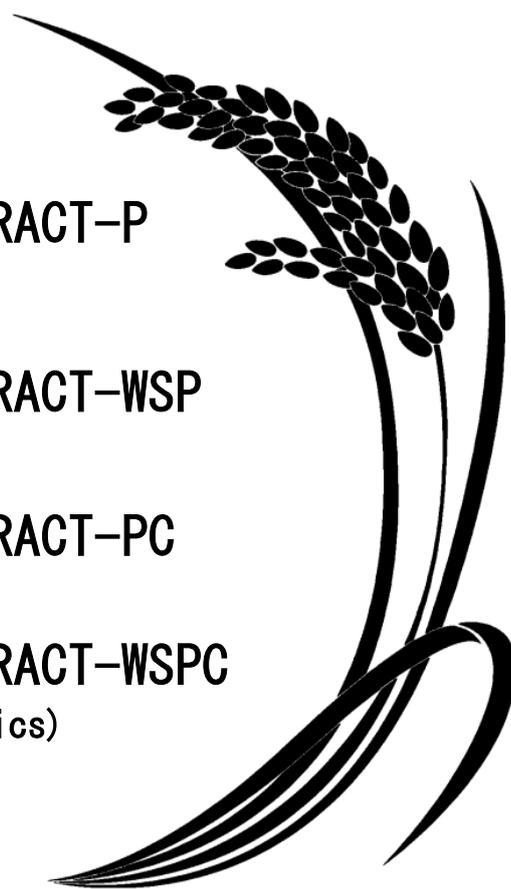


Fermented Rice Germ Extract

Immunostimulatory Activity

- **FERMENTED RICE GERM EXTRACT-P**
(Powder, for food)
- **FERMENTED RICE GERM EXTRACT-WSP**
(Water-soluble powder, for food)
- **FERMENTED RICE GERM EXTRACT-PC**
(Powder, for cosmetic)
- **FERMENTED RICE GERM EXTRACT-WSPC**
(Water-soluble powder, for cosmetics)



FERMENTED RICE GERM EXTRACT

Health Food Ingredients

1. Introduction

Rice (*Oryza sativa*) have been widely grown in the Southeast Asia, not only as a chief crop but also as acts an integral part of traditional culture and lifestyle of some Asian countries. In recent years, much attention have been focused on rice bran and rice germ, which are discharged in the process of the polished rice production, because of it contains many unique bioactive compounds.

In the course of our investigation on rice bran and rice germ for a long time, some products were developed by utilizing functional compounds containing in it, and have been used as medicines, cosmetics, health foods, and food additives.

Fermentation is a traditional technique for food processing. At ORYZA OIL & FAT CHEMICAL CO., LTD., we have found that fermented rice germ generates functional components that successfully stimulate the immune system. Using our extraction and purification technique, “FERMENTED RICE GERM EXTRACT” has been produced on a commercial basis as a potential ingredient for foods.

2. Fermentation

Fermentation has traditionally been used as a food processing technique in Japan. Various fermented foods such as *Miso* paste, soy sauce, *Natto* (fermented soybeans), dried bonito flesh and pickles have been produced from rice, soybeans or other ingredients by utilizing microorganisms, including yeasts, *Bacillus natto* (a strain of *B. subtilis*), lactic acid bacteria and *Aspergillus*. In Western countries, some fermented foods such as cheeses, breads, beer and wine have been made to extend shelf life.

These fermented foods have recently attracted attention not merely as food items but also as food with beneficial health-promoting functions.

During fermentation, *Aspergillus* generates various enzymes that trigger de novo syntheses of certain components, which might exhibit a novel biological activity.

(1) *Aspergillus*

As shown in Fig. 1 *Aspergillus* is classified. The *Aspergillus* has been utilized in Japan to produce indigenous fermented foods such as alcoholic beverages (*Sake* and *Awamori*), *Miso* paste, soy sauce, *Mirin* (sweet rice wine used for cooking) and pickles.

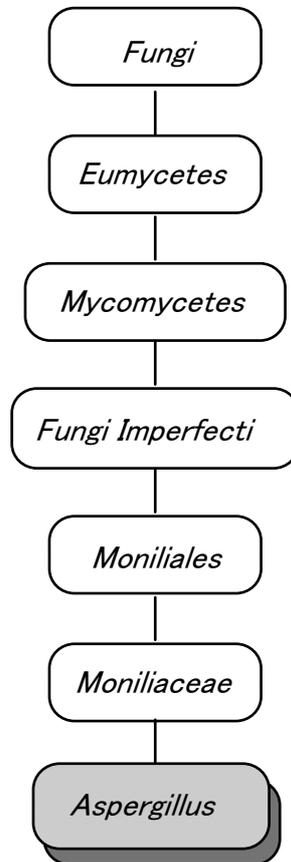


Fig. 1 Classified of *Aspergillus*

(2) Components enriched by the fermentation of rice germ

In the process of rice germ fermentation by *Aspergillus*, newly produced enzymes cause catabolic changes, including digestion of starches and sugars as well as hydrolysis of proteins yielding amino acids and low molecular peptides.

In addition, concentrations of phosphoserine (a phosphorylated serine), cysteine and cystine are increased. Ferulic acid content is elevated, leading to the synthesis of diferulic acid.

It has been demonstrated that a phosphoserine-containing peptide exhibits mitogenic activity in cultured cells derived from the spleen, Peyer's patches and thymus (H. Otani, Faculty of Agriculture, Shinshu University, Proceedings of the fifth conference of Japanese Society for Mastitis).

