Evaluation of the Chemical and Biological Characteristics of Sake Lees

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Abstract
Sake lees (SLs), produced from brewing Japanese Sake and known to be rich in yeast, is expected to be an alternative of feed protein sources for ruminants. Previous studies showed that yeast improved the digestibility of fibers in ruminants. However, the nutrient composition and the numbers of live and dead yeast in the SLs, have large diversity because of the different brewing materials and processes. The objective of this study was to evaluate the chemical and biological characteristics of SLs with diverse brewing processes and storing periods (0-23 weeks). Seven types of SLs (SLs 1-7) were collected from two breweries. SLs 1, 2, 5 and 6 were made from liquefied rice under high-temperature saccharification method and SLs 3, 4 and 7 were made from steamed rice under general multiple parallel fermentation method. The crude protein (CP) contents of SLs from liquefied rice were higher than those from steamed rice (59.3-69.9% vs 32.8-51.4%DM). Ethanol concentrations were in the range of 6.1 to 11.2%FM in SLs 1-5. The numbers of live yeast ($\times 10^4$ CFU/ FM g) were ranged from 1,462 to 6,109 before storing, which dramatically decreased to 0-145 at 4 weeks' storing at 4℃ in SLs 1-5. The numbers of dead yeast ($\times 10^9$ / FM g) were ranged from 0.7 to 3.0 before storing, which were stable during 4 weeks' storing showing 0.5-4.6 in SLs 1-5. These results suggested that SLs, especially from liquefied rice, had high CP contents and substantial amounts of ethanol. The live yeast observed drastically disappeared, on the other hand, the numbers of dead yeast were stable under refrigeration. Further study is needed to evaluate the effect of supplementary SLs on digestibility in ruminants especially in roughage feeding condition.

Introduction
Sake is a traditional Japanese alcohol beverage. The main sources of Sake are rice and yeast (*Saccharomyces cerevisiae*) for brewing. After brewing Sake, a great deal of leftover materials called Sake lees (SLs) was generated 39,000 tons/year in Japan (NTA 2018), and also in Europe and USA. Sake lees is rich in nutrients such as yeast, crude protein, vitamins and amino acids. Therefore, SLs is expected to be an alternative of feed materials for ruminants in various regions. Previous studies suggested that yeast culture increased the number of cellulolytic microorganisms in rumen (Dawson et al. 1990; Harrison et al. 1988) and improved the digestibility of fibers in Holstein cows (Wiedmeier et al. 1987). However, the nutrient composition and the numbers of live and dead yeast of SLs have large diversity due to the different brewing materials and processes. The nutrient diversity should be considered when using SLs as feed for ruminants. The objective of this study was to investigate the chemical and biological characteristics of SLs with diverse brewing processes and storing periods at 4℃.

Materials and Methods
Seven types of SLs (SLs 1-7) were collected from brewery A in Kyoto and brewery B in Hyogo, Japan. The SLs 1, 2, 5 and 6 were made from liquefied rice under high-temperature saccharification method and SLs 3, 4 and 7 were made from steamed rice under general multiple parallel fermentation method. The samples were stored at 4℃ until subsequent analyses up to a maximum of 23 weeks. The dry matter (DM), crude protein (CP) and ether extract (EE) contents of SLs were determined using the methods described by the Standards of the Association of Official Analytical Chemists (AOAC 2016). The neutral detergent fiber assayed without a heat-stable amylase and expressed exclusive of residual ash (NDFom) and acid detergent fiber expressed exclusive of residual ash (ADFom) were analyzed according to the procedure described by Van Soest et al. (1991). The ethanol concentration was determined using a F-kit (UV-method; J.K. International Co. Ltd. Tokyo Japan). The SLs were spread and incubated at 28 ℃ for 72 h on potato dextrose agar (FUJIFILM Wako Pure Chemical Corporation Osaka Japan), and the numbers of live yeast (colony forming units; CFU) were counted macroscopically. The numbers of dead yeast were counted using a buffered methylene blue solution

(0.02%MB solution in phosphate buffer pH 4.6) according to the staining method by Painting and Kirsop (1990).

Results

Chemical compositions and ethanol concentrations

Chemical compositions of SLs are presented in Table 1. The DM contents of SLs were in the range of 43.3 to 57.5%FM in SLs 1-7. The CP and NDFom contents of SLs from liquefied rice were higher than those from steamed rice (59.3-69.9% vs 32.8-51.4%DM; 20.2-22.9% vs 7.0-12.7%DM). Ethanol concentrations were in the range of 6.1 to 11.2%FM in SLs 1-5 (Table 2).

Table1. Chemical compositions (%) of Sake lees.

