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Original Research Article

Characterization of concentrated agave saps and storage effects on browning, antioxidant capacity and amino acid content

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ABSTRACT

Sap from agave plants ("aguamiel") is traditionally consumed in Mexico as a fresh beverage, fermented or concentrated. Concentrated agave sap (CAS) is used as a sweetener but, due to heating, a brown color develops and intensifies during storage. Browning varies among CAS batches and this work was focused on its correlation with the chemical composition changes observed during 20 weeks of storage. The browning index (BI), measured as the optical density at 490 nm ($OD_{490\ nm}$) per gram of sample, increased 54.4% in the batch that initially had 57 $OD_{490\ nm}$/g but in the other two batches that had a lower BI, the increase was less than 26.1%. Antioxidant capacity only increased in the batch with the highest BI going from 18 to 23 Trolox equivalent μmol/g dry weight. Saponin content was different in the three batches (224.2–434.7 protodioscin equivalents/gram dry weight) but did not change after 20 weeks of storage. Browning index and antioxidant capacity were negatively correlated with free amino acid concentration, particularly serine, phenylalanine and lysine decreased 29.4, 50 and 30%, respectively. Browning was positively correlated to furosine, an early Maillard reaction derivative of lysine previously reported as a free radical scavenger.

1. Introduction

Agave (*Agave* spp.) is a genus of monocotyledon, monocarpic plants, distributed throughout the American continent (Ortiz-Basurto et al., 2008) and among the 293 species, 75% may be found in Mexico (Good-Avila et al., 2006). This plant can be used as a raw material for the manufacture of traditional beverages, such as tequila and mezcal (Narváez-Zapata and Sánchez-Teyer, 2009). It is also considered as a source of functional food ingredients, such as prebiotic fructans, and as a medicinal plant. Agave leaves and

Abbreviations: ACE, acetonic extract; ACN, acetonitrile; AOXC, antioxidant capacity; ASX, asparagine/aspartic acid; AUC, area under curve; B1, batch 1; B2, batch 2; B3, batch 3; CAS, concentrated agave sap; DDMP, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; dw, dry weight; EI, electron impact ionization; ESI, electrospray ionization; fw, fresh weight; GLX, glutamine/glutamic acid; HMF, 5-hydroxymethylfurfural; LYS, lysine; OD, optical density; ORAC, oxygen raidcal absorbance capacity; PHE, phenylalanine; PRO, proline; SER, serine; TE, Trolox equivalents; TP, total phenols; TSS, total soluble solids; u, units.

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stems contain bioactive metabolites conferring a diversity of effects on health, such as anticancer, anti-inflammatory and immunomodulatory (Santos-Zea et al., 2012). Saponins, in particular, have been previously reported in fresh agave sap (Leal-Díaz et al., 2015), and these compounds have shown pharmacological properties, such as anti-inflammatory and ulceroprotective effects (Mina et al., 2014).

The plant sap, called "aguamiel" in Mexico, is a liquid composed mainly of sugars, including fructo-oligosaccharides (Ortiz-Basurto et al., 2008). This fluid is harvested exclusively from certain agave species known as "agaves pulqueros", including *Agave americana*, *A. atrovirens*, *A. ferox*, *A. mapisaga* and *A. salmiana* (Escalante et al., 2008). When the plant reaches maturity (8–12 years), a cavity is made in the center by cutting the floral stem, where sap is manually collected twice a day (Gutierrez-Uribe and Serna-Salvdivar, 2013). It may be directly consumed as a beverage but due to its composition and the presence of endophytic microorganisms, it is not suitable for long-term storage. In fact, more than 300 endophytic bacteria strains have been isolated from *Agave tequilana* leaf base (Martínez-Rodríguez et al., 2015). Agave sap is also traditionally fermented to produce a mild ethanolic drink named "pulque" (Narváez-Zapata and Sánchez-Teyer, 2009) or submitted to a thermal process to yield a product denominated concentrated agave sap (CAS) (Gutierrez-Uribe and Serna-Salvdivar, 2013). CAS production is an artisanal process and we consider that the information included in this manuscript would be very helpful to understand the importance of setting time and temperature set points and study the agave sap characteristics that must be analyzed for its use as raw material.

As in other foods with a high concentration of sugars and phytochemicals, including honey, agave sap concentrate may show changes in the concentration or transformation of antioxidants and other phytochemicals with beneficial effects on health. Moreover, a relationship between antioxidant capacity and a diversity of compounds generated or released during honey storage has been demonstrated (Brudzynski and Miotto, 2011). Browning due to formation of Maillard compounds, deteriorative reactions and complexing of polyphenols with amino acids and proteins has been observed in honey. Citrus honey physicochemical and organoleptic properties changed during one-year storage because of the loss of volatile compounds and sugars and Maillard derivatives formation (Castro-Vázquez et al., 2008). Maillard reaction is known to occur during storage in products containing sugar and free amino acids, such as honey, juice concentrates and jams (Aslanova et al., 2010; Bulut and Kilic, 2009; Selen Burdurlu and Karadeniz, 2003). Some of the markers used to analyze this complex system of reactions in food products include furosine, carboxymethyllysine and hydroxymethylfurfural (Bastos and Gugliucci, 2015).

