Greening in sunflower butter cookies as a function of egg replacers and baking temperature

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Abstract Chlorogenic acid (CGA) binding to proteins in alkaline conditions results in the production of green trihydroxy benzacradine (TBA) derivatives. The formation of TBA derivatives could decrease product quality due to the potential losses in soluble protein and antioxidants and the production of an undesirable green color. To determine how cookie formulation affected the formation of TBA derivatives in sunflower butter cookies, two egg replacers (chia and banana) and two baking temperatures (162.8 and 190.6 °C) were used. Moisture, greening intensity, CGA content and antioxidant capacity were measured. Cookies made with egg and baked at 162.8 °C had the highest moisture, internal greening intensity, and TBA derivative formation, in addition to lower CGA content and antioxidant capacity. Cookies made with banana baked at 190.6 °C produced the opposite outcome with 35, 4, and 23% less internal greening, moisture, and TBA derivatives, respectively, and 90 and 76% higher CGA and antioxidant capacity. Internal greening was positively correlated with moisture and adduct concentration, and negatively correlated with spread factor and CGA content. Moisture had a significant impact on greening, which indicates that baking temperature and cookie dough formulation can be modified to produce a less green cookie with more unreacted antioxidants and protein.

Keywords Chlorogenic acid · Greening · Oxidation · Sunflower · Trihydroxy benzacradine derivatives

Introduction

Plant-based butters are popular vegan alternatives to regular dairy butter; however, soy, peanuts, and tree nuts are all major allergens, limiting their applications in foods for those with allergies. In the United States, approximately 0.60 and 0.45% of the population have peanut and tree nut allergies, respectively (Boyce et al. 2010). In Europe, a reported 0.4 and 1.3% of the population have peanut and tree nut allergies, respectively (Nwaru et al. 2014). Due to the high number of people who cannot consume these allergenic products and the demand for vegan protein sources, there is a current need for a minimally allergenic butter product, such as sunflower butter (Radnitz et al. 2015). In addition to being vegan and minimally allergenic, other benefits of sunflower butter include a lower calorie content of 80 kcal/tablespoon, compared to other plant-based butters (ranging from 80 to 106 kcal/tablespoon), and a protein content of 3.0 g/tablespoon, surpassed only by peanut butter (3.8 g/tablespoon) and soy butter (4.0 g/tablespoon) (Gorrepati et al. 2015). Sunflower butter also has more than twice as much phosphorous, selenium, copper, and iron as peanut butter (USDA 2017). Therefore, sunflower butter can be a minimally allergenic seed butter alternative to both dairy and other plant-based butters.

Despite sunflower butter being both a health-beneficial and minimally allergenic alternative to other plant-based butters, it can negatively affect sensory qualities that can limit its application in baking. For example, if sunflower seeds are not roasted properly, the resulting butter is bitter, firm, and difficult to spread (Lima and Guraya 2005).

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While the textural qualities related to roasting can be mitigated, one unresolved concern is the green color produced by this seed butter under aqueous and alkaline conditions. Sunflower seeds have a high chlorogenic acid (CGA) content (1.9–3.1%). Under moist and alkaline conditions, CGA-quinones covalently bind with proteins to form green trihydroxy benzacradine (TBA) derivatives, which may be perceived as undesirable by consumers (Weisz et al. 2009; Wildermuth et al. 2016). Not only could this greening reaction be unappealing in foods, but it may also lead to decreases in unbound protein content and antioxidant capacity since protein and phenolic compounds are the two key reactants (Rohn 2014). Consumer acceptance of sunflower butter as a nutritious, non-allergenic substitute for other plant-based butters in baking is predicated upon a product with exceptional nutritional and sensory value in comparison to other plant-based butters. This standard cannot be met if the formation of TBA derivatives causes unfavorable changes in color, and lowers available protein, and antioxidant capacity.

