Protein-Nutritive Assessment of Sake Lees Obtained by Brewing from Liquefied Rice

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(Received August 24, 1997)

Summary Sake lees obtained by brewing from liquefied rice were deprived of water and alcohol by lyophilization, and then examined for nutritional availability with the aid of proximate food analysis, amino acid analysis and animal experiment. Freeze-dried sake lees powder was comprised of 44.6% protein, 37.4% carbohydrate, 2.5% fat, 6.7% fiber, 1.8% ash and 7.2% moisture (alcohol <0.1%), of which the nutritive value (amino acid score) was estimated as 89.6 when compared with the amino acid requirements for preschool children (FAO/WHO/UNU, 1985). Sake lees protein had been, however, appreciably improved in the limiting amino acid “lysine” relative to polished rice protein. As a result of an animal experiment, the rats fed a 50% sake lees powder diet proved to be equal in growth to those fed a 20% casein (control) diet, although the former diet had to be supplemented with vitamins and minerals, which were in shortage as compared to the control diet. On the other hand, the feeding of sake lees powder was effective in lowering the serum triacylglycerol concentration. Accordingly, sake lees powder can be assessed as a favorable candidate for not only protein-rich but also hypolipidemic provisions.

Key Words sake brewing, sake lees protein, liquefied rice, nutritive value, hypolipidemic effect

Sake lees abundant in protein, carbohydrate and alcohol have not only been formerly eaten as toasted cakes or ‘kasu’ soup, but are still used as flavoring materials for the pickling of vegetables, fish and meats (1, 2). Great variation in current eating habits, however, has progressively lessened the consumption of sake lees, and significant quantities amounting to 100,000t/y are changing into waste

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foodstuffs for unprofitable reasons (3–5). What is worse, sake-brewing employees have become far advanced in age. Under such situations, a new technique of liquefying rice with the aid of a thermostable saccharifying amylase was introduced in the sake-brewing process, availing to raise productivity and eliminate or reduce labor (6–9). Sake lees produced in this manner may differ somewhat in composition. If a food shortage were to occur in the future, one ready choice could be to use sake lees efficiently as provisions. This investigation was designed to re-evaluate sake lees of ‘liquefied rice’ origin from a protein-nutritive point of view.

MATERIALS AND METHODS

Preparation of dried sake lees. Pressed cakes of sake lees, produced by rice liquefaction* in the early spring of 1995 and kindly offered from Saito Sake Brewing Co., Kyoto, Japan, were homogenized with 3 volumes of distilled water in a blender and immediately lyophilized with a laboratory freeze-dryer. A portion of the sake lees powder was sampled to determine ethanol content. The ethanol content estimated by UV measurement with NAD⁺ and alcohol dehydrogenase (EC 1.1.1.1) (10) was less than 1 mg/g on a dry weight basis.

All other commercially available chemicals and assay kits were used as such without further purification.

Proximate analysis of sake lees powder. Crude protein, crude fat and crude fiber were determined by the micro-Kjeldahl, Soxhlet’s and acid-alkali extraction methods, respectively (11). Crude ash was obtained as the residue after combustion at 600°C, and moisture was due to the difference before and after dehydration at 120°C. The remainder subtracted by the above sum was conveniently defined as carbohydrate.

Amino acid analysis of sake lees protein. Sake lees protein was hydrolyzed with 6N HCl at 110°C for 23 h in a deaerated tube as usual, and its clarified aliquot after pH adjustment was applied to a Hitachi 835 analyzer equipped with a computer to determine amino acids. Tryptophan was colorimetrically determined by the use of p-dimethylaminobenzaldehyde after alkaline hydrolysis in the usual manner.

