The anti-glycation potential of rice

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Abstract

Introduction: Glycation is a non-enzymatic reaction between reducing sugars and amino group proteins which leads to the formation of advanced glycation end products (AGEs). The buildup of AGEs in the body is a key factor in the onset of aging and lifestyle-related diseases. *Oryza sativa*, or rice is one of the most widely consumed staple food for a large part of the world's population. It contains two major subspecies; *Japonica* and *Indica*. Rice water is the water left over after you cook rice, or obtained by soaking rice in water. It has long been used traditionally in some Asian countries for improving skin, face and hair. However, the benefits of rice water are not supported by enough scientific research although cosmetic manufacturers greatly assert its benefits. The main aim of this study was to investigate the anti-glycation potential of rice water, and to identify the substances responsible for the anti-glycation property of rice.

Chapter 1: Rice water was prepared following three traditional methods; type 1, type 2, and type 3. The human serum albumin (HSA) glycation model was used to evaluate the inhibitory effect on the formation of fluorescent AGEs by rice water. The total phenolic content (TPC) in rice water was determined according to the Folin–Ciocalteau procedure. All rice water samples showed an inhibitory effect on the formation of fluorescent AGEs, and positive correlations were observed between the inhibitory effect and TPC; type 1 ($r = 0.906$), type 2 ($r = 0.918$), type 3 ($r = 0.765$).

Chapter 2: Rice water extracts were prepared and the HSA glycation model was used to measure the inhibition of the production of fluorescent AGEs, pentosidine, and glycation intermediates; 3-deoxygluocosone (3-DG), methylglyoxal (MGO), and glyoxal (GO). Pentosidine and glycation intermediates were analyzed by high-performance liquid chromatography (HPLC). One sample each of *Japonica* black, *Japonica* red, and *Indica* red was selected for further investigation after initial screening. It was found that the bran layer's contribution towards inhibition was higher than the endosperm. The bran samples were fractionated by fractional purification using Oasis HLB Plus to water, 5% acetonitrile (ACN), 10% ACN, and 75% ACN fractions. The inhibition of pentosidine was stronger by the 5% ACN fraction of *Indica* red rice bran, therefore clean-cut peaks were collected and purified. Two stable fractions were identified, and their contribution towards the inhibition of fluorescent AGEs was

calculated at 20.1% and 22.8%, and towards the inhibition of pentosidine was calculated at 25.0% and 31.2% respectively. These fractions were further purified and analyzed by Capillary Electrophoresis-Mass Spectrometry (CE-MS). Glutamic acid and guanosine was identified as representative anti-glycative compounds in *Indica* red rice bran.

Chapter 3: The subjective symptoms and changes in skin condition of university students that consumed sub-aleurone layer residual rinse-free rice (SARFR) for one month was compared with polished rice. There were 37 subjects in the SARFR group (24 males, 13 females 21.0 ± 1.5 years), and 22 in the control group (13 males, 9 females 22.0 ± 1.2 years). In the SARFR group, 150 g or more of the test meal was ingested once a day for one month. In the control group, polished rice was taken freely. Skin age was significantly improved in the SARFR. This effect was remarkable in male and home students, and was not observed in boarding house students. There was no significant difference in skin AGEs fluorescence.

Chapter 4: The subjective symptoms and changes in skin condition of university students and staff that consumed dewaxed brown rice (DBR) for one month was compared in comparison with polished rice. There were 43 subjects in the DBR group (25 males, 18 females 23.8 ± 8.8 years) and 22 in the control group (13 males, 9 females 22.0 ± 1.2 years). In the DBR group, a test meal of 150 g or more was ingested once a day for one month. In the control group, polished rice was taken freely. Skin age was significantly improved in the DBR group but there was no significant difference in skin AGE fluorescence. Gender analysis showed that wrinkles and porphyrin levels were significantly improved in the DBR group in women.

Conclusion: The study shows that rice water has a strong inhibitory efficacy against various AGEs and the bran layer of rice is mainly responsible for its anti-glycation activity. There are many unknown bioactive compounds in bran with anti-glycation potential. Thus, stable bioactive compounds responsible for anti-glycation of rice bran are yet to be identified. The clinical data suggests that DBR or SARFR when compared with polished rice, contributes to health promotion, including skin condition, by reducing the indigestibility of brown rice and ensuring nutrition, which facilitates continuous intake.

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1. Introduction

1.1 Glycative stress and advanced glycation end products (AGEs)

Aging is the progressive accumulation of damage over time, which leads to disturbed function on the cellular, tissue and organ level and ultimately to disease and death. Aging is a complex, multifactorial process where genetic, endogenous and environmental factors play a role (1). Glycation is a non-enzymatic chemical reaction between amino acids or proteins and reducing sugars, which was discovered by Louis-Camille Maillard, a French scientist. This reaction is also called the Maillard reaction (2, 3). Ever since the first description of glycation by Louis-Camille Maillard in 1912 and its association with food browning during thermal processing by John E. Hodge in 1953 (4), its presence in living systems and connection to various pathologies of the human body, including aging and diabetes, have been an intensive area of research.

Glycated proteins formed in the process of glycation lead to the formation of advanced glycation end products (AGEs) that are accumulated in various tissues and organs. AGEs induce inflammation, pigmentation, and deterioration of physiological function that lead to aging (5). The phenomenon of the accumulation of these cytotoxic and irreversibly-formed sugar-derived AGEs that contribute to aging, age-related diseases, and other metabolic diseases such as diabetes, cancer, kidney, ocular, and cardiovascular diseases is known as glycative stress (6). Therefore, the discovery of AGE inhibitors would offer a potential preventive and therapeutic approach for reducing the risks of pathogenic complications triggered by AGE formation.

Recently, both synthetic and natural compounds have been evaluated in terms of their potential to inhibit the formation of AGEs. Strong inhibitory effects on the formation of AGEs have been observed by some synthetic products, however, they have the possibility to cause severe side effects (7). Thus, naturally occurring products such as plant extracts that are relatively safer for human consumption have been evaluated for their effect on the formation of AGEs (8-11). Compounds from natural products therefore have huge potential for future applications as functional foods, preventive drugs, or even as cosmetic products against AGE associated diseases and disorders.

The formation of AGEs is a complicated molecular process involving simple and complex multi-path reactions as seen in Figure 1. In the Maillard reaction, electrophilic carbonyl groups of glucose or other reactive sugars such as fructose or glucose-6-phospahte react with free amino groups of amino acids (basic lysine or arginine residues), forming a Schiff base, which is an unstable and reversible N-substituted glycosylamine. Further rearrangement leads to the formation of a more stable and reversible ketoamine called Amadori product (12). Although they are reversible reaction products, they can react irreversibly with amino acid residues of peptides or proteins to form protein crosslinks or protein adducts. They can also undergo further oxidation, dehydration, polymerization and oxidative breakdown reactions to produce several other AGEs (13, 14).

Glycation reactions can be divided into early stage, intermediate stage, and late stage reactions. 3-deoxyglucosone (3-DG), glyoxal (GO), methylglyoxal (MGO), glyceraldehyde and glycolaldehyde are some of the intermediates for AGEs. 3-DG is an α-dicarbonyl compound formed from glycated proteins. GO is formed by the auto-oxidation of glucose and the peroxidation of lipids. MGO is formed in the glycolytic pathway and polyol pathway in cells. The glycation pathway of intermediates for AGEs is different depending on the compound, therefore the kinds of AGEs ultimately formed are different (15).

AGEs are classified in to different groups based on their chemical structures and ability to emit fluorescence as follows:

1. fluorescent cross-linked AGEs such as pentosidine, crossline, etc. (Figure 2),
2. non-fluorescent non-cross-linked AGEs such as carboxyethyl-lysine; CEL, pyrraline, etc. (Figure 3),
3. non-fluorescent protein cross-linked AGEs such as glyoxal-lysine dimer; GOLD, methylglyoxal-lysine dimer; MOLD, etc. (Figure 4),
4. fluorescent non-cross-linked AGEs such as argopyrimidine (Figure 5) (16).

AGEs can be exogenously ingested through the consumption of food. They can also be produced endogenously. Endogenous AGE formation is increased in patients with diabetes, however, they are

also formed at lower rates by normal metabolic processes (17). Environmental factors, such as diet and smoking (18) and genes (19) influence the rate of AGE formation.

1.2 Basis of research

Rice is one of the most widely consumed staple foods for a large part of the world's population, mainly in Asia. It is also an important beautifying agent as women from Japan, China, and some Southeast Asian countries such as Sri Lanka have used rice water to beautify their face, skin, and hair traditionally. *Oryza sativa* is the plant species most commonly referred to as rice. It contains two major subspecies; the sticky, short-grained *Japonica* variety, and the non-sticky, long-grained *Indica rice* variety (20). Rice also occurs in a variety of colors, including white, brown, black or purple, and red. Rice water is the water left over after you cook rice, or is obtained by soaking rice in water. It is said to soothe and tone your skin, and even improve different skin conditions. It is also said to repair damaged hair.

Rice water is used to lighten the skin and a lot of commercial products contain rice water. Studies have shown that fermented rice water can help improve skin damage from the sun (21), keep your skin supple and help prevent wrinkling, has natural sunscreen properties, and anti-aging benefits because of its antioxidant properties. Rice water is known to help with skin irritation caused by sodium laurel sulfate (SLS), which is an ingredient found in many personal care products. Hair that's been bleached can be helped by inositol, a substance present in rice water. Some people also recommend drinking rice water if you get food poisoning. Plenty of people claim that applying rice water topically can soothe the skin, clear up blemishes caused by skin conditions like eczema, and help it heal. Drinking rice water or eating certain types of rice is said to help fix eye problems like macular degeneration. However, the benefits of rice water are not supported by enough scientific research although cosmetic manufacturers greatly assert the benefits of rice water.

Recently, the search for new, natural, and most importantly organic bioactive compounds to prevent skin aging has greatly increased. Rice water is natural, economical and simple, and could be

easily included in skincare products. Different types of rice are readily available as it is a large part of the human diet, and it can easily be obtained from the rice industry as well.

1.3 Structure of rice and its bioactive compounds

A rice grain is covered in an inedible protective layer called the hull. It is followed by the bran layer which has higher levels of the majority of the bioactive compounds in rice. The bran layer is composed of the pericarp, tegmen and aleurone layer, followed by the embryo and the starchy endosperm as seen in Figure 6 (22). Previous studies have shown that pigmented rice contains higher amount of phenolic compounds. The pigment is located in the aleurone layer of rice bran (23, 24). Rice is composed of phytochemicals and nutrients. It is a source of a variety of bioactive compounds including:

1. flavonoids (specially anthocyanin and proanthocyanidin)
2. carotenoids (such as α−, β−carotene, lutein, and lycopene)
3. phenolic compounds (such as caffeic acid, ferulic acid, etc.)
4. phytosterols (such as β-sitosterol, stigmasterol, and capesterol)
5. the vitamin E isoforms (α−, γ−, δ−tocotrienols and tocopherols)
6. γ-oryzanol
7. coumaric acid
8. phytic acid
9. tricin, etc. (25).

Rice bran oil is recognized for containing antioxidant-rich components such as ferulic acid, γ-oryzanol, and phytic acid (26). These components have been established and used in the cosmetic industry, and in the management of skin diseases (27). Rice bran bioactive compounds have been found to be efficient in the treatment of alopecia (28) and also have an anti-aging potential (29). Starch, which is the major component in rice grains, is a biodegradable polymer with safe application in the pharmaceutical industry. Starch is recommended to be added to bath water as it is said to help with the treatment of atopic dermatitis or other skin diseases associated with pruritus (30). A study done in

Portugal presented *in vitro* biological antioxidant activity and elastase inhibitory effects by rice water. Their clinical study done using a topical gel formulation containing 96 % rice water was shown to be biocompatible with the human skin and presented suitable cosmetic properties (31). It has also been shown that the bioactive compounds present in rice have huge potential for many health benefits and has anti-tumor, anti-atherosclerosis, anti-diabetic, anti-allergic agents, alleviation of gallstones, anti-cancer activity, anti-inflammatory, and other effects (32).

1.4 Sub-aleurone layer residual rinse-free rice (SARFR)

In recent years, diseases related to high glycative stress such as obesity, and type 2 diabetes mellitus (T2DM) have been increasing. Longitudinal medical surveys of Japanese Americans have shown that those who were originally obese are at increased risk of developing T2DM due to excessive fat intake (33). Even if the persons are lean, excessive carbohydrate intake increases the risk of obesity. It has been pointed out that rice-based food has advantages over wheat-based food, such as being gluten-free and having less fecal incontinence in the elderly (34). Although most of the rice food is polished rice, it is obvious that brown rice is rich in vitamins, minerals and dietary fiber and is nutritionally superior. However, there are many individuals who cannot eat brown rice for an extended period of time, and only a few people continue to eat it.

Regarding brown rice, it is known that there is a problem in compliance with continuous intake, and there are few reports of clinical trials so far. Compliance means "observing the instructions", and in this study, it represents the rate at which brown rice can be continuously ingested as instructed. Various measures have been attempted to reduce the disadvantage of indigestion while utilizing the nutritional advantages of brown rice.

Sub-aleurone layer residual rinse-free rice (SARFR), also known as Kinmemai rice, is the rice produced by the special rice processing as follows; the rice hulls (chaff), wax layer (rind), and bran layer, in order from the outside, were scraped off from the whole chaff, leaving the sub-aleurone layer, scutella of the germinal base (kinme) and the endosperm boundary to which the scutellum adheres (Figure 7). The rice husk is inedible, the wax layer hinders water absorption and the bran layer is not

tasty. The sub-aleurone layer is located between the starch layer and the bran layer, and is rich in the nutritional components of brown rice; it has a unique flavor and sweetness with a favorable taste. The nutritional components vary depending on the amount of soil microbes and the method of processing. In general, SARFR contains twice as much vitamin B1 and E as conventional polished rice, 1.5 times more dietary fiber, and more carbohydrate (probiotics) that adjusts the gastrointestinal environment by immersion in the cooking process or other factors; maltose reaches about 60 times, oligosaccharides 12 times, and lipopolysaccharide (LPS) reaches 6 times that of polished rice

1.5 Dewaxed brown rice (DBR)

It has been shown that whole grains such as brown rice may be effective in preventing lifestyle-related diseases such as obesity, type 2 diabetes (T2DM), and coronary artery disease (35). A brown rice diet is rich in vitamins, minerals and dietary fiber, and is undoubtedly nutritionally better than polished rice. However, there are some problems with continuous intake of brown rice. Problems include the need for long-term water immersion on the cooking side, the time required for digestion on the physical side, and the need for sufficient chewing. Various measures have been attempted to reduce the disadvantage of indigestibility while utilizing the nutritional advantages of brown rice. Brown rice from which the wax bran layer has been removed is called dewaxed brown rice (DBR), and there are reports of ingredients (36), improvement of constipation (37) and amelioration of fatty liver (38) in animal experiments.

DBR is brown rice from which rice husks have been removed from whole rice that have been dried after harvesting, and the wax bran layer that covers the surface of the brown rice has been removed by special processing (Figure 8). The non-edible part of the rice husk, the wax layer, hinders water absorption, while the bran layer contains various nutrients. The sub-glue powder layer is located between the starch layer and the bran layer, the nutritional components of brown rice are concentrated, it has a unique flavor and sweetness, and the taste is also preferable. The nutritional components in rice differ depending on the amount of cultivated soil bacteria and the rice milling process. In general, DBR is four times higher in vitamin B1, fourteen times higher in vitamin E, and seven times higher in dietary fiber than ordinary polished rice.

Brown rice is covered with a highly waterproof layer (wax layer) so that it does not germinate as seeds by absorbing water until the growing conditions are met. The strong waterproofing of the wax layer hinders water absorption, and the rice grains do not swell sufficiently, which can make them difficult to eat. It usually takes about 20 hours to soak before cooking rice. This time is longer than the immersion of polished rice for about one hour. Since the presence of the wax layer hinders water absorption, the rice does not expand sufficiently even when cooked, and the rice grains are hard. Therefore, it has low digestibility even though it contains high nutritional value. On the other hand, since DBR removes the wax on the surface, water is easily absorbed during soaking and cooking rice, as with polished rice. The starch of rice is pre-gelatinized and swells well when heated during cooking, so the color is whitish like polished rice, and the rice is cooked into plump rice with good digestion and absorption.

1.6 Objective of study

The main purpose of this study is to verify the presence or absence of anti-glycation effect as a new function of rice water, and to identify the specific substances responsible for the anti-glycation property of rice. The main purpose of the clinical studies conducted is to compare the subjective symptoms and changes in skin condition of university students that consumed dewaxed brown rice (DBR) or sub-aleurone layer residual rinse-free rice (SARFR) in comparison with polished rice.

2. Materials and methods

2.1 Chapter 1: Inhibition of the production of AGEs by traditional rice water and the correlation with its total phenolic content (TPC)

2.1.1 Sample and reagents

The 14 rice samples used in this part of the study were purchased randomly at readily available stores across Japan and Sri Lanka (Table 1). Human serum albumin (HSA) was purchased from Sigma-Aldrich (Tokyo, Japan). Folin-Ciocalteau's phenol reagent was purchased from MP Biomedicals LLC (California, USA). Catechin was purchased from Funakoshi Co. Ltd. (Tokyo, Japan). Difco™ nutrient agar and Difco™ nutrient broth were purchased from Becton, Dickinson and Company (MD, USA; #21152). All other chemicals were analytical grade and purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) or nacalai tesque, INC. (Kyoto, Japan).

2.1.2 Rice water preparation by three different methods

Based on the traditional usage in Sri Lanka, the following three types of rice water samples were prepared.

2.1.2.1 Type 1

Three grams of rice was boiled in 36 mL of distilled water at 100 ºC for 30 minutes. Samples were centrifuged at 2500 rpm for ten minutes at room temperature, and filtered.

2.1.2.2 Type 2

Five grams of rice was oven-dried at 160 ºC for 30 minutes. Three grams of oven-dried rice was boiled in 36 mL of distilled water at 100 ºC for 30 minutes. Samples were centrifuged at 2500 rpm for ten minutes at room temperature, and filtered.

2.1.2.3 Type 3

Three grams of rice was soaked in 9 mL of distilled water at room temperature for six hours. Samples were centrifuged at 2500 rpm for ten minutes at room temperature, and filtered.

A solid concentration (mg/mL) was obtained after 3 mL of rice water was placed on an aluminum tray and the weights were measured before and after dehydration at 120 ºC for 120 minutes. Samples were prepared for the experiments by adjusting the concentration with distilled water to 1 mg/mL of solid concentration.

2.1.3 Preparation of glycated proteins

The HSA glycation model (39) was used to evaluate the effect of type 1, type 2, and type 3 rice water on glycation. 25 μL of the samples were added to 125 μL of 0.1 mol/L phosphate buffer solution (pH 7.4), 25 μL of distilled water, 50 μL of 40 mg/mL HSA, and 25 μL of 2.0 mol/L glucose (solution A). Distilled water was added instead of the glucose of solution A as a blank (solution B). At the same time, distilled water was added instead of the samples of solution A as a blank (solution C). A mixed solution containing purified water instead of the glucose solution of solution C was also prepared (solution D). 1 mg/mL aminoguanidine (AG) (40), a known AGE formation inhibitor, was used as a positive control. The reaction mixtures were incubated at 60 ºC for 40 hours.

2.1.4 Measurement of fluorescent AGEs in rice water

The volume of fluorescent AGEs after incubation was measured as reported previously (39). Concisely, 200 μL of the reaction mixture was used to measure fluorescence by a Varioscan® Flash microplate reader (Thermo Scientific, Waltham, MA) at an excitation wavelength of 370 nm and an emission wavelength of 440 nm. The fluorescence intensity was calculated as a relative value when the fluorescence intensity of 5 μg/mL quinine sulfate was 1000. The production inhibition rate (%) of fluorescent AGEs was calculated by the following formula.

Inhibition of fluorescent AGEs formation (%) = [1− (A−B) / (C−D)] × 100

2.1.5 Measurement of total phenolic content (TPC) of rice water

TPC was determined according to the Folin–Ciocalteau procedure (41) with some modifications that do not change the final result. Concisely, 50 μL of the reaction mixture (5 μL sample, 20 μL distilled water and 25 μL ethanol) was mixed with 250 μL of 400 mmol/L sodium carbonate

followed by 25 µL of 50 % Folin–Ciocalteau reagent. Distilled water was used instead of Folin–Ciocalteau reagent as a blank. The mixtures were well mixed and incubated at 30 ºC for 30 minutes. The mixtures were then allowed to stand at room temperature for another 30 minutes. Absorbance was measured at 660 nm by a Varioscan® Flash microplate reader (Thermo Scientific, Waltham, MA). TPC was expressed as catechin equivalent (µM catechin eq) determined by a calibration curve, which was graphed following the same procedure using catechin as the standard phenolic compound (41).

2.1.6 Microbial colony isolation and measurement of fluorescent AGEs

2.1.6.1 Sample preparation and serial dilution

Red *Indica* rice variety; Pachchaperumal, and white *Indica* rice variety; Ma Vee were used to prepare two types of rice water samples. Three grams of rice was soaked in 9 mL of sterile distilled water at room temperature for twelve hours to prepare the first type. Three grams of rice was washed in 9 mL of sterile distilled water to prepare the second type. Samples were centrifuged at 2500 rpm for ten minutes at room temperature, and filtered using sterile filter paper. Serial dilutions of the samples were prepared using 0.85 % sterile saline solution. Ten-fold dilutions up to a dilution factor of 10^{-3} was prepared by adding 1 mL of sample to 9 mL of diluent.

2.1.6.2 Nutrient agar (NA)

Nutrient agar (NA) solution was prepared by mixing the manufacturer's powder with sterile distilled water for the desired volume of materials to a concentration of 23 g/L. The solution was then autoclaved at 121 ºC for 15 minutes. Before cooling, about 15 mL of the autoclaved solution was poured into 10 cm diameter culture plates and left to cool overnight at room temperature and then stored at 4 ºC.

2.1.6.3 Spread plate method

The NA plates were inoculated with 100 µL of prepared rice water samples of dilution factors 10^{0}, 10^{-1}, 10^{-2} and 10^{-3} using the spread plate method. The inoculum was placed on the center of the

plate and spread uniformly using a bent glass rod. The plates were then incubated in an inverted position at 37 ºC for 24 to 48 hours depending on growth.

2.1.6.4 Nutrient broth (NB)

Nutrient broth (NB) solution was prepared by mixing the manufacturer's powder with sterile distilled water for the desired volume of materials to a concentration of 8 g/L. The solution was then autoclaved at 121 ºC for 15 minutes and stored at 4 ºC until use.

2.1.6.5 Colony isolation and purity confirmation

Colonies were picked based on colony characteristics such as shape, edge, elevation, and color after 24 and 48 hours (see Figure 9 for example). The isolated colonies were suspended in 5 mL of NB and incubated at 37 ºC for 24 hours at 250 rpm in a shaking incubator. Purity of colonies was confirmed after plating in NA using the spread plate method as described above (refer section 2.1.6.3).

2.1.6.6 Measurement of fluorescent AGEs in isolated colonies

Microbial solutions of pure colonies were centrifuged at a gravitational force of 6000 for 15 minutes and the supernatants were obtained. The supernatants effect on glycation was evaluated using the HSA glycation model as described above (refer sections 2.1.3 and 2.1.4)

2.1.7 Statistical analysis

The measured values are shown as mean ± standard deviation (Excel add-in, Microsoft Excel 2013, Microsoft, Redmond, USA). Linear regression analysis was performed using Microsoft Excel (Excel add-in, Microsoft Excel 2013, Microsoft, Redmond, USA). Comparisons between groups were performed in measurements using the Tukey's test (Excel add-in, Microsoft Excel 2016, Microsoft, Redmond, USA). Correlation analysis between the measured values was performed using the Pearson correlation coefficient. Differences were considered significant at P values less than 0.05.

2.2 Chapter 2: Identification of bioactive compounds in rice responsible for its anti-glycation activity

2.2.1 Sample and reagents

The 16 rice samples used in this part of the study were purchased randomly at readily available stores across Japan and Sri Lanka (Table 2). Human serum albumin (HSA) and methylglyoxal solution were purchased from Sigma-Aldrich (Tokyo, Japan). 3-deoxyglucosone and 2.3-diaminonaphthalene (DAN) were purchased from Dojindo Laboratories Co., Ltd. (Kumamoto, Japan). 40 % glyoxal solution was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Pentosidine-trifluoroacetic acid salt (pentosidine-TFA salt) was used as the standard pentosidine and was purchased from Peptide Research Institute (Ibaraki, Osaka). All other chemicals were analytical grade and purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) or nacalai tesque, INC. (Kyoto, Japan).

2.2.2 Initial screening of rice water extracts

2.2.2.1 Rice water extract preparation

Two grams of rice was added to 40 mL of distilled water and kept in a water bath of 80 ºC for 60 minutes. Samples were centrifuged at 2500 rpm for ten minutes at room temperature, and filtered. Samples were prepared for the experiments by adjusting the concentration with distilled water to 0.5 mg/mL of solid concentration.

2.2.2.2 Measurement of fluorescent AGEs in rice water extracts

Same procedure as above was followed (refer sections 2.1.3 and 2.1.4).

2.2.2.3 Measurement of intermediates for AGEs in rice water extracts

The intermediates for AGEs were measured as reported previously by reversed-phase high-performance liquid chromatography (RP-HPLC) assay with 2, 3-diaminonaphthalene (DAN) pre-column derivation (15, 42, 43). Concisely, a 3-DG, GO and MGO standard solution was prepared by mixing 100 µL each of 1 mg/mL 3-DG, 1 mg/mL GO, 1 mg/mL MGO and distilled water. Standards

of concentration 3, 1, 0.3, 0.1, 0.03, and 0.01 μg/mL were prepared by serial dilution with distilled water. 100 μL of each standard was mixed with 50 μL of 200 mmol/L phosphate buffer (pH 7.4) and 15 μL of distilled water. 100 μL of the HSA glycation model reaction mixtures were mixed with 65 μL of distilled water. Next, all samples were deproteinized by adding 85 μL of 6 % perchloric acid. After centrifugation at 12,000 rpm for 10 minutes, 200 μL of the supernatant was instantaneously neutralized with 175 μL of saturated sodium bicarbonate. Then, 3-DG, GO and MGO in the samples were labeled with 25 μL of 1 mg/mL DAN at 4 ºC for 24 hours. After centrifugation at 15,000 rpm for 10 minutes, supernatants were analyzed by RP-HPLC.

The HPLC conditions were as follows; column: Unison UK-Phenyl (3 μm, 75 × 3 mm) ZC173 (Imtakt Corporation, Kyoto, Japan), eluent; solution A: 50 mmol/L phosphoric acid, solution B: 100 % acetonitrile (ACN), column temperature: 40 ºC, flow rate: 1.0 mL/min, UV detection: 268 nm, fluorescence detection (excitation wavelength: 271 nm, emission wavelength: 503 nm), injection volume: 20 μL. The measurement was performed using the HPLC system (degassing unit; DGU-20A_{3R}, liquid chromatograph; LC-20AT, communications bus module; CBM-20A, fluorescence detector; RF-20A_{XS}, UV/VIS detector; SPD-20A, auto sampler; SIL-20AC, column oven; CTO-20AC) and LabSolutions data analysis system (Shimadzu Corporation, Kyoto, Japan).

2.2.2.4 Measurement of pentosidine in rice water extracts

Pentosidine was measured as reported previously by RP-HPLC assay (44-46). Concisely, 50 μL of the HSA glycation model reaction mixtures were reduced in advance to prevent pentosidine formation during the hydrolysis process by mixing with 50 μL of 200 mmol/L sodium borohydride (pH 9.2) and allowed to stand for 30 minutes at room temperature. Next the reaction solution was mixed with 100 μL of 6 N iron-free hydrochloric acid and hydrolyzed at 105 ºC for 18 hours in a block incubator.

2.2.2.4.1 MonoSpin column pretreatment method

After hydrolysis, 200 μL of 1.5 mol/L Tris (hydroxymethyl) amino-methane (Tris) solution was added to dilute the hydrochloric acid in the reaction solution. Impurities in the hydrolyzed solution was

removed by centrifugation at a gravitational force of 5,000 for 1 minute at 4 ºC using a centrifugal filtration filter of polytetrafluoroethylene (PTFE) membrane pore size 0.45 μm (Centricut ultra mini W-MR; Kurabo, Chuo-ku, Osaka).

MonoSpin AG column (GL Sciences, Shinjuku-ku, Tokyo) was used to perform pretreatment for pentosidine measurement by spin column. The MonoSpin AG column is characterized by having a carrier in which a mixed cation exchange group and hydrophobic group is bonded to monolithic silica. It is a high-purity silica gel with a large surface area with pores in the silica skeleton. The solution was added to the Monospin AG column in each step of the pretreatment, and the solution was centrifuged at a gravitational force of 5,000 for 1 minute at 4 ºC. The steps were as follows; conditioning with 200 μL of 0.1 mol/L citric acid/ACN (990/10 v/v) solution, equilibration with 400 μL of 0.1 mol/L citric acid, addition of 150 μL of sample, washing with 500 μL of 0.1 mol/L citric acid, first elution using 75 μL of 1 mol/L ammonium formate solution, and second elution with 75 μL of 1 mol/L ammonium formate/ACN (50/50 v/v) solution. The elutes obtained in the final two steps were uniformly mixed and used as the sample for measurement by RP-HPLC.

Pentosidine-TFA salt was dissolved in an appropriate amount of distilled water, and the pentosidine concentration (pmol/mL) was calculated based on the description in the attached document (molecular weight: 378.43). The prepared pentosidine solution was divided into aliquots and stored at - 20 ºC until usage. 10 ng/mL pentosidine stock solution was used to prepare standards of concentrations 3, 1, 0.3, 0.1, and 0.03 ng/mL by serial dilution with distilled water. Standards were also measured by RP-HPLC. The HPLC conditions were as follows; column: Inert Sustain AG (3 μm, 4.6 I.D. × 100 mm) 20B0091799 (GL Sciences Inc., Tokyo, Japan), eluent; solution A: 0.1 % (v/v) formic acid aqueous solution, solution B: 100 % ACN, column temperature: 20 ºC, flow rate: 1.0 mL/min, fluorescence detection (excitation wavelength: 325 nm, emission wavelength: 385 nm), injection volume: 20 μL. The same HPLC system mentioned above was used to perform the measurement (refer section 2.2.2.3).

2.2.2.4.2 Citric acid eluent method

After hydrolysis, the samples were evaporated to dryness using a centrifugal concentrator, and the pellet was dissolved in 400 µL of 0.1 mol/L citric acid and acetonitrile in a ratio of 995 to 5 (solution A). The solution was then centrifuged at a gravitational force of 15,000 for 10 minutes at 4 ºC and 300 µL of the supernatant was used as the sample for measurement by RP-HPLC. 100 ng/mL pentosidine stock solution was used to prepare standards of concentrations 10, 3, 1, 0.3, and 0.1 ng/mL by serial dilution with distilled water. Standards were also measured by RP-HPLC.

The HPLC conditions were as follows; column: Unison US-C18 (5 µm, 150 x 4.6 mm) US005 (Imtakt Corporation, Kyoto, Japan), eluent; solution A: 0.1 mol/L citric acid/acetonitrile (995/5 v/v), solution B: 100 % ACN, column temperature: 20 ºC, flow rate: 1.0 mL/min, fluorescence detection (excitation wavelength: 325 nm, emission wavelength: 385 nm), injection volume: 15 µL. The same HPLC system mentioned above was used to perform the measurement (refer section 2.2.2.3).

2.2.3 Analysis of rice bran water extracts

2.2.3.1 Rice milling process

Rice milling machine Clean One Pass CBS 2200 (Satake Corporation, Hiroshima, Japan) was used to separate the bran from rice. Polished rice (endosperm) and rice bran were collected separately.

2.2.3.2 Rice bran water extract and polished rice (endosperm) water extract preparation

Two grams of rice bran or polished rice (endosperm) was added to 40 mL of distilled water and kept in a water bath of 80 ºC for 60 minutes. Samples were centrifuged at 2500 rpm for ten minutes at room temperature, and filtered. Samples were prepared for the experiments by adjusting the concentration with distilled water to 0.5 mg/mL of solid concentration.

2.2.3.3 Measurement of fluorescent AGEs in rice bran and polished rice water extracts

Same procedure as above was followed (refer sections 2.1.3 and 2.1.4).

2.2.3.4 Fractional purification of rice bran water extracts

Oasis® HLB Plus 60 µm LP Extraction Cartridge (Waters Corporation, MA, USA) was used for the fractional purification of the bran water extracts. These cartridges contain the Oasis HLB sorbent, which is a universal polymeric reversed-phase sorbent for extraction of a wide range of acidic, basic, and neutral compounds from various matrices using a simple, standard protocol. They can attach easily to syringes for reliable, positive-pressure flow to speed up the sample processing time.

Concisely, the steps are as follows. First the cartridge is conditioned with 5 mL of 100 % ACN and 10 mL of distilled water. Next 1 mL of sample is loaded followed by 2 mL of distilled water to obtain the water fraction. Then 3 mL of 5 % ACN is loaded to obtain the 5 % ACN fraction. Similarly 3 mL of 10 % ACN and 3mL of 75 % ACN is loaded to obtain the 10 % and 75 % ACN fractions. The cartridge is discarded after use.

2.2.3.5 Measurement of fluorescent AGEs in fractions of rice bran water extracts

Rice bran extracts were diluted with distilled water to a dilution factor of 3 for comparison purposes and fluorescent AGEs of the diluted samples along with the fractions (water, 5 % ACN,10 % ACN, and 75 % ACN) were measured following the same procedure as before (refer sections 2.1.3 and 2.1.4).

2.2.3.6 Ultraviolet-visible (UV) spectroscopy of fractions of rice bran water extracts

UV spectroscopy was performed on the fractions of rice bran water extracts. Absorbance was measured at 200 to 800 nm by a Varioscan® Flash microplate reader (Thermo Scientific, Waltham, MA) and distinct peaks were identified.

2.2.3.7 Measurement of cyanidin 3-glucoside (C3G) in fractions of rice bran water extracts

C3G in fractions were measured by RP-HPLC assay. C3G stock solution was used to prepare standards of concentration 300, 3, and 0.3 µg/mL by serial dilution with distilled water. Next 50 µl of standard or sample was mixed with 50 µL of 100 mmol/L phosphate buffer (pH 2.6) and analyzed by RP-HPLC. The HPLC conditions were as follows; column: Cadenza CD-C18 (3 µm, 75 x 4.6 mm)

CD003 (Imtakt Corporation, Kyoto, Japan), eluent; solution A: 100 mmol/L phosphate buffer (pH 2.6), solution B: 100 % ACN, column temperature: 40 ºC, flow rate: 1.0 mL/min, UV detection; 270 nm and 510 nm, injection volume: 20 µL. The measurement was performed using the HPLC system (degasser; DGU-20A$_3$, liquid chromatograph; LC-20AT, communications bus module; CBM-20A, fluorescence detector; RF-20AXS, UV/VIS detector; SPD-20A, auto sampler; SIL-20AC, column oven; CTO-20AC) and LabSolutions data analysis system (Shimadzu Corporation, Kyoto, Japan).

2.2.3.8 Ferulic acid and phytic acid

2.2.3.8.1 UV spectroscopy of ferulic acid and phytic acid

UV spectroscopy was performed to confirm the absorbance wavelength of 0.01 mg/mL ferulic acid and 5 mg/mL phytic acid. Same procedure as above was followed (refer section 2.2.3.6).

2.2.3.8.2 Measurement of ferulic acid and phytic acid in rice bran water extracts

Ferulic acid and phytic acid in fractions were measured by RP-HPLC assay. Samples were mixed with 1 % formic acid in a ratio of 9:1 respectively. Then samples were centrifuged at a gravitational force of 5000 at 4 ºC for 5 minutes. Next 200 µL of supernatant was obtained to be analyzed by HPLC. The HPLC conditions were as follows; column: Inert Sustain C18 (5 µm, 6.0 I.D. × 150 mm) 21C0191457 (GL Sciences Inc., Tokyo, Japan), eluent; solution A: 1 % (v/v) formic acid aqueous solution, solution B: 100 % ACN, column temperature: 40 ºC, flow rate: 1.0 mL/min, UV detection: 270 nm and 340 nm, injection volume: 100 µL. The same HPLC system mentioned above was used to perform the measurement (refer section 2.2.3.7). The chromatograms of the samples were then compared with standard chromatograms of 0.01 mg/mL ferulic acid and 5 mg/mL phytic acid.

2.2.3.9 Isolation of glycation inhibitory compounds in water fractions of rice bran water extracts

2.2.3.9.1 Fraction collection by HPLC

HPLC conditions were the same as before (refer section 2.2.3.8.3). Fraction collector; FRC-10A (Shimadzu Corporation, Kyoto, Japan) was used to collect fractions from 0 to 34 minutes at a rate

of 1 mL/min. The collected fractions were evaporated to dryness using the centrifugal concentrator; CC-105 (Tomy Seiko Co., Ltd., Tokyo, Japan) and re-dissolved in 250 µl of 30 % ACN.

2.2.3.9.2 Measurement of fluorescent AGEs in fractions collected by HPLC

The fluorescent AGEs in the fractions re-dissolved in 30 % ACN was measured. Same procedure as above was followed (refer sections 2.1.3 and 2.1.4).

2.2.3.9.3 Purification of HPLC fractions with high inhibition rates and confirming stability

Fractions with a high inhibition rate of fluorescent AGEs that showed clean-cut peaks in the chromatogram were selected for further purification. HPLC conditions were the same as before (refer section 2.2.3.8.3). Screened fractions were hand-collected at their specific retention times. The collected fractions were then evaporated to dryness using the centrifugal concentrator and re-dissolved in 250 µl of 30 % ACN.

Next the re-dissolved samples' chromatograms were checked using HPLC by comparing their retention times with the original chromatograms to confirm stability. HPLC conditions were the same as before (refer section 2.2.3.8.3). Fluorescent AGEs were measured again to reconfirm stability following the same procedure as before (refer sections 2.1.3 and 2.1.4).

