Industrial Crops & Products 118 (2018) 30–37

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

# Varietal differences in nutrient, amino acid and mineral composition and *in vitro* rumen digestibility of grape (*Vitis vinifera*) pomace from the Cape Winelands vineyards in South Africa and impact of preservation techniques

Obert C. Chikwanha[a], Emiliano Raffrenato[a], Voster Muchenje[b], Hannibal T. Musarurwa[c], Cletos Mapiye[a,*]

[a] Department of Animal Sciences, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa
[b] Department of Livestock and Pasture Science, Faculty of Science and Agriculture, University of Fort Hare, P. Bag X1314, Alice, 5700, South Africa
[c] Department of Human Biology, Faculty of Health Sciences, Walter Sisulu University, Private Bag X1, Mthatha, 5117, South Africa

ARTICLE INFO

*Keywords:*
Drying treatments
Grape pomace
*In vitro* digestibility
Nutrient composition

ABSTRACT

Grape pomace, a by-product of the winemaking industry, is a potential source of animal feed but its nutrient and chemical composition is not sufficiently studied in South Africa. The current study investigated the changes in nutrient, amino acid and mineral composition, and *in vitro* digestibility of neutral detergent fiber of grape (*Vitis vinifera* var. Pinotage, Shiraz and Sauvignon Blanc) pomace after three drying treatments: sun (7d), freeze (72 h) and oven drying (72 h at 60 °C). Oven-dried Shiraz had the greatest dry matter, crude protein and the least ash content ($P < 0.05$), while sun-dried Shiraz had the highest neutral detergent fiber, acid detergent fiber and acid detergent lignin compared to other grape pomace treatments ($P < 0.05$). Freeze-dried Sauvignon Blanc had the highest starch content, whereas freeze-dried Shiraz had the highest ether extract content relative to other grape pomace treatments ($P < 0.05$). The *in vitro* digestibility of neutral detergent fiber at 24- and 48 h were highest in freeze-dried Pinotage followed by freeze-dried Shiraz and Sauvignon Blanc treatments ($P < 0.05$). Freeze-dried Pinotage had the majority of amino acids in highest concentration, followed by freeze-dried Shiraz ($P < 0.05$). Freeze- and oven-dried Pinotage exhibited higher contents of potassium, magnesium, sulfur, sodium, iron and aluminum than other treatments. Overall, sun- and oven-dried Shiraz had the higher dry matter, crude protein, neutral detergent fiber, acid detergent fiber and acid detergent lignin contents, freeze- and oven-dried Pinotage had the best mineral composition, while freeze-dried Pinotage had the best amino acid profile and *in vitro* digestibility of neutral detergent fiber at 24 and 48 h.

## 1. Introduction

The wine growing region of the Western Cape Province of South Africa has a Mediterranean climate, ideal for the wine grape, *Vitis vinifera*. The South African wine industry produces close to 1.5 million tonnes of grapes per annum (Beres et al., 2017) of which 20–25% ends up as grape pomace (GP) after juice or wine extraction (Yu and Ahmedna, 2013). The quantity of pomace, however, varies with grape cultivar, pressing process and fermentation steps (Beres et al., 2017). The main constituents of GP are skins (50–65%), seeds (38–52% DM) and stems (2–8%) produced after pressing the crushed grapes in white wine production or fermented grapes in red wine production (Maier et al., 2009; Mendes et al., 2013; Naziri et al., 2014; Spigno et al., 2013).

