1-31-2018

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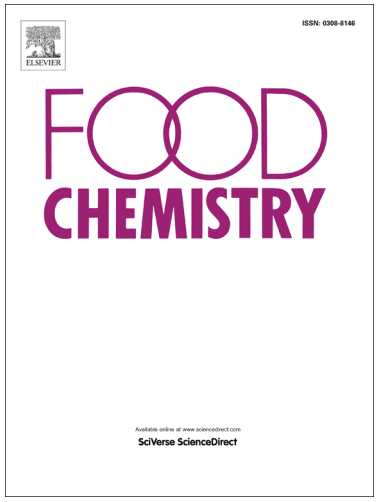

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PII: S0308-8146(18)30133-X
DOI: https://doi.org/10.1016/j.foodchem.2018.01.118
Reference: FOCH 22307

To appear in: *Food Chemistry*

Received Date: 8 August 2017
Revised Date: 12 December 2017
Accepted Date: 17 January 2018

Please cite this article as: Liang, S., Tran, H.L., Were, L., Lowering greening of cookies made from sunflower butter using acidic ingredients and effect on reducing capacity, tryptophan and protein oxidation, *Food Chemistry* (2018), doi: https://doi.org/10.1016/j.foodchem.2018.01.118

Lowering greening of cookies made from sunflower butter using acidic ingredients and effect on reducing capacity, tryptophan and protein oxidation

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ABSTRACT

Lowering of greening formed from oxidized chlorogenic acid (CGA) and amino groups, and favoured at alkaline pH, was investigated using acidic ingredients (sour cream, buttermilk, yoghurt, and honey) in sunflower butter cookies. Cookies with maple syrup added were used as a positive control. Changes in greening intensity, greening reactants (total phenols, CGA, protein), antioxidant capacity, tryptophan and Schiff base fluorescence were measured. Percentage greening, pH and a_w of cookies followed the same order: maple syrup> sour cream≥ buttermilk> yoghurt> honey. pH was positively correlated with greening intensity (r=0.77) and negatively correlated with CGA (r=-0.96). Total phenolic content, antioxidant capacity, tryptophan and Schiff bases were similar among cookies. The results suggest it is possible to decrease greening by minimizing storage time and using acidic ingredients. Minimal greening with acidic ingredients can extend the application of sunflower butter as a baking ingredient without loss of free radical-scavenging capacity, or higher protein oxidation.

Keywords: acidic ingredients; chlorogenic acid; greening, pH; sunflower butter, water activity

1. Introduction

Legume and nut butters and spreads are a good source of plant based protein and fat. However, allergies are a concern that both manufacturers and consumers must contend with. Peanuts are the major cause of food related anaphylactic shock, while tree nuts are also a major source of allergies in many western countries (Sicherer, Munoz-Furlong, Godbold, & Sampson, 2010). Sunflower seed spread, and butter, is not a major source of allergies compared to legume or nut butters and spreads (Hsu & Katelaris, 2007). Sunflower butter can thus be consumed by the estimated 1 % of those allergic to peanuts and tree nuts in the U.S population (Sicherer et al., 2010). Unlike milk, egg, and wheat allergies which children can outgrow, allergies to peanut and tree nuts can be lifelong (Boyce et al., 2011). An allergy to one food does not translate to an allergy for other foods (Sicherer et al., 2010). For adults not allergic to dairy (Nwaru et al., 2014), but who are allergic to peanut and tree nuts without co-allergies to sunflower seeds, use of sunflower butter is an alternative in baking applications. Sunflower seeds used to make butter and spreads contain up to 20 % crude protein (Dorrell & Vick, 1997) in addition to a high antioxidant potential due to the high chlorogenic acid (CGA) content (Weisz, Kammerer, & Carle, 2009), making sunflower butter a source of both protein and phenols.

Greening in baked products with sunflower butter is attributed to the high CGA content in sunflower seeds compared to other plant based butters. This high CGA content results in green to blue pigment formation when CGA dimerizes with itself under alkaline conditions. The CGA dimer then oxidizes to form highly reactive *o*-quinones which can subsequently react with amino acids in an aqueous, alkaline environment to form green trihydroxy benzacridine (TBA) derivatives (Liang & Were, 2018a, 2018b). The rate of greening increases with pH and heat, which can limit the application of sunflower butter in bakery applications (Wildermuth, Young,

& Were, 2016). Lowering greening is thus paramount if sunflower butter is to be commercialized or adopted by consumers for baking applications where greening is unwanted.

Use of maple syrup, a higher pH sweetener in baking encourages greening (Liang & Were, 2018a). High pH in maple syrup is due to the filtration process, which removes sugar sand and organic acids, resulting in a higher pH compared to unfiltered maple syrup. Lower organic acids content is also attributed to the acid conversion to flavour compounds and maple syrup's mineral content (Stuckel & Low, 1996) .

As alkaline pH favours formation of green pigments from amino group-chlorogenic acid interactions (Yabuta, Koizumi, Namiki, Hida, & Namiki, 2001), the use of acidic dairy ingredients in baking could potentially decrease greening. Acidic dairy ingredients commonly used in bakery products, include yogurt, buttermilk, and sour cream (Dineen, Takeuchi, Soudah, & Boor, 1998). Lactic acid fermentation of milk consumes carbohydrates such as lactose to produce lactic acid and lower the pH of dairy products such as yoghurt, sour cream and buttermilk (Shiby & Mishra, 2013).