Rice bran extracts were diluted with distilled water to a dilution factor of 2.5 for comparison purposes and fluorescent AGEs of the diluted samples were measured following the same procedure as before (refer sections 2.1.3 and 2.1.4). Finally, the percentage contribution towards the rice bran water extract by the purified fractions of fluorescent AGEs was calculated by the following formula.

$$\text{Percentage contribution (\%)} = \frac{\text{Inhibition by purified fraction}}{\text{Inhibition by rice bran water extract}} \times 100$$

2.2.3.10 Measurement of pentosidine in fractions of rice bran water extracts

Rice bran extracts, originally diluted by a dilution factor of 3, were diluted again with distilled water by a dilution factor of 2.5 for comparison purposes. Pentosidine of the diluted samples were measured following the same procedure as above (refer section 2.2.2.4).

2.2.3.11 Isolation and identification of glycation inhibitory compounds in 5 % ACN fraction of *Indica* red rice bran water extract

2.2.3.11.1 Fraction collection by HPLC

HPLC chromatograms of 5 %, 10 % and 75 % ACN fractions of *Indica* red rice bran water extract were compared to identify and collect fractions present only in the 5 % ACN fraction that could be the reason for its high inhibition of pentosidine. HPLC conditions were the same as before (refer section 2.2.3.8.3). The screened fractions were hand-collected, evaporated to dryness using the centrifugal concentrator and re-dissolved in 250 µl of 30 % ACN.

2.2.3.11.2 Purification of collected fractions and confirming stability

The re-dissolved samples' chromatograms were checked using HPLC by comparing their retention times with the original chromatograms to confirm stability. HPLC conditions were the same as before (refer section 2.2.3.8.3). Fluorescent AGEs were measured to confirm stability following the same procedure as before (refer sections 2.1.3 and 2.1.4). Pentosidine was also measured to confirm stability following the same procedure as before (refer section 2.2.2.4).

Rice bran extracts were diluted with distilled water to a dilution factor of 2.5 for comparison purposes and fluorescent AGEs of the diluted samples were measured following the same procedure as before (refer sections 2.1.3 and 2.1.4). Finally, the percentage contribution towards the rice bran water extract by the purified fractions of both fluorescent AGEs and pentosidine was calculated by the following formula.

$$\text{Percentage contribution (\%)} = \frac{\text{Inhibition by purified fraction}}{\text{Inhibition by rice bran water extract}} \times 100$$

2.2.3.11.3 UV spectroscopy

UV spectroscopy was performed to check the absorbance wavelength of the isolated fractions from the 5 % ACN fraction of *Indica* red rice bran water extract. Same procedure as above was followed (refer section 2.2.3.6).

2.2.3.11.4 Capillary Electrophoresis-Mass Spectrometry (CE-MS) analysis

A total of 11 injections (1100 µL) of each fraction was collected. HPLC conditions were the same as before (refer section 2.2.3.8.3). The hand-collected fractions were evaporated to dryness using the centrifugal concentrator and re-dissolved in 110 µl of 30 % ACN.

CE-MS analyses were performed for identification of the structure of the compounds. We used the technical services of Human Metabolome Technologies Inc. (Yamagata, Japan). The analytical conditions were not disclosed.

2.2.3.11.5 Measurement of fluorescent AGEs and pentosidine in glutamic acid and guanosine

Glutamic acid stock solution and dilutions were prepared using distilled water. Guanosine is insoluble in water so guanosine stock solution was prepared using dimethyl sulfoxide (DMSO) and dilutions were prepared using 80% ethanol. 10, 5, 3, and 1 mmol/L dilutions of glutamic acid, and 50, 20, and 5 mmol/L dilutions of guanosine were used to measure fluorescent AGEs following the same procedure as before (refer sections 2.1.3 and 2.1.4). 10 and 5 mmol/L dilutions of glutamic acid, and 3 and 1 mmol/L dilutions of guanosine were used to measure pentosidine following the same procedure as above (refer section 2.2.2.4). After glycation, all samples were filtered using Amicon® Ultra-0.5 mL 30kDa centrifugal filter devices (Merck Millipore Ltd., Ireland) before measuring fluorescent AGEs and pentosidine to remove the reaction products between glucose and glutamic acid or guanosine. HPLC chromatograms of commercialized glutamic acid and guanosine were also checked. HPLC conditions were the same as before (refer section 2.2.3.8.3).

2.2.4 Analysis of samples provided by Toyo Rice Co. Ltd.

Samples provided by Toyo Rice Co. Ltd. included 4 rice samples and 3 layer samples (Figure 10). The rice samples were brown rice, dewaxed brown rice (DBR), which is brown rice without the wax layer, Kinmemai (SARFR), which is rice without the bran layer, and white rice which is fully polished rice. The layer samples were the bran layer, the sub-aleurone layer, and a mix of these two layers. DBR and SARFR were used in the clinical studies described in chapters 3 and 4. The rice variety was Koshihikari produced in Nagano prefecture which is a type of brown rice, but also eaten as white rice after polishing.

2.2.4.1 Measurement of fluorescent AGEs in samples provided by Toyo Rice Co. Ltd.

Same procedure as above was followed (refer sections 2.1.3 and 2.1.4).

2.2.4.2 Fractional purification of bran layer sample provided by Toyo Rice Co. Ltd.

Same procedure as above was followed (refer section 2.2.3.4).

2.2.4.2.1 Measurement of fluorescent AGEs in fractions of bran layer

Same procedure as above was followed (refer sections 2.1.3 and 2.1.4).

2.2.4.2.2 Measurement of pentosidine in fractions of bran layer

Same procedure as above was followed (refer section 2.2.2.4).

2.2.4.2.3 Measurement of glycation intermediates in fractions of bran layer

Same procedure as above was followed (refer section 2.2.2.3).

2.2.5 Statistical analysis

The measured values are shown as mean ± standard deviation (Excel add-in, Microsoft Excel 2013, Microsoft, Redmond, USA).

2.3 Chapter 3: Effects on skin by sub-aleurone layer residual rinse-free rice (Kinmemai rice): An open label test

2.3.1 Subjects

The subjects were students (men and women) from a university in Tokyo. This study was conducted with 61 subjects who agreed to participate in this trial in advance in writing. They met the selection criteria, did not conflict with the exclusion criteria, and were judged to be appropriate to participate by the principal investigator. The selection criteria are shown below.

1. Men and women 18 to 60 years old at the time when the consent for participation in the study was obtained.
2. Persons in good health without chronic physical illness, including skin diseases.
3. Persons with the ability to give consent after receiving an adequate explanation of the purpose and content of the study, and who volunteer to participate at their own accord after proper understanding and provide a written consent to participate in this study.
4. Persons who can come on the designated examination dates to undergo examination.
5. Persons determined to be suitable as a subject of this study by the principal investigator.

The exclusion criteria are shown below.

1. Persons who are currently receiving medication due to an illness.
2. Persons with a history of, or currently suffering from impaired glucose tolerance, mental illness, sleep disorders, hypertension, diabetes mellitus, dyslipidemia, or any other serious illness.
3. Persons who have been taking drugs for the treatment of a disease for the past one month (excluding those with a history of taking temporary-relief medication for headaches, menstrual pain, and cold).
4. Persons with a history of, or currently suffering from a severe disease of the liver, kidney, heart, lungs, digestive organs, or a hematologic disease.
5. Persons with a history of, or currently suffering from a severe disease of the gastrointestinal tract, excluding appendicitis.

6. Persons with a body mass index (BMI) of more than 30 kg/m^2.
7. Those who may have allergic reactions to the test food, and those who may have serious allergic reactions to other foods or drugs.
8. Women pregnant, lactating or possibly pregnant.
9. Currently, and within the past 3 months, those who have a habit of continuously ingesting functional foods and health foods that claim to be related to skin quality improvement, and those who plan to take them during the test period (ingestion for the purpose of maintaining health was permitted).
10. Persons with photosensitivity.
11. Persons who have been determined by the principal investigator as not suitable to be a subject of this study.

The transition of the number of test subjects can be seen in Figure 11. The breakdown of 59 subjects analyzed was 37 in the SARFR group (24 men and 13 women) and 22 in the control group (13 men and 9 women). The average age of each group was 21.0 ± 1.5 years in the SARFR group (men 21.4 ± 1.2 years, women 20.2 ± 1.7 years) and 22.0 ± 1.2 years in the control group (men 22.4 ± 1.2 years, women 21.4 ± 1.0 years).

2.3.2 Exam design

This study was an open label study between parallel groups. The SARFR group ingested SARFR, and the control group freely ingested normal polished rice without SARFR. The SARFR group ingested the test product once a day during the test period by any of the following methods.

1. Cook the SARFR in the cafeteria, weigh it, and prepare at least 150 g or more for ingestion.
2. Heat the packaged SARFR (160 g) in a microwave oven and ingest it.

The test product was provided by Toyo Rice Co., Ltd. (Wakayama, Japan). The subjects participated in the pre-ingestion test for one day, the ingestion period for 33 days, and the post-intake test for one day.

Pre-intake tests for each group included a background survey by filling out a questionnaire, survey of lifestyle and skin condition by filling out a questionnaire, skin condition measurement by a device Clreo-Pro (Fujitex, Shinjuku-ku, Tokyo), skin AGEs score by an AGEs sensor device (RQ-AG01J: Sharp Life Sciences, present Air Water Biodesign, Kobe, Hyogo, Japan).

Post-ingestion tests included a survey of lifestyle-related and skin conditions, skin condition measurement, and AGEs score. In both the pre-ingestion test and the post-intake test, female subjects washed their face 20 minutes or more prior to the test to remove cosmetics, and performed skin diagnostic measurements after acclimation. The test period was from June 2019 to July 2019.

2.3.3 Evaluation item

2.3.3.1 Skin index

The skin indices included functions, skin age, overall skin health, pores, wrinkles, moisture, elasticity, skin tone, pigmentation (epidermis layer, dermis layer), oil content, and porphyrin, that were measured by Clreo-Pro.

2.3.3.2 AGEs measurement

The AGEs score was measured using AGEs sensors (47, 48).

2.3.3.3 Subjective symptoms

The contents of the questionnaire were questions about changes in eating habits, lifestyle habits (exercise, sleep, bowel movements, drinking, smoking, and mental stress), and skin conditions. The average value of compliance (the intake rate of the test product) was calculated based on the questionnaire.

2.3.4 Statistical analysis

For analysis, a software SPSS (Statistics25: IBM Japan, Chuo-ku, Tokyo, Japan) was used to perform paired-t test or Wilcoxon signed rank test. A risk rate of less than 5 % was defined as significantly different.

2.3.5 Ethical standards

This study was conducted in compliance with the Declaration of Helsinki (revised at the 2013 WMA Fortaleza General Assembly) and the ethical guidelines for human-based medical research (notification by Ministry of Education, Culture, Sports, Science and Technology [MEXT] and Ministry of Health, Labor and Welfare [MHLW]). This research obtained the approval of the Ethical Committee of the Society for Glycative Stress Research (GSE 2019-004), which has discussed the ethics and validity of the study. The clinical trial for this study was pre-registered (UMIN #000037017).

2.4 Chapter 4: Effects on skin by dewaxed brown rice: An open label test

2.4.1 Subjects

The subjects were male and female students and staff from a university in Tokyo. This study was conducted with 66 subjects who agreed to participate in this trial in advance in writing, who met the selection criteria, did not conflict with the exclusion criteria, and were judged to be appropriate to participate by the principal investigator. The subjects enrolled in this study were randomly designated by the assigners to the DBR group and the control group. The selection criteria and the exclusion criteria were the same as above (refer section 2.3.1).

The transition of the number of test subjects is shown in Figure 12. The breakdown of 65 subjects analyzed was 43 in the DBR group (25 men and 18 women) and 22 in the control group (13 men and 9 women). The DBR group includes 7 university staff (5 men and 2 women). The average age of each group was 23.8 ± 8.8 years in the DBR group (men 24.2 ± 8.8 years, women 23.1 ± 9.1 years) and 22.0 ± 1.2 years in the control group (men 22.4 ± 1.2 years, women 21.4 ± 1.0 years). The control group was the same as in the previous report (49).

2.4.2 Exam design

This study was an open label study between parallel groups. The DBR group ingested brown rice from which the wax layer had been removed, and a control group was set in which normal polished rice without DBR was ingested freely. The DBR group always ingested the test product once a day during the test period by any of the following methods.

1. DBR was cooked in the on-campus cafeteria, weighed, served at 150 g or more, and ingested.
2. Packaged DBR (150 g) was heated in the microwave and ingested.

The test product was provided by Toyo Rice Co., Ltd. (Wakayama, Japan). The subjects participated in the pre-ingestion test 1 day, the ingestion period 33 days, and the post-intake test 1 day.

Pre-intake tests for each group included a background survey via questionnaire on lifestyle and skin condition, skin condition measurement via device Clreo-Pro (Fujitex, Shinjuku-ku, Tokyo), and skin AGEs score via AGEs sensor device (RQ-AG01J: Sharp Life Sciences, present Air Water Biodesign, Kobe, Hyogo, Japan).

Post-ingestion tests included a survey of lifestyle-related and skin conditions, skin condition measurement, and AGEs score. In both the pre-ingestion test and the post-intake test, female subjects washed their face 20 minutes or more prior to the test to remove cosmetics, and performed skin diagnostic measurements after acclimation. The test period was from June 2019 to July 2019.

2.4.3 Evaluation item

2.4.3.1 Skin index

The skin indices included functions, skin age, overall skin health, pores, wrinkles, moisture, elasticity, skin tone, pigmentation (epidermis layer, dermis layer), oil content, and porphyrin, that were measured via Clreo-Pro as in the previous report (49).

2.4.3.2 AGEs measurement

The AGEs score was measured using AGEs sensors (47, 48).

2.4.3.3 Subjective symptoms

The contents of the questionnaire were questions about changes in eating habits, lifestyle habits (exercise, sleep, bowel movements, drinking, smoking, and mental stress), and skin conditions. The average value of compliance (the intake rate of the test product) was calculated based on the questionnaire.

2.4.4 Statistical analysis

For analysis, a software SPSS (Statistics25: IBM Japan, Chuo-ku, Tokyo, Japan) was used to perform paired-t test or Wilcoxon signed rank test. A risk rate of less than 5 % was defined as significantly different.

2.4.5 Ethical standards

This study was conducted in compliance with the Declaration of Helsinki (revised at the 2013 WMA Fortaleza General Assembly) and the ethical guidelines for human-based medical research (notification by the Ministry of Education, Culture, Sports, Science and Technology [MEXT] and Ministry of Health, Labor and Welfare [MHLW]). This research obtained the approval of the Ethical Committee of the Society for Glycative Stress Research (GSE 2019-004), which has discussed the ethics and validity of the study. The clinical trial for this study was pre-registered (UMIN #000037017).

3. Results

3.1 Chapter 1: Inhibition of the production of AGEs by traditional rice water and the correlation with its total phenolic content (TPC)

3.1.1 Inhibitory effect on formation of fluorescent AGEs by traditional rice water samples

The inhibitory effect on the formation of AGEs by type 1, type 2 and type 3 rice water prepared using 14 rice samples (n=42), and aminoguanidine (AG) are shown in Figure 13. All 42 rice water samples inhibited the formation of fluorescent AGEs. Sample number 1 showed the highest inhibition on the formation of fluorescent AGEs among type 1 rice water samples (66.4 ± 0.4 %, Figure 13. (a)). Sample number 1 also showed the highest inhibition on the formation of fluorescent AGEs among type 2 rice water samples (66.6 ± 0.7 %, Figure 13. (b)). Sample number 12 showed the highest inhibition on the formation of fluorescent AGEs among type 3 rice water samples, and among all 42 rice water samples (69.6 ± 0.8 %, Figure 13. (c)).

There was a significant difference ($P < 0.05$) between the different types of rice water samples except in sample number 1, as shown in Figure 14. Comparing within the groups, sample numbers 2 and 3 showed the highest inhibition on the formation of fluorescent AGEs in type 1 rice water, sample number 1 showed the highest inhibition in type 2 rice water, and sample numbers 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 showed the highest inhibition in type 3 rice water. Among the 14 rice samples studied, all rice water samples (n = 42) showed lower inhibition that that of aminoguanidine (AG).

3.1.2 Total phenolic content (TPC) in traditional rice water samples

The total phenolic content (TPC) in type 1, type 2 and type 3 rice water prepared using 14 rice samples (n=42) are shown in Figure 15. All 42 rice water samples contained phenolic compounds. Sample number 1 showed the highest TPC among type 1 rice water samples (94.7 ± 4.9 µM catechin eq), type 2 rice water samples (124.8 ± 7.5 µM catechin eq), and type 3 rice water samples (176.2 ± 3.4 µM catechin eq). Comparing within the groups, sample numbers 3 and 11 had the highest TPC in type 1 rice water, and sample numbers 1, 2, 4, 5, 6, 7, 8, 9, 10, 12, 13, and 14 had the highest TPC in type 3 rice water.

3.1.3 Correlation analysis

Figure 15 shows the correlation results between the total phenolic content (TPC) and the inhibitory effect on the formation of fluorescent AGEs. Figure 16. (a) shows that there is a strong positive correlation between TPC and inhibition of fluorescent AGEs in type 1 rice water ($r = 0.906$). Similarly, a strong positive correlation was observed between TPC and inhibition of fluorescent AGEs in type 2 rice water ($r = 0.918$, Figure 16. (b)). A moderately strong positive correlation was seen between TPC and inhibition of fluorescent AGEs in type 3 rice water ($r = 0.765$, Figure 16. (c)).

3.1.4 Inhibitory effect on formation of fluorescent AGEs by colonies isolated from rice water

None of the supernatants of the 32 isolated colonies from traditional rice water showed inhibition of fluorescent AGEs as seen in Figure 17.

3.2 Chapter 2: Identification of bioactive compounds in rice responsible for its anti-glycation activity

3.2.1 Inhibitory effect on formation of fluorescent AGEs by rice water extracts

Figure 18 shows the inhibitory effect on the formation of AGEs by the 16 rice water extracts and aminoguanidine (AG). *Japonica* black, *Japonica* red, and *Indica* red samples showed a higher inhibitory effect compared to *Japonica* brown, *Japonica* white and *Indica* white samples. An inhibition rate greater than 50 % was observed in samples 1, 2, 3, and 12 at 66.4 ± 2.2 %, 60.5 ± 3.9 %, 57.6 ± 2.0 %, and 74.2 ± 2.4 % respectively. Among the 16 rice samples studied, all showed lower inhibition that that of AG.

3.2.2 Inhibitory effect on formation of glycation intermediates by rice water extracts

The inhibitory effect by rice water extracts on the formation of glycation intermediates, 3-deoxyglucosone (3-DG), glyoxal (GO), and methylglyoxal (MGO) is shown in Figure 19. A majority of the samples did not inhibit the formation of 3-DG. An inhibition rate greater than 20 % was observed in samples 2, 4, 11, and 13 at 26.0 ± 5.4 %, 23.4± 2.2 %, 20.1 ± 2.4 %, and 25.7 ± 2.5 % respectively. All samples had inhibited the formation of GO and an inhibition rate greater than 70 % was observed in all samples. The highest inhibition of GO was observed in sample 12 at 94.4 ± 0.4 % followed by samples 13 and 3 at 93.1 ± 0.4 % and 90.0 ± 0.7 % respectively. *Japonica* black, *Japonica* red, and *Indica* red samples were able to inhibit the formation of MGO. However, *Japonica* brown, *Japonica* white and *Indica* white samples showed no or little inhibition. The highest inhibition was seen in sample 12 at 51.6 ± 0.9 %.

3.2.3 Inhibitory effect on formation of pentosidine by rice water extracts

Figure 20 shows the inhibitory effect on the formation of pentosidine by the rice water extracts. Similar to the inhibition of fluorescent AGEs, *Japonica* black, *Japonica* red, and *Indica* red samples showed a higher inhibitory effect compared to *Japonica* white and *Indica* white samples. However, unlike the inhibition of fluorescent AGEs, *Japonica* brown samples also showed high inhibition rates

of pentosidine formation. An inhibition rate greater than 65 % was observed in samples 1, 2, 3, 12, and 13 at 66.8 ± 1.1 %, 75.0 ± 7.9 %, 65.7 ± 3.8 %, 74.9 ± 1.4 %, and 77.8 ± 8.9 %.

3.2.4 Screening of 16 rice water extracts

Sample 2 (*Japonica* black), sample 3 (*Japonica* red), and sample 12 (*Indica* red) were chosen for further analysis based on the following screening conditions.

1. High inhibition rate of fluorescent AGEs
2. High inhibition of pentosidine
3. High inhibition of glycation intermediates
4. Subspecies and color of rice sample
5. Sample availability

3.2.5 Inhibitory effect on formation of fluorescent AGEs by rice bran and polished rice water extracts

Inhibitory effects of whole rice, rice bran and polished rice (endosperm) on the formation of fluorescent AGEs were compared in the samples chosen above (refer section 3.2.4). Figure 21 shows that the inhibition rate was significantly higher in the rice bran samples than the polished rice samples suggesting that most of the bioactive compounds or the strongest bioactive compound responsible for its anti-glycation potential is located in the rice bran. Inhibition rate of *Japonica* black rice bran was 48.4 ±1.0 %, *Japonica* red rice bran was 43.0 ± 2.2 %, and *Indica* red rice bran was 24.3 ± 2.0 %. Inhibition rate of *Japonica* black polished rice was 2.3 ± 3.1 %, *Japonica* red polished rice was 5.7 ± 3.0 %, and *Indica* red polished rice was 17.6 ± 1.0 %.

3.2.6 Inhibitory effect on formation of fluorescent AGEs by fractions of rice bran water extracts

Fractions of rice bran water extracts obtained after fractional purification by Oasis® HLB Plus is shown in Figure 22. The inhibitory effect by the fractions on the formation of fluorescent AGEs is shown in Figure 23. When compared with the original rice bran water extract, the water fractions of all 3 samples had the highest inhibition rates than the acetonitrile (ACN) fractions. ACN fractions of

Japonica black rice bran water extract showed inhibition rates less than 30 %. However, its water fraction had an inhibition rate of 64.8 ± 1.7 %. ACN fractions of *Japonica* red rice bran water extract showed inhibition rates less than 15 %. However, its water fraction had an inhibition rate of 62.1 ± 0.6 %. ACN fractions of *Indica* red rice bran water extract showed inhibition rates less than 5 %. However, its water fraction had an inhibition rate of 33.9 ± 1.4 %. Based on these data, the water fractions of all 3 rice bran water extracts were chosen for further analysis.

3.2.7 UV spectroscopy of fractions of rice bran water extracts

Low absorbance was observed in the UV spectroscopy data of all fractions of the rice bran water extracts. However, 3 distinct peaks were seen in most samples at around 270, 340, and 500 nm (Figure 24). Common compounds found in rice with corresponding wavelengths are ferulic acid, phytic acid, and anthocyanins (50).

3.2.8 RP-HPLC assay of cyanidin 3-glucoside (C3G) in fractions of rice bran water extracts

RP-HPLC assay detected C3G only in the original *Japonica* black rice bran water extract. The amount of C3G detected was 0.000353 mg/mL. C3G was not detected in any of the other samples or fractions.

3.2.9 Ferulic acid and phytic acid

3.2.9.1 UV spectroscopy of ferulic acid and phytic acid

UV spectroscopy data showed peaks at around 340 nm for ferulic acid and 270 nm for phytic acid.

3.2.9.2 RP-HPLC assay of ferulic acid phytic acid in rice bran water extracts

The HPLC chromatogram of *Japonica* black (Figure 25), *Japonica* red (Figure 26) and *Indica* red (Figure 27) rice bran water extracts comparison with the chromatograms of ferulic acid and phytic acid chromatograms show that neither ferulic acid nor phytic acid are the main bioactive compounds responsible for rice bran's anti-glycation activity as the corresponding peaks in the samples are very small.

3.2.10 HPLC fractionation of water fractions of rice bran water extracts and their inhibitory effect on formation of fluorescent AGEs

Fractions for further purification and analysis were chosen based on the following conditions.

1. High inhibition of fluorescent AGEs
2. Clean-cut peaks

Fraction #15 and #16 were chosen from the water fraction of *Japonica* black rice bran extract (Figure 28). Fraction #8, #15 and #16 were chosen from the water fraction of *Japonica* red rice bran extract (Figure 29). Fraction #8, #15 and #16 were chosen from the water fraction of *Japonica* black rice bran extract (Figure 30).

3.2.10.1 Purification of HPLC fractions and confirming stability

Unfortunately fraction #8 of *Japonica* red and *Indica* red was found to be unstable after evaporating to dryness and re-dissolving in 30 % ACN. This was found by comparing the retention time of the purified fraction's HPLC chromatogram with its original chromatogram. Fraction #16 of *Japonica* black, *Japonica* red and *Indica* red was also found to be unstable after evaporating to dryness and re-dissolving in 30 % ACN because of the same reason mentioned before. Fraction #15 showed a very small change in retention time but was more stable compared to the other fractions (Figure 31).

Fraction #15 of *Japonica* black and *Japonica* red was not able to inhibit the formation of fluorescent AGEs when measured again to confirm stability. However, fraction #15 of *Indica* red showed an inhibition rate of 10.2 % (Figure 32). *Indica* red rice bran water extract showed an inhibition rate of 48.8 % after being diluted 2.5-fold. Thereby, the percentage contribution by fraction #15 towards the rice bran water extract was calculated at 20.9 %.

3.2.11 Inhibitory effect on formation of pentosidine by fractions of rice bran water extracts

The inhibitory effect by the fractions obtained after fractional purification by Oasis® HLB Plus on the formation of pentosidine is shown in Figure 33. All fractions of *Japonica* black and *Japonica* red rice bran extracts was able to inhibit the formation of pentosidine. Interestingly, in the *Indica* red

rice bran extract, only the 5 % ACN fraction was able to inhibit the formation of pentosidine. The water fraction, 10 % ACN fraction and 75 % ACN fraction was not able to inhibit the formation of pentosidine. This suggests that the bioactive compounds in the 5 % ACN fraction is responsible for the anti-glycation activity of *Indica* red rice bran.

3.2.12 Isolation and identification of glycation inhibitory compounds in 5 % ACN fraction of *Indica* red rice bran water extract

HPLC chromatograms of 5 %, 10 % and 75 % ACN fractions of *Indica* red rice bran water extract were compared and two peaks present only in the 5 % ACN fraction were identified; fraction #10 and fraction #14 (Figure 34). After purification, fraction #10 and #14 was found to be stable as retention times were not changed as shown in Figure 35 and Figure 36.

Fraction #10 showed an inhibition rate of 9.8 % for fluorescent AGEs and fraction #14 showed an inhibition rate of 11.1 % for fluorescent AGEs (Figure 37). *Indica* red rice bran water extract showed an inhibition rate of 48.8 % after being diluted 2.5-fold. Thereby, the percentage contribution by fraction #10 towards the rice bran water extract was calculated at 20.1 % and the percentage contribution by fraction #14 was calculated at 22.8 %.

Fraction #10 showed an inhibition rate of 20.1 % for pentosidine and fraction #14 showed an inhibition rate of 25.2 % for pentosidine (Figure 38). *Indica* red rice bran water extract showed an inhibition rate of 80.6 % after being diluted 2.5-fold. Thereby, the percentage contribution by fraction #10 towards the rice bran water extract was calculated at 25.0 % and the percentage contribution by fraction #14 was calculated at 31.2 %.

UV spectroscopy analysis of fraction #10 showed a band around 250 nm (Figure 39) and fraction #14 showed a band around 310 nm (Figure 40). However, the bands were not clearly visible as there seemed to be impurities or background noise.

From the CE-MS analysis, guanosine was identified in fraction #10 at *m/z* 284.101 and a retention time of 10.02, and glutamic acid was identified in fraction #14 at *m/z* 148.061 and a retention time of 8.74 (Figure 41). Five other unknown compounds were seen in both fractions at *m/z* 141.017,

151.062, 273.069, 202.181, and 316.213 at retention times 2.71, 4.69, 4.69, 7.43, and 8.26 respectively (Table 3).

The C18 HPLC chromatogram of 0.01 mmol/L guanosine showed that it has a retention time very similar to that of fraction #10 confirming the presence of guanosine in fraction #10 (Figure 42). The C18 HPLC chromatogram could not be used to identify the peak of different concentrations of glutamic acid at 230 and 270 nm.

Both glutamic acid and guanosine were able to inhibit fluorescent AGEs at concentrations higher than 5 mmol/L (Figure 43). Both glutamic acid and guanosine were able to inhibit pentosidine (Figure 44), and guanosine showed higher inhibition rates at lower concentrations when compared with fluorescent AGEs inhibition. Although aminoguanidine shows high inhibition of fluorescent AGEs, its inhibition of pentosidine hasn't been established yet so negative values can be observed.

3.2.13 Analysis of samples provided by Toyo Rice Co. Ltd.

3.2.13.1 Inhibitory effect on formation of fluorescent AGEs in samples

As expected brown rice showed the highest inhibition among all samples, followed by dewaxed brown rice. Interestingly, the bran layer showed higher inhibitions rates compared to white rice and SARFR (Figure 45). These results tally with the previous results where bran showed higher inhibition rates than the polished rice (refer section 3.2.5).

3.2.13.2 Inhibitory effect on formation of fluorescent AGEs in fractions of bran layer

The highest inhibition of fluorescent AGEs was seen by the water fraction and the 75 % ACN fraction (Figure 46).

3.2.13.3 Inhibitory effect on formation of pentosidine in fractions of bran layer

Only the 5 % and 75 % ACN fractions inhibited pentosidine but with inhibition rates less than 20 % (Figure 47).

3.2.13.4 Inhibitory effect on formation of glycation intermediates in fractions of bran layer

Only the 75 % ACN fraction inhibited all 3 intermediates. Only the 75 % ACN fraction inhibited GO and MGO but in rates less than 10%. All except the 10 % ACN fraction inhibited 3-DG (Figure 48).

3.3 Chapter 3: Effects on skin by sub-aleurone layer residual rinse-free rice (Kinmemai rice): An open label test

3.3.1 Compliance

Compliance in the SARFR group was 84 %; 2 out of 39 dropped out (dropout rate 5 %) and there was no dropout due to test product intake (Figure 11). The reason for the dropout was due to personal circumstances. In the control group, 0 out of 61 dropped out.

3.3.2 Subjective symptoms

There were no significant findings regarding lifestyle habits (*i.e.*, exercise, sleep, bowel movements, drinking, smoking, stress), and subjective symptoms regarding skin conditions.

3.3.3 Skin index

Table 4 shows the results of the skin index. Regarding the skin age (variation), the value in the SARFR group was significantly larger than that in the control. Regarding the skin age (variation), the SARFR group showed a significantly larger decrease than the control, being more improved ($p = 0.034$, Figure 49. a)). There was no significant difference in women (Figure 49. c)).

There was a significant difference between the groups in the change of wrinkles, which was significantly improved in the SARFR group (Figure 50. a)). A significant difference was observed only in men (Figure 50. b)), but not in women (Figure 50. c)). No significant changes were noted in other items.

3.3.4 Measurement of AGEs

The intensity of skin AGEs fluorescence did not change significantly before and after the test in both groups, and there was no significant difference in the variation between groups (Table 4).

3.3.5 Subclass analysis

Since there are differences in lifestyle habits between boarding house students and home students, subclass analysis was performed separately for both. The number of home students was 28 (20.9 ± 1.5

years) in the SARFR group and 12 (22.0 ± 1.1 years) in the control group. The boarding house students were 8 (21.0 ± 1.6 years old) in the SARFR group and 8 (22.3 ± 1.3 years old) in the control group. In the home students, the SARFR group showed significant improvement in pores, pigmentation (polarized light), total skin health score, and skin age after ingestion (Figure 51). In boarding house students, only ultraviolet (UV) pigmentation was significantly improved in the SARFR group (Figure 52). Overall, the effect on skin quality was more pronounced in the home students.

3.3.6 Safety

There were no adverse events attributable to the test product during the observation period.

3.4 Chapter 4: Effects on skin by dewaxed brown rice: An open label test

3.4.1 Compliance

The compliance of the DBR group was 87.5%, 1 out of 45 patients (dropout rate 2.2%), and no dropouts were caused by ingestion of the test product (Figure 12). There were no dropouts in the control group. The reason for dropping out was due to personal reasons.

3.4.2 Subjective symptoms

No notable findings were found regarding lifestyle-related symptoms (exercise, sleep, bowel movements, drinking, smoking, stress) and subjective symptoms related to skin condition. No adverse symptoms were observed.

3.4.3 Skin index

Table 5 shows the measurement results of the skin index. The rejuvenation in skin age (change amount) was significantly greater in the DBR group than in the control, and was improved in the DBR group ($p = 0.023$, Figure 53. a)). Skin age was significantly improved in the DBR group in women ($p = 0.011$, Figure 53. c)), but not significantly in men (Figure 53. b)).

There was a significant difference in the change rate in wrinkles between the groups, which was significantly improved in the DBR group ($p = 0.039$, Figure 54. a)). No significant difference was observed in males (Figure 54. b)), but in females ($p = 0.049$, Figure 54. c)). Regarding the change rate in porphyrin, there was no significant difference between the groups for men and women (Figure 55. a), b)), but there was a significant improvement for women in the DBR group ($p = 0.044$, Figure 55. c)).

3.4.4 Measurement of AGEs

There was no significant change in skin AGE fluorescence intensity before and after the test in both groups, and there was no significant difference in the amount of change between the groups (Table 5).

3.4.5 Safety

No adverse events thought to be caused by the test product were observed during the observation period.

4. Discussion

4.1 Chapter 1: Inhibition of the production of AGEs by traditional rice water and the correlation with its total phenolic content (TPC)

4.1.1 Research background

In this study, the anti-glycative effect and the total phenolic content of three different rice water preparations using 14 rice samples were studied. The 14 rice samples included two black rice varieties, five red rice varieties, two brown rice varieties, four white rice varieties, and one mixed rice variety. Different pigmented rice varieties were chosen as previous studies have shown various health benefits of pigmented rice compared to white rice. Although white rice makes a major contribution to the calorific intake of most Asian populations and other populations around the world, its nutritional quality is low compared to that of pigmented rice varieties (51). Pigmented rice have been reported as a potent source of phytonutrients including antioxidant compounds, and that they have marked health benefits in preventing diabetic complications as well (23, 52). Pigmented rice contains a range of bioactive compounds including phenolic acids and flavonoids (53). Several epidemiological studies have suggested that a high dietary consumption of polyphenols is associated with a decreased risk of various diseases including cardiovascular disease (54, 55) and neurodegenerative diseases (56). It has been shown that the concentration of phenolic compounds are positively associated with the antioxidant activity and could aid in the prevention of cancer (57-59).

4.1.2 Anti-glycation effect as a new functionality

In this study, rice water, which is a natural, economical and simple product was used as the primary test sample. Rice water is prepared by different methods with different purposes globally, however, it always involves rice grains in contact with water. In order to examine which rice water preparing method would result in rice water with a higher anti-glycative effect, three traditional methods from Sri Lanka were replicated in the lab and used in this study to prepare the rice water. Type 1 was made by boiling, type 2 by oven-drying and boiling, and type 3 by soaking in water. A study conducted

in Japan has shown hair care benefits by rinsing with water obtained from the washing of rice (60), which is quite similar to the type 3 rice water of this study.

The anti-glycative effect of rice water was verified by measuring the inhibitory effect on the formation of fluorescent AGEs using the HSA glycation model (39). Rice water was incubated with HSA and glucose at 60 ºC for 40 hours to evaluate its anti-glycative effect. This glycation reaction is a non-enzymatic one controlled by reaction temperature and time. Previous studies show that the formation of fluorescent AGEs at 60 ºC for 40 hours was equivalent to that obtained from a reaction time of about 60 days at 37 ºC (61). The positive control used when measuring the inhibitory effects of AGEs, aminoguanidine, is an inhibitory agent of the glycation reaction. Aminoguanidine is an AGE formation inhibitor developed with the aim of curing diabetic complications (62). Aminoguanidine blocks the carbonyl group in molecules of the intermediate products of glycative reactions. However, aminoguanidine is not approved as a medical product in the domestic market, because a side effect was recognized in Japan. All 42 rice water samples showed an inhibitory effect on the formation of fluorescent AGEs. This could be due to the transference to the water of several phenolic compounds identified in rice, such as tocopherols, tocotrienols and γ-oryzanol (63).

4.1.3 Relationship with total phenolic content (TPC)

Rice water application has been a popular folk remedy for skin lightening, increasing elasticity of skin, and soothing sun damage. This study verifies the anti-glycative effects of rice water from a viewpoint of skin aging prevention. However, there is a significant difference of inhibition values between the different types of rice water preparations, and between the different varieties of pigmented rice. Type 3 rice water showed higher inhibition values in average compared to type 1 and type 2 rice water. Black and red rice varieties showed higher inhibition values in average compared to brown, mixed, and white rice varieties. This suggests that the pigment, which is located in the aleurone layer of rice contains higher amount of phenolic compounds such as anthocyanins (23). A previous study has shown that the total phenolic content (TPC) was positively correlated to the anti-AGEs formation capacity of different pigmented Thai rice varieties (64). These results also agree with previous studies that have reported pigmented rice having higher phenolic compounds (23, 24).

Also in this study, strong positive correlation results can be seen between the total phenolic content (TPC) and inhibitory effect on the formation of fluorescent AGEs by all three types of rice water. Similar results have been observed in another study that shows a positive correlation of the total phenolic content, antioxidant activities, and anti-AGEs formation capacity of rice bean in China (65). Additionally, similar relationships have been observed in garcinol from *Garcinia indica* fruit rind (8), and in *Camellia sinensis* (66). These results indicate that the inhibition of AGEs formation depend on the total phenolic content. Type 3 rice water showing higher inhibition of fluorescent AGEs could be due to the fact that boiling in water led to the loss of soluble phenolic compounds in type 1 and type 2 rice water. In most rice samples, the type 2 rice water preparation method led to lower inhibition rates. This could be due to the extreme heat applied to the rice over a longer period of time (160 ºC for 30 minutes and 100 ºC for another 30 minutes) when compared to the other two rice water preparing methods. The extreme heat over a longer period of time might have led to a higher loss or degradation of phenolic compounds, thus decreasing the inhibition potential of type 2 rice water. A previous study has shown that cooking of rice led to a significant decrease in inhibitory capacity of angiotensin I-converting enzyme (ACE) accompanied by a relative reduction in the soluble phenolic content (67). These results also agree with another study that shows the total phenolic content was lower in rice water prepared by the boiling process (31). It has also been shown that heat significantly affected the amount of phenolic compounds in other foods (68-70). This could also be the reason for type 3 rice water to show a moderately strong positive correlation with its TPC. It could also be that another factor in addition to the TPC is playing a role in the inhibition of AGEs in type 3 rice water.

