Grass silage for biorefinery – Separation efficiency and aerobic stability of silage and solid fraction

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Introduction

A green biorefinery concept involves processing of green biomass into a range of products (Mandl, 2010). Grasses have an excellent potential for biomass production under Boreal conditions and they provide versatile properties as a raw material for a green biorefinery. Ensiling allows the green biomass to be processed all year round. Usually the first step of the green biorefinery approach is the separation of liquid and solid fractions by mechanical liquid-solid separation. These fractions can be further processed to produce other products (McEniry *et al*., 2011). Solid fraction from green biorefinery can be used e.g. as feed for ruminants (to be published in Kautto *et al*., 2018), to produce insulation boards (Grass, 2004) or hydrolysed into simple sugars for further processes (Niemi *et al*., 2017). Liquid fraction can be used as feed for pigs and cows (to be published in Rinne *et al*., 2018). Another possible application of liquid fraction may be raw material for extraction of lactic acid and amino acids (Ecker *et al*., 2012).

The yield and composition of the liquid and solid fractions vary significantly depending on raw material quality and processing technology. There is a high correlation between silage quality and liquid yield and composition (to be published in Franco *et al*., 2018), which provides an opportunity to predict the biorefinery potential of silages. Furthermore, the silage production system can be manipulated in order to create feed that best meets the requirements of a particular green biorefinery process.

A variety of technological solutions can be used for liquid-solid separation and the method has a great impact on the liquid yield and composition. A further challenge is the preservation of solid and liquid fractions after silage pressing, since they can potentially deteriorate in a few days. Aerobic stability reflects the stability of the feed over the time after exposure to the air. Short aerobic stability of the diet may influence dry matter (DM) intake, quantity of feed refusals and will affect the production costs by increasing the feed losses. Preservatives can be added to materials to preserve and/or enhance the quality, such as increasing aerobic stability (Seppälä *et al*., 2016). The aim of this experiment was to compare three liquid-solid separation methods of grass silage on liquid yield, composition and retained compounds in liquid. Effect of preservatives on aerobic stability of silage, solid fraction and solid fraction added with water used as such or in total mixed ration (TMR) was also assessed using two methods (increase in temperature and visual inspection).

Materials and Methods

The grass silage was produced in Jokioinen, Finland, from a first cut (21st and 22nd of June 2017) mixed timothy and meadow fescue with the following composition: 193 g/kg DM, 132 g/kg DM of crude protein (CP) and organic matter digestibility 749 g/kg OM. Grass was precision chopped and ensiled into a clamp using a formic acid based additive with target application rate of 5 l/ton (AIV 2 Plus, Eastman Chemical Company, Oulu, Finland).

The silage was separated into liquid and solid fractions using three pressing methods as follows:

- Farm scale twin screw press (FTS; Haarslev Industries A/S, Søndersø, Denmark). Liquid-solid separation was made by feeding the press by a TMR wagon equipped with a scale. The liquid was pumped into 1000 l containers and the amount of liquid was estimated based on volume. This allowed the mass balances (liquid and solid yields from the original silage) to be calculated. In 8 batches with one week intervals, a total of 34 410 kg of silage was processed. Each batch was processed over two days. Before starting the measurements the FTS was running for 15 min to fill the press and reach an optimal performance. The solid fraction was fed to dairy cows in a feeding trial (to be published in Kautto *et al*., 2018);
- Laboratory scale twin screw press (LTS; Angel Juicer Ltd., Busan, South Korea). Batches of 300 g were pressed. Before starting the measurements the LTS was filled with 150 g of silage in order to reach an optimal performance. Liquid was quantitatively collected and weighed; and
- Laboratory scale pneumatic press (LPP, Luke in-house built equipment, Jokioinen, Finland). The liquid-solid separation was made placing 150 g of sample into a mesh bag and then pressed between two piston plates during 2 minutes with 6 bars (× 100 kPa) pressure. The mesh bags were wetted and pressed before the actual samples were processed. Liquid was quantitatively collected and weighed. There were 3 replicates per sample and a mean value was used for statistical analyses.