Eggs and egg replacers differ in protein, moisture, and phospholipid content, which may affect the rate of greening (USDA 2017; Wu et al. 2012). Changes in baking temperature may also affect moisture content and the rate of various reactions that occur during baking (Sablani et al. 1998). While many studies have investigated the effect of pH and polyphenol oxidase on greening, few have focused on how greening is affected in a food product by changes in formulation (Liang and Were 2018). Therefore, the objective of this study was to determine how variations in sunflower butter cookie formulation (eggs vs. vegan egg replacers such as chia seeds or banana) and baking temperature (162.8 vs. 190.6 °C) would affect greening, soluble protein content, and antioxidant capacity. Once the variables that have the greatest effect on greening are identified and minimized, sunflower butter has the potential to become widely accepted by consumers as a vegan, low-allergy alternative in baking applications.

Materials and methods

Materials

The chemicals used included hydrochloric acid (37%), 0.1 M sodium hydroxide, ethanol (95%), ethyl acetate (99.5%), trichloroacetic acid (TCA), guanidine hydrochloride (99.5%), glacial acetic acid, formic acid (88%), HPLC-grade water, HPLC-grade acetic acid, and bovine serum albumin (BSA) from Fisher Scientific (Hampton, New Hampshire, USA). Chlorogenic acid, L-lysine (98%), 2,4-dinitrophenylhydrazine (DNPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic

acid), ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), and potassium persulfate (99+%) were obtained from Sigma Aldrich (St. Louis, Missouri, USA).

Experimental design

The cookie formulation was modified with two different egg replacers (chia seeds and blanched Cavendish bananas compared to egg as a control) and cookies were baked at 162.8 and 190.6 °C. Chia seeds and bananas are minimally allergenic vegan alternatives for eggs in the baking industry because chia seed mucilage acts as a stabilizer and emulsifier while banana has demonstrated good foaming capacity (Agrahar-Murugkar et al. 2016). Baking temperatures of 162.8 °C (325 °F) and 190.6 °C (375 °F) were chosen because they are within the range of commonly used baking temperatures for optimal textural characteristics and to ensure microbial safety of cookies (USDA 2004). All treatments were made in duplicate for a total of 12 batches of sunflower butter cookies. Before formulating the dough, approximately 15 eggs were whisked together in a bowl and 100 g of egg mixture was weighed out for each batch. Chia seeds were prepared by mixing 25 g of chia seeds with 75 g of water for each batch. The mixture was then allowed to sit for approximately 15 min until a thick gel formed. Slightly unripe Cavendish bananas (green approximately 6.0 cm down from the stem and 2.0 cm from the bottom) were blanched by slicing and steaming for 10 min at 60 °C (Taiwo and Adeyemi 2009) to deactivate polyphenol oxidase, which could affect greening through formation of quinones (Bongartz et al. 2016). Bananas were then tested for peroxidase activity by adding 2–3 drops of guaiacol and 2–3 drops of 0.08% H₂O₂ to 1–2 g of the blanched banana. The absence of a brick red color indicated that enzymes were denatured.

Sunflower butter cookies were made with 278 g all-purpose flour (39.6%), 170 g unsalted and unsweetened sunflower butter (24.2%), 150 g Grade A amber maple syrup (21.4%), and 4 g baking soda (0.6%) as constant variables and 100 g blanched Cavendish bananas, 100 g whole mixed grade A eggs, or 100 g chia seed and water mixture (14.2%) as independent variables. Cookie dough was hand-mixed, rolled out with a rolling pin, and then cut into round cookies with a height of 0.5 cm and a diameter of 4 cm. Cookies were baked at 162.8 or 190.6 °C in a Doyon, JA12SL convection oven for 7 min. The cookies were then stored in plastic Ziploc® bags for 24 h at room temperature. After 24 h, the cookies were stored in a – 80 °C freezer until chemical analysis. Two cookies were tested from each treatment replicate to ensure repeatability for all assays.

pH

Higher pH can increase greening in sunflower butter cookies. Therefore, the pH of the supernatant obtained after homogenizing 3.0 g of dough or cookie in 10 mL of DI water for 1 min was measured as outlined by Liang and Were (2018).

Moisture and protein content

Moisture content was measured according to Liang and Were (2018). Each sample (3.0 g) was placed in a disposable aluminum pan, which was then placed into a vacuum oven (Fisher Isotemp™ Model 281) at 60 °C with a pressure of −70 kPa for 24 h. The samples were then removed from the oven and cooled to room temperature in a desiccator.