4.1.4 Microbial study

It was hypothesized that the higher inhibition by type 3 rice water could be due to the presence of microbes in the rice water in addition to its high amount of phenolic compounds. As previous research has shown that microorganisms in fermented rice are probiotic and beneficial for health (71, 72), the anti-glycation potential of these microbes were investigated. Fermented rice water's said benefits are quite popular in Sri Lanka so the micro flora in a couple of the *Indica* rice samples were chosen as the initial investigation. The rice packages ordered from Sri Lanka were opened directly inside the clean

bench to prevent the exposure to the micro flora in Japan. In this study, none of the isolated colonies (n=32) had the ability to inhibit the formation of AGEs, therefore the microbes in these rice samples were concluded to have no anti glycation activity, and a bioactive compound to be responsible for the anti-glycation activity of rice water. However, the micro flora of rice varies largely depending on the growth conditions, environment, species, location, soil conditions, etc. Thus, there could be other microbes in rice that possess anti-glycation ability. So far this remains unknown therefore future research efforts should be focused on identifying microbes in rice that could potentially inhibit the formation of AGEs.

4.2 Chapter 2: Identification of bioactive compounds in rice responsible for its anti-glycation activity

4.2.1 Initial screening of rice samples

This study is composed of a series of screening steps to identify novel bioactive compounds in rice responsible for its anti-glycation activity. In this study, 16 rice samples were used for the initial screening. The 16 rice samples included two black *Japonica* rice varieties, two red *Japonica* rice, three brown *Japonica* rice varieties, three white *Japonica* rice varieties, three red *Indica* rice varieties, and three white *Indica* rice varieties. Pigmented rice (black and red) showed relatively higher inhibition rates than brown or white rice in the initial screening. These results were also similar to the results observed in the first chapter where pigmented rice varieties showed higher inhibition rates for fluorescent AGEs. Various health beneficial bioactive compounds from pigmented rice were previously reported which include sterols, γ-oryzanol, tocopherols, tocotrienols, and phenolic compounds that are present in the outer layer of grains such as the pericarp and the aleurone (73).

Nutrient content of several pigmented rice varieties have created new interests and reinforced for its utilization in nutraceuticals, and has attracted the food and cosmetic industries. The antioxidant activity and scavenging capacity of anthocyanins in pigmented rice has undoubtedly revealed the potential role in food formulations. More research work related to pigmented rice and its components will further explain the mechanisms about the usefulness of the bioactive compounds and help to understand its various roles such as anti-glycation, antioxidant and free radical scavenging, anti-tumor, anti-atherosclerosis, anti-allergic, anti-influenza, anti-obesity activities against various chronic and degenerative diseases of human. Taking this into consideration along with the high inhibition rates and sample availability, one sample each of *Japonica* black, *Japonica* red, and *Indica* red were chosen for further screening and analysis.

In the initial screening process, samples were screened based on their inhibition rates on the formation of fluorescent AGEs, glycation intermediates (3-DG, GO, MGO), and pentosidine. It has been reported that AGE intermediates such as 3-DG, GO, MGO mediate the AGE generation pathway

(74). Thus, to inhibit the formation of AGEs, intermediates lead to inhibit the AGE formation. AGE formation pathways start with the reaction between reducing sugars and amino groups. After the formation of a Schiff base and Amadori rearrangement, Amadori products undergo subsequent dehydration and rearrangement to form highly reactive dicarbonyl compounds (75) including 3-DG, GO, and MGO. The reactions between these AGE precursors and amino, sulfhydryl and guanidine groups of intracellular and extracellular proteins (76, 77) would result in the formation of stable and irreversible AGEs. Pentosidine has been recognized as a fluorescent protein cross-link from human extracellular matrix, which involves lysine and arginine residues (78). It has been reported that 3-DG, fructose, ascorbate and Amadori compounds act as precursors for the formation of pentosidine (79, 80).

4.2.2 Screening of rice bran

Japonica black, *Japonica* red, and *Indica* red rice samples were separated into its bran and endosperm using a simple rice milling machine in a farming household of an acquaintance. As it was not a very sophisticated machine, the rice was found to be slightly broken and the separation was not very fine. However, the bran was removed to a satisfactory degree as shown in Figure 58. The inhibition of fluorescent AGEs by the bran of all 3 samples was higher than the polished rice (endosperm). This suggests that most of the bioactive compounds or the strongest bioactive compound with anti-glycation potential is present in the bran of pigmented rice. These results tally with the results of the samples received from Toyo Rice Company Ltd. This is important because the rice polishing method we used was less sophisticated compared to the more complex polishing methods used by the company. The results were similar despite the difference in the rice milling or polishing conditions.

To investigate if the bioactive compounds in rice bran are hydrophilic or hydrophobic compounds, fractional purification by Oasis HLB plus was performed, which is a type of reversed-phase chromatography. Oasis HLB Plus Short cartridges contain the Oasis HLB sorbent, which is a universal polymeric reversed-phase sorbent for extraction of a wide range of acidic, basic, and neutral compounds from various matrices using a simple, standard protocol. Since the Oasis HLB sorbent is water wettable, it maintains its capability for higher retention and excellent recoveries even if the sorbent runs dry, which means there is no need to take unusual steps to keep the sorbent

beds from drying out during the critical steps prior to sample loading. According to the company, the 60 µm particle size is more suitable for viscous samples but the bran samples were not too viscous. These cartridges are versatile and can attach easily to pumps and syringes for consistent, positive-pressure flow to speed up the sample processing time. A water fraction, 5 % ACN, 10 % ACN and 75 % ACN was obtained for each bran sample.

Inhibition of fluorescent AGEs was significantly higher by the water fractions suggesting that some of the bioactive compounds responsible for the anti-glycation capacity of rice bran is probably present in the water fraction. An interesting observation was made in the inhibition of pentosidine by the *Indica* red rice bran water extract; only the 5% ACN extract inhibited the formation of pentosidine whereas the other ACN fractions and the water fraction did not inhibit pentosidine formation. This suggests that the 5% ACN fraction is solely responsible for the inhibition of pentosidine of *Indica* red rice bran. Therefore this fraction was analyzed by HPLC to identify and isolate the bioactive compound responsible.

UV spectroscopy of the bran water extracts and their fractions showed bands at around 270 nm, 340 nm, and 500 nm. Common compounds found in rice with these wavelengths are ferulic acid, phytic acid, and anthocyanins (32, 81, 82). RP-HPLC assay detected ferulic acid, phytic acid, and cyanidin-3-glucoside only in very small amounts in the samples suggesting that these compound are not responsible for rice bran's anti-glycation activity.

A stable fraction with a retention time of 15 minutes (fraction #15) was purified from the water fraction of *Indica* red rice bran water extract. Two stable fractions (fraction #10 and fraction #14) with retention times of 10 and 14 minutes respectively were purified from the 5% ACN fraction of *Indica* red rice bran extract. Fraction #15 only inhibited fluorescent AGEs whereas fraction #10 and #14 had the ability to inhibit both pentosidine and fluorescent AGEs and had a combined percentage contribution rate greater than 50 %. Thus, fraction #10 and #14 was chosen for further analysis by UV spectroscopy and mass spectroscopy.

UV spectroscopy data showed a band at around 250 nm for fraction #10 and at around 310 nm for fraction #14. This suggests that the possible structure of the compounds in these fractions are flavonoids. The UV spectrum of flavonoids typically shows a band at 210-290 nm region (band I), which is due to the absorption of the benzoyl system (A-ring) and a second band in the 300-400 nm region (band II), which is associated with the cinnamoyl system (rings B and C) as shown in Figure 59 (83).

Capillary Electrophoresis-Mass Spectrometry (CE-MS) was chosen as the analytical method to analyze fraction #10 and fraction #14. Most metabolites are hydrophilic and ionic small molecules. CE-MS is an analytical technique that combines capillary electrophoresis (CE) and mass spectrometry (MS), which is suitable for measuring such metabolites. This method was also the most suitable analytical method in terms of time and cost. The CE-MS analysis data identified glutamic acid in fraction #14 and guanosine in fraction #10. Five other compounds were detected in both fractions #10 and #14, but could not be identified possibly due to low purity or low concentration of samples.

4.2.3 Glutamic acid and guanosine

Glutamic acid ($C_5H_9NO_4$) is an α-amino acid that is used by most living beings in the biosynthesis of proteins. It is a non-essential amino acid in humans, and it is also the most abundant excitatory neurotransmitter in the vertebrate nervous system. Glutamic acid also functions as the precursor for the synthesis of the inhibitory gamma-aminobutyric acid (GABA) (84). Glutamic acid is present in foods that contain protein, however it can only be tasted when it is present in an unbound form. Significant amounts of free glutamic acid are present in a wide variety of foods, including cheeses and soy sauce. Glutamic acid is also responsible for umami, which is one of the five basic tastes of the human sense of taste. Glutamic acid is often used as a food additive and flavor enhancer as monosodium glutamate (MSG), which is its sodium salt (85). Meat, fish, poultry, eggs, dairy products, and kombu are great sources of glutamic acid. Some protein-rich plant foods are also sources of glutamic acid.

Amino acids are known to be therapeutic agents in conditions such as respiratory physiology, cardiology, renal failure, neurological disorders, and congenital defects (86). Some of the regulatory

roles of amino acids comprise of gene expression, nutrient metabolism and oxidative defense, synthesis and secretion of hormones, intracellular protein turnover, immune function and others (87). In terms of glycation, free amino acids have been found to mitigate the glycation of lens protein, delay progression of cataract and also bring down blood sugar levels in diabetic rats. Some amino acids inhibit or reduce glycation by impeding the binding of glucose to proteins by competitive inhibition, thus offering protection, while some amino acids influence pathological pathways that results in increased tissue sensitivity towards insulin (88).

Guanosine ($C_{10}H_{13}N_5O_5$) is a purine nucleoside consisting of guanine attached to a ribose (ribofuranose) ring via a β-N9-glycosidic bond. It can be phosphorylated to become guanosine monophosphate (GMP), cyclic guanosine monophosphate (cGMP), guanosine diphosphate (GDP), and guanosine triphosphate (GTP) (89). These forms play important roles in many biochemical processes such as synthesis of nucleic acids and proteins, muscle contraction, photosynthesis, and intracellular signal transduction (cGMP). Guanosine can be found in pancreas, coffee plant, clover, and pollen of pines. Some other foods high in guanosine are elderberry, malus (crab apple), acerola, and arrowhead.

Guanosine is thought to have neuroprotective properties. It is released in the brain under physiological conditions, especially during pathological events. Thereby it reduces neuroinflammation, oxidative stress, and excitotoxicity. It also exerts trophic effects in neuronal and glial cells. Guanosine was also found to be protective in several in vitro and/or in vivo experimental models of several central nervous system (CNS) diseases such as ischemic stroke, Parkinson's disease, Alzheimer's disease, spinal cord injury, and depression (89). There is not much research related to the anti-glycation potential of guanosine. However, a study conducted in Brazil observed age-dependent changes in glutamate uptake, glutamine synthetase (GS) activity, the glutathione (GSH) system, pro-inflammatory cytokine (tumor necrosis factorα (TNF-α) and interleukin 1β(IL-1β)) release, and the transcriptional activity of nuclear factor kB (NFkB) were prevented by guanosine in an HO-1-dependent manner. This suggests guanosine to be a favorable therapeutic agent that could provide glioprotection during the aging process (90). In contrast, a major product found in reaction mixtures of guanosine and glucose is N^2-[1-(1-carboxy) ethyl] guanosine (CEG), has been used as a marker of advanced glycation of DNA in some

studies (91, 92). As the findings from the current study confirm the anti-glycation potential of guanosine in terms of the inhibition of fluorescent AGEs and pentosidine, future research should focus on investigating the mechanism of the anti-glycation potential of guanosine.

4.3 Chapter 3: Effects on skin by sub-aleurone layer residual rinse-free rice (Kinmemai rice): An open label test

4.3.1 Pros and cons of brown rice

This study compared the changes in skin condition and skin AGEs between two groups of students who took SARFR as a test product once a day for one month and ordinary polished rice. SARFR was processed so as to be easy to eat by removing only the epidermal wax layer and the outer layer containing a large amount of indigestible fiber from brown rice to leave the nutrients. The reported effects of brown rice include suppression of elevated blood triglyceride or total cholesterol (93, 94), reduction of postprandial blood glucose (95), improvement of fasting blood glucose (96), reduction of HbA1c (97), prevention of bone density reduction in the elderly (98), visceral fat reduction in persons with metabolic syndrome (99), increased bone density (98), and improved vascular endothelial function (100).

It is not easy to prove because human intestinal flora varies greatly among individuals (101), however it has been pointed out that brown rice intake may have a positive effect on intestinal flora. When the effect of brown rice on human intestinal flora was examined, a significant increase in the number of *Bifidobacterium adolescentis* and *Enterococcus faecalis*, a significant decrease in the total number of bacteria, *Bacteroides*, *Eubacterium aerofaciens*, and *Escherichia coli* were observed by individuals ingesting brown rice (102). Also, decreased detection rates of *Clostridium paraputrificum* and *Clostridium perfringens* were observed during brown rice intake. It has also been reported that brown rice eaters with a high subjective health have a high occupancy rate of butyric acid-producing bacteria, such as *Faecalibacterium prausnitzii*, *Roseburia* genus, *Bilophila* genus (103).

However, it cannot be said that a brown rice diet is widely popular, although it is expected to be effective in preventing and improving lifestyle-related diseases. The reasons are that the taste and texture are different from that of polished rice, the cooking time is long, the digestion or absorption is poor due to the contained cellulose, digestion is poor, and it can cause abnormal intestinal fermentation. Anxiety about iron deficiency anemia due to the chelating effect of phytic acid has also been pointed

out, but it is not a problem if a normal amount of brown rice is eaten. It was also said that some people were worried about pesticide residue on brown rice. Although it was detected in some brands of brown rice in the past, there is almost no problem nowadays with local and/or organic products. The biggest problem is probably the difficulty of digestion and absorption. To solve this problem, easy-to-eat processed products have been developed, including pre-germinated brown rice also known as “hatsuga genmai” (95), surface-treated brown rice (104), ultra-high hydrostatic pressurizing brown rice (98), and SARFR. Also the use of brown rice with a reduced mixing ratio such as 50% barley and brown rice is recommended (99). Alternatively, brown rice extract is used as a supplement.

4.3.2 Research background

Typical functional ingredients of brown rice are dietary fiber and lipophilic ingredients such as ferulic acid and γ-oryzanol. When γ-oryzanol is given to animal fat-fed mice, it has been shown to relieve endoplasmic reticulum stress in the hypothalamus and attenuate the animal fat preference (105). Furthermore, by the epigenomic mechanism, γ-oryzanol acts to restore the decreased expression of dopamine receptors in the brain's reward system (ventral striatum) which recognizes the pleasure and satisfaction of eating mitigates, thus reducing the animal fat preference, followed by exhibiting the effect of transforming an "unsatisfied brain" into a "satisfied brain” (106). As a result, it eliminates animal fat dependence, improves hyperglycemia, and ameliorates metabolic syndrome (107). Furthermore, for the triterpene alcohol and sterol preparation (TASP) contained in the oil-soluble fraction in rice bran, there is evidence that TASP suppresses the increase in postprandial blood concentration of GIP (glucose-dependent insulinotropic polypeptide), a digestive tract hormone, and that it inhibits translocation of SGLT1 (glucose transporter sodium-dependent glucose transporter (33) to the cell membrane (108). As an overall result of these actions, it is considered that glycative stress is reduced.

To date, there has been only one clinical trial report for SARFR. In that study, 25 people (6 men, 19 women) who stayed in an elderly facility participated and ingested rinse-free rice for two months for every meal (control group), followed by intake of SARFR for 4 months (test group) in a non-crossing manner (109). The results showed significant reduction of systolic blood pressure ($p =$

0.008) and a tendency to decrease diastolic blood pressure (p = 0.079), body fat percentage (p = 0.064), and HbA1c (p = 0.050) in the SARFR group. There is a case report on the relationship between meal and mileage during the 118 days when Mr. Hazama Kanpei, a comedian, ran 50 km a day for 118 days (110). The entire process consisted of 15 cycles, with a basic rule of running for five days and resting for one day. The breakfast on the day of running was 33 days of Japanese food and 66 days of Western food, and the dinner on the previous day was 30 days of Japanese food, 25 days of Western food, and 23 days of Chinese food. During the process, SARFR was served for Japanese food. The results of mileage and running time for each cycle show that the days when SARFR was ingested in the morning and afternoon, the running time was extended by 20 minutes, the mileage was 5.8 km longer, and the average speed was 0.84 km/day faster than that of the Western food diet. On the day he consumed SARFR for breakfast, the running time and average speed were significantly longer, the mileage being 51.4 km for Japanese food and 45.6 km for Western food ($p < 0.05$).

There are several conference presentations on SARFR. It has been pointed out that the GI value of SARFR is lower than that of common white rice (111), and this may be involved in the downward trend of HbA1c observed in the previous report of Kyo H. *et al.* (109). It is also reported that angiotensin intracellular signal transduction in the vascular smooth muscle was suppressed (112), and it may be involved in the hypotensive effect seen in the report of Kyo H *et al*. (109). In addition, the possibility of activation of immune function (111), health promotion (113), and constipation amelioration (114) have been reported.

4.3.3 SARFR as an alternative to brown rice

In young subjects in this study, some positive effects of SARFR intake were confirmed. First is the high level of compliance and no dropout due to the test food intake. If brown rice intake had been imposed on subjects, they usually would not provide such high compliance (115, 116). This reflects that there is no problem in the taste and flavor of the test product, nor with digestive system symptoms due to indigestion and malabsorption. Rather, positive effects such as improved bowel movements were seen. The second is the effect of improving skin function. In general, young subjects in their early twenties have a slight decrease in skin function and are better than middle-aged subjects. Nonetheless,

it is significant that the test product intake showed a significant improvement effect on the skin age as compared with the control. In contrast, there was no significant change in skin AGEs fluorescence, its reason may be that the pre-value was close to the normal range and there was little room for improvement in young subjects.

Most skin function problems are said to be due to photo aging, which is caused by oxidative stress due to ultraviolet (UV) exposure. Next is the influence of glycative stress. This involves yellowing due to AGEs deposition on the skin and a decrease in skin elasticity due to the formation of glycated cross-links of collagen protein (117). Also, it is associated with a decline in moisturizing function due to glycation and reduced production of filaggrin, a natural moisturizing factor (NMF) (118, 119). Additionally AGEs act on pigment cells (melanocytes) to enhance melanin production, thus inducing spot formation (120). It is speculated that these symptoms were alleviated as a result of reducing glycative stress by ingesting the test product (Figure 56).

Regarding gender differences, almost no effect of SARFR was observed in women, but differences were observed in men. The reason may be that the skin of women in their early twenties was in almost normal health, thus being no room for further improvement. Comparing boarders and home students, it has been reported that boarders have a lower nutritional satisfaction rate and especially a lower protein intake (121, 122). Also, boarders have a larger amount of drinking (123), a higher rate of skipped breakfast (124), and more men with abnormal taste sense (125). The elevated glycative stress due to disturbance of nutritional balance was more remarkable in male boarders, those who in this study might be in a similar situation.

4.3.4 Safety of SARFR

Brown rice, SARFR, and polished rice are basically staple foods that have been used in meals for many years, and can therefore be considered to have sufficient food safety. However, the problems of indigestion, abnormal intestinal fermentation, and residual pesticides have been pointed out regarding brown rice intake. The reason for this is based on the insufficient expansion due to hindered water absorption by the wax layer located on the surface of brown rice during steam cooking. SARFR is made

by removing the wax layer and bran layer from brown rice by special processing, its color looks almost the same as that of polished rice (conventional white rice) and its taste reserved, resulting in the improvement of problems that are present in brown rice. Furthermore, it has been reported that SARFR contains almost no anti-mutagenic substance (126). There are no reports of adverse events related to SARFR in this and past clinical trials. It was judged that there was no problem regarding the safety of SARFR.

4.4 Chapter 4: Effects on skin by dewaxed brown rice: An open label test

4.4.1 Historical background

Whole-grain brown rice contains a variety of nutrients. When brown rice was the major staple food in Japan, vitamins such as vitamin B1, could be supplemented, however, during the Genroku era (1688-1704), beriberi became popular in Edo, where polished rice was prevalent. This caused what was called "Edo illness." Later, from the Meiji era (1868-1912) to the Taisho era (1912-1926), beriberi developed in so many people that it was said to be one of the two major national diseases along with tuberculosis. In the Showa era (1926-1989), when the "Funpu Rice Milling Method" was developed in 1955, over-polished rice began to spread, beriberi and other illnesses had then begun to be seen mainly among young people with unbalanced diets and persons with chronic diseases. This method (Funpu meaning blasts) is a modification of the conventional friction type rice milling machine, which uses a high pressure inside the machine (200 to 300 g/cm2) to increase the frictional force between rice particles and polishes them, followed by blowing off the adhered bran and residues with a blast (30 m/sec or more).

4.4.2 Functional ingredients in brown rice

Although brown rice contains various ingredients and is clearly superior to polished rice in terms of nutrition, it is difficult to cook, has a taste and texture different from that of polished rice, and is difficult to digest. Brown rice has moved away from its main staple food position. Thus, we conducted a clinical trial for university students using DBR, which was developed to reduce the disadvantageous characteristics of brown rice and obtain its advantages. In the group (43 subjects) who took 150 g of DBR or more per day for 1 month, intake compliance was maintained as high as 87.5%, no adverse events were observed, and skin age, an index of skin condition, was improved significantly compared with the control (22 subjects). These actions are due to the functional ingredients contained in brown rice. Brown rice contains B vitamins such as vitamins B1, B2, B6, nicotinic acid, patent acid, inositol, choline, folic acid and other components that are described below.

4.4.2.1 γ-Oryzanol

γ-Oryzanol is a combination of ferulic acid and food sterols and is the main component of rice bran oil. Excessive intake of animal fat causes ER stress in pancreatic β cells and nerve cells in the hypothalamus, and γ-oryzanol has the effect of relieving these ER stresses (107). As a result, it exerts a central effect that helps improve tension, anxiety, depression, and withdrawal from animal fat dependence. In the pancreas, it enhances β-cell glucose-responsive insulin secretion and reduces α-cell glucagon hyper secretion. These actions lead to the reduction of glycation stress. It is known that the intake of brown rice diet improves animal fat addiction, and it is presumed that γ-oryzanol plays an important role in this (127). Supplements containing γ-oryzanol have also been developed (128).

Brown rice may contain unknown functional ingredients. It has been reported that the triterpene alcohol and sterol fractions (TASP) contained in the oil-soluble fractions in rice bran have an effect of ameliorating postprandial hyperglycemia (129) and an effect of suppressing high-fat diet-dependent obesity (130). As its mechanism of action, it is supposed that TASP suppresses the increase in postprandial blood concentration of glucose-dependent insulinotropic polypeptide (GIP), a gastrointestinal hormone, and interferes with the translocation of sodium-dependent glucose transporter 1 (SGLT1), a glucose transporter, on the cell membrane (108).

4.4.2.2 Ferulic acid

Ferulic acid has strong antioxidant power and weak cytotoxicity (131). It is experimentally known to have an effect of reducing amyloid β oligomers, and is expected to have an anti-dementia effect in humans (132, 133).

4.4.2.3 Dietary fiber

Rice bran and germ are rich in dietary fiber. It is an essential substance for "healthy intestinal-brain correlation" that contributes to the healthy growth of the intestinal flora and the enhancement of brain mental function. It is an essential substance for the healthy growth of the intestinal flora and the homeostasis of the "gut-brain axis" that contributes to the enhancement of brain mental function. Dietary fiber acts as a probiotic to promote the growth of beneficial bacteria in the intestinal flora, and

increases, in particular, the number of bacteria that produce short-chain fatty acids, i.e., acetic acid, butyric acid, and propionic acid. Short-chain fatty acids promote body temperature elevation, heart rate increase, lipolysis in adipose tissue via specific receptors (i.e., GRP41), and have the effect of increasing basal metabolism when overdosed (134). Dietary fiber has the effect of suppressing postprandial hyperglycemia (135). Both actions can be expected to have the effect of reducing glycative stress

GABA or gamma-Aminobutyric acid is contained in a trace amount in brown rice and acts as an inhibitory neurotransmitter in the body. It is related to long-term object recognition memory and working memory, and it has been reported that it has an effect of reducing mental and physical stress in animal experiments (136, 137) and an effect of improving memory deterioration after head injury (137).

4.4.2.4 Phytic acid

Phytic acid, also called inositol 6-phosphate, has an antioxidative effect, and is known to suppress the increase in plasma uric acid levels, prevent renal stone formation, and reduce serum cholesterol (138). In addition, it has been pointed out that it may reduce the risk of carcinogenesis (139). Although phytic acid has a chelating effect, the content of brown rice does not deplete the useful minerals.

4.4.2.5 Lipopolysaccharide (LPS)

Brown rice contains LPS. This is a component common to the surface membranes of *Escherichia coli* and *Salmonella*, however, LPS is not pathogenic. When a small amount of LPS enters the intestinal tract together with brown rice, the bowel movement becomes active and activates macrophages having a bacterial phagocytosis (140). LPS is known to increase the secretion of mucus and bactericidal substances from the intestinal mucosa. As a result, innate immunity is strengthened. Also, LPS prevents the onset of allergic diseases such as pollinosis and reduces the symptoms (141). It has been reported that it stimulates microglial cells to stimulate the phagocytosis of amyloid β in the brain (142).

4.4.3 The mechanism and components involved in improving skin condition

In general, skin condition changes due to aging and various environmental factors. Among these factors, the effect of ultra violet (UV) exposure is the largest. This is called photo aging, and the main mechanism is oxidative stress (117). As menopause approaches, complaints of skin problems increase as estrogen secretion decreases. However, since the subjects in this study are young people, the effect of these factors is small. It is presumed that the effect of glycative stress is greater than these. When collagen and elastin that make up the skin are denatured by glycative stress, elasticity is reduced, thus causing the sagging of skin. Accumulation of AGEs causes yellowing in skin. Glycation of keratin and filaggrin (a natural moisture factor) increases transepidermal water loss (TEWL) and reduces the moisturizing function. A decrease in filaggrin promotes the breakdown of the skin barrier and increases the risk of developing atopic dermatitis (143).

Effects known for brown rice, including processed brown rice, include the amelioration of triglyceridenia and total cholesterolemia (94, 144), improvement of postprandial hyperglycemia (95, 129), reduction of fasting blood glucose (96) and HbA1c (97, 107) and reduction of visceral fat in metabolic syndrome (99), which are all actions that reduce glycative stress. Dietary fiber is known to have an improving effect on the intestinal flora (102), and promotes basal metabolism via elevated production of short-chain fatty acids (134), thus contributing to the reduction of glycative stress. The restoration of the intestinal flora leads to the improvement of skin condition via improvement of bowel movement (145). In addition, for photo aging (117), which is a major cause of skin disorders, antioxidants such as ferulic acid reduce oxidative stress associated with UV exposure. As a result of these mechanisms acting in an integrated manner, it is possible that they had a favorable effect on skin condition (Figure 57).

4.4.4 The effect of antioxidant ferulic acid and dietary fiber that regulates the intestinal environment

There are also some reports on the effects of DBR. It contains about 100 times more LPS than polished rice, and LPS activates macrophages mainly via TLR4 (36). LPS is derived from symbiotic

bacteria and is also abundant in the bran layer. Animal experiments have shown that ingestion of DBR powder into antibiotic-induced constipation model mice significantly suppressed weight loss and improved bowel movements compared to control mice to which antibiotics alone were administered (146). In pollinosis model mice, an anti-allergic effect was observed by the supplemented feed of DBR subglue powder layer extract as compared with the control (mice administered with no additive feed) (141). In a clinical trial in humans, the cognitive ability evaluation scale, HDS-R (Revised Hasegawa's Dementia Scale), improved as a result of ingesting DBR for six months in the elderly, where an association was found between DBR intake and the scale scores (147). Brown rice, which is rich in LPS, is expected to be useful as a staple food that helps maintain good health.

One of the causes of skin disorders is allergic diseases. Adult atopic dermatitis has been on the rise in the last 30 years, it is said that it has finally reached a plateau in recent years. There are many cases with a latent predisposition to easily produce the IgE antibody, and it is expected that it will affect the skin condition of young people in particular. It is possible that the allergic symptom-reducing effect of LPS in brown rice (141) had a positive effect on this problem. The details of the action mechanism of LPS for allergy relief remains unclear. Further research is needed in the future.

4.4.5 Comparison of DBR and SARFR

The test product (DBR) is processed from brown rice to be easy to eat, leaving nutrients by removing only the wax layer of the epidermis and the outer layer containing a large amount of indigestible fiber. In SARFR, the wax layer and part of the bran layer are removed, while in DBR only the wax layer is removed, which is closer to brown rice and rich in nutrients. Since dietary fiber is contained in a slightly large amount, the effect varies from person to person, and some people can accept the positive effect, while others may exhibit discomfort due to dietary fiber.

Comparing the previous report using SARFR (49) with this study, there was no difference in intake compliance between DBR and SARFR, and there was no safety problem in either case. Furthermore, both showed improvement in skin condition (skin age as an index), and there was no difference in the magnitude of the effect between the two. The feeding period was one month in this

study. If DBR was taken for a longer period of several years or more, it might be more effective. When individuals want to start a processed brown rice diet, they should choose DBR or SARFR according to their taste and physical condition.

4.4.6 Safety of DBR

Brown rice, DBR, and polished rice are basically staple foods. Therefore, they have abundant food uses and are fully guaranteed for food safety. There was a time when pesticide residues in brown rice were regarded as a problem, but all recent test results are within the standard values (148, 149). Even if a small amount of foreign matter adheres to the outer layer, it will be scraped off by special rice milling, so no problem will occur. No adverse events related to DBR have been reported in this study or in past clinical studies (145). After all, it was judged that there was no problem with the safety of DBR.

Abscisic acid is known as a phytohormone that regulates plant growth and physiological activity, and is present in all plants including brown rice. It has an anti-inflammatory effect and is a food ingredient expected for impaired glucose tolerance and inflammatory bowel disease (150, 151). In a report (152) that integrated information and evaluated the safety of the ingestion of brown rice, indicating that the harm event was not reported, it is considered that danger is hardly a problem and the health effect is greater.

5. Conclusion

AGEs can be formed both inside the human body through normal metabolism and aging, and in food by the heating of sugars with lipids or proteins. In either way, AGE accumulation *in vivo* has been found to be closely related to the pathogenesis of diabetic complications, atherosclerosis, osteoporosis, dementia, and infertility. Therefore, there is a need for natural, low toxic AGE inhibitors to be developed.

This study is the first report on a correlation between the inhibition of AGEs formation and the total phenolic content (TPC) in rice water of various pigmented and non-pigmented *Japonica* and *Indica* varieties. This study shows that rice water has an anti-glycation effect (AGE production inhibitory effect) as a novel function. This study also shows that rice water produced using pigmented rice varieties have a higher inhibitory effect against fluorescent AGE formation, and that there is a strong positive correlation between the inhibitory effect and the total phenolic content of rice water.

Our findings suggest that rice water extracts have a strong inhibitory efficacy against various AGEs such as fluorescent AGEs, and pentosidine, and also glycation intermediate such as 3-DG, GO and MGO. We have also found that the bran layer of rice is mainly responsible for its anti-glycation activity. However, there are many unknown bioactive compounds in bran with anti-glycation potential. In this study, two stable fractions from *Indica* red rice bran water extract were purified and analyzed by CE-MS. Two compounds; glutamic acid and guanosine, were identified as compounds with anti-glycation potential in *Indica* red rice bran.

The clinical data suggests that DBR or SARFR when compared with polished rice, contributes to health promotion, including skin condition, by reducing the indigestibility of brown rice and ensuring nutrition, which facilitates continuous intake.

References

1. Viña J, Borrás C, Miquel J. Theories of ageing. IUBMB Life. 2007;59(4):249-54.

2. Zhang Q, Ames JM, Smith RD, Baynes JW, Metz TO. A Perspective on the Maillard Reaction and the Analysis of Protein Glycation by Mass Spectrometry: Probing the Pathogenesis of Chronic Disease. Journal of Proteome Research. 2009;8(2):754-69.

3. Maillard LC. Action des acides amines sur les sucres : Formation des melanoidines par voie Methodique. C R Acad Sci (Paris). 1912;154:66-8.

4. Hodge JE. Dehydrated Foods, Chemistry of Browning Reactions in Model Systems. Journal of Agricultural and Food Chemistry. 1953;1(15):928-43.

5. Nagai R, Mori T, Yamamoto Y, Kaji Y, Yonei Y. Significance of Advanced Glycation End Products in Aging-Related Disease. ANTI-AGING MEDICINE. 2010;7(10):112-9.

6. Rowan S, Bejarano E, Taylor A. Mechanistic targeting of advanced glycation end-products in age-related diseases. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2018;1864(12):3631-43.

7. Williams ME. Clinical studies of advanced glycation end product inhibitors and diabetic kidney disease. Current Diabetes Reports. 2004;4(6):441-6.

8. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and Anti-Glycation Activity of Garcinol fromGarcinia indicaFruit Rind. Journal of Agricultural and Food Chemistry. 2000;48(2):180-5.

9. Lee GY, Jang DS, Lee YM, Kim JM, Kim JS. Naphthopyrone glucosides from the seeds ofCassia tora with inhibitory activity on advanced glycation end products (AGEs) formation. Archives of Pharmacal Research. 2006;29(7):587-90.

10. Ma H, Johnson S, Liu W, Dasilva N, Meschwitz S, Dain J, et al. Evaluation of Polyphenol Anthocyanin-Enriched Extracts of Blackberry, Black Raspberry, Blueberry, Cranberry, Red Raspberry, and Strawberry for Free Radical Scavenging, Reactive Carbonyl Species Trapping, Anti-Glycation, Anti-β-Amyloid Aggregation, and Mic. International Journal of Molecular Sciences. 2018;19(2):461.

11. Liu W, Wei Z, Ma H, Cai A, Liu Y, Sun J, et al. Anti-glycation and anti-oxidative effects of a phenolic-enriched maple syrup extract and its protective effects on normal human colon cells. Food & Function. 2017;8(2):757-66.

12. Ahmed N. Advanced glycation endproducts—role in pathology of diabetic complications. Diabetes Research and Clinical Practice. 2005;67(1):3-21.

13. Thorpe SR, Baynes JW. Maillard reaction products in tissue proteins: New products and new perspectives. Amino Acids. 2003;25(3-4):275-81.

14. Paul RG, Bailey AJ. Glycation of collagen: the basis of its central role in the late complications of ageing and diabetes. The International Journal of Biochemistry & Cell Biology. 1996;28(12):1297-310.

15. Masayuki Y, Yoshikazu Y. Glycative stress and anti-aging: 2. The Evaluation of Glycative Stress: Measurement of blood glucose, glycated proteins and intermediates. Glycative Stress Research. 2016;3(4):205-9.

16. Perrone A, Giovino A, Benny J, Martinelli F. Advanced Glycation End Products (AGEs): Biochemistry, Signaling, Analytical Methods, and Epigenetic Effects. Oxidative Medicine and Cellular Longevity. 2020;2020:1-18.

17. Fleming TH, Humpert PM, Nawroth PP, Bierhaus A. Reactive Metabolites and AGE/RAGE-Mediated Cellular Dysfunction Affect the Aging Process – A Mini-Review. Gerontology. 2010.

18. Cerami C, Founds H, Nicholl I, Mitsuhashi T, Giordano D, Vanpatten S, et al. Tobacco smoke is a source of toxic reactive glycation products. Proceedings of the National Academy of Sciences. 1997;94(25):13915-20.

19. Leslie RD, Beyan H, Sawtell P, Boehm BO, Spector TD, Snieder H. Level of an advanced glycated end product is genetically determined: a study of normal twins. Diabetes. 2003;52(9):2441-4.

20. Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S. Genetic structure and diversity in Oryza sativa L. Genetics. 2005;169(3):1631-8.

21. Binic I, Lazarevic V, Ljubenovic M, Mojsa J, Sokolovic D. Skin ageing: natural weapons and strategies. Evid Based Complement Alternat Med. 2013;2013:827248-.

22. Cozzano Ferreira S, Fernández AM, del Castillo Bilbao MD, Medrano Fernández A. New Functional Ingredients From Agroindustrial By-Products for the Development of Healthy Foods. In: Ferranti P, Berry EM, Anderson JR, editors. Encyclopedia of Food Security and Sustainability. Oxford: Elsevier; 2019. p. 351-9.

23. Yawadio R, Tanimori S, Morita N. Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chemistry. 2007;101(4):1616-25.

24. Itani T, Tatemoto H, Okamoto M, Fujii K, Muto N. A Comparative Study on Antioxidative Activity and Polyphenol Content of Colored Kernel Rice. NIPPON SHOKUHIN KAGAKU KOGAKU KAISHI. 2002;49(8):540-3.

25. Verma DK, Srivastav PP. Bioactive compounds of rice (Oryza sativa L.): Review on paradigm and its potential benefit in human health. Trends in Food Science & Technology. 2020;97:355-65.

26. Pal YP, Pratap AP. Rice Bran Oil: A Versatile Source for Edible and Industrial Applications. Journal of Oleo Science. 2017;66(6):551-6.

27. Lerma-García MJ, Herrero-Martínez JM, Simó-Alfonso EF, Mendonça CRB, Ramis-Ramos G. Composition, industrial processing and applications of rice bran γ-oryzanol. Food Chemistry. 2009;115(2):389-404.