Silage from batches 4 and 7 of FTS were used for LTS and LPP. The average composition of liquid and solid fractions and the original silages are shown in Table 1. Retained proportion of DM, CP and ash in liquid were calculated for each liquid-solid separation method.

Samples for typical chemical analysis were taken along the experiment and analysed as described by Seppälä *et al*. (2016) at the laboratory of Luke in Jokioinen, Finland.

Table 1 Chemical composition of original silages, and solid and liquid fractions.

	FTS			LTS			LPP	
	Silage	Solid	Liquid	Silage	Solid	Liquid	Solid	Liquid
Dry matter, g/kg	204	430	63	214	497	85	310	70
In dry matter, g/kg								
Ash	71	42	197	70	43	183	55	229
Crude protein	142	107	279	144	99	262	118	271
Neutral detergent fibre	609	727	-*	609	Nd**	-*	Nd**	-*
Ammonium-N, g/kg N	30	16	3	30	Nd**	Nd**	Nd**	Nd**
Organic matter digestibility	724	695	-*	724	Nd**	-*	Nd**	-*

FTS: farm scale twin screw press; LTS: laboratory scale twin screw press; LPP: laboratory scale pneumatic press. *By definition liquid is totally soluble. **Not determined.

Further, an aerobic stability experiment was arranged using a 3 × 2 × 3 factorial design, where first factor was three types of raw material (silage, solid fraction or solid fraction with added water to reach the DM content of the silage), second factor was the form that the raw material was used (as such or as part of TMR) and third factor was three preservative treatments, including a control without preservative, a formic and propionic acid based preservative (FAPA, AIV Ässä Na, Eastman Chemical Company, Oulu, Finland) and a propionic acid based preservative (PA, Eastman Stabilizer Crimp, Eastman Chemical

Company, Oulu, Finland) both at a rate of 3 l/t of fresh matter. The diets recipes are shown in Table 2.

Aerobic stability was evaluated according to Luke standard temperature method, where thermocouple wires in polystyrene boxes were connected to a data logger. Temperature from each of the treatments was automatically recorded at 10-minute intervals. Aerobic stability was defined as the time taken to increase the temperature for 2 °C above the ambient temperature (Seppälä et al., 2016).

Aerobic stability through visual inspection of the silage and solid fraction of FTS was also evaluated . Silage and solid fraction were placed (3 cm layer) in a plastic container which was covered with a slightly perforated plastic film and kept at +20 ºC. The visual inspection was conducted once a day using a score scale: 0 = no spoilage; 1 = slight spoilage; 2 = moderate spoilage; and 3 = severe spoilage. Aerobic stability through visual inspection of spoilage was defined as hours to the sample reach score 3, which were then discarded.

Table 2 Composition of the diets used to measure aerobic stability through increase in temperature.

	As such			As total mixed ration		
	Silage	Solid	Solid+water	Silage	Solid	Solid+water
Water, g			723			566
In g/kg dry matter						
Grass silage	1000			502		
Solid fraction		1000	277		500	218
Barley				160	160	69
Oats				160	160	69
Rapeseed meal				160	160	69
Minerals				18	20	9

The data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability. Effect of liquid-solid separation method on liquid yield, composition and retained compounds in liquid was evaluated using a Tukey test. The factorial scheme to evaluate the aerobic stability was partitioned into contrasts, where type of raw material, used form and preservative were included as fixed effects in the model, while replicate was considered a random effect. Effect of preservatives on aerobic stability measured through visual inspection was tested taking replicates as a random effect into the model.

Results and Discussion

The grass silage (Table 1) used in this experiment was representative for typical grass silage used in Northern Europe (Huhtanen *et al*., 2006).

Separation of grass silage into fractions resulted in solid fraction with higher DM and NDF concentrations and lower CP and ash than the original silage (Table 1). Also Wachendorf *et al*. (2009) reported that separation resulted in solid fraction with lower ash concentration and higher fibre than the original silage. In the present study the concentrations of CP and ash in the solid fraction decreased on average 0.33 (SD = 0.110) and 0.50 (SD = 0.132) relatively to base grass silage, respectively. Similarly, McEniry *et al.* (2012) reported 0.55 decreases of CP and ash in the solid fraction compared to the fresh material pre-ensiling. McEniry &

O`Kiely (2013) reported decreasing of CP concentration of 0.66 on average in solid fraction compared to the fresh material pre-ensiling. Larger reduction of CP in McEniry & O`Kiely (2013) compared to present study may be explained by pretreatment of material with deionized water at 60 °C with a detergent (sodium dodecyl sulphate) to enhance the separation process.

