Original Paper

Change in chemical composition and fermentation characteristics of drift seaweed during ensiling

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Abstract This study considered the change in chemical composition and fermentation quality of drift seaweed during ensiling. The drift seaweeds were pre-dried for 2 h and subsequently packed into plastic bag for 0, 15, 30 or 60 days. The moisture, ether extract, neutral detergent fiber, and non-fibrous carbohydrate content of seaweed silage for each storage period did not differ significantly. We found positive correlations between organic acid concentrations (acetic, propionic, lactic acids, and total organic acid) and storage duration (P<0.001). The V-score of each ensiling period was 100 points. These results show that drift seaweed silage can keep 60 days good fermentation condition, and there is a possibility that it may be used as a feed resource.

Key words: Chemical composition, drift seaweed, fermentation characteristics, silage

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Introduction

Seaweeds are the dominant organisms in coastal waters and distributed, widely along the coasts [1]. Large amounts of drift seaweed reach the coastline due to strong winds or typhoons [2,3]. These drift seaweeds accumulate on beaches and in coastal waters, causing significant social and economic losses such as providing rotting odor, and damaging boats due to the entanglement with propellers. Hence, the huge quantities of drift seaweed need to be removed from beaches. Generally, the seaweed removed has been disposed by burning or burying. Seaweeds are rich in nutrients [4]. It has been reported that functional components such as viscous polysaccharides are abundant in seaweed [5]. Therefore, it is important to utilize drift seaweed as a resource for sustainable environmental conservation.

In recent years, utilization of seaweed for different purposes such as a feed resource and functional food ingredient are examined [6-8]. It has been reported that feeding seaweed to

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pigs and cattle enhances immune function [7,8]. Therefore, using seaweed as the feed may improve the health of animals due to immune activation and could even contribute to enhance animal welfare.

In feed preparation, it is necessary to consider the cost, nutritive value, and storage stability. Commonly, the prepare of feed with high water content is ensiled in terms of the cost, storage stability, and so on. In ensiling, it is important that lactic acid fermentation is performed stably. It has been reported that seaweed is low levels of water-soluble carbohydrates (WSC) and high lactic buffering capacity [9]. The viscous polysaccharide rich in brown seaweed do not include within the WSC fraction [10]. However, it is utilized as a substrate for lactic acid bacteria during the ensiling process [11]. Therefore, it is necessary to study the change in chemical composition and fermentation quality associated with silage for making a stable good quality silage at low cost.

This study considered the change in chemical composition and fermentation characteristics of drift seaweed during ensiling at laboratory-scale with plastic bags.

Materials and methods

1. Seaweed Sampling and Ensiling Process

Drifting seaweeds of approximately 20 kg fresh weight were collected on October 7, 2021, at Noto-cho, Ishikawa Prefecture, Japan (37.303984 N, 137.240112 E). Processing and ensiling of seaweeds were conducted immediately after collection. Collected seaweed was washed with cold tap water to remove adherent sand and impurities. Seaweed samples were classified by species and all seaweed species were pre-dried in an airy place for 2 hours to wilt to a moisture content around 70%. Then, seaweed was chopped to approximately 30 mm with a straw cutter in length, mixed thoroughly, and divided into 100 g (fresh weight, FW) sub-samples. The seaweed sample was placed in a plastic pouch (20 × 30 cm × 0.04 mm, polyethylene; Bonsenkitchen, China) and sealed by a vacuum sealer (VS3911; Bonsenkitchen, China). The silage was stored at room temperature (21.7±0.04°C) and different periods (0, 15, 30, and 60 days). It was not so gas production that the plastic bag expand. After the storage period, the samples were frozen at -20°C until analyzed. The samples were prepared at three for each storage period.

2. Chemical Composition and Fermentation Characteristics

This study investigated the changes in chemical composition (dry matter (DM), crude ash (CA), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and non-fibrous carbohydrate (NFC)), and fermentation quality (pH, organic acid concentrations (acetic, propionic, butyric, isovaleric and lactic acids), ammonia nitrogen/total nitrogen (NH_3-N/TN), and V-score). The samples were dried at 60°C for 48 h. NDF was measured according to Van Soest et al [12]. The chemical composition other than NDF was analyzed according to Milledge et al. [13]. The pH of each sample was determined on an extract prepared by soaking silage in distilled water for 24 h at 5°C. This extract was use too for determination of organic acid concentration. The concentration of organic acids in extracts were quantified by gas chromatography (GC-1700; Shimadzu Co., Ltd., Japan), equipped with an DB-WAX column (30 m × 0.53 μm × 1 μm) and FID detector. Helium was used as carrier gas at a constant flow of 4.74 mL/min. Injector temperature was 200°C and detector temperature was 250°C. NH_3-N content of silage extracts was measured using a RQflex reflectometer (Merck, Germany) and a Reflectoquant Ammonium Test kit (16977-1M; Merck, Germany) by Nessler's method [14]. The V-score was calculated from the NH_3-N/TN and volatile fatty acid contents in the silage [15]. NH_3-N was used as the volatile basic nitrogen (VBN) in calculating the V-score because most of the VBN in silage is NH_3 [16].

