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Impact of dehydration on retention of bioactive profile and biological activities of different grape (*Vitis vinifera* L.) pomace varieties

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ABSTRACT

The effects of drying method [sun (7 days), oven (72 h at 60 °C) and freeze (~72 h)] and grape (*Vitis vinifera* L. cv. Pinotage, Shiraz and Sauvignon Blanc) variety on pomace fatty acid composition, polyphenolic content and antioxidant capacity were evaluated. Furthermore, the influence of sun-dried pomaces on rumen microbial diversity was assessed *in vitro*. Freeze-dried Shiraz had the highest proportions of 18:1n-9, 18:2n-6, total monounsaturated fatty acid (MUFA), polyunsaturated fatty acids (PUFA) and content of polyphenolics compared to other drying × variety interactions ($P \leq 0.05$). Freeze-dried Sauvignon Blanc had the highest proanthocyanidin content and antioxidant activity for both 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) relative to other drying × variety interactions ($P \leq 0.05$). Regardless of variety and inclusion level, grape pomace variety reduced bacterial species abundance, but improved species diversity, evenness and richness compared to the control ($P \leq 0.05$). Overall, freeze-dried Shiraz had the best fatty acid profile and highest polyphenolic content, while freeze-dried Sauvignon Blanc had the highest proanthocyanidin content and antioxidant activity.

1. Introduction

Grape pomace (GP) is the major by-product of the wine industry, which equates to 250 g/kg of the pressed grapes and on a dry matter (DM) basis contains stalks (~20 g/kg), seeds (~470 g/kg), skin and pulp (~510 g/kg) (Beres et al., 2017; Zhang et al., 2017). The wine industry is doubtful about the value of GP, and thus treat it as waste. Even though GP is not a hazardous waste *per se*,

Abbreviations: ADFom, ADF expressed exclusive of residual ash; aNDFom, NDF assayed with a heat stable amylase and expressed exclusive of residual ash; ANOSIM, Analysis of similarity; ARISA, Automated Ribosomal Intergenomic Spacer Analysis; DM, dry matter; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FA, Fatty acids; FRAP, Ferric reducing antioxidant power; GP, Grape pomace; MUFA, Monounsaturated fatty acids; n-3, omega- 3; n-6, omega- 6; PCA, principal component analysis; PUFA, Polyunsaturated fatty acids; RSA, Radical scavenging activity; SFA, Saturated fatty acids

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disposal may be detrimental to the environment, due to the phenolic compounds, which decrease the pH of the pomace and increase its resistance to biological degradation (Beres et al., 2017). Generally, GP has low nutritional value because of the high content of phenolic compounds and fiber, particularly lignin (Chikwanha et al., 2018a). However, it may be used as ruminant feed because of their well-developed and specialized digestion mechanism that allows better utilization of polyphenolic-rich and fibrous diets (Mlambo and Mapiye, 2015).

Globally, only 3% of GP is currently used as livestock feed, with Australia, a major wine producing country, using 13% as animal feed (Beres et al., 2017; Zhang et al., 2017). From a nutritional point of view, GP is an abundant and inexpensive source of polyphenols that have high antioxidant and antimicrobial properties (García-Lomillo and González-SanJosé, 2017). Furthermore, its lipid fraction presents an interesting fatty acid (FA) profile rich in polyunsaturated FA (PUFA) ranging between 600–800 g/kg (Lutterodt et al., 2011; Yi et al., 2009), notably, linoleic acid (18:2n-6) (García-Lomillo and González-SanJosé, 2017). Feeding ruminants diets containing elevated levels of 18:2n-6 and moderate levels of polyphenols (i.e., 20–60 g/kg proanthocyanidins DM) increases tissue accumulation of PUFA and their biohydrogenation intermediate products (Mapiye et al., 2015; Vasta et al., 2010), including rumenic acid [*cis*-9, *trans*11-18:2, the most abundant conjugated linoleic acid (CLA)] and its precursor vaccenic acid (*trans*11-18:1), which seem to have human health benefits (Shokryazdan et al., 2017; Wannamethee et al., 2018). Thus, modulation of ruminal FA metabolism by polyphenols facilitates higher forestomach output of PUFA and their biohydrogenation intermediate products for absorption and incorporation into animal tissues. In that regard, if digested and absorbed GP has the potential to improve meat FA profile and enhance its shelf life through the antioxidative and antibacterial properties of polyphenols.

