Effect of Prompt-Delayed Packaging and Ensiling Time on Fermentation and Aerobic Stability of Soybean Curd Residue

Ajmal Wali
Okayama University, Japan

Takeshi Tsuruta
Okayama University, Japan

Naoki Nishino
Okayama University, Japan

The XXIV International Grassland Congress / XI International Rangeland Congress (Sustainable Use of Grassland and Rangeland Resources for Improved Livelihoods) takes place virtually from October 25 through October 29, 2021.

Proceedings edited by the National Organizing Committee of 2021 IGC/IRC Congress

Published by the Kenya Agricultural and Livestock Research Organization

Effect of prompt-delayed packaging and ensiling time on fermentation and aerobic stability of soybean curd residue

Wali Ajmal*;Tsuruta Takeshi, Nishino Naoki

Graduate School of Environmental and Life Science, Okayama University, Okayama

Key words: amplicon sequencing; bacteria; silage; soybean curd residue

Abstract

Wet soybean curd residue (SCR) obtained from two tofu factories (F1 and F2) was anaerobically stored with or without added beet pulp (BP). Sealing was performed on the day of tofu production (prompt sealing [PS]) or 2 days after SCR was piled and unprocessed (delayed sealing [DS]). Predominant lactic acid fermentation was observed regardless of the sealing time and BP addition. *Acinetobacter* spp. were the most abundant (>67%) bacteria in pre-ensiled SCR, regardless of the factory and sealing time. In PS silage, the abundances of typical lactic acid-producing bacteria, such as *Lactobacillus*, *Pediococcus*, and *Streptococcus* spp. reached >50%. In DS silage, *Acinetobacter* spp. were the most abundant in F1 products, whereas *Bacillus* spp. were the most abundant in long-stored F2 products. These results indicated that owing to preceding processing, including heating, distinctive microbiota may have participated in the ensiling of wet by-products. Lactic acid fermentation was observed even in DS silage, and an association of *Bacillus* spp. was suggested.

Introduction

A significant amount of soybean curd residue (SCR) is generated as a by-product from the manufacturing of tofu, a traditional Asian food prepared by coagulating soy milk. The sum of SCR in China, Korea, and Japan is approximately 3.9 million tons per year, equivalent to the annual amount of tomato pomace generated globally (Knoblich et al., 2005). Although the low dry matter (DM) content (about 200 g/kg) of wet SCR poses a challenge to its handling and transportation, its high crude protein content (about 250 g/kg DM) makes it an attractive ingredient for use in animal feed. In addition, the storability of SCR can vary greatly between factories and seasons because of large differences in the handling procedures executed by vendors.

The industrial scale of tofu manufacturing varies widely across factories. In addition, their locations are scattered, and are separate from the livestock sector. Daily on-site ensiling is difficult to perform, thus sealing is often delayed in practice. This lowers the opportunity for the stable production of a high-quality feed and increases the risk of spoilage due to rancidity, especially in hot summers.

In this study, the effect of prompt-delayed packaging and ensiling time on fermentation and aerobic stability of soybean curd residue was examined. The objective was to characterize the microbiota associated with SCR silage stored under PS and DS conditions. Both bacterial and fungal microbiota were assessed using high-throughput amplicon sequencing.

Methods and Study Site

Two sets of SCRs were obtained from F1 and F2, and each was further divided into 2 subsets. The first and second subsets of SCR were subjected to PS and DS ensiling, respectively. Briefly, 300 g of wet SCR was sealed in a plastic bag (Hiryu BN-12; Asahi Kasei Pax, Tokyo, Japan) in triplicate, with or without dried BP (60 g). Air was removed using a vacuum sealer (SQ-303; Asahi Kasei Pax, Tokyo, Japan), and the bags were stored at room temperature. The silos containing the samples were opened after 2 weeks and 3 and 6 months to examine the fermentation products and evaluate the bacterial and fungal microbiota.