28. Choi J-S, Park JB, Moon W-S, Moon J-N, Son SW, Kim M-R. Safety and Efficacy of Rice Bran Supercritical CO₂ Extract for Hair Growth in Androgenic Alopecia: A 16-Week Double-Blind Randomized Controlled Trial. Biological & Pharmaceutical Bulletin. 2015;38(12):1856-63.

29. Kanlayavattanakul M, Lourith N, Chaikul P. Jasmine rice panicle: A safe and efficient natural ingredient for skin aging treatments. Journal of Ethnopharmacology. 2016;193:607-16.

30. De Paepe K, Hachem JP, Vanpee E, Roseeuw D, Rogiers V. Effect of rice starch as a bath additive on the barrier function of healthy but SLS-damaged skin and skin of atopic patients. Acta Derm Venereol. 2002;82(3):184-6.

31. Marto J, Neves Â, Gonçalves L, Pinto P, Almeida C, Simões S. Rice Water: A Traditional Ingredient with Anti-Aging Efficacy. Cosmetics. 2018;5(2):26.

32. Samyor D, Das AB, Deka SC. Pigmented rice a potential source of bioactive compounds: a review. International Journal of Food Science & Technology. 2017;52(5):1073-81.

33. Sugihiro T, Yoneda M, Ohno H, Oki K, Hattori N. Associations of nutrient intakes with obesity and diabetes mellitus in the longitudinal medical surveys of Japanese Americans. J Diabetes Investig. 2019;10(5):1229-36.

34. Nakano K, Takahashi T, Tsunoda A, Matsui H, Shimizu Y. Dietary trends in patients with fecal incontinence compared with the National Health and Nutrition Survey. Journal of the Anus, Rectum and Colon. 2019;3(2):69-72.

35. Malik VS, Sudha V, Wedick NM, Ramyabai M, Vijayalakshmi P, Lakshmipriya N, et al. Substituting brown rice for white rice on diabetes risk factors in India: a randomised controlled trial. British Journal of Nutrition. 2019;121(12):1389-97.

36. Inagawa H, Saika T, Nisizawa T, Kohchi C, Uenobe M, Soma G-i. Dewaxed Brown Rice Contains a Significant Amount of Lipopolysaccharide Pointing to Macrophage Activation via TLRs. Anticancer research. 2016;36 7:3599-605.

37. Improvement Effect of Dewaxed Brown Rice on Constipation in Antibiotic-treated Mice. In Vivo. 2017;31(4):573-7.

38. Inagawa H, Saika T, Nishiyama N, Nisizawa T, Kohchi C, Uenobe M, et al. Dewaxed Brown Rice Feed Improves Fatty Liver in Obese and Diabetic Model Mice. Anticancer Res. 2018;38(7):4339-45.

39. Ishioka Y, Yagi M, Ogura M, Yonei Y. Antiglycation effect of various vegetables: Inhibition of advanced glycation end product formation in glucose and human serum albumin reaction system. Glycative Stress Research. 2015;2:22-34.

40. Hammes HP, Martin S, Federlin K, Geisen K, Brownlee M. Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. Proceedings of the National Academy of Sciences. 1991;88(24):11555-8.

41. Stevanato R, Fabris S, Momo F. New Enzymatic Method for the Determination of Total Phenolic Content in Tea and Wine. Journal of Agricultural and Food Chemistry. 2004;52(20):6287-93.

42. Yamada H, Miyata S, Igaki N, Yatabe H, Miyauchi Y, Ohara T, et al. Increase in 3-deoxyglucosone levels in diabetic rat plasma. Specific in vivo determination of intermediate in advanced Maillard reaction. Journal of Biological Chemistry. 1994;269(32):20275-80.

43. Yamaguchi T, Takabe W, Yagi M, Yonei Y. Analysis of anti-glycative components in Smallanthus sonchifolius (yacon)

Glycative Stress Research. 2018;5(1):36-44.

44. Yagi M, Isshiki K, Takabe W, Ishizaki K, Yonei Y. Measurement of pentosidine in human plasma by the high performance liquid chromatography. Glycative Stress Research. 2018;5(2):119-28.

45. Yagi M, Inoue K, Sato Y, Ishizaki K, Sakiyama C, Yonei Y. Antiglycative effect of black galangal, Kaempferia parviflora Wall. Ex. Baker (Zingiberaceae) Glycative Stress Research. 2021;8(1):1-7.

46. Sakiyama C, Yagi M, Miyazaki S, Sato A, Ishizaki K, Yonei Y. A rapid pretreatment method for the determination of pentosidine in human plasma by high performance liquid chromatography. Glycative Stress Research. 2020;7(3):211-9.

47. Yamanaka M, Matsumura T, Ohno R-i, Fujiwara Y, Shinagawa M, Sugawa H, et al. Non-invasive measurement of skin autofluorescence to evaluate diabetic complications. Journal of Clinical Biochemistry and Nutrition. 2016;advpub.

48. Morita Y, Yagi M, Ishizaki K, Takabe W, Komatsu T, Nakazawa M, et al. Evaluation of the glycative stress by non-invansive skin AGEs measurement devices. Glycative Stress Research. 2019;6(2):92-102.

49. Wickramasinghe UPP, Uenaka S, Tian Z, Kawakami M, Yamaguchi R, Nishiyama N, et al. Effects on skin by sub-aleurone layer residual rinse-free rice (Kinmemai rice): An open label test. Glycative Stress Research. 2020;7(3):248-57.

50. Goufo P, Trindade H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ -oryzanol, and phytic acid. Food Science & Nutrition. 2014;2(2):75-104.

51. Mbanjo EGN, Kretzschmar T, Jones H, Ereful N, Blanchard C, Boyd LA, et al. The Genetic Basis and Nutritional Benefits of Pigmented Rice Grain. Frontiers in Genetics. 2020;11.

52. Morimitsu Y, Kubota K, Tashiro T, Hashizume E, Kamiya T, Osawa T. Inhibitory effect of anthocyanins and colored rice on diabetic cataract formation in the rat lenses. International Congress Series. 2002;1245:503-8.

53. Wongsa P. Phenolic Compounds and Potential Health Benefits of Pigmented Rice: IntechOpen; 2021.

54. Lo LMP, Kang MY, Yi SJ, Chung SI. Dietary supplementation of germinated pigmented rice (Oryza sativaL.) lowers dyslipidemia risk in ovariectomized Sprague–Dawley rats. Food & Nutrition Research. 2016;60(1):30092.

55. Ling WH, Cheng QX, Ma J, Wang T. Red and Black Rice Decrease Atherosclerotic Plaque Formation and Increase Antioxidant Status in Rabbits. The Journal of Nutrition. 2001;131(5):1421-6.

56. Vargas CG, Da Silva Junior JD, Rabelo TK, Moreira JCF, Gelain DP, Rodrigues E, et al. Bioactive compounds and protective effect of red and black rice brans extracts in human neuron-like cells (SH-SY5Y). Food Research International. 2018;113:57-64.

57. Hu C, Zawistowski J, Ling W, Kitts DD. Black Rice (Oryza sativaL.indica) Pigmented Fraction Suppresses both Reactive Oxygen Species and Nitric Oxide in Chemical and Biological Model Systems. Journal of Agricultural and Food Chemistry. 2003;51(18):5271-7.

58. Hyun JW, Chung HS. Cyanidin and Malvidin fromOryza sativacv. Heugjinjubyeo Mediate Cytotoxicity against Human Monocytic Leukemia Cells by Arrest of G2/M Phase and Induction of Apoptosis. Journal of Agricultural and Food Chemistry. 2004;52(8):2213-7.

59. Chen P-N, Kuo W-H, Chiang C-L, Chiou H-L, Hsieh Y-S, Chu S-C. Black rice anthocyanins inhibit cancer cells invasion via repressions of MMPs and u-PA expression. Chemico-Biological Interactions. 2006;163(3):218-29.

60. Inamasu S, Ikuyama R, Fujisaki Y, Sugimoto K-I. The Effect of Rinse Water Obtained from the Washing of Rice (YU-SU-RU) as a Hair Treatment. Journal of Society of Cosmetic Chemists of Japan. 2010;44(1):29-33.

61. Yagi M, Yonei Y. Glycative stress and anti-aging4. The evaluation of glycative Stress: Evaluation for anti-glycative effect. Glycative Stress Research. 2017;4:87-92.

62. Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, et al. Randomized Trial of an Inhibitor of Formation of Advanced Glycation End Products in Diabetic Nephropathy. American Journal of Nephrology. 2004;24(1):32-40.

63. Walter M, Marchesan E. Phenolic compounds and antioxidant activity of rice. Brazilian Archives of Biology and Technology. 2011;54(2):371-7.

64. Daiponmak W, Senakun C, Siriamornpun S. Antiglycation capacity and antioxidant activities of different pigmented Thai rice. International Journal of Food Science & Technology. 2014;49(8):1805-10.

65. Yao Y, Cheng X-Z, Wang L-X, Wang S-H, Ren G. Major Phenolic Compounds, Antioxidant Capacity and Antidiabetic Potential of Rice Bean (Vigna umbellata L.) in China. International Journal of Molecular Sciences. 2012;13(3):2707-16.

66. Ho S-C, Wu S-P, Lin S-M, Tang Y-L. Comparison of anti-glycation capacities of several herbal infusions with that of green tea. Food Chemistry. 2010;122(3):768-74.

67. Massaretto IL, Madureira Alves MF, Mussi De Mira NV, Carmona AK, Lanfer Marquez UM. Phenolic compounds in raw and cooked rice (Oryza sativa L.) and their inhibitory effect on the activity of angiotensin I-converting enzyme. Journal of Cereal Science. 2011;54(2):236-40.

68. Ghafoor K, Ahmed IAM, Doğu S, Uslu N, Fadimu GJ, Juhaimi FA, et al. The Effect of Heating Temperature on Total Phenolic Content, Antioxidant Activity, and Phenolic Compounds of Plum and Mahaleb Fruits. International Journal of Food Engineering. 2019;15(11-12).

69. Xu G, Ye X, Chen J, Liu D. Effect of Heat Treatment on the Phenolic Compounds and Antioxidant Capacity of Citrus Peel Extract. Journal of Agricultural and Food Chemistry. 2007;55(2):330-5.

70. Maghsoudlou Y, Asghari Ghajari M, Tavasoli S. Effects of heat treatment on the phenolic compounds and antioxidant capacity of quince fruit and its tisane's sensory properties. Journal of Food Science and Technology. 2019;56(5):2365-72.

71. Varnakulendran N, Ravimannan N, Navaneetha S, Shiyamala V. Study on the Fermented Boiled Srilankan Red Rice- Scientific and Traditional View. International Journal of Advanced Ayurveda and Naturopathy. 2016;1(1):1-5.

72. Jeygowri N, Parahitiyawa N, Jeyatilake S, Ranadheera S, Madhujith T. Study on isolation of potentially probiotic Lactobacillus species from fermented rice. Tropical Agricultural Research. 2015;26(3):428-40.

73. Samad N. Rice bran oil prevents neuroleptic-induced extrapyramidal symptoms in rats: Possible antioxidant mechanisms. J Food Drug Anal. 2015;23(3):370-5.

74. Takeuchi M, Yamagishi S-i. Involvement of Toxic AGEs (TAGE) in the Pathogenesis of Diabetic Vascular Complications and Alzheimer's Disease. Journal of Alzheimer's Disease. 2009;16:845-58.

75. Bierhaus A, Hofmann MA, Ziegler R, Nawroth PP. AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. Cardiovasc Res. 1998;37(3):586-600.

76. Lo TW, Westwood ME, McLellan AC, Selwood T, Thornalley PJ. Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N alpha-acetylarginine, N alpha-acetylcysteine, and N alpha-acetyllysine, and bovine serum albumin. J Biol Chem. 1994;269(51):32299-305.

77. Frye EB, Degenhardt TP, Thorpe SR, Baynes JW. Role of the Maillard reaction in aging of tissue proteins. Advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins. J Biol Chem. 1998;273(30):18714-9.

78. Peng X, Ma J, Chao J, Sun Z, Chang RC, Tse I, et al. Beneficial effects of cinnamon proanthocyanidins on the formation of specific advanced glycation endproducts and methylglyoxal-induced impairment on glucose consumption. J Agric Food Chem. 2010;58(11):6692-6.

79. Wautier MP, Massin P, Guillausseau PJ, Huijberts M, Levy B, Boulanger E, et al. N(carboxymethyl)lysine as a biomarker for microvascular complications in type 2 diabetic patients. Diabetes Metab. 2003;29(1):44-52.

80. Hori M, Yagi M, Nomoto K, Ichijo R, Shimoda A, Kitano T, et al. Experimental models for advanced glycation end product formation using albumin, collagen, elastin, keratin and proteoglycan. Anti-Aging Medicine. 2012;9:125-34.

81. Huang Y-P, Lai H-M. Bioactive compounds and antioxidative activity of colored rice bran. Journal of Food and Drug Analysis. 2016;24(3):564-74.

82. Moongngarm A, Daomukda N, Khumpika S. Chemical Compositions, Phytochemicals, and Antioxidant Capacity of Rice Bran, Rice Bran Layer, and Rice Germ. APCBEE Procedia. 2012;2:73-9.

83. Halbwirth H. The Creation and Physiological Relevance of Divergent Hydroxylation Patterns in the Flavonoid Pathway. International Journal of Molecular Sciences. 2010;11(2):595-621.

84. Fenalti G, Law RH, Buckle AM, Langendorf C, Tuck K, Rosado CJ, et al. GABA production by glutamic acid decarboxylase is regulated by a dynamic catalytic loop. Nature Structural & Molecular Biology. 2007;14(4):280-6.

85. Kobayashi K, Tanaka M, Tanabe S, Yatsukawa Y, Tanaka M, Suzuki T. Distinguishing glutamic acid in foodstuffs and monosodium glutamate used as seasoning by stable carbon and nitrogen isotope ratios. Heliyon. 2018;4(9):e00800-e.

86. Shantharam CS, Suyoga Vardhan DM, Suhas R, Sridhara MB, Gowda DC. Inhibition of protein glycation by urea and thiourea derivatives of glycine/proline conjugated benzisoxazole analogue - synthesis and structure-activity studies. European Journal of Medicinal Chemistry. 2013;60:325-32.

87. Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids. 2009;37(1):1-17.

88. Anuradha CV. Aminoacid support in the prevention of diabetes and diabetic complications. Current Protein & Peptide Science. 2009;10(1):8-17.

89. Bettio LEB, Gil-Mohapel J, Rodrigues ALS. Guanosine and its role in neuropathologies. Purinergic Signal. 2016;12(3):411-26.

90. Souza DG, Bellaver B, Bobermin LD, Souza DO, Quincozes-Santos A. Anti-aging effects of guanosine in glial cells. Purinergic Signal. 2016;12(4):697-706.

91. Seidel W, Pischetsrieder M. Immunochemical detection of N2-[1-(1-carboxy)ethyl]guanosine, an advanced glycation end product formed by the reaction of DNA and reducing sugars or L-ascorbic

acid in vitro. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 1998;1425(3):478-84.

92. Li H, Nakamura S, Miyazaki S, Morita T, Suzuki M, Pischetsrieder M, et al. N2-carboxyethyl-2′-deoxyguanosine, a DNA glycation marker, in kidneys and aortas of diabetic and uremic patients. Kidney International. 2006;69(2):388-92.

93. Yokoyama C, Maeda Y, Ishikawa Y, Suzuki T, Kobayashi K-I, Tsujii Y, et al. Verification of reducing effect on serum cholesterol level by the long-term consumption of the brown rice. Journal for the Integrated Study of Dietary Habits. 2017;28(2):89-95.

94. Suzuki M. Repressive Effect of Dietary Fiber Fractions in Unpolished Rice on the Increase in Cholesterol and Triglyceride. Eiyo To Shokuryo. 1982;35(3):155-60.

95. Ito Y, Mizukuchi A, Kise M, Aoto H, Yamamoto S, Yoshihara R, et al. Postprandial blood glucose and insulin responses to pre-germinated brown rice in healthy subjects. The Journal of Medical Investigation. 2005;52(3-4):159-64.

96. Hamano-Nagaoka M, Nishimura T, Matsuda R, Maitani T. Evaluation of a Nitric Acid-based Partial-digestion Method for Selective Determination of Inorganic Arsenic in Rice. Journal of the Food Hygienic Society of Japan (Shokuhin Eiseigaku Zasshi). 2008;49(2):95-9.

97. Hayakawa T, Suzuki S, Kobayashi S, Fukutomi T, Ide M, Ohno T, et al. Effect of Pre-Germianted Brown Rice on Metabolism of Glucose and Lipid in Patients with Diabetes Mellitus Type 2. JOURNAL OF THE JAPANESE ASSOCIATION OF RURAL MEDICINE. 2009;58(4):438-46.

98. Matsuzaki K, Yano S, Sumiyoshi E, Shido O, Katsube T, Tabata M, et al. Long-Term Ultra-High Hydrostatic Pressurized Brown Rice Intake Prevents Bone Mineral Density Decline in Elderly Japanese Individuals. Journal of Nutritional Science and Vitaminology. 2019;65(Supplement):S88-S92.

99. Maeda-Yamamoto M, Hirosawa T, Mihara Y, Kuranuki S, Nakamura T, Kawamoto S, et al. Randomized, Placebo-controlled, Clinical Study to Investigate Anti-metabolic Syndrome Effects of Functional Foods in Humans. Nippon Shokuhin Kagaku Kogaku Kaishi. 2017;64(1):23-33.

100. Kondo K, Morino K, Nishio Y, Ishikado A, Arima H, Nakao K, et al. Fiber-rich diet with brown rice improves endothelial function in type 2 diabetes mellitus: A randomized controlled trial. PLOS ONE. 2017;12(6):e0179869.

101. Nemoto H, Ikata K, Arimochi H, Iwasaki T, Ohnishi Y, Kuwahara T, et al. Effects of fermented brown rice on the intestinal environments in healthy adult. The Journal of Medical Investigation. 2011;58(3,4):235-45.

102. Benno Y, Endo K, Miyoshi H, Okuda T, Koishi H, Mitsuoka T. Effect of rice fiber on human fecal microflora. Microbiol Immunol. 1989;33(5):435-40.

103. Fukushima-Hirakawa A. Study on relationship between self-rated health and intestinal microbiota. International Journal of Human Culture Studies. 2019;2019(29):101-11.

104. Araki R, Matsuura A, Fujie K, Nakata Y, Suzuki H, Hashimoto K. An Exploratory Randomized Crossover Trial to Investigate the Palatability of Partially Abraded Brown Rice. Nippon Eiyo Shokuryo Gakkaishi. 2016;69(5):249-55.

105. Kozuka C, Yabiku K, Sunagawa S, Ueda R, Taira S, Ohshiro H, et al. Brown rice and its component, γ-oryzanol, attenuate the preference for high-fat diet by decreasing hypothalamic endoplasmic reticulum stress in mice. Diabetes. 2012;61(12):3084-93.

106. Kozuka C, Kaname T, Shimizu-Okabe C, Takayama C, Tsutsui M, Matsushita M, et al. Impact of brown rice-specific γ-oryzanol on epigenetic modulation of dopamine D2 receptors in brain striatum in high-fat-diet-induced obesity in mice. Diabetologia. 2017;60(8):1502-11.

107. Masuzaki H, Kozuka C, Okamoto S, Yonamine M, Tanaka H, Shimabukuro M. Brown rice-specific γ-oryzanol as a promising prophylactic avenue to protect against diabetes mellitus and obesity in humans. J Diabetes Investig. 2019;10(1):18-25.

108. Shimotoyodome A, Okahara F. Anti-obese and Anti-hyperglycemic Effects of Dietary Triterpene Alcohols and Sterols from Rice Bran Oil. Oleoscience. 2017;17(6):269-76.

109. Kyo H, Suzuki N, Enomoto T, Urata T, Sutoh K, Usumi K, et al. Influence of the Special Processing Rice on the Factors of Lifestyle Related Disease. Japanese Journal of Complementary and Alternative Medicine. 2018;15(2):103-8.

110. Hirakawa A, Miyachi M, Watanabe S. Effects of different meals for the around-the-world marathon-run. Clinical and Functional Nutriology. 2015;7:152-6.

111. Uenobe M, Nakata K, Inagawa H. Benefits of subaleurone layer of milled rice (Kinmemai): Analysis of Kinmemai from the view point of glycemic index food. : ACN2015 Abstract Book; 2015. 276 p.

112. Utsunomiya H, Shirai H, Eguchi S. Inhibitory effect of kinmemai (sub-aleurone layer residual rinse-free rice) on angiotensin intracellular signal transduction in vascular smooth muscle cells. Proceedings of the Japanese Society of Pathology. 2008;97:277.

113. Okuda K. Positive effects on health due to continuous intake of kinmemai (sub-aleurone layer residual rinse-free rice). . Proceesings of Lectures of Japan Society of Nutrition and Food Science. 2007;61:223.

114. Nishiyama N, Inejima T, Heya Y. The ameliorating effect on bowel movements by sub-aleurone layer residual rinse-free rice. The Japanese Journal of Nutrition and Dietetics. 2018;76:208.

115. Wedick NM, Sudha V, Spiegelman D, Bai MR, Malik VS, Venkatachalam SS, et al. Study design and methods for a randomized crossover trial substituting brown rice for white rice on diabetes risk factors in India. Int J Food Sci Nutr. 2015;66(7):797-804.

116. Zhang G, Malik VS, Pan A, Kumar S, Holmes MD, Spiegelman D, et al. Substituting brown rice for white rice to lower diabetes risk: a focus-group study in Chinese adults. J Am Diet Assoc. 2010;110(8):1216-21.

117. Ichihashi M, Yagi M, Nomoto K, Yonei Y. Glycation Stress and Photo-Aging in Skin. ANTI-AGING MEDICINE. 2011;8(3):23-9.

118. Cadau S, Leoty-Okombi S, Pain S, Bechetoille N, André-Frei V, Berthod F. In vitro glycation of an endothelialized and innervated tissue-engineered skin to screen anti-AGE molecules. Biomaterials. 2015;51:216-25.

119. Khmaladze I, Österlund C, Smiljanic S, Hrapovic N, Lafon-Kolb V, Amini N, et al. A novel multifunctional skin care formulation with a unique blend of antipollution, brightening and antiaging active complexes. J Cosmet Dermatol. 2020;19(6):1415-25.

120. Abe Y, Takabe W, Yagi M, Uwaya A, Isami F, Yamagishi S-i, et al. Melanin synthesis induction by advanced glycation end-products (AGEs) without α-melanocyte stimulating hormone (α-MSH) or UV exposure. Glycative Stress Research. 2016;3(4):229-35.

121. Kaneko T, Sato M, Sasayama R. Actual state of eating habits and Night-Eating Syndrome in medical students. Bulletin of Yamanashi Medical College Bulletin of Yamanashi Medical University. 2001;18:7-10.

122. Ohira M, Saegusa K, Sawai S. A study on nourishmentintake condition of nursing college students. . Journal of Mie Prefectural College of Nursing 2002;6:1-9.

123. Iwamoto M. A study of awareness and behavior of nursing students regarding drinking, smoking and related factors. Nursing Journal of Kagawa Medical University 2003;7(39-47).

124. Nagahata T, Nakade M, Hasegawa J. The relationship between skipping breakfast and the living arrangements of university students. . Jpn J Nutr Diet. 2014;72:32-9.

125. Kojima C, Susaki H, Ishida S. The relationship between accurate conditions of taste and diet for the School of Nutritional Sciences' students. . Annual Report of Institute of Health and Nutrition Nagoya University of Arts and Sciences. 2010;4:19-26.

126. Mori I, Natsui S, Takeda K. Search for antimutagenic substances contained in kinmemai (sub-aleurone layer residual rinse-free rice). . Proceedings of the Japanese Society of Pathology. 2008;97(1):312.

127. Shimabukuro M, Higa M, Kinjo R, Yamakawa K, Tanaka H, Kozuka C, et al. Effects of the brown rice diet on visceral obesity and endothelial function: the BRAVO study. Br J Nutr. 2014;111(2):310-20.

128. Masuzaki H, Fukuda K, Ogata M, Kinjo A, Matsuo T, Nishihira J. Safety and efficacy of nanoparticulated brown rice germ extract on reduction of body fat mass and improvement of fuel metabolism in both pre-obese and mild obese subjects without excess of visceral fat accumulation. Glycative Stress Research. 2020;7(1):1-12.

129. Okahara F, Suzuki J, Hashizume K, Osaki N, Shimotoyodome A. Triterpene alcohols and sterols from rice bran reduce postprandial hyperglycemia in rodents and humans. Mol Nutr Food Res. 2016;60(7):1521-31.

130. Misawa K, Jokura H, Shimotoyodome A. Rice bran triterpenoids improve postprandial hyperglycemia in healthy male adults: a randomized, double-blind, placebo-controlled study. Food Nutr Res. 2018;62.

131. Ogiwara T, Satoh K, Kadoma Y, Murakami Y, Unten S, Atsumi T, et al. Radical scavenging activity and cytotoxicity of ferulic acid. Anticancer Res. 2002;22(5):2711-7.

132. Kikugawa M, Tsutsuki H, Ida T, Nakajima H, Ihara H, Sakamoto T. Water-soluble ferulic acid derivatives improve amyloid-β-induced neuronal cell death and dysmnesia through inhibition of amyloid-β aggregation. Biosci Biotechnol Biochem. 2016;80(3):547-53.

133. Kikugawa M, Ida T, Ihara H, Sakamoto T. Ferulic acid and its water-soluble derivatives inhibit nitric oxide production and inducible nitric oxide synthase expression in rat primary astrocytes. Bioscience, Biotechnology, and Biochemistry. 2017;81(8):1607-11.

134. Shimizu H, Ohue-Kitano R, Kimura I. Regulation of host energy metabolism by gut microbiota-derived short-chain fatty acids. Glycative Stress Research. 2019;6(3):181-91.

135. Uenaka S, Ogura M, Yagi M, Takabe W, Yonei Y. Examination of postprandial blood glucose prediction model using food nutrition component values. Glycative Stress Research. 2020;7(4):268-77.

136. Thanapreedawat P, Kobayashi H, Inui N, Sakamoto K, Kim M, Yoto A, et al. GABA affects novel object recognition memory and working memory in rats. J Nutr Sci Vitaminol (Tokyo). 2013;59(2):152-7.

137. Takeshima K, Yamatsu A, Yamashita Y, Watabe K, Horie N, Masuda K, et al. Subchronic toxicity evaluation of γ-aminobutyric acid (GABA) in rats. Food Chem Toxicol. 2014;68:128-34.

138. Schlemmer U, Frølich W, Prieto RM, Grases F. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. Mol Nutr Food Res. 2009;53 Suppl 2:S330-75.

139. Vucenik I, Shamsuddin AM. Protection against cancer by dietary IP6 and inositol. Nutr Cancer. 2006;55(2):109-25.

140. Nakata K, Inagawa H, Nishizawa T, Kohchi C, Soma GI. Specific messenger RNA expression for signal transduction molecules by lipopolysaccharide in intestinal macrophages. Clin Exp Immunol. 2006;143(3):484-93.

141. Tamura Y, Inagawa H, Nakata Y, Kohchi C, Soma G. Effects of the Subaleurone Layer of Rice on Macrophage Activation and Protection of Pollen Allergy in a Murine Model. Anticancer Res. 2015;35(8):4467-72.

142. Kobayashi Y, Inagawa H, Kohchi C, Okazaki K, Zhang R, Soma G. Effect of Lipopolysaccharide Derived from Pantoea agglomerans on the Phagocytic Activity of Amyloid β by Primary Murine Microglial Cells. Anticancer Res. 2016;36(7):3693-8.

143. Furue M, Chiba T, Tsuji G, Ulzii D, Kido-Nakahara M, Nakahara T, et al. Atopic dermatitis: immune deviation, barrier dysfunction, IgE autoreactivity and new therapies. Allergol Int. 2017;66(3):398-403.

144. Yokoyama C, Maeda Y, Ishikawa Y. Verification of reducing effect on serum cholesterol level by the long-term consumption of the brown rice. Journal for the Integrated Study of Dietary Habits. 2017;28:2017.

145. Mori N, Kano M, Masuoka N, Konno T, Suzuki Y, Miyazaki K, et al. Effect of probiotic and prebiotic fermented milk on skin and intestinal conditions in healthy young female students. Bioscience of Microbiota, Food and Health. 2016;35(3):105-12.

146. Inagawa H, Saika T, Nishiyama N, Nisizawa T, Kohchi C, Uenobe M, et al. Improvement Effect of Dewaxed Brown Rice on Constipation in Antibiotic-treated Mice. In Vivo. 2017;31(4):573-7.

147. Uenobe M, Saika T, Waku N, Ohno M, Inagawa H. Efficacy of continuous ingestion of dewaxed brown rice on the cognitive functions of the residents of elderly welfare facilities: A pilot test using crossover trial. Food Sci Nutr. 2019;7(11):3520-6.

148. Hirai C, Sakai Y, Hozuki T. Survey of Neonicotinoid Insecticides in Honey and Brown Rice in Fukui Prefecture. . Annual report of Fukui Prefectural Institute of Public Health and Environmental Science 2017;15:55-9.

149. Sakuraba K, Konno R, Ugajin R. Development of simultaneous analysis method for residual pesticides in food and its results (2014-2016 fiscal year). . Annual Report of Akita Research Center for Public Health and Environment 2017;13:58-61.

150. Bassaganya-Riera J, Guri AJ, Lu P, Climent M, Carbo A, Sobral BW, et al. Abscisic acid regulates inflammation via ligand-binding domain-independent activation of peroxisome proliferator-activated receptor gamma. J Biol Chem. 2011;286(4):2504-16.

151. Lievens L, Pollier J, Goossens A, Beyaert R, Staal J. Abscisic Acid as Pathogen Effector and Immune Regulator. Front Plant Sci. 2017;8:587-.

152. Maeda Y, Tsujii Y, Yatomi S. Information On Safety of Brown Rice Meal. Food Preservation Science. 2015;41:273-5.

153. Yonei Y, Uenaka S, Yagi M, Wickramasinghe UPP, Kawakami M, Yamaguchi R, et al. Effects on skin by dewaxed brown rice: An open label test. Glycative Stress Research. 2021;8(1):29-38.

Table 1. Rice samples used in chapter 1.

Sample number	Sample name	Subspecies	Color	Country of origin and purchase	Additional details regarding source of material
1	Asamurasaki	*Japonica*	Black	Japan	Purchased from Tomizawa Shoten (TOMIZ) Product No. 00347802
2	Black Kodaimai	*Japonica*	Black	Japan	Purchased from Asuka Kanko
3	Dik Vee	*Indica*	Red	Sri Lanka	Purchased from Siriketha Products, Ethkandura
4	Hitomebore	*Japonica*	Brown	Japan	Purchased from Rice Friend, Takashimaya Osaka store
5	Koshihikari	*Japonica*	Brown	Japan	Purchased from Rice Friend, Takashimaya Osaka store
6	Kuruluthuda	*Indica*	Red	Sri Lanka	Purchased from Siriketha Products, Ethkandura
7	Ma Vee	*Indica*	White	Sri Lanka	Purchased from a local farmer in the Gampaha district
8	Madathavalu	*Indica*	White	Sri Lanka	Purchased from a local farmer in the Gampaha district
9	Martin Samba	*Indica*	White	Sri Lanka	Purchased from a local farmer in the Gampaha district
10	Mixed Kodaimai	*Japonica*	Mixed (black,	Japan	Purchased from Asuka Kanko

			green, red)		
11	Pachchaperumal	*Indica*	Red	Sri Lanka	Purchased from a local farmer in the Gampaha district
12	Red Kodaimai	*Japonica*	Red	Japan	Purchased from Asuka Kanko
13	Suduru Hel	*Indica*	White	Sri Lanka	Purchased from a local farmer in the Gampaha district
14	Yuyakemochi	*Japonica*	Red	Japan	Purchased from Tomizawa Shoten (TOMIZ) Product No. 00347901

Table 2. Rice samples used in chapter 2.

Sample number	Sample name	Subspecies	Color	Country of origin and purchase	Additional details regarding source of material
1	Asamurasaki	*Japonica*	Black	Japan	Purchased from Tomizawa Shoten (TOMIZ) Product No. 00347802
2	Black Kodaimai	*Japonica*	Black	Japan	Purchased from Asuka Kanko
3	Red Kodaimai	*Japonica*	Red	Japan	Purchased from Asuka Kanko
4	Yuyakemochi	*Japonica*	Red	Japan	Purchased from Tomizawa Shoten (TOMIZ) Product No. 00347901
5	Hitomebore	*Japonica*	Brown	Japan	Purchased from Rice Friend, Takashimaya Osaka store
6	Koshihikari	*Japonica*	Brown	Japan	Purchased from Rice Friend, Takashimaya Osaka store
7	Sasanishiki	*Japonica*	Brown	Japan	Purchased from Rice Friend, Takashimaya Osaka store
8	Hitomebore	*Japonica*	White	Japan	Purchased from Rice Friend, Takashimaya Osaka store
9	Koshihikari	*Japonica*	White	Japan	Purchased from Rice Friend, Takashimaya Osaka store
10	Sasanishiki	*Japonica*	White	Japan	Purchased from Rice Friend, Takashimaya Osaka store
11	Kuruluthuda	*Indica*	Red	Sri Lanka	Purchased from Siriketha Products, Ethkandura

12	Pachchaperumal	*Indica*	Red	Sri Lanka	Purchased from a local farmer in the Gampaha district
13	Madathavalu	*Indica*	Red	Sri Lanka	Purchased from a local farmer in the Gampaha district
14	Ma Vee	*Indica*	White	Sri Lanka	Purchased from a local farmer in the Gampaha district
15	Suduru Hel	*Indica*	White	Sri Lanka	Purchased from a local farmer in the Gampaha district
16	Martin Samba	*Indica*	White	Sri Lanka	Purchased from a local farmer in the Gampaha district

Table 3. CE-MS analysis of fraction #10 and #14. HMT DB †, human mitochondrial database; m/z, mass-to-charge ratio; MT, migration time; RT, retention time.

ID	HMT DB †		m/z	MT/RT	Relative Area	
	Compound name	Pathway label			Fraction #10	Fraction #14
					1	2
C_0001	Glutamic acid	Glu	148.06	8.74	-	2.50E-02
C_0004	Guanosine	Guanosine	284.1	10.02	1.50E-02	-
A_0001			141.02	2.71	1.20E-01	1.50E-01
A_0002			151.06	4.69	1.10E-01	1.30E-01
A_0004			273.07	4.69	1.10E-01	1.10E-01
C_0003			202.18	7.43	9.40E-02	1.30E-01
C_0005			316.21	8.26	2.00E-02	2.20E-02

Table 4. Results of skin condition and AGEs fluorescence. SARFR, sub-aleurone layer residual rinse-free rice; AGEs, advanced glycation end products; PL, polarized right; UV, ultraviolet; SD, standard deviation; skin condition measured by Clreo-Pro (Fujitex); AGEs score measured by AGEs sensor (Sharp); SARFR group, n = 37; the control group, n = 22; Statistical analysis by Student's t test.

		Before ingestion			One month after ingestion			Comparison between groups (by variation)
		Mean		SD	mean		SD	vs. control
Age (years)	SARFR	21.0	±	1.5				
	Control	22.0	±	1.2				
Pores (%)	SARFR	-0.9	±	12.1	-3.0	±	12.2	0.589
	Control	-2.0	±	13.5	-4.6	±	11.3	
Wrinkles (%)	SARFR	2.8	±	8.2	2.6	±	8.5	0.558
	Control	0.5	±	6.1	-0.4	±	4.9	
Pigmentation (PL) stratum corneum (%)	SARFR	2.9	±	5.3	1.8	±	5.7	0.942
	Control	1.9	±	4.1	0.8	±	3.7	
Pigmentation (UV) stratum corneum (%)	SARFR	6.3	±	7.9	4.3	±	8.3	0.419
	Control	3.9	±	5.3	2.8	±	5.0	
Porphyrin	SARFR	34.0	±	8.5	32.1	±	6.8	0.702
	Control	32.8	±	9.9	30.1	±	10.6	
Skin tone	SARFR	62.0	±	5.5	62.8	±	4.9	0.658
	Control	61.7	±	4.7	62.8	±	4.8	
Elastisity (angle)	SARFR	45.8	±	5.6	44.4	±	9.2	0.250
	Control	42.7	±	7.7	43.6	±	5.2	
Skin health score	SARFR	43.2	±	10.8	49.2	±	12.6	0.340
	Control	47.3	±	9.6	50.9	±	10.0	
Skin age (year)	SARFR	21.5	±	1.9	21.1	±	1.7	**0.034**
	Control	22.2	±	1.6	22.0	±	1.6	
AGEs score	SARFR	0.43	±	0.07	0.44	±	0.06	0.421
	Control	0.44	±	0.21	0.46	±	0.05	

Table 5. Results of skin condition and AGE fluorescence. DBR, dewaxed brown rice; AGE, advanced glycation end product; PL, polarized right; UV, ultraviolet; SD, standard deviation; skin condition measured by Clreo-Pro (Fujitex); AGE score measured by AGEs sensor (Sharp); DBR group, n = 43; the control group, n = 22; Statistical analysis by Student's t test.

