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Title

Chlorogenic acid induced colored reactions and their effect on carbonyls, phenolic content, and antioxidant capacity in sunflower butter cookies

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Abstract:

The high chlorogenic acid (CGA) content of sunflower seeds causes a greening reaction in sunflower butter baked products which can deter application of sunflower butter as an allergen-free alternative to other plant and dairy based butters. This study focused on how greening intensity of sunflower butter cookies made with different sweeteners (maple, agave, corn syrups, honey and xylitol) affected greening, protein oxidation products, Folin and $ABTS^{\bullet+}$ radical scavenging ability. Cookies made with maple syrup and xylitol had higher pH and resulted in more greening. The dough made with agave syrup had highest total carbonyls caused by its highest reducing sugar content resulting in more Maillard reaction during dough preparation, while after baking cookies with highest greening (maple syrup) and highest reducing sugar (agave syrup) had higher carbonyls than other sweetener treatments. Cookies made with maple syrup and xylitol also had lower folin-ciocalteau reagent reducing capacity and tryptophan fluorescence. The greening reaction did not affect Schiff bases from oxidation and antioxidant capacity in cookies made with different sweeteners. Higher pH sweeteners thus enhanced greening intensity, tryptophan loss and lowered the total phenolic content after baking and storage, but did not influence the $ABTS^{\bullet+}$ capacity of sunflower butter cookies.

Keywords: Antioxidant capacity; carbonyls; chlorogenic acid; greening

1. Introduction

Sunflower butter offers an alternative nut butter for people allergic to legume and tree nut butters. Compared to peanut and almond butter, sunflower seed butter offers additional nutritional benefits. It is an excellent source (≥ 20% of Daily Value) of minerals, such as phosphorus, magnesium, copper and selenium (FDA, 2013; Thomas & Gebhardt, 2010), which are essential for building up bones and muscles, and are essential in formation of metabolic enzymes (NIH, 2017). Sunflower seed's lipids are 90g unsaturated fatty acids/100g total fatty acids with kernels containing 270-289 mg phyto-sterols/100g (Phillips, Ruggio, & Ashraf-Khorassani, 2005; USDA, 2016).

In addition, sunflower butter is rich in phenolic compounds that have antioxidant health benefits (Olthof, Hollman, & Katan, 2001). In particular, sunflower seeds have approximately 3.0 g/100 g chlorogenic acid (CGA) of the 4.2 g/100 g total phenolic content (dry matter) in kernels (Weisz, Kammerer, & Carle, 2009). This high total phenolic content in sunflower seeds is almost 84 times higher than that in peanut butter, which has about 0.05g/100 g (Ma et al., 2013). Chlorogenic acid prevents lipid oxidation reactions (Budryn, Nebesny, Zyzelewicz, & Oracz, 2014) by reducing free radical formation (Liang & Kitts, 2016), inhibiting low-density lipoprotein (LDL) oxidation and DNA damage in vitro (Budryn et al., 2017; Olthof, Hollman, & Katan, 2001). However, the high free CGA content induces a greening reaction in sunflower seed products which can hinder the application of sunflower butter in the bakery industry (Wildermuth, Young, & Were, 2016). The greening reaction also consumes free CGA, protein and primary amino acids, and thus may affect the nutritional properties of sunflower butter bakery products.

Different sweeteners have different sugar composition, pH and moisture (St-Pierre et al., 2014), which can influence the extent of Maillard and greening reactions (Devi & Khatkar, 2016; Yabuta, Koizumi, Namiki, Hida, & Namiki, 2001). Lower moisture, higher pH and reducing sugar cause more browning products (Pereyra Gonzales, Naranjo, Leiva, & Malec, 2010). Besides color, the Maillard reaction can produce unhealthy products, for instance acrylamide, α-dicarbonyls, and advanced glycation end products (AGEs) or healthy compounds such as antioxidant reductones (de Oliveira, dos Reis Coimbra, de Oliveira, Giraldo Zuniga, & Garcia Rojas, 2016). The effect of the greening reaction on formation of compounds with nutritional effects warrants investigation. The higher moisture ingredients and higher pH in baked products using baking soda promote formation of green and blue pigments when free CGA and primary amino acids and/or proteins interact (Yabuta et al., 2001). This study focused on whether the greening reaction as a function of different sweeteners affected appearance in sunflower butter cookies in addition to antioxidant capacity, total phenolic content, and protein oxidation products (total carbonyl, tryptophan fluorescence and Schiff bases) before and after baking. Correlation between greening and changes in total phenols, antioxidant capacity and loss of tryptophan in cookies made with sunflower butter were determined.

