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APPENDICES

APPENDIX A

MANIPULATION OF THE TESTER STRAINS

1. Preparation of Stock Solutions and Media (Maron and Ames, 1983)

1.1 Vogel-Bonner Medium E Stock Salt Solution (VB salts)

Use: Minutesimal gar

| <u>Ingredient</u> | Per liter |
|---|-----------|
| Warm distilled water (45 °C) | 670 ml |
| Magnesium sulfate (MgSO ₄ .7H ₂ O) | 10 g |
| Citric acid monohydrate | 100 g |
| Potassium phosphate, dibasic (anhydrous) (K ₂ HPO ₄) | 500 g |
| Sodium ammonium phosphate (NaNH ₄ HPO ₄ .4H ₂ O) | 175 g |

Add salts in the order indicated to warm water in a 2-liter beaker placed on a magnetic stirring hot plate. Allow each salt to dissolve completely before adding the next. Adjust the volume to 1 liter and filter the solutions into the glass bottles with screw caps and then autoclave at 121°C for 20 minutes.

1.2 Minimal Glucose Agar Plate

Use: Mutagenicity assay

| | |
|-------------------|--------|
| <u>Ingredient</u> | 300 ml |
| Bacto agar | 4.5 g |
| Distilled water | 279 ml |
| VB salts | 6 ml |
| 40% glucose | 15 ml |

Agar was added to distilled water in a glass bottle and then autoclaved at 121°C for 20 minutes. When the solution has cooled slightly, sterile VB salts and sterile 40% glucose were added, stirred thoroughly and poured 30 ml into each sterile petri dish. Minimal glucose agar plates were kept in an incubator at 37°C for 48 hours before using.

1.3 Oxoid Nutrient Broth No. 2

Use: Growing culture

| | |
|----------------------|--------|
| <u>Ingredient</u> | 100 ml |
| Nutrient broth No. 2 | 2.5 g |
| Distilled water | 100 ml |

2.5 g of nutrient broth No.2 was dissolved in 100 ml distilled water. Mixed it until dissolve completely and 12 ml of nutrient broth was transferred into each 50 ml Erlenmeyer flask (covered with sterile gauze). They were autoclave at 121°C for 20 minutes.

1.4 0.1 M L-Histidine HCl Stock

Use: Preparation of 1 mM L-histidine HCl stock

| | |
|-------------------|---------|
| <u>Ingredient</u> | 100 ml |
| L-histidine HCl | 2.096 g |
| Distilled water | 100 ml |

Dissolve L-histidine HCl (MW 209.63) in distilled water and then autoclave at 121°C for 20 minutes. The solution was stored in a glass bottle at 4°C until use.

1.5 1 mM L-Histidine HCl Stock

Use: Preparation of 0.5 mM L-histidine/biotin solution

| | |
|-----------------------|--------|
| <u>Ingredient</u> | 100 ml |
| 0.1 M L-histidine HCl | 1 ml |
| Distilled water | 99 ml |

Dilute 1 ml of 0.1 M L-histidine HCl in 99 ml of distilled water and then autoclave at 121°C for 20 minutes.

1.6 1 mM Biotin Stock

Use: Preparation of 0.5 mM L-histidine/biotin solution

| | |
|-------------------|----------|
| <u>Ingredient</u> | 100 ml |
| Biotin | 24.43 mg |
| Distilled water | 100 ml |

Dissolve biotin (MW 244.3) in distilled water. Warm it until dissolve completely and then autoclave at 121°C for 20 minutes.

1.7 0.5 mM L-Histidine/Biotin Solution

Use: Mutagenicity assay (add in to Top agar)

| | |
|----------------------|--------|
| <u>Ingredient</u> | 200 ml |
| 1 mM L-histidine HCl | 100 ml |
| 1 mM Biotin | 100 ml |

Mix all ingredients and autoclave at 121°C for 20 minutes.

1.8 Top Agar

Use: Mutagenicity assay

| | |
|-------------------|--------|
| <u>Ingredient</u> | 100 ml |
| Bacto-agar | 0.6 g |
| Sodium chloride | 0.5 g |
| Distilled water | 100 ml |

Dissolve ingredients in distilled water. Store the solution in a glass bottle and autoclave at 121°C for 20 minutes and then add 10 ml of 0.5 mM L-histidine/biotin solution.

1.9 1 M Potassium Chloride (KCl)

Use: Preparation of Na₃PO₄-KCl buffer

| | |
|--------------------------|-----------|
| <u>Ingredient</u> | per liter |
| Potassium chloride (KCl) | 74.56 g |
| Distilled water | 1000 ml |

Dissolve potassium chloride in distilled water and autoclave at 121°C for 20 minutes.

1.10 0.5 M Sodium Dihydrogen Phosphate (NaH₂PO₄)

Use: Preparation of 0.5 M sodium phosphate pH 7.4

| | |
|---|--------|
| <u>Ingredient</u> | 500 ml |
| Sodium dihydrogen phosphate (NaH ₂ PO ₄) | 30 g |
| Distilled water to | 500 ml |

Dissolve sodium dihydrogen phosphate (MW 120) in distilled water. Stir it until dissolve completely and adjust the final volume to be 500 ml.

1.11 0.5 M Sodium Phosphate (Na₃PO₄) pH 7.4Use: Preparation of Na₃PO₄-KCl buffer

| | |
|---|--------|
| <u>Ingredient</u> | 500 ml |
| Disodium hydrogen phosphate dihydrate (Na ₂ HPO ₄ ·2H ₂ O) | 44.5 g |
| Distilled water to | 500 ml |

Dissolve disodium hydrogen phosphate dihydrate in distilled water. Add NaH₂PO₄ until to pH 7.4 and adjust the final volume to be 500 ml. Autoclave at 121°C for 20 minutes.

1.12 Na₃PO₄-KCl Buffer

Use: Mutagenicity assay

| | |
|--|----------|
| <u>Ingredient</u> | 330 ml |
| 0.5 M Na ₃ PO ₄ pH 7.4 | 100 ml |
| 1 M KCl | 16.5 ml |
| Distilled water | 213.5 ml |

All ingredients were mixed and autoclave at 121°C for 20 minutes.

