FERMENTATION QUALITY AND DRY MATTER LOSSES OF GRAPE POMACE SILAGES WITH UREA ADDITION

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Abstract

The aim of this study was to determine the effect of urea as silage additive on the fermentation parameters and dry matter losses of grape pomace silages. Grape pomace (Vitis vinifera L.) Pinot Gris variety with 45 % dry matter content was ensilaged in laboratory conditions. Grape pomace was ensilaged in two variants: without additive as a control and with addition of urea applied at a rate of 4kg.t^{-1}. Mini silo bags were filled by grape pomace matter from both variants and hermetically sealed. The mini silo bags were stored in air-conditioned laboratory conditions (t 22±1 °C). Average samples from each variant (n=3) were taken for laboratory analysis after 5 weeks of storage. Dry matter was analysed after drying of the sample at (103±2 °C). Content of lactic, acetic and butyric acid on analyzer EA 100 (Villa Labeco, SR) by ionic electrophoresis method was determined. Contents of alcohols were detected by Conway microdiffusion method and active acidity by the electrometric method. For evaluation of statistical parameters SPSS Statistics 20.0 (IBM) (ANOVA-Tukey test) were used. Dry matter losses in control silages 19.1% and in silages with urea 11.2% (P<0.05) were observed. After addition of urea a significantly (P<0.05) higher lactic acid content in grape pomace silages in comparison with control was found. The lactic acid content was lower than 10g of the original matter in all samples of both variants. There were no significant differences in acetic acid content. The ratio of lactic to acetic acid was favorable in both variants (control- 4.9:1 and additive- 5.7:1). Undesirable butyric acid was not found in any samples. Addition of urea non-significantly (P>0.05) increased value of pH in grape pomace silages. The alcohol content was significantly (P<0.05) higher in silages without an additive.

Keywords: *grape pomace silage, urea, quality, fermentation*

1. INTRODUCTION

Grape pomace is a by-product of the wine industry, which represents around 25% of total grape weight used in the winemaking process (Beres et al., 2017). The environmental and economic impact associated with their storage, transformation and disposal can be reduced by addition of wine-making industry by-products in animal feed (Brenes et al., 2016). The advantage of wine-making industry by-products is low cost compared with the alternative of expensive purchased feeds (Moate et al., 2014; Manso et al., 2016). Grape pomace consists of grape skins (50%), stalks (25%), and seeds (25%) (Duba and Fiori, 2015). From the perspective of nutrition grape pomace contains moderate levels of crude protein, crude fat and minerals (Clifford, 2015; Chikwanha et al., 2018) and lower energy content (Baumgartel et al., 2007). Grape pomace is therefore nutritionally valuable feedstuff manly for ruminants. Additionally, grape pomace contains biological active substances which affect health status (Brindza et al., 2015) and feed efficiency (Clifford, 2015). For example, condensed tannins, which have significant potential to improve protein supply to the animal (Clifford, 2015). The content of total tannins is variable and ranges from 39.1 to 105.8 g.kg^{-1} dry matter (Ky et al., 2014). The grape pomace can be used in animal nutrition either fresh (Molina-Alcaide et al., 2008), dried (Pirmohammadi et al., 2007; Bahrami et al., 2010), or as silage (Dinić et al., 2015; Winkler et al., 2015; Caetano et al., 2016). Additives have been available for enhancing silage preservation for decades (Muck et al., 2018). Urea is used like nutrient additive (Ebrahim Pour et al., 2014; Yaghoubi et al., 2014; Dinić et al., 2015). Beneficial effect of urea addition is economical source of crude protein, improve the aerobic stability of silage at feedout (Yitbarek and

Tamir, 2014). The highest pH values and ammonia-N levels associated to higher anaerobic proteolytic bacterial populations are associated with urea application (Singh et al., 1996; Doležal et al., 2017).