		Before ingestion			One month after ingestion			Comparison between groups (by variation)
		Mean		SD	Mean		SD	vs. control
Age (year)	DBR	23.8	±	8.7				
	Control	22.0	±	1.2				
Pores (%)	DBR	-0.3	±	12.6	-2.8	±	11.9	0.885
	Control	-2.0	±	13.5	-4.6	±	11.3	
Wrinkles (%)	DBR	3.5	±	8.8	1.4	±	7.3	0.300
	Control	0.5	±	6.1	-0.4	±	4.9	
Pigmentation (PL) stratum corneum (%)	DBR	2.6	±	4.7	1.3	±	4.4	0.533
	Control	1.9	±	4.1	0.8	±	3.7	
Pigmentation (UV) stratum corneum (%)	DBR	6.2	±	8.8	4.6	±	7.7	0.615
	Control	3.9	±	5.3	2.8	±	5.0	
Porphyrin	DBR	31.4	±	7.7	30.3	±	6.4	0.393
	Control	32.8	±	9.9	30.1	±	10.6	
Skin tone	DBR	61.8	±	4.7	62.6	±	4.4	0.630
	Control	61.7	±	4.7	62.8	±	4.8	
Elasticity (angle)	DBR	43.8	±	4.9	44.8	±	5.0	0.973
	Control	42.7	±	7.7	43.6	±	5.2	
Skin health score	DBR	45.0	±	12.9	50.5	±	12.4	0.405
	Control	47.3	±	9.6	50.9	±	10.0	
Skin age (year)	DBR	24.2	±	9.2	23.8	±	8.8	**0.023**
	Control	22.2	±	1.6	22.0	±	1.6	
AGE Score	DBR	0.44	±	0.06	0.46	±	0.05	0.260
	Control	0.44	±	0.21	0.46	±	0.05	

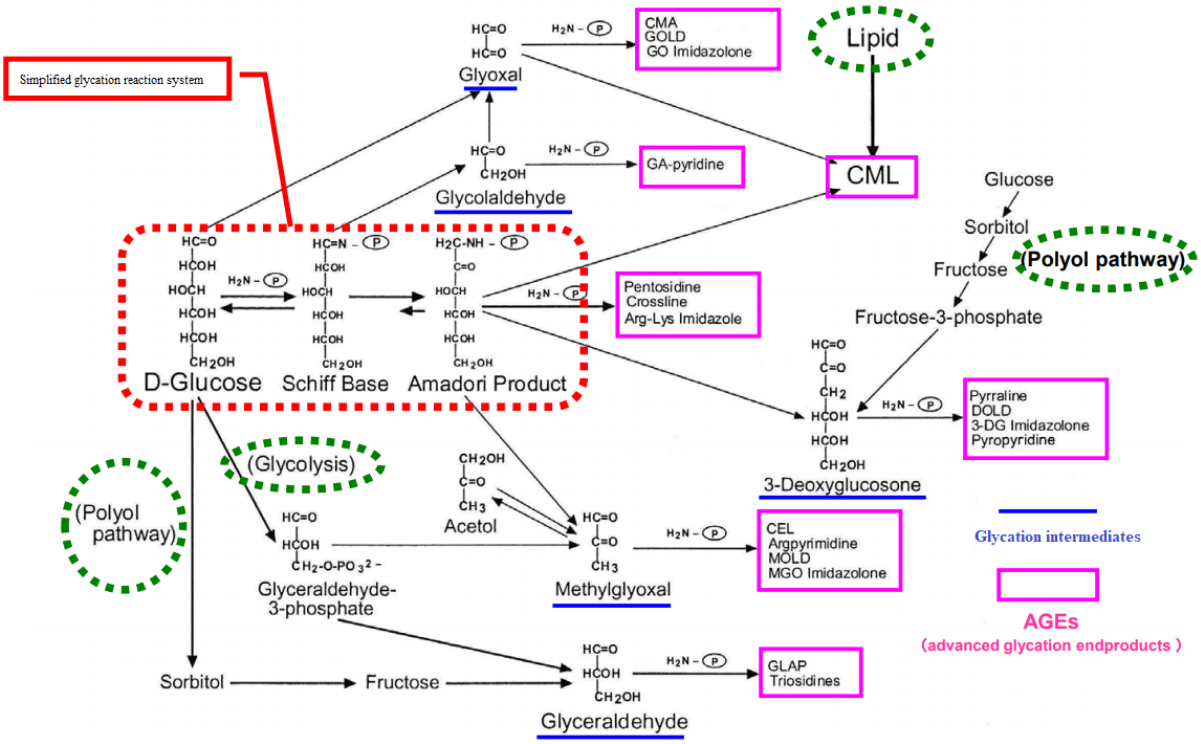


Figure 1. Glycation pathway. A complicated molecular process involving simple and complex multi-path reactions leading to the formation of AGEs (74).

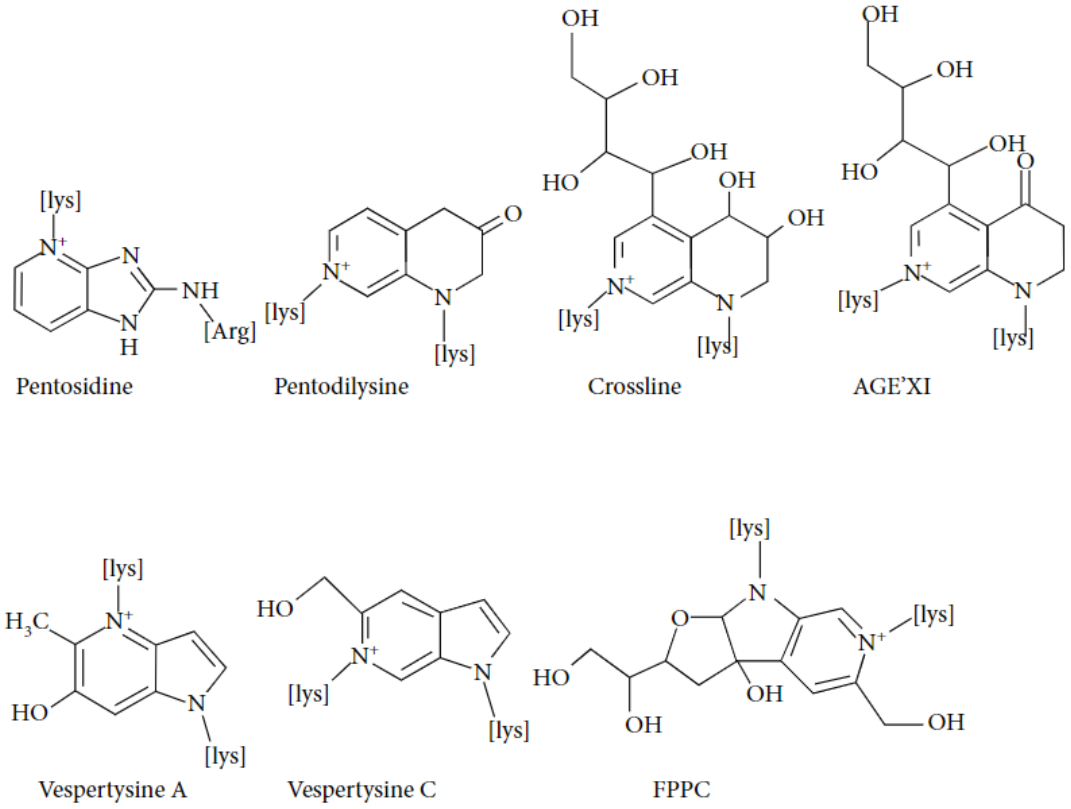


Figure 2. Examples of fluorescent cross-linked AGEs (16).

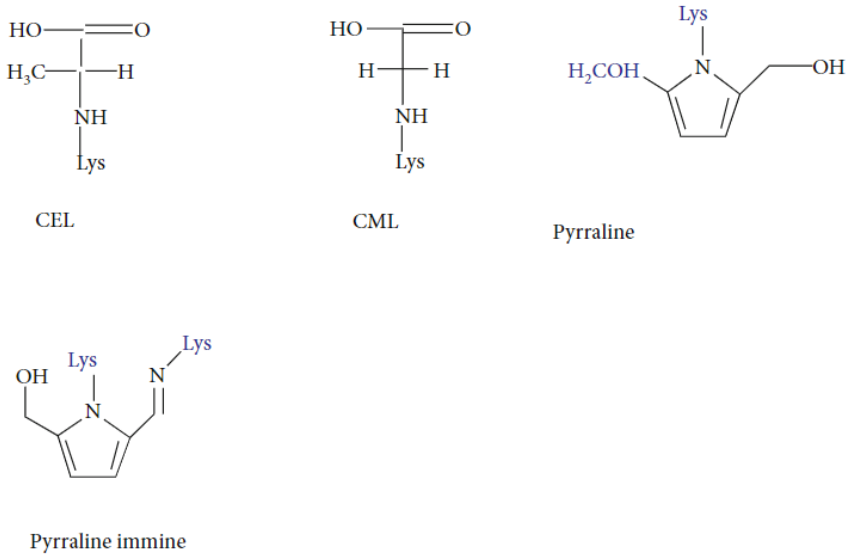


Figure 3. Examples of non-fluorescent non-cross-linked AGEs (16).

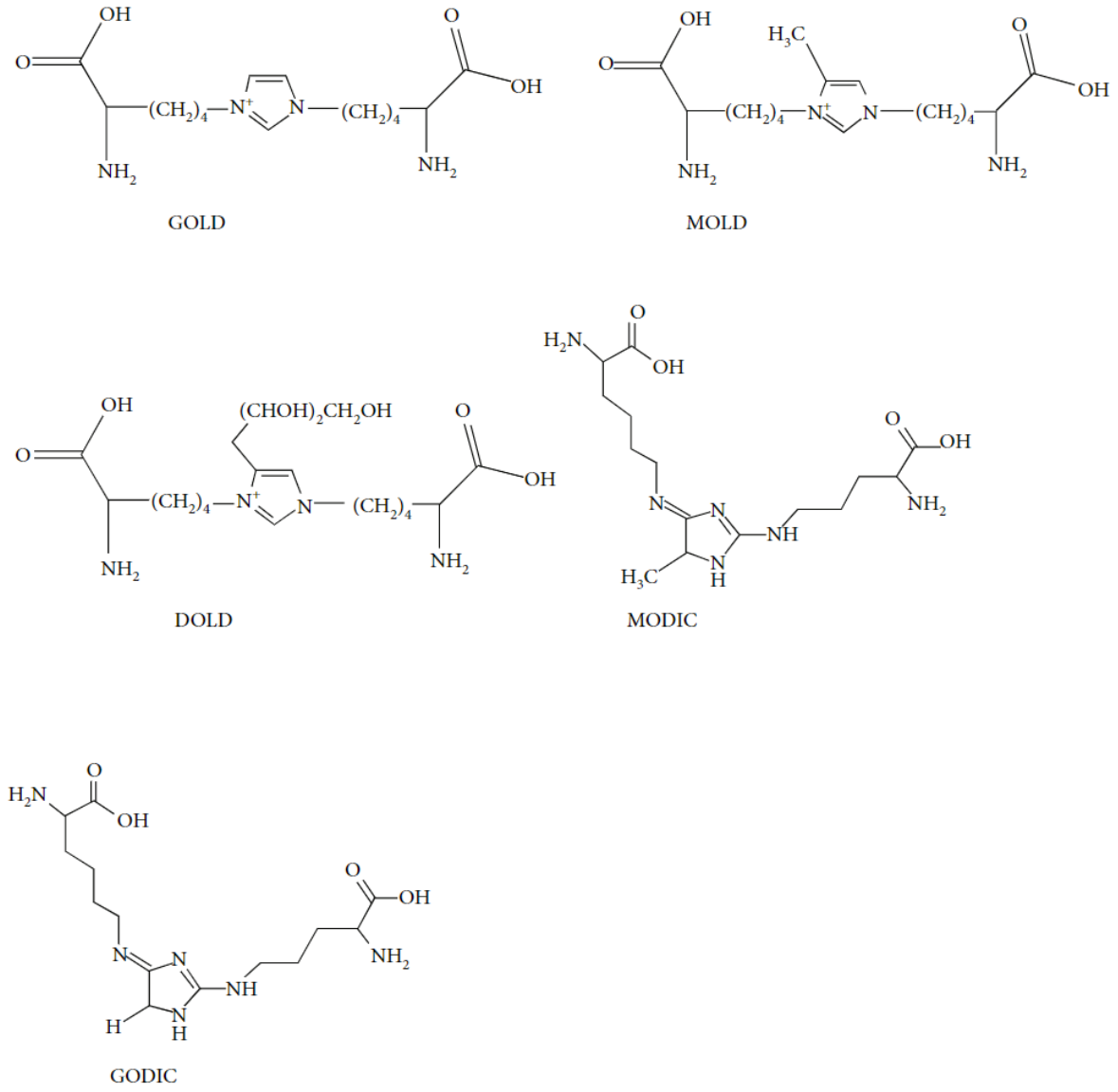


Figure 4. Examples of non-fluorescent cross-linked AGEs (16).

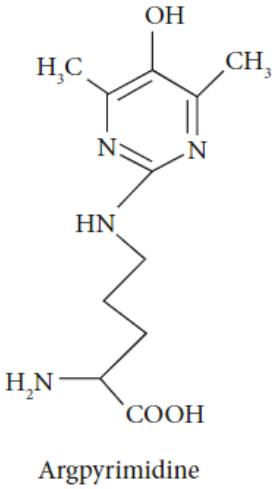


Figure 5. Example of fluorescent non-cross-linked AGEs (16).

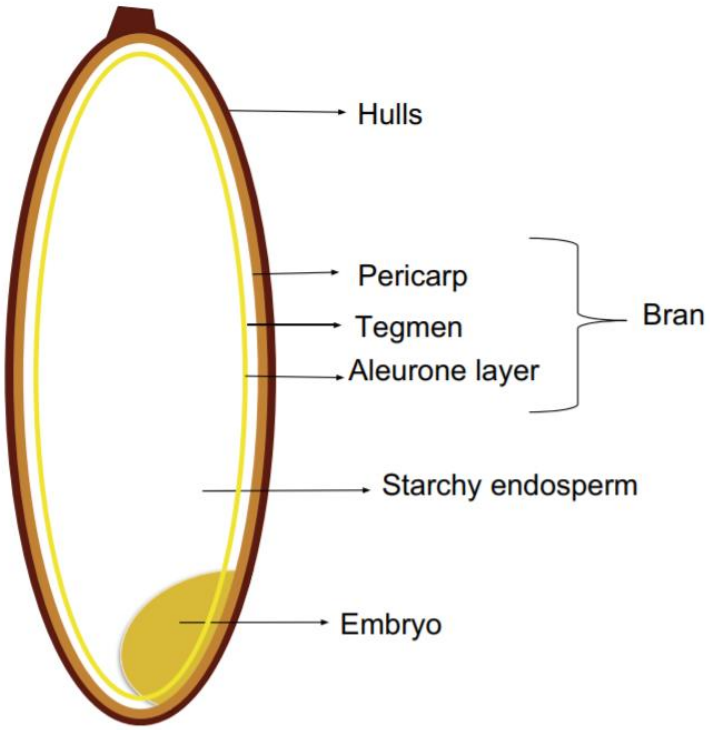


Figure 6. Structure of a rice grain (22).

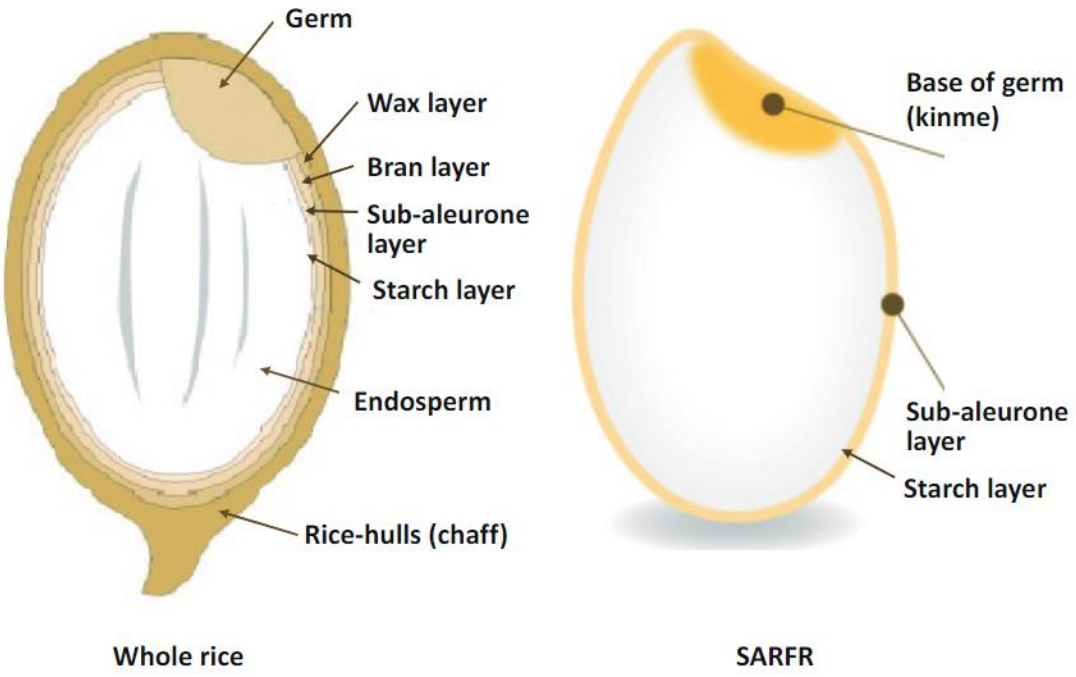


Figure 7. Structure of rice and SARFR (49).

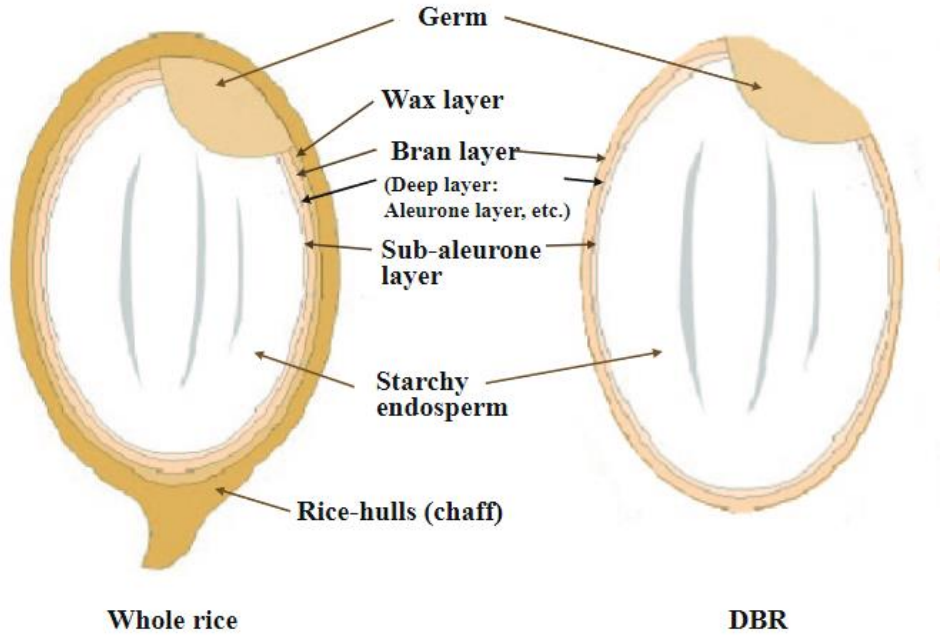


Figure 8. Structure of rice and DBR (153).

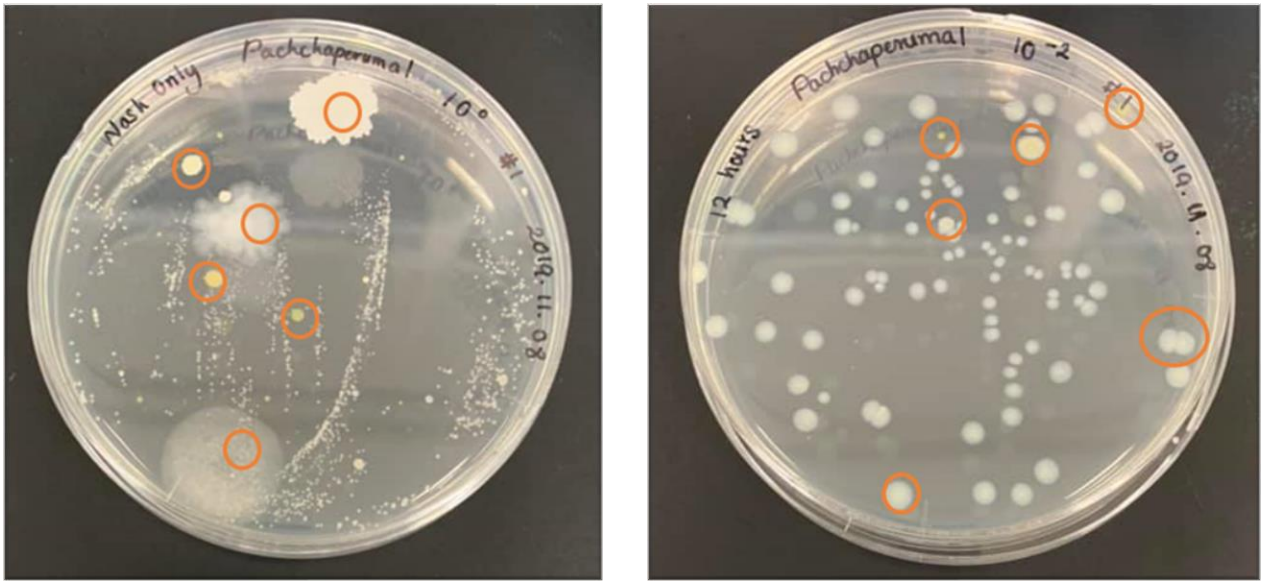


Figure 9. Some of the isolated colonies shown as an example.

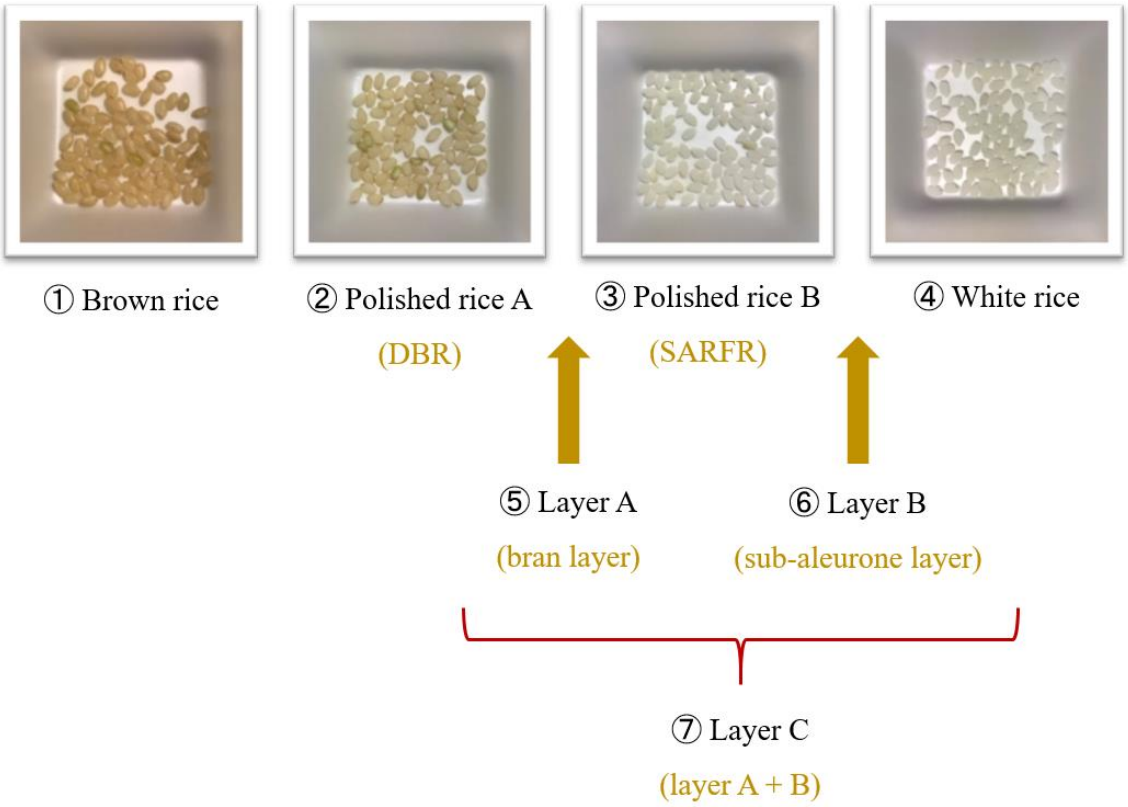


Figure 10. Samples provided by Toyo Rice Co. Ltd.

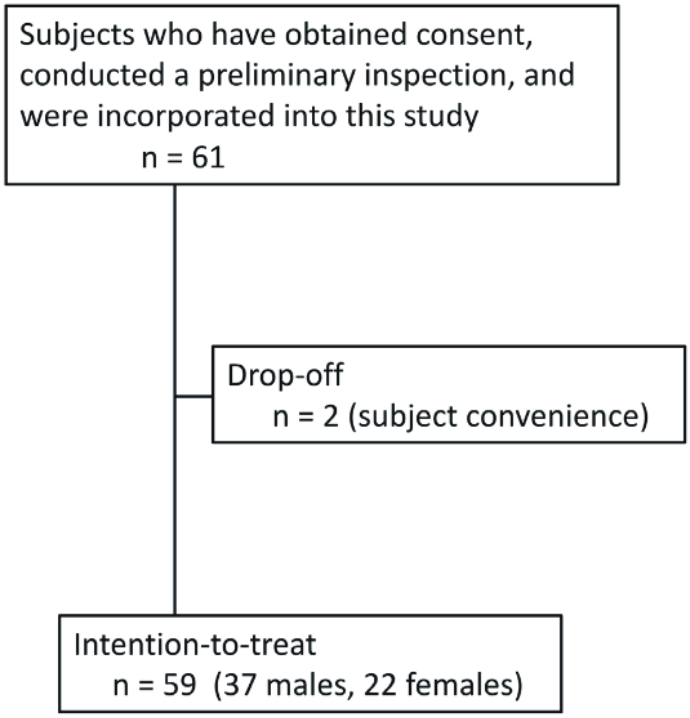


Figure 11. Changes in the number of test subjects in study of chapter 3 (49).

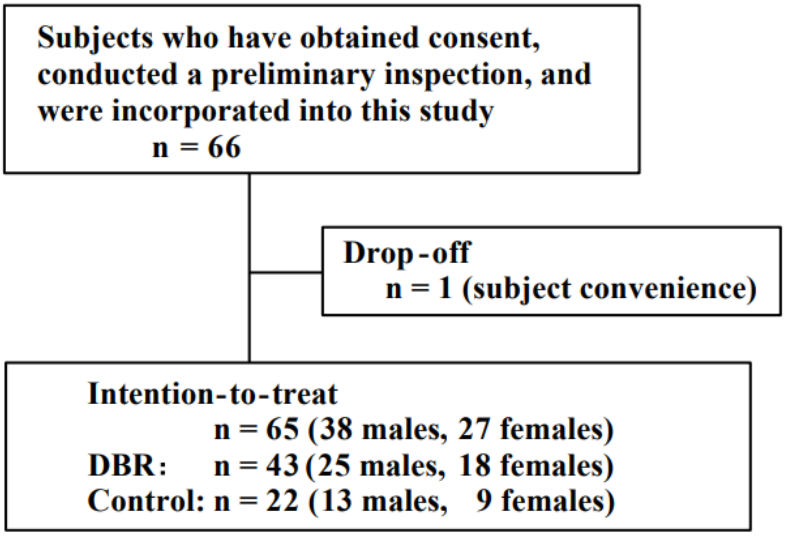


Figure 12. Changes in the number of test subjects in study of chapter 4 (153).

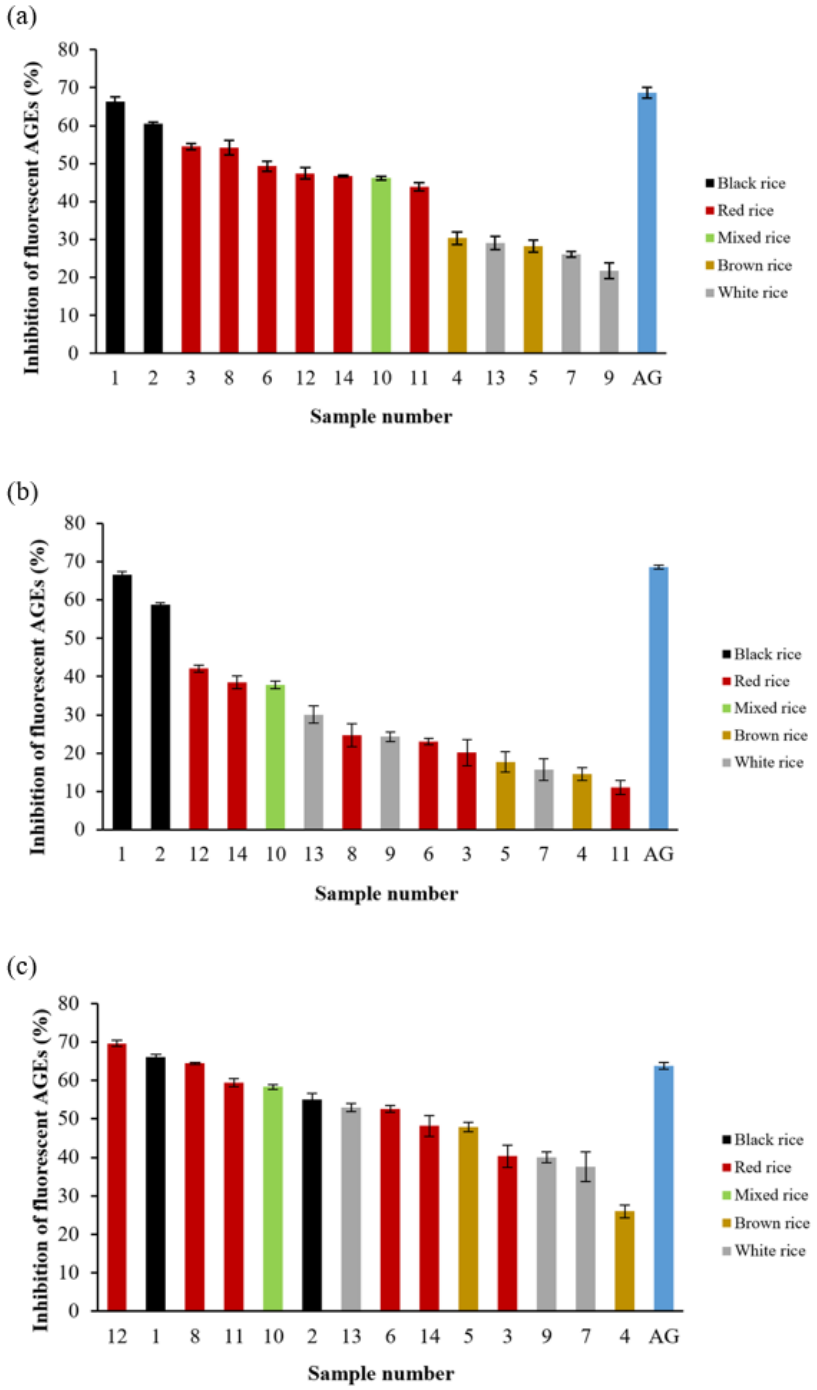


Figure 13. Inhibition of fluorescent AGEs formation by (a) type 1 rice water and AG, (b) type 2 rice water and AG, (c) type 3 rice water and AG. The final concentration of samples was 1 mg/mL. Results are expressed as mean ± standard deviation, n=3. AGEs, advanced glycation end products; AG, aminoguanidine. Sample number; 1, Asamurasaki, 2, Black Kodaimai, 3, Dik Vee, 4, Hitomebore, 5, Koshihikari, 6, Kuruluthuda, 7, Ma Vee, 8, Madathavalu, 9, Martin Samba, 10, Mixed Kodaimai, 11, Pachchaperumal, 12, Red Kodaimai, 13, Suduru Hel, 14, Yuyakemochi.

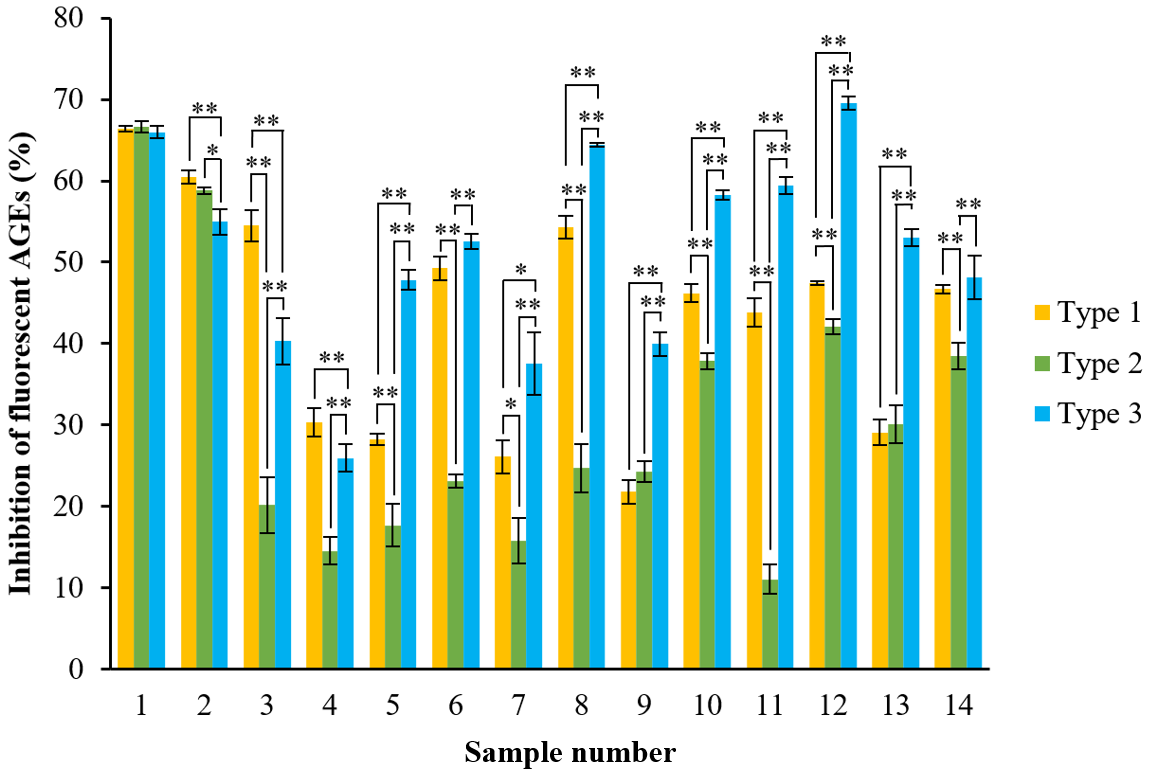


Figure 14. Comparison of the inhibition of fluorescent AGEs formation by the 3 types of rice water. The final concentration of samples was 1 mg/mL. Results are expressed as mean ± standard deviation, n = 3, *p < 0.05, **p < 0.01 by Tukey's test. AGEs, advanced glycation end products. Sample number; 1, Asamurasaki, 2, Black Kodaimai, 3, Dik Vee, 4, Hitomebore, 5, Koshihikari, 6, Kuruluthuda, 7, Ma Vee, 8, Madathavalu, 9, Martin Samba, 10, Mixed Kodaimai, 11, Pachchaperumal, 12, Red Kodaimai, 13, Suduru Hel, 14, Yuyakemochi.

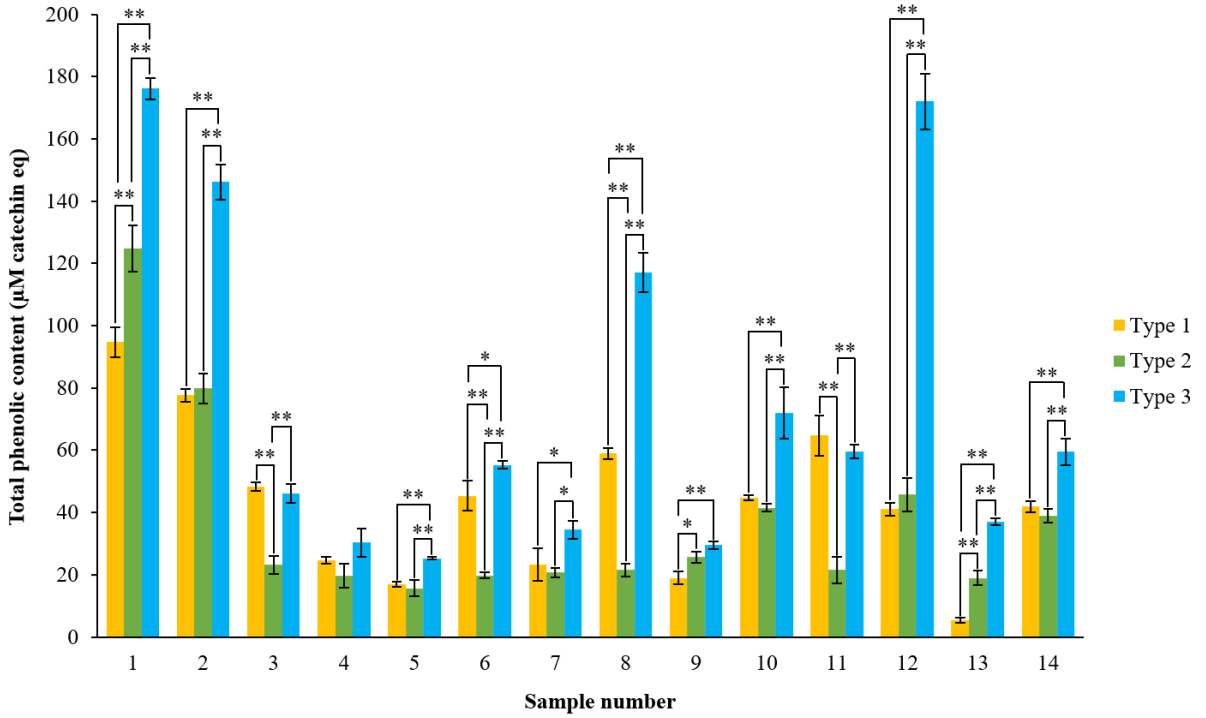


Figure 15. Comparison of the total phenolic content (TPC) in the 3 types of rice water. The final concentration of samples was 1 mg/mL. Results are expressed as mean ± standard deviation, n = 3. *p < 0.05, **p < 0.01 by Tukey's test. TPC, total phenolic content. Sample number; 1, Asamurasaki, 2, Black Kodaimai, 3, Dik Vee, 4, Hitomebore, 5, Koshihikari, 6, Kuruluthuda, 7, Ma Vee, 8, Madathavalu, 9, Martin Samba, 10, Mixed Kodaimai, 11, Pachchaperumal, 12, Red Kodaimai, 13, Suduru Hel, 14, Yuyakemochi.