2. Preparation of Some Reagents**2.1 2 M Sodium Nitrite**

Use: Mutagenicity assay (Nitrosation)

| | |
|--------------------|--------|
| <u>Ingredient</u> | 10 ml |
| Sodium nitrite | 1.38 g |
| Distilled water to | 10 ml |

Dissolve sodium nitrite in distilled water and adjust the final volume to be 10 ml. Store in a glass bottle with a screw cap (wrap the bottle with metal foil to protect from light) and autoclave at 121°C for 20 minutes.

2.2 2 M Ammonium Sulfamate

Use: Mutagenicity assay (Reaction mixture)

| | |
|--------------------|--------|
| <u>Ingredient</u> | 10 ml |
| Ammonium sulfamate | 2.28 g |
| Distilled water to | 10 ml |

Dissolve ammonium sulfamate in distilled water and adjust the final volume to be 10 ml. Store in a glass bottle with a screw cap and autoclave at 121°C for 20 minutes.

2.3 0.2 N Hydrochloric Acid

Use: Mutagenicity assay (Reaction mixture)

| <u>Ingredient</u> | 100 ml |
|-------------------------|----------|
| Conc. hydrochloric acid | 1.66 ml |
| Sterile distilled water | 98.34 ml |

Dissolve conc. hydrochloric acid in sterile distilled water and store in sterile glass bottle with screw cap. (Use sterile technique and hydrochloric acid cannot be autoclaved)

2.4 0.075 mg/ml 1-Aminopyrene

Use: Standard solution for mutagenicity assay

| <u>Ingredient</u> | 2 ml |
|-------------------------|---------------|
| 0.3 mg/ml 1-aminopyrene | 500 μ l |
| Acetonitrile | 1,500 μ l |

Dissolve 3 mg of 1-aminopyrene in 300 μ l of acetonitrile and mix (3 mg/ml 1-aminopyrene). Subsequently dilute this 300 μ l solution in 2,700 μ l of acetonitrile (0.3 mg/ml 1-aminopyrene). Then dilute 500 μ l of 0.3 mg/ml 1-aminopyrene in 1,500 μ l of acetonitrile. Store the solution in sterile glass vial with screw cap in a freezer. (Use sterile technique)

2.5 8 mg/ml Ampicillin Solution

Use: Test of ampicillin resistance (to confirm R-factor strains)

| <u>Ingredient</u> | 10 ml |
|---------------------|-------|
| Ampicillin (sodium) | 80 mg |
| Distilled water | 10 ml |

Ampicillin was dissolved into water and stored in glass bottle with screw cap at 4°C. (Use sterile technique)

2.6 0.1% Crystal Violet

Use: Test for crystal violet sensitivity (to confirm *rfa* mutation)

| <u>Ingredient</u> | 10 ml |
|-------------------|-------|
| Crystal violet | 10 mg |
| Distilled water | 10 ml |

Crystal violet was dissolved into water and stored in glass bottle with screw cap at 4°C (wrap the bottle with metal foil to protect from light).

3. Procedure for Re-isolation and Growing Culture (Maron and Ames, 1983)

Tester strains, TA 98 and TA 100 were grown in Oxoid nutrient broth No. 2 and incubated overnight in a 37°C in shaking water bath. The growth period should not exceed 16 hours. These cultures were re-isolated by streaking the bacteria on minimal glucose agar plates which the surface were spreaded with 0.1 ml of 8 mg/ml ampicillin, 0.3 ml of 0.1 M histidine HCl and 0.1 ml of 1 mM biotin. These plates were incubated at 37°C for 48 hours. After incubation, the 4 single colonies per strain of TA98 and TA100 were picked up using sterile wire loop and grown in Oxoid nutrient broth No. 2 and shaken overnight at 37°C in shaking water bath. Each culture was confirmed genotypes of the strains and kept the cultures as the source of bacteria for mutagenicity testing. For each 1 ml of culture, 0.09 ml of spectrophotometric grade DMSO was added. Combine the culture and DMSO in a sterile tube and distribute 200 µl of the culture aseptically into sterile cryotube. Store the tubes in a freezer at -80°C.

4. Confirming Genotype of Tester Strains

Broth cultures of TA98 and TA100 were used to confirm genotypes in the following ways.

4.1 Histidine requirement

The his⁻ character of the strains was confirmed by demonstrating the histidine requirement for growth on the minimal glucose agar plates enriched with histidine and biotin.

| | | |
|-------------------|---------|--|
| Procedure: | plate a | no histidine and biotin |
| | plate b | 0.1 ml of 1mM biotin |
| | plate c | 0.3 ml of 0.1 M his-HCl |
| | plate d | 0.3 ml of 0.1 M his-HCl + 0.1 ml of 1mM biotin |

Four minimal glucose agar plates were required for each tester strains and applied on the surface with 0.1 ml of 1mM biotin, 0.3 ml of 0.1 M his-HCl, 0.3 ml of 0.1 M his-HCl plus 0.1 ml of 1mM biotin and no application (plate b, c, d, a respectively). A single streak of each strain was made across these plates. Four strains could be tested on the same plate. The plates were incubated at 37°C for 48 hours. The growing of bacteria on histidine plus biotin plate (plate d) was the result of histidine requirement.

4.2 R-Factor

The R-factor strains (TA97, TA98, TA100 and TA102) should be tested routinely for the presence of the ampicillin resistance factor because the plasmid is somewhat unstable and can be lost from the bacteria.

Procedure: For each tester strain (TA98 and TA100), 0.3 ml of fresh overnight culture was added to a tube containing 0.1 ml of 0.1 M histidine-HCl, and then 2 ml of molten top agar containing 0.5 mM histidine-HCl and 0.5 mM biotin were added, mixed and poured on a minimal agar plate. The plate was rotated in order to distribute the mixtures and allowed several minutes for agar to become firm. R-factor and *rfa* mutation (see the next section) are performed in the same plate by dividing the plate into 2 parts, one for R-factor and the other for *rfa* mutation. For R-factor, filter paper disc (1/4 inch) containing 8 mg/ml ampicillin was placed on the surface of the agar by using sterile forceps. The disc was pressed lightly to embed in the overlay. The plates were incubated at 37°C for 24 hours. The absence of the clear zones of inhibition around the disc was indicated resistance to ampicillin.