The same homogenates prepared for measuring pH were used to determine changes in soluble protein content, which could be affected by the greening and browning reactions, protein-oxidation, and formation of lipid-oxidation protein adducts (Hawkins et al. 2009). For each treatment, sample homogenate (250 µL) and 2.5 M HCl (250 µL) were mixed in a microcentrifuge tube. The samples were vortexed every 5 min for a total of 15 min. TCA (125 µL, 50% w/v) was then added to each tube. The samples were incubated at −20 °C for 15 min and then centrifuged at 4 °C for 15 min at 9000×g (Fisher, accuSpin™ Micro 17R Microcentrifuge). After removing the supernatant, the protein pellet was dissolved in 1 mL of 6 M guanidine-HCl. Once the protein pellet was fully dissolved, the absorbance of the solution was measured at 280 nm. A solution of BSA in 6 M guanidine-HCl (0–1 mg/mL) was used as a standard.

Spread factor, hunter color lab, and image analysis

Three cookies were used to measure spread factor as outlined by Liang and Were (2018). Cookies were cut in half to measure the internal color. A Hunter Lab colorimeter (CM-2500D, Konica Minolta, Tokyo, Japan) with a D65 illumination, 10° standard observer, and 8-mm view aperture equipped with SpectraMagic NX color data software was used to measure the L^* (lightness and darkness), a^* (redness and greenness), and b^* (yellowness and blueness) of the cookies 0.25, 0.75, 1.5, 4, and 6 h after baking (Liang and Were 2018).

The percentage of surface greening on sunflower butter cookies was measured using an image analyzer (CV-X422A Vision System, Keyence, Itasca, IL, USA) equipped with CA-H1D8 Vision Database Ver1.2 analysis software as outlined by Liang and Were (2018).

Chlorogenic acid

Chlorogenic acid standards at concentrations ranging from 0 to 0.24 mg/mL were used to quantify CGA in 0.3 g of cookie/10 mL of HPLC-grade water (same solution used for pH measurements) as outlined by Liang and Were (2018).

Trihydroxy benzacradine derivatives

LC–MS was used to detect the presence of TBA derivatives in sunflower butter cookie samples according to the method of Bongartz et al. (2016) with modifications. An Ultimate3000 HPLC instrument (Dionex, Sunnyvale, CA, USA) equipped with an autosampler and diode array detector was used. The same samples and column used for CGA quantification were used for LC–MS. The mobile phase consisted of 0.1% formic acid in HPLC-grade water (A) and 0.1% formic acid in HPLC-grade acetonitrile (B). The flow rate was 0.4 mL/min and a wavelength of 320 nm was used. The gradient used was increased from 2 to 17.7% B over 20 min, 17.7 to 100% B for 0.5 min, remained at 100% B for 2 min, before decreasing it from 100 to 2% B for 9.5 min. A mass spectrometer (Bruker Impact II™ UHR-QqTof, Bruker, Billerica, MA, USA) equipped with an electrospray interface was used for MS. The MS parameters were as follows: end plate offset, 500 V; capillary voltage, 4500 V, nebulizer, 5.8 psi; dry gas, 4.0 L/min; dry temperature, 150 °C. Positive ion mass spectra of the column eluate were recorded in the range of 50–1300 m/z.

TBA derivatives were quantified by running a visible spectrum scan using a flat-bottom Falcon 96 microplate and a FLUOstar Omega Microplate Reader (BMG Labtech, Cary, NC, USA). The supernatants of the homogenized samples were compared against a CGA-lysine adduct standard, which was prepared as described by Bongartz et al. (2016). The λ_{max} of the standard (680 nm) was used to quantify the green pigmented CGA-lysine adducts in the samples.

Schiff bases

The same samples used for HPLC analysis were placed in a 4-mL quartz cuvette and Schiff bases formed by the Maillard reaction were measured at 294 nm (Benjakul et al. 2005) using an Ocean Optics™ Red Tide UV–Vis spectrophotometer (Vernier Software & Technology, Beaverton, OR, USA) equipped with Logger Pro 3.8.5 software.