To eliminate greening of sunflower products, studies have focussed on separating the protein and CGA to minimize the green discolouration during protein extraction or during the application of sunflower protein (Pickardt, Eisner, Kammerer, & Carle, 2015). Our approach was however, to leave both reactants in, and lower greening by formulation changes as reported by Liang and Were (2018a). Lower pH could affect CGA, lipid and protein oxidation, with interactions between CGA and protein potentially affecting both nutrition and appearance. As CGA is a phenolic antioxidant compound, its reducing capacity in sunflower butter cookies, in addition to its action as a reactant in the greening reaction, was investigated to determine how CGA-induced greening could affect the overall reducing capacity of cookies. The combined

effects of typical acidic baking ingredients and storage time on greening of cookies, reducing capacity, Schiff bases and tryptophan were measured. This was done to determine potential impact of greening on nutrition, and reactants and products formed. Differences between two storage conditions (vacuum-sealed container and zip-lock bag) were also investigated to determine how oxygen post-baking affected greening of sunflower butter cookies.

2. Materials and methods

2.1. Cookie formulation and baking conditions

Sunflower butter dough (400 g in total) was formulated using all-purpose flour (39.7 %), unsweetened and salt and sugar-free sunflower butter (24.3 %), egg (13 %), baking soda (0.6 %), vanilla extract (0.6 %), salt (0.4 %) and four acidic ingredients: honey and dairy ingredients (21.4 %) versus maple syrup. To eliminate moisture as a variable, which may enhance greening during baking and storage, the moisture of the four acidic ingredients was adjusted to that of maple syrup, which has approximately 32.03 % water content (USDA, 2016). The same moisture content in cookie doughs was achieved by adding 13.73 g of water to honey and 50.60, 55.14, and 53.81 g of sucrose to sour cream, buttermilk and yogurt, respectively, according to the moisture content recorded in US Department of Agriculture (USDA), Branded Food Products Database, Release 28 document.

Two cookie dough batches for each treatment were prepared and mixed separately, rolled out and cut into 0.5±0.1 cm thick and 4.5 cm width cookie doughs, and baked at 177 ˚C (350˚F) for 7 min in a conventional oven (JA12SL, DOYON, Inc. Saint-Côme-Linière, Canada). The water activity, greening intensity and percentage greening were monitored after 1, 4, 8, 16, 24, and 72 h. Moisture content, CGA, greening TBA, Folin-Coicalteu and ABTS-scavenging

capacity and tryptophan and Schiff base fluorescence were tested before and after baking the cookies.

2.2. pH, a_w, proximates (moisture, lipid, protein) and sugar content

The pH of sweeteners and acidic ingredients (1 g/4 ml of deionized water), and dough and cookies mixtures (0.5 g/5 ml of deionized water) was measured (AACC, 1999). Sample mixtures were homogenized at $6.8x10^2$*g for 45 s, using a multi-prep rapid homogenizer (Multi-prep Homogenizer, PRO Scientific Inc., Oxford, CT. USA) and incubated for 4 h before centrifugation at 4 ˚C and $3.3x10^3$* g for 30 min, using a AccuSpin 1R-75003449 centrifuge (Thermo Fisher Scietific, Inc. CA. USA). The supernatant was collected to determine the pH of cookies and doughs (AACC, 1999), using a Vernier® pH sensor connected to Labquest 2 Version 2.7.1 software (Vernier Software & Technology).

AACC method 44-15.02 (AACC, 1999) was used to measure the moisture content in doughs and cookies. Samples (3 g) were placed in aluminium pans and put into a vacuum oven (Model 281, Thermo Fisher Scientific, Inc. CA. USA), kept at 60 ˚C and a pressure of -70 kPa. The samples were taken out after 24 h and cooled in a desiccator for 5 h. The mass of cookies was recorded before and after drying, and percentage difference in masses was used to calculate moisture content on a wet basis. The changes in a_w were monitored using an AQUALAB water activity meter (Pullman, WA USA).

Lipid content was measured after Soxhlet extraction according to AACC method 30.26 (AACC, 1999). Crude protein of defatted and dehydrated samples was quantified using a nitrogen conversion factor of 5.7 according to AACC Method 46-12.01 (AACC, 1999), using a Kjeltec™ 8100 system (Eden Prairie, MN USA).

Sugar compositions of dehydrated-defatted cookies were quantified using HPLC at a column temperature of 70 ºC as described by Liang and Were (2018a) using standards prepared at 0-2.5 mg/ml.

*2.3. Hunter L*a*b* and image analysis*

Greening was measured using a spectrophotometer (CM-2500d) equipped with SpectraMagic NX® colour data software (Konica Minolta, Inc. CA. USA) with the CIE L*a*b* system as described by Liang and Were (2018a and 2018b)

The percentage greening of cookies was carried out using an image analyzer (Keyence Corporation, CA. USA) equipped with a camera (CV-X422A) which had 2.0 megapixel. The percentage greening was calculated by equation 1, using Vision Database (CA-H1DB, Keyence Corporation, CA. USA).

$$\text{percentage } greening = \frac{Total\ green\ area}{Total\ cookie\ area\ (brown\ +\ green\ area\ )} \times 100 \quad \text{(equation. 1)}$$

Browning index (BI) was used to compare the internal browning of cookies after 0.25 h of baking and was calculated using equations 2 and 3 (Isleroglu et al., 2012).

$$Browning\ Index = \frac{X-0.31}{0.17} \times 100 \quad \text{(equation. 2)}$$

where

$$X = \frac{(a*+1.75L*)}{5.645L*+a*-3.012b*} \quad \text{(equation. 3)}$$

2.4. Chlorogenic and caffeic acids, and tryhydroxyl benzacridine derivatives

Chlorogenic acid and caffeic acid (Sigma-Aldrich, Saint Louis, MO. USA) standard solutions were prepared (0.00-0.06 mg/ml) in HPLC water. Sample solutions of doughs or cookies (0.3 g in 10 ml of HPLC water) were homogenized at 0.68 x 10^3 g for 45 s and

centrifuged at $3.3*10^3$ g for 30 min (AccuSpin 1R-75003449, Thermo Fisher Scientific, Inc. CA. USA) at 4 °C. The solutions were filtered, using Whatman® glass microfibre filters (Grade GF/A circles, diam. 90 mm), and then filtered, with a 0.45 µm nylon syringe filter. The CGA of the supernatant was quantified, as decribed by Liang and Were (2018b). Chlorogenic acid-lysine standard solutions were prepared according to our previous study (Liang & Were, 2018b) and TBA was quantified at 680 nm, considering that all the CGA was reacted with lysine in the standard solutions. The results were expressed as mmol TBA/g cookies.