In vitro digestion of sake lees protein. Two 10% suspensions of sake lees powder in 0.05 M sodium phosphate buffer (pH 7.5) were incubated at 30°C for 18 h together with and without 700 units of Arthrobacter tunicase-R70 (Daiwa Kasei Co., Osaka, Japan). Then, these suspensions were adjusted to pH 2 with HCl and incubated at 37°C in the presence of 33 mg/L porcine pepsin (Sigma Chemical Co., St. Louis,

* Rice-liquefying conditions: Seventy percent polished rice (Nihonbare) was immersed in mashing water at 40°C, to which saccharifying amylase “Amano AY-2” was added at a ratio of 1 : 2,000. The mixture was allowed to warm with stirring from 40 to 90°C (i.e., for 2 h at 40–70°C, 1 h at 70–80°C, 45 min at 80–90°C, and 5 min at 90°C), followed by cooling to 35°C. At that time, the viscous liquid showed 18.2 Be with a Baume hydrometer, and a viscosity of 200 cps.

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Table 1. Composition of experimental diets (%).

| Ingredient              | A   | B   | C   | D   |
|-------------------------|-----|-----|-----|-----|
| Sake lees powder        | —   | 50  | 94  | 100 |
| Bovine casein           | 20  | —   | —   | —   |
| x-Corn starch           | 66  | 42  | —   | —   |
| Soybean oil             | 4   | 3   | 2   | —   |
| Mineral mixture¹        | 5   | 4   | 3   | —   |
| Vitamin mixture²        | 1   | 1   | 1   | —   |
| Cellulose powder        | 4   | —   | —   | —   |

¹,² AIN-76 likeness, products of Oriental Yeast Co., Tokyo.

MO, USA). Eighteen hours later, the suspensions were neutralized with NaOH, to which porcine pancreatin (Sigma Chemical) was added to a final concentration of 33 mg/L. At appropriate intervals during incubation at 37°C, aliquots of the suspension were taken out, acidified with one-tenth volume of 25% TCA and centrifuged to remove the resulting precipitate. As an indication of protein digestibility, the nitrogen content in the TCA-soluble fraction was determined by the micro-Kjeldahl method.

Animal feeding and diets. This experimental design was approved by the Animal Experiment Committee of Kyoto Prefectural University in line with the Guideline for Care and Use of Laboratory Animals.

Weanling male rats of the Wistar strain, were purchased from Shimizu Laboratory Supplies Ltd., Kyoto, Japan, and housed in the animal care facility at room temperature of 24±1°C with a half-day light/dark cycle. The rats weighing about 45 g, after being acclimated to a powdered stock diet for a week, were divided into 4 groups (n=5) and fed ad libitum for a further 3 weeks; their respective diets are shown in Table 1. Daily changes in body weight gain were recorded over a period of 2 weeks. At 3 weeks, the rats were subject to blood-collecting under ether anesthesia.

Serum triacylglycerol, cholesterol and glucose assays. Each blood sample collected was immediately centrifuged to separate the plasma, which was stored in a freezer until use. After removal of fibrous precipitates following thawing, the serum triacylglycerol, cholesterol and glucose concentrations were measured according to their protocols with assay kits (Wako Pure Chemical Industries Ltd., Osaka, Japan).

Statistical analysis. Data for each dietary group in the animal experiment were obtained as the mean ±SE for 5 animals. Evaluation as to whether or not there were significant differences among these three (A, B and C) groups was conducted using the Student-Neuman-Keuls test (12).
RESULTS

Table 2 shows a comparison of the proximate food composition of sake lees powder from liquefied rice with that of traditional sake lees. There was a noteworthy difference between them; that is, protein was the highest content in the former in contrast to carbohydrate in the latter. Besides carbohydrate, fat and fiber were somewhat reduced in content.

Table 3 shows the amounts of essential amino acids in sake lees powder from

Table 2. Proximate food composition of sake lees powder.

| Ingredient   | Sake lees powder by rice liquefication | Sake lees powder as of old* |
|--------------|----------------------------------------|-----------------------------|
| Protein      | 44.6                                   | 36.7                        |
| Carbohydrate | 37.4                                   | 44.1                        |
| Fat          | 2.3                                    | 3.7                         |
| Fiber        | 6.7                                    | 7.1                         |
| Ash          | 1.8                                    | 1.2                         |
| Moisture     | 7.2                                    | 7.2                         |

*Calculated in moisture content of 7.2% from Standard Tables of Food Composition in Japan, 4th revised edition—1982—, Resources Council, Science and Technology Agency, Japan.