Fluorescence of advanced Maillard reaction products and soluble tryptophan (FAST)

Fluorescence of advanced Maillard reaction products (AMPs) and soluble tryptophan (FAST) was measured periodically over a week using the method of Birlouez-

Aragon et al. (2002). The homogenized sample used for HPLC (0.5 mL) was diluted with 2.5 mL DI water and acidified to a pH of 4.6. Excitation and emission wavelengths of 330 and 420 nm, respectively were used for monitoring AMPs. Soluble tryptophan fluorescence was measured at an excitation of 290 nm and an emission of 340 nm. The data was presented as a ratio of AMP fluorescence to tryptophan fluorescence.

Carbonyl content

The same homogenates used for HPLC analysis were used to measure carbonyls as described by Hawkins et al. (2009). The sample preparation was the same as the method described for protein content, however, instead of adding 250 μ L of 2.5 M HCl to the microcentrifuge tube, 250 μ L of 10 mM DNPH in 2.5 M HCl was added. In addition, after centrifuging, the supernatant was removed and the protein pellet was washed three times with ice-cold ethanol: ethyl acetate (1:1). Equation 1 was used to calculate the carbonyl content measured at 370 nm using a FLUOstar Omega Microplate Reader.

$$\text{carbonyl concentration} = \frac{\text{absorbance at 370 nm}}{22,000 \text{ M}^{-1} \text{ cm}^{-1}} \quad (1)$$

Trolox equivalent antioxidant capacity (TEAC)

TEAC in sunflower butter cookies was measured as described by Zhang et al. (2014) with modifications. The stock radical solution was prepared by reacting 7.0 mM of ABTS with 2.45 mM potassium persulfate. The solution was allowed to react in the dark at room temperature for 24 h. The working radical solution was then prepared by diluting the stock radical solution with DI water to an absorbance of 0.7 ± 0.05 at 734 nm. Ground cookies were dispersed in 30% ethanol for a final concentration of 100 mg/mL. Samples were vortexed every 10 min for 30 min and then centrifuged at $3300 \times g$ for 30 min (Fisher, accuSpinTM Micro 17R Microcentrifuge). The supernatant (100 μ L) was combined with 3.8 mL of ABTS radical solution. Then, 100 μ L of the combined solution was pipetted into a flat-bottom Falcon[®] 96 microplate and tested using a FLUOstar Omega Microplate Reader at 734 nm. The Trolox standard was prepared in the same way as the samples with concentrations ranging from 0.1 to 1.0 mM.

Statistical analysis

It was hypothesized that egg replacers and baking temperature would significantly impact greening, antioxidant capacity, and soluble protein due to changes in moisture

content. Two-way ANOVA models were used to determine the effect of egg replacers and baking temperature on moisture content, protein content, spread factor, CGA content, and antioxidant capacity. A linear mixed effects model was used to determine the effect of egg replacers, baking temperature, and time on greening. Tukey's HSD was used to determine which pairs of treatment groups were significantly different ($P \leq 0.05$) if ANOVA showed a significant difference. Correlation coefficients were then determined between moisture, spread factor, internal greening intensity, CGA content, and adduct concentration.

Results and discussion

pH

The dough had significantly lower pH (6.79 ± 0.09) than cookies (7.52 ± 0.04). During baking, baking soda produces CO_2 and Na_2CO_3 . The presence of Na_2CO_3 in the cookies caused the pH to rise in comparison to the dough (Czernohorsky and Hooker 2005). There was, however, no significant difference between the pH of cookies under different baking treatments (Fig. 1). It was important for pH to remain constant for all cookie treatments in this study because pH is positively correlated with greening (Liang and Were 2018). Since pH was similar amongst all cookie treatments, pH was eliminated as a factor affecting greening, which allowed for the effect of egg replacers and baking temperature on greening to be observed.