2.5. Folin-Ciocalteu reagent and trolox equivalent antioxidant capacity

The same supernatants and standards (0.2 ml) as from the CGA quantification (section 2.4) were mixed with 0.1 M Folin-Ciocalteu reagent (1.5 ml). After 5 min, the supernatants were neutralized, using 1.5 ml of Na_2CO_3 (0.6 g/10 ml of deionized water). Solutions were incubated for 30 min at room temperature (Liang & Were, 2018a). The absorbance, at 760 nm, of sample solutions was recorded, using a SpectroVis® plus spectrophotometer (Vernier Software & Technology. USA).

TEAC assay was conducted according to Liang and Were (2018a). The results were expressed as nmol trolox/g cookies. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), potassium peroxosulfate, and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid/ABTS), were purchased from Sigma-Aldrich (St. Louis, MO. USA) and ethanol was from Thermo Fisher Scientific (Huntington Beach, CA. USA). Trolox equivalent antioxidant capacity (TEAC) assay was used to measure the scavenging of $ABTS^{\bullet+}$, as $ABTS^{\bullet+}$ can fully dissolve in both organic and aqueous solutions with minimal interference by ionic strength, so multiple solvents can be used to test the antioxidant capacity for a complex food matrix, such as cookies. In contrast to the DPPH assay, which is also used in determination of antioxidant capacity in

bakery foods, TEAC's $ABTS^{\bullet+}$ radical is also more light and temperature-stable than DPPH's radical (Marecek et al., 2017).

2.6. Tryptophan and Schiff base fluorescence intensity

Supernatants from ground dough and cookie mixtures (0.3 g/10 ml of nano-filter water) were incubated for 1 h before centrifugation and filtration using 0.45 µm nylon filters. Tryptophan (Trp) and Schiff base fluorescence intensity (FI) of supernatants were recorded using a spectrofluorometer (Fluoromax®-4, Horiba Scientific, CA. USA) with λ_{ex}= 280 nm, λ_{em}=300-500 nm and λ_{ex}= 350 nm, λ_{em}= 380-600 nm, respectively (Estevez, Kylli, Puolanne, Kivikari, & Heinonen, 2008). The maximum intensity at λ_{max}=364 nm and λ_{max}=475 nm for Trp and Schiff bases, respectively, were normalized using the equation. 4:

$$Normalized\ data = \frac{Intensity\ of\ treatment - minimum\ intensity\ of\ treatment}{Overall\ maximum\ intensity\ of\ treatment - minimum\ intensity\ of\ treatment}$$

(equation. 4)

2.7. Statistical analysis

The effects of treatments (maple syrup, honey, buttermilk, sour cream, and yoghurt), and storage time (1, 4, 8, 11, 24, and 72 h) on greening of cookies, water activity, pH, CGA content, TBA, Folin-Ciocalteu reducing capacity, ABTS-scavenging capacity, tryptophan and Schiff base fluorescence intensity were analyzed. Analysis of variance (ANOVA) with version 9.3 Statistical analysis software (, SAS institute Inc. NC. USA) and Duncan's multiple range test was used to determine level of the statistical significance of variables.

3. Results and discussion

3.1. pH, a_w, proximates (moisture, lipid, protein) and sugar content

The pH increased in cookies compared to the corresponding doughs, possibly due to release of carbon dioxide and increase in hydroxyl ions during baking (Tables 1 and 2; Gokmen,

Acar, Serpen, & Morales, 2008). After 1 h post-baking, the cookies made with honey had the lowest pH (7.84), while cookies made with maple syrup had the highest (pH=8.69), with dairy - containing cookies having a pH in between the two sweeteners. Differences in pH of cookies were attributed to initial pH differences of ingredients used, with a higher pH of the maple syrup compared to acid containing ingredients. The pH was strongly negatively correlated with Hunter a* (r=-0.918, $P<0.05$, supplementary materials Fig. S1A), which indicated that higher pH, resulted in lower Hunter a*, which led to more internal greening in cookies.

All dough samples and all cookie samples had similar moisture contents (Table 1 and 2). This ruled out moisture as a variable in this study. Cookies made with sour cream had the highest lipid content (Table 2), which was consistent with a higher initial lipid content in sour cream (8.33%), according to the US Department of Agriculture (USDA), Branded Food Products Database, Release *28* document.

Cookies made with maple syrup, yogurt and buttermilk had higher sugar contents than cookies made with sour cream. There was no reducing sugar (glucose, fructose or lactose) detected in cookies except in cookies made with honey, which had 42.4 % and 49.6 % glucose and fructose (Table 1), which resulted in more surface browning of cookies (supplementary materials Table 1; Ajandouz, Tchiakpe, Dalle Ore, Benajiba, & Puigserver, 2001).

The a_w values of cookies after baking and 24 h post storage were in the order: maple syrup >sour cream≈buttermilk>yogurt>honey (Table 2). The higher reducing sugar content in honey caused a significant lowering in the water activity post-baking of cookies made with honey. Lower a_w in cookies made with sour cream and yogurt compared to those with maple syrup could be due to their larger pore size (Table 2, supplementary materials Table S1) which may cause greater release of water vapour. More surface greening of cookies, after 24 h post -

baking storage under higher humidity condition was observed (Table 2, Table S1) and a_w decreased with time under the low relative humidity conditions (21%RH) of storage, which was consistent with our previous study (Liang & Were, 2018b).