Cysteine and cystine are antioxidative agents. Besides being a component of glutathione, which is an anti-cancer substance, cysteine has been found to enhance detoxification activity in the liver and inhibit melanin synthesis, which facilitates the metabolism of the skin epidermal tissue. Some studies have demonstrated that both ferulic and diferulic acids have anti-oxidative and ultraviolet (UV)-absorbing properties. Ferulic acid has been recently investigated as a cancer preventive agent (First international symposium on disease prevention by IP-6 and other rice components).

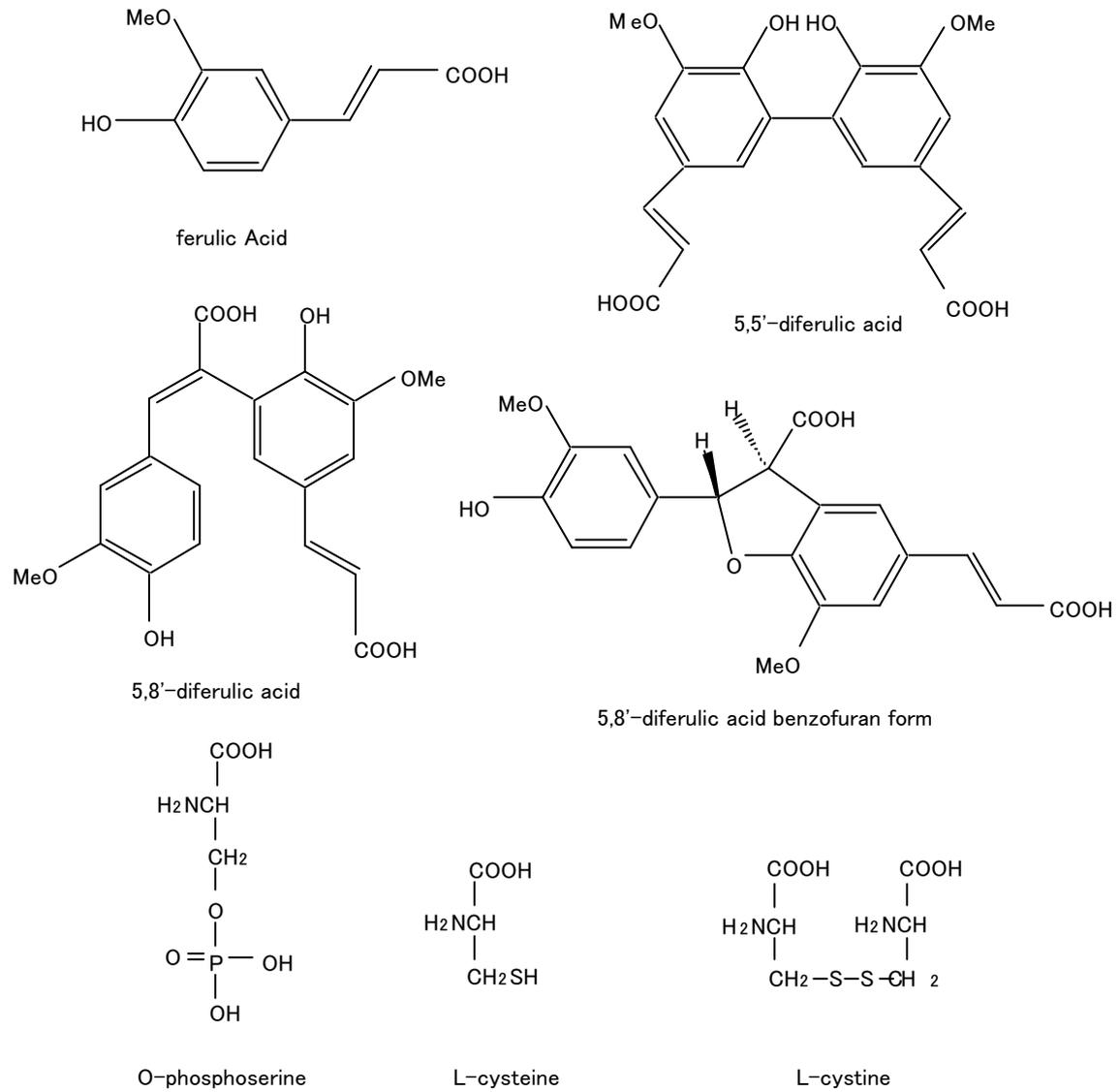


Fig. 2 Components enriched by the rice germ fermentation

3. Immune system

The body system that responds to a foreign substance such as pathogen or virus is called the immune system. Some substances have “immunostimulatory activity”, namely, a biological property to enhance this system.

The immune system is mainly mediated by white blood cells. In particular,

granulocytes (neutrophils, eosinophils and basophils), macrophages and lymphocytes (natural killer NK cells, T cells and B cells) play important roles in the system.

Macrophages act as scavengers. Once a macrophage engulfs a foreign substance including pathogens and viruses by phagocytosis, it detoxifies the substance. Moreover, the macrophage displays information about the substance and activates other immune cells.

An activated NK cell spontaneously attacks the infected cells as well as the cancer cells and causes lethal damage to the targets by destroying the cell membrane.

