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Chlorogenic acid oxidation-induced greening of sunflower butter cookies as a function of different sweeteners and storage conditions

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ABSTRACT

Sunflower butter use as an allergen-free alternative to tree and legume nut butter in baking is limited by chlorogenic acid induced greening that occurs at alkaline pH. Limited information is available on controlling this greening in a food matrix. This study examined how different liquid sweeteners and relative humidity influenced greening of sunflower butter cookies. Doughs had similar initial pH (7.52–7.66) which increased to 8.44–9.13 after baking as ranked: xylitol > maple syrup > corn syrup > honey > agave syrup. Cookies made with maple syrup had the highest moisture and greening corresponding with lowest free chlorogenic acid. The % greening followed the same trend as greening intensity, and was positively correlated (r = 0.9101) with chlorogenic-lysine adduct content. Our findings provide an ingredient solution to controlling greening, as results demonstrate that greening can be promoted with high relative humidity storage, and use of high moisture and pH ingredients. Unwanted greening can be inhibited by simply changing the liquid sweetener.

1. Introduction

Sunflower seed butter can act as an alternative to legume and tree nut based plant butters, which are members of the “big 8” allergens affecting an estimated 0.6–1.3 and 0.4–0.6% people in USA who suffer from peanut and tree nut allergies respectively (FDA, 2016; Peabody, 2016). Sunflower nut butter contains a higher phenolic content (1–5%) than other nut butters. Of the total phenols, 50–70% is chlorogenic acid (CGA), a substrate in both browning and greening reactions with sunflower protein during aqueous processing or under alkaline conditions (Bekedam, Schols, Van Boekel, & Smit, 2008; Weisz, Kammerer, & Carle, 2009; Yabuta, Koizumi, Namiki, Hida, & Namiki, 2001).

Use of sunflower seed butter can cause bitterness and firmness, and it is difficult to spread compared to peanut butter, which can make it less acceptable to consumers (Lima & Guraya, 2005). Due to the bitterness, sweetened versions of sunflower butter are commercially available (NPI, 2014). These sweeteners differ in pH, moisture, and phenolic content and this could affect the visual appeal of sunflower butter bakery products. For instance, the higher pH of maple syrup compared to honey could impact post-baking color reactions (Ball, 2007) such as the greening reaction in sunflower seed based products. Texture and taste are challenges the industry has overcome, but the green color remains a problem when using sunflower butter in bakery applications when greening is considered undesirable. The oxidation product of CGA dimer: *o*-quinone reacts with amino acids and side chains of proteins to form green trihydroxy benzacridine (TBA) derivatives under alkaline conditions. Yabuta et al. (2001) showed that pH influences the binding of CGA to sunflower protein. Increasing pH from 5 to 9 results in a color change from yellow to blue-green. The role of pH and moisture in polyphenoloxidase induced greening has been determined (Vaintraub & Kratch, 1989), however, the role of moisture in non-enzymatic greening reactions has not been fully investigated in a food matrix.

2. Materials and methods

2.1. Cookie formulation and experimental design

Two batches of sunflower butter cookie dough with different sweeteners were prepared separately and baked at 149 °C (300 °F). After mixing flour (39.7%), baking soda (0.6%) and salt (0.6%), egg (13%), sweeteners (21.2%), sunflower butter (24.3%) and vanilla extract (0.6%) were then added. The doughs (0.5 ± 0.2 cm thick) were cut with a 4.5 cm diameter cutter. Baking was carried out using a convection oven (JA12SL, Doyon, Inc. Saint-Côme-Linière, Canada) at 149 °C for 7 min, with the temperature monitored using a thermocouple. Three desiccator cabinets (Fisherbrand™) containing NaOH, K_2CO_3, and $(NH_4)_2SO_4$ solutions were prepared, and had RH of 75, 79, and 84% RH respectively measured using a LogTag® humidity and

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temperature recorder.