Table 3. Amino acid composition of sake lees protein obtained by brewing from liquefied rice.

| Essential amino acid | Content (g/16 g N) | Scoring pattern for preschool age* |
|----------------------|--------------------|------------------------------------|
| His                  | 4.1                | 1.9                                |
| Ile                  | 5.7                | 2.9                                |
| Leu                  | 9.7                | 6.6                                |
| Lys                  | 5.2                | 5.8                                |
| Met + Cys            | 7.3                | 2.5                                |
| Phe + Tyr            | 10.9               | 6.3                                |
| Thr                  | 6.2                | 3.4                                |
| Trp                  | 1.1                | 1.1                                |
| Val                  | 7.1                | 3.5                                |

Score (%) 89.6

*This is the amino acid-scoring pattern (g/16 g N) proposed for preschool children in 1985 by the Joint FAO/WHO/UNU Expert Consultation. Analytical data on amino acids other than “essential” were as follows (in g/16 g N); Asp 11.5, Ser 6.7, Glu 18.5, Gly 6.0, Ala 9.2, and Arg 8.0.

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liquefied rice origin, which are accompanied with their individual requirements for preschool children (FAO/WHO/UNU, 1985). As a result of comparison, sake lees protein was still insufficient in lysine but its amino acid score was about 1.5-fold that of polished rice protein. This fact implied that sake lees protein did not all originate from rice protein but from microbial protein to some extent. In this connection, and the effect of the cell wall of yeast, sake lees protein was examined for treatment with Arthrobacter tunicase, followed by digestion with pepsin and pancreatin. As illustrated in Fig. 1, the presence and absence of tunicase didn't affect the in vitro digestibility of sake lees protein. The gain of TCA-soluble nitrogen is considered to be caused by proteolytic enzymes derived from ‘koji’ and yeast in sake lees powder (Fig. 1a). It seemed unlikely that the cell wall of yeast withstood the attack by tunicase and remained in sake lees without additional great destruction. Similarly, in the pepsin-pancreatin digestion experiment, the digestibility of sake lees protein never exceeded 50% (Fig. 1b); although casein, for reference, underwent satisfactory digestion (>90%) under the same assay conditions.

Figure 2 depicts the growth curves of rats fed, ad libitum, the respective diets given in Table 1. The 50% sake lees powder-based diet was regarded as equivalent to the 20% casein-based (control) diet from the viewpoint of amino acid score, simultaneously being corrected for its oil, mineral and vitamin contents. Moreover, two kinds of diets which completely resorted to sake lees powder for both protein and carbohydrate were set up for comparison. There was no significant difference

![Figure 1](image_url)

Fig. 1. Digestibility of lyophilized sake lees protein in vitro. (a) Preincubation (pH 7.5, 37°C, 18 h) in the absence (−) or presence of tunicase (+). None, TCA-soluble nitrogen content of 10% suspension of sake lees powder in 0.05 M sodium phosphate buffer (preincubation time, 0 h). (b) Pepsin digestion (pH 2, 18 h) and its following pancreatin digestion (pH 7, 18 h) after preincubation in the absence (∅) and presence of tunicase (●). Values are the means of triplicate measurements (SD within 8%).

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Fig. 2. Growth curves of rats fed the experimental diets: A, control; B, 50% sake lees powder (supplemented with starch, oil, minerals and vitamins); C, 94% sake lees powder (supplemented with oil, minerals and vitamins); D, 100% sake lees powder.

Fig. 3. Serum triacylglycerol, total cholesterol and glucose levels in rats fed the experimental diets (A, B and C) for 3 weeks. The serum level of each parameter was determined by the use of commercially available assay kits. ** Significantly different from control at $p<0.01$.

in growth between both groups fed the 50% sake lees powder and 20% casein diets. A similar effect on growth was observed for the group given only a sake lees powder supplemented with oil, minerals and vitamins, while such was not the case with the group given only a sake lees powder without any supplementation. Five rats of the latter group showed little gain in body weight. Setting aside those nutrients which are deficient in sake lees, the satisfactory growth in the 50% sake lees powder group has led to the view that the nutritive value of sake lees protein is comparable to that of casein, despite its incomplete digestibility in vitro.