2. Materials and methods

2.1. Materials

Sucrose (≥ 99.5%), fructose (≥ 99.0%), glucose (≥ 99.5%), sodium carbonate (≥ 99.0%), (+)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (≥ 98.0%), monosodium phosphate (≥ 99.0%), 2,4-dinitrophenylhydrazine (97.0%), and disodium phosphate (≥ 98.0%) were purchased from Sigma-Aldrich (St. Louis, MO. USA). HPLC-grade water and ethanol were

obtained from Thermo Fisher Scientific (Huntington Beach, CA. USA). Folin-Ciocalteu reagent was obtained from MP Biochemicals (Santa Ana, CA. USA).

2.2. Cookie formulation and experimental design

Sunflower butter cookie dough treatments contaning one of four sweeteners (maple syrup/ UPC 096619955886, xylitol granules/ UPC 875002000033 diluted xylitol: water=8:2 (w:w), light corn syrup/ UPC: 761720051108, organic blue agave syrup/ UPC 012511204419 and honey/ UPC 073299000075) were prepared using the formulation presented in Fig. 1. The doughs were formed into disks of 4.5 cm diameter and 0.5 ± 0.2 cm thickness and baked at 149 °C (300 °F) using a convection oven (JA12SL, Doyon, Inc. Saint-Côme-Linière, Canada) for 7 min. The baking temperature was monitored using a thermocouple thermometer (Nicety® K-type DT 1312). After baking, the cookies were stored uncovered at room temperature (20 ± 5 ºC) for 24 h.

2.3. Sugar composition

Dough and cookie samples (0.9 ± 0.01 g) with 30 mL HPLC water were homogenized (Multi-prep Homogenizer, PRO Scientific Inc., Oxford, CT. USA) at $1.3x10^3$*g for 1 min. After centrifugation for 15 min at $0.3\ x10^3$*g and filtration using 0.45 µm nylon membrane filters, the filtrates were stored at 4 °C for later use. Sucrose, glucose and fructose standards (0.3-2.8 mg/mL) were used to create a standard curve. The sugar content was quantified using a Shodex® Sugar SP0810 column (300 mm x 8 mm i.d., 8.0 mm, Shodex, Colorado Springs, CO. USA) with a Shodex® Sugar SP-G 6B (50 mm x 6 mm i.d 6.0 mm) guard column. An Agilent HPLC 1100 series with a refractive index detector was used. The flow rate was 0.6 mL/min with an isocratic elution with HPLC water at a run time of 25 min (Wang, Yagiz, Buran, Nunes, & Gu, 2011).

100 *2.4. pH and Hunter L*a*b**

101 pH of sample mixtures prepared by dissolving 0.5 g of dough and cookies in 5 mL nano
102 filtered water was measured according to AACCI method 02-52.01 (1999). After 1 min
103 homogenization (Multi-prep Homogenizer, PRO Scientific Inc., Oxford, CT. USA) at speed of
104 $1.3x10^3$*g, mixtures were incubated for 1 h, centrifuged (AccuSpin 1R-75003449, Thermo
105 Fisher Scientific, Inc. CA. USA) at $9x10^3$*g at 4 °C for 30 min, before pH testing using a pH
106 meter (Vernier Software & Technology, OR. USA).

107 Internal greening intensity of cookies (lateral cut) was measured using a Hunter L*a*b*
108 spectrophotometer (CM-2500d, Konica Minolta, Inc. Japan) where negative and positive a*
109 value represents greeness and redness respectively (Zhang, Chen, & Wang, 2014).

110 *2.5. Carbonyl content*

111 Total carbonyl content was measured as outlined by Hawkins, Morgan, and Davies
112 (2009) using 2,4-dinitrophenylhydrazine (DNPH) as the derivatization agent.