3.2. Greening and browning in cookies

Monitoring of Hunter -a* and -b* values, which are important indices for evaluating green to blue colour changes that complemented the percentage greening, using an image analyzer was initially done to compare different oxygen storage conditions (vacuum versus Ziploc® bags) post-baking. Under vacuum storage conditions, the a* values were not significantly different from those of cookies stored in Ziploc® bags, because CGA quinone formation, needed for greening, promoted by the presence of oxygen, had probably mostly occurred during baking at high temperature. The differences in cookies stored under vacuum compared to those exposed to oxygen post-baking were negligible over the duration of storage, so subsequent trials omitted storage of cookies under vacuum (supplemental material Fig. S2).

Cookies made with maple syrup as a greening control had the highest percentage internal greening and greening intensity (Table 2, supplementary material Table S1, Fig. S1) and turned blue-green after 16 h (Table. 2, Fig. 1D). The order of internal greening was: cookies made with maple syrup>sour cream> buttermilk>yoghurt>honey (Fig. 1), which was due to the highest pH in maple syrup (Table 1 and 2) that favoured greening (Liang & Were 2018a, 2018b). The low pH of honey is due to organic acids, such as gluconic, citric, levulinic, and formic acids (da Silva, Gauche, Gonzaga, Costa, & Fett, 2016). This higher acidity did not favour green pigment formation, as also illustrated by the the suppression of green derivatives measured at 680 nm (Table 1). The observed A_{max} at 680 nm, in the lysine-chlorogenic acid adducts solution, was attributed to trihydroxy benzacridine (TBA) derivatives, consistent with observations in model

systems (Iacomino et al., 2017), in our previous study (Liang & Were, 2018b), and that of cookies in the current study (Table 2, Fig. 1, supplementary materials Table S1). Lysine cross-linking with CGA has been associated with higher green pigment formation, as the ε-amino side group of lysine makes it highly reactive in the greening reaction (Bongartz et al., 2016). The dairy ingredients had a decolourizing effect, but they were generally not as effective as honey in preventing greening, attributed to the higher initial pH of dairy ingredients when compared to honey (Table 1).

The increase in absorbance at 680 nm corroborated with image analysis and the Hunter - a* values (Table 1, Fig. 1), with the higher pH ingredients resulted in more greening in sunflower butter cookies, corresponding to formation of the green TBA pigment (Yabuta et al., 2001; Liang & Were, 2018b), with maximum absorbance at 680 nm (supplementary materials Fig. S1D). The greening observed in the cookies results from the alkaline conditions caused by high pH ingredients (baking soda and maple syrup), favouring oxidation of CGA in the sunflower seed butter and subsequently reacting with amino acids (Bongartz et al., 2016).

In addition to the effect of pH, greening intensity and percentage green cookie area increased with time, and cookies made with maple syrup had nearly 100 % green area coverage after 24 h (Table 2, supplementary materials Table S1). The changes in greening over time were rapid in the first 8 h, with a rate of 1.2 to 5.7 percentage internal greening per hour, in cookies made with maple syrup and dairy ingredients, where greening displaced the brown regions on the cookies. The cookies made with honey, however, were not green (Table 2, Fig. 1, supplementary materials Table S1). Increased greening over time may have occurred due to increased contact time between reactants. Higher internal greening of cookies compared to surface greening (Fig.1) was due to greater moistness in the interior compared to the top or bottom of the cookies consistent with our earlier findings (Liang & Were, 2018b).

The browning of cookies could have been from oxidized CGA reacting with amines and other polyphenols, or with itself (Bongartz et al., 2016). The reducing sugar content in honey also contributed to the browning through the Maillard reaction. The internal browning index (BI) of cookies showed BIs of 76.2, 76.7, 78.3, 76.8 and 80.2 in cookies formulated with maple syrup, sour cream, buttermilk, yoghurt and honey, 1 h post-baking, respectively. The BI indicated that cookies made with honey had the most browning (Isleroglu et al., 2012; Table 2 and supplementary materials Table S1). In addition, cookies made with honey had the highest A_{294}/A_{420} ratio, which indicated more Maillard Schiff bases compared to melanoid polymers after 1 h post-baking (Table 1; Ajandouz, Tchiakpe, Dalle Ore, Benajiba, & Puigserver, 2001) that resulted in more browning compared to other cookies.

Given the presence of protein, carbohydrates and phenolic compounds in various foods, there are various colour reactions that occur simultaneously during baking. Some of the key reactants, such as CGA, are similar for both Maillard browning and greening reactions. Browning differences observed were attributed to differences in types of sugars. Cookies made with honey was comprised of approximately 92 % reducing sugars (Table 1), while maple syrup and the cookies made with dairy ingredients had sucrose, a non Maillard reactive sugar as the main sweetener (St-Pierre et al., 2014). This resulted in cookies made with honey having greater surface browning that resembled gingerbread snap cookies in colour, in contrast to a lighter colour in other cookies where sucrose would need to be inverted by acid and/or heat to participate in Maillard browning reactions (Golon, Kropf, Vockenroth, & Kuhnert, 2014).

3.3. Changes in chlorogenic acid and protein content

There were no significant differences among the CGA contents of all the doughs (P= 0.19), which was attributed to the same amount of sunflower butter in all cookie formulations.

After baking, cookies had a lower CGA content due to CGA reacting with the amino groups to form TBA derivatives, and Maillard reaction products after baking (Narita & Inouye, 2013; Yabuta et al., 2001). The ratio of CGA to its hydrolysis product (caffeic acid/CA) in cookies made with maple syrup, sour cream, buttermilk, yogurt, and honey decreased after baking compared to their corresponding doughs (Table 1). This indicated the loss of CGA (forming CA) during heating. Cookies made using maple syrup, sour cream, and buttermilk had lower CGA after baking, while cookies made with honey and yogurt had higher CGA (Table 1, Fig. 2), which was attributed to their lower pH. Chlorogenic acid dimers oxidize, leading to *o*-quinones formation, which readily react with amino groups to form TBA derivatives (Yabuta et al., 2001). These reactions lead to the loss of CGA in favour of increased CGA- amino acid adduct contents (Table 1, Bongartz et al., 2016; Narita & Inouye, 2013),

Cookies made with sour cream had the highest protein content, as expected (Table 2), based on amounts used in our cookie formulations and published higher protein contents in dairy ingredients of 6.7, 3.3, and 4.7 g/100 g in sour cream, buttermilk and yogurt, respectively (USDA, 2016).