2. MATERIALS AND METHODS

2.1. Materials

Grape pomace (*Vitis vinifera* L.) Pinot Gris variety with 45 % dry matter content was ensilaged in laboratory conditions. Grape pomace was ensilaged in two variants: without additive as a control and with addition of urea applied at a rate of 4kg.t^{-1}. Mini silo bags were filled with grape pomace matter from both variants and hermetically sealed. The mini silo bags were stored in air-conditioned laboratory conditions (t 22±1 °C). Average samples from each variant (n=3) were taken for laboratory analysis, after 5 weeks of storage.

2.2. Methods

Laboratory testing was carried out in Laboratory of Quality and Nutritive Value of Feeds at Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra according to standard laboratory techniques. Dry matter was analysed after drying of the sample at (103±2 °C). The dry matter losses during fermentation were calculated as the percentage difference between the dry matter of the mass at ensiling and the dry matter of the silage at the end of conservation. Content of fermentation acids: lactic, acetic and butyric on analyzer EA 100 (Villa Labeco, SR) by ionic electrophoresis method was determined. Contents of alcohols and ammonia (NH_3) were detected by Conway microdiffusion method and active acidity by the electrometric method. Degree of proteolysis was calculated (NH_3-N / total N * 100). Fermentation products were calculated by count of fermentative acids with alcohols. The determination of the acidity of water extract was carried out by alkalimetric titration of the silage extract to pH 8.5 (mg KOH.100g^{-1}).

2.2.1. Statistical analysis of results

For evaluation of statistical parameters SPSS Statistics 20.0 (IBM) (ANOVA-Tukey test) an independent samples T-test were used.

3. RESULTS

Statistically significant higher dry matter content (by 6.9%) in grape pomace silages with urea addition was found ($P<0.05$). Higher dry matter content was observed due to lower dry matter losses in experimental variant compared with a variant without an additive (by 7.9%). Higher lactic acid concentration by 12.0% in grape pomace silages with urea was observed ($P<0.05$). On the other hand, concentration of acetic acid was higher in silages without additive. The differences in acetic acid content between the variants were nonsignificant. The ratio of lactic acid to acetic acid was relatively wide with values 4.9:1 for control silages and 5.7:1 for silages with urea. The presence of undesired butyric acid was not observed. A higher proportion of lactic acid and lower proportion of acetic acid from the total acid content was observed in silages with urea addition in comparison with control (85.1:14.9% vs. 83.1:16.9%). The pH value in grape pomace silages between variants was similar and the differences were very small (0.06) and nonsignificant. The grape pomace silages had high content of alcohols. In silages with urea addition, a lower content of alcohols in comparison with silages without an additive by 17.7% ($P<0.05$) was found. The application of urea significantly ($P<0.05$) influenced the value of acidity of water extract (AWE) and the degree of proteolysis in grape pomace silages (higher value by 7.9%; and by 59.3%). A lower content of fermentation products by 11.4% in silages with urea (with lower dry matter) was detected.

Variant	**Control**		**With urea**	
Parameter	Mean	± S.D.	Mean	± S.D.
Dry matter*	366.60[a]	7.90	391.98[a]	15.09
Dry matter losses %	19.10[a]	1.79	11.20[a]	3.31
Lactic acid**	16.78[a]	2.13	18.79[a]	1.00
Acetic acid**	3.41	0.90	3.28	1.31
Butyric acid**	ND	/	ND	/
pH	4.15	0.01	4.21	0.04
Degree of proteolysis %	1.50 [a]	0.07	2.39 [a]	0.21
Alcohols**	69.14[a]	7.09	56.91[a]	4.25
Acidity of water extract***	458.83[a]	16.42	494.83[a]	12.67
Fermentation products**	91.26[a]	9.54	80.86[a]	5.54

Table 1. Fermentation quality and dry matter losses in grape pomace silages

Abbreviations: *$g.kg^{-1}$, **$g.kg^{-1}$ of dry matter, ***mg KOH.$100g^{-1}$,
[a]values with the same index in row are significant at P<0.05