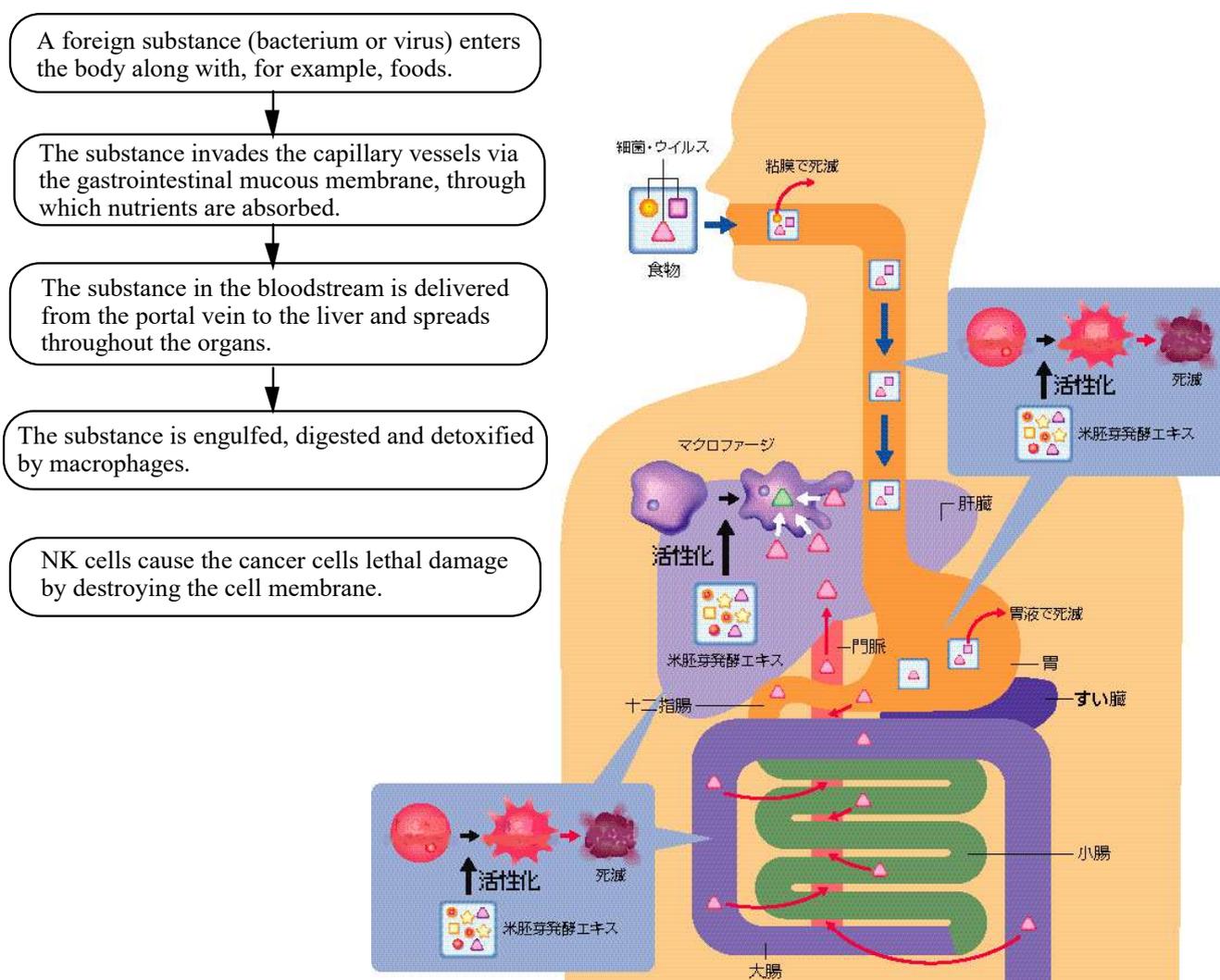


Fig. 3 The immune system in the body

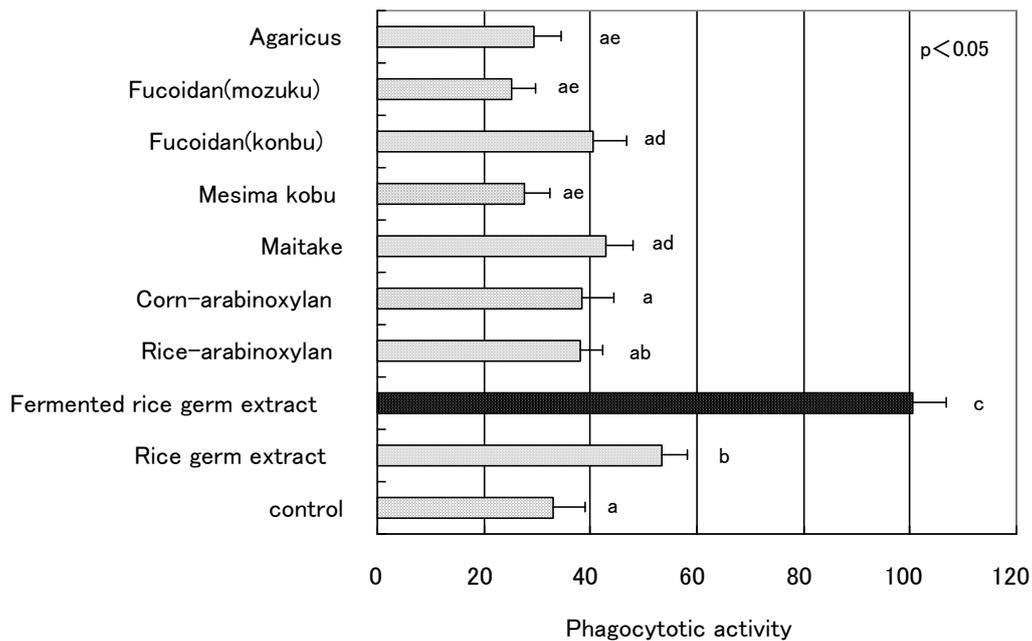
4. Potential function of FERMENTED RICE GERM EXTRACT

1) Immunostimulatory activity

(1) Preventing infections by enhancing the phagocytotic capacity of macrophages (*in vitro*)

FERMENTED RICE GERM EXTRACT stimulates phagocytosis in macrophages, by which foreign viruses and bacteria are digested and detoxified.