Traditionally, exploitation of GP for economic and environmental reasons has focused in recycling for soil conditioning, recovery of bioactive compounds and production of ethanol, flour, food colorings, grape seed oil and animal feed (Arvanitoyannis et al., 2006; Beres et al., 2017; Brenes et al., 2016; García-Lomillo and González-SanJosé, 2017). These applications have limited markets and can absorb only a small portion of the generated GP waste and the large portion of GP is discarded (El Achkar et al., 2016). Disposal of GP is usually carried out by external companies, thus adding to the production costs for the wine industry (Spigno et al., 2017). Besides economic costs, there are many environmental costs associated with the disposal of GP. For example the application of GP as an organic fertilizer can have phenolic content related phytotoxic effects that inhibit seed germination and root growth (Barbera et al., 2013; Devesa-Rey et al., 2011). The low pH of GP linked to delayed mineralization and long-term loss of carbonate from the top soil (Barbera et al., 2013; Nendel and Reuter, 2007) can have negative

* Corresponding author.
*E-mail address:* cmapiye@sun.ac.za (C. Mapiye).

https://doi.org/10.1016/j.indcrop.2018.03.026
Received 15 September 2017; Received in revised form 13 November 2017; Accepted 12 March 2018

effects on soil chemistry. Other environmental problems include surface and groundwater pollution; foul odors; attraction of flies and pests which may spread diseases; and leachates of tannins and other compounds with the possibility of oxygen depletion in the soil and ground waters, affecting surrounding flora and fauna (Arvanitoyannis et al., 2006; Dwyer et al., 2014). Thus, identification of alternative use for GP will result in increased demand and consequently reduce environmental problems posed by its disposal.

Grape pomace has been included in ruminant diets either in the fresh, dried or ensiled form mostly to meet maintenance requirements (Baumgärtel et al., 2007). Wet GP typically has high moisture content (65%) and water activity, which makes it highly perishable (Goula et al., 2016). The seasonality of GP availability, high energy costs for dehydration, variable chemical composition and presence of undesirable contaminants, such as mycotoxins, biogenic amines, pesticides and heavy metals (e.g., cadmium and lead; Bustamante et al., 2008; Moncalvo et al., 2016) often pose challenges for its utilization as an animal feed. Nutritional composition and digestibility of GP is mainly influenced by environmental conditions, grape variety (Baumgärtel et al., 2007; Spanghero et al., 2009) and drying methods (Basalan et al., 2011; Zalikarenab et al., 2007) among other factors. The effects of different drying methods on nutrient and chemical composition of GP from locally grown varieties in South Africa, particularly the locally bred variety, Pinotage, are not known. Lack of such fundamental information currently limits the inclusion of GP in ruminant diets. The objective of the current study was, therefore, to evaluate the effects of drying method on nutrient and chemical composition and *in vitro* neutral detergent fiber digestibility (*iv*NDFd) of pomace from three grape varieties commonly grown in South Africa.

## 2. Materials and method

### 2.1. Preparation of grape pomace samples

Fresh grape (*Vitis vinifera* L. var. Pinotage, Sauvignon Blanc and Shiraz) pomace were provided by Stellenbosch University Winery (Stellenbosch, South Africa). Sauvignon Blanc is a white grape variety, while Pinotage and Shiraz are red varieties. Fresh pomace (2 kg) of each variety were collected after each pressing (n = 6), pooled and moisture content determined. The pooled samples for each variety were divided into three batches of 500 g each and randomly allocated to three drying treatments. The three drying treatments were sun-drying (7 days at temperatures between 25 and 33 °C), freeze-drying and oven drying at 60 °C until no further weight loss. Frozen GP were freeze dried using a freeze dryer (VirTis Co., Gardiner, NY, USA) at a vacuum pressure of 7 millitorr and condenser temperature of −88.7 °C. The dried samples were ground 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. Proximate analyses

Dry matter (DM), ash and ether extract (EE) contents were determined according to the AOAC (2002) procedures. Total nitrogen content was analyzed using the Dumas method with a macro-Nitrogen analyzer (LECO® FP528, LECO Corporation, Miami, USA). Crude protein (CP) was calculated by multiplying the nitrogen content by a factor of 6.25. Starch was measured using a commercial assay (Total Starch Megazyme kit KTSTA, Megazyme International Ireland Ltd., Wicklow, Ireland), following the method for samples containing glucose and/or maltodextrins (Hall, 2009). Neutral detergent fiber (aNDFom) was determined using heat-stable alpha-amylase and addition of sodium sulfite (Mertens et al., 2002). Acid detergent fiber (ADFom) was performed according to AOAC (2002). Acid detergent lignin (ADL) was analyzed according to Goering and Van Soest (1970) as modified by Raffrenato and Van Amburgh (2011). Neutral detergent fiber, ADFom and ADL were expressed exclusive of ash. All analyses were performed in triplicate.