Separation methods affected liquid yield and liquid DM concentration (Table 3), with significantly lower liquid yield for LPP and higher liquid DM for LTS. There was a significant effect of separation method on DM, CP and ash retained in liquid. The LPP resulted in significantly lower DM, CP and ash retained in liquid. Higher DM retained in liquid was obtained for LTS, while CP and ash retained in liquid were not significantly different among FTS and LTS.

Table 3 Effect of pressing methods on liquid yield, composition and retained compounds in liquid.

	FTS	LTS	LPP	SEM
Liquid yield	0.576[a]	0.601[a]	0.345[b]	0.0218
Liquid dry matter (DM), g/kg	71[b]	84[a]	69[b]	1.4
In liquid DM, g/kg				
Crude protein (CP)	270[a]	263[a]	271[a]	1.2
Ash	189[a]	178[a]	218[a]	11.7
Amount retained in liquid as proportion from original silage				
DM	0.193[b]	0.237[a]	0.112[c]	0.0056
CP	0.361[a]	0.422[a]	0.209[b]	0.0112
Ash	0.535[a]	0.606[a]	0.351[b]	0.0308

FTS: farm scale twin screw press; LTS: laboratory scale twin screw press; LPP: laboratory scale pneumatic press. SEM: standard error of the mean. Means within the same row without same superscript differ significantly (P<0.05, Tukey test).

The effects of preservatives and different combinations of raw material in the diet (Table 2) on aerobic stability measured through increase in temperature are presented in Figure 1.

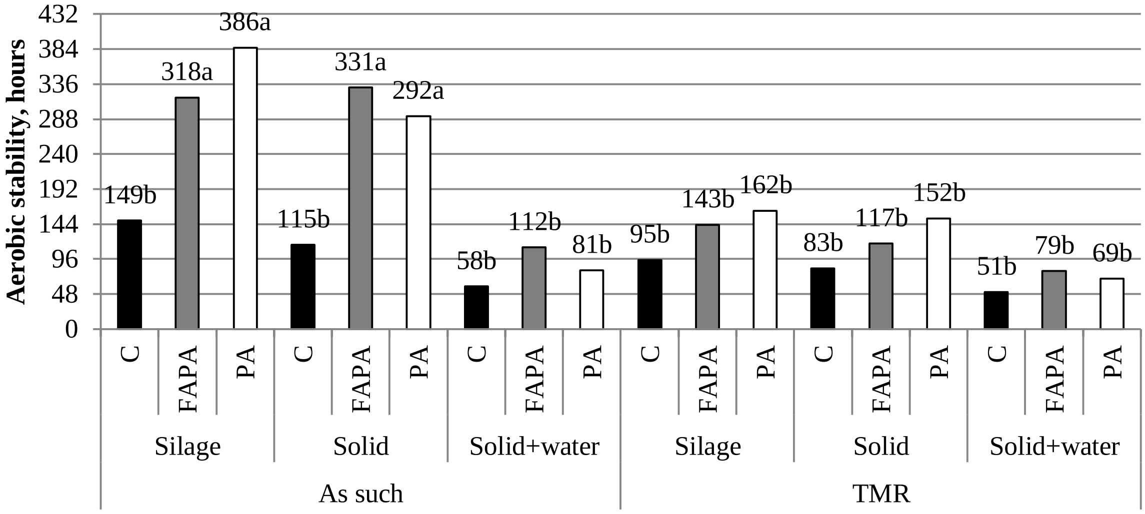

Figure 1. Effect of preservatives on aerobic stability of grass silage, solid fraction and solid fraction + water used as such or in TMR assessed through increasing in temperature. Preservative <0.001; Silage vs Solid used as such =0.060; Silage vs Solid in TMR =0.417; Silage as such vs Silage in TMR <0.001; Solid as such vs Solid

in TMR <0.001; Silage vs Solid+water as such <0.001; Silage vs Solid+water in TMR =0.001; As such vs TMR <0.001. C: control without preservative; FAPA: formic and propionic acid based preservative; PA: propionic acid based preservative. Means without same letter differ significantly (P<0.05, Tukey test).