Using an *in vitro* assay system, we have found that FERMENTED RICE GERM EXTRACT markedly enhances the phagocytotic capacity of rat hepatic macrophages compared to extracts derived from unfermented rice germ as well as from other plants. The results suggest that FERMENTED RICE GERM EXTRACT has a preventive effect against viral and bacterial infections *in vivo*.



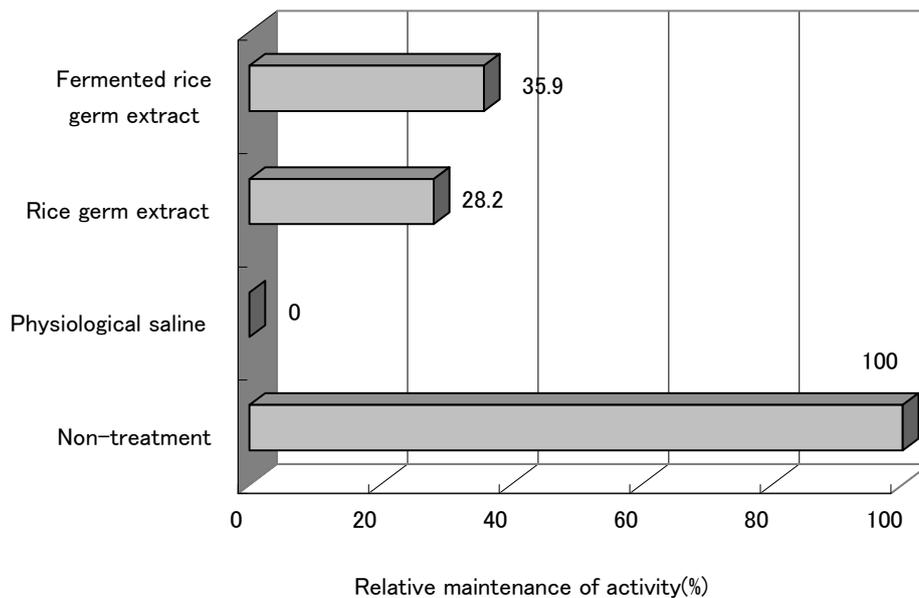
There were no significant differences observed among groups indicated by the same alphabetic character on the right side of bar. Phagocytotic capacity was indicated as a percent of the baseline value measured for each sample using intact macrophages whose phagocytotic capacity was not blocked.

Fig. 4 The phagocytotic capacity of rat hepatic macrophages

(final concentration of each substance being examined: 200 μ g/ml)

(in vivo)

During a two-week experimental period, Sprague-Dawley (SD) rats aged five weeks were administered daily oral dose of 200 mg of “FERMENTED RICE GERM EXTRACT” dissolved in 1 ml of saline, and exposed to light irradiation and water swim stress. To examine the phagocytotic capacity, peritoneal macrophages were mixed with latex beads, subsequently dissolved and analyzed the turbidity in comparison with the saline-treated control group. The results demonstrate that “FERMENTED RICE GERM EXTRACT” attenuates the stress-induced damage in phagocytotic capacity of macrophages. This product will improve the immune system, which is vulnerable to aging, stress and environmental pollution, and prevent infections with invading viruses or bacteria.



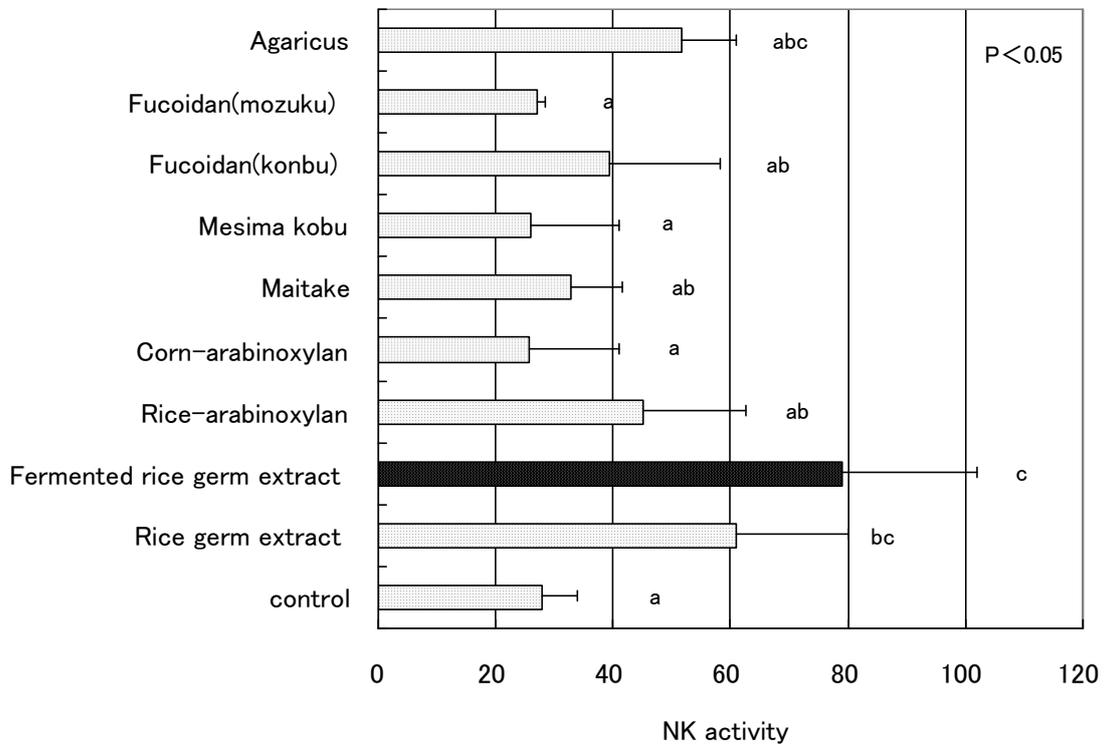
Relative maintenance of phagocytotic capacity was estimated as follows. The difference in values between an extract-treated group and the saline-treated control was calculated (a). The mean difference in values between the intact group and the saline-treated control was also estimated (b). By defining “b” as 100%, we expressed a percentage of “a” as the relative maintenance of phagocytotic capacity (%).

