Comparison of Ingestive Effects of Brewer's Yeast, Casein, and Soy Protein on Bioavailability of Dietary Iron

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Summary The effects of brewer's yeast, casein, and soy protein intakes on the absorption and retention as well as the incorporation into hemoglobin and systemic iron stores of dietary iron were examined in an animal experiment with growing rats. Relative biological values (RBV) of iron in the rats fed casein (C), soy protein (SP), and yeast (Y) diets were 1.00, 0.31, and 1.77, respectively. The apparent absorption of iron in Y-diet-fed rats was significantly higher than that in C- or SP-diet-fed rats. The hemoglobin regeneration efficiency (HRE) of iron in Y group was significantly higher than those in C and SP groups. As a result of search for iron-absorptive enhancers (IAE) in yeast, RBV and HRE of the yeast-cell-wall-including diet turned out to be significantly higher than those of its lacking diet. These results suggest that IAE occurring in the yeast cell wall may be effective for iron absorption.

Key Words brewer's yeast, yeast cell wall, soy protein, iron bioavailability, iron-absorptive enhancers

Iron-deficient anemia is still a major nutritional problem worldwide (1, 2). Infants, school-age children, and women of reproductive age are most critically affected. Approximately 50% of populations in developing countries suffer from anemia, where diets are rich in iron absorption inhibitors or low in iron-absorptive enhancers (3). A useful means of preventing iron-deficient anemia is to fortify foods with iron (4). An excessive iron intake, however, may be detrimental to health and increase the incidence of atherosclerosis (5) or cancer (6) because of oxidative stress. To avoid risks of both iron deficiency and iron excess, it is desirable to fortify food with components that can enhance the absorption of natural dietary iron instead of iron fortification.

Several investigators have found that brewer's yeast (yeast) can improve iron-deficient anemia in pregnant rats (7, 8). The effect of soy protein on dietary iron bioavailability was higher than that of egg yolk (9), and casein did not affect the bioavailability of ferrous sulfate (10). Moreover, soy protein and casein have been widely used as protein sources in daily life, and yeast is a cheap source for protein, vitamins, and minerals. If yeast intake enhances the intestinal absorption of dietary iron, yeast will serve not only as a good source of protein, vitamins, and minerals, but also as a preventive against malnutrition anemia in developing countries.

From this viewpoint, the present study was designed to compare the effects of yeast intake on the absorption and retention of dietary iron with those of casein and soy protein.

MATERIALS AND METHODS

Materials. Casein was obtained from Wako Pure Chemical Industries Ltd., Japan (Protein (P), 86.2%; Carbohydrate (C), 0.0%; Lipid (L), 1.5%; and Iron (I), 0.8 mg%). Soy protein was a gift from Fuji Seiyu, Ltd. (Tokyo, Japan) (P, 90.0%; C, 4.1%; L, 5.5%; and I, 10.0 mg%). Dried brewer's yeast (yeast) as whole cells (P, 51.5%; C, 30.5%; L, 4.3%; and I, 6.5 mg%), yeast cytoplasm after its enzymatic proteolysis (P, 67.5%; C, 17.6%; L, 0.5%; and I, 1.4 mg%), and centrifugally separable yeast cell wall (P, 30.0%; C, 50.0%; L, 8.5%; and I, 3.9 mg%), were obtained from Asahi Breweries, Ltd. (Tokyo, Japan). These preparations were used without further purification. Other chemicals were of analytical grade.

Animals and diets. Experiment I. Comparison of the effects of brewer's yeast, casein, and soy protein on the absorption and incorporation into hemoglobin. Male 10-wk-old Wistar rats were individually housed in stainless steel cages in a room maintained at 23±2°C with a 12 h light-dark cycle. The rats were allowed free access to a stock diet (CE-2, containing 31.5 mg iron/kg, CLEA Japan, Tokyo) and distilled water for 3 d. After blood was drawn from the tail vein for measurements of he-
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Table 1. Compositions of iron-deficient diet and iron-containing diets.

