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Short communication

Variability in spectrophotometric pyruvate analyses for predicting onion pungency and nutraceutical value

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ABSTRACT

Onion pyruvate concentration is used as a predictor of flavor intensity and nutraceutical value. The protocol of Schwimmer and Weston (SW) (1961) is the most widespread methodology for estimating onion pyruvate. Anthon and Barret (AB) (2003) proposed modifications to this procedure. Here, we compared these spectrophotometry-based procedures for pyruvate analysis using a diverse collection of onion cultivars. The SW method always led to over-estimation of pyruvate levels in colored, but not in white onions, by up to 65%. Identification of light-absorbance interfering compounds was performed by spectrophotometry and HPLC analysis. Interference by quercetin and anthocyanins, jointly, accounted for more than 90% of the over-estimation of pyruvate. Pyruvate determinations according to AB significantly reduced absorbance interference from compounds other than pyruvate. This study provides evidence about the mechanistic basis underlying differences between the SW and AB methods for indirect assessment of onion flavor and nutraceutical value.

1. Introduction

Onion (*Allium cepa* L.) is cultivated and consumed worldwide, mainly due to its distinctive odor and taste. In addition, various health-promoting effects have been associated with the consumption of onion and other *Allium* species, such as garlic and leek. Among them, antiplatelet, antihypertensive, antioxidant, hypoglycemic, anticancer, and hypolipidemic properties have been reported (reviewed by Corzo-Martínez, Corzo, & Villamiel, 2007; and Block, 2010).

Allium flavor (pungency) constituents arise from interaction of the vacuolar enzyme alliinase with the cytoplasmic flavor precursors S-alk(en)yl-L-cysteine sulfoxides (ACSOs) after cutting or crushing of fresh tissues (Lancaster & Boland, 1990). Alliinase-mediated cleavage of the ACSOs produces volatile thiosulfinates (TSs), pyruvate and ammonia (Block, 2010). TSs are responsible for the pungency (Macpherson et al., 2005) and, together with other sulfur-compounds derived from TSs degradation (for a comprehensive review on *Allium* biochemistry see Block, 2010), they contribute greatly to most of the health-enhancing properties of *Allium* (Corzo-Martínez et al., 2007). Because pyruvate and TSs are formed stoichiometrically in the ACSOs-alliinase reaction, pyruvate content correlates positively with pungency intensity (Wall & Corgan, 1992), and is used commonly as an estimator of the total TS content (Goldman, Kopelberg, Debaene, & Schwartz, 1996). Therefore, pyruvate content is also used to predict both flavor intensity and nutraceutical value in onion and garlic.

To date, the most widespread method for estimating pyruvate levels in *Allium* has been the spectrophotometry-based protocol of Schwimmer and Weston (1961) (SW). For example, in onion, at least 56 scientific papers have used this procedure (Suppl. Table 1). Despite its general use, variation in pyruvate results among and within laboratories, using the SW technique and the same lot of onion samples, was reported in a previous study, although the observed variations were attributed to factors other than the analytical method (Havey et al., 2002). The SW method

Abbreviations: AB, Anthon and Barrett (2003); SW, Schwimmer and Weston (1961); ACSOs, S-alk(en)yl-L-cysteine sulfoxides; TSs, thiosulfinates; fw, fresh weight; CV, coefficient of variation; w/v, weight/volume; DNPH, 2,4-dititrophenylhidrazine.

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is relatively rapid and inexpensive, advantages that have led to its widespread adoption. The method determines total 2,4-dinitrophenyl-hydrazine-reacting carbonyls, resulting from the addition of excess 2,4-dinitrophenyl-hydrazine (DNPH) to pyruvate-containing aqueous onion extracts. Color development in the solution, due to the formation of chromogenic DNPH-pyruvate adducts, is measured at 420 nm.

Anthon and Barrett (2003) (AB) proposed modifications to the SW method, specifically changes in reagent concentrations and the use of 515 nm (instead of 420 nm). The authors proposed that such modifications could improve linearity and sensitivity of the assay, and eliminate interferences at 420 nm from other compounds that may be present in onion bulbs.

Given the relevance of pyruvate determinations for inferring indirectly onion flavor and functional value, it is important to compare the two methods in a consistent and systematic way. Also, the identification of interfering compounds in onion bulbs (as proposed by AB), and quantification of their relative contribution to such interferences, would shed light on the mechanistic basis for this source of variation, providing a rationale for predicting the extent of methodology-based variations that can be expected when using either method for estimating onion pyruvate levels. Thus, the objectives of the present study were to: (1) compare the SW and AB spectrophotometry-based procedures for pyruvate analysis in a diverse collection of onion cultivars over three growing seasons; and (2) investigate sources of variation due to interfering compounds and, once identified, estimate their magnitude and contribution for different onion color types. Our results highlight the importance of examining critically the method of choice for estimating pyruvate levels, which is used to predict onion flavor and putative health benefits.