4.3 *rfa* mutation

Strains having the deep rough (*rfa*) character should be tested for crystal violet sensitivity.

Procedure: 0.1 μ l of 0.1% crystal violet solution was pipetted to the sterile filter paper disc (1/4 inch) and transferred the disc to the seeded plates (the procedure was similar to R-factor). The plates were incubated at 37°C for 48 hours. The clear zone appeared around the disc indicated the presence of the *rfa* mutation (crystal violet transferred into the cell and kill bacteria).

5. Spontaneous Reversion

Spontaneous reversion of the tester strains to histidine independence is measured routinely in mutagenicity experiments and is expressed as the number of spontaneous revertants per plate. The revertant colonies are clearly visible in a uniform background lawn of auxotrophic bacteria. Each tester strain reverts spontaneously at a frequency that is characteristic of the strain. Nevertheless, there is variability in the number of spontaneous revertants from one experiment to another and from one plate to another, and it is advisable to include at least 2-3 spontaneous mutation control plates for each strain in a mutagenicity assay.

Procedure: 0.1 ml of DMSO was added to capped culture tube, then 0.5 ml of $\text{Na}_3\text{PO}_4\text{-KCl}$ buffer pH 7.4 and 0.1 ml of fresh overnight culture of TA98 or TA100 was added. The mixture was incubated in shaking water bath at 37°C for 20 minutes. After that 2.0 ml of molten top agar was added to the mixture, mixed and then poured on the minimal glucose agar plate. Plates were rotated and left it to become harden and incubated at 37°C for 48 hours. The his⁺ revertants colonies that grown on the minimal glucose agar plate were counted.

6. The Response to Standard Mutagen

Standard mutagens or positive mutagens are used routinely in mutagenicity experiments to confirm the reversion property and specificity of each strain. The standard mutagen, which used in this experiment, was nitrosated-aminopyrene. Tester strains that highly response to positive mutagens will be chosen.

Procedure: 0.01 and 0.02 ml of 0.0375 mg/ml aminopyrene in acetonitrile were pipetted to sterile capped tube. Then, 0.55 ml of 0.2 N HCl were added respectively, and followed by 0.25 ml of 2 M NaNO_2 . The final concentration of aminopyrene was 0.6 and 1.2 mg respectively, and the final concentration of nitrite was 0.5 M. The solution was mixed and shaken in water bath at 37°C for 4 hours. The tube was placed in an ice bath and 0.25 ml of 2 M ammonium sulfamate ($\text{NH}_4\text{SO}_3\text{NH}_4$) was added and standard for 10 minutes in ice bath. 0.1 ml of each mixture was pipetted to capped culture tube for testing the stock culture TA98 and TA100, equal to 0.06 and 0.12 mg of aminopyrene/plate, respectively). Then, the evaluation of their mutagenicity was tested as described in spontaneous reversion.

The characteristic properties of the stock culture for TA98 and TA100 as the source of bacteria for mutagenic testing are contain R-factor (pKM 101) and *rfa* mutation, his⁺ requirement, low spontaneous reversion and highly response to standard carcinogen. The experiment was performed only when the characteristic properties of bacterial strain were done.

APPENDIX B

ANTIOXIDANT ASSAY

1. Preparation of the reagents for antioxidant assay

1.1 DPPH Reagent:

Chemicals

1. 150 μ l DPPH^{*} (2,2'-Diphenyl-1-picrylhydrazyl) in 80% Methanol
2. 1.28 mM Trolox in 80% Methanol

Standard Trolox was run in triplicate using several concentrations (1.28, 0.64, 0.32, 0.16 and 0.08 mM).

1.2 FRAP Reagent:

Chemicals

1. 300 mM Acetate buffer (pH 3.6)
3.1 g of sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$) plus 16 ml of glacial acetic acid and made up to 1 liter with distilled water.
2. 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl
3. 20 mM $FeCl_3 \cdot 6 H_2O$
4. 1,000 μ M $FeSO_4 \cdot 7 H_2O$

Mixing the reagent from 1-3 before use (300 mM acetate buffer: 10 mM TPTZ: 20 mM $FeCl_3 \cdot 6 H_2O$; ratio 10:1:1) and heated to 37°C. Standard $FeSO_4 \cdot 7 H_2O$ was run in triplicate using several concentrations (1000, 500, 250, 125 and 62.5 μ M).

1.3 Phenolic Reagent:

Chemicals

1. Folin-Ciocalteu reagent
2. Saturated sodium carbonate solution
3. 800 mg/l gallic acid

Standard gallic acid was run in triplicate using several concentrations (800, 400, 200, 100, 50, 25 and 12.5 mg/l).