Fig. 5 The phagocytotic capacity of rat peritoneal macrophages

(2) Preventing cancer by enhancing the cancericidal capacity of NK cells

(in vitro)

FERMENTED RICE GERM EXTRACT intensifies the cancericidal capacity of NK cells. We confirmed *in vitro* that FERMENTED RICE GERM EXTRACT effectively stimulates rat hepatic NK cells (Pit cells) to kill cancer cells compared to that induced by extracts derived from unfermented rice germ and other plants.



There was no significant difference observed among groups having the same alphabetic character on the right side of bar.

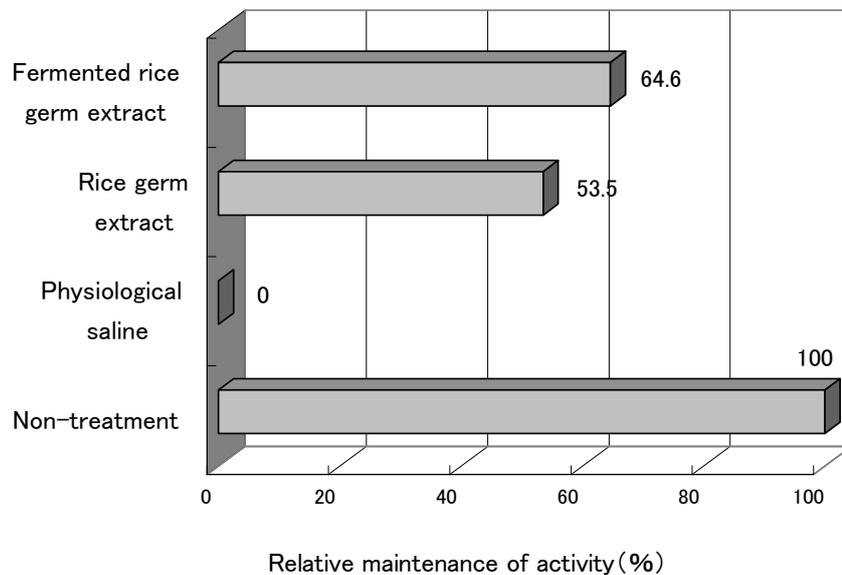
NK cell activity is indicated as a percentage of lysed (killed) cells in the total target cancer cells.

Fig.6 The cancericidal capacity of rat hepatic NK cells

(final concentration of each substance being examined: 200 μg/ml)

(in vivo)

Five-week-old SD rats were orally administered either 200 mg of FERMENTED RICE GERM EXTRACT dissolved in 1 ml of saline or an equal volume of saline, and subjected to water swim and light irradiation stress for two weeks. Splenic NK cells were obtained and co-cultured with mouse lymphoma cells (YAC-1). Cytotoxicity to YAC-1 cells was evaluated by analyzing lactate dehydrogenase (LDH) activity in the culture medium. The enzyme activity was expressed as a percent of the maximal LDH activity, which was measured when all YAC-1 cells were lysed. We concluded from the results that FERMENTED RICE GERM EXTRACT improves the cancericidal capacity of NK cells under stress situations. Namely, this extract will enhance immunity to cancer by stimulating NK cell activity, which is vulnerable to aging, stress and environmental pollution.



Relative maintenance of cancericidal capacity was estimated as follows. The difference in values between an extract-treated group and the saline-treated control was calculated (a). The mean difference in values between the intact group and the saline-treated control was also estimated (b). By defining “b” as 100%, we expressed a percentage of “a” as the relative maintenance of cancericidal capacity (%)

Fig. 7 The cancericidal capacity of rat splenic NK cells

2) Antioxidative activity (1,1-diphenyl-2-picrylhydrazyl [DPPH] radical scavenging activity and superoxide dismutase [SOD]-like activity)

Enhanced DPPH radical scavenging and SOD-like activities were observed during rice germ fermentation by *Aspergillus*. Both activities peaked on the third day of fermentation.

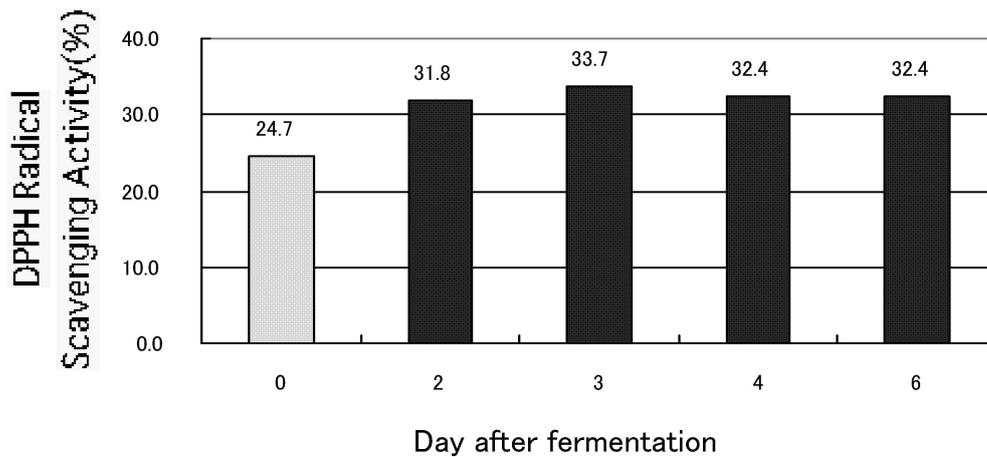


Fig.8 Sequential changes in DPPH radical scavenging activity during fermentation

(final concentration of the substance being examined: 100 μ g/ml)

