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Fermentation characteristics, aerobic stability, proteolysis and lipid composition of alfalfa silage ensiled with apple or grape pomace

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ABSTRACT

The effects of adding 100 g of either dried apple pomace (AP), unground grape pomace (GP) or ground grape pomace (GGP) per kg wilted alfalfa (wet basis) on silage characteristics, aerobic stability, proteolysis and lipid composition were studied. The three combinations as well alfalfa without addition were ensiled in 1.8 L- jars for 60 d, with 4 jars per treatment. Addition of AP and GGP decreased pH ($P<0.05$). Pomace-treated silages contained more lactic acid than the control silage. Pomace treatments decreased aerobic stability of the ensiled alfalfa. The nonprotein nitrogen (NPN) concentrations in the ensiled alfalfa were decreased by 54%, 67% and 69% after being ensiled with AP, GP and GGP, respectively. Total fatty acid concentrations in the control silage and AP silage were comparable, but in GP or GGP-treated silages it was more than double that of the control silage. Application of pomace markedly increased ($P<0.05$) the proportions of oleic acid and linoleic acid in the ensiled alfalfa due to high proportions of these fatty acids in both pomaces. Silages treated by GP or GGP had a much greater proportion of linoleic acid than the control or AP treated silages ($P<0.05$); this fatty acid accounted for half of the fatty acids in GP or GGP-treated silages. The proportion of α-linolenic acid was lower in GP or GGP silages than in control silage or AP silage ($P<0.05$). In conclusion, application of apple or grape pomace at ensiling of alfalfa could not only employ this industrial waste as feed, but also inhibit proteolysis and alter the fatty acid composition of ensiled alfalfa.

1. Introduction

Extensive proteolysis of alfalfa during silage fermentation is a prevalent problem. Attempts to reduce proteolysis in ensiled forage have been focused mainly on application of chemical additives, such as formic acid (Nagel and Broderick, 1992) and tannins (Salawu et al., 1999; Tabacco et al., 2006) or of biological additives like bacteria inoculants and enzymes (Loucka et al., 1999; Pys and Migdal, 2002). No satisfactory, consistent and feasible means of inhibiting proteolysis in ensiled

Abbreviations: CP, crude protein; DM, dry matter; aNDF, amylase-treated neutral detergent fiber expressed inclusive of residual ash; ADF, acid detergent fiber expressed inclusive of residual ash; NPN, non-protein N; TCA, trichloroacetic acid; FA, fatty acid; VFA, volatile fatty acids; WSC, water soluble carbohydrates; PUFAs, polyunsatured fatty acids; CLA, conjugated linoleic acid; AP, apple pomace; GP, unground grape pomace; GGP, ground grape pomace.

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alfalfa has been found. Although formic acid is one of the most effective additives in inhibiting proteolysis, cost and logistics of this chemical additive limit its widespread use (Guo et al., 2011). Recently, changes of fatty acid composition in forages after ensiling has become an additional concern, especially with respect to losses of C18:2n-6 and C18:3n-3 during ensiling (Alves et al., 2011); intake of polyunsaturated fatty acids can increase their concentration in ruminant products slightly (Wood and Enser, 1997) and, consequently be beneficial in human health (Simopoulos, 2001). A recent study showed that application of inoculant or of calcium formate affected the FA composition of ryegrass silages, largely by increasing the proportions of short chain fatty acid, but these two additives had no effect on FA composition of corn silage (Alves et al., 2011). Polyphenols have been shown to reduce proteolysis and lipolysis in red clover through deactivating proteolytic and lipolytic enzymes or through formation of protein–phenol–lipid complexes (Lee et al., 2008).

The cider and red wine industries generate huge amounts of apple and grape pomace as an industrial waste that generally is disposed in open areas and often causes environmental problems (Botella et al., 2005). Apple pomace has been used widely as animal feed after fermentation or drying (Joshi and Sandhu, 1996). It also is known as a valuable material for pharmacological or cosmetic purposes because it is rich in polyphenols. The major phenols in apple pomace are cinnamic acids (chlorogenic and caffeic acids), flavanols, dihydrochalcones and flavonols (García et al., 2009). Comparatively, grape pomace contains much more polyphenol including phenolic acids, flavonoids, anthocyanins, proanthocyanidins and other phenols (Lu and Yeap Foo, 1999). According to Baydar et al. (2007), the oil concentration of grape pomace varied from 54.7 to 86.6 g/kg DM and was rich in oleic and linoleic acids; the degree of unsaturation in the oils was over 850 g/kg total fatty acids. Based on the polyphenol compounds in apple or grape pomaces, its low pH ranging from 3.4 to 4.2 (Pirmohammadi et al., 2006), and the beneficial lipid composition in grape pomace, we hypothesized that application of these two materials at ensiling may inhibit proteolysis, depress pH and improve fatty acid composition of ensiled alfalfa. The objective of our study was to investigate fermentation quality, aerobic stability, protein degradation and alterations in lipid composition of alfalfa when ensiled with the by-products of cider and red wine production.

