The utilisation of two detectors for the determination of water in honey using headspace gas chromatography

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A B S T R A C T

A headspace gas chromatography (HSGC) method was developed for the determination of water content in honey. This method was shown to work with five different honey varieties which had a range of water from 14–16%. It also utilised two different detectors, the thermal conductivity detector (TCD) and the barrier discharge ionisation detector (BID). This method needs no heating pretreatment step as in the current leading method, (i.e. the measurement of refractive index). The solvent-free procedure negates the possibility of solvent–compound interactions as well as solubility limitations, as is common with Karl Fischer titrations. It was also apparent that the classic loss on drying method consistently and substantially produced results that were lower than the correct values. This approach is shown to be rapid, with an analysis time of 4 min when using the TCD detector and under 3 min when utilising the BID detector. HSGC is feasible for the determination of water due to the new PEG-linked geminal dicationic ionic-liquid-coated GC capillary column. In addition it provides accurate and precise determinations of the water content in honey. When using the sensitive BID detector, other trace volatile compounds are observed as well.

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1. Introduction

Honey is a natural, viscous, stable sweetener, consisting mainly of fructose, glucose and water. Since honey is a saturated sugar solution it is able to absorb moisture from the atmosphere (Gallina, Stocco, & Mutinelli, 2010). The water content in honey is influenced by a number of factors, including botanical origin, atmospheric conditions and seasonal variations (Gallina et al., 2010; Gergen, Radu, Bordean, & Isengard, 2006; Leach, 1903; Ruoff, 2006). In addition to natural variations, assorted human modifications (e.g., treatments, production and storage conditions) also affect the water content (Gallina et al., 2010). The content of water affects the quality, marketability, and physical properties of the honey. The moisture content of honey must not exceed 21%; however, it should remain above 14% water (Gallina et al., 2010; Molan, 1992). When the water content is below 14%, the viscosity is increased and crystalline entities appear. However, if the water content exceeds 21%, it can support microbial growth. In these cases the contaminated honey can cause severe illness if consumed by humans (Gallina et al., 2010; Ruoff, 2006; Sanchez, Baeza, Ciappini, Zamora, & Chirife, 2010).

The water content of honey has traditionally been analyzed in one of three ways, refractive index (RI) measurement, gravimetric determination of water loss after drying (LOD), or Karl Fischer titration (KFT). Refractive index measurement is the most common method for water determination in honey. While this is simple, fast and reproducible, there are some problems with this approach (Sanchez et al., 2010). The procedure requires a thermal pretreatment step for the honey sample, which leads to some loss of water content and therefore inaccurate results (Gallina et al., 2010). In order for the refractive index to be used as a method for water determination a relative conversion table has to be utilised as well. The conversion table however may not be accurate for all types of honey, due to significant variations in the ratio of different sugars and other minor components (Sanchez et al., 2010). These differences in composition are known to affect the refractive index, thereby decreasing the accuracy of the method (Gallina et al., 2010; White, 1992).

LOD is not used nearly as often, due to difficulties with this method (Isengard, Schultheiß, Radović, & Anklam, 2001; Sanchez et al., 2010). After heating, a highly viscous product is formed, leading to slow diffusion of water, and tightly bound water, which is difficult to vaporise. This approach usually produces numbers that are lower than the true water content of the samples (Isengard & Präger, 2003). Also, this method is time-consuming and labour-intensive. Additionally, honey can have other volatile components which can vaporise, leading to errors in the estimation of water content (Isengard et al., 2001; Sanchez et al., 2010).

More recently, attempts have been made to adapt Karl Fischer titration (KFT) for determining the water content in honey. While KFT has better reproducibility, compared to LOD or RI, it does have an increased cost in solvents and can be time consuming (Sanchez et al., 2010). Furthermore, honey has limited solubility in the KFT medium (typically methanol solutions). In order to overcome this problem, formamide and methanol are combined with the working solvent, and the titration cell is heated. This method is known to have poor laboratory-to-laboratory reproducibility, making it a less than ideal technique.

The water content in a few select foods has been determined with a fourth method, headspace gas chromatography (HSGC). In this method the samples are first dissolved in methylglycol (i.e., 2-methoxyethanol). Since few samples can be completely dissolved suspensions are usually obtained. It is a labour-intensive method because of the need for standard addition, multiple standards and multiple headspace extractions (Kolb & Auer, 1990). GC has been utilised in the past with packed columns in order to measure water, however there were numerous problems (e.g., broad tailing peaks, peak overlap, irreproducibility) (Knight & Weiss, 1962; Quiram, 1963). In addition many of these columns degraded in the presence of water. In 1999, a new class of open tubular column stationary phases was introduced, which consisted of ionic liquids (Armstrong, He, & Liu, 1999; Breitbach & Armstrong, 2008; Huang, Han, Zhang, & Armstrong, 2007; Payagala, Sidisky, & Armstrong 2009; Payagala et al., 2009). These stationary phases are stable in the presence of water and oxygen. Further, water is easily separated from other solvents and volatile substances as a relatively efficient, symmetrical peak. The water peak had improved peak area reproducibility due to the narrowing of the peak and improved peak symmetry (Jayawardhana, Woods, Zhang & Armstrong, 2011). Consequently water analysis using capillary GC became feasible. Recently, this approach was used to measure the water content of active pharmaceutical ingredients and the, water/ethanol content of various consumer products (Frink, Weatherly, & Armstrong, 2014; Weatherly, Woods, & Armstrong, 2014).