The current study was carried out to determine the effect of storage on the main characteristics of concentrated agave sap. Physicochemical parameters (water activity, dry matter, pH, soluble solids, browning) and chemical composition (content of sugars, protein, amino acids, saponins, phenolic acids, Maillard reaction products and antioxidant capacity) were evaluated over the course of 20 week storage. Correlations among the analyzed parameters were established.

2. Materials and methods

2.1. Concentrated agave sap preparation and storage conditions

Three different batches of concentrated agave sap (CAS), elaborated by artisanal means, were obtained from a commercial supplier (AGMEL, Monterrey, Mexico). Each batch was produced as follows: agave sap (aguamiel) was collected from mature agaves (over 7 years of age) from the species *Agave salmiana* by traditional means. It was then pooled and concentrated to nearly 10% of its original volume by heating in a stove until boiling point was reached (Gutierrez-Uribe and Serna-Salvdivar, 2013). The brown-colored syrup obtained by this process was then stored in plastic containers until delivery to the laboratory. First batch (B1) was produced in the state of Hidalgo, Mexico on July 2011. Second (B2) and third (B3) batches were acquired from the same region on November and December 2013, respectively.

The storage study started 14 days after CAS was produced. Each batch was separated into 50 mL aliquots and stored at 25 ± 2 °C and 85% relative humidity in darkness. Every two weeks, an aliquot was collected until 20 weeks were completed. Aliquots were stored at −20 °C until analysis.

2.2. Physicochemical tests

Dry matter was determined by gravimetric means (AOAC 425.45) drying at 60 °C in vacuum. Liquid sample (2 g) was mixed with 0.5 g dry silicon dioxide (Celite 545, Desarrollo de Especialidades Químicas, Monterrey, Mexico) and left to dry during 24 h. Weight was recorded before and after drying to obtain dry matter percentage. Water activity was measured using the instrument Aqualab with dew point sensor (4TEV, Decagon Devices, Pullman, WA, USA) at 25 °C, pH with a digital pH meter (Mettler-Toledo AG, Schwerzenbach, Switzerland) and total soluble solids (TSS) with a manual refractometer in scale 58–90 °Brix (ATAGO, Tokyo, Japan). Browning index was determined according to a procedure used to evaluate color for tequila "cooking honey" (Waleckx et al., 2008), with some modifications. Concentrated agave sap was diluted 50-fold (w/v) with distilled water, vortexed and 200 μL were plated in 96-well microplates. Optical density at 490 nm (OD_{490}) was recorded in a microplate reader (Synergy HT, Bio-Tek, Winooski, VT, USA). Results were reported as OD_{490} per gram of CAS fresh weight (OD_{490}/g fw). Protein content was determined according the AOAC 978.02 micro Kjeldahl standardized method and results were reported as % dry weight (% dw), using conversion factor $N \times 6.25$.

2.3. Sugars and free amino acid determination

To quantitate sugars and amino acids, CAS was diluted 10-fold (~7 °Brix) with distilled water, and centrifuged at 13,800 *g* to eliminate suspended matter (Centrifuge 5804 R, Eppendorf, Hamburg, Germany). The supernatant was then filtered through 0.22 μm nylon membranes (VWR International LLC, Radnor, PA, USA) and kept at −20 °C until determination was carried out.

Sugar concentrations were quantitated using liquid chromatography with evaporative light scattering (HPLC-ELSD) detection (Agilent Technologies, 1200 Series, Santa Clara, CA, USA) according to application XBridge Amide WA64101 (Waters Corporation, Milford, MA, USA). Separation was carried out in XBridge Amide column, particle size 3.5 μm, 250 mm × 4.6 mm i.d. (Waters Corporation, Milford, MA, USA) with mobile phase A: 80% acetonitrile (ACN) (BDH, Poole, UK) and 20% water (BDH, Poole, UK) with 0.2% triethylamine (Sigma–Aldrich, St. Louis, MO, USA) (v/v/v) and B: 30% ACN and 70% water with 0.2% triethylamine (v/v/v) at a flow rate of 1 mL/min, using the following gradient: 0→16 min, 10→70% B; 16→17 min, 70→10% B; 17→30 min, isocratic elution at 10% B. Conditions for ELSD were: gain 2, pressure 2 bar, temperature 50 °C, filter: 0.5 s and sampling time 100–10 Hz. D-Glucose (Sigma–Aldrich, St. Louis, MO, USA), D-fructose (Merck KgaA, Darmstadt, Germany), and sucrose (Fluka, St. Louis, MO, USA) were used as standards, in a range from 0.5 to 10 mg/mL for each sugar. Before injection (10 μL), samples were further diluted with HPLC grade water to ~1 °Brix. Results were reported as % dw.