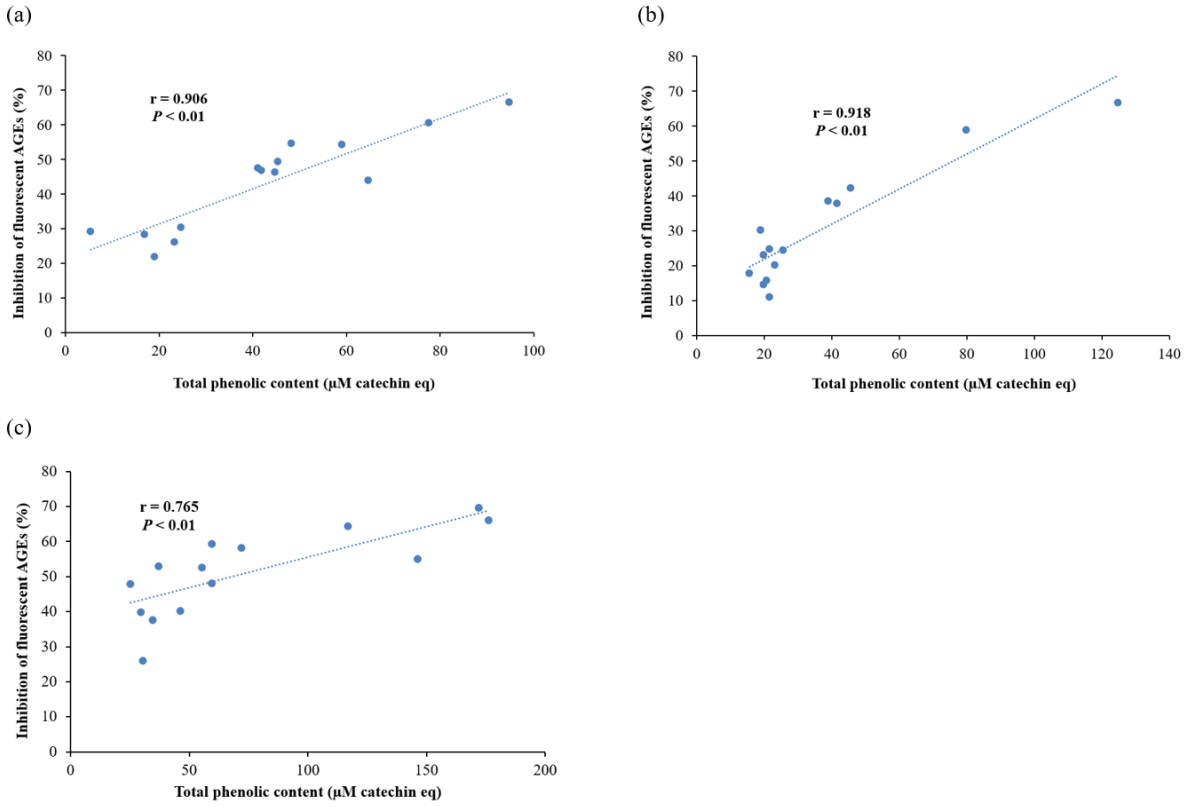


Figure 16. Correlation of inhibition of fluorescent AGEs formation to TPC of (a) type 1 rice water, (b) type 2 rice water, (c) type 3 rice water. $p < 0.01$, r = Pearson correlation coefficient. AGEs, advanced glycation end products; TPC, total phenolic content.

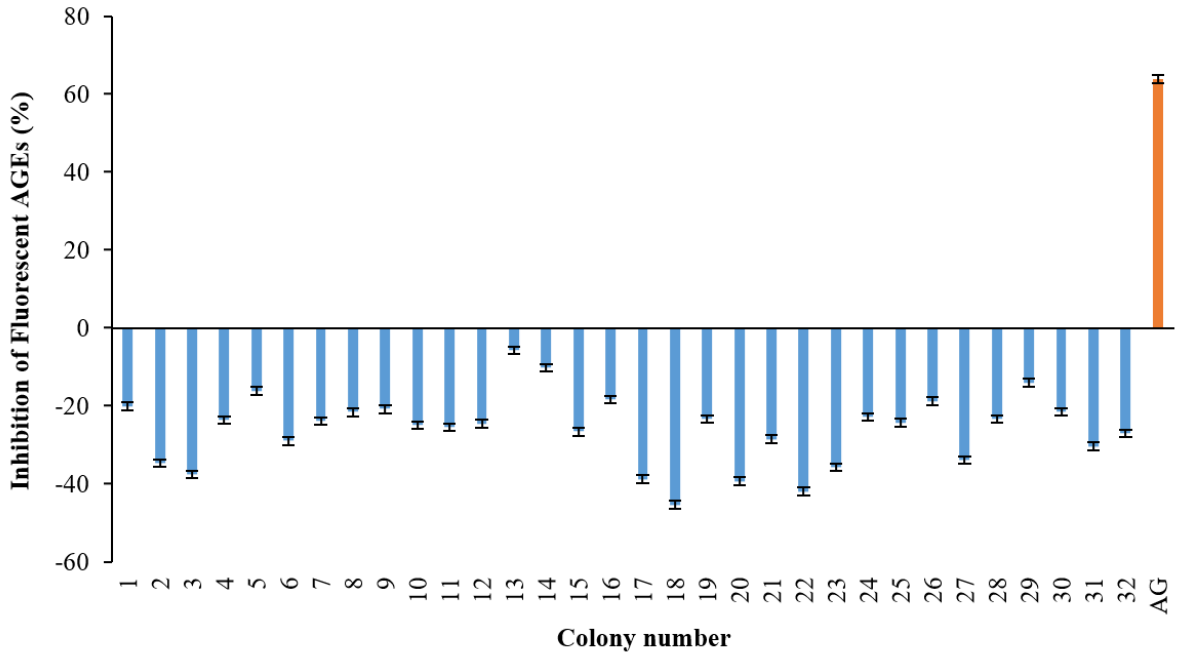


Figure 17. Inhibition of fluorescent AGEs formation by supernatants of isolated colonies from rice water. The final concentration of AG was 0.1 mg/mL. Results are expressed as mean ± standard deviation, n=3. AGES, advanced glycation end products; AG, aminoguanidine.

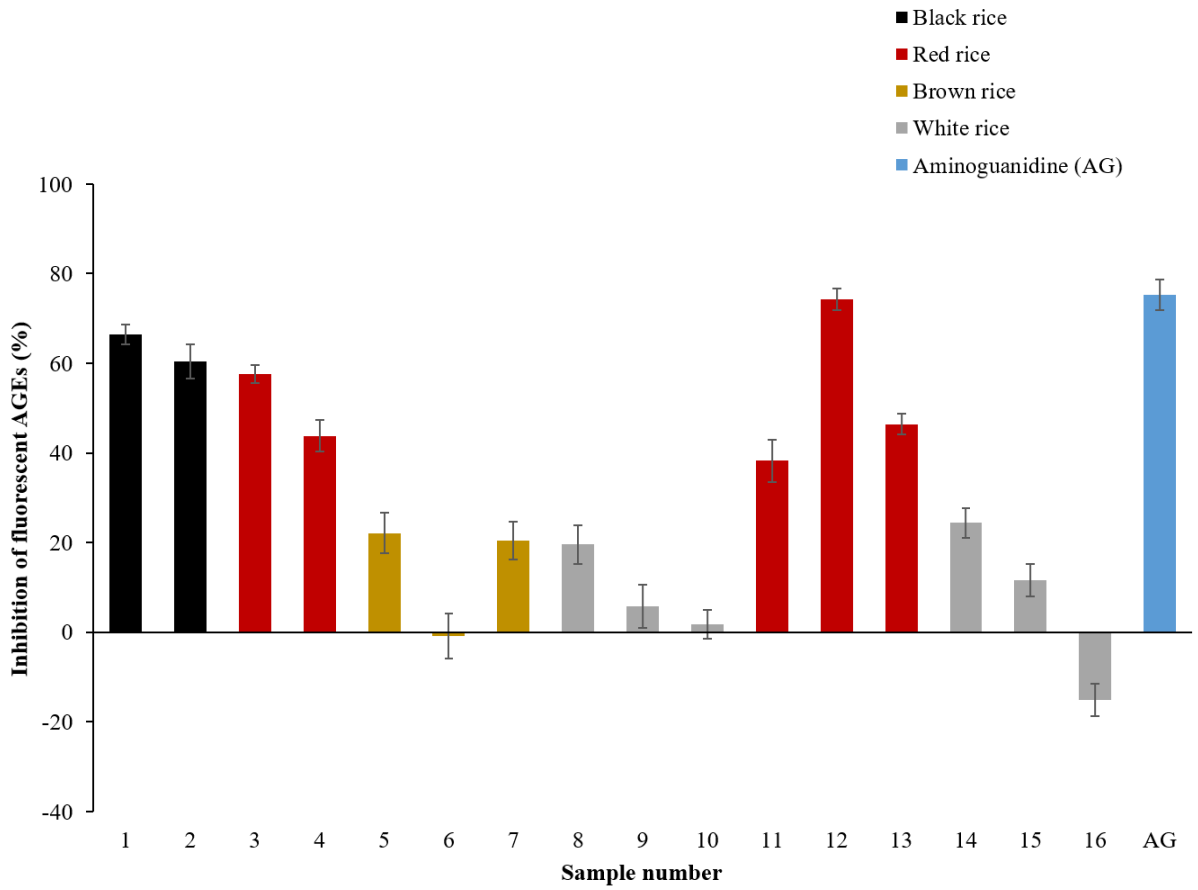


Figure 18. Inhibition of fluorescent AGEs formation by rice water extracts and AG. The final concentration of samples was 0.5 mg/mL and that of AG was 0.1 mg/mL. Results are expressed as mean ± standard deviation, n=3. AGEs, advanced glycation end products; AG, aminoguanidine. Sample number; 1, Asamurasaki, 2, Black Kodaimai, 3, Red Kodaimai, 4, Yuyakemochi, 5, Hitomebore (brown), 6, Koshihikari (brown), 7, Sasanishiki (brown), 8, Hitomebore (white), 9, Koshihikari (white), 10, Sasanishiki (white), 11, Kuruluthuda, 12, Pachchaperumal, 13, Madathavalu, 14, Ma Vee, 15, Suduru Hel, 16, Martin Samba.

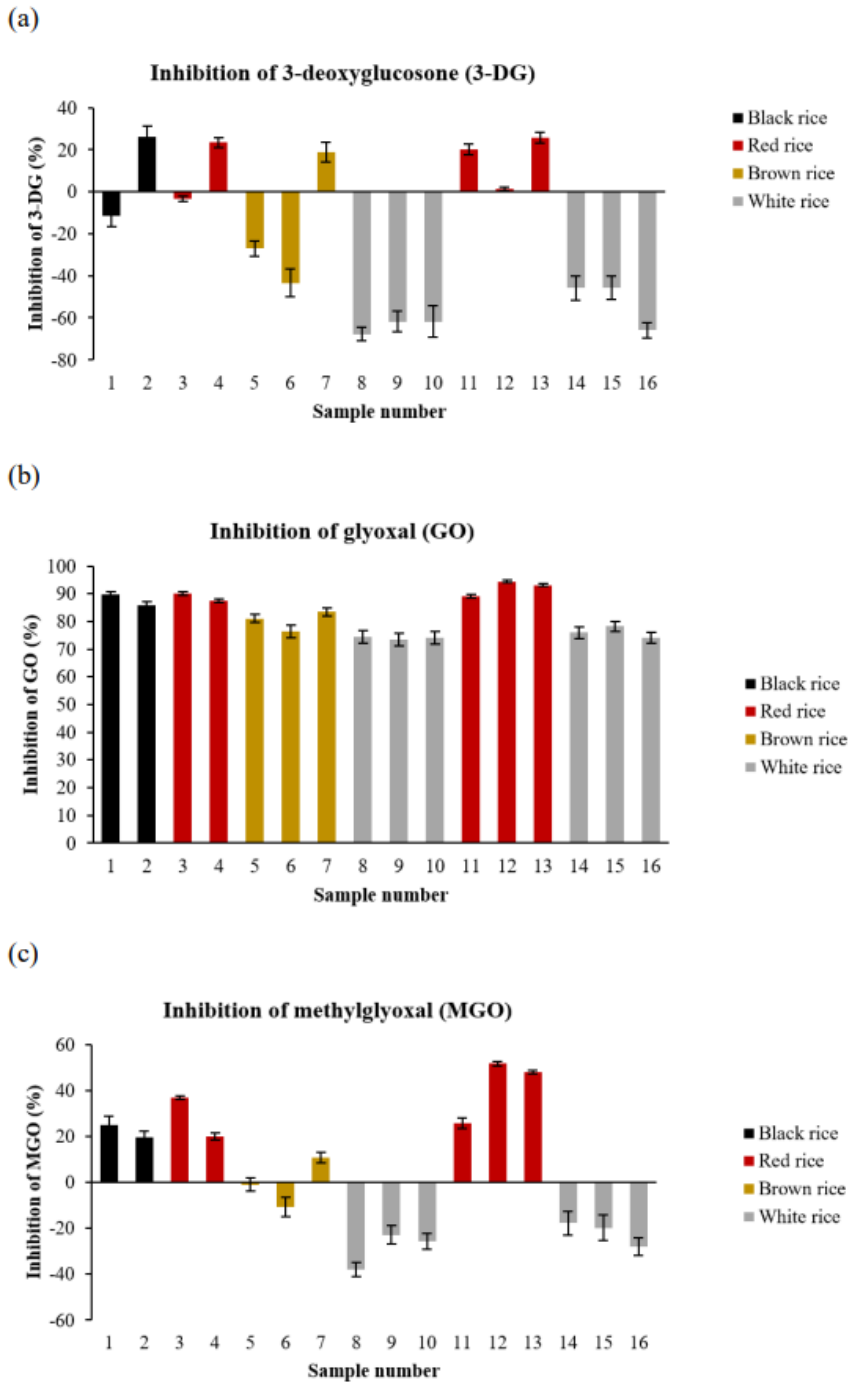


Figure 19. Inhibition of glycation intermediates by rice water extracts. The final concentration of samples was 0.5 mg/mL. Results are expressed as mean ± standard deviation, n=3. 3-DG, 3-deoxyglucosone, GO, glyoxal, MGO, methylglyoxal. Sample number; 1, Asamurasaki, 2, Black Kodaimai, 3, Red Kodaimai, 4, Yuyakemochi, 5, Hitomebore (brown), 6, Koshihikari (brown), 7, Sasanishiki (brown), 8, Hitomebore (white), 9, Koshihikari (white), 10, Sasanishiki (white), 11, Kuruluthuda, 12, Pachchaperumal, 13, Madathavalu, 14, Ma Vee, 15, Suduru Hel, 16, Martin Samba.

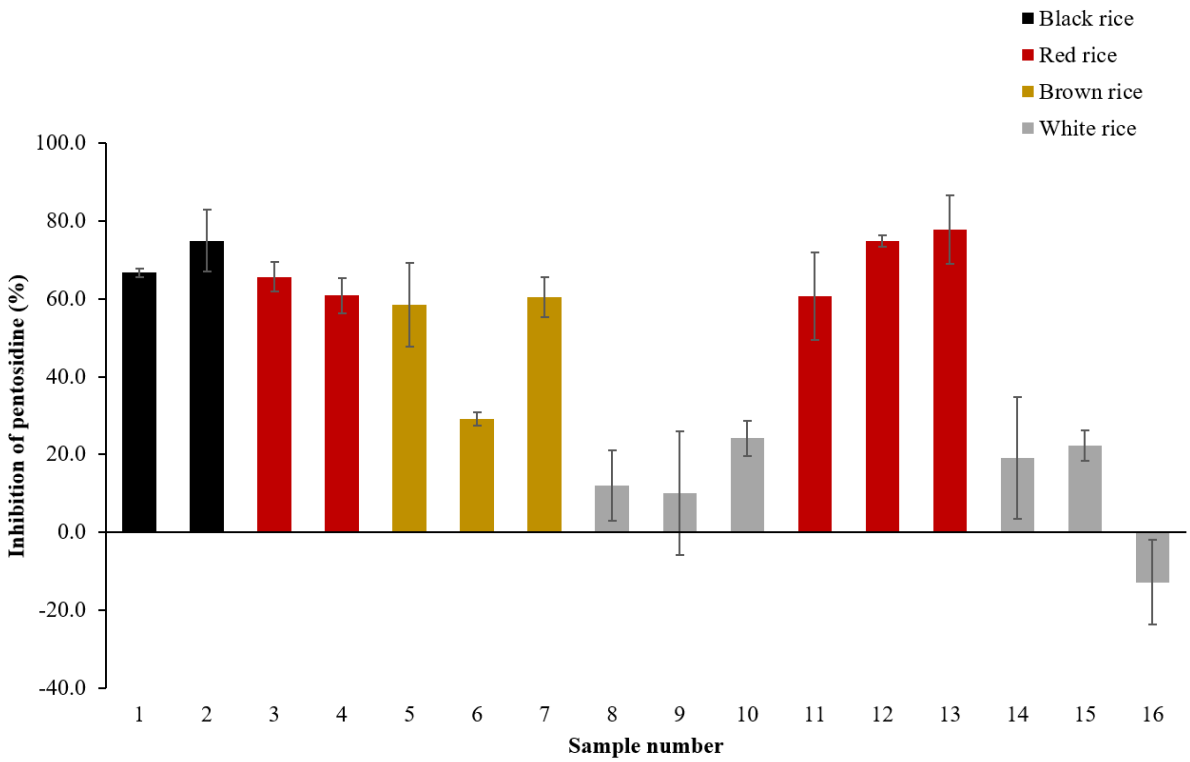


Figure 20. Inhibition of pentosidine by rice water extracts. The final concentration of samples was 0.5 mg/mL. Results are expressed as mean ± standard deviation, n=3. Sample number; 1, Asamurasaki, 2, Black Kodaimai, 3, Red Kodaimai, 4, Yuyakemochi, 5, Hitomebore (brown), 6, Koshihikari (brown), 7, Sasanishiki (brown), 8, Hitomebore (white), 9, Koshihikari (white), 10, Sasanishiki (white), 11, Kuruluthuda, 12, Pachchaperumal, 13, Madathavalu, 14, Ma Vee, 15, Suduru Hel, 16, Martin Samba.

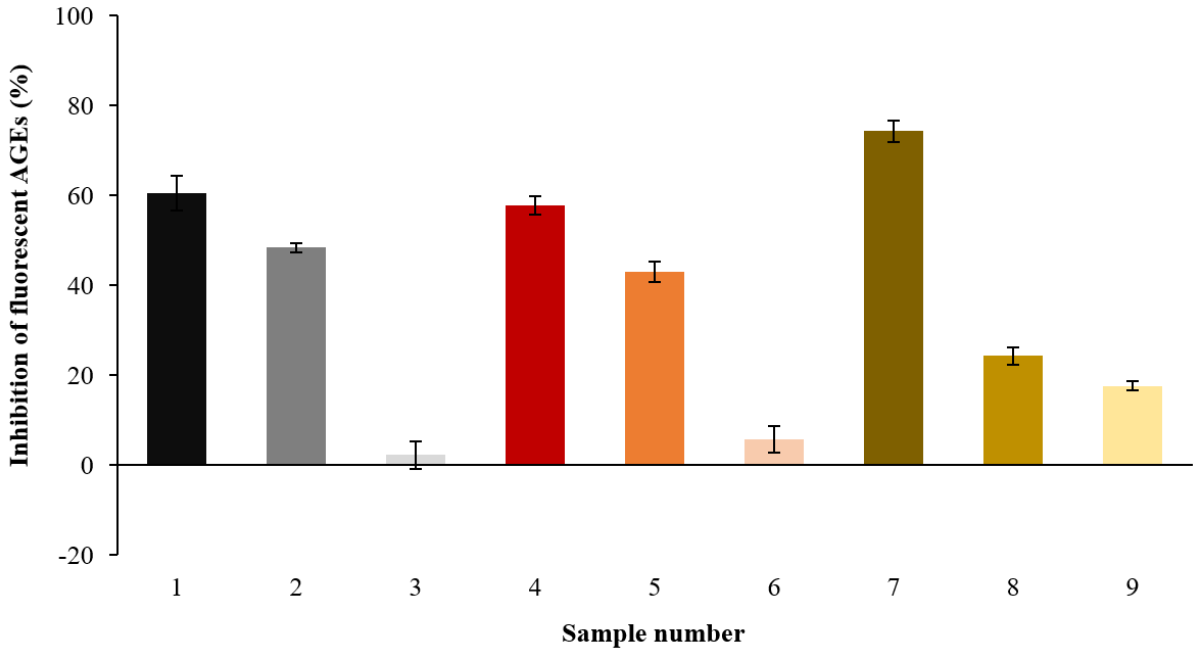


Figure 21. Inhibition of fluorescent AGEs by rice bran water extract compared with whole rice and polished rice. The final concentration of samples was 0.5 mg/mL. Results are expressed as mean ± standard deviation, n=3. AGEs, advanced glycation end products. Sample number; 1, *Japonica* black whole rice, 2, *Japonica* black rice bran, 3, *Japonica* black polished rice, 4, *Japonica* red whole rice, 5, *Japonica* red rice bran, 6, *Japonica* red polished rice, 7, *Indica* red whole rice, 8, *Indica* red rice bran, 9, *Indica* red polished rice.

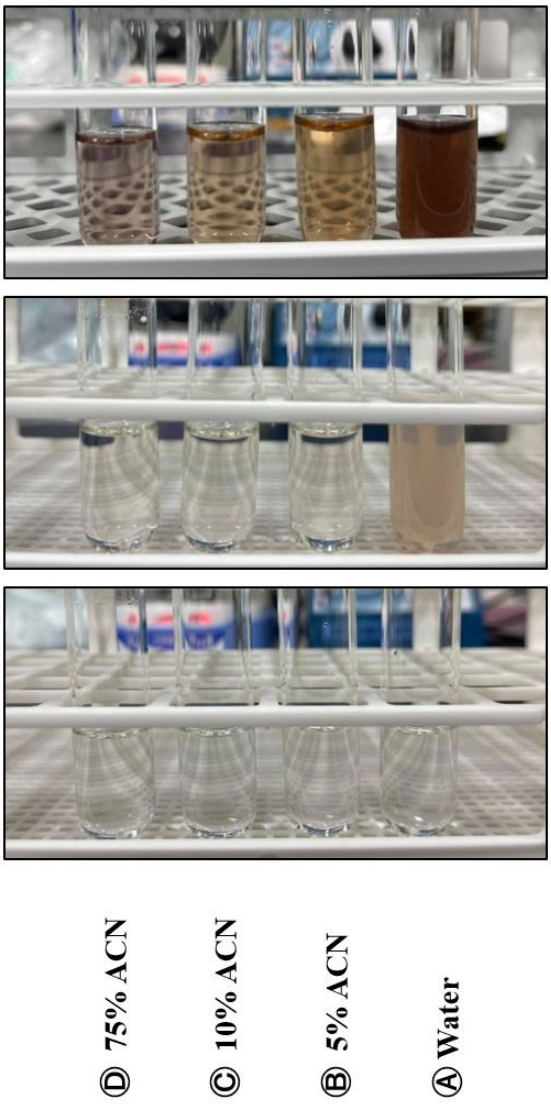


Figure 22. Fractional purification of rice bran water extracts by Oasis® HLB Plus.

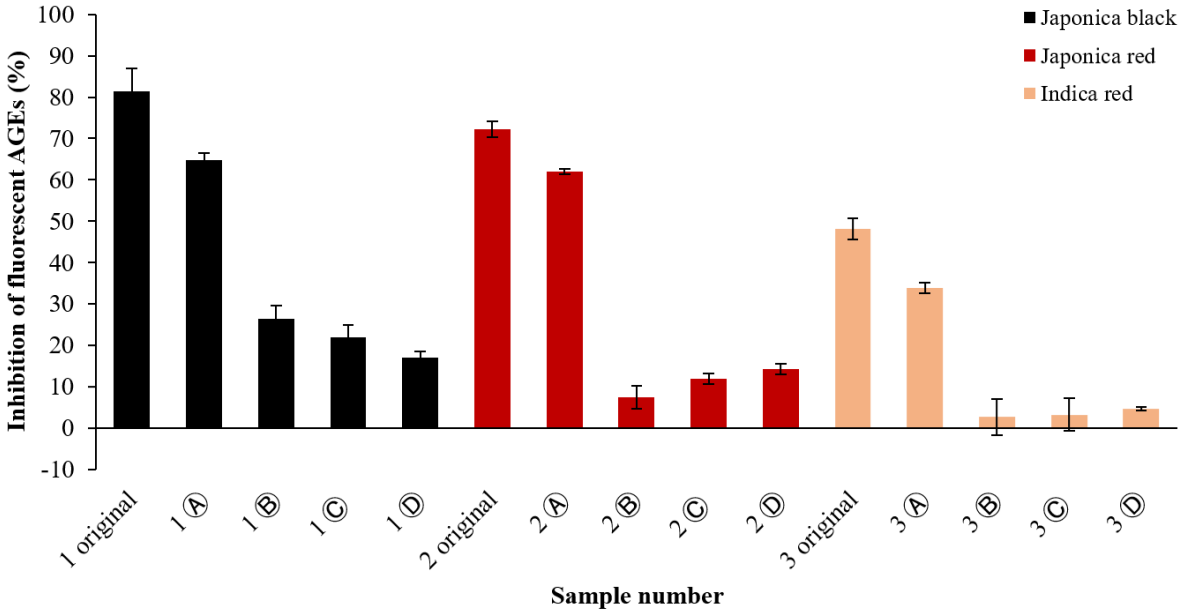


Figure 23. Inhibition of fluorescent AGEs by fractions of rice bran water extracts. The final concentration of samples was a 3-fold dilution of the original concentration (0.5 mg/mL). Results are expressed as mean ± standard deviation, n=3. AGEs, advanced glycation end products; ACN, acetonitrile. Sample number; 1 original, *Japonica* black rice bran water extract, 1 Ⓐ, *Japonica* black rice bran water extract water fraction, 1 Ⓑ, *Japonica* black rice bran water extract 5 % ACN fraction, 1 Ⓒ,*Japonica* black rice bran water extract 10 % ACN fraction, 1 Ⓓ, *Japonica* black rice bran water extract 75 % ACN fraction, 2 original, *Japonica* red rice bran water extract, 2 Ⓐ, *Japonica* red rice bran water extract water fraction, 2 Ⓑ, *Japonica* red rice bran water extract 5 % ACN fraction, 2 Ⓒ,*Japonica* red rice bran water extract 10 % ACN fraction, 2 Ⓓ, *Japonica* red rice bran water extract 75 % ACN fraction, 3 original, *Indica* red rice bran water extract, 3 Ⓐ, *Indica* red rice bran water extract water fraction, 3 Ⓑ, *Indica* red rice bran water extract 5 % ACN fraction, 3 Ⓒ,*Indica* red rice bran water extract 10 % ACN fraction, 3 Ⓓ, *Indica* red rice bran water extract 75 % ACN fraction.

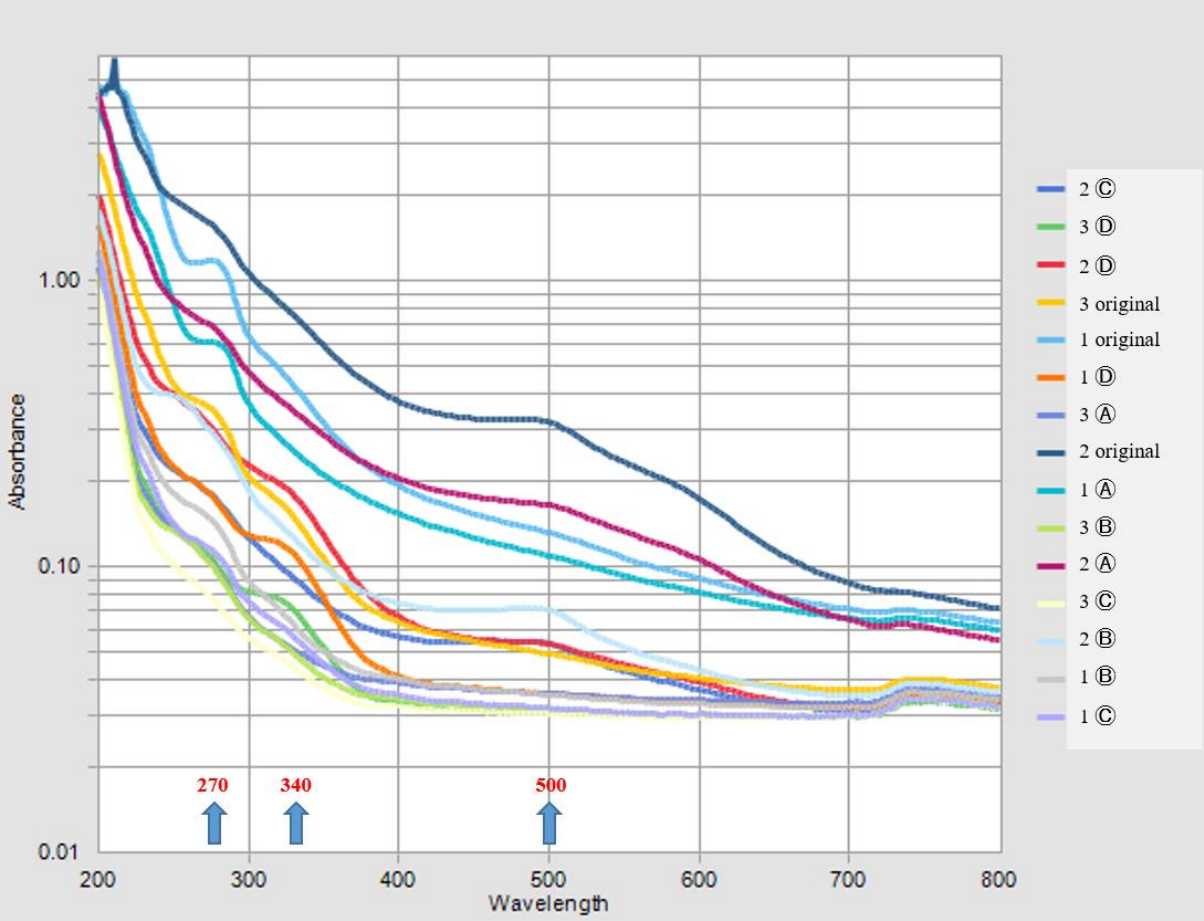


Figure 24. UV spectroscopy data of fractions of rice bran water extracts. ACN, acetonitrile. Sample number; 1 original, *Japonica* black rice bran water extract, 1 Ⓐ, *Japonica* black rice bran water extract water fraction, 1 Ⓑ, *Japonica* black rice bran water extract 5 % ACN fraction, 1 Ⓒ,*Japonica* black rice bran water extract 10 % ACN fraction, 1 Ⓓ, *Japonica* black rice bran water extract 75 % ACN fraction, 2 original, *Japonica* red rice bran water extract, 2 Ⓐ, *Japonica* red rice bran water extract water fraction, 2 Ⓑ, *Japonica* red rice bran water extract 5 % ACN fraction, 2 Ⓒ,*Japonica* red rice bran water extract 10 % ACN fraction, 2 Ⓓ, *Japonica* red rice bran water extract 75 % ACN fraction, 3 original, *Indica* red rice bran water extract, 3 Ⓐ, *Indica* red rice bran water extract water fraction, 3 Ⓑ, *Indica* red rice bran water extract 5 % ACN fraction, 3 Ⓒ,*Indica* red rice bran water extract 10 % ACN fraction, 3 Ⓓ, *Indica* red rice bran water extract 75 % ACN fraction.

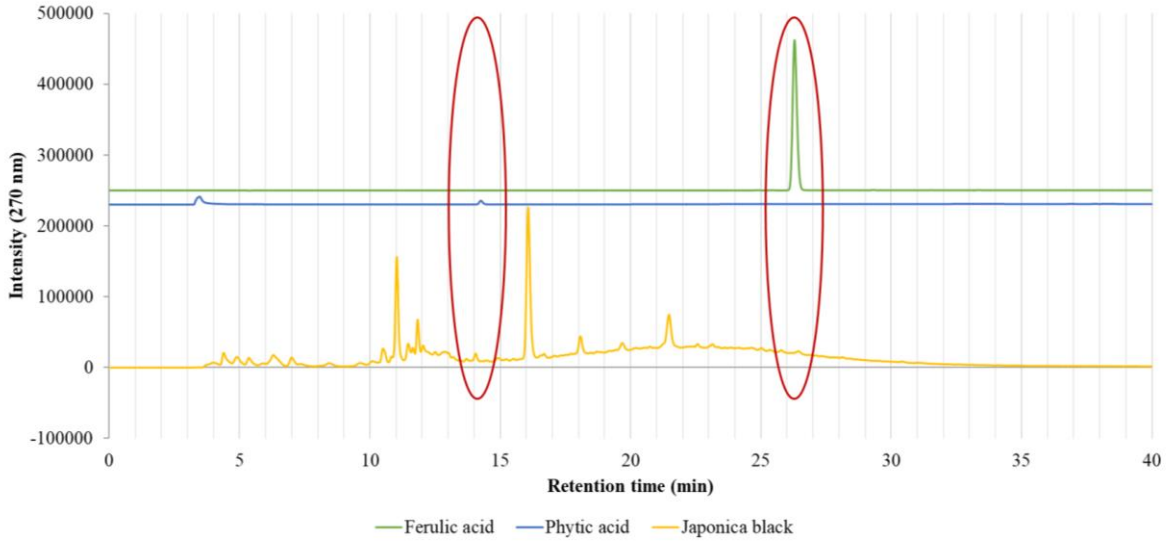


Figure 25. C18 HPLC chromatogram of Oasis HLB cartridge column of *Japonica* black rice bran extract compared with ferulic acid and phytic acid.

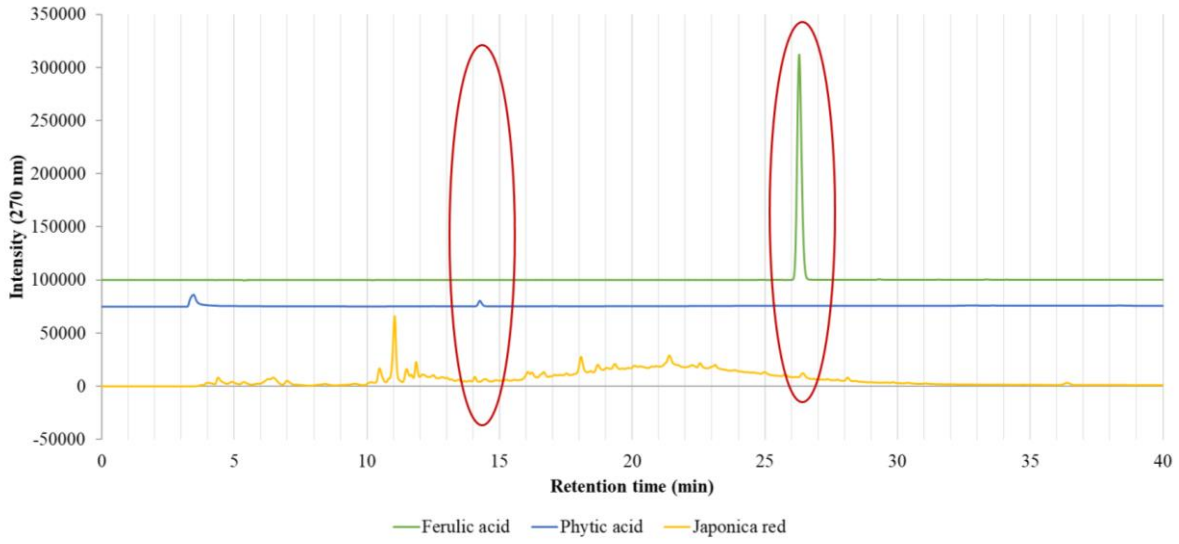


Figure 26. C18 HPLC chromatogram of Oasis HLB cartridge column of *Japonica* red rice bran extract compared with ferulic acid and phytic acid.

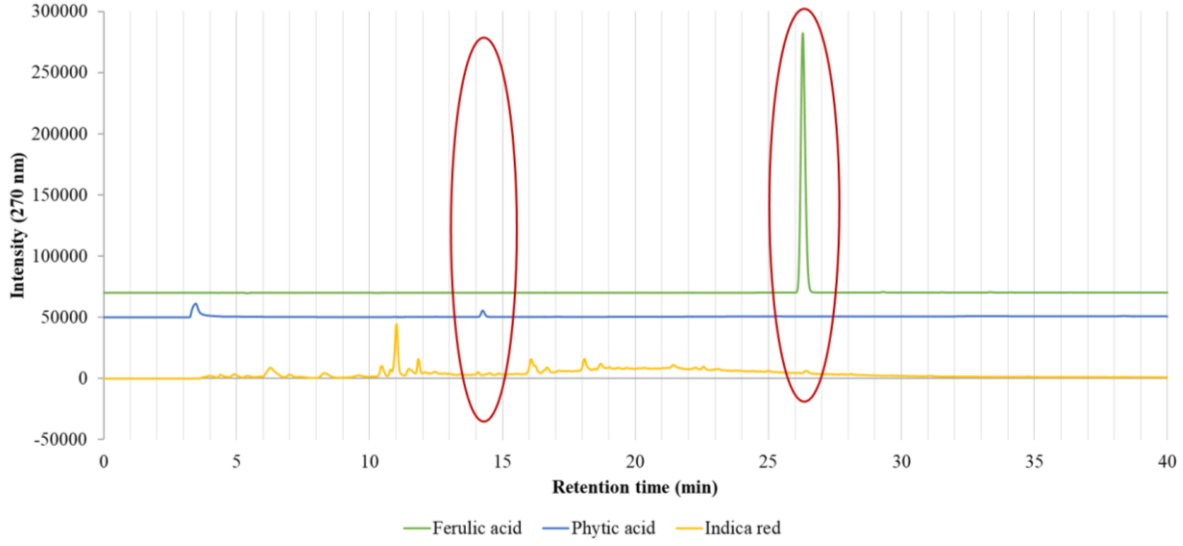


Figure 27. C18 HPLC chromatogram of Oasis HLB cartridge column of *Indica* red rice bran extract compared with ferulic acid and phytic acid.