| Ingredients (g/kg food) | ID<sup>1</sup> diet |
|-------------------------|---------------------|
|                         | Experiment I       |
|                         | Experiment I-A   |
|                         | Experiment I-B   |
|                         | Experiment I-C   |
|                         | C<sup>2</sup> SP<sup>3</sup> Y<sup>4</sup> | C | SP | Y |
| Casein                  | 200.0             | 200.0 | 200.0 | 200.0 |
| Soy protein             | —                 | 160.0 | —     | 160.0 |
| Yeast                   | —                 | —     | 250.0 | —     |
| YC                      | —                 | —     | —     | 250.0 |
| YC+W                   | —                 | —     | —     | 190.0 |
| Egg white<sup>7</sup>   | —                 | 35.0  | 52.0  | 35.0  |
| Corn starch             | 420.0             | 420.0 | 318.0 | 420.0 |
| Sucrose                 | 235.0             | 235.0 | 235.0 | 235.0 |
| Soybean oil            | 50.0              | 50.0  | 50.0  | 50.0  |
| Vitamin mix<sup>8</sup> | 10.0              | 10.0  | 10.0  | 10.0  |
| Mineral mix<sup>9</sup> | 35.0              | 35.0  | 35.0  | 35.0  |
| Cellulose               | 50.0              | 50.0  | 50.0  | 50.0  |
| Iron<sup>6</sup> (mg)   | 9.2               | 9.2   | 25.6  | 24.2 |
| FeSO<sub>4</sub> (mg)   | 1640              | 147.6 | 149.0 | 9.2  |
| Total iron<sup>10</sup> | 9.2              | 173.2 | 173.2 | 60.2 |

| Iron-containing diets |
|-----------------------|
| Experiment I-A       |
| Experiment I-B       |
| Experiment I-C       |
| C         | Y         | YC<sup>5</sup> | YC+W<sup>6</sup> |
| C         | Y         | YC         | YC+W       |
| C         | Y         | YC         | YC+W       |

<sup>1</sup> ID, iron-deficient diet; <sup>2</sup> C, casein; <sup>3</sup> SP, soy protein; <sup>4</sup> Y, yeast; <sup>5</sup> YC, yeast cytoplasm; <sup>6</sup> YC+W, yeast cytoplasm+yeast cell wall. <sup>7</sup> Egg white was added to the test diet to adjust the protein content. <sup>8</sup> Vitamin and mineral mixtures were prepared as described in reference from AIN 93. <sup>9</sup> Iron from materials. <sup>10</sup> Total iron in the diet=added FeSO<sub>4</sub> + iron from materials.

moglobin (Hb), hematocrit (Ht), and serum iron (SI), the rats were divided into two groups; one (n=15) was used for the iron absorption experiment (experiment I-A), and the other (n=20) for Hb regeneration efficiency (HRE) and iron balance experiments (experiment I-B and I-C).

Iron absorption experiment (experiment I-A). This experiment was carried out to investigate the effects of diets on iron absorption. Rats were meal-fed with 10 g of an iron-deficient diet (Table 1) for 10 d from 09:00 to 10:00 every day. The rats were then assigned to casein (C), soy protein (SP), and yeast (Y) groups that had been similar to one another at the Hb level (Table 2). After the rats fasted for 1 d, the initial blood sample (T<sub>0</sub>) was drawn. Within 1 h later, the rats were fed test diets containing casein, soy protein, or yeast as a main protein source. These three diets were adjusted with egg white to compensate for the protein content. FeSO<sub>4</sub> was added to the diets to give approximately 173 mg of iron per kg (Table 1). Blood was withdrawn from the tail vein at 30, 90, and 240 min after the feeding of test diets for 1 h.

Hemoglobin regeneration experiment (Experiment I-B). The effect of each diet on iron incorporation was evaluated by Hb repletion assay in anemic rats (12). For this purpose, the rats were fed an iron-deficient diet. On day 28, twenty rats were weighed and subjected to measurements of Hb, Ht, SI, and total iron-binding capacity (TIBC); every 5 rats were allotted to 3 repletion diets (C, SP, or Y) and control (Co) diet. The iron contents in the internal organs of the Co group were first determined and regarded as calculative initial values in the organ iron regeneration efficiencies (OIRE) of the repletion groups (C, SP, Y) (Table 2). Iron from materials. Total iron in the diet=added FeSO<sub>4</sub> + iron from materials.