4. DISCUSSION

The dry matter content is a decisive factor for silage quality (Borreani et al., 2018). Higher dry matter content was observed in grape pomace silages with urea addition (P<0.05), which resulted in lower dry matter losses (P<0.05). In silages of both variants a higher content of dry matter greater than 300 $g.kg^{-1}$ was observed, which is the minimum value for inhibition of effluent loses (Bíro et al., 2014). The average dry matter losses in experimental variant were lower by 7.9% compared with variant without an additive (11.20 vs.19.10%). The urea was efficient in the reduction of dry matter losses when compared to the control silages in study of Sousa et al. (2008). Köhler et al. (2013) reported average dry matter losses for maize silages 10% (min.4 and max.19%), for grass silages 9% (min.2 and max.26%) and for alfalfa silages 12% (min.6 and max.15%). The average concentrations of lactic acid were 6.2 g in control silages and 7.4 g in kg of original matter in silages with urea. The silages of both variants had a lower content of lactic acid, less than 10 g in kg of original matter, which is one of the parameters for top quality silage (Bíro et al., 2014). The application of a urea dose of $4kg.t^{-1}$ of original matter resulted in a significant (P<0.05) increase of the lactic acid content. This finding is consistent with the study realized by Sousa et al. (2008), but contradicts the finding by Doležal et al. (2017). The acetic acid content was not affected by treatment, with lower concentration in experimental silages. Doležal et al. (2017) recorded the same result in maize silages with urea addition. The results confirmed, that the concentration of acetic acid is inversely related to dry matter content (Kung et al., 2018). The ideal ratio of lactic acid to acetic acid should not be less than 3:1 (Jalč, et al., 2010). In all silages higher ratio as 3:1 was detected (for control silages 4.9:1 and for silages with urea 5.7:1). This finding is in concordance with Doležal et al. (2017) who reported higher ratio of lactic acid to acetic acid in maize silages with urea addition ($5 kg.t^{-1}$) compared to the control sample. The presence of undesired butyric acid was not observed. Lactic acid should be at least 65 to 70% of the total silage acids in a good silage (Kung and Shaver, 2001). The silages of both variants had higher proportion of lactic acid than previous recommendation. Higher proportion of lactic acid from the total acid content was determined in silages with urea addition (85.1 vs. 83.1%). These findings are in agreement with the study by Doležal et al. (2017), where similar effect was reported with urea application ($5 kg.t^{-1}$) in maize silages. The pH value was not influenced by urea addition, with nonsignificantly higher pH in experimental silages. The same results after application of urea were also observed by Yaghoubi et al. (2014). For silages with dry matter

content 30-40% made from matter with difficult and very difficult ensilability is the optimal pH value < 4.5 (Bíro et al., 2014). Silages of both variant had pH value lower than 4.5. All grape pomace silages were characterized by high alcohol content. According to Mitrík (2006) the content of alcohols in silages < 2% in dry matter is acceptable. High concentrations of ethanol are usually an indicator of excessive metabolism by yeasts (Shaver, 2013). All tested silages had an alcohol content higher than 2% in dry matter. Compared with the control silages, the urea addition resulted in reduced ($P<0.05$) alcohol content. These findings are in agreement with the study by Sousa et al. (2008) where a similar effect was reported with urea application (1% of fresh forage) in sugarcane silages. The titration acidity of the water extract is considered an important parameter of the quality of the silage fermentation (Doležal et al., 2012). The use of additive significantly ($P<0.05$) influenced the value of acidity of water extract (AWE) and degree of proteolysis in grape pomace silages. The variant with urea had the highest value of AWE and degree of proteolysis in comparison with the control variant. The concentration of fermentation products in silages affects the dry matter content (Bencová, 1999). Lower content of fermentation products in silages with urea (with lower dry matter) was observed.

5. CONCLUSIONS

Urea addition influenced the fermentation quality of grape pomace silages after 5 weeks of storage with statistically higher lactic acid content, acidity of the water extract and degree of proteolysis. However, the lactic acid content was lower than 10g of the original matter in all samples of both variants. The acetic acid content and pH value were not affected by treatment. The ratio of lactic to acetic acid was favorable in both variants (control- 4.9:1 and urea- 5.7:1). Undesirable butyric acid was not found in any samples. The application of urea statistically and significantly decreased alcohol content and content of fermentation products. The results confirmed, that the addition of urea decreased dry matter losses during fermentation of grape pomace silages.

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