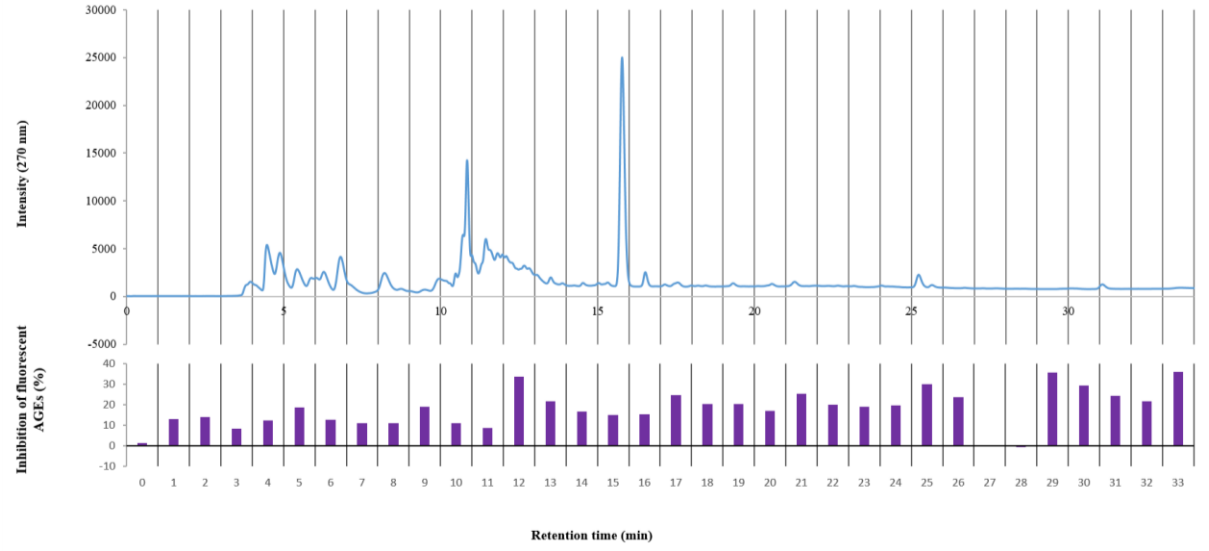


Figure 28. C18 HPLC chromatogram of Oasis HLB cartridge column of the water fraction of *Japonica* black rice bran extract and inhibition of fluorescent AGEs by the respective HPLC fractions. Results are expressed as mean (standard deviation not shown for better visual clarity), n=3. AGEs, advanced glycation end products.

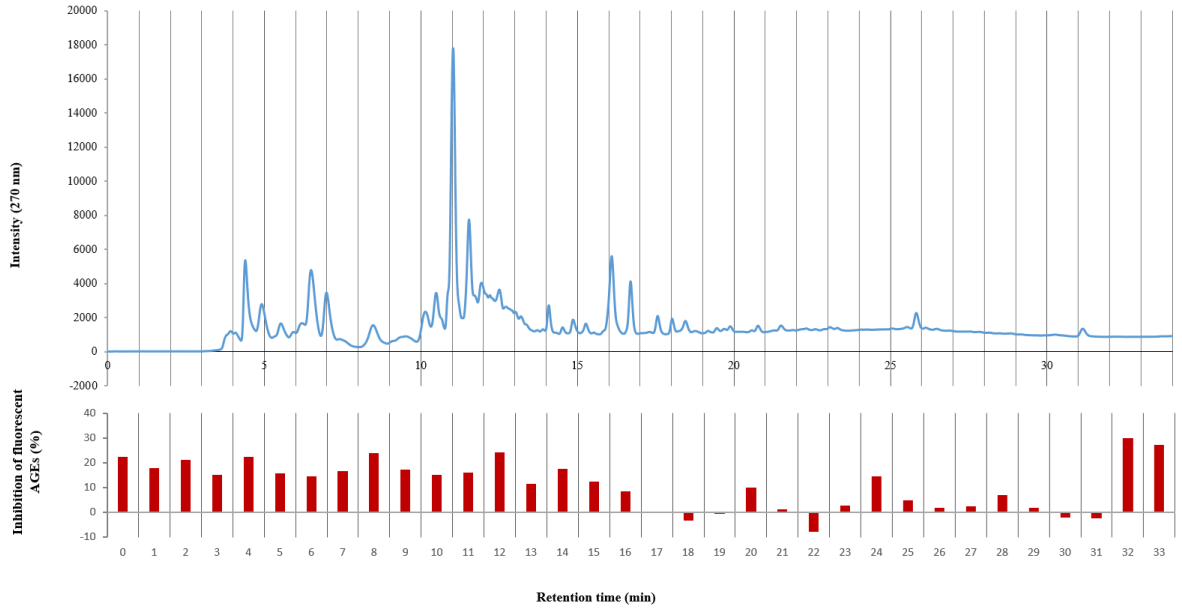


Figure 29. C18 HPLC chromatogram of Oasis HLB cartridge column of the water fraction of *Japonica* red rice bran extract and inhibition of fluorescent AGEs by the respective HPLC fractions. Results are expressed as mean (standard deviation not shown for better visual clarity), n=3. AGEs, advanced glycation end products.

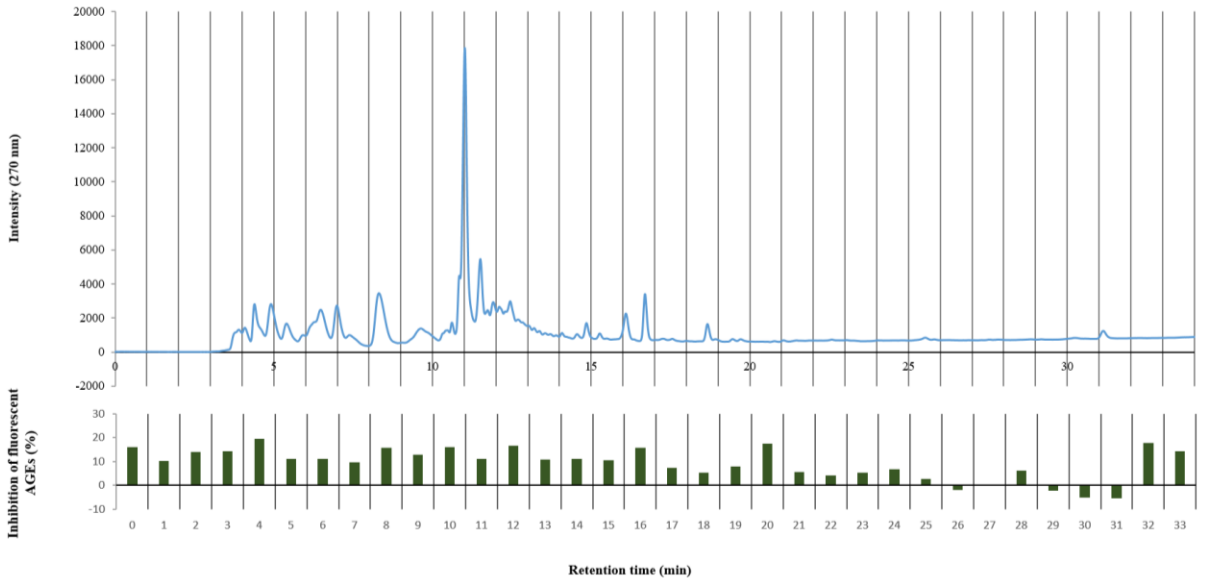


Figure 30. C18 HPLC chromatogram of Oasis HLB cartridge column of the water fraction of *Indica* red rice bran extract and inhibition of fluorescent AGEs by the respective HPLC fractions. Results are expressed as mean (standard deviation not shown for better visual clarity), n=3. AGEs, advanced glycation end products.

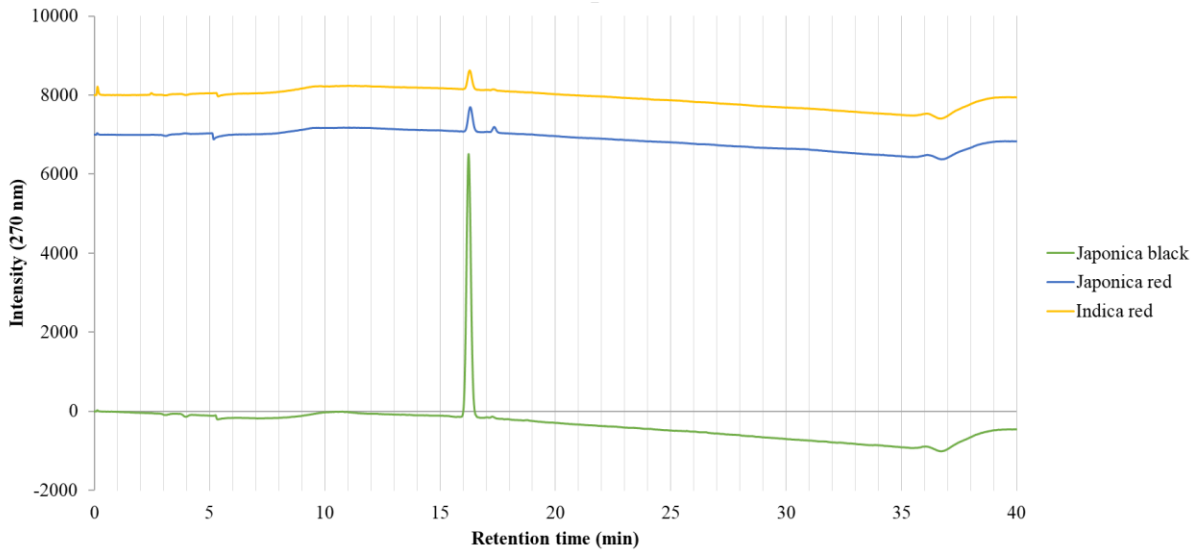


Figure 31. C18 HPLC chromatogram of Oasis HLB cartridge column of purified fraction #15.

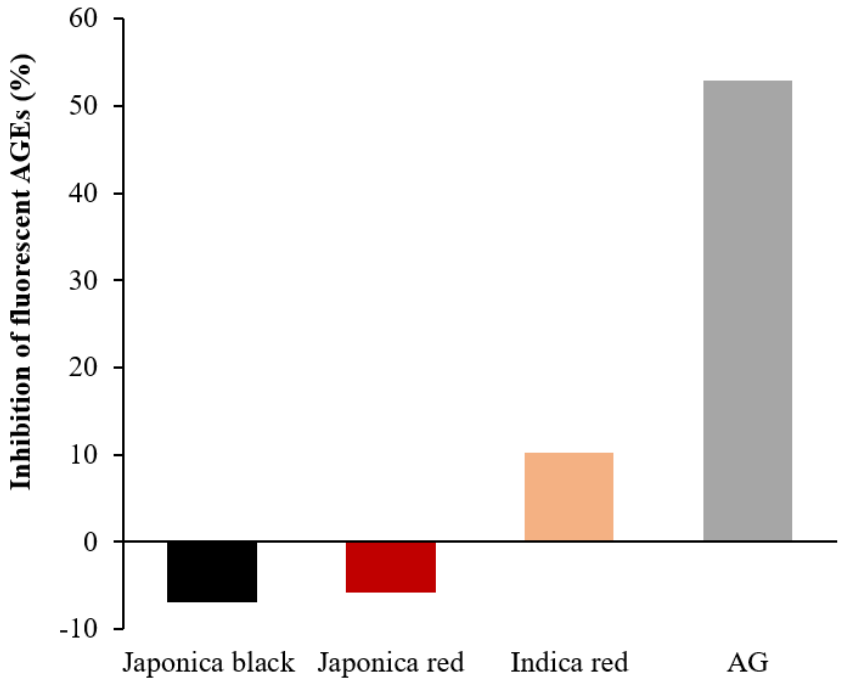


Figure 32. Inhibition of fluorescent AGEs by fraction #15. n=2. AGEs, advanced glycation end products; AG, aminoguanidine.

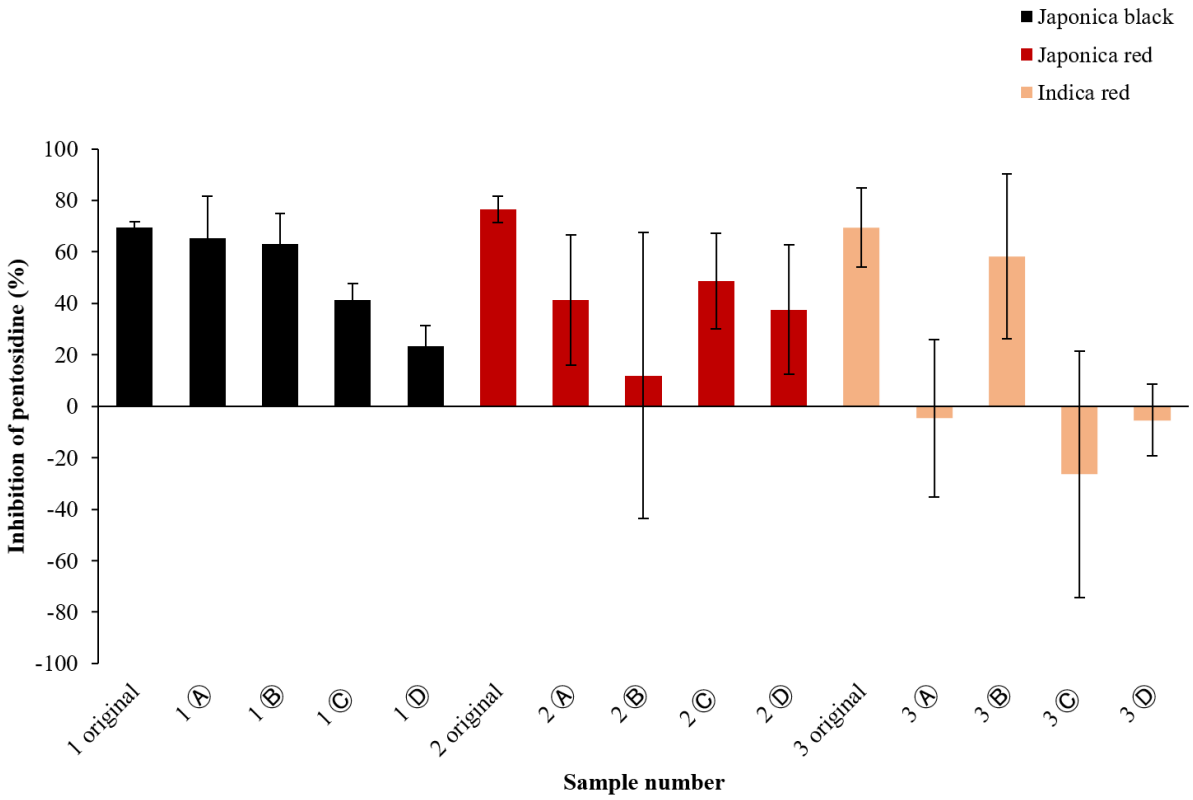


Figure 33. Inhibition of pentosidine by fractions of rice bran water extracts. The final concentration of samples was a 3-fold and a 2.5-fold dilution of the original concentration (0.5 mg/mL). Results are expressed as mean ± standard deviation, n=3. ACN, acetonitrile. Sample number; 1 original, *Japonica* black rice bran water extract, 1 Ⓐ, *Japonica* black rice bran water extract water fraction, 1 Ⓑ, *Japonica* black rice bran water extract 5 % ACN fraction, 1 Ⓒ,*Japonica* black rice bran water extract 10 % ACN fraction, 1 Ⓓ, *Japonica* black rice bran water extract 75 % ACN fraction, 2 original, *Japonica* red rice bran water extract, 2 Ⓐ, *Japonica* red rice bran water extract water fraction, 2 Ⓑ, *Japonica* red rice bran water extract 5 % ACN fraction, 2 Ⓒ,*Japonica* red rice bran water extract 10 % ACN fraction, 2 Ⓓ, *Japonica* red rice bran water extract 75 % ACN fraction, 3 original, *Indica* red rice bran water extract, 3 Ⓐ, *Indica* red rice bran water extract water fraction, 3 Ⓑ, *Indica* red rice bran water extract 5 % ACN fraction, 3 Ⓒ,*Indica* red rice bran water extract 10 % ACN fraction, 3 Ⓓ, *Indica* red rice bran water extract 75 % ACN fraction.

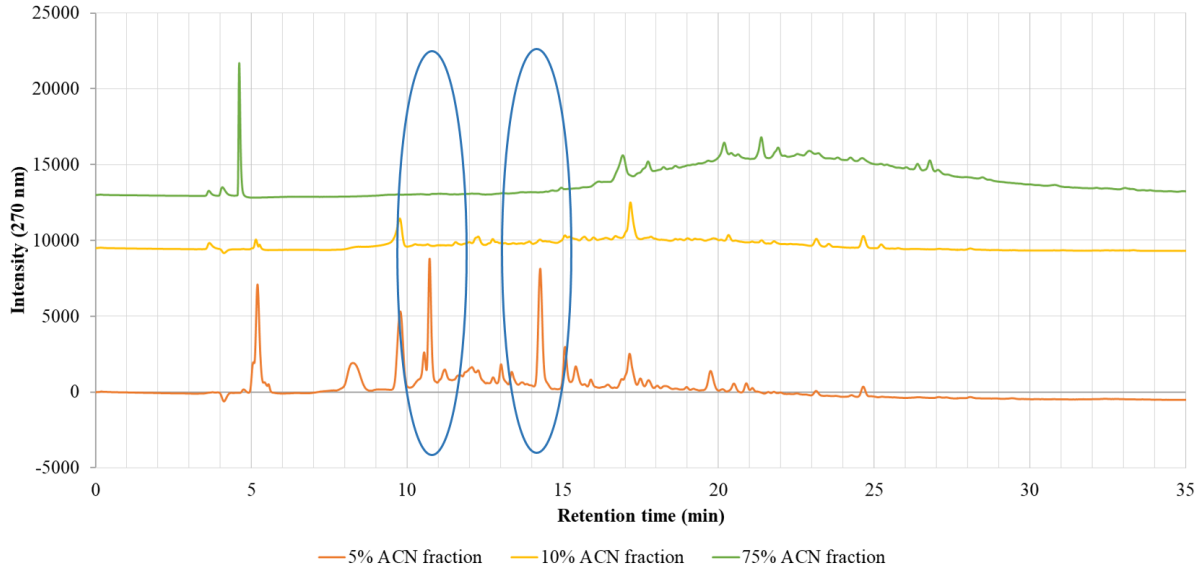


Figure 34. C18 HPLC chromatogram of Oasis HLB cartridge column of 5 % ACN fraction, 10 % ACN fraction, and 75 % ACN fraction of *Indica* red rice bran water extract.

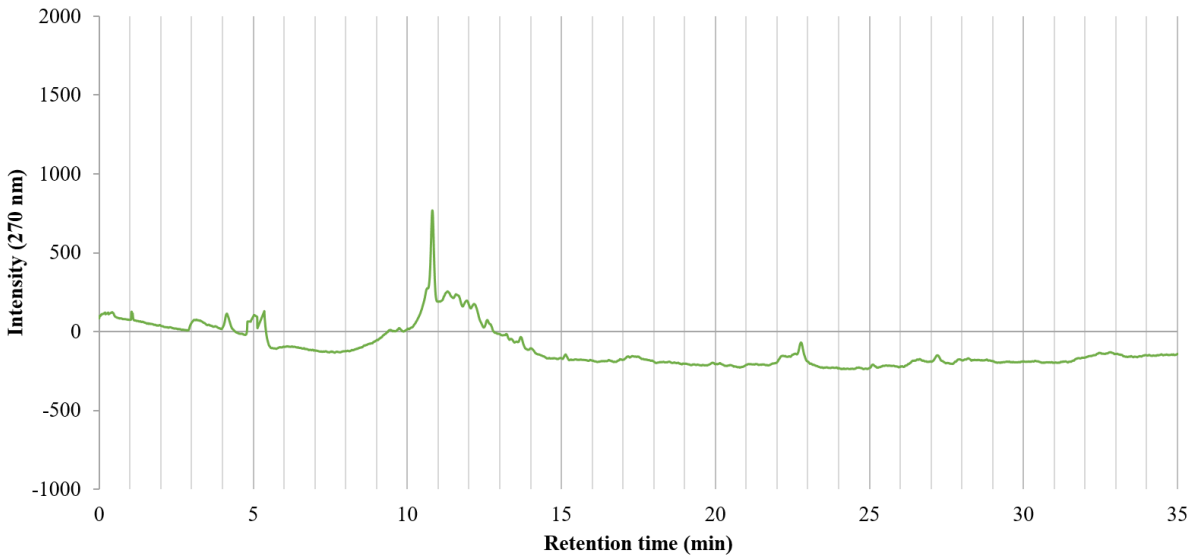


Figure 35. C18 HPLC chromatogram of Oasis HLB cartridge column of purified fraction #10.

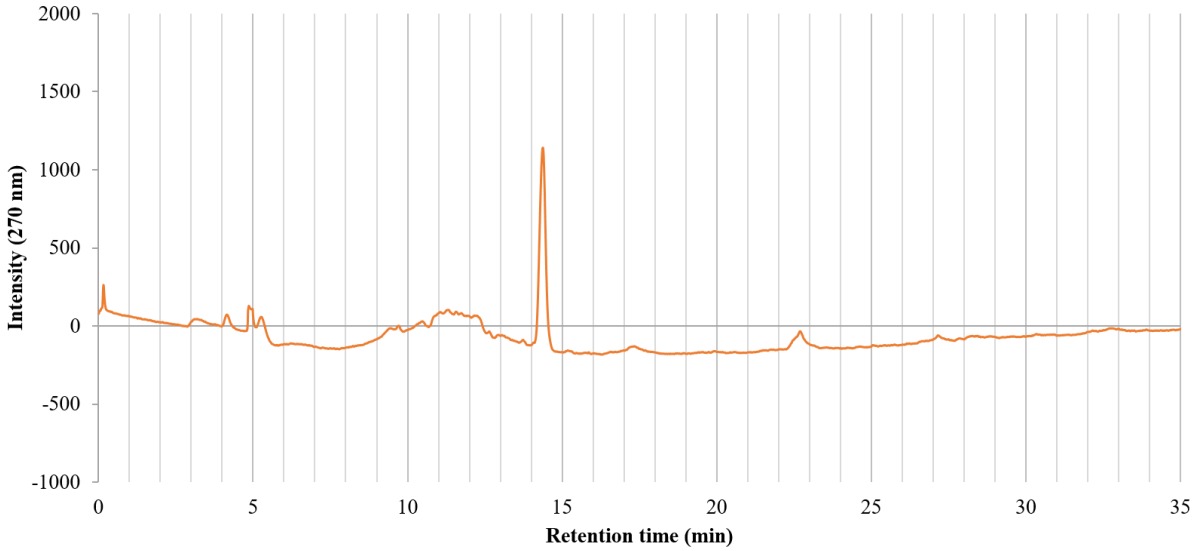


Figure 36. C18 HPLC chromatogram of Oasis HLB cartridge column of purified fraction #14.

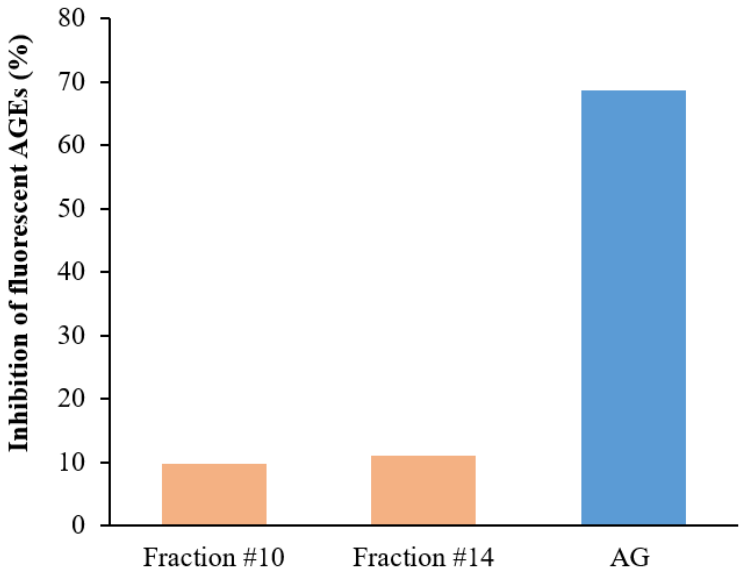


Figure 37. Inhibition of fluorescent AGEs by fraction #10, fraction #14, and AG. n=2. AGEs, advanced glycation end products; AG, aminoguanidine.

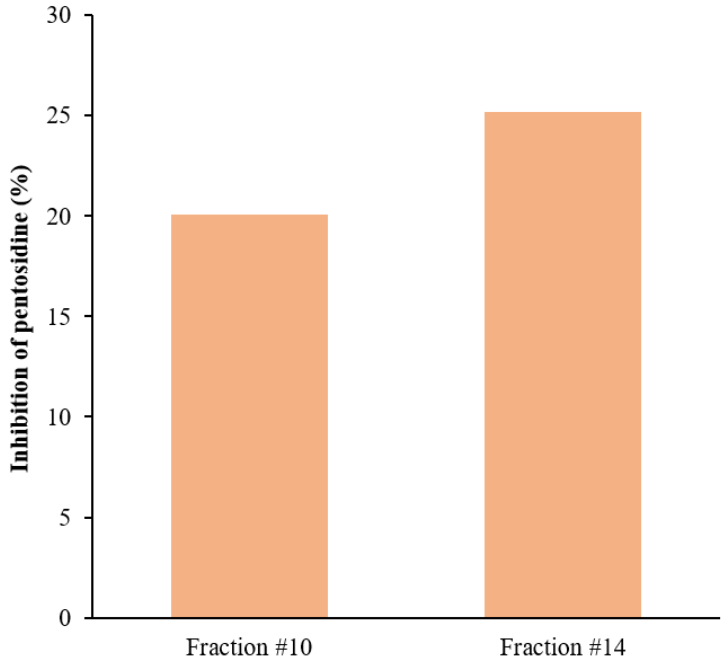


Figure 38. Inhibition of pentosidine by fraction #10 and fraction #14. n=2.

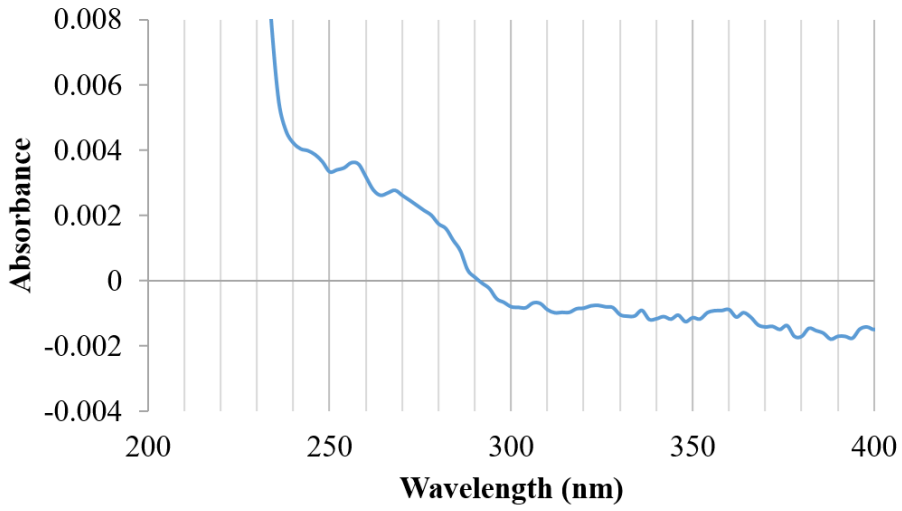


Figure 39. UV spectroscopy data of fraction #10.

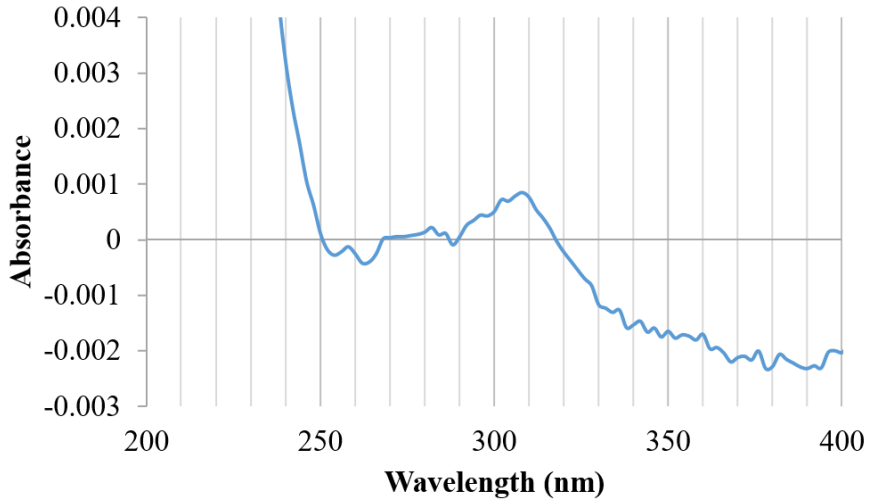


Figure 40. UV spectroscopy data of fraction #14.

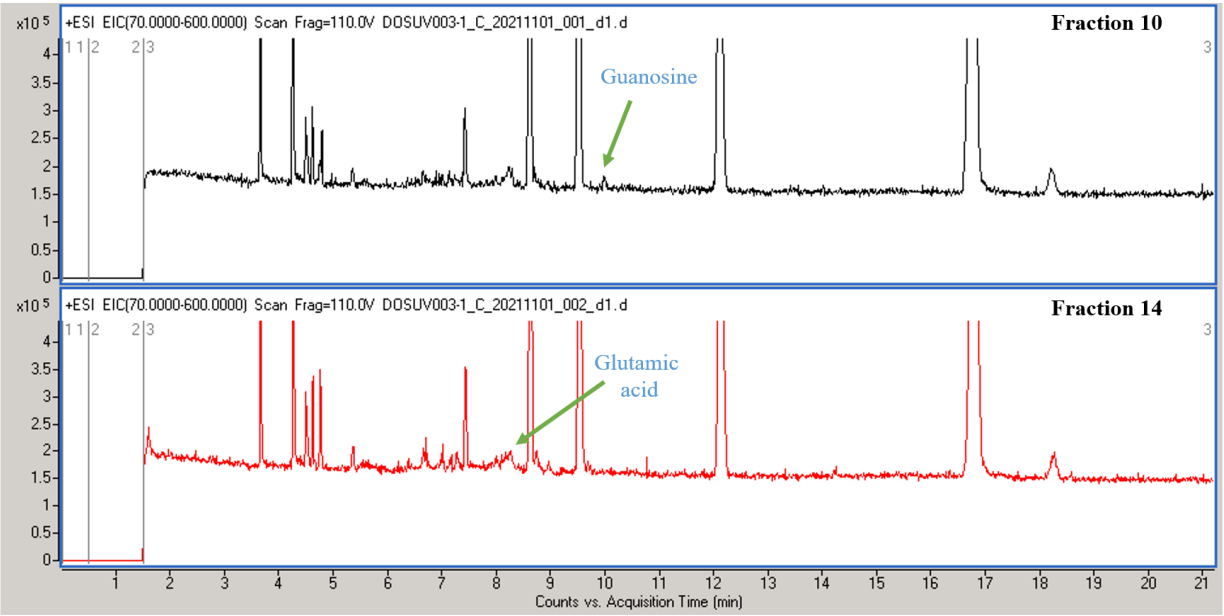


Figure 41. Extracted ion chromatograms (EIC) of fraction #10 and fraction #14.

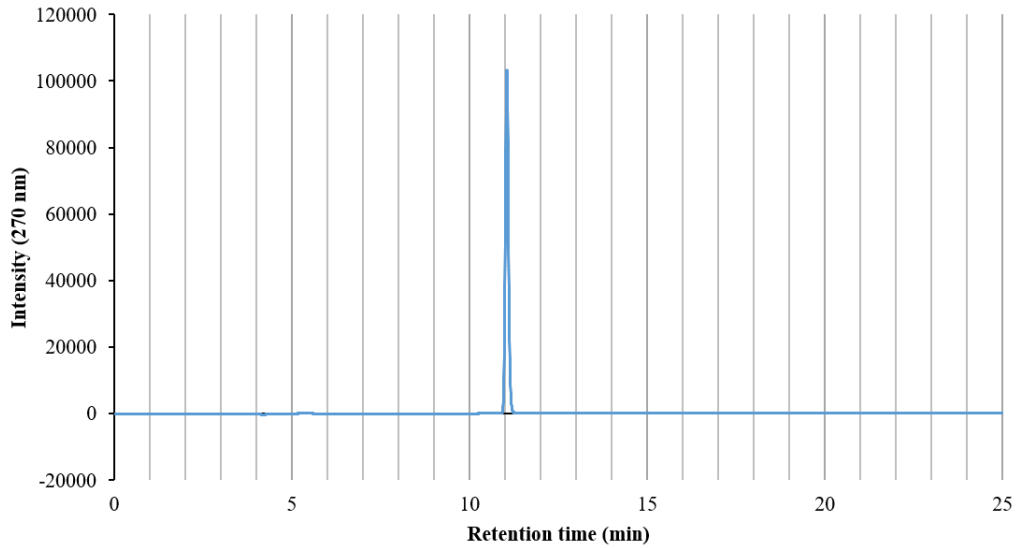
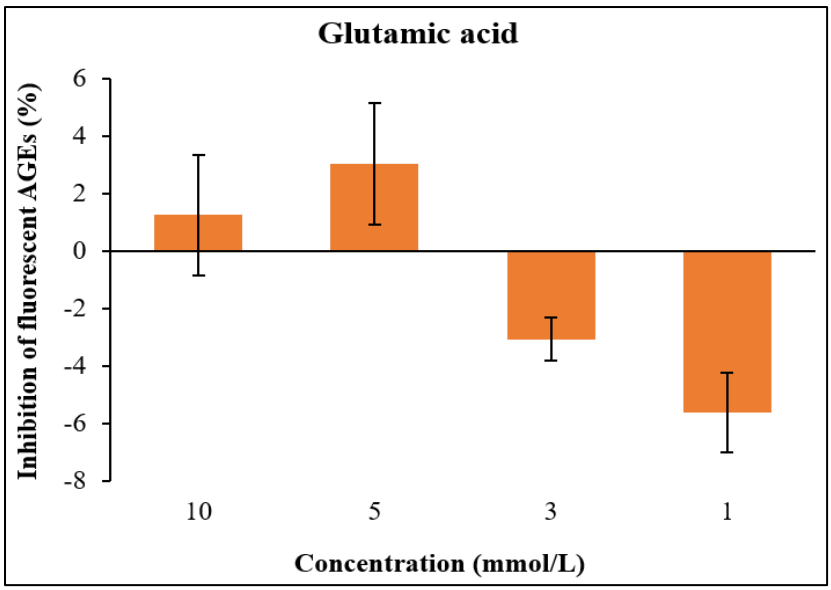


Figure 42. C18 HPLC chromatogram of Oasis HLB cartridge column of 0.01 mmol/L guanosine.



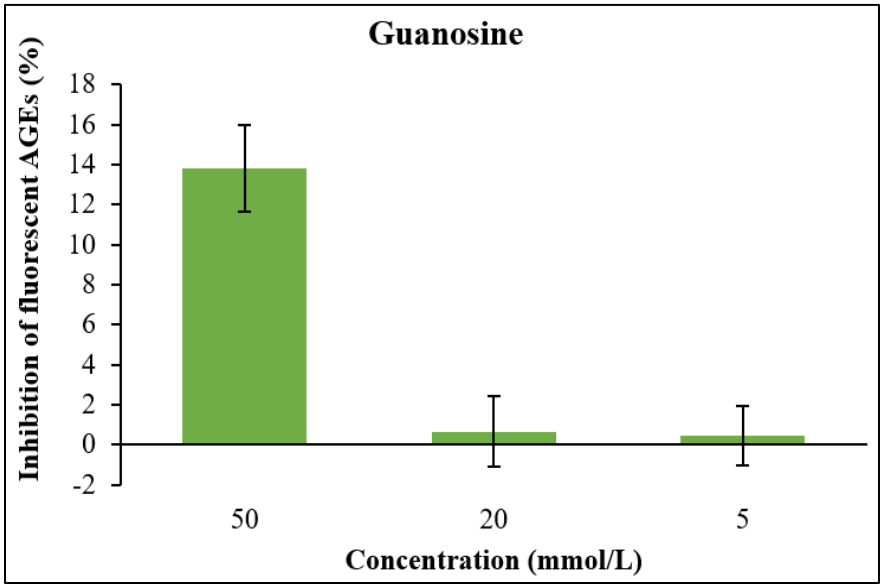


Figure 43. Inhibition of fluorescent AGEs by glutamic acid and guanosine. Results are expressed as mean ± standard deviation, n=3. AGEs, advanced glycation end products.