In this work, we developed a simple HSGC method for the determination of the water content in honey. Since this method directly quantifies water, it does not require a conversion table, is not impacted by solid particles, has no preheating treatment, does not have any solubility issues and does not require multiple extractions. In addition, the method is fast and straightforward.

2. Materials and methods

2.1. Materials

Fructose was obtained from Sigma–Aldrich (St. Louis, MO). Buckwheat blossom honey was purchased from Dutch Gold (Littleton, NH). Organic white raw honey was obtained from Whole Foods Market (Austin, TX). Wild flower honey was acquired from Madhava Natural Sweeteners (Longmont, CO). Mandarin blossom honey was bought from Rigoni di Asiago (Miami, FL). Raw honey was obtained from Mountain Gold Honey (Ogden, UT).

The 15 × 45 mm, 1-dram vials were purchased from Fisher Scientific (Waltham, MA). The screw-on moulded plastic covers were obtained from SKC Inc. (Eighty Four, PA). White silicone/TFE septa were obtained from Sigma–Aldrich. The 22 × 75 mm screw-thread vials and the magnetic screw-thread covers for the autosampler were purchased from Restek (Bellefonte, PA).

2.2. Apparatus and conditions

All manual injections were performed utilising a 6890 N gas chromatograph (Agilent Technologies Inc., Wilmington, DE) with thermal conductivity detector (TCD). The 6890 N GC-TCD was equipped with Chemstation Plus software (Rev.B.01.03). The oven temperature was held isothermally at 110 °C while a split ratio of 10:1 was used. The injection port and detector were set to 280 °C and 250 °C, respectively. Helium at 1 mL/min was used for all runs. A typical analysis was completed in 7 min. A 1-mL gas tight syringe (SGE Analytical Science, Melbourne, Australia) was used for all manual injections. A Tracera GC-2010 Plus gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a barrier ionisation discharge detector (BID), LabSolutions (version 5.71 SP1) and an AOC-5000 Plus autosampler (Shimadzu Scientific Instruments) was used for all automated sampling. The oven was kept isothermally at 110 °C with a split ratio of 100:1. The injection port was set at 280 °C and the detector was set at 200 °C. A 2.5-mL headspace HD-type syringe (Hamilton, Reno, NV) was used for all automated injections. The analysis was completed in 5 min. A 30 m × 0.25 mm ID × 0.2 μm film coat thickness SLB-IL107 fused silica capillary column (Huang et al., 2007) is now commercially available as the Watercol™ 1910 column from Supelco (Bellefonte, PA). All samples were measured on an AR1140 Adventurer balance (Ohaus Corp., Pine Brook, NJ).

The refractive index samples were heated to 50 °C. Once the samples were heated the honey was added to the measuring cell kept at 20 °C without any air bubbles (Isengard & Schultheiß, 2003). The dry material (DM) was determined by using a formula DM = 78 + 390.7 (RI − 1.4768) (Auerbach & Borries, 1924). The water content was then determined by subtracting the DM from 100 (Auerbach & Borries, 1924). The samples were measured in triplicate and the percent water was calculated from a conversion table.

The gravimetric determination after drying was performed by first weighing 0.5 g of honey into a vial and the sample was heated at 70 °C for 24 h. The sample was then cooled and reweighed. It was then heated again for 2 h to assure a constant mass was achieved (Herrick, 1995).

2.3. Sample preparation

The samples analysed on the GC-TCD were prepared by adding 400 mg of the honey to the vial. All weights were recorded to 0.1 mg using an analytical balance. The vials were then purged with dry argon for 2 min using a 20 G 1½″ needle and were immediately capped with the moulded plastic covers containing two white silicone/PTFE septa. The capped samples were then purged again using a smaller 25 G, 5/8″ long needle with dry argon for 15 s, while a second 25 G, 5/8″ long needle was inserted into the septum. The two purging needles were removed and the sample was heated to 55 °C for 30 min. Finally, 600 μL of headspace were manually extracted with a gas-tight syringe and injected into the GC-TCD. When honey samples were analysed using the Tracera GC-BID, 500 mg of the analyte honey were added to the vial. All weights were recorded to the nearest 0.1 mg. The vials were then purged in the same way as the vials used for manual analysis. After purging the vials, the samples were heated at 55 °C for 20 min and had 250 μL of headspace automatically injected with the autosampler (Section 2.1) into the GC-BID. The samples analysed with the GC-BID had different equilibrium times due the difference in agitation and heating. The difference in the amount of headspace vapour injected in the two different approaches is attributed to the increased sensitivity of the BID detector compared to the TCD detector.

The calibration curve for the manual injection on the GC-TCD was produced by using solutions of 0.36, 0.32, 0.28, 0.24, and 0.2 g fructose plus 0.04, 0.08, 0.12, 0.16, and 0.2 g water respectively each in 15 × 45 mm vials. The calibration curve for the analysis with the autosampler on the GC-BID was produced using solutions of 0.45, 0.4, 0.35, and 0.3 g fructose plus 0.05, 0.1, 0.15