APPENDIX C
RESULTS OF ANTIOXIDANT ASSAY
AND MUTAGENICITY ASSAYS

1. The standard curves of antioxidant assays

1.1 2,2'-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

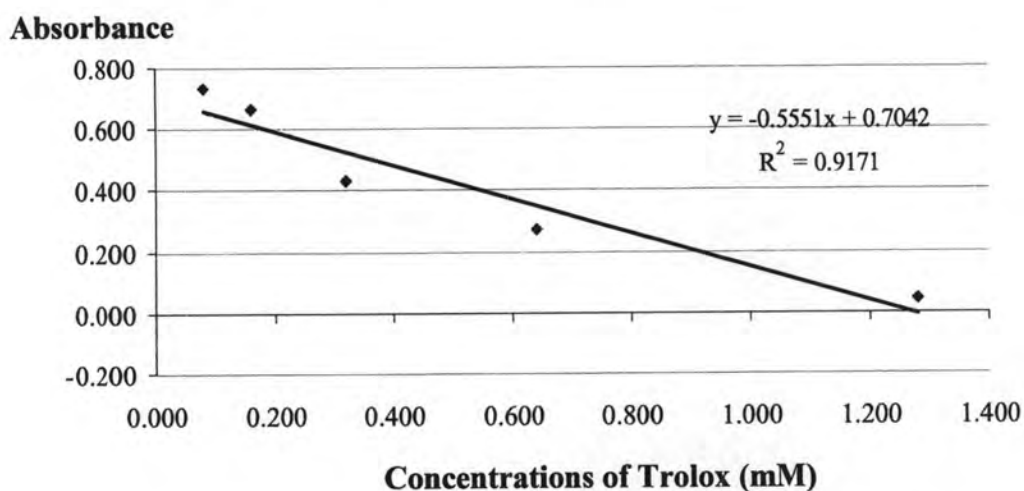


Figure C-1 Standard curves of Trolox

1.2 Ferric Reducing Antioxidant Power (FRAP) Assay

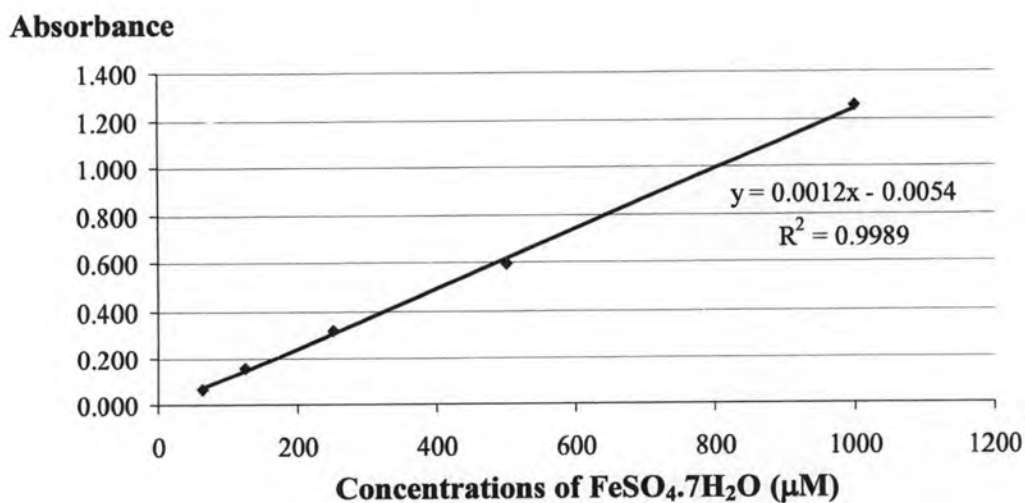


Figure C-2 Standard curves of FeSO₄·7H₂O

1.3 Determination of Total Phenolic Contents

Absorbance

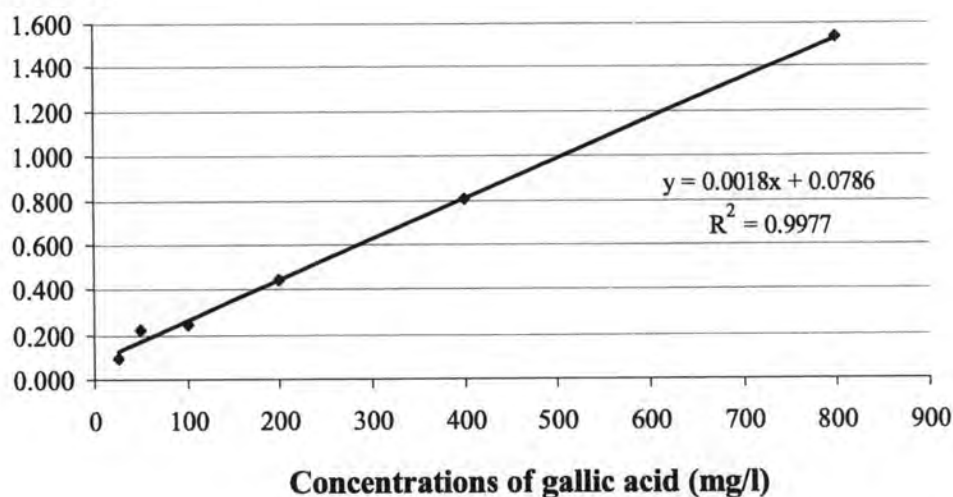


Figure C-3 Standard curves of gallic acid

2. The results of antioxidant assays

Table C-1 Antioxidant activity and total phenolic contents of each sample and extraction yield

| Sample | DPPH assay | | FRAP ^b value | Total polyphenol content (GAE) ^c | %Yield (dry weight) |
|-----------------------------|-------------------|--------------|-------------------------|---|---------------------|
| | TEAC ^a | % Scavenging | | | |
| Hom Nil rice | | | | | |
| Raw | 1.189 | 95.03 | 5214.44 | 272.07 | 0.8 |
| Cooked | 1.173 | 93.99 | 2744.36 | 235.42 | 0.6 |
| Fermented | 0.117 | 24.26 | 360.87 | 38.56 | 27.3 |
| Black glutinous rice | | | | | |
| Raw | 1.245 | 98.41 | 6797.89 | 458.37 | 1.7 |
| Cooked | 1.236 | 98.19 | 6352.16 | 452.75 | 0.6 |
| Fermented | 1.032 | 84.71 | 1461.40 | 65.21 | 12.75 |

All values are the means of three measurements.

^aTEAC (Trolox equivalent antioxidant capacity) in mg/g dry weight of sample.

^bFRAP assay = The FRAP values of the extracts were expressed as mg of ferrous iron (Fe (II))/g dry weight of sample.

^cGAE (The gallic acid equivalent) in mg/g dry weight (DW) of sample.

3. The results of mutagenicity assays

Table C-2 Mutagenicity of chicken extract without and with sodium nitrite treatment in acid condition (pH 3.0-3.5) on *Salmonella typhimurium* TA98 and TA100

| <i>Salmonella typhimurium</i> strains | Amount (mg/plate) | No. revertants/plate ^a | | Mutagenicity Index ^b (MI) | |
|---------------------------------------|-------------------|-----------------------------------|---------------------|--------------------------------------|---------------------|
| | | Without sodium nitrite | With sodium nitrite | Without sodium nitrite | With sodium nitrite |
| TA98 | 0 ^c | 24 ± 4 | - | 1.00 | - |
| | 3.6 | 21 ± 2 | 75 ± 12 | 0.84 | 3.10 |
| | 5.4 | 16 ± 3 | 107 ± 9 | 0.64 | 4.38 |
| | 7.2 | 21 ± 3 | 139 ± 5 | 0.84 | 5.71 |
| TA100 | 0 ^c | 105 ± 3 | - | 1.00 | - |
| | 3.6 | 112 ± 20 | 337 ± 25 | 1.06 | 3.20 |
| | 5.4 | 132 ± 21 | 432 ± 36 | 1.25 | 4.10 |
| | 7.2 | 141 ± 51 | 706 ± 102 | 1.34 | 6.70 |

10 µ and 20 µl of 1-aminopyrene (0.075 mg/ml) interacted with excess amount of sodium nitrite induced 2103 ± 95 and 884 ± 64 revertants/plate for TA98 and TA100 respectively.