113 *2.6. Tryptophan and Schiff bases fluorescence*

114 Sweeteners (0.12 g) were dissolved in 20 mL nano filtered water to obtain the same
115 sweetener concentration in cookies after baking. The sweetener solutions were placed into a
116 convection oven for 7 min at 140 °C to account for tryptophan fluorescence from sweeteners
117 alone. Dough and cookie samples (0.3 g) were dissolved in 10 mL nano filtered water. After
118 homogenization, centrifugation and filtration (0.45 μm nylon syringe filter), the filtrates (0.5 mL)
119 were diluted (1:10) using nano-filtered water. Tryptophan fluorescence intensity was measured at
120 $\lambda_{excitation}$=280 nm and $\lambda_{emission}$=300-500 nm, while Schiff bases produced from protein and lipid
121 oxidation were measured at $\lambda_{excitation}$=350 nm and $\lambda_{emission}$=380-600 nm (λ_{max}=475 nm), with a slit

width of 5 nm using a Fluoromax-4 Spectrofluorometer (Horiba Scientific, CA. USA) as outlined by Utrera, Rodriguez-Carpena, Morcuende, and Estevez (2012).

2.7. Folin-Ciocalteu Reagent reducing and trolox equivalent antioxidant capacity (TEAC) assay

The supernatant for determination of Folin-Ciocalteu Reagent/FCR reducing capacity in sunflower butter cookies was prepared according to Yu, Nanguet, and Beta (2013). The sample supernatant (0.2 mL) and CGA standard solutions (0.05-0.4 mg/mL) were mixed with 1.5 mL 0.1 M Folin-Ciocalteu reagent and neutralized after 5 min using 1.5 mL of 6 g Na2CO3:100g water before incubating for 30 min at 4 °C. Sample solutions (100 µL) were pipetted into a flat bottom microplate (Falcon®) for reading at λ760 using a FLUOstar Omega Microplate Reader (BMG Labtech, Inc. Cary, NC. USA).

Antioxidant capacity of samples (0.3 g) dissolved in 30 mL ethanol/water (30/70, v/v) was also measured according to Zhang et al. (2014) using 0.16-0.67 mg/mL (+)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid as a standard.

2.8. Statistics analysis

The effect of 5 liquid sweeteners, storage time and their interactions were evaluated in a mixed model by a two-way analysis of variance (ANOVA): two fixed effects (sweeteners and storage time) as variables were repeated at 3 different time points. Where a significant effect of treatment was detected, the Student t-test was used to determine the levels of significance between dependent variables and two-way interactions of independent variables. All values were reported as the mean ± standard deviation from duplicate batches of dough made on the same day. Differences were considered significant when $P<0.05$. Correlation between variables was calculated using Statistics Analysis Software (SAS Institute Inc. NC. USA).

3. Results and discussion

3.1. Sugar composition

As expected (USDA, 2016), the sugar composition of dough and cookies presented in Table 1 showed that dough and cookies made with maple syrup had the most sucrose, followed by corn syrup, honey and agave syrup. Before baking there was no glucose and fructose in doughs made with maple syrup, but after baking, 0.5 mg/g fructose was measured, indicating some hydrolysis of sucrose occurred (Andrews, Godshall, & Moore, 2002; Eggleston & Amorim, 2006). Total sugars were higher in dough compared to the corresponding cookies. Decreased glucose and fructose after baking was attributed to reducing sugars being used up in the Maillard reaction (Martins, Jongen, & Boekel, 2001). Sugars from the other carbohydrate containing ingredients (wheat flour and sunflower butter) in cookies were negligible as they were below detection limit in dough and cookies made with xylitol. Given that xylitol is a sugar alcohol, the source of the reducing sugars in all doughs was thus the liquid sweeteners, since flour and butter did not contribute to sugar content.

*3.2. Hunter L*a*b* and total carbonyls*

The greening intensity represented by Hunter a* value, showed that cookies made with maple syrup had the most greening (lowest a* value) among all other cookies at all time points tested (Table 2; $P<0.01$), which was due to higher pH and moisture content (Yabuta et al., 2001).

Total carbonyls content was similar in all doughs except the dough made with agave syrup, which had higher carbonyls than other doughs ($P<0.01$; Table 3). These differences in carbonyl content in sunflower butter doughs were attributed to differences in reducing sugar and free amino groups content from different sweeteners, and their corresponding reactions (Yabuta et al., 2001; Yamaki, Kato, & Kikugawa, 1992). The doughs made with agave syrup and honey

had the highest reducing sugars. Agave syrup however had higher moisture compared to honey that may have also favored carbonyl product formation during dough preparation (Table 2; Mellado-Mojica & Lopez, 2015; Yamaki et al., 1992).