2. Materials and methods

2.1. Plant materials

Eleven Argentine onion cultivars (Galmarini, 2000) were characterized for their bulb pyruvate concentration using the methods of Schwimmer and Weston (1961) (SW) and Anthon et al., (2003) (AB). Pyruvate levels were determined during three growing seasons, 2012, 2014 and 2015. Nine cultivars were grown at the experimental station of INTA La Consulta (Mendoza, Argentina), using conventional agricultural practices, whereas cultivars "Morada1" and "Morada2", two phenotypically different red onions were obtained from a local market during 2012 and 2015.

2.2. Processing of samples

Sample processing and preparation of aqueous extracts from onion bulbs were performed as previously described by Galmarini, Goldman, and Havey (2001) and Cavagnaro and Galmarini (2012). Briefly, three replicates composed of five bulbs were used. The outer dry scales were removed and the onions cut in half longitudinally. One half of each bulb was bulked and juiced in a 1:1 vol (w/v) of distilled water using a blender (Braun MR 400 Plus, Kronberg, Germany). The homogenates were then filtered, centrifuged, and the clear supernatants were stored at −20 °C.

2.3. Pyruvate content analyses

Both the methods of SW and the AB were used strictly as indicated by the authors. Briefly, for the SW method, 2 mL of diluted extracts were added to 1 mL of 0.0125% DNPH in 2 M HCl and incubated at 37 °C for 10 min before 5 mL of NaOH 0.6 mol/l was added. The absorbance at 420 nm was measured using a Beckman DU Series 500 UV/Visible spectrophotometer (Beckman Coulter Inc., Brea, USA). Pyruvate concentrations were calculated using standard curves for sodium pyruvate (Sigma ultra 99%, Sigma-Aldrich, Buenos Aires, Argentina), performed independently for each method, and expressed as μmol pyruvate/g fw.

The AB procedure included the following modifications to the SW protocol: DNPH concentration was increased from 0.0125% to 0.25%; final sample volume was reduced; the NaOH concentration, which is used to stop the DNPH-pyruvate reaction, modified from 2.5 mL of 0.6 mol/l NaOH (in SW) to 1 mL of 1.5 mol/l NaOH; and the absorbance wavelength used was 515 nm not 420 nm.

2.4. Analysis of total flavonoids

Total flavonoids content was estimated in 11 onion cultivars (Table 1) during 2012 and 2015, according to Yang, Meyers, Van der Heide, and Liu (2004). Flavonoid content was calculated using a quercetin standard curve and expressed as mg quercetin equivalents % g fw.

2.5. Light absorbance interference by compounds different from pyruvate

In order to quantify the absorbance of compounds other than pyruvate (i.e., the absorbance of onion compounds that may interfere with pyruvate determinations), aqueous extracts from three white (Refinta 20, Alfredo, Antártica), three yellow (Grano de Oro, Valcatorce, Navideña), and two red (Morada1, Morada2) cultivars were used. Five replicates per cultivar were prepared as described above. Absorbance was measured as described above but without the addition of DNPH to the reaction mixture, in order to avoid formation of yellow DNPH-pyruvate adducts. Since colorless pyruvate (i.e., pyruvate not bond to DNPH) does not absorb light at the wavelengths used by either methods, only compounds absorbing light at 420 nm (SW) and 515 nm (AB) contributed to the readings. These compounds represent a source of error in pyruvate determination. Absorbance values obtained with both methods were compared. In addition, absorption spectra (400–550 nm) for white, yellow and red onion extracts, as well as for the flavonoid quercetin, with and without the addition of NaOH (SW, 0.6 N and AB, 1.5 M), were characterized.

2.6. Identification of light-absorbance interfering compounds

To test if the interference observed in pyruvate determinations of colored onion extracts was due to flavonoid compounds, the following was performed: Extracts of a white onion cultivar (Refinta20), characterized for its very low quercetin content and no anthocyanins, were spiked with quercetin and/or anthocyanins to a final concentration equivalent to that found for these flavonoids in a yellow (Valcatorce; 41.7 mg quercentin % g fw) and a red (Morada1; 80.7 mg quercentin % g fw, 23 mg cyanidine % g fw) onion cultivars. A commercial standard of cyanidine-3 glucoside (Sigma-Aldrich, St. Louis, USA) was used for the anthocyanin assay. Absorbance was measured at 420 nm. Total anthocyanins content was measured spectrophotometrically according to Fuleki and Francis (1968). Quercetin was considered to represent ~85% and ~30% of the total flavonoids content of yellow and red onions, respectively, as indicated in a previous study (Slimestad, Fossen, & Molund, 2007).

2.7. HPLC analysis of interfering compounds

Pure standards (Sigma > 95%, Sigma-Aldrich, St. Louis, USA) for seven phenolic compounds commonly found in onion [quercetin,