3. Statistical Analyses

In each item, differences due to storage duration were determined using unpaired one-way analysis of variance and the Bonferroni multiple comparison test. The relation between the storage duration and each item was statistically analyzed using Pearson's correlations coefficient. The statistical analyses were performed with SPSS 25.0 (IBM Corp., USA.).

Results

The collected drifting seaweed contained 5 types of seaweed. And these seaweeds were all *Sargassum* species. The collected seaweed species has been distributed commonly at the area in this season [17]. The weight ratio of the seaweeds sampled in this study is presented in Table 1. The change of chemical composition of the seaweeds silage in accordance with storage days is presented in Table 2. DM loss did not occur in any silages.

Table1 Species composition of seaweed silage material.

Species	Weight ratio (% FM)
Sargassum piluliferum	31.7
Sargassum ringgoldianum ssp. Coreanum	29.5
Sargassum confusum	27.4
Sargassum macrocarpum	8.2
Sargassum siliquastrum	3.2

FM, fresh matter.

Table2 Chemical composition of seaweed silage.

Item	Storage days 0	15	30	60
DM (%)	35.8±0.8	34.0±0.8	34.1±0.8	34.2±0.8
CA (% DM)	19.6±0.2^a	20.1±0.2^a	19.8±0.2^a	18.7±0.2^b
CP (% DM)	5.1±0.1^a	5.6±0.1^b	5.0±0.1^a	5.6±0.1^b
EE (% DM)	1.4±0.3	1.7±0.3	1.7±0.3	1.9±0.3
NDF (% DM)	9.5±1.8	11.6±1.8	11.6±1.8	13.0±1.8
NFC (% DM)	64.4±2.0	61.0±2.0	61.8±2.0	60.9±2.0

Each value represents the average ± standard error (n=3); DM, dry matter; CA, crude ash; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; NFC, non fibrous carbohydrate; ^{a,b} Means with different superscripts are significantly different (P < 0.05)

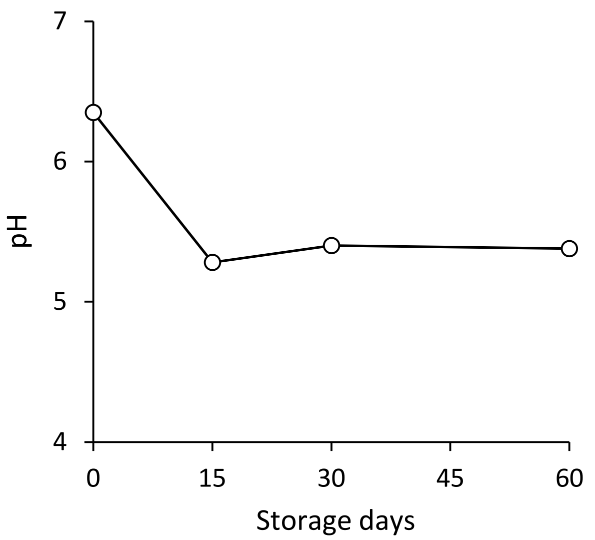

Figure1. pH on 0 to 60 days of strage time.

The chemical composition was relatively stable, DM, EE, NDF, and NFC contents averaged 34.5±0.3, 1.7±0.1, 11.4±0.7, and 62.0±0.7%, respectively. On the other hand, CA content was significantly lower at 60 days than 0, 15, and 30 days of storage (P<0.05). CP contents

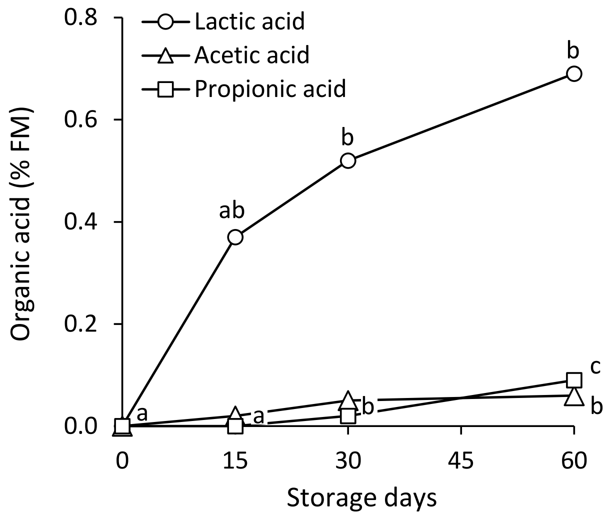

Figure 2. Organic acid concentration on 0 to 60 days of storage time. Butyric and isovaleric acids were not detected. a,b: means within the same acid with different letters differ significantly (P<0.05).

Table3 Pearson correlation between the storage time and chemical composition of seaweed silage.