Moisture and protein content

Two-way ANOVA indicated that temperature ($P = 3.8 \times 10^{-5}$), egg replacers ($P = 1.4 \times 10^{-7}$), and their two-way interactions ($P = 1.3 \times 10^{-4}$) all had significant effects on the moisture content in cookies. Raw bananas had a starting moisture content of $73.1 \pm 1.0\%$; however, the bananas gained 3.3% moisture during blanching from the steam in the surrounding atmosphere. Blanched bananas had the highest moisture content ($76.4 \pm 0.5\%$), followed by hydrated chia seeds ($75.7 \pm 1.0\%$), and finally raw egg ($69.6 \pm 2.6\%$) prior to adding them to the dough. Despite blanched bananas having the highest moisture content and raw eggs having the lowest moisture content, cookies made with egg contained the highest moisture content and those made with banana contained the lowest moisture content (Fig. 1). The cookies made with egg had the highest moisture content due to egg phospholipids' emulsification capacity. Egg yolk contains 3.0% lecithin, which traps moisture into the cookie during baking, while some cookies made with banana cracked, allowing water vapor to escape (Wu et al.

	Egg	Chia	Banana	Egg	Chia	Banana
	162.8 °C			190.6 °C		
pH	7.53±0.02	7.47±0.08	7.57±0.02	7.56±0.01	7.51±0.08	7.50±0.08
Moisture (%)	17.71±0.41	16.13±0.58	13.31±0.80	14.65±0.78	14.85±0.43	13.62±0.51
Spread factor 0.25 h	2.38±0.08	4.50±0.30	4.84±0.08	2.49±0.06	4.07±0.02	4.70±0.00
a						
b						
6 h						
24 h						

Fig. 1 Physical characteristics of sunflower butter cookies as a function of temperature, egg replacers, and time. ^a % surface greening, ^b internal greening intensity

2012; Czernohorsky and Hooker 2005; Azizi et al. 2003). The cookies made with banana solely relied on sunflower butter emulsifiers, which may not have been as effective as egg phospholipids in trapping moisture. Additionally, cookies baked at 162.8 °C had a higher moisture content than cookies baked at 190.6 °C because heat during baking causes moisture to evaporate (Sablani et al. 1998).

Two-way ANOVA indicated that only egg replacers had a significant effect ($P = 1.4 \times 10^{-3}$) on protein content. Cookies made with egg and banana had the highest and lowest soluble protein content, respectively (Fig. 2). The differences in protein content were related to the higher

protein content inherent in raw eggs (12.6%) compared to hydrated chia seeds (4.1%) and bananas (1.1%) (USDA 2017). Cookies baked at 162.8 °C had less soluble protein than dough or cookies baked at 190.6 °C because protein reactivity with CGA, reducing sugars, and lipids was increased (Friedman 1996). Cookies baked at 162.8 °C resulted in a lower protein content than cookies baked at 190.6 °C because cookies baked at 162.8 °C had a 0.4–12% higher moisture content than cookies baked at 190.6 °C, resulting in increased greening and browning reactions (Fig. 1) from greater mobility of the reactants (Liang and Were 2018).

Fig. 2 Protein content of sunflower butter dough and cookies

The formation of green and brown reaction products, lipid-oxidation protein adducts, and protein-oxidation products lowers soluble protein content. When proteins are bound or oxidized, the absorbance of aromatic tryptophan and tyrosine at 280 nm decreases (Hawkins et al. 2009). It is important to note that, although phenolic compounds also absorb at 280 nm, they did not interfere with this assay because the protein was precipitated out using TCA, while the phenolic compounds remained in the supernatant (Rajalingam et al. 2009).

Spread factor

No significant difference in spread factor due to a 27.8 °C variation in baking temperature was observed. However, egg replacers had a significant impact on spread factor ($P = 6.7 \times 10^{-7}$). Cookies made with egg had a significantly lower spread factor than cookies made with chia or banana (Fig. 1). Eggs' emulsifying abilities prevent collapse during baking more than banana or chia seeds due to the lecithin in egg yolks lining the outside of air bubbles and acting as a binder (Czernohorsky and Hooker 2005). The lecithin in sunflower seeds may not have been enough to significantly increase the volume in sunflower butter cookies made with chia seeds or bananas (Wu et al. 2012). However, an additional 3.0% lecithin in egg yolks created a significantly greater volume in cookies made with egg than in the other egg replacer treatments.