Internal organs of the Co group were first determined and regarded as calculative initial values in the organ iron regeneration efficiencies (OIRE) of the repletion groups (C, SP, Y) (Table 2). Egg white was used also for the adjustment of protein content. Each diet was supplemented with FeSO<sub>4</sub> at a level of 60 mg/kg diet (Table 1). Rats were pair-fed against the C group for a repletion period. On day 14, they were anesthetized with 0.1 mL of 1/20 sodium pentobarbital solution/100 g body weight. Blood was collected from the abdominal aorta for measurements of Hb, Ht, SI, and TIBC. The liver, spleen, and kidney were excised to measure organ iron concentration.

HRE and OIRE were calculated in the same manner as previously described, and transferrin saturation (TS) was done according to the American Dietetic Association method.

Iron balance experiment (Experiment I-C). On days 4–7 and 10–13 during the repletion period, daily feces were collected from each rat to measure iron retention. The iron levels in urine, perspiration, and sloughed skin were considered negligible. FeSO<sub>4</sub> was added to the diets to give approximately 173 mg of iron per kg (Table 1). Blood was withdrawn from the tail vein at 30, 90, and 240 min after the feeding of test diets for 1 h.

Iron absorption enhancers (IAE) in brewer’s yeast. Male 8 wk-old Wistar rats (n=45) were divided into 9 groups of five rats each; 4 groups such as casein (C), whole yeast cells (Y), yeast cytoplasm (YC) and a mixture of yeast cytoplasm and yeast cell wall (YC+W) were used for iron absorption
Table 2. The status of rats at the beginning of experiment I and experiment II. Values are expressed as mean ± SD.

| Parameters          | Experiment I-A | Experiment I-B | Experiment II-A | Experiment II-B |
|---------------------|----------------|---------------|----------------|----------------|
| Weight (g)          | 227 ± 9        | 227 ± 9       | 227 ± 9        | 227 ± 9        |
| HB (g/l)            | 145 ± 9        | 165 ± 9       | 165 ± 9        | 165 ± 9        |
| SI (mg/dl)          | 47 ± 2.2       | 48 ± 2.2      | 47 ± 2.2       | 47 ± 2.2       |
| TIBC (mg/dl)        | 14.3 ± 0.1     | 14.3 ± 0.1    | 14.3 ± 0.1     | 14.3 ± 0.1     |
| TS (%)              | 14.3 ± 0.1     | 14.3 ± 0.1    | 14.3 ± 0.1     | 14.3 ± 0.1     |
| LI (mg/dl)          | 3.0 ± 0.1      | 3.0 ± 0.1     | 3.0 ± 0.1      | 3.0 ± 0.1      |
| SpI (mg/dl)         | not detected   | not detected  | not detected   | not detected   |
| KI (mg/dl)          | not detected   | not detected  | not detected   | not detected   |

Values are expressed as mean ± SD. 

Experiment I-A: Comparison of the effects of brewer’s yeast, casein and soy protein intakes on iron absorption, and its incorporation into hemoglobin or retention within the body. Results and discussion.

The rate of iron absorption was evaluated from experiment I-A; Fig. 1 and Table 3 show the results of slope-ratio statistical analyses. RBV of iron from C, SP, or Y diets were obtained as 1.00, 0.31, and 1.77, respec-
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Fig. 1. Dose-response curves for serum iron concentration in rats fed with casein, soy protein, or yeast diets. Y, yeast; C, casein; SP, soy protein.