### 2.3. In vitro digestibility

Two rumen-cannulated Holstein dairy cows were used as rumen content donors for the *in vitro* NDF digestibility (*iv*NDFd). The animals were fed a total mixed ration consisting of Lucerne hay (40%), concentrate (60%) at 07:00 and 16:00. Rumen fluid from each cow was collected before the morning feeding and mixed in pre-warmed insulated Thermos flask. The rumen fluid was filtered through four layers of cheesecloth, 100 µm mesh and glass wool prior to inoculation. Ground and dried GP (~0.5 g) was weighed into 125-mL Erlenmeyer flask (in duplicate) before the addition of 40 ml of Van Soest buffer as described by Goering and Van Soest (1970). The flasks were placed in a heated (39.5 °C) shaking water bath under $CO_2$ positive pressure to ensure an anaerobic environment, before addition of 10 ml of rumen fluid. *In vitro* NDF digestibility was estimated as the difference between the NDF weight incubated and the NDF weight of the filter obtained using 50-mL sintered Gooch crucible porosity 2, with added Whatman glass microfiber filters(934-AH®, GE Healthcare, Pittsburgh, PA, USA), expressed as a proportion of DM weight incubated. Residual NDF were measured at 24 and 48 h. The analyses was performed in two runs, with each treatment analyzed in duplicate.

### 2.4. Amino acid analyses

Amino acid separation and detection was performed using Waters Acquity Ultra Performance Liquid Chromatograph fitted with a photodiode array detector (UPLC-PDA). Briefly, 100 mg of dried ground GP was used for the extraction of amino acids using acid hydrolysis extraction (0.5 ml of 6 M HCl). L-Norvaline was used as the standard amino acid. Derivatization of the amino acids was performed using AccQ Fluor reagent Kit, Waters (En Yvelines Cedex, France). For derivatization, 10 µl of standard/sample was mixed in vials with 70 µl buffer solution (0.2 M borate buffer) and 20 µl of derivatization reagent (2 mg/ml AQC). The capped vials were transferred into an oven at 55 °C for 10 min to build stable derivatives. A volume of 1 µl of standard/sample solution was injected into the mobile phase onto the Waters UltraTag $C_{18}$ column (2.1 × 50 mm x 1.7 µm) at 60 °C. Analytes eluting off the column were detected by the PDA detector, with each amino acid coming off the column at a unique retention time. Data acquisition was performed by MassLynx V4.1 2011 software (Waters, Milford, USA). The peak areas and retention times were used to plot calibration curves and subsequent quantification of amino acid concentration. All analyses were performed in triplicate.

### 2.5. Mineral analysis

The mineral content of the dried GP were first exposed to microwave acid digestion and dissolution of the sample. Minerals were quantified by inductively coupled plasma-atomic emission spectrometry (ICP-AES) according to Sah and Miller (1992). All analyses were performed in triplicate.

### 2.6. Statistical analyses

The data was analyzed as a completely randomized designed with a 3 × 3 factorial arrangement of treatments using the generalized linear model procedure of SAS v.9.3 (SAS Institute Inc., Cary, NC, USA). The statistical model for used for analysis was as follows:

$$Y_{ijk} = \mu + V_i + D_j + VD_{ij} + \varepsilon_{ijk}$$

$Y_{ijk}$ = nutrient composition (*in vitro* NDF digestibility, nutrient composition), µ = overall mean, $V_i$ = effect of $i^{th}$ variety (i = Pinotage,