Fresh GP has high moisture content (~600 g/kg) and if not preserved within a week of pressing, it spoils resulting in pungent odors that attract flies and pests, which transmit pathogenic organisms (Zhang et al., 2017). Overall, high moisture content in feeds and foods is usually associated with elevated water activity, which accelerates microbial spoilage and deteriorative biochemical reactions including oxidation of lipids and degradation of phenolic compounds (Choe and Oh, 2013). Thus, moisture levels in GP should be lowered to preserve it against microbial spoilage and undesirable biochemical reactions.

Dehydration as opposed to fermentation (ensiling) is by far the most common method used to extend the shelf life of GP bioactive compounds for off-season use by decreasing the amount of water available for microbes and deteriorative biochemical reactions (Gan et al., 2017). Dehydration also reduces the bulkiness of GP, which in turn decreases costs of packaging, storage and transportation (Gan et al., 2017). However, some adverse effects on GP quality caused by dehydration including degradation of valuable nutrients (Chikwanha et al., 2018a) and loss of bioactive compounds and consequently antioxidant and antimicrobial activities should not be ignored (Gan et al., 2017; Tseng and Zhao, 2012). These losses vary with dehydration technique (Çoklar and Akbulut, 2017; Tseng and Zhao, 2012). Varietal differences among GP are also responsible for the varying content and composition of bioactive compounds (This et al., 2006), which have a bearing on the resultant biological properties.

In South Africa, GP has relatively been unexploited by the local meat industry as a feed supplement rich in PUFA, natural antioxidants and antibacterials. Overall, GP seasonality is among the main obstacles for its standardization as a continuous and steady ingredient in ruminant diets. Furthermore, there is scant literature regarding the effects of dehydration on the retention of bioactive profile and biological activities of different grape (*Vitis vinifera* L.) pomace varieties, particularly the locally bred red variety, Pinotage. The first objective of the current study was to evaluate the effects of drying method and grape variety on pomace FA profile, phenolic composition and antioxidant activity. Secondly, the *in vitro* digestion of sun-dried grape pomaces on ruminal bacterial diversity was investigated.

2. Materials and methods

2.1. Preparation of grape pomace

The three most commonly produced grape (*Vitis vinifera* L.) varieties (i.e., Pinotage, Sauvignon Blanc and Shiraz) in South Africa were sourced at Stellenbosch University's Welgevallen farm (Stellenbosch, South Africa). Sauvignon Blanc is a white grape variety, while Pinotage and Shiraz are red varieties. Pinotage was harvested in January, Sauvignon Blanc in February and Shiraz in March 2017. All the varieties were harvested over six consecutive days (6 pressings). Each day, about eight tons of each variety were harvested, pressed and a representative sample (2 kg) of fresh pomace collected ($n = 6$ pressings). The sample from each day's pressing for each variety was divided into three fractions of 500 g and randomly allocated to three drying treatments: sun-drying for 7 days at temperatures between 25 and 33 °C, oven drying at 60 °C for 72 h and freeze-drying for 72 h (vacuum pressure of 7 mTorr and condenser temperature of −88.7 °C; VirTis Co., Gardiner, NY, USA). The dried samples were ground into fine powders using a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) with a 1-mm sieve and stored at −20 °C pending analyses.

2.2. Extraction of phenolic compounds

The dried GP powders were defatted using n-hexane (10:1; v/w) with subsequent filtration using a Whatman® # 1 filter. Polyphenols were extracted in acidic acetone [i.e., 70% acetone, 29.9% mL water and 0.1% hydrochloric acid (v/v/v)] according to Tseng and Zhao (2012) in an ultrasonic water bath (Branson B-220H, SmithKline Co., USA) at a solvent to pomace ratio of 10:1 for 20 min at 20 °C. The extracts were centrifuged at 12 857 × *g* for 15 min at 4 °C and the supernatant recovered and stored at −80 °C pending analyses.