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Ensilage of seaweeds from an integrated multi-trophic aquaculture system

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ABSTRACT

This study aimed to evaluate the feasibility of ensiling preservation of three seaweed species (*Gracilaria vermiculophylla*, *Ulva rigida* and *Saccharina latissima*) produced in an integrated multi-trophic aquaculture system without or with a lactic acid bacteria inoculant after 24–48 hours wilting. The course of silage fermentation for a storage period up to nine weeks was also studied. The characteristics of fresh seaweeds suggested ensiling to be challenging. Overall, seaweeds presented a pH above 7.0, low dry matter (DM) content, and a non-structural polysaccharides content (DM basis) ranging from 14% (*U. rigida*) to 43% (*S. latissima*). The highest buffering capacity was recorded for *U. rigida* while the fermentation coefficient was higher for *S. latissima*, lower for *U. rigida*, and intermediate for *G. vermiculophylla.* The ensiling of *S. latissima* resulted in a pronounced lactic acid formation and a lower pH in comparison to the red and green seaweed silages. The ensilage of *U. rigida* and of *G. vermiculophylla* failed to decrease pH below 5.0 up to nine weeks, fermentation products suggesting the occurrence of a heterolactic fermentation or the promotion of heterofermentative bacteria and clostridia growth, respectively. The use of a lactic acid bacteria inoculant prior to ensiling had only minor effects on fermentation. Composition of seaweeds was generally preserved by ensilage, but *in vitro* digestibility of *G. vermiculophylla* was severely reduced. More research is needed to test different inoculants and dosages, and to assess the effects of seaweed silages on animal feed intake and performance.

1. Introduction

Seaweeds have been occasionally or systematically used for thousands of years to feed livestock. Recently, Makkar et al. [30] reviewed the state-of-the-knowledge on seaweed use as animal feed and concluded that while some might contribute to fulfil the protein and energy requirements of livestock, others contain bioactive compounds thus having the potential to be used as prebiotic to improve production and health status of monogastric and ruminant animals. The identification of novel feeds (as seaweeds) would therefore contribute for the expansion of feed resource base, playing a vital role in the sustainability of the livestock sector.

The nutritive value of seaweeds varies widely with the species, season, habitat, and external conditions (*e.g.*, water temperature and nutrient concentration, light intensity) [37]. In the integrated multi-trophic aquaculture (IMTA) system the by-products (wastes) from fish production are recycled to become fertilizers and food for another species (*e.g.*, macroalgae), originating a marketable product for little or no additional input costs, decreasing waste outputs from overall farming activities, and thus promoting a more environmentally sustainable fish farming. The growth rate and nutritive value of seaweeds can largely change with the cultivation conditions [1]. Harvesting of seaweeds naturally produced or from an IMTA system can be performed at optimal times for attaining the maximum biomass production and nutritive value. Nevertheless, this seasonal harvesting creates the challenge of preservation as seaweeds degrade rapidly if left untreated due to their high content of water (70–90%; [29]).

Drying can successfully preserve macroalgae, sun-drying being the main method used [16]. Although no fossil energy is required, sun-drying is strongly dependent on weather and seaweed volume [36]. Other methods of water evaporation comprise intensive energy processes due to the high water content of most seaweeds at harvest [3]. Alternatively, seaweeds might be preserved with comparatively low energy input through ensiling. There is very little information on ensiling of seaweeds. In an earlier study, Black [7] evaluated the ensiling of four brown seaweeds (*Laminaria hyperborea, Ascophyllum nodosum*, *Laminaria digitata*, and *Saccharina latissima*) to be used as animal feed and despite these seaweeds have supported lactic acid fermentation, silage pH did not drop below 4.7. In 2013, a patent for ensilage methods of seaweeds was applied [53] and, more recently,

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Herrmann et al. [20] investigated the ensiling of five seaweed species (*Ulva lactuca*, *A. nodosum*, *L. digitata*, *Saccorhiza polyschides*, and *S. latissima*) and its storage up to 90 days as a preservation method of their methane yield potential. The authors observed a difficult natural lactic acid fermentation due to high buffering capacities, low content on rapidly fermentable carbohydrates, and low numbers of lactic acid bacteria in the fresh seaweeds.