^a Data are expressed as mean ± SD of triplicate plates from two independent experiments (N=6)

^b Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the chicken extract divided by that of spontaneous revertants. Bold number indicates the positive result.

^c Spontaneous revertants.

Table C-3 Mutagenicity of rice extracts without and with sodium nitrite treatment in acid condition (pH 3.0-3.5) on *S. typhimurium* TA98

| Rice extract | Amount ^a (mg/plate) | No. of revertants/plate ^b | | Mutagenicity Index ^c | |
|-----------------------------|-----------------------------------|--------------------------------------|---------------------|---------------------------------|---------------------|
| | | Without sodium nitrite | With sodium nitrite | Without sodium nitrite | With sodium nitrite |
| Hom Nil rice | | | | | |
| Raw | 0 ^d | 16 ± 2 | - | 1.00 | - |
| | 0.8 | 15 ± 4 | 144 ± 22 | 0.92 | 8.98 |
| | 1.2 | 17 ± 2 | 160 ± 16 | 1.05 | 10.01 |
| | 1.6 | 14 ± 4 | 162 ± 26 | 0.86 | 10.10 |
| Cooked | 0 ^d | 23 ± 1 | - | 1.00 | - |
| | 0.8 | 35 ± 10 | 165 ± 23 | 1.50 | 7.17 |
| | 1.2 | 32 ± 4 | 187 ± 41 | 1.38 | 8.13 |
| | 1.6 | 36 ± 11 | 209 ± 39 | 1.54 | 9.07 |
| Fermented | 0 ^d | 22 ± 1 | - | 1.00 | - |
| | 0.8 | 23 ± 5 | 35 ± 7 | 1.05 | 1.61 |
| | 1.2 | 24 ± 6 | 41 ± 35 | 1.08 | 1.85 |
| | 1.6 | 24 ± 7 | 43 ± 9 | 1.08 | 1.96 |
| Black glutinous rice | | | | | |
| Raw | 0 ^d | 15 ± 4 | - | 1.00 | - |
| | 0.8 | 14 ± 2 | 87 ± 21 | 0.89 | 5.67 |
| | 1.2 | 15 ± 4 | 113 ± 18 | 0.98 | 7.38 |
| | 1.6 | 20 ± 4 | 116 ± 13 | 1.32 | 7.58 |
| Cooked | 0 ^d | 19 ± 1 | - | 1.00 | - |
| | 0.8 | 19 ± 5 | 64 ± 3 | 1.02 | 3.40 |
| | 1.2 | 18 ± 5 | 123 ± 20 | 0.98 | 6.88 |
| | 1.6 | 18 ± 4 | 135 ± 16 | 0.95 | 7.23 |
| Fermented | 0 ^d | 15 ± 1 | - | 1.00 | - |
| | 0.8 | 16 ± 3 | 30 ± 4 | 1.06 | 1.97 |
| | 1.2 | 16 ± 2 | 47 ± 5 | 1.09 | 3.10 |
| | 1.6 | 15 ± 4 | 50 ± 9 | 1.01 | 3.33 |

10 µ of 1-aminopyrene (0.075 mg/ml) interacted with excess amount of sodium nitrite induced 2103±95 revertants/plate for TA98.

^a Amount per plate of rice extracts.

^b Data are expressed as mean ± SD of triplicate plates from two independent experiments (N=6).

^c Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the rice extracts divided by that of spontaneous revertants. Bold number indicates the positive result.

^d Spontaneous revertants.

Table C-4 Mutagenicity of rice extracts without and with sodium nitrite treatment in acid condition (pH 3.0-3.5) on *S. typhimurium* TA100

| Rice extract | Amount ^a (mg/plate) | No. of revertants/plate ^b | | Mutagenicity Index ^c | |
|-----------------------------|-----------------------------------|--------------------------------------|---------------------|---------------------------------|---------------------|
| | | Without sodium nitrite | With sodium nitrite | Without sodium nitrite | With sodium nitrite |
| Hom Nil rice | | | | | |
| Raw | 0 ^d | 117 ± 8 | - | 1.00 | - |
| | 0.8 | 122 ± 12 | 684 ± 94 | 1.05 | 5.86 |
| | 1.2 | 120 ± 7 | 700 ± 73 | 1.03 | 6.00 |
| | 1.6 | 120 ± 16 | 793 ± 93 | 1.03 | 6.33 |
| Cooked | 0 ^d | 144 ± 9 | - | 1.00 | - |
| | 0.8 | 139 ± 11 | 819 ± 172 | 0.96 | 5.70 |
| | 1.2 | 134 ± 12 | 824 ± 123 | 0.93 | 5.73 |
| | 1.6 | 130 ± 15 | 827 ± 126 | 0.91 | 5.76 |
| Fermented | 0 ^d | 119 ± 15 | - | 1.00 | - |
| | 0.8 | 109 ± 24 | 149 ± 14 | 0.92 | 1.25 |
| | 1.2 | 101 ± 17 | 172 ± 34 | 0.85 | 1.44 |
| | 1.6 | 120 ± 24 | 200 ± 42 | 1.10 | 1.68 |
| Black glutinous rice | | | | | |
| Raw | 0 ^d | 123 ± 11 | - | 1.00 | - |
| | 0.8 | 111 ± 18 | 356 ± 70 | 0.90 | 2.89 |
| | 1.2 | 128 ± 17 | 414 ± 39 | 1.04 | 3.37 |
| | 1.6 | 133 ± 32 | 447 ± 29 | 1.08 | 3.63 |
| Cooked | 0 ^d | 127 ± 16 | - | 1.00 | - |
| | 0.8 | 120 ± 26 | 379 ± 29 | 0.95 | 2.99 |
| | 1.2 | 131 ± 14 | 541 ± 44 | 1.03 | 4.27 |
| | 1.6 | 123 ± 16 | 568 ± 77 | 0.97 | 4.49 |
| Fermented | 0 ^d | 120 ± 6 | - | 1.00 | - |
| | 0.8 | 109 ± 12 | 205 ± 14 | 0.91 | 1.70 |
| | 1.2 | 110 ± 17 | 241 ± 19 | 0.91 | 2.00 |
| | 1.6 | 116 ± 20 | 299 ± 30 | 0.96 | 2.48 |