Sake lees powder contains a quantity of fiber. It was therefore examined to what extent its intake would affect triacylglycerol, cholesterol and glucose concentrations in the serum. The results of measurements with assay kits are diagrammed in Fig. 3. The sake lees powder group without any supplementation
was excluded from the object of assays on the account of a considerable difference in body weight. The serum triacylglycerol concentrations in the two groups fed the 50% and 94% sake lees powder diets were significantly lower than in the control group. There were rather reverse tendencies, although not significantly, with regard to the serum cholesterol and glucose concentrations. Taken altogether, dried sake lees exclusive of alcohol were favorably evaluated as not only protein-rich but also a hypolipidemic provision.

DISCUSSION

Traditionally manufactured sake lees are scarcely available these days because mashing with liquefied rice predominates in sake brewing. For this reason, the proximate food composition in the name of ‘sakekasu’ was cited from “Standard Tables of Food Composition in Japan (4th revised edition)” published in October 1982, and then corrected for the moisture content of the present freeze-dried sake lees powder. Comparison between both sake lees powder preparations with and without previous rice-liquefying revealed that a significant increase in the protein content, probably by efficient fermentation, was characteristic of sake lees powder brewed from liquefied rice. According to the Tables of Food Composition, the protein content of polished rice was approximately 6.8%, which amounted to more than 40% by at least 6-fold concentration. Incidentally, the yield of sake lees was approximately 16% (in dry weight) of the original polished rice (the analytical value of Saito Sake Brewing Co.). So long as sake lees are a fermented food, however, such an increase must have been overlapped with microbial protein. To prove this possibility, the amino acid composition of our freeze-dried sake lees powder was compared with that of polished rice protein described in “Revised Tables of Amino Acid Composition of Foods in Japan—1986—,” Resources Council, Science and Technology Agency, Japan. The individual essential amino acid levels were evaluated on the basis of amino acid requirements for preschool children proposed in 1985 by the Joint FAO/WHO/UNU Expert Consultation. The limiting amino acid in polished rice protein was lysine. Its amino acid score was 60.3 by moderate calculation, while that of sake lees protein was 89.6 (Table 3). Sake lees protein ought to be lacking in lysine equal to that of polished rice irrespective of previous rice-liquefying, provided that protein bodies occurring in polished rice undergo little proteolysis and remain insoluble through the process of sake brewing (13). As a matter of fact, the sake lees protein appreciably improved in lysine content although it was still the limiting amino acid. The peptide bond adjacent to basic amino acids such as lysine and arginine is rather susceptible to proteinase. It thus seems unlikely that lysine-rich rice protein body fragments are preferentially left behind. Yeast protein is more abundant in lysine than rice protein (14–16). Accordingly, an improvement in the nutritive value of sake lees protein may be accounted for by the supply of lysine from microbial protein.

In this feeding experiment with sake lees powder, an ordinary 20% casein diet

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was used as the control. Casein is a satisfactory protein for preschool children in nutritional aspects. The experimental diet consisting of 50% sake lees powder includes protein of 89.6 in amino acid score at the rate of 22.3% on a dry weight basis, being a good match in protein nutrition for the 20% casein diet. This assessment was made toward preschool children but may possibly be inappropriate for very young animals. For example, the lysine requirement for growing albino rats has been formerly proposed as 9.0 (Rama Rao, 1961), 7.5 (NRC, 1972) or 6.6 (Miyazaki, 1965) in g/16 g N. There were great variances among these values, each of which was much higher relative to the 5.8 g/16 g N for preschool children. Because of the actual difficulty in setting up a proper standard, the experimental diet was no longer fortified with amino acids. Vitamins, minerals and fat were compensated for their respective shortages, but no attention was paid to a cellulose supplement in consideration of fibered sake lees. Apparently as shown in Fig. 2, the 50% sake lees powder group was not significantly different in growth from the control group for the first 2 weeks. Quite similar growth was observed for the 94% sake lees powder group; nevertheless, non-supplemented sake lees powder did little to increase body weight gain during the same period as above. On the other hand, growth was not impaired in the case of no supplementation with fat (data not shown). Hence, it can be said that sake lees powder including good quality protein is regrettably insufficient in terms of both vitamins and minerals. It remains to be further investigated what kind of nutrient should be supplemented.