Hunter color lab and image analysis

Temperature, egg replacers, and time all had a significant effect on internal green and blue color with egg replacers and time ($P < 2 \times 10^{-16}$) having a larger effect than temperature ($P = 7.13 \times 10^{-6}$ for greenness and $P = 4.01 \times 10^{-15}$ for blueness). Cookies made with

banana and baked at 190.6 °C underwent the least greening (a^* value of -7.52 after 6 h) because they contained 1.1–4.1% less moisture than all other cookie treatments except for cookies made with banana baked at 162.8 °C (Fig. 3). Cookies made with egg and baked at 162.8 °C had the highest moisture content due to their low spread factor so they had the most greening (a^* value of -11.53). Internal greening had a high positive correlation with moisture ($r = 0.88$) and a moderate negative correlation with the spread factor ($r = -0.78$). These correlations indicate that as spread factor decreased, moisture content and internal greening increased. Because the pigments were somewhat blue-green (Fig. 1), the same trend for greening was observed for blueness of cookies (Fig. 3).

Like the internal green and blue color intensity, temperature, egg replacers, and time all significantly affected the percentage of surface greening ($P < 2e-16$). Cookies made with banana and baked at 190.6 °C had the lowest percentage of surface greening after 6 h (Fig. 3), which was attributed to their low moisture content (Fig. 1). While cookies made with egg and baked at 162.8 °C had the highest moisture content and highest internal green and blue color, these cookies did not have the highest percentage of surface greening after 6 h. At 162.8 °C, cookies made with egg had 26.8% less surface greening after 6 h than cookies made with chia seeds (Fig. 3) attributed to the lower spread factor of cookies made with egg. Because cookies made with chia had a significantly higher spread factor than the cookies made with egg, cookies made with chia had more surface area than the cookies made with egg. Even though cookies made with chia seeds had less moisture than cookies made with egg, this difference in surface area accounted for the resulting higher percentage of surface greening of cookies made with chia compared to cookies made with egg. In addition, individual cookies of the same treatment varied in surface area and texture from ridges, holes, or slight differences in spread factor. Cookie shape variability led to possible areas within the cookie matrix that were more hygroscopic due to variations in moisture content (Romani et al. 2016). Regions of the cookie that were moister had more greening than in dry areas, leading to large standard deviations in cookie surface greening that could not be minimized despite the repeated runs conducted.

Chlorogenic acid

Free CGA content was inversely proportional to cookie greening intensity ($r = -0.73$). In addition, two-way ANOVA determined that both egg replacers and baking temperature had a significant effect on CGA content in both dough and cookies with baking temperature ($P = 1.1 \times 10^{-7}$) having a greater effect than egg

Fig. 3 Changes in the internal green color ($-a^*$) (a), the internal blue color ($-b^*$) (b), and the percentage of surface greening (c) of cookies as a function of time, temperature, and egg replacer

replacers ($P = 9.4 \times 10^{-3}$) due to the significant decreases in CGA content after baking. Dough samples, which were not yet green, had significantly higher soluble CGA than corresponding cookie treatments because baking increases formation of highly reactive *o*-quinones (Liang and Were 2018). As *o*-quinone formation increased, free CGA content decreased and greening increased. Cookies made with egg baked at 162.8 °C had the lowest free CGA content (0.034 mg/g) because the high moisture content created a favorable aqueous environment for TBA derivative formation (Liang and Were 2018). Cookies made with banana also had significantly less moisture than cookies made with egg or chia so less greening occurred while higher free CGA was retained. Cookies made with banana had 65.1–88.3% more CGA than cookies made with egg and 73.1–77.6% more CGA than cookies made with chia (Fig. 4). Cookies made with egg baked at 190.6 °C had up to 72.0% more free CGA than cookies made with egg baked at 162.8 °C because cookies baked at higher temperatures had less moisture and therefore less greening.

Trihydroxy benzacradine derivatives

The most prominent peak found using LC–MS was the CGA-lysine adduct at m/z 700. MS² of the m/z 700 peak gave a fragment at m/z 526 confirming that this was the CGA-lysine adduct as the m/z 526 fragment was the mass of the precursor ion without a quinic acid (Bongartz et al. 2016). In addition, adduct polymers were found in cookie samples. Peaks with differences of m/z 44 were found ranging from m/z 300–1300; m/z 44 is the mass of the carboxyl groups on proteins, which were used to cross-link adducts (Rohn 2014).