The total carbonyls increased after baking, as heat induces protein and lipid oxidation in addition to accelerating browning and greening reactions (Dean, Fu, Stocker, & Davies, 1997; Martins et al., 2001; Wildermuth et al., 2016). Lipid oxidation as a result of baking forms aldehydes and ketones such as malonaldehyde, hexanal and ketodienes, which could partially account for the higher carbonyls in cookies compared to dough in addition to carbonyls from Maillard reaction and protein oxidation (Dean et al., 1997; Martins et al., 2001; Yamaki et al., 1992). The total carbonyl content after baking was ranked as follows: cookies made with xylitol≈agave syrup>maple syrup>honey>corn syrup (Table 2), potentially due to more phenols and protein oxidation at higher pH in cookies made with xylitol and the higher reducing sugar content in cookies made with agave syrup (Damodaran, 2008). Given that xylitol is a non-reducing sugar and does not participate in Maillard browning, carbonyls in xylitol cookies were not from reducing sugars and Maillard reactions. In the presence of oxygen, amino acids and protein can easily react with free radicals to form carbonyl compounds (Dean et al., 1997). Cookies made with xylitol had the highest pH. High pH can induce oxidation of polyphenols leading to oxidized products that may have enhanced greening reactions from oxidized polyphenols and protein interaction (Damodaran, 2008). Higher carbonyl content was found in cookies that were most green (those made with maple syrup and xylitol) than the cookies made with honey and corn syrup (Dean et al., 1997; Yabuta et al., 2001). The higher carbonyl content in cookies made with agave syrup (Table 3) was due to a balance between its moisture and reducing sugar content. The lowest pH of cookies was detected in cookies with agave syrup

190 which also resulted in less greening and thus more free reactants/reducing sugars (Table 1 and 2).
191 Greening and total carbonyl content was influenced by the various pH and moisture
192 combinations, and could also be influenced by processing and storage history of the sugar syrups
193 which were not determined in the current study.

194 *3.3. Schiff base fluorescence*

195 Schiff bases formed from oxidation were similar amongst dough and cookies made with
196 different sweeteners. The Schiff bases however increased after baking (Fig. 2), which was due to
197 the heat accelerating oxidation (Dean et al., 1997; Martins et al., 2001). Greening however had
198 no effect on Schiff base formation.

199 *3.4. Tryptophan fluorescence*

200 The profile of fluorophores present in sweeteners showed a peak around 320 nm in
201 honey, maple and agave syrups (Fig. 3a) that was absent in corn syrup and xylitol which could
202 be from polyphenols (Papoti & Tsimidou, 2009). A higher tryptophan (Trp) fluorescence
203 intensity was detected in honey compared to other sweeteners after heating (Fig. 3a). Chen et al.
204 (2017) quantified 1-3.6 mg/kg Trp using HPLC-FLD, and some Trp is thus inherently present in
205 honey. Overall contribution of Trp fluorescence from the different sweetener ingredients was
206 however neglible (Fig. 3a) compared to that in dough (Fig. 3b) and cookies (Fig. 3c-d). When
207 UV-Vis spectra of the different dough and cookie solutions were compared, the highest
208 absorbances at the maximum excitation wavelengths of tryptophan was the honey and lowest
209 was maple and xylitol. Quenching of the tryptophan fluorescence in cookies with maple and
210 xylitol was thus not from the sugars or inner filter effect (Gu & Kenny, 2009), and was attributed
211 to the greening reaction.

The doughs made with different sweeteners had similar Trp fluorescence, which was higher than that of the corresponding cookies (Fig. 3b-c). The doughs' higher Trp fluorescence is from protein maintaining the native structure and less protein oxidation occuring before baking. After baking, Trp fluorescence in cookies was ranked as honey>corn syrup>agave syrup>xylitol>maple syrup (Fig.3). Cookies made with maple syrup had the largest decrease (40.3% lower) in Trp fluorescence compared to corresponding doughs, while there was only a 14.0% and 20.2% trp decrease in cookies made with honey and corn syrup compared to their corresponding doughs. Trp fluorescence loss was attributed to Trp cross-linking with monomer CGA, Trp oxidation and Maillard reaction (Friedman, 1996; Utrera et al., 2012).