Chemical composition	R	P-value
Moisture	0.430	n.s.
CA	-0.697	0.012
CP	0.429	n.s.
EE	0.543	n.s.
NDF	0.518	n.s.
NFC	-0.420	n.s.
Organic acid		
Acetic acid	0.889	<0.001
Propionic acid	0.943	<0.001
Lactic acid	0.830	<0.001
Total	0.876	<0.001

n.s., not significant (P >0.05); CA, crude ash; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; NFC, non fibrous carbohydrate

were significantly higher at 15 and 60 days than 0 and 30 days of storage (P<0.05).

The pH of seaweed silages is shown in Figure 1. The pH declined rapidly until the 15th day of ensiling, and then remained stable to the end of the 60th day (approximately 5.4). Figure 2 illustrate the Organic acid concentrations of seaweed silages. Lactic, acetic, and propionic acids were detected from day 15 onwards, lactic acid was the dominant organic acid. Lactic acid was reaching concentrations of approximately 0.7 % FM on day 60. Butyric and isovaleric acids were not detected in any sample. NH_3-N was also not detected in any silage. Therefore, the NH_3-N/ TN of each silage were 0%. The V-score of silage in each duration period were all 100 points.

Table 3 shows the correlation between each item and the storage period. There was a significant negative correlation between CA content and storage duration (P<0.05). Organic acids (acetic, propionic, lactic acids, and total organic acid) concentration and the storage time exhibited positive linear and quadratic relationships (P<0.001)

Discussion

Generally, it is considered appropriate that the water content of silage is about 60-70% [18]. In this study, the moisture contents of seaweed silage were approximately 65% through the ensiling period. This result suggests that the seaweed silage was prepared properly.

The CA content decreased gradually as the number of ensiling days increased in the present study. It has been reported that some trace minerals can reduce according to species of seaweed [19]. In this study, some species constituting the seaweed silage can be in line with the result of Campbell et al. [19]. For this reason, the CA content of seaweed silage may reduce. In this study, we did not find a correlation between the CP content of silage and storage duration. Whereas CP content significantly changed in storage duration. It is reported that the increase and decrease of CP content in seaweeds during the ensiling varies from species to species [20]. In this study, the seaweed silage consisted of the multiple species. We considered that it was one of the factors.

In our research, the pH of seaweed silage declined significantly until the 15th day of ensiling and stabled after the day. However, the pH did not decline below 5.2 during ensiling. This result agrees with the report by Herrmann et al. [21]. In general, seaweeds have high buffering capacity due to their high anions content, mainly chloride and sulphate [20-22]. The drift seaweed in this study also has much ash content, and the anion content was high probably. Therefore, the high buffering capacity due to high anions content of this drift seaweed might have counteracted a decrease of the pH value. On the other hand, it is also possible that lactic acid bacteria could not assimilate the viscous polysaccharides due to the pH did not decrease sufficiently. However, quadratic relationships were observed between the organic acid concentrations and storage time, suggested changes in chemical composition of the seaweeds silage with consumption of the fermentation substrate. In the future, it is necessary to examine the relationship between consumption of the fermentation substrate and fermentation quality with storage time.

In this study, the V-score to evaluate the quality of silage fermentation were 100 points in any ensiling time. Additionally, NH_3-N and butyric acid were not detected in the seaweed silage. The content of NH_3-N and butyric acid in silage is used as an indicator of poor fermentation. Therefore, these concentrations usually show a low value in the case of good fermentation [23,24]. The silage fermentation characteristics of the drift seaweed used in this study can be stable and good during the whole period. However, it holds potential for lab-scale evaluations of silage. Therefore, it needs to consider in large-scale demonstrations.

In conclusion, drift seaweed silage can be good quality for long periods, and there is a possibility that it may be used as a feed resource. However, we have warranted caution when considering the supplementation of livestock diets with seaweeds because seaweed can include of toxic substances such as As and heavy metal [1]. In addition, we need to study that seasonal change of chemical composition and species of seaweed.

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原　著

漂着海藻を用いたサイレージの化学成分および発酵品質の経時的変化

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本研究では沿岸に漂着する海藻の飼料化を目的として、漂着海藻を用いて調製したサイレージの化学成分および発酵品質の経時的変化について検討した。供試試料は沿岸に漂着する新鮮な海藻とし、採取後約2時間予備乾燥させ、真空密閉して0、15、30および60日間室温（約20℃）で貯蔵した。調査項目は、化学成分（水分、粗灰分、粗蛋白質、粗脂肪、中性デタージェント繊維および非繊維性炭水化物含量）および発酵品質（pH値、有機酸含量（酢酸、プロピオン酸、n-酪酸、イソ吉草酸、乳酸）アンモニア態窒素/全窒素およびV-スコア）とした。水分、粗脂肪、中性デタージェント繊維および非繊維性炭水化物含量は貯蔵期間の違いによる有意差はみられなかった。有機酸含量（酢酸、プロピオン酸、乳酸、全有機酸）と貯蔵期間に有意な正の相関関係が認められた。サイレージのV-スコアは15、30および60日間のいずれ貯蔵期間においても100点となった。以上のことから、漂着海藻サイレージは、60日間良好な発酵状態が保持され、飼料として活用できる可能性があることが示唆された。

キーワード：成分組成、漂着海藻、発酵品質、サイレージ

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