The visible scans of the CGA-lysine adduct standards and samples had a peak absorbance at 680 nm. Cookies made with egg and banana had the highest and lowest absorbance at 680 nm, respectively. These results indicate that cookies made with egg formed the greenest adducts, while cookies made with banana formed the least green adducts (Fig. 5). Cookies baked at 162.8 °C had a higher CGA-lysine adduct absorbance than cookies baked at 190.6 °C, confirming that cookies with higher moisture

Fig. 4 mg of chlorogenic acid per g of dough or cookie (a), Trolox equivalent mM of antioxidant capacity per mg of dough or cookie (b), and ng of carbonyls per g of dough or cookie (c)

Fig. 5 Peak absorbance at 680 nm signifying chlorogenic acid-lysine adducts formation in cookies

formed more adducts than less moist cookies. The adduct formation had the highest positive correlation with internal greening ($r = 0.90$), which indicates that color changes are mainly caused by CGA-lysine adducts.

Schiff bases

At 162.8 °C, cookies made with egg, chia, and banana had absorbance values of 0.85, 0.86, and 0.79, respectively. At 190.6 °C, cookies made with egg, chia, and banana had absorbance values of 0.78, 0.86, and 0.73, respectively. Like the greening reaction, increased mobility of reactants when exposed to moisture leads to greater reactivity and therefore more Schiff bases (Acevedo et al. 2008). Schiff bases are intermediates formed when reducing sugars covalently bind with the amino groups on amino acids and proteins to form glycated proteins (Benjakul et al. 2005). Considering that reducing sugars and proteins are key reactants in glycation, the differing quantities of reactants and moisture content in eggs, banana, and chia seeds affected Schiff base formation in sunflower butter cookies. While the higher amount of reducing sugars (12.2%) in bananas contributes to Schiff base formation through the Maillard reaction (USDA 2017), cookies made with egg and chia both formed more Schiff bases due to the high protein content of eggs and the high moisture content of both cookies made with egg and chia.

Fluorescence of advanced Maillard reaction products and soluble tryptophan (FAST)

While the greening reaction was the main focus of this study, Maillard browning reactions must also be accounted for since Maillard reactions also consume protein and carbonyls. FAST results represent the ratio of AMPs to free soluble tryptophan. The dough samples had lower FAST values (Fig. 6) because they had not been exposed to heat, which speeds up production of AMPs and CGA-protein adducts. As storage time increased, the FAST values in dough samples increased more rapidly than cookie samples because they had much more available tryptophan to be used up in the reactions than the cookies. A higher FAST value indicates lower nutritional content due to more AMPs and less free soluble tryptophan (Birlouez-Aragon et al. 2002). Cookies made with banana and egg had increased FAST values from 162.8 to 190.6 °C, while cookies made with chia did not. In addition to having a greater reducing sugar content, which increases AMP

production, cookies made with banana formed less CGA-protein adducts than cookies made with chia. Therefore, cookies made with banana had more remaining tryptophan to be used in the formation of AMPs. AMP formation increases with heat, so the FAST index was higher at 190.6 °C. Cookies made with chia were completely green from adduct formation even at 190.6 °C (Fig. 1) and had less starting tryptophan in comparison to cookies made with egg. As a result, cookies made with chia may have had less free tryptophan to react in the formation of AMPs than cookies made with banana or egg. As a result of limited tryptophan availability, changes were not seen from 162.8 to 190.6 °C in cookies made with chia. Cookies made with banana had a higher FAST index at 190.6 °C than 162.8 °C due to loss of the remaining free tryptophan in AMP formation. Because eggs have a higher initial tryptophan content in comparison to chia seeds or banana (USDA 2017), cookies made with egg had more available tryptophan to be used in the formation of AMPs despite losses from the formation of CGA-tryptophan adducts. Like

Fig. 6 Effect of egg replacers [egg (a), chia seed (b), and banana (c)], baking temperature, and time on fluorescence of advanced Maillard reaction products (AMPs) and soluble tryptophan (FAST) index calculated by $(F_{AMP}/F_{Trp}) \times 100$ determined over 1 week

cookies made with banana, more tryptophan was used in the formation of AMPs at 190.6 °C than 162.8 °C.