2.2. pH and °brix index of sweeteners

A xylitol solution was prepared by dissolving xylitol granules (4.4 g) in 5 ml nano-filtered water at 75 °C (88% w/v) to obtain a similar moisture content as honey. The same ratio of sweeteners in cookies were used to make sweetener-water solutions by weighing 0.1 g of the liquid sweeteners and adding 5 ml nano filtered water. Sweetener solutions (2% w/v) were vortexed for 30 s and then incubated at room temperature for 3 h on a shaker (Rocker II, 260350, Boekel Industries, Inc. PA) at a speed of 1.5*g. The pH was tested after incubation using a LabQuest 2® pH meter (Vernier Software & Technology, OR, USA). The °Brix index of 0.3 ml of each sweetener was tested using a PAL-α, ATAGO refractometer (Nova Tech International, Inc. TX, USA).

2.3. Physical tests of cookie and dough

2.3.1. pH and water activity (a_w)

Dough and cookie sample solutions (10%, w/v) were prepared with nano filtered water (Miller, Graf, & Hoseney, 1994), and incubated for 3 h on a shaker at a speed of 1.5*g. After incubation, cookie and dough samples were centrifuged (AccuSpin 1R-75003449, Thermo Fisher Scientific, Inc. CA) at 9000*g at 4 °C for 15 min and the supernatant was used for testing (AACCI, 1999a). Water activity of cookies was measured right after each color testing (Section 2.5) using a water activity meter (Model 3ET, Aqua Lab Technologies, Inc. CA, USA) according to manufacturer's instructions.

2.3.2. Moisture content and spread factor

Moisture content was determined using a vacuum oven as outlined in AOAC method 925.09 (AOAC, 2005) with modifications. Samples (3 g) were placed into pre-dried aluminum pans and put in a vacuum oven (Model 281, Thermo Fisher Scientific, Inc. CA), maintained at 60 °C and pressure of -70 kPa for 24 h. After drying, samples were cooled in a desiccator for 6 h. The weights of samples were recorded before and after drying.

The spread factor was measured following AACCI Method 10-50.05 (1999b) by randomly selecting 6 cookies (three from each batch). A vernier caliper (Mecanic Type 6911. KWB, Inc. Switzerland) was used to measure the width and thickness of cookies. The spread factor was calculated by dividing the width by the thickness of the cookies.

2.4. Protein content

Supernatants (250 µl) from section 2.3 were prepared following the DNPH assay as outlined by Hawkins, Morgan, and Davies (2009), and protein content was determined at 280 nm using a spectrophotometer (Vernier Software & Technology, OR, USA).

2.5. Color changes

Color changes were measured after 0.25, 1, 4, 7, 11, and 24 h post-baking under uncovered storage and 1, 4, and 7 days after three relative humidity/RH conditions (75, 79, and 84%). Greening changes were measured using a spectrophotometer (CM-2500d, Konica Minolta, Inc. Japan) and analyzed using SpectraMagic NX color data software. The spectrophotometer aperture size was 8 mm and the scan number was twice per sample. The illuminant was D_{65} and the radius of illumination area was 8 mm, with an observation angle of 10°. Data was collected as L^* [darkness to lightness (0–100)], a^* [greenness ($-a^*$) to redness (a^*)], and b^* [blueness ($-b^*$) to yellowness (b^*)]. Two different surface locations (upper and bottom) of whole cookie samples were placed under a 2 mm cylinder probe and L^*, a^*, and b^* was recorded. The cookie samples were then sliced to measure internal greening.

The percent internal greening of cookies was measured using an image analyzer CV-X422A and CA-H1DB VisionDatabase Ver 1.2 (Keyence America, Corp. CA, USA). Samples were placed under a camera with 2 M pixels with 16 speed color change-coupled devices (CA-HX200C) and the image was captured for analysis. The distance from the camera to cookie was 28 cm. The image size was set as 1600 × 1200, shutter speed was 1/30, and the sensitivity was 6.3. The binary luminosity was 180 for greening and 85 for browning. The radius of the cookies averaged 2.3 cm and the preset area on the image was kept at a radius of 1.5 cm. The mouse pointer was used for selecting green areas on cookies. The percent greening was calculated by dividing the green area by the whole selected cookie area (Ishak & Hudzari, 2010).