Carbohydrate exclusive of fiber occupies more than one-third of the sake lees powder and its bulk seems to be made available as starch. Strictly speaking, however, there is no evidence to show whether carbohydrate is virtually equivalent to starch from the standpoint of digestibility. Since rice starch in liquefying is pretreated at high temperature with thermostable Bacillus amylase, it is highly possible that such a structural change as to affect in vivo digestibility occurs in starch during heating and cooling. In addition to rice liquefaction, the process of fermentation will inevitably concentrate digestion-resistant starch. This “resistant starch” is looked upon as a sort of dietary fiber because of its indigestibility in the small intestine. Similarly, microbial remnants participate in broadly defined “dietary fiber.” It is not doubtful that sake lees have plenty of dietary fiber. Dietary fiber is generally accepted to play an important role in relieving constipation, sweeping out septic matters and alleviating risk factors for cardiovascular diseases. One of its various physiological functions is to improve serum lipid levels in normal animals. In this respect, the rats arranged for growth test were fed for a further 1 week in each successive state and examined for their serum lipid levels. The results of animal experiments are often stretched in human favor. We are careful not to eat sugar and/or cholesterol too much in ordinary eating habits. The use of a cholesterol-supplemented sucrose diet was consciously avoided in this experiment. It is reasonable to observe no significant difference in serum cholesterol concentration between the 20% casein and 50 and 94% sake lees powder groups under such dietary conditions. Unexpectedly, a significant difference was observed for serum
triacylglycerol concentration. The reason for the serum triacylglycerol-lowering effect of these sake lees powder groups is not clear at present.

When the layout of this manuscript drew to a close 16 mon after our last oral presentation at the 34th Annual Meeting of the Kinki Branch of Japanese Society of Nutrition and Food Science (1995), we came across an article concerning the serum cholesterol-lowering effect of sake lees powder (17). Both proximate food and amino acid compositions of hot air-dried sake lees powder were also items mentioned in the article. Interestingly, the protein content amounted to 72.1% on a dry weight basis, and the scores of the first and second limiting amino acids were estimated as 66.3 (lysine) and 72.8 (tryptophan), respectively, by comparison with the proposal for preschool children (FAO/WHO/UNU, 1985). As compared with our analytical data, the striking protein content was more reflected in a sharp drop in the carbohydrate content. This difference is mainly dependent on the efficiency of rice liquefaction by the use either of polished grains or their crushed pieces. In the case of liquefying with polished rice grains, alcohol fermentation proceeds at an easy pace but the yeast smoothly proliferates to produce a peculiar flavor. Sake of provincial reputation is brewed in this manner at the cost of productivity. With respect to high-protein sake lees powder, a diet containing 33.3% was comparable to the 25% casein diet in rat growth, never conflicting with our observation. We have different views on the serum cholesterol-lowering effect of sake lees powder. There is a clear discrepancy in dietary composition between their animal experiments and ours. Namely, a diet with cholesterol (+ sodium cholate) and sucrose (instead of starch) was used in the latter experiment. Starch and fiber will probably interfere in micelle formation as well as cholesterol absorption in the small intestine, even though dietary protein also serves as an impermeant component. Polished rice includes protein in the form of protein bodies which are little liable to in vitro proteolysis by digestive enzymes. Sake lees protein is, for the most part, originated from rice protein bodies, yet it is not necessarily inferior to casein in bioavailability. "Resistant protein," if any, should be duly considered a member of dietary fiber. The tendency for increased lipid and cholesterol excretions into the feces of rats fed the sake lees powder diet are so distinctly illustrated in the above article; proving that the hypocholesterolemic action of sake lees powder can be interpreted in terms of the obstruction of intestinal cholesterol absorption and its resultant lowering of serum cholesterol concentration.

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