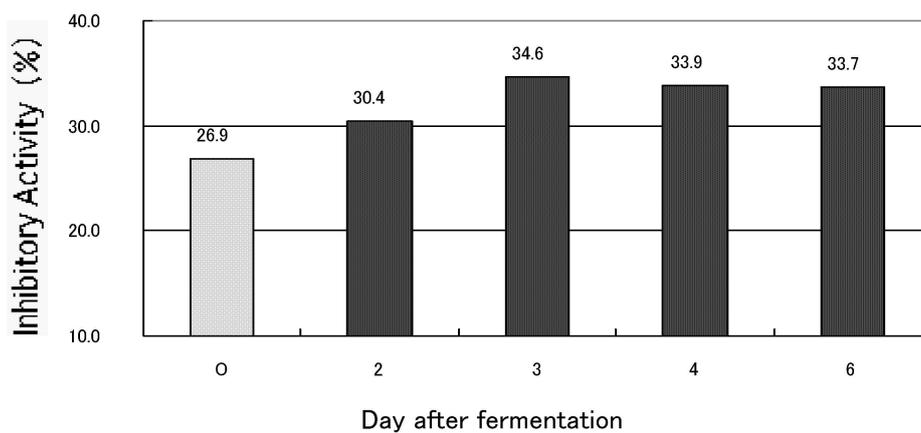


Fig.9 Sequential changes in SOD-like activity during fermentation

(final concentration of the substance being examined: 1,000 μ g/ml)

3) Prolyl endopeptidase (PEP)-inhibitory activity

It has been reported that PEP accumulates in the brain and causes functional impairment in patients with Alzheimer’s disease. We found that the rice germ fermentation by *Aspergillus* a strong PEP-inhibitory activity.

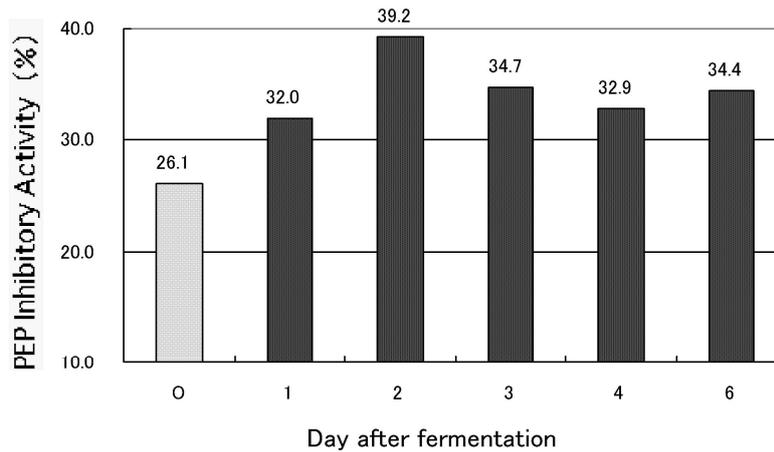


Fig.10 Sequential changes in PEP-inhibitory activity during fermentation (final concentration of the substance being examined: 1.9 mg/ml)

4) Polyphenol content

During fermentation by *Aspergillus*, rice germ yields an increased amount of polyphenols, the concentration of which was maximized on the third day of fermentation.

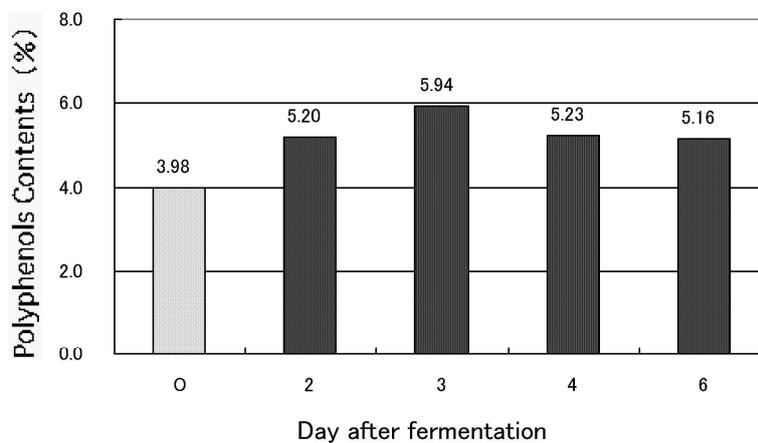
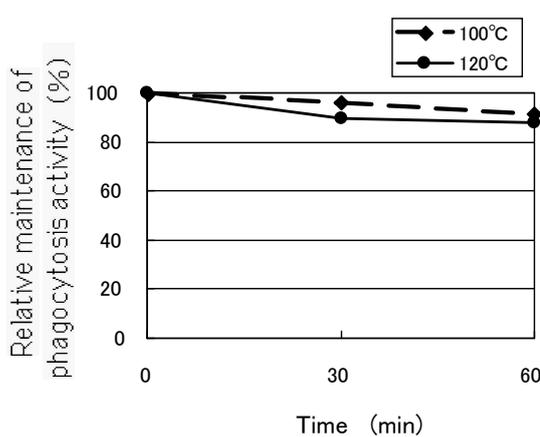


Fig.11 Sequential changes in polyphenol content during fermentation

5. Stability of FERMENTED RICE GERM EXTRACT

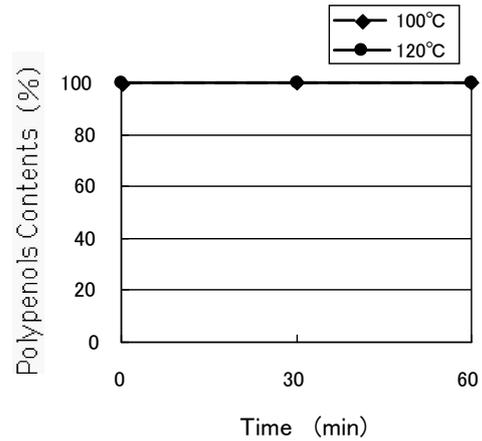
1) Thermal Resistance

The pyrolysis of FERMENTED RICE GERM EXTRACT dose not occur at a normal food processing temperature for 60 minutes.