3.4. Folin-Ciocalteu reagent reducing and trolox equivalent antioxidant capacity (TEAC) assay

Doughs made with honey after 1 h had the highest Folin-Ciocalteu reagent/FCR reducing capacity (Fig. 3A) due to its higher reducing capacity (from the phenols) and highest reducing sugar content (Ball, 2007). After 24 h of storage, the FCR reducing capacities were similar in doughs. However, cookies made with honey had a significant increase in FCR reducing capacy due to the production of intermediate and/or final Maillard reaction products (Fig. 3A; Gu, Kim, Hayat, Xia, Feng, & Zhang, 2009; Michalska, Amigo-Benavent, Zielinski, & del Castillo, 2008).

The FCR reducing capacity decreased after baking, except in cookies made with honey, where it increased. Amongst the treatments, cookies made with yogurt and honey had higher FCR reducing capacity, as these were the treatments with less greening due to their lower pH (Table 1 and 2). The loss of FCR reducing capacity in cookies made with maple syrup, sour cream and butter milk was due to the loss of CGA, which oxidized and bound with amino groups and formed TBA (Bongartz et al., 2016). Cookies, after 24 h of storage, had similar FCR reducing capacity to cookies after 1 h of storage, due to continual Maillard reaction with MRPs compensating for the loss of CGA (Gu et al., 2009; Michalska et al., 2008).

The antioxidant capacities (AOX) of all doughs after 1 h were similar except for doughs made with maple syrup, which had lower AOX (Fig. 3B). Similar to FCR reducing capacity, the loss of AOX in doughs made with maple syrup after 1 h of storage was due to oxidized CGA at higher pH reacting with protein (Liang & Were, 2018b). Cookies after 1 and 24 h post-baking storage had higher AOX compared to their corresponding doughs (Fig. 3B). Increased AOX in cookies was caused by reducing capacity of formed MRPs (Ateea, Omayma, Mohammed, & Ahmed, 2012)

3.5. Tryptophan fluorescence intensity

To complement the TBA derivative complexes measured at 680nm, intrinsic Trp fluorescence was measured to further confirm conjugation between the protein-containing ingredients (sunflower, egg and/or dairy ingredients), chlorogenic acid and sugars. Most of the tryptophan would be expected to come from the egg used which was the same in all formulations. Solutions of the sweeteners and acidic dairy ingredients in the ratios present in the dough were first brought to the same moisture content by adding water to honey and sucrose to the dairy ingredients, prior to heating, to determine their role in the overall Trp fluorescence

intensity (FI) in the cookies. As expected, the dairy ingredients had a higher Trp FI compared to the sweeteners (Supplementary materials Fig. S3A).

When doughs were tested, the addition of dairy ingredients increased initial Trp FI as the dairy containing dough had higher initial Trp FI when unheated (Fig. 4A). The λ_{max} emission wavelength was 364 ± 1 nm for all treatments, except for cookies made with honey which had a slight red shift with λ_{max} at 367 nm, accompanied by higher FI (supplementary material Fig. S3). Doughs, after 24 h storage, had decreased FI, attributed to Trp oxidation during storage. Decreased Trp FI could also be due to covalently-bound protein-polysaccharide complexes that have been noted to be more hydrophilic than is unbound protein; therefore, they will have lower electron excitation due to quenching and thus, lower electron excitation due to quenching (Gu & Kenny, 2009). The FI of doughs after 24 h of storage and baking decreased, due to conjugate formation during Maillard reaction (Wang & Xiong, 2016).

Cookies tested after 1 and 24 h of storage had lower Trp FI than had their corresponding doughs. Trp FI decrease with baking was due to the various heat-induced reactions. In addition, the Trp FI decreased with increased pH (Fig. 4A), attributed to the oxidation of Trp, which is favoured under high pH conditions (Liang & Were, 2018a). Direct glycation of reducing sugars with the Trp indole group has been noted to destroy Trp in cookies when glucose rather than sucrose was used in baking (Morales, Acar, Serpen, Arribas-Lorenzo, & Gokmen, 2007). Our results, however, showed that cookies with sucrose as the main sweetener had lower Trp FI. These cookies with lower Trp, excepting those with maple syrup, had dairy as an ingredient, so this was possibly due to the fat oxidation products from added dairy ingredients interacting with Trp (Table 1; Horvatic & Vedrina-Dragojevic, 2000). To what extent this hypothesis is valid

needs to be further investigated to distinguish the contribution of Trp FI quenching from the various concurrent reactions taking place with heat.

3.6. Schiff base formation

Schiff base FI values in dough amongst the different treatments after 1 and 24 h of storage were similar (Table 1, Fig. 4B). After baking, cookies made with honey had the highest Schiff bases while cookies made with sour cream had the lowest after 1 h of storage. Higher Schiff bases could be from protein and lipid oxidation, along with Maillard reaction (Charissou et al., 2007; Yamaki, Kato, & Kikugawa, 1992). Cookies made with honey had the most reducing sugar and resulted in more Schiff bases forming from the Maillard reaction. In addition, higher pH, as observed in the doughs and cookies made with maple syrup, creates a favourable condition for formation of Schiff bases (Ajandouz, Tchiakpe, Dalle Ore, Benajiba, & Puigserver, 2001). Schiff bases are, however, an intermediate product of the Maillard reaction, and the higher reducing sugars may have resulted in the high Schiff bases observed initially in the cookies made with honey, which later declined after 24 h due to Schiff bases participating in further Maillard reactions (Fig. 4). The lower pH of cookies made with the dairy ingredients or honey, may have contributed to lower Schiff bases in cookies after 24 h, given that baking time and temperature, leavening agent and moisture contents were similar.