Silage and solid fraction treated with preservatives had the highest aerobic stability compared to remaining treatments in this study (Figure 1). Similarly as reported by Seppälä *et al*. (2016) sole silage had longer stability than TMR. It should be noted that in both cases, the silage was of high hygienic quality and preserved with a formic acid based additive. Seppälä *et al*. (2016) indicated that high yeast count in brewer's grain is likely causing low aerobic stability of TMR. Also in current study, the concentrate components may have had lower hygienic quality. Addition of water to solid fraction (to the same DM as in silage) reduced aerobic stability. It may be speculated that high DM of solid fraction may restrict microbial growth. Also Seppälä *et al*. (2016) suggested that high DM of the silage may restrict growth of spoiling microbes. But on the other hand, high DM silage are more prone to heating, probably due to better air ingress and lower concentrations of fermentation end products, which may be quite efficient in preventing aerobic spoilage. There was no difference between silage and solid fraction used as such or in a TMR on aerobic stability measured through increasing in temperature. Both preservatives improved the aerobic stability of diets.

Figure 2 presents the effect of preservatives on aerobic stability of grass silage and solid fraction assessed through visual inspection. There was an interaction between raw material and preservative. Preservatives provided higher aerobic stability than control and the effect of preservative was stronger in silage than in solid fraction of grass silage.

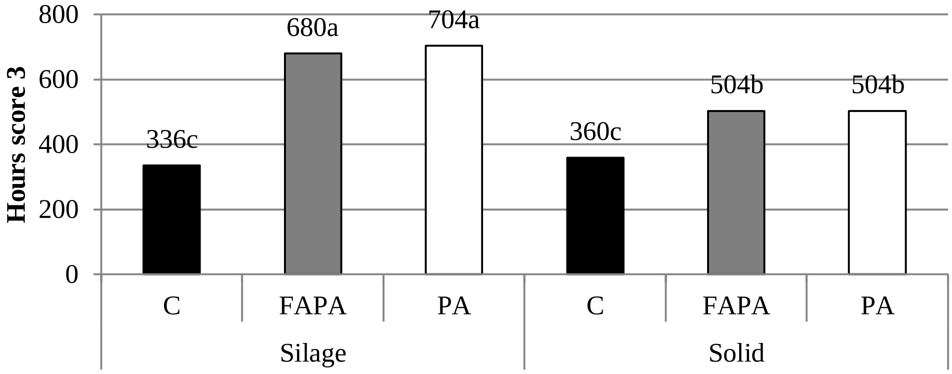

Figure 2. Effect of preservatives on aerobic stability of grass silage and solid fraction of grass silage assessed through visual inspection. Silage vs Solid <0.001; Preservative in silage <0.001; Preservative in solid <0.001; Preservative <0.001; Raw material*Preservative <0.001; FAPA vs PA = 0.458. C: control without preservative; FAPA: formic and propionic acid based preservative; PA: propionic acid based preservative. Means without same letter differ significantly (P<0.05, Tukey test).

Both methods (increasing in temperature and visual inspection) indicated that preservatives were effective in increasing aerobic stability of grass silage and solid fraction from a biorefinery process. However visual inspection resulted in longer aerobic stability than that evaluated through increasing in temperature, which indicates the need of a smaller threshold for visual inspection, so that both methods would match. There was a high correlation between methods (R^2 = 0.798), indicating that both techniques are suitable to measure aerobic stability of silage and solid fraction.

Conclusions

Twin screw presses, farm and laboratory scale, resulted in higher liquid yield and greater amount of retained compounds in liquid fraction than a pneumatic press. Preservatives extended aerobic stability of silage, solid fraction and solid fraction added with water used as such or in a total mixed ration diet.

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