	Process	Brewery	DM	CP†	EE†	NDFom†	ADFom†
SLs 1	liquefied	A	55.0	69.9	6.0	22.9	15.8
SLs 2	liquefied	A	51.4	62.3	3.9	20.2	9.8
SLs 3	steamed	A	46.9	34.8	1.0	7.0	N.A
SLs 4	steamed	A	43.8	32.8	1.7	12.7	N.A
SLs 5	liquefied	A	57.5	64.4	5.1	20.2	N.A
SLs 6	liquefied	B	43.3	59.3	N.A	N.A	N.A
SLs 7	steamed	B	43.6	51.4	N.A	N.A	N.A

† on a Dry matter basis; N.A, not analyzed.

Table2. Ethanol concentrations (%FM) in Sake lees.

	Process	Brewery	2 weeks	4 weeks	6 weeks	16 weeks	23 weeks
SLs 1	liquefied	A	11.2	7.6	7.5	N.A	N.A
SLs 2	liquefied	A	6.6	10.6	8.0	N.A	N.A
SLs 3	steamed	A	7.4	11.0	8.8	N.A	N.A
SLs 4	steamed	A	9.9	8.1	7.7	N.A	N.A
SLs 5	liquefied	A	N.A	N.A	6.4	7.4	6.1
SLs 6	liquefied	B	N.A	N.A	N.A	N.A	N.A
SLs 7	steamed	B	N.A	N.A	N.A	N.A	N.A

N.A, not analyzed.

Numbers of live and dead yeast

The numbers of live yeast and dead yeast are presented in Tables 3 and 4, respectively. The numbers of live yeast ($\times 10^4$ CFU/ FM g) ranged from 1,462 to 6,109 before storing but, then dramatically decreased to 0-145 at 4 weeks' storing in SLs 1-5 (Table 3). The numbers of dead yeast ($\times 10^9$ / FM g) ranged from 0.7 to 3.0 before storing, which were stable during 4 weeks' storing showing 0.5-4.6 in SLs 1-5 (Table 4).

Table3. The numbers of live yeast in Sake lees.

	Process	Brewery	The numbers of live yeast ($\times 10^4$CFU/FMg)							
			0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks	7 weeks	10 weeks
SLs 1	liquefied	A	1462.3	38.3	8.5	0.2	0.1	N.A	N.A	N.A
SLs 2	liquefied	A	6108.6	9959.8	74.3	20.2	0.7	N.A	N.A	N.A
SLs 3	steamed	A	1492.1	2532.2	492.7	449.8	144.7	N.A	N.A	N.A
SLs 4	steamed	A	2552.2	177.0	10.5	0.9	0.9	N.A	N.A	N.A
SLs 5	liquefied	A	1755.4	N.A	N.A	188.5	N.A	244.5	1.4	0.01
SLs 6	liquefied	B	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A
SLs 7	steamed	B	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A

N.A, not analyzed.

Table4. The numbers of dead yeast in Sake lees.

	Process	Brewery	The numbers of dead yeast ($\times 10^9$number/FMg)				
			0 week	1 week	2 weeks	3 weeks	4 weeks
SLs 1	liquefied	A	2.4	2.3	2.3	1.8	2.7
SLs 2	liquefied	A	3.0	3.5	2.7	4.0	4.6
SLs 3	steamed	A	0.7	0.6	0.8	0.7	0.9
SLs 4	steamed	A	0.8	0.8	0.8	0.8	0.5
SLs 5	liquefied	A	2.0	2.4	2.2	1.9	2.1
SLs 6	liquefied	B	N.A	N.A	N.A	N.A	N.A
SLs 7	steamed	B	N.A	N.A	N.A	N.A	N.A

N.A, not analyzed.

Discussion

The rice undergoes washing and steaming before fermentation in making Sake in the ordinary process (multiple parallel fermentation method; SLs 3, 4 and 7). On the other hand, under the new process (high-temperature saccharification method), the rice is not steamed, but liquefied with heat stable enzymes before fermentation (SLs 1, 2, 5 and 6). In the new process, the starch from the rice appears to be converted into glucose by the enzyme preparation; the glucose is easily assimilated by yeast and thereby the yeast content in SLs increases as the fermentation progresses. The high total yeast contents (live and dead yeast) of SLs obtained from liquefied rice may be attributed to the difference of brewing process in this study. A previous study suggested that protein contents of SLs may result from yeast protein (Tsutsui et al. 1998). Therefore, the high CP contents of SLs from liquefied rice might have reflected the yeast content. In a previous study (Emery et al. 1959), ethanol did not affect rumen fermentation and digestibility in cows; 800mL of 47.5% ethanol was administered via rumen fistula during the once-daily feeding. Ethanol concentration in SLs was 8.3% on an average, which might not result in adverse effects in ruminants in a small amount of feeding. In the present study, the numbers of live yeast observed drastically decreased over time, whereas the numbers of dead yeast were stable under refrigeration. However, it should be noticed that information on the effect of live and dead yeasts in SLs in ruminants is still limited. Further study would be needed to evaluate the effect of supplementary SLs on *in vivo* digestibility and rumen fermentation especially in roughage (high fibre contents) feeding condition in ruminants.

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