4. Conclusions

The results suggest that it is possible to decrease greening with changes in formulation and by limiting storage time to address concerns about greening in sunflower butter when it is used as a replacement for allergen containing plant butters. Honey which had the lowest pH and a_w of the investigated acidic ingredients, prevented post-baking greening to the greatest extent. The cookies made with honey were, however, darker brown, which was attributed to their higher

reducing sugar content, despite their lack of surface and internal greening. Further research could focus on sensory evaluation and consumer acceptance of green sunflower butter cookies. The effects of greening induced oxidation on the levels of other Maillard reaction products, such as acrylamide and HMF concentrations, should be assessed to optimize formulations for lowering of green pigment formation and enhancing nutrition to complement sensory effects (taste, mouthfeel and potential aftertaste).

Conflict of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors would like to thank Chapman University for financially supporting this research, and Pham Vu with the preliminary data collection.

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Figure Captions

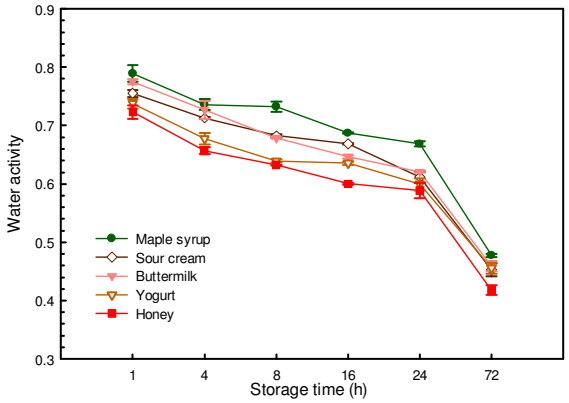
Fig. 1. Changes in water activity (A), percent greening (B), Hunter a* (C), and b* values (D) with time.

Fig. 2. Changes in chlorogenic and caffeic acid in sunflower butter doughs (A) and cookies (B).

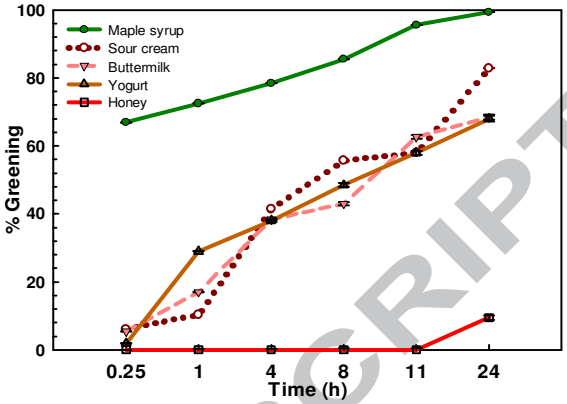
Fig. 3. Folin-Ciocalteau Reagent reducing capacity (A) and trolox equivalent antioxidant capacity (B) of sunflower butter doughs and cookies at different storage times.

Fig. 4. Normalized fluorescence intensity of tryptophan at λ_{max}=356 nm (A) and Schiff bases at λ_{max}=475 nm (B).

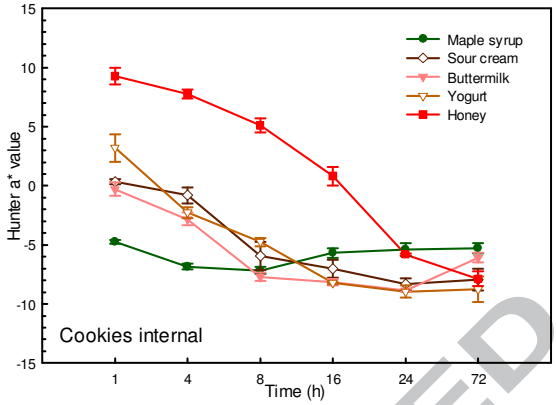
A) water activity changes along with time



B) % greening changes along with time



C) Cookie internal Hunter a* value changes along with time



D) Cookie internal Hunter b* value changes along with time

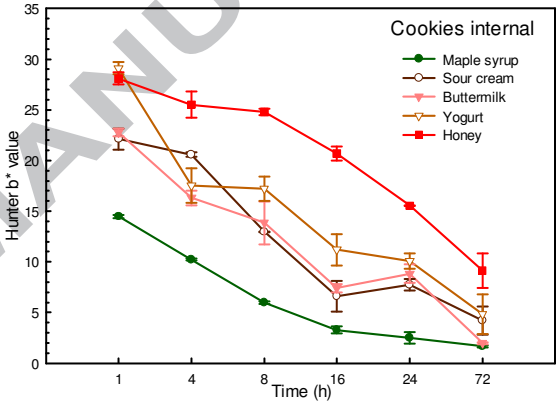


Fig. 1.