2. Materials and methods

2.1. Preparation of laboratory scale silos

Alfalfa (*Medicago sativa* L. cv. Golden Empress) was harvested by hand clippers in the late bud to early bloom stage leaving a stubble of 5 cm. The fresh forage samples were taken into the laboratory, wilted to a DM content of approximately 350 g/kg FW, and chopped to about 1–2 cm using a paper-cutter. There were four treatments in the ensiling trial *i.e.*, the chopped forage was either untreated (control) or mixed with either apple pomace (AP, dried pomace after mashing apple for juice from cider industry), unground grape pomace (GP, dried and integrated particles of grape skin and seeds after fermentation of grapes from the red wine industry) and ground grape pomace (GGP, ground by a mill with 1 mm screen), respectively, at an application rate of 100 g dried pomace (with moisture content of 112–120 g/kg) per kg wilted alfalfa forage according to Xue et al. (2009). The purpose of grinding grape pomace was to increase the contact to the chopped alfalfa material. Dried pomaces were obtained from cider and red wine factories. To insure the pomaces adhered to alfalfa during mixing and have similar moisture content to wilted alfalfa at ensiling, the dried apple and grape pomaces were moistened by spraying distilled water to yield a moisture content of 650 g/kg of pomace before mixing. The moistened apple or grape pomaces were mixed thoroughly with the chopped forage by hand before packing into silos. The silos consisted of 1.8 L-glass jars (Nanjing Junya Industry and Trade Co., LTD, Nanjing, China) packed at a density of 500 g/l, sealed with a screw cap and stored at room temperature (~25 °C) for 60 d. There were 4 jars per treatment. Each glass jar was filled with about 900 g ensiling materials. In the ensiled materials, the mixed amounts of moistened apple or grape pomace to wilted alfalfa were 180–720 g (equal to the application rate of 100 g dried pomace per kg wilted alfalfa). No gas ventage was allocated.

2.2. Chemical and fatty acid analyses

Mini silos from each treatment were opened after 60 d of ensiling with a portion of silage immediately frozen (−20 °C) in sealed plastic bags until further analyzed chemically. Initial fresh forage samples were taken before samples were ensiled. A 20 g (fresh weight) sample from each silo was placed in a blender jar, diluted with distilled water to 200 g, and macerated for 30 s in a high-speed blender, and filtered through four layers of medical gauze. The filtrate pH was measured immediately. After acidification with 7.14 M H_2SO_4, the filtrate was centrifuged for 15 min at 10,000 × *g* and filtered with a 0.45 μm dialyzer. Lactic acid, acetic acid, propionic acid, and butyric acid were analyzed by High Performance Liquid Chromatography (HPLC, KC-811 column, Shodex; Shimadzu; Japan; oven temperatures 25 °C; flow rate 1 ml/min; SPD 210 nm). One milliliter of 250 g/l (w/v) trichloroacetic acid (TCA) was added to 4 ml of the filtrate from each silo; this solution was allowed to stand at room temperature for 1 h or was held overnight at 4 °C to precipitate the protein. The solution then was centrifuged at 4 °C, 18,000 × *g* for 15 min, and the supernatant fluid was analyzed for ammonia nitrogen (NH_3-N) according to Broderick and Kang (1980). Non protein nitrogen (NPN) content of forage samples were analyzed after precipitating protein by using TCA as described by Licitra et al. (1996). Enumeration of lactic acid bacteria, yeast and mold in fresh alfalfa, apple and grape pomace, ensiled forage was performed according to methods described by Reich and Kung (2010). Briefly, samples (10 g) were homogenized in 100 ml of sterile Ringer's solution (Oxide BR52) for 1 min and serially diluted (10-fold). The number of LAB was enumerated on spread plates using Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK) and incubated at 30 °C for