The objectives of this study were to evaluate the feasibility of ensiling preservation of three different seaweed species produced in an IMTA system without or with a lactic acid bacteria inoculant after 24–48 hours wilting. The pattern of silage fermentation up to nine weeks of storage was also evaluated.

2. Material and methods

2.1. Seaweeds

Seaweed from three distinct phyla, *Gracilaria vermiculophylla* (Rhodophyta), *Ulva rigida* (Chlorophyta) and *S. latissima* (Phaeophyta), cultivated in an IMTA system and supplied by ALGAPLUS Lda. (Aveiro, Portugal) and CIIMAR, Faculty of Sciences, University of Porto (for *S. latissima*) were investigated for preservation by ensiling. After harvesting, seaweeds were washed with cold tap water in order to remove impurities.

2.2. Ensiling process

All seaweed species were laid out in a shed on a dry concrete floor to wilt at room temperature (18–20 °C) during 24 h to increase the dry matter (DM) content. *Ulva rigida* was also dried for 48 h. Prior to ensilage, each seaweed was cut separately by hand to a particle size of 10–20 mm long/wide. Seaweeds were then spread thinly on a polyethylene sheet and two samples of each species prepared: (a) a control sample, and (b) a sample with an additive (LALSIL CL Forage, Lallemand Inc., Blagnac, France) consisting of an inoculant of freshly cultured *Lactobacillus plantarum* ($> 5.00 \times 10^{10}$ cfu g^{-1}), and *Pediococcus acidilactici* ($> 1.50 \times 10^{10}$ cfu g^{-1}) applied at 2 g ton^{-1} seaweed. The additive was sprayed directly onto the cut seaweeds and thoroughly mixed. Seaweed biomasses were filled into 1 L glass preservation jars (Bormioli Rocco Glass Co Inc., Fidenza, Italy) fitted with glass lids, gas-tight rubber seals, and spring retaining clips to ensure anaerobic conditions. Glass silos were manually filled and seaweed compressed to remove the air between seaweed particles; no headspace was left in the jars. Silos were wrapped with aluminum foil to protect from light and stored at 18–20 °C. The silage fermentation pattern was evaluated by opening replicate silos after three, six, and nine weeks of storage. For the silages from 48-h dried *U. rigida*, silos were opened after six and nine weeks of storage. Ensilage was prepared in duplicate per seaweed, per inoculant (control and additive) and per time of ensilage, thus resulting in a total of 44 silage jars.

To determine silage losses, silos were weighed immediately after filling and after each of the three storage periods. At opening, the contents of each jar were thoroughly mixed and sampled for further chemical analyses. Fresh seaweeds were sampled on day zero for chemical analyses.

2.3. Chemical analysis

The pH of each fresh seaweed and silage was measured immediately after opening the jars (electrode 5202, GLP22 + pH meter; Crison Instruments SA, Spain).

Fresh seaweeds and seaweed silages were dried in a forced-air oven at 65 °C until constant weight for DM determination. These samples were then ground to pass a 1-mm screen and dried again (at 103 °C for 6 h) to express their chemical composition in a DM basis [5], and analyzed for ash ([5], ID 942.05), ether extract (EE; [5], ID 920.39), Kjeldahl N ([5], ID 954.01), neutral detergent fiber (NDF, with α-amylase and without sodium sulfite), and acid detergent lignin (ADL; [41,48]). Crude protein (CP) was determined as Kjeldahl N × 5.0 [4]. Neutral detergent fiber and ADL were expressed exclusive of residual ash. Gross energy (GE) of seaweeds was determined in an adiabatic bomb calorimeter (Werke C2000, IKA, Staufen, Germany). Non-structural polysaccharides (NSP) were calculated subtracting ash, CP, EE, and NDF to DM.