On days 3 and 4, FAST values decreased 0.6–2.0% in cookies made with chia baked at 162.8 °C and cookies made with egg baked at 190.6 °C. A decrease in FAST values occurred because, initially, only the tryptophan on the surface of the protein reacted in oxidation and color reactions. However, these reactions cause protein conformational changes to occur. When conformational changes occur, tryptophan that was initially quenched is able to fluoresce, leading to increases in tryptophan fluorescence intensity and decreases in FAST values (Ghisaidoobe and Chung 2014). When the internal tryptophan is exposed to the surface, it may form AMPs and CGA-amino acid adducts, causing the tryptophan fluorescence to decrease again and the FAST values to increase.

Carbonyl content

Dough made with banana had the highest carbonyl content (103.26 nmol/g) due to higher reducing sugar (12.2 g/100 g) content (USDA 2017). Because the DNPH method reacts with ketones and aldehydes, reducing sugars are highly reactive (Kadam et al. 2012), causing cookies made with banana to have a greater carbonyl content than treatments with less reducing sugars. The dough had higher quantities of unreacted reducing sugars than cookies, so dough treatments had more carbonyls than cookies (Fig. 4). During baking, reducing sugars form AMPs causing a loss of reactive carbonyls. From 162.8 to 190.6 °C, carbonyls in cookies made with banana and chia increased by 65.4 and 28.7%, respectively, possibly due to the increased hydrolysis of sucrose into glucose and fructose at higher temperatures (Moore 2002). The increase in carbonyls at higher temperatures could additionally be due to increased protein and lipid oxidation caused by increased heat (Friedman 1996). Considering cookies made with banana had the highest carbonyl content, reducing sugars may have played a larger role in carbonyl content than protein and lipid oxidation in these cookies.

Trolox equivalent antioxidant capacity (TEAC)

ANOVA determined that both baking temperature and egg replacers had a significant impact on the antioxidant capacity with baking temperature ($P = 3.3 \times 10^{-4}$) having a greater effect than egg replacers ($P = 5.5 \times 10^{-3}$). Cookies baked at 190.6 °C had significantly higher antioxidant capacity than cookies baked at 162.8 °C (Fig. 4). Antioxidant capacity in cookies made with egg decreased by 64.7% from 190.6 to 162.8 °C due to possible losses in phenolics from adduct formation. Another reason for the higher antioxidant capacity at 190.6 °C could be

due to higher temperatures increasing the release of bound phenolic compounds (Žilić et al. 2016).

At 162.8 °C, cookies made with banana were 35% higher in antioxidant capacity than cookies made with chia and 68% higher than cookies made with egg. At 190.6 °C, cookies made with banana were 32% higher in antioxidant capacity than cookies made with egg and 47% higher than cookies made with chia. The significantly higher antioxidant capacity in cookies made with banana may be attributed to the high quantities of unreacted CGA compared to other treatments (Fig. 4).

Conclusion

The formulations resulting in the highest and the lowest greening were cookies baked with egg at 162.8 °C and cookies baked with banana at 190.6 °C, respectively. In addition to limiting greening, sunflower butter cookies baked with banana at 190.6 °C had higher antioxidant capacity and lower losses in soluble protein than other cookie treatments. The treatments with the most greening had significantly higher moisture content than other treatments. This may be a result of increased mobility of CGA and protein when exposed to an aqueous medium. Limiting storage time, baking at higher temperatures, increasing spread factor, and using less moist egg replacers are all effective ways to limit greening in sunflower butter cookies.

Besides moisture and pH, polyphenol oxidase (PPO) and certain nutritional components of egg replacers such as lysine or lecithin, can affect the greening reaction. Therefore, future studies could analyze the effect these factors have on sunflower butter quality in baked goods. This could be done by adding lysine into the cookie formulation at various quantities or by analyzing the effect of banana ripeness on the greening reaction as a function of PPO activity and moisture content.

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