Table 3. The effects of iron-containing diets on response parameters of experiment 11.

| Variables                                | Dietary group |
|------------------------------------------|---------------|
|                                          | C             | SP             | Y              |
| Experiment I-A                           |               |                |                |
| Iron intake (mg)                         | 0.82±0.27     | 0.85±0.31      | 0.85±0.11      |
| Relative biological value2,1             | 1.00          | 0.31           | 1.77           |
| Experiment I-B                           |               |                |                |
| Iron intake (mg)                         | 15.6±0.6      | 15.4±0.9       | 15.6±0.9       |
| Feed intake (g)                          | 259.7±10.0    | 256.0±15.0     | 259.6±12.0     |
| Weight gain (g)                          | 26.6±9.0      | 24.4±2.7       | 24.4±8.3       |
| Feed efficiency ratio (%)                | 10.2±0.9      | 9.5±0.2        | 9.4±0.7        |
| Hemoglobin regeneration efficiency (%)   | 11.9±2.9b     | 10.6±1.8a      | 18.2±2.1b      |
| Hemoglobin gain (g/L)                    | 11.2±3.1      | 9.6±3.2        | 18.5±4.2       |
| Hematocrit gain (%)                      | 6.3±1.9       | 5.5±1.2        | 6.9±3.6        |
| Transferrin saturation gain (%)          | 17.3±7.0      | 11.1±5.0       | 20.6±8.0       |
| Liver iron regeneration efficiency (%)   | 2.6±1.2       | 3.3±0.9        | 3.5±0.6        |
| Spleen iron regeneration efficiency (%)  | 1.2±0.5       | 0.7±0.3        | 0.9±0.4        |
| Kidney iron regeneration efficiency (%)  | 0.06±0.06     | 0.07±0.03      | 0.10±0.05      |
| Experiment I-C                           |               |                |                |
| Apparent iron absorption (%)             | 14.7±3.0a     | 15.3±4.2a      | 25.7±3.5b      |

Values are means±SD (n=5). 1 Experiment I-A, iron absorption experiment; Experiment I-B, hemoglobin regeneration experiment; Experiment I-C, iron balance experiment. 2 Relative biological values (RBV) were calculated by the use of slopes from the regression lines of the casein, soy protein, and yeast diets according to the method of Finney (16). All serum iron parameters were significantly correlated with iron intake and met requirements for the bioavailability estimation based on multiple linear regression models. 3 C was chosen as standard diet in RBV calculation, and the RBV value of iron from C diet was considered as 1. Values not sharing a common superscript letter in the same row are significantly different at p<0.05.

...tively, indicating that yeast might enhance iron uptake and its transport into plasma.

Incorporation of dietary iron into storage

Iron storage was evaluated by an iron repletion experiment (experiment I-A). In this connection, there are four biochemical indices, which represent three stages of iron depletion: namely, serum ferritin at the first stage, TS and free erythrocyte protoporphyrin at the second stage, and Hb at the last stage (21).

Table 3 shows average HRE and OIRE in the liver, spleen and kidney, as well as gains in Hb, Ht, and TS at the finish of repletion period. HRE was slightly high in Y group relative to C group, but it became significantly higher than in SP group, as shown in Table 3. Hb and Ht data did not necessarily correlate with each other. This tendency was the same as observed by Anderson et al. (22) and Kim and Atallah (23).

In contrast, neither TS gain nor OIRE in Y group significantly differed from those in both C and SP groups (Table 3). A similar phenomenon has been observed by Hunter (24) and Hou et al. (25), who found that Hb levels were restored in 1 wk or so during the repletion period. On the other hand, TS and organ iron levels somewhat increased at the 2nd wk, but a 2-wk supplementa-
tion with iron seems to be not enough to produce significant differences in TS or organ iron levels among the above three dietary groups.

Iron retention

This assessment was based on the iron balance experiment (experiment I-C). AIA in Y group was significantly higher than in C and SP groups. These values were obtained as 25.7%, 14.7%, and 15.3%, respectively (Table 3).

From these results, it is highly probable that yeast intake enhances iron absorption and its transport into plasma or body iron store, thereby raising the retention of dietary iron.