Chemical composition was performed as described by (Tran et al., 2014). Silage samples were added to a 20× volume of sterilized phosphate-buffered saline (pH 7.4). DNA extraction was performed as described by (Yu et al., 2014). Both bacterial and fungal microbiota were assessed using high-throughput amplicon sequencing (Ni et al., 2017)

Results

Chemical composition and fermentation

When F1 SCR was ensiled, lactic acid fermentation was observed regardless of PS and DS (Table 1). The lactic acid levels were 37.5 and 55.5 g/kg DM at 2 weeks in PS and DS silage, respectively. After storage for 6 months, the lactic acid content increased by about 40% compared to that at 2 weeks. BP addition suppressed lactic acid production, and this effect was manifested in DS silage. The acetic acid content was 6–20% of the lactic acid content, with levels higher than PS silage. The addition of BP increased the acetic acid content in PS silage stored for 3 and 6 months. The ethanol content was lower than the acetic acid content and greater in DS than in PS silage. Although the NH_3-N content was as low as 0.13 g/kg DM even in long-stored PS silage, the level was substantially high (as much as 2.0 g/kg DM) in DS silage. The addition of BP lowered the NH_3-N level in DS silage but not in PS silage.

When F2 SCR was ensiled, lactic acid fermentation was predominant, and DS enhanced the lactic and acetic acid contents (Table 2). Similar to F1 SCR silages, BP addition suppressed the lactic acid content in DS silage and increased the acetic acid content in PS silage. Likewise, the NH_3-N content attained at 2.0 g/kg DM in DS silage and BP addition only lowered the NH_3-N levels in PS silage. Unlike F1 SCR ensiling, however, substantial amounts of propionic and butyric acid were produced, and the levels of 20 g/kg DM at 6 months were greater than those of acetic acid in PS silage.

Table 1. Composition of fermentation products of factory 1 soybean curd residue silage prepared with prompt and delayed sealing (S) and with and without beet pulp (BP) addition.

Item		Prompt sealing		Delayed sealing			Two-way ANOVA		
		Control	+BP	Control	+BP	MSE	S	BP	S X BP
pH	2W	4.39	4.18	4.28	4.32	0.08	NS	NS	NS
	3M	4.16	4.20	4.24	4.09	0.02	NS	*	**
	6M	4.12	4.08	4.22	4.08	0.02	NS	**	NS
Lactic acid (g/kg DM)						1.02	**	**	**
	2W	37.5	33.9	55.5	41.2				
	3M	48.2	39.6	79.8	53.8	1.79	**	**	**
	6M	54.5	39.1	75.6	50.7	2.12	**	**	NS
Acetic acid (g/kg DM)						0.37	**	NS	NS
	2W	2.65	3.98	7.04	6.94				
	3M	4.45	8.16	14.9	13.1	0.80	**	NS	**
	6M	7.69	10.7	12.6	10.1	0.70	*	NS	**
Propionic + Butyric						0.00	NS	NS	NS
	2W	0.00	0.00	0.00	0.00				
acids (g/kg DM)	3M	0.00	0.00	0.00	0.00	0.00	NS	NS	NS
	6M	0.00	0.00	2.08	0.00	0.42	*	*	*
Ethanol (g/kg DM)						0.25	**	NS	*
	2W	2.33	3.24	4.04	3.67				
	3M	1.32	3.19	2.61	1.97	0.25	NS	*	**
	6M	0.00	0.00	3.68	2.79	0.17	**	*	*
NH_3-N (g/kg DM)						0.12	**	*	*
	2W	0.06	0.10	1.98	1.35				
	3M	0.13	0.12	1.08	0.48	0.09	**	**	**
	6M	0.13	0.07	2.05	0.83	0.17	**	**	*

2W; 2 weeks, 3M; 3 months, 6M; 6 months. NS; not significant, *; $P<0.05$, *; $P<0.01$