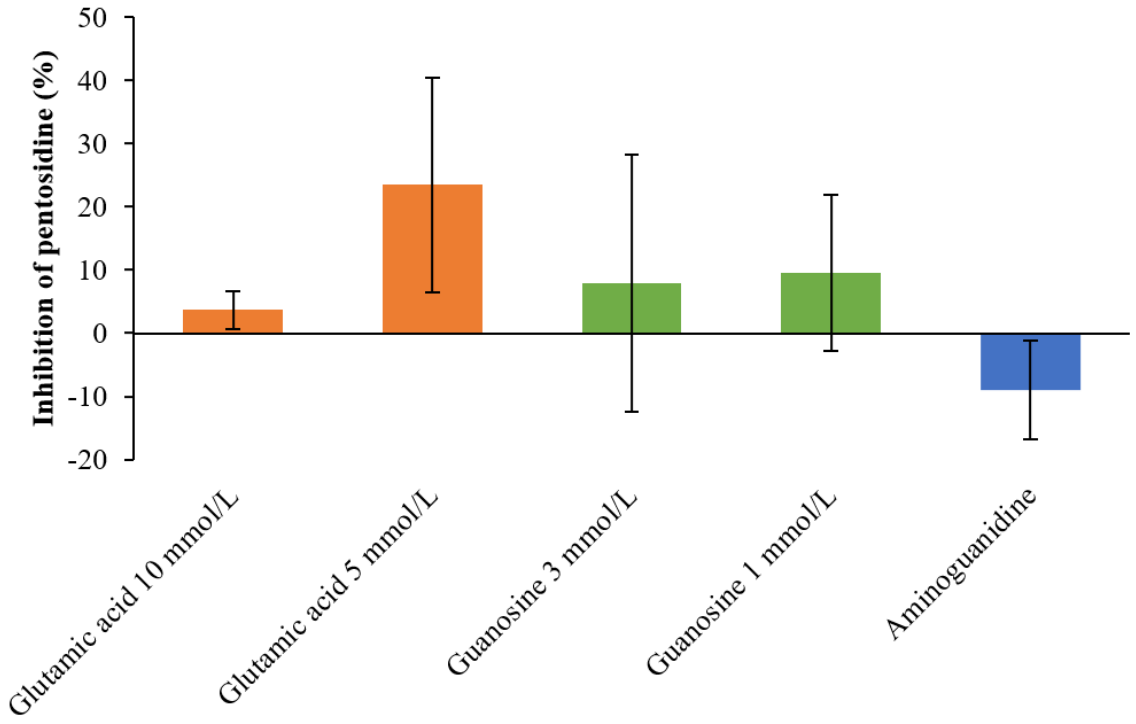


Figure 44. Inhibition of pentosidine by glutamic acid and guanosine. Results are expressed as mean ± standard deviation, n=3.

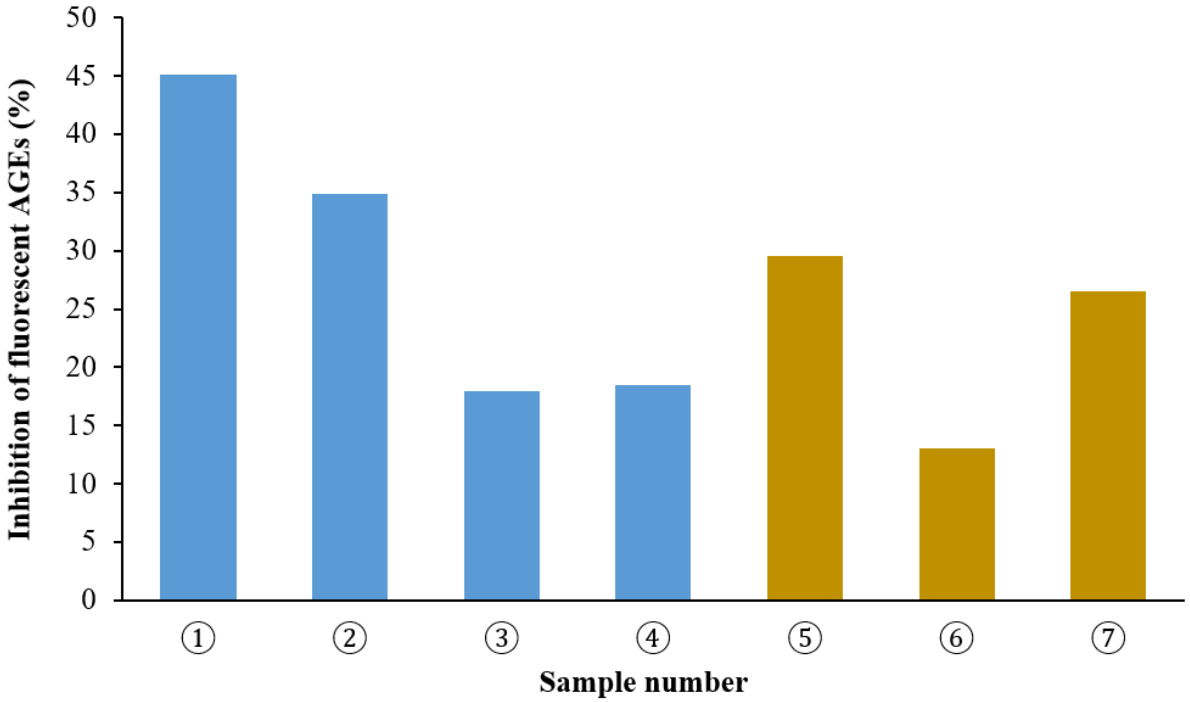


Figure 45. Inhibition of fluorescent AGEs by samples provided by Toyo Rice Co. Ltd. The final concentration of samples was 0.6 mg/mL. n=2. AGEs, advanced glycation end products. Sample number; ①, brown rice, ②, polished rice A (DBR), ③, Polished rice B (SARFR), ④, white rice, ⑤, layer A (bran layer), ⑥, layer B (sub-aleurone layer), ⑦, layer C (layer A + B).

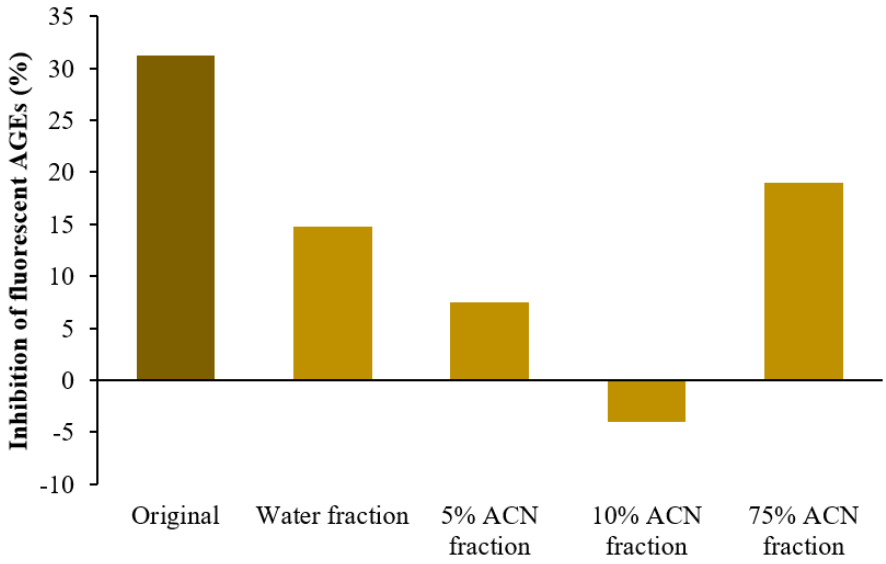


Figure 46. Inhibition of fluorescent AGEs by fractions of bran layer sample provided by Toyo Rice Co. Ltd. The final concentration of samples was a 3-fold dilution of the original concentration (0.6 mg/mL). n=2. AGEs, advanced glycation end products; ACN, acetonitrile.

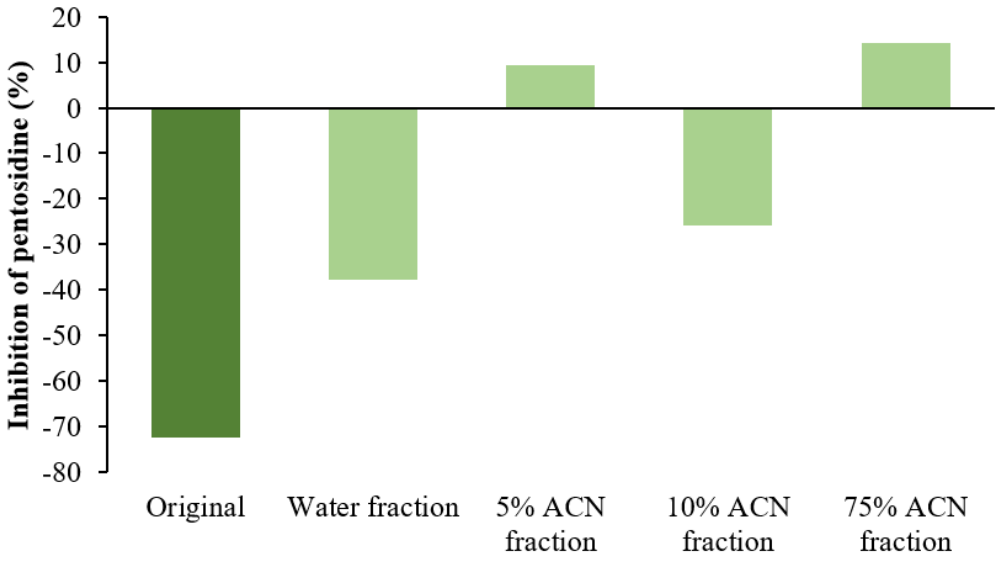


Figure 47. Inhibition of pentosidine by fractions of bran layer sample provided by Toyo Rice Co. Ltd. The final concentration of samples was a 3-fold dilution of the original concentration (0.6 mg/mL). n=2. ACN, acetonitrile.

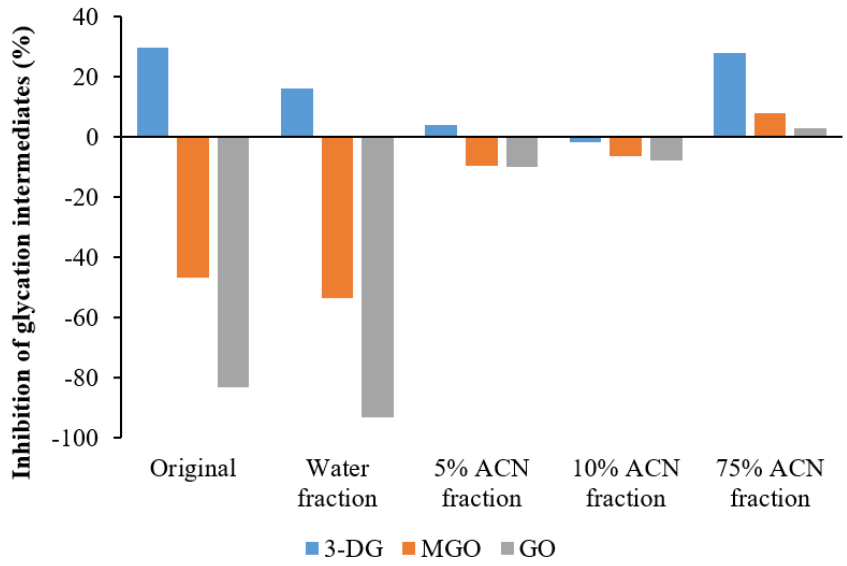


Figure 48. Inhibition of glycation intermediates by fractions of bran layer sample provided by Toyo Rice Co. Ltd. The final concentration of samples was a 3-fold dilution of the original concentration (0.6 mg/mL). n=2. ACN, acetonitrile; 3-DG, 3-deoxyglocosone; MGO, methylglyoxal; GO, glyoxal.

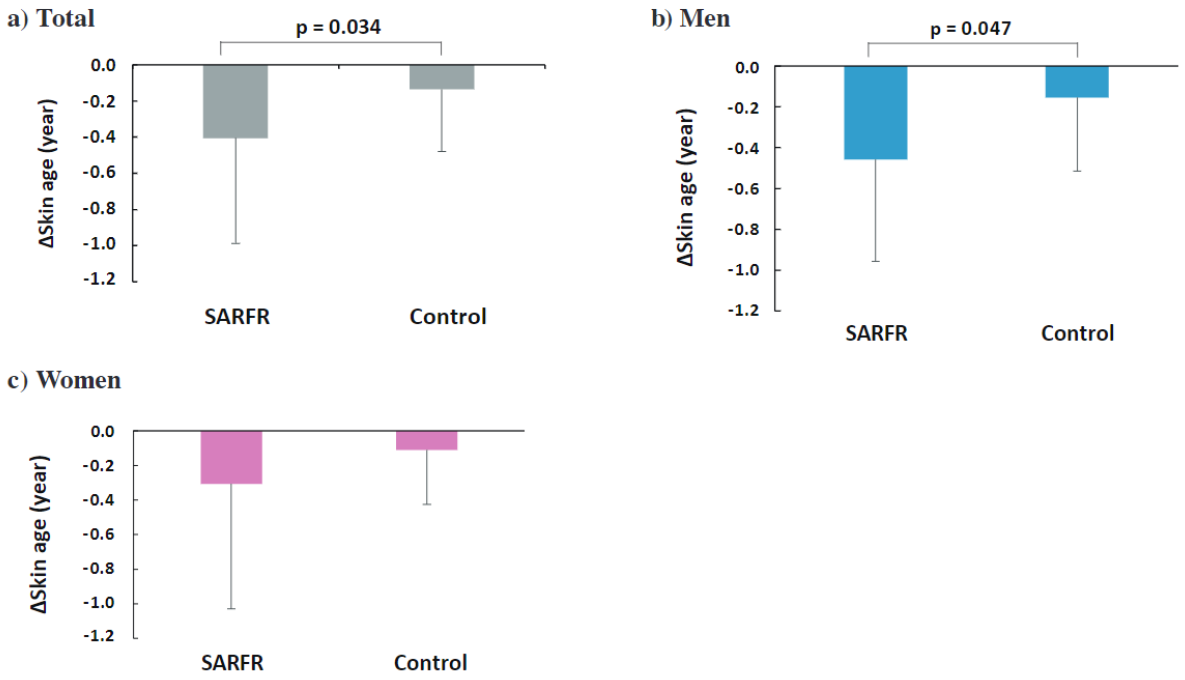


Figure 49. Change of skin age. a) Total, SARFR group, n = 37; the control group, n = 22. b) Men, SARFR group, n = 24; the control group, n = 13. c) Women SARFR group, n = 13; the control group, n = 9. Skin age evaluated by Clreo-Pro (Fujitex). Results are expressed as mean ± SEM; Statistical analysis by Wilcoxon signed rank test. SARFR, sub-aleurone layer residual rinse-free rice; SEM, standard error mean.

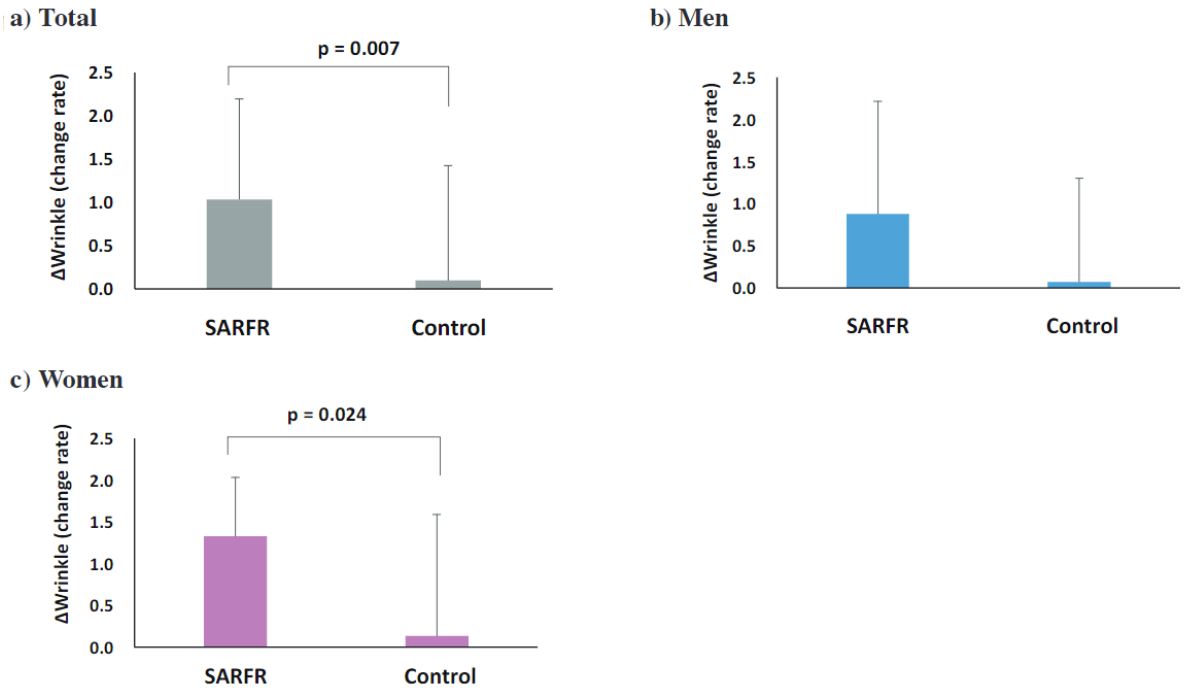


Figure 50. Change of wrinkles. a) Total, SARFR group, n = 37; the control group, n = 22. b) Men, SARFR group, n = 24; the control group, n = 13. c) Women SARFR group, n = 13; the control group, n = 9. Winkles are evaluated by Clreo-Pro (Fujitex). Results are expressed as mean ± SEM; Statistical analysis by Wilcoxon signed rank test. SARFR, sub-aleurone layer residual rinse-free rice; SEM, standard error mean.

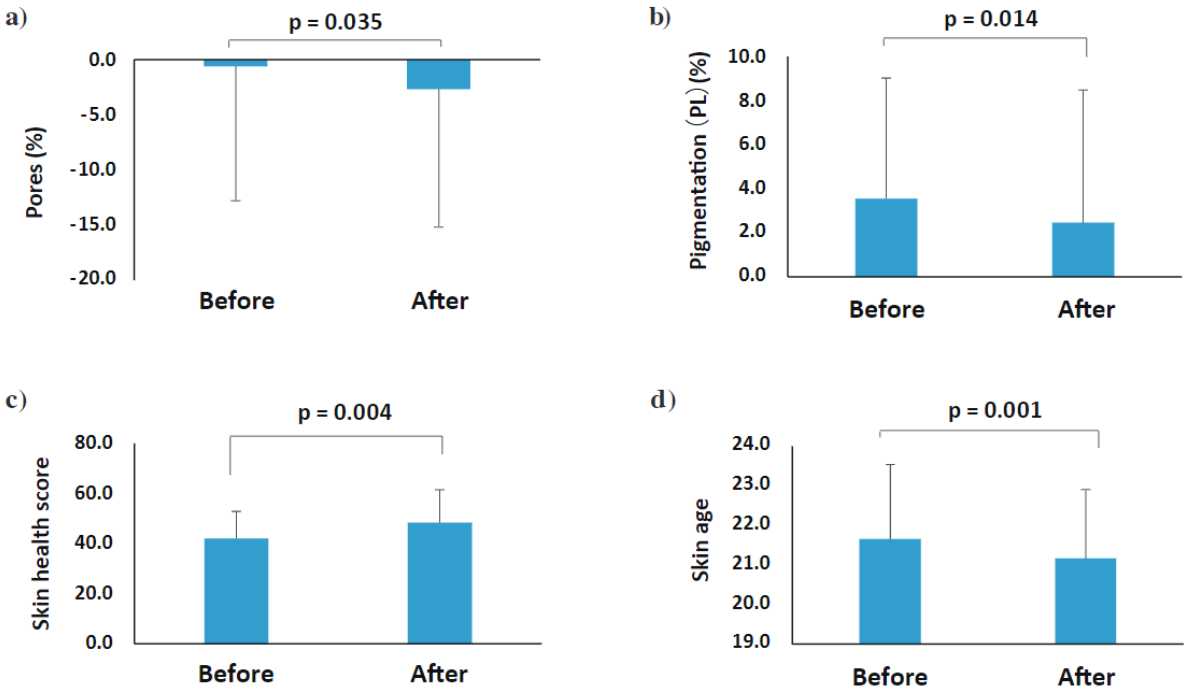


Figure 51. Effects of SARFR on skin condition: Subclass analysis in home students. a) Pores. b) Pigmentation by PL. c) Total skin health score. d) Skin age. Skin conditions are evaluated by Clreo-Pro (Fujitex). Results are expressed as mean ± SEM; number of the home students, n = 12; Statistical analysis by paired-t test. SARFR, sub-aleurone layer residual rinse-free rice; PL, polished rice; SEM, standard error mean.

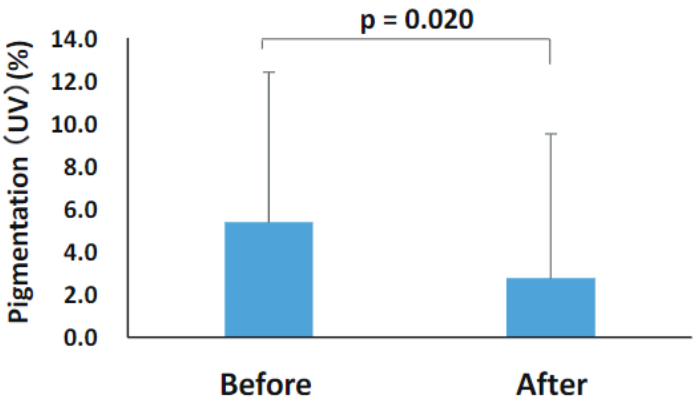


Figure 52. Effects of SARFR on skin condition: Subclass analysis in the boarding house students.

Skin conditions are evaluated by Clreo-Pro (Fujitex). Results are expressed as mean ± SEM; number of the boarding house students, n = 8; Statistical analysis by paired-t test. SARFR, sub-aleurone layer residual rinse-free rice; PL, polished rice; SEM, standard error mean.

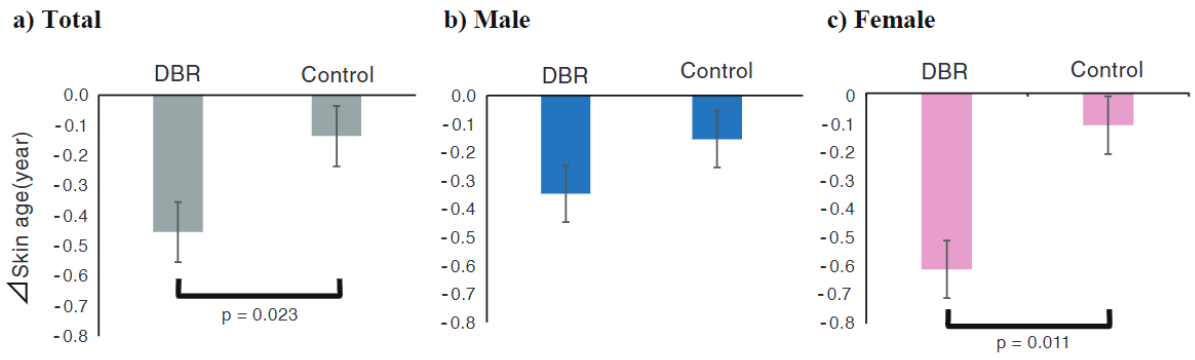


Figure 53 Change of skin age. a) Total, DBR group, n = 43; the control group, n = 22. b) Male, DBR group, n = 25, the control group, n = 13. c) Female, DBR group, n = 18 the control group, n = 9. Skin age evaluated by Clreo-Pro (Fujitex). Results are expressed as mean ± SEM; Statistical analysis by Wilcoxon signed rank test. DBR, dewaxed brown rice; SEM, standard error mean.

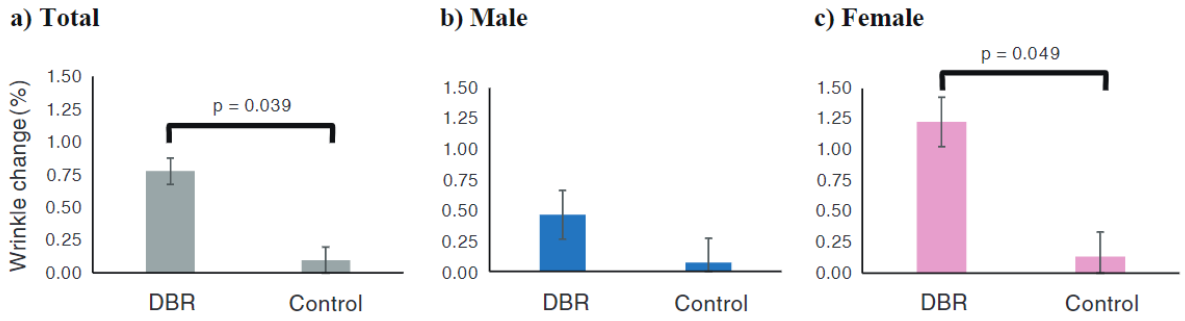


Figure 54. Change of wrinkles. a) Total, DBR group, n = 43; the control group, n = 22. b) Male, DBR group, n = 25, the control group, n = 13. c) Female, DBR group, n = 18 the control group, n = 9. Winkles are evaluated by Clreo-Pro (Fujitex). Results are expressed as mean ± SEM; Statistical analysis by Wilcoxon signed rank test. DBR, dewaxed brown rice; SEM, standard error mean.

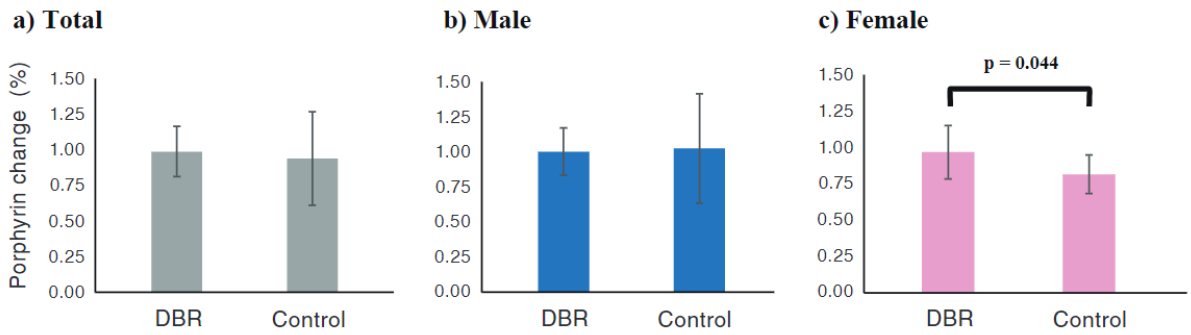


Figure 55. Change of porphyrin. a) Total, DBR group, n = 43; the control group, n = 22. b) Male, DBR group, n = 25, the control group, n = 13. c) Female, DBR group, n = 18, the control group, n = 9. Winkles are evaluated by Clreo-Pro (Fujitex). Results are expressed as mean ± SEM; Statistical analysis by Wilcoxon signed rank test. DBR, dewaxed brown rice; SEM, standard error mean.

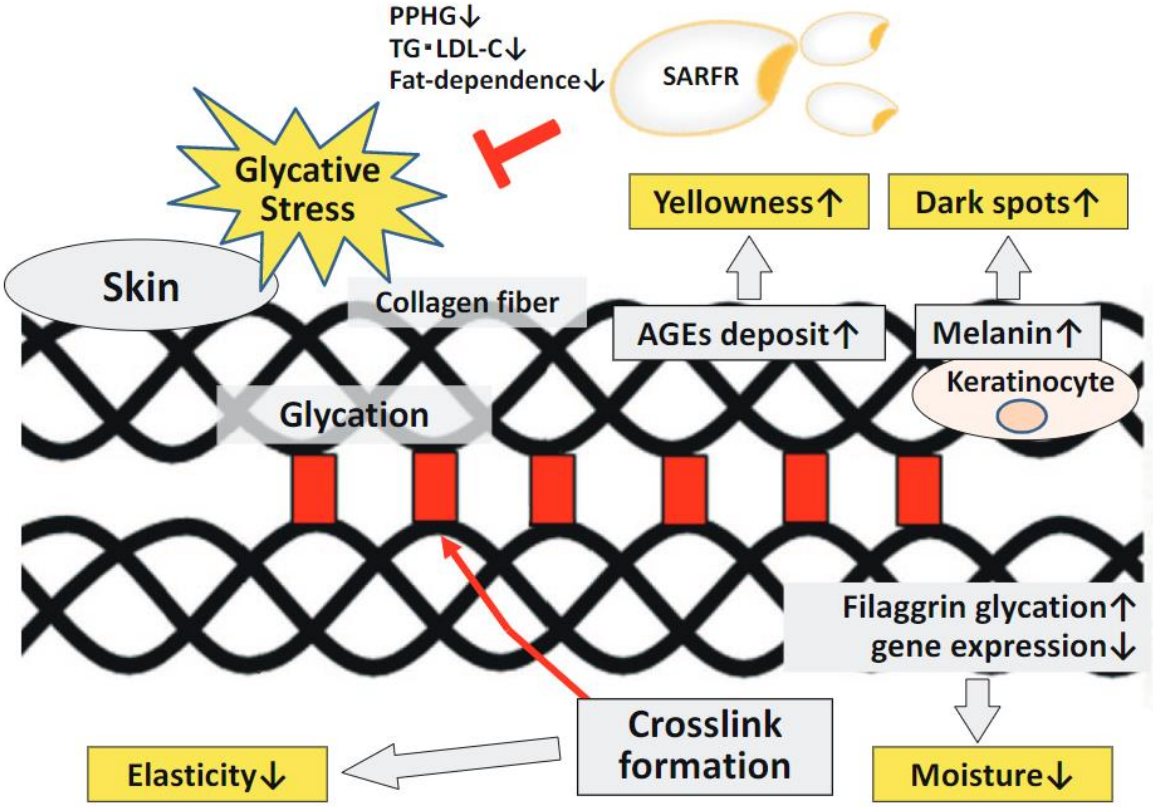


Figure 56. Glycative stress-induced skin damages and the possible suppressive actions by SARFR.

SARFR, sub-aleurone layer residual rinse-free rice; AGEs, advanced glycation end products; PPHG, post-prandial hyperglycemia: TG, triglyceride, LDL-C, low-density lipoprotein-cholesterol.

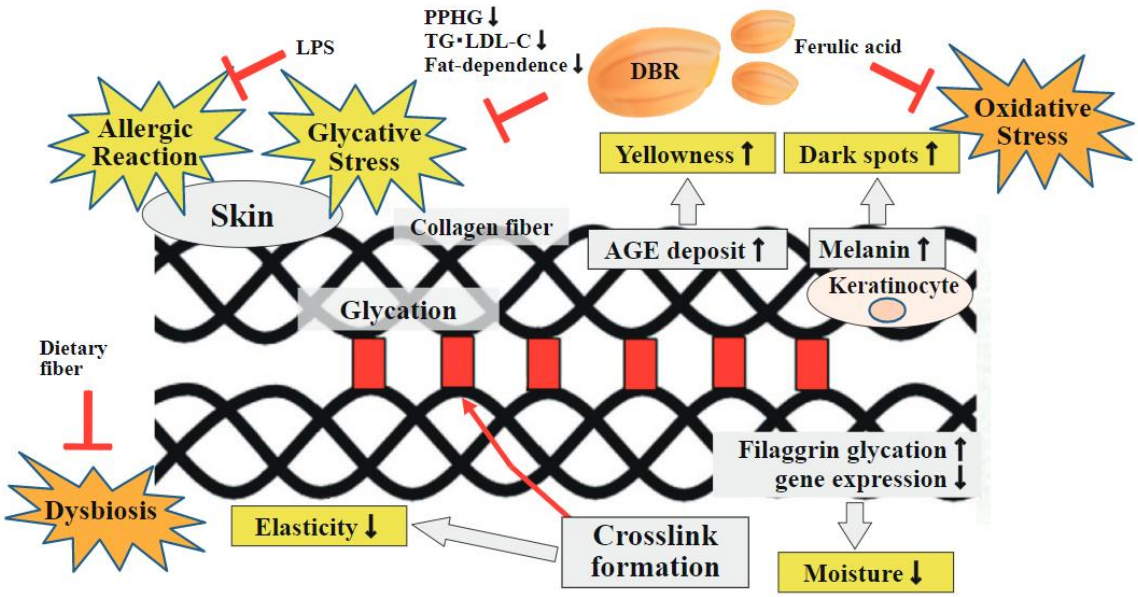


Figure 57. The possible suppressive actions by DBR. DBR, dewaxed brown rice; AGEs, advanced glycation end products; PPHG, post-prandial hyperglycemia: TG, triglyceride, LDL-C, low-density lipoprotein-cholesterol; LPS, lipopolysaccharide.

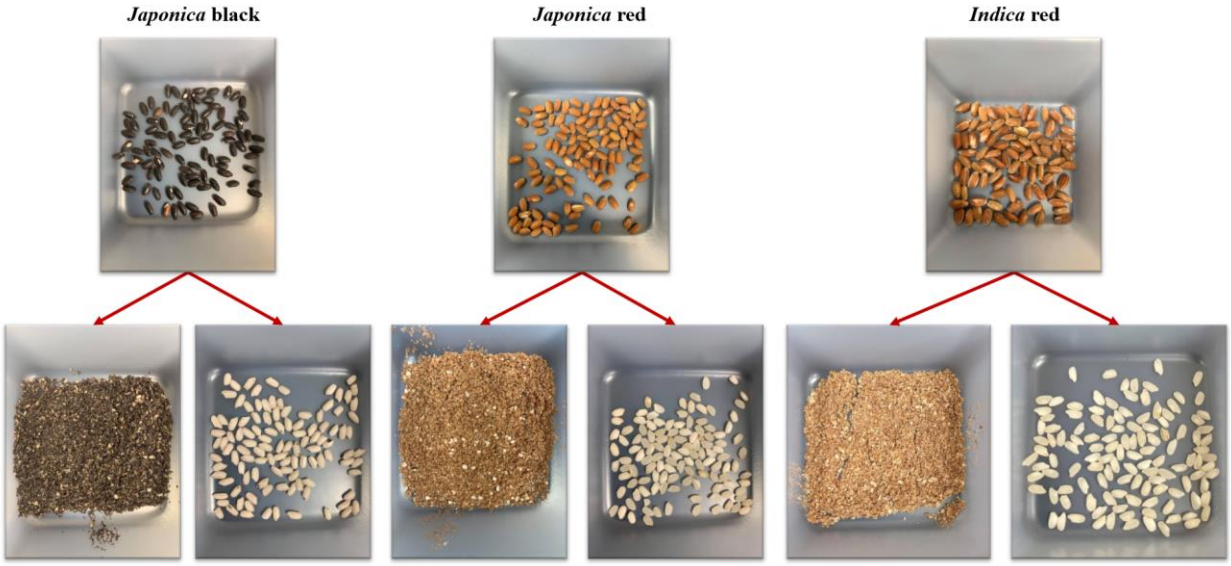


Figure 58. Rice samples separated into bran and endosperm using a simple rice milling machine.

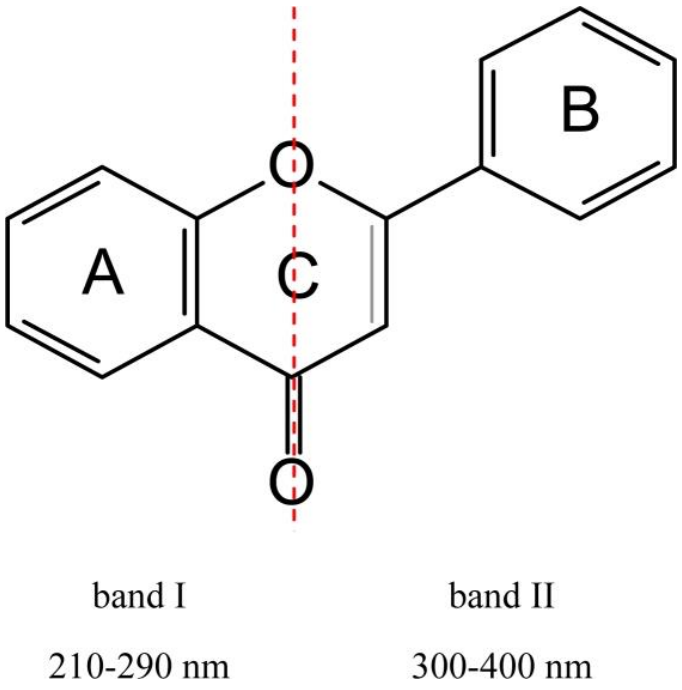


Figure 59. Structural base for flavonoid light absorbance (83).

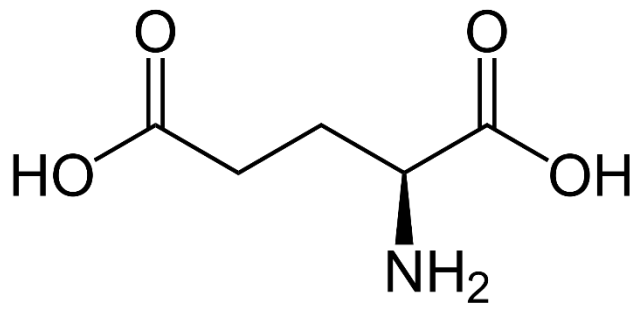


Figure 60. Structure of L-glutamic acid (glutamic acid in its non ionic form).

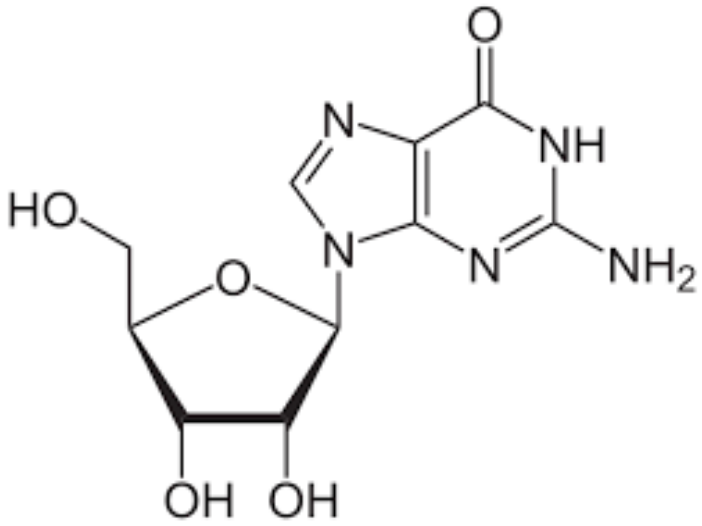


Figure 61. Structure of guanosine.