Cookie samples (0.6 g in 20 ml) were then homogenized (Multi-prep Homogenizer, PRO Scientific Inc., Oxford, CT, USA) at $1.3x10^{3*}$g for 1 min. The solutions were filtered using Double Rings® No. 102 filter papers and then through a 0.45 µm nylon filter. Green and brown color of solutions were determined using a SpectroVis® Plus spectrophotometer (Vernier Software & Technology, OR, USA) at λ_{680} and λ_{420}, respectively.

2.6. Chlorogenic acid and trihydroxy benzacridine derivatives content

Dough or cookies (0.9 g) were dissolved in 30 ml HPLC water. After homogenization (Multi-prep Homogenizer, PRO Scientific Inc., Oxford, CT, USA) at $1.3 \times 10^{3*}$g for 1 min, sample solutions were centrifuged at $9 \times 10^{3*}$g (AccuSpin 1R-75003449, Thermo Fisher Scientific, Inc. CA, USA) for 20 min at 4 °C. The supernatant was first filtered through Double Rings® No. 102 filter paper and then filtered through a 0.45 µm nylon filter for HPLC analysis. Chlorogenic acid purchased from Sigma-Aldrich (Saint Louis, MO, USA) was used to make a standard curve (0–0.060 mg/ml in HPLC water).

Chlorogenic acid quantification was carried out on an Agilent 1100 series HPLC (Agilent Technologies, Inc. Santa Clara, CA, USA) with a Phenomenex® Luna 5µ C8 (2) 100 Å (150 × 2 mm, 1.5 µm particle size) column using a modified method from Jully, Toto, & Were (2016). The UV-Vis detector was operating at 320 nm. Mobile phases were 0.1% glacial acetic acid/water (A) and 0.1% glacial acetic acid/acetonitrile (B). The gradient used was 0 min, 6.0% B; 2 min, 7.0% B; 2.5 min, 7.2% B; 3 min, 7.3% B; 4 min, 7.4% B; 5 min, 7.5% B; 5.5 min, 7.7%; 6 min, 10% B; 7 min, 6.0% B; 8 min 6.0% B at a flow rate of 0.8 ml/min with column temperature at 30 °C.

Chlorogenic acid-lysine standard was prepared by mixing 5 ml of 112 mM lysine with 5 ml of 28 mM CGA solutions. The pH of CGA-lysine solution was adjusted to 9.0 and stirred for 20 h at room temperature for greening reaction (Bongartz et al., 2016; Prigent et al., 2008). The original adduct solution was diluted 1:2, 1:4, 1:6, 1:8 and 1:10 (v:v) for quantifying CGA-lysine adducts. The CGA control solution was diluted 1:1 with HPLC water, and was also stirred for 20 h without adjusting pH. Adducts were determined using a Phenomenex® Luna 5µ C8 (2) 100 Å (150 × 2 mm, 1.5 µm particle size) column with LC/MS (LC: Ultimate 3000 series, Thermo Fisher Scientific, Inc. CA; MS: Impact II, Bruker, CA, USA) according to the method of Bongartz et al. (2016) with modification: the gradient program was 0–20 min, 2% B; 20–20.5 min, 17.7% B; 20.5–22.5 min, 100% B and 22.5–36 min 2% B. MS detection used Electrospray Ionization source (ESI) with positive polarity, while the end plate offset and capillary voltage was kept at −500 and 4500 V, respectively. The ion and collision energy were 4.0 and 25.0 eV. For ion cooler, the transfer and prepulse storage time were 220.0 and 20.0 µs, respectively. The dry gas temperature was kept at 180 °C with a flow rate of 4 L/min. The UV–Vis detector was monitored at 280, 320, and 631 nm. The mass spectra of the column eluate for positive ion ranged from m/z 50 to 1200.

2.7. Statistical analysis

The effect of liquid sweetener type, storage conditions, storage time