Bacterial microbiota

The MiSeq sequencing resulted in non-chimeric sequence reads with an average of 68,820 and 70,626 for F1 and F2 samples, respectively. *Acinetobacter* spp. were the most abundant bacteria (67.4%) in pre-ensiled F1 SCR (Figure 1). In F1 PS silages, the abundance of *Acinetobacter* spp. was 7.6–26.1% at 3 months, growing to >40% at 6 months. Although *Lactobacillus* spp. were undetectable in pre-ensiled F1 SCR, the abundance increased to 8.99% and >30% at 2 weeks and 3 months, respectively, in F1 PS silage. *Streptococcus* spp. were found at >20% in pre-ensiled F1 SCR. The abundance was retained until 3 months before decreasing to <10% at 6 months. After 6 months of storage, *Bacillus* (2.7–4.9%), *Enterococcus* (1.2–2.5%), and *Clostridium* spp. (3.0–4.4%) in F1 PS silage became non-negligible.

The abundance of *Acinetobacter* spp. in SCR increased to 88% during piling and unprocessing for 2 days. Although the abundance decreased during prolonged ensiling, levels of 57.2–65.2% were observed in F1 DS silage even after 6 months. BP addition did not affect the abundance of *Acinetobacter* spp. The abundance of *Lactobacillus* spp. was maintained at <6% throughout the 6 months in F1 DS silage, regardless of BP addition. Likewise, *Streptococcus* spp. were found at 5.3% after ensiling, however, the abundance was almost unchanged throughout ensiling. Meanwhile, the abundance of *Bacillus* spp. increased to 16.7–19.7% at 6 months from <1% at the time of ensiling in F1 DS silage.*Acinetobacter* spp. were by far the most

abundant bacteria (91.9%) in pre-ensiled F2 SCR, followed by *Enhydrobacter* spp. (4.47%). In F2 PS silage, the abundance of *Acinetobacter* spp. was <10% at 3 months, and increased to >20% at 6 months, regardless of BP addition. Similar to pre-ensiled F1 SCR, although *Lactobacillus* spp. were initially undetectable at ensiling in F2 PS silage, the abundance increased to 7.19–16.6% at 2 weeks and 32.5–50.7% at 3 months. *Streptococcus* spp. were observed at <0.5% at ensiling, >20% at 2 weeks, and 8.5–18.0% at 6 months in F2 PS silage. As a result of long-term storage, *Bacillus* (1.7–2.1%) and *Enterococcus* spp. (4.0–5.6%) were also observed in F2 PS silage. Unlike the F1 SCR silages, *Clostridium* spp. were found at around <1.0% in F2 PS silage stored for 6 months. Keeping SCR piled and unprocessed for 2 days decreased the abundance of *Acinetobacter* spp. (69.2%) in pre-ensiled F2 SCR. Although *Acinetobacter* spp. remained the most abundant taxon at 2 weeks, *Bacillus* spp. became apparent after prolonged ensiling and were the most abundant bacteria (46.8–65.3%) at 6 months in F2 DS silage. The abundance of *Lactobacillus* spp. in F2 DS silage remained low (<10%), regardless of BP addition.

Table 2. Composition of fermentation products of factory 2 soybean curd residue silage prepared with prompt and delayed sealing (S) and with and without beet pulp (BP) addition.

Item		Prompt sealing		Delayed sealing			Two-way ANOVA		
		Control	+BP	Control	+BP	MSE	S	BP	S X BP
pH	2W	4.38	4.26	4.19	4.05	0.02	**	**	NS
	3M	4.31	4.25	4.14	4.01	0.04	**	*	NS
	6M	4.36	4.15	4.12	3.99	0.03	**	**	NS
Lactic acid (g/kg DM)	2W	35.5	29.3	78.7	53.3	1.48	**	**	**
	3M	50.9	42.0	88.5	61.8	1.95	**	**	**
	6M	42.9	34.4	78.9	54.3	2.35	**	**	**
Acetic acid (g/kg DM)	2W	2.78	3.74	16.4	7.51	0.38	**	**	**
	3M	7.68	6.87	24.7	13.6	0.92	**	**	**
	6M	4.91	6.62	18.4	10.5	0.81	**	**	**
Propionic + Butyric	2W	0.00	0.00	0.00	0.00	0.00	NS	NS	NS
acids (g/kg DM)	3M	2.52	0.87	0.00	0.00	0.12	**	**	**
	6M	21.7	17.2	3.38	1.91	0.80	**	**	NS
Ethanol (g/kg DM)	2W	0.73	2.08	2.20	0.96	0.30	NS	NS	**
	3M	0.00	2.65	0.00	0.00	0.03	**	**	**
	6M	0.76	0.45	0.00	0.00	0.23	*	NS	NS
NH3-N (g/kg DM)	2W	0.15	0.13	2.05	1.20	0.11	**	**	**
	3M	0.20	0.13	1.45	0.73	0.05	**	**	**
	6M	0.31	0.11	2.05	1.09	0.10	**	**	**