X-axis: % of initial Phagocytotic activity

Fig.12



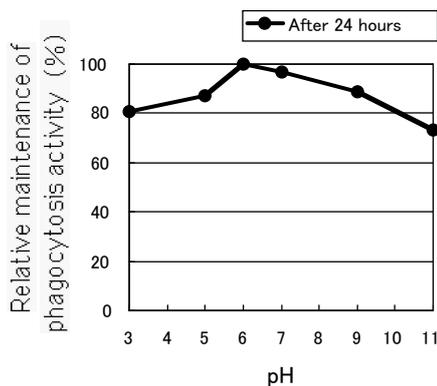
X-axis: % of initial polyphenols

Fig.13

Heat-Resistance of FERMENTED RICE GERM EXTRACT

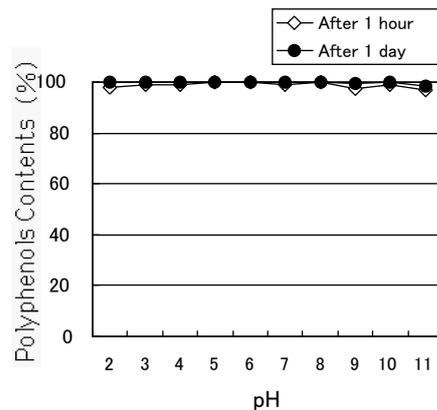
2) pH Stability

FERMENTED RICE GERM EXTRACT remained stable at wide range of pH-field .



X-axis: % of non-treatment Phagocytotic activity in 4% solution.

Fig.14



X-axis: % of non-treatment polyphenols in 0.2% solution.

Fig.15

Influence of pH on FERMENTED RICE GERM EXTRACT

6. Daily Dosage of FERMENTED RICE GERM EXTRACT

FERMENTED RICE GERM EXTRACT (powder) 200~500mg/day

7. Nutrition facts of FERMENTED RICE GERM EXTRACT

Items Analyzed	Result
Water	3.4 g/100g
Protein* ¹	18.4 g/100g
Fat	1.6g/100g
Ash	10.2 g/100g
Available carbohydrate* ²	61.6 g/100g
Energy* ³	334 kcal/100g
Dietary Fiber	4.8 g/100g
Sodium	38.2 mg/100g

*1 $N \times 5.95$

*2 $100 - (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash} + \text{Dietary fiber})$

*3 Factors for calculating the energy value:

Protein - 4, Fat - 9, Available carbohydrate - 4

Tested by : Japan Food Research Center Foundation

Research result issue number : 301080468-001

8. Acute toxicity of FERMENTED RICE GERM EXTRACT

Five weeks old mice were orally given FERMENTED RICE GERM EXTRACT (5000mg/kg) and then fed a laboratory chow for two weeks. No toxic effect were observed, thus the LD50 (mice) is more than 5000 mg/kg.

9. Practical Applications of FERMENTED RICE GERM EXTRACT

Applications	Examples
Health foods	Soft-capsule, Tablet, Hard-capsule, and so on .
Food	Candy, Gum, Gummi, Cookies, Chocolate, Wafers, Jelly, Drink, Soup, Dried Noodles, Seasoning, Bean paste, Soy sauce, Bread, Yogurt and so on.

10. Packaging

FERMENTED RICE GERM EXTRACT-P

5kg Interior packaging: aluminum-coated plastic bag

Exterior packaging: cardboard box

FERMENTED RICE GERM EXTRACT-WSP

5kg Interior packaging: aluminum-coated plastic bag

Exterior packaging: cardboard box

11. Storing Method

Store in cool, dry place. Avoid humidity

12. Expression of FERMENTED RICE GERM EXTRACT

FERMENTED RICE GERM EXTRACT

※ Please refer to your nation's standards.

Methods of Experiments

Fig. 4 The phagocytotic capacity of rat hepatic macrophages (*in vitro*)

Hepatic macrophages were obtained from Wistar rats (12-14 weeks old). After liver perfusion with collagenase solution via the portal vein, macrophages were isolated from the perfusate using an elutriation rotor and adhered to a culture platform.

Prior to adding an extract to the culture medium, the phagocytotic capacity was blocked. To measure phagocytosis, latex beads were added to the medium and the number of beads engulfed by one macrophage was counted under a phase contrast microscope.

Phagocytotic capacity was indicated as a percent of the baseline value measured for each sample using intact macrophages whose phagocytotic capacity was not blocked.

Fig. 5 Methods are described in the text.

Fig. 6 The cancericidal capacity of rat hepatic NK cells (*in vitro*)

Wistar rats (12-14 weeks old) were used in this study. Hepatic NK cells were collected via the portal vein using a hyperbaric liver perfusion system, and purified through a column.