20 µl of 1-aminopyrene (0.075 mg/ml) interacted with excess amount of sodium nitrite induced 872±14 revertants/plate for TA100.

^a Amount per plate of rice extracts.

^b Data are expressed as mean ± SD of triplicate plates from two independent experiments (N=6).

^c Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the rice extracts divided by that of spontaneous revertants. Bold number indicates the positive result.

^d Spontaneous revertants.

Table C-5 Antimutagenicity of rice extracts against sodium nitrite treated chicken extract on *S. typhimurium* TA98 and TA100

| Rice extract | | TA98 | | | | TA100 | | | |
|---------------------|-----------------------------------|--------------------------------------|---|--------------------------|--------------------------------------|-------|--------------------------|-----|--------|
| Name | Amount ^a (mg/plate) | No. of revertants/plate ^b | | %Inhibition ^c | No. of revertants/plate ^b | | %Inhibition ^c | | |
| Hom Nil rice | | | | | | | | | |
| Raw | Spontaneous ^d | 15 | ± | 1 | - | 112 | ± | 11 | - |
| | 0 ^e | 144 | ± | 9 | - | 609 | ± | 45 | - |
| | 0.8 | 128 | ± | 19 | +12.72 | 634 | ± | 36 | -5.16 |
| | 1.2 | 133 | ± | 20 | +8.52 | 627 | ± | 55 | -3.64 |
| | 1.6 | 137 | ± | 12 | +5.47 | 621 | ± | 47 | -2.55 |
| Cooked | Spontaneous | 36 | ± | 1 | - | 110 | ± | 7 | - |
| | 0 | 174 | ± | 3 | - | 938 | ± | 14 | - |
| | 0.8 | 174 | ± | 22 | -0.12 | 795 | ± | 72 | +17.30 |
| | 1.2 | 191 | ± | 7 | -12.35 | 725 | ± | 82 | +25.71 |
| | 1.6 | 193 | ± | 16 | -13.92 | 716 | ± | 67 | +26.84 |
| Fermented | Spontaneous | 21 | ± | 5 | - | 112 | ± | 1 | - |
| | 0 ^e | 173 | ± | 7 | - | 891 | ± | 63 | - |
| | 0.8 | 134 | ± | 26 | +25.55 | 804 | ± | 158 | +11.24 |
| | 1.2 | 139 | ± | 33 | +22.37 | 788 | ± | 157 | +13.25 |
| | 1.6 | 154 | ± | 20 | +12.50 | 699 | ± | 198 | +24.71 |

Table C-5 Antimutagenicity of rice extracts against sodium nitrite treated chicken extract on *S. typhimurium* TA98 and TA100 (continued)

| Rice extract | | TA98 | | TA100 | |
|-----------------------------|-----------------------------------|--------------------------------------|--------------------------|--------------------------------------|--------------------------|
| Name | Amount ^a (mg/plate) | No. of revertants/plate ^b | %Inhibition ^c | No. of revertants/plate ^b | %Inhibition ^c |
| Black glutinous rice | | | | | |
| Raw | Spontaneous ^d | 15 ± 5 | - | 157 ± 18 | - |
| | 0 ^e | 141 ± 15 | - | 655 ± 10 | - |
| | 0.8 | 138 ± 24 | +2.77 | 507 ± 74 | +29.75 |
| | 1.2 | 132 ± 9 | +7.78 | 448 ± 69 | +41.51 |
| | 1.6 | 129 ± 15 | +9.50 | 435 ± 46 | +44.18 |
| Cooked | Spontaneous | 18 ± 3 | - | 111 ± 12 | - |
| | 0 | 141 ± 9 | - | 703 ± 37 | - |
| | 0.8 | 140 ± 10 | +0.14 | 674 ± 46 | +4.90 |
| | 1.2 | 135 ± 13 | +5.04 | 655 ± 98 | +8.16 |
| | 1.6 | 134 ± 17 | +5.72 | 652 ± 47 | +8.56 |
| Fermented | Spontaneous | 18 ± 3 | - | 140 ± 17 | - |
| | 0 ^e | 145 ± 5 | - | 703 ± 41 | - |
| | 0.8 | 110 ± 16 | +27.69 | 447 ± 45 | +45.15 |
| | 1.2 | 96 ± 19 | +39.24 | 444 ± 25 | +45.65 |
| | 1.6 | 93 ± 20 | +41.08 | 439 ± 68 | +46.52 |

^a Amount per plate of rice extracts. ^b Data are expressed as mean ± SD of triplicate plates from two independent experiments (N=6). ^c + or - indicates that the extract decreased or increased the mutagenicity of the model respectively. ^d Spontaneous revertants. ^e No rice extract was added to the standard mutagen, namely sodium nitrite treated chicken extract (7.2 mg/plate for *Salmonella typhimurium* TA98 and TA100).