2W; 2 weeks, 3M; 3 months, 6M; 6 months.NS; not significant, *; P<0.05, *; P<0.01.

Discussion

Tofu is a traditional Asian food, and many studies have been conducted to examine the process of soybean fermentation and identify additives for SCR ensiling (Amaha et al., 1999). Because of its milled and mashed physical properties, SCR is regarded as an easy material to ensile. As a result, the fermentation of this legume can be performed by lactic acid without the need for additives. The microbiota associated with SCR ensiling have also been examined in a number of studies (Wang et al., 2008). However, except for the total mixed ration silage, containing SCR as an ingredient, most of the previous findings have been derived from plate culture, which enumerated limited taxa. The rancidity arising from piling and unprocessing is an important obstacle to the widespread use of SCR. Although this study examined 2 types of wet SCRs derived from 2 different factories (F1 and F2), the microbiota associated with SCR ensiling were found to be different from those associated with forage ensiling.

Our finding that lactic acid dominated the fermentation of SCR silage with PS is in agreement with other studies (Wang et al., 2008). Although the soluble sugar content was low, the lactic acid content reached >30 g/kg DM after 2 weeks. Although the initial abundances of lactic acid-producing bacteria were quite small, *Lactobacillus* and *Streptococcus* spp. were the main bacteria in F1 PS silage, while *Lactobacillus*, *Streptococcus*, *Enterococcus*, and *Pediococcus* spp. were the main bacteria in F2 PS silage. Based on the PCoA results, the bacterial microbiota of PS silage was found to set up and remain separated from that of DS silage. Interestingly, although *Acinetobacter* and *Bacillus* spp. remained the most abundant bacteria during ensiling, lactic acid was prevalent in DS silage. Since *Acinetobacter* spp. lack the ability to produce lactic acid, *Bacillus* spp. could have been involved in lactic acid fermentation in DS silage. Although *Bacillus* spp. are generally regarded as strictly aerobic bacteria, facultatively anaerobic species are also present, and their ability to produce lactic acid has been acknowledged (Lara et al., 2016).

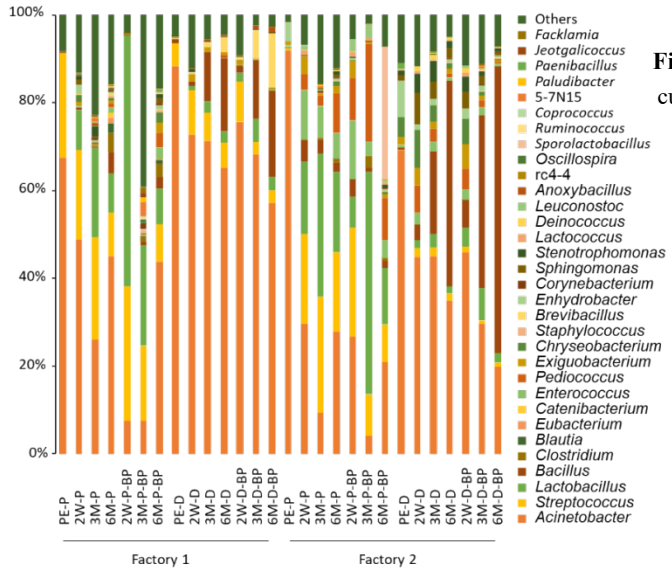

Figure 1. Genus-level bacterial microbiota in soybean curd residue silage prepared with prompt and delayed sealing and with and without beet pulp addition. PE, 2W, 3M, and 6M indicate pre-ensiled material and silage stored for 2 weeks, 3 months, and 6 months, respectively. P, D, and BP after the hyphenation denote prompt sealing, delayed sealing, and beet pulp addition, respectively.