Experiment II. IAE occurrence in yeast and its effect on iron absorption and storage

To ascertain the possibility of IAE occurring in yeast, we compared the effects of whole yeast cell (Y), yeast cytoplasm (YC), and a mixture of yeast cytoplasm plus cell wall (YC+W) on the absorption and storage of dietary iron. The iron bioavailability in C- and Y-fed groups was obtained from the iron absorption velocity by the use of slope-ratio statistics (15–18), as shown in Fig. 2. As a result of calculation, it turned out that the RBV in Y and YC+W groups was much higher than those in C and YC groups (Table 4 and Fig. 2). Moreover, the incorporation into storage forms indicated by HRE and OIRE

![Graph](image)

Fig. 2. Dose-response curves for serum iron concentration in rats fed with casein or yeast-containing diets (yeast, yeast cytoplasm, and a mixture of yeast cytoplasm with yeast cell wall). Y, yeast; YC+W, yeast cytoplasm+yeast cell wall; YC, yeast cytoplasm; C, casein.

Table 4. The effects of iron-containing diets on response parameters of experiment II.

| Variables                                      | Dietary group |
|------------------------------------------------|---------------|
| Iron intake (mg)                               | C             |
| Relative biological value<sup>2,3</sup>        | 0.69±0.01     |
|                                                | 0.68±0.03     |
|                                                | 0.68±0.01     |
|                                                | 0.67±0.01     |
| Experiment II-B                                |               |
| Iron intake (mg)                               |               |
| Food intake (g)                                |               |
| Weight gain (g)                                |               |
| Feed efficiency ratio (%)                      |               |
| Hemoglobin regeneration efficiency (%)         |               |
| Hemoglobin gain (g/L)                          |               |
| Hematocrit gain (%)                            |               |
| Transferrin saturation gain (%)                |               |
| Liver iron regeneration efficiency (%)         |               |
| Spleen iron regeneration efficiency (%)        |               |
| Kidney iron regeneration efficiency (%)        |               |

Values are means±SD (n=5).<sup>1</sup> Experiment II-A, iron absorption experiment; Experiment II-B, hemoglobin regeneration experiment. See footnotes 2 and 3 in Table 3. Values not sharing a common superscript letter in the same row are significantly different at p<0.05.
revealed that these values in the YC+W group were significantly higher than those in both C and YC groups (Table 4). From these results, it is estimated that an IAE exists in yeast cell walls.

It is reasonably inferred from the above data that yeast cell walls stimulate not only iron absorption, but also its incorporation into storage forms. The mechanism by which yeast cell walls enhance iron absorption is not clear. However, the process of iron absorption can be divided into 3 stages according to Conrad et al. (26): (1) iron uptake by enterocytes, (2) storage, and (3) transfer to extracellular. Yeast cell walls may be involved in iron uptake as iron chelating factors, reductants connecting ferric to ferrous and fermentative sources generating low-molecular organic acids. Chelators facilitate solubilization of iron; this step is essential so that iron can be absorbed in the lumen (26). Yeast contains 1.2% histidine (data from Asahi Breweries); therefore the Y diet contains 25% yeast, i.e., 0.3% histidine, thereby providing enough histidine to reduce ferric to ferrous iron (27). Besides histidine, glutathione constituting 1.5% of the yeast cell is also expected to have a similar effect on iron absorption (28). Incidentally, Ishikawa et al. (29) have found that yeast has almost the same antioxidative potency as glutathione and cysteamine; suggesting that the antioxidative potency of yeast may play a role in the mechanism of ferrous sulfate absorption.

Iron chelators play an important role in iron absorption in the small intestine. Furthermore, indigestible carbohydrates such as fructosaccharides, inulin, and guar gum hydrolysate stimulate iron absorption in the large intestine (30). Forty-five percent of the yeast cell wall is polysaccharides such as glucan and mannan (data from Asahi Breweries). The fermentation of these polysaccharides in the large intestine will produce lactate (31) that lowers pH of the luminal contents, forms a complex with ferric iron, and raises the solubility of iron (32).

In conclusion, it may safely be said that brewer’s yeast, exactly yeast cell wall, promotes the intestinal absorption of dietary iron and its incorporation into hemoglobin or other storage forms. Therefore we speculate that yeast may serve as an iron-absorptive enhancer and as an actual preventive against anemia in developing countries because of its cheap source of protein, vitamins, and minerals.

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