A) Doughs after 1 h storage

B) Cookies after 1 h storage

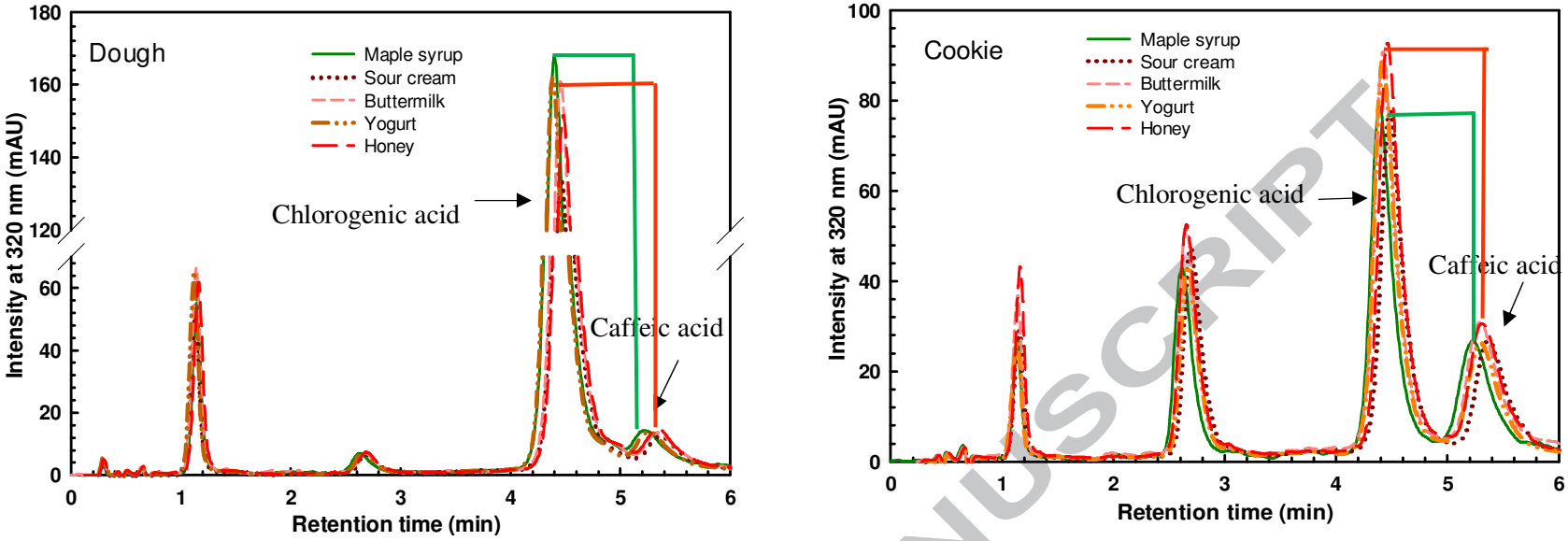
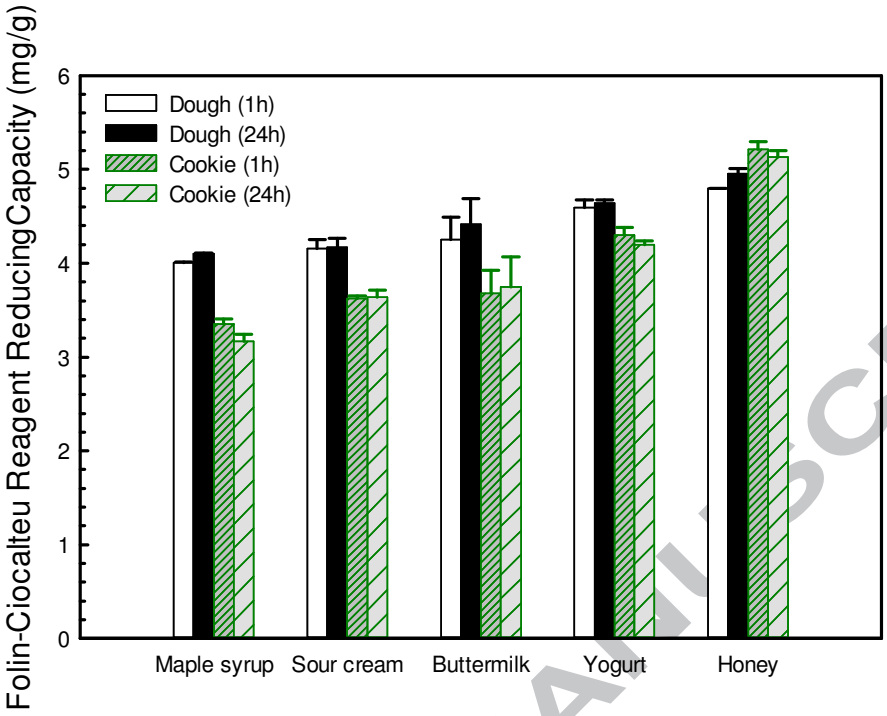


Fig. 2.

A) Folin-Ciocalteau reagent reducing capacity in doughs and cookies after 1 and 24 h



B) Trolox equivalent antioxidant capacity in doughs and cookies after 1 and 24 h storage