Potential decrease in surface hydrophobicity may have caused Trp loss and a 6 nm blue shift of Trp (Jiang, Xiong, Newman, & Rentfrow, 2012) in cookies made with maple syrup and xylitol, possibly caused by dipole-dipole interactions under slightly higher pH conditions (Vivian & Callis, 2001), which required less energy to quench protein. The blue shift in cookies made with xylitol due to the higher pH may have resulted in more Trp quenching (Ehrig, Muhoberac, Hurley, & Bosron, 1992).

3.5. Folin-Ciocalteu Reagent reducing and trolox equivalent antioxidant capacity (TEAC)

There was no significant difference in FCR reducing capacity in dough as a function of the different sweeteners (P>0.05; Fig. 4.), which was attributed to sunflower butter being the major contributor to the total phenols measured. Folin-Ciocalteu reagent reducing capacity however increased after baking compared to dough, except for cookies made with xylitol. Increased FCR reducing capacity with heat has been noted by other investigators such as Zou, Yang, Zhang, He, and Yang (2015). Increased FCR reducing capacity from dough to cookies could be related to the reductones formed from Maillard reaction (Zilic et al., 2016), as the cookies made with higher

235 reducing sugars (honey and agave syrup) had the highest FCR reducing capacity post-baking
236 compared to cookies made with maple syrup and xylitol. Baking also may have increased the
237 phenols' solubility by releasing the bound phenols (Zilic et al., 2016). The decrease in FCR
238 reducing capacity after 24 h storage at 20-25°C could be due to loss of free CGA in greening and
239 browning reactions (Martins et al., 2001; Yabuta et al., 2001).

240 When comparing the different treatments, cookies made with agave syrup had the highest
241 total soluble phenols, from the higher reducing compounds (sugars and polyphenol) and the
242 corresponding Maillard reaction (Everette et al., 2010). Lower soluble phenolics in cookies made
243 with maple syrup and xylitol could be due to its higher pH consuming more CGA, the
244 predominant phenol in sunflower butter, when reacted with amino groups to form green TBA
245 pigments (Yabuta et al., 2001). Xylitol, a sugar alcohol does not undergo Maillard reaction
246 resulting in the least browning (Table 1). Lowest FCR reducing capacity were in cookies made
247 with xylitol (Borrelli, Esposito, Napolitano, Ritieni, & Fogliano, 2004), and carbonyl containing
248 CGA in cookies may have reacted with reactive amino acids such as proline, tryptophan to cause
249 some browning, in addition to any hydrolysis products of CGA (quinic acid and caffeic acid)
250 cross-linking with amino groups in cookies also contributing to the browning (Bongartz et al.,
251 2016).

252 Antioxidant capacity is a balance between the loss of CGA antioxidants and formation of
253 Maillard antioxidant products in cookies. Antioxidant capacity as assessed with $ABTS^{\bullet+}$ radical
254 scavenging ability was highest in doughs and decreased after baking (Fig.4). The FCR reducing
255 capacity was positively correlated with $ABTS^{\bullet+}$ radical scavenging ability in doughs ($r=0.9516$,
256 $P=0.01$), attributed to CGA's antioxidant capacity in doughs. In contrast to many studies where
257 higher FCR reducing capacity is associated with higher $ABTS^{\bullet+}$ radical scavenging ability, our

findings did not find a strong correlation between FCR reducing capacity and $ABTS^{\bullet+}$ radical scavenging ability (r=0.5538, *P*=0.33). Formation of Maillard reaction products and their associated antioxidant capacity did not compensate for the loss of phenolics/CGA in cookies with baking, as doughs had higher antioxidant capacity compared to cookies. The time/temperature combinations used maintained sufficient antioxidant capacity in the sunflower butter cookies, with the remaining CGA contributing significantly to the overall antioxidant capacity compared to Maillard reaction products (MRPs) formed during baking (Delgado-Andrade, Rufian-Henares, & Morales, 2005). The lower antioxidant capacity after baking may have been caused by CGA-related greening reactions, which consumed free CGA during baking and storage (Yabuta et al., 2001), which could explain why cookies made with maple syrup had the lowest antioxidant capacity. Lower antioxidant capacity in cookies made with maple and xylitol could also be due to lower reducing compound content due to lower content of reducing sugars. The percent contribution of CGA compared to MRP to reducing ability in sunflower butter cookies warrants further investigation in addition to quantifying how MRP compounds such as dicarbonyls are affected (supplementary material).