Table C-6 Antimutagenicity of rice extracts against sodium nitrite treated 1-aminopyrene on *S. typhimurium* TA98 and TA100

| Rice extract | | TA98 | | TA100 | |
|---------------------|-----------------------------------|--------------------------------------|--------------------------|--------------------------------------|--------------------------|
| Name | Amount ^a (mg/plate) | No. of revertants/plate ^b | %Inhibition ^c | No. of revertants/plate ^b | %Inhibition ^c |
| Hom Nil rice | | | | | |
| Raw | Spontaneous ^d | 16 ± 2 | - | 117 ± 8 | - |
| | 0 ^e | 2543 ± 210 | - | 764 ± 83 | - |
| | 0.8 | 843 ± 101 | +67.27 | 757 ± 107 | +1.08 |
| | 1.2 | 647 ± 110 | +75.05 | 739 ± 154 | +3.91 |
| | 1.6 | 643 ± 173 | +75.18 | 683 ± 112 | +12.51 |
| Cooked | Spontaneous | 23 ± 1 | - | 144 ± 9 | - |
| | 0 | 2040 ± 63 | - | 1341 ± 69 | - |
| | 0.8 | 1059 ± 105 | +48.63 | 1201 ± 174 | +11.72 |
| | 1.2 | 962 ± 102 | +53.43 | 1055 ± 113 | +23.86 |
| | 1.6 | 741 ± 170 | +64.40 | 963 ± 112 | +31.60 |
| Fermented | Spontaneous | 22 ± 1 | - | 119 ± 15 | - |
| | 0 ^e | 2088 ± 396 | - | 888 ± 65 | - |
| | 0.8 | 2031 ± 316 | +2.76 | 826 ± 95 | +8.07 |
| | 1.2 | 1851 ± 279 | +11.49 | 806 ± 106 | +10.62 |
| | 1.6 | 1739 ± 99 | +16.92 | 769 ± 53 | +15.44 |

Table C-6 Antimutagenicity of rice extracts against sodium nitrite treated 1-aminopyrene on *S. typhimurium* TA98 and TA100 (continued)

| Rice extract | | TA98 | | TA100 | |
|-----------------------------|-----------------------------------|--------------------------------------|--------------------------|--------------------------------------|--------------------------|
| Name | Amount ^a (mg/plate) | No. of revertants/plate ^b | %Inhibition ^c | No. of revertants/plate ^b | %Inhibition ^c |
| Black glutinous rice | | | | | |
| Raw | Spontaneous ^d | 15 ± 4 | - | 123 ± 11 | - |
| | 0 ^e | 2089 ± 410 | - | 689 ± 63 | - |
| | 0.8 | 987 ± 145 | +53.17 | 465 ± 77 | +39.67 |
| | 1.2 | 738 ± 127 | +65.16 | 416 ± 58 | +48.26 |
| | 1.6 | 596 ± 157 | +71.99 | 390 ± 56 | +52.80 |
| Cooked | Spontaneous | 19 ± 1 | - | 127 ± 16 | - |
| | 0 | 2639 ± 360 | - | 1060 ± 102 | - |
| | 0.8 | 1414 ± 260 | +46.76 | 827 ± 40 | +25.01 |
| | 1.2 | 1110 ± 220 | +58.38 | 726 ± 89 | +35.81 |
| | 1.6 | 851 ± 198 | +68.24 | 649 ± 73 | +44.09 |
| Fermented | Spontaneous | 15 ± 1 | - | 120 ± 6 | - |
| | 0 ^e | 2300 ± 111 | - | 1052 ± 106 | - |
| | 0.8 | 891 ± 257 | +61.67 | 784 ± 222 | +28.82 |
| | 1.2 | 785 ± 222 | +66.33 | 748 ± 258 | +32.63 |
| | 1.6 | 525 ± 180 | +77.67 | 696 ± 204 | +38.21 |

^a Amount per plate of rice extracts. ^b Data are expressed as mean ± SD of triplicate plates from two independent experiments (N=6). ^c + or - indicates that the extract decreased or increased the mutagenicity of the model respectively. ^d Spontaneous revertants. ^e No rice extract was added to the standard mutagen, namely sodium nitrite treated 1-aminopyrene (0.12 and 0.24 µg/plate for *Salmonella typhimurium* TA98 and TA100 respectively).

Table C-7 Mutagenicity of chicken extract treated sodium nitrite in the presence of each rice extract on *S. typhimurium* TA98 and TA100

| Rice extract | | TA98 | | TA100 | |
|---------------------|-----------------------------------|--------------------------------------|-----------------|--------------------------------------|-----------------|
| Name | Amount ^a (mg/plate) | No. of revertants/plate ^b | MI ^c | No. of revertants/plate ^b | MI ^c |
| Hom Nil rice | | | | | |
| Raw | Spontaneous ^d | 15 ± 1 | - | 112 ± 11 | - |
| | 0 ^e | 144 ± 9 | 9.60 | 609 ± 45 | 5.44 |
| | 0.8 | 184 ± 10 | 12.27 | 868 ± 69 | 7.75 |
| | 1.2 | 224 ± 30 | 14.93 | 933 ± 77 | 8.33 |
| | 1.6 | 235 ± 22 | 15.67 | 968 ± 86 | 8.64 |
| Cooked | Spontaneous | 36 ± 1 | - | 110 ± 7 | - |
| | 0 ^e | 174 ± 3 | 4.83 | 938 ± 14 | 8.53 |
| | 0.8 | 235 ± 15 | 6.53 | 1148 ± 135 | 10.44 |
| | 1.2 | 279 ± 14 | 7.67 | 1157 ± 127 | 10.52 |
| | 1.6 | 296 ± 15 | 8.22 | 1188 ± 77 | 10.80 |
| Fermented | Spontaneous | 21 ± 5 | - | 112 ± 1 | - |
| | 0 ^e | 173 ± 7 | 8.24 | 891 ± 63 | 7.96 |
| | 0.8 | 155 ± 25 | 7.38 | 735 ± 113 | 6.56 |
| | 1.2 | 160 ± 33 | 7.62 | 754 ± 157 | 6.73 |
| | 1.6 | 162 ± 25 | 7.71 | 800 ± 80 | 7.14 |