NK cells were co-cultured with mouse lymphoma cells (YAC-1) in a medium containing an extract. Cytotoxicity to YAC-1 cells was evaluated by analyzing lactate dehydrogenase (LDH) activity in the culture medium. When all YAC-1 cells were lysed, LDH activity was regarded as 100%.

Fig. 7 Methods are described in the text.

Fig. 8 Sequential changes in DPPH radical scavenging activity during fermentation

DPPH Radical Scavenging Activity was measured in terms of absorbance.

Fig. 9 Sequential changes in SOD-like activity during fermentation

The sample was assayed by using SOD-Test Wako. SOD-like Activity was measured as inhibitory.

Fig. 10 Prolyl endopeptidase (PEP)-inhibitory activity

The sample was assayed by using Method of Yoshimoto et al.

Enzyme : Prolyl endpeptidase (PEP) derive from *Flavobacterium meningosepticum*

Substrate : Z-Gly-Pro-pNA

Fig. 11 Sequential changes in polyphenol content during fermentation

The sample was measured by the Folin-Denis method described in the Food Function Study Method. Gallic acid was used as a standard reference material.

Fig. 12 Heat-Resistance of FERMENTED RICE GERM EXTRACT

The sample was compared with the phagocytotic capacity of rat hepatic macrophages (*in vitro*)

Fig. 13 Heat-Resistance of FERMENTED RICE GERM EXTRACT

The sample was compared with content of polyphenol that were measured by the Folin-Denis method described in the Food Function Study Method.

Fig. 14 Influence of pH on FERMENTED RICE GERM EXTRACT

The sample was compared with the phagocytotic capacity of rat hepatic macrophages (*in vitro*)

Fig. 15 Influence of pH on FERMENTED RICE GERM EXTRACT

The sample was compared with content of polyphenol that were measured by the Folin-Denis method described in the Food Function Study Method.



PRODUCT STANDARD

PRODUCT NAME

FERMENTED RICE GERM EXTRACT-P

(FOOD)

This product is extracted with aqueous ethanol from fermented rice germ of *Oryza sativa* Linne (*Gramineae*). It contains minimum of 3.0 % polyphenols.

Appearance Slightly brown powder with slightly unique smell.

Content of Polyphenols Min. 3.0 % (Folin-Denis method)

Loss on Drying Max. 10.0 % (Analysis for Hygienic Chemists, 1g, 105°C, 2h)

Purity Test

(1) Heavy Metals (as Pb) Max. 10ppm (Sodium Sulfide Colorimetric Method)

(2) Arsenic (as As₂O₃) Max. 1ppm (Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)

Standard Plate Counts Max. 3×10^3 cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10^3 cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Fermented rice germ extract	70 %
Dextrin	30 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

FERMENTED RICE GERM EXTRACT-WSP

(FOOD)

This product is extracted with aqueous ethanol from fermented rice germ of *Oryza sativa* Linne (*Gramineae*). This powder is water-soluble.

<u>Appearance</u>	Slight yellowish powder with slightly unique smell.	
<u>Content of Polyphenols</u>	Min. 0.4 %	(Folin-Denis method)
<u>Loss on Drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105°C, 2h)
<u>Purity Test</u>		
(1) Heavy Metals (as Pb)	Max. 10 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As ₂ O ₃)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
<u>Standard Plate Counts</u>	Max. 3 × 10 ³ cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1 × 10 ³ cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Fermented rice germ extract	10 %
Dextrin	90 %
Total	100 %



PRODUCT STANDARD

PRODUCT NAME

FERMENTED RICE GERM EXTRACT-PC

(COSMETIC)

This product is extracted from fermented rice germ of *Oryza sativa* Linne (*Gramineae*) with aqueous ethanol. It contains minimum of 3.0 % polyphenols.

Appearance Slight brown powder with slight unique aroma.

Content of Polyphenols Min. 3.0 % (Folin-Denis method)

Loss on Drying Max. 10.0 % (1 g, 105 °C, 2 h)

Purity Test

(1) Heavy Metals (as Pb) Max. 10 ppm (The Second Method of The Japanese Standards of Quashi-Drug Ingredients)

(2) Arsenic (as As₂O₃) Max. 1 ppm (The Third Method of The Japanese Standards of Quashi-Drug Ingredients)

Standard Plate Counts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Aspergillus/Rice Germ Fermented Extract Filtrate	70 %
Dextrin	30 %
Total	100 %



PRODUCT STANDARD

PRODUCT NAME

FERMENTED RICE GERM EXTRACT-WSPC

(COSMETIC)

This product is extracted from fermented rice germ of *Oryza sativa* Linne (*Gramineae*) with aqueous ethanol. It contains minimum of 0.4 % polyphenols. This powder is water-soluble.

Appearance Slight yellowish powder with slight unique aroma.

Content of Polyphenols Min. 0.4 % (Folin-Denis method)

Loss on Drying Max. 10.0 % (1 g, 105 °C, 2 h)

Purity Test

(1) Heavy Metals (as Pb) Max. 10 ppm (The Second Method of The Japanese Standards of Quashi-Drug Ingredients)

(2) Arsenic (as As₂O₃) Max. 1 ppm (The Third Method of The Japanese Standards of Quashi-Drug Ingredients)

Standard Plate Counts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Dextrin	90 %
Aspergillus / Rice Germ Ferment Extract Filtrate	10 %
Total	100 %



ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

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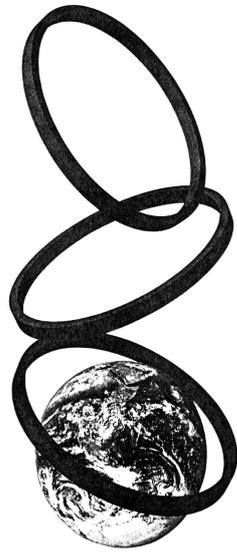
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