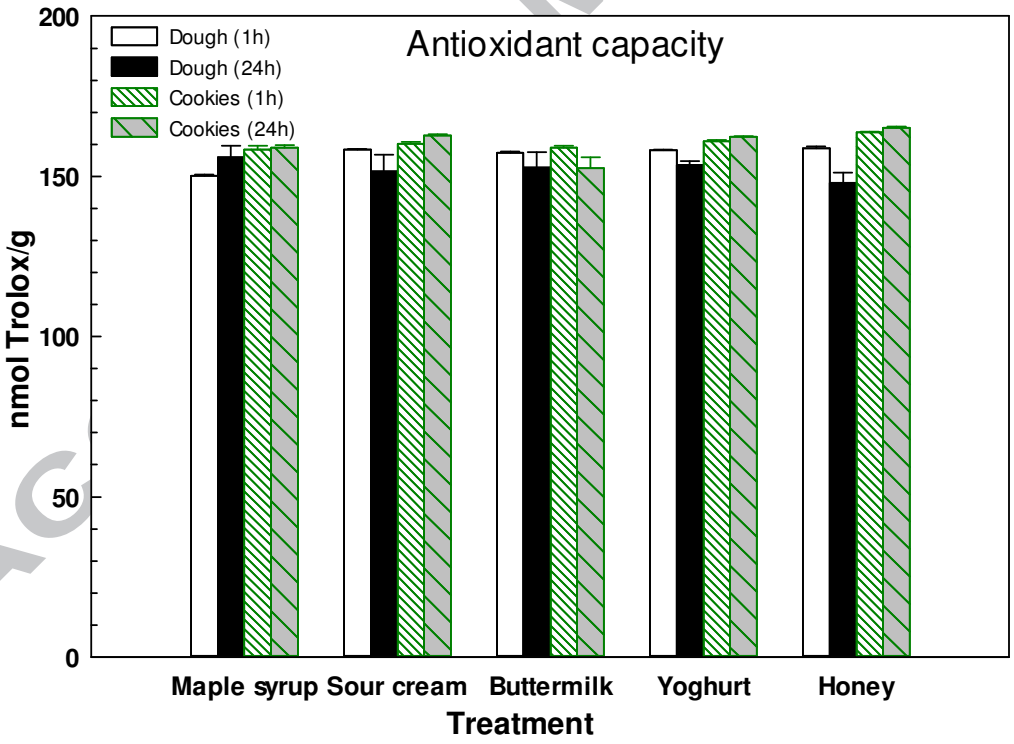
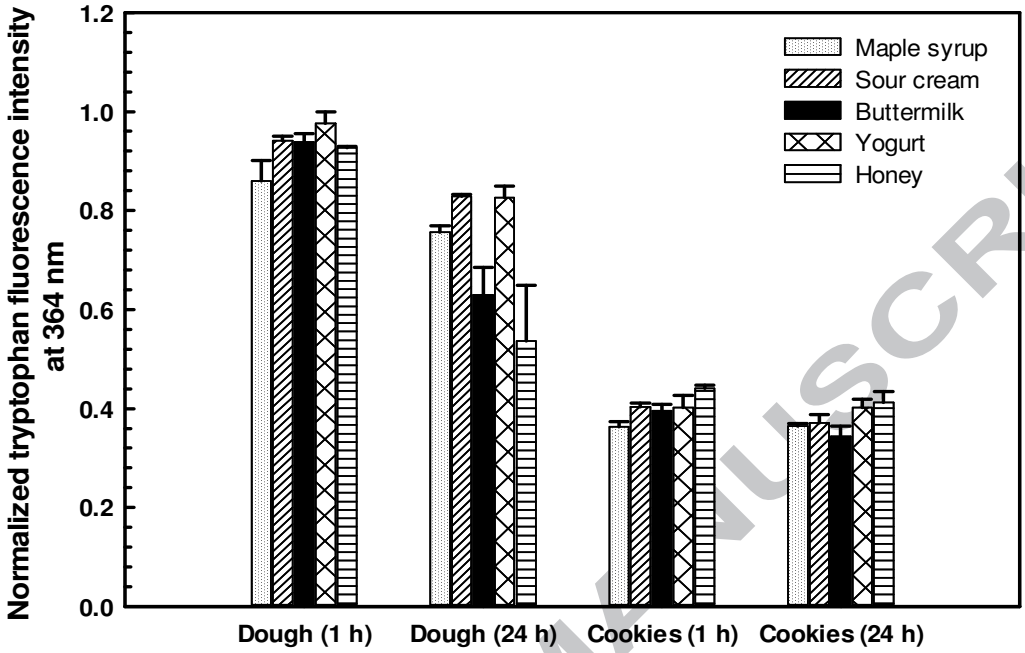


Fig. 3.

A) Tryptophan fluorescence intensity of doughs and cookies after 1 and 24 h storage



B) Schiff bases fluorescence intensity of doughs and cookies after 1 and 24 h storage

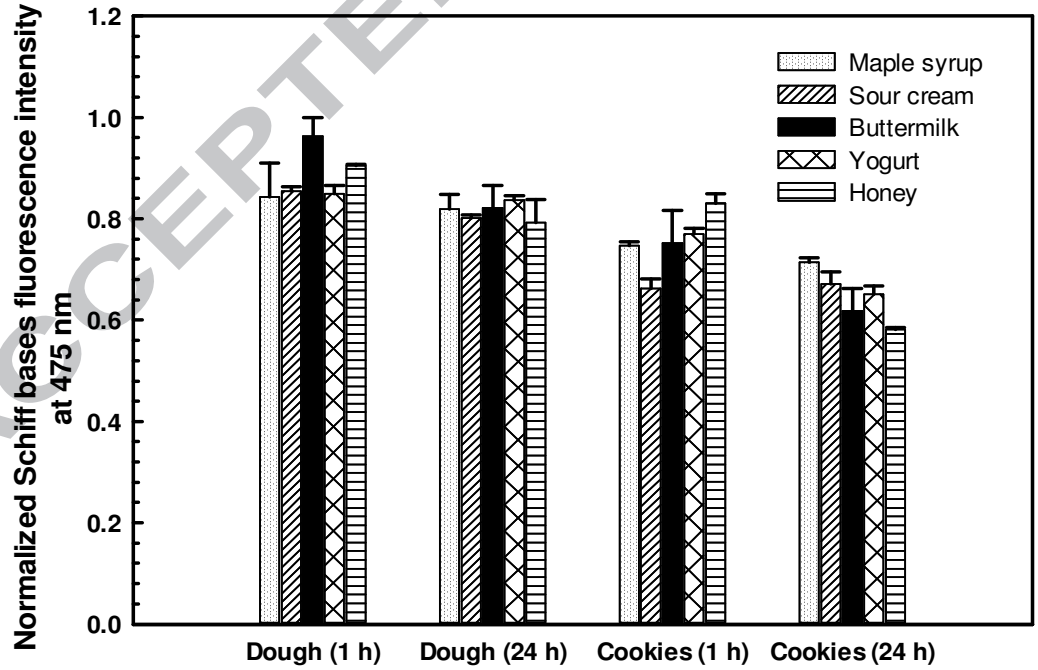


Fig. 4.

Highlights

- Use of acidic ingredients decreased greening in cookies made with sunflower butter
- Greening intensity was positively correlated to pH (r=0.77)
- pH was negatively correlated with chlorogenic acid (r=-0.96)
- Most green cookies had the most tryptophan quenching
- Greening reaction did not negatively affect antioxidant capacity