Table C-7 Mutagenicity of chicken extract treated sodium nitrite in the presence of each rice extract on *S. typhimurium* TA98 and TA100 (continued)

| Rice extract | | TA98 | | TA100 | |
|--------------------------------------|-----------------------------------|--------------------------------------|-----------------|--------------------------------------|-----------------|
| Name | Amount ^a (mg/plate) | No. of revertants/plate ^b | MI ^c | No. of revertants/plate ^b | MI ^c |
| Black glutinous rice | | | | | |
| Raw | Spontaneous ^d | 15 ± 5 | - | 157 ± 18 | - |
| | 0 ^e | 141 ± 15 | 9.40 | 655 ± 10 | 4.17 |
| | 0.8 | 164 ± 22 | 10.93 | 793 ± 58 | 5.05 |
| | 1.2 | 173 ± 21 | 11.53 | 931 ± 84 | 5.93 |
| | 1.6 | 253 ± 53 | 16.87 | 1044 ± 43 | 6.65 |
| Cooked black glutinous rice | Spontaneous | 18 ± 3 | - | 111 ± 12 | - |
| | 0 ^e | 141 ± 9 | 7.83 | 703 ± 37 | 6.33 |
| | 0.8 | 209 ± 6 | 11.61 | 897 ± 75 | 8.08 |
| | 1.2 | 238 ± 22 | 13.22 | 955 ± 65 | 8.60 |
| | 1.6 | 245 ± 21 | 13.61 | 960 ± 97 | 8.65 |
| Fermented black glutinous rice | Spontaneous | 18 ± 3 | - | 140 ± 17 | - |
| | 0 ^e | 145 ± 5 | 8.06 | 703 ± 41 | 5.02 |
| | 0.8 | 134 ± 18 | 7.44 | 622 ± 78 | 4.44 |
| | 1.2 | 135 ± 28 | 7.50 | 625 ± 109 | 4.46 |
| | 1.6 | 153 ± 34 | 8.50 | 687 ± 40 | 4.91 |

^a Amount per plate of rice extracts. ^b Data are expressed as mean ± SD of triplicate plates from two independent experiments (N=6). ^c Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the rice extracts divided by that of spontaneous revertants. ^d Spontaneous revertants. Bold number indicates the higher result compared with that of no extract. ^e No extract was added to the standard mutagens, nitrite treated chicken extract.

Table C-8 Mutagenicity of 1-aminopyrene treated sodium nitrite in the presence of each rice extract on *S. typhimurium* TA98 and TA100

| Rice extract | | TA98 | | TA100 | |
|---------------------|-----------------------------------|--------------------------------------|-----------------|--------------------------------------|-----------------|
| Name | Amount ^a (mg/plate) | No. of revertants/plate ^b | MI ^c | No. of revertants/plate ^b | MI ^c |
| Hom Nil rice | | | | | |
| Raw | Spontaneous ^d | 16 ± 2 | - | 117 ± 8 | - |
| | 0 ^e | 2543 ± 310 | 158.94 | 764 ± 83 | 6.53 |
| | 0.8 | 3446 ± 267 | 215.38 | 1076 ± 75 | 9.20 |
| | 1.2 | 2616 ± 577 | 163.50 | 917 ± 61 | 7.84 |
| | 1.6 | 2093 ± 535 | 130.81 | 912 ± 54 | 7.79 |
| Cooked | Spontaneous | 23 ± 1 | - | 144 ± 9 | - |
| | 0 ^e | 2040 ± 63 | 88.70 | 1341 ± 69 | 9.31 |
| | 0.8 | 1485 ± 142 | 64.57 | 1302 ± 198 | 9.01 |
| | 1.2 | 1125 ± 245 | 48.91 | 1123 ± 123 | 7.80 |
| | 1.6 | 896 ± 118 | 38.96 | 983 ± 135 | 6.83 |
| Fermented | Spontaneous | 22 ± 1 | - | 119 ± 15 | - |
| | 0 ^e | 2088 ± 396 | 94.91 | 888 ± 65 | 7.46 |
| | 0.8 | 5871 ± 516 | 266.86 | 1690 ± 149 | 14.20 |
| | 1.2 | 5349 ± 585 | 243.14 | 1502 ± 211 | 12.62 |
| | 1.6 | 5069 ± 429 | 230.41 | 1392 ± 145 | 11.70 |

Table C-8 Mutagenicity of 1-aminopyrene treated sodium nitrite in the presence of each rice extract on *S. typhimurium* TA98 and TA100 (continued)

| Rice extract | | TA98 | | TA100 | |
|-----------------------------|-----------------------------------|--------------------------------------|-----------------|--------------------------------------|-----------------|
| Name | Amount ^a (mg/plate) | No. of revertants/plate ^b | MI ^c | No. of revertants/plate ^b | MI ^c |
| Black glutinous rice | | | | | |
| Raw | Spontaneous ^d | 15 ± 4 | - | 123 ± 11 | - |
| | 0 ^e | 2089 ± 410 | 139.27 | 689 ± 63 | 5.60 |
| | 0.8 | 3128 ± 493 | 208.53 | 791 ± 87 | 6.43 |
| | 1.2 | 2830 ± 583 | 188.67 | 786 ± 79 | 6.39 |
| | 1.6 | 2315 ± 404 | 154.33 | 765 ± 70 | 6.22 |
| Cooked | Spontaneous | 19 ± 1 | - | 127 ± 16 | - |
| | 0 ^e | 2639 ± 360 | 138.89 | 1060 ± 102 | 8.35 |
| | 0.8 | 3612 ± 264 | 190.11 | 1030 ± 217 | 8.11 |
| | 1.2 | 3447 ± 269 | 181.42 | 1017 ± 115 | 8.01 |
| | 1.6 | 2852 ± 280 | 150.11 | 997 ± 155 | 7.85 |
| Fermented | Spontaneous | 15 ± 1 | - | 120 ± 6 | - |
| | 0 ^e | 2300 ± 111 | 153.33 | 1052 ± 106 | 8.77 |
| | 0.8 | 3683 ± 164 | 245.53 | 1479 ± 168 | 12.33 |
| | 1.2 | 3262 ± 139 | 217.47 | 1403 ± 161 | 11.96 |
| | 1.6 | 3171 ± 278 | 211.40 | 1304 ± 109 | 10.87 |

^a Amount per plate of rice extracts. ^b Data are expressed as mean ± SD of triplicate plates from two independent experiments (N=6). ^c Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the rice extracts divided by that of spontaneous revertants. ^d Spontaneous revertants. Bold number indicates the higher result compared with that of no extract. ^e No extract was added to the standard mutagens, nitrite treated 1-aminopyrene.

BIOGRAPHY

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