Buffering capacity (BC) of seaweeds prior to ensiling was measured by suspending 1 g of dried sample in 100 mL of ultra-pure water (maximum conductivity of 0.055 mS cm^{-1}; Sartorius arium pro water purification system, Goettingen, Germany), and the suspension titrated to pH 4.0 with 0.1 M lactic acid [50]. A fermentation coefficient (FC) was calculated from DM content, NSP and BC of fresh seaweeds to assess the ensilability of seaweed biomass according to Weißbach [51], but replacing the concentration of water-soluble carbohydrates by NSP content.

Volatile fatty acids (VFA), ammonia-N (NH_3-N), and lactic acid were analyzed on aqueous silage extracts. Extracts were obtained from steeping 30 g of fresh silage in 120 mL of ultra-pure water for 16 h at 4 °C in a sealed flask, after shaken for 20 min in an orbital shaker at room temperature, followed by filtration through filter paper (Whatman 2; GE Healthcare Europe, Carnaxide, Portugal). For VFA analysis, 1 mL of 0.15 M oxalic acid solution with internal standard (15 mM 3-methyl valeric acid; Sigma-Aldrich Inc., St. Louis, MO) was added to 4 mL of silage extract, mixed and filtered through a 25 mm polyethersulfone syringe filter (0.45 μm pore size; VWR International - Material de Laboratório, Lda., Carnaxide, Portugal) to a chromatographic sample vial and analyzed in a gas chromatograph (GC-2010 Plus; Shimadzu Corporation, Kyoto, Japan) equipped with a capillary column (HP-FFAP, 30 m × 0.25 mm × 0.25 μm; Agilent Technologies, Santa Clara, CA), and a flame ionization detector. Injector and detector temperatures were held at 260 °C. Initial oven temperature was 80 °C for 1 min, increased at 20 °C/min to 120 °C, then increased at 6 °C/min to 205 °C and finally increased at 20 °C/min to 240 °C. Helium was used as a carrier gas at a flow rate of 0.86 mL/min. The injection volume was 1 μL and the split of 50:1. Volatile fatty acids were quantified with the internal standard (3-methyl valeric acid) and identified by comparison of retention times with a standard (Volatile Free Acid Mix, Sigma-Aldrich Inc., St. Louis, MO).

For NH_3-N analysis, 5 mL of silage extract was added to 5 mL of 0.1 N HCl solution, steam distilled (Vapodest 40, distiller unit; C. Gerhardt GmbH & Co. KG, Germany), and N content determined through titration ([5], ID 954.01).

Total D-/L-lactic acid content of silage extracts was determined enzymatically using a UV based commercial kit (AK00141; NZYTech, Lisbon, Portugal).

All chemical analyses were run in duplicate.

2.4. In vitro digestibility

In vitro DM digestibility (DMD) of seaweeds and silages was determined according to Tilley and Terry [45] method modified by Goering and Van Soest [18]. The *in vitro* digestibility was measured using three separate batches of ruminal fluid collected from non-lactating Holstein cows fitted with rumen cannula (10 cm diameter; Bar Diamond Inc., Parma, ID). Two Holstein cows were used. First, one cow was fed a diet (486 g kg^{-1} DM; 248 g kg^{-1} starch, 428 g kg^{-1} NDF, and 119 g kg^{-1} CP, DM basis) comprising (DM basis) 48% corn silage, 20% wheat straw, and 32% compound feed, then one cow was fed a diet (845 g kg^{-1} DM; 610 g kg^{-1} NDF, and 145 g kg^{-1} CP, DM basis) comprising (DM basis) 80% wheat straw and 20% soybean meal, and other cow a diet (700 g kg^{-1} DM; 557 g kg^{-1} NDF, and 146 g kg^{-1} CP, DM basis) comprising (DM basis) 87% ryegrass hay-silage and 13% soybean meal. Diets were offered at 1.2 times the maintenance with fresh supplies of feed offered twice a day at 0930 and