient China, 2000 years or more, and spreads to Japan from there. The AMAZAKE that got warm drinking was winter drinks at the Heian era. It is existence not to allow it to lack in the New Year's visit to a shrine now. However, the people were familiar in Edo period as the summer beverage. A nutritious AMAZAKE seems to have played the role of the summer heat prevention. It is possible to look for such a long history from "AMAZAKE festival" of nationwide various places. We dedicate AMAZAKE to the god and drink it and offer it each other and wishes good health and good harvest of staple grains. People knew the AMAZAKE to be nutritious for a long time.

The AMAZAKE is called, "Japanese yogurt" from the probiotic action in recent years, and is remarkable. We paid attention to a further functionality of the AMAZAKE, and examined the possibility as the healthy drink. We paid attention to the AMAZAKE made by SAKE Cake this time though there was an AMAZAKE made only from the molded rice as a raw material, too. It is thought by the AMAZAKE made like this including various active ingredients that have the functionality of each material origin shown. The synergy effect of the combination of sake lees and molded rice, the generation of a new element when interacting with one another, and the compound element can be expected. Then, the various functionalities of the AMAZAKE were evaluated, using the mouse *in vivo*.

We have investigated some physiological functions of AMAZAKE, Anti-obesity, Anti-hyperlipidemia, Anti-hypertensive and Anti-amnestic, etc.

1) Anti-Amnestic effect of AMAZAKE (Sweet drink made from fermented rice)

Anti-Amnestic effect was investigated using *AMAZAKE* that is the traditional Japanese food made from fermented rice. We used water-maze and ICR mouse for that test. It significantly controlled the scopolamine inducement type amnesia in this test.

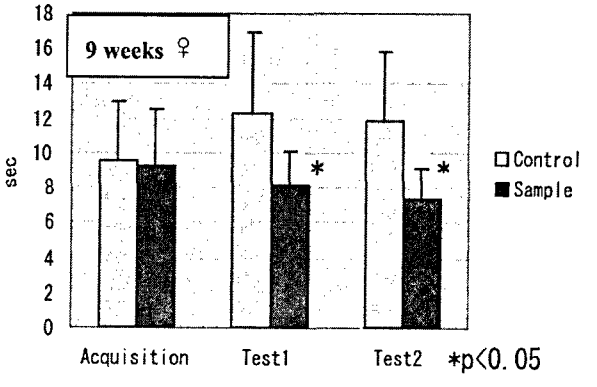

Fig. 3 Anti-amnestic effect of AMAZAKE