Bacillus spp. are known to grow well in soybean products, exhibiting various enzyme activities, including protease, amylase, cellulase, and pectinase (Chen et al., 2011). In the present study, when SCR was unprocessed and unsealed for 2 days, the abundance of *Bacillus* spp. increased from 0.03–0.05% to 0.49–0.90%, while maltose became detectable, and a substantial amount of NH_3-N (0.64–1.37 g/kg DM) was produced. *Paenibacillus*, *Exiguobacterium*, *Enhydrobacter*, *Stenotrophomonas*, and *Deinococcus* spp. also increased their abundance during piling and unprocessing for 2 days. Further, *Acinetobacter* spp., the most abundant bacteria in pre-ensiled SCR, are also shown to exert both proteinase and deaminase activities (Ashwini et al., 2015). Hence, the intensive production of NH3-N observed in the DS materials could be attributed to the collective activities of bacteria, including *Bacillus* and *Acinetobacter* spp. The NH_3-N content of PS silage without added BP was as low as 0.31 g/kg DM, indicating that the proteolysis and deamination activities were efficiently suppressed under PS conditions. Although *Acinetobacter* spp. were exclusively found in wet SCR at the time of generation in the tofu factories, predominant lactic acid fermentation was obtained in SCR silage by low levels of *Lactobacillus* spp., *Streptococcus* spp., and *Pediococcus* spp. Further, *Bacillus* spp. were involved in lactic acid production in DS silage and long-stored PS silage. The rancidity arising from piling and unprocessing in wet SCR could be attributed to the collective activities of bacteria, including *Bacillus* spp. and *Acinetobacter* spp. Owing to preceding processing, including heating, distinctive microbiota may have participated in the ensiling of wet by-products.

References

Amaha, K.; Sasaki, Y.; Segawa, T. Utilization of tofu (soybean curd) by-products as feed for cattle. FFTC Publication, 1999, http://www.agnet.org/htmlarea_file/library/20110716100439/eb419.pdf (accessed on 30th June, 2020)

Ashwini, M.S.; Shilpa, S.M.; Smita, B.P.; Balu, A.C. Isolation, biotyping, biochemical and physiological characterization of marine *Acinetobacter* isolated from west coast of India. *Int. J. Curr. Microbiol. App. Sci.***2015**, *2*, 277-301.

Chen, T.; Wang, M.; Jiang, S.; Xiong, S.; Zhu, D.; Wei, H. Investigation of the microbial changes during koji-making process of Douchi by culture-dependent techniques and PCR-DGGE. *Int. J. Food Sci. Technol.* **2011**, *46*, 1878-1883.

Knoblich, M.; Anderson, B.; Latshaw, D. Analyses of tomato peel and seed byproducts and their use as a source of carotenoids.*J. Sci. Food Agric.***2005**, *85*, 1166-1170.

Lara, E.C.; Basso, F.C.; de Assis, F.B.; Souza, F.A.; Berchielli, T.T.; Reis, R.A. Changes in the nutritive value and aerobic stability of corn silages inoculated with *Bacillus subtilis* alone or combined with *Lactobacillusplantarum*. *Anim. Prod. Sci.***2016**, *56*, 1867-1874.

Tran, T.M.T.; Nguyen, H.V.; Nishino, N. A pilot examination of the fermentation products, aerobic stability and bacterial community of total mixed ration silage produced in Vietnam. *Grassl. Sci.***2014**, *60*, 63-68

Wang, F.; Nishino, N. Ensiling of soybean curd residue and wet brewers grains with or without other feeds as a total mixed ration. *J. Dairy Sci.***2008**, *91*, 2380-2387.

Yu, Z.; Morrison, M. Improved extraction of PCR-quality community DNA from digesta and fecal samples.*Biotechniques***2014**, *36*, 808-812.