4. Conclusion

Lower pH sweeteners with more reducing sugars, for instance, agave syrup and honey maintained more phenols, antioxidant capacity, and tryptophan than maple syrup, which has higher pH and low reducing sugar content. The study showed that higher pH and moisture sweeteners enhanced the greening reaction and resulted in lower total phenol, antioxidant capacity, and tryptophan fluorescence. The chemistry behind interactions between CGA, proteins and Maillard reaction products is largely unknown in sunflower based products. Further research could focus on determining specific advanced glycation products, such as pentosidine, N(ε)-

carboxymethyl-lysine and fluorescent advanced glycation end products content to confirm the effect of greening reaction on formation of Maillard reaction products in food products where both reactions occur simultaneously.

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Conflicts of interest:

None.

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Table

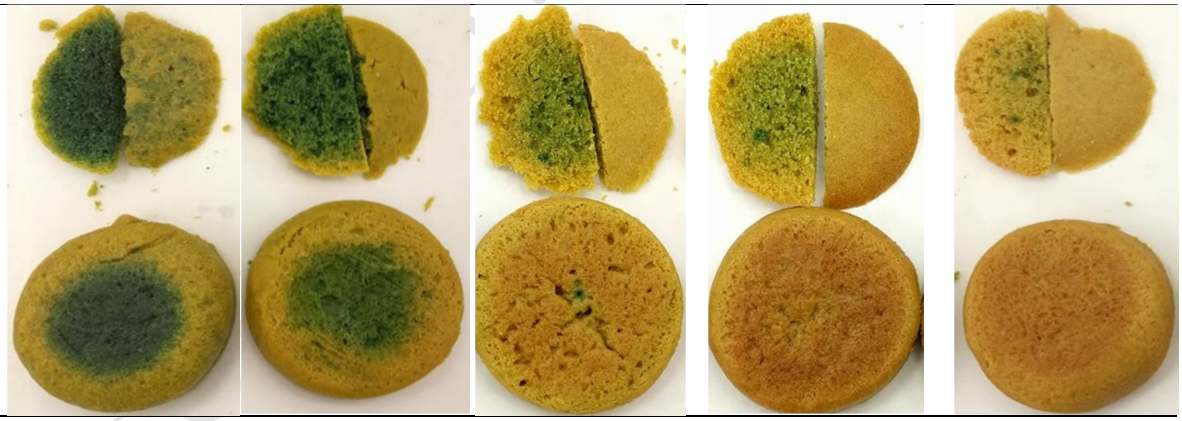
Table 1. Sugar composition of doughs and cookies made with different sweeteners.

	Sugar composition (mg/g, wb) in dough				**Sugar composition (mg/g, wb) in cookies after 0.25h**			
	Sucrose	**Glucose**	**Fructose**	**Total**	**Sucrose**	**Glucose**	**Fructose**	**Total**
Maple syrup	13.76±0.002[a]	bdl	bdl	13.76	13.02±0.001[a]	bdl	0.50±0.000[d]	13.37
Xylitol	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Corn syrup	6.77±0.000[b]	2.55±0.000[b]	0.16±0.000[c]	9.47	4.16±0.000[b]	1.83±0.000[b]	0.63±0.001[c]	6.62
Agave syrup	0.68±0.001[d]	0.11±0.000[c]	15.22±0.001[a]	16.00	0.53±0.002[d]	0.28±0.002[c]	10.67±0.000[a]	11.84
Honey	1.76±0.002[c]	6.88±0.001[a]	7.16±0.000[b]	15.79	1.31±0.000[c]	6.27±0.001[a]	7.36±0.002[b]	14.93

Values are the average of four replicates ± standard deviation. Same letters in each column are not significantly different (P>0.05)

bdl refers to below detection limit.

Table 2. Photos representing relationship between greening of cookies as a function of storage time and sweeteners differing in moisture, pH and time.

	Maple syrup pH=8.99±0.01[b] MC=14.06	Xylitol pH=9.13±0.01[a] MC=8.97	Corn syrup pH=8.69±0.05[c] MC=11.34	Agave syrup pH=8.44±0.04[d] MC=12.09	Honey pH=8.63±0.02[c] MC=11.45
-a* value after 0.25 h post-baking	-9.55±0.31[d]	-3.65±0.22[c]	3.07±0.95[b]	7.97±0.68[a]	6.22±0.11[a]
					
-a* value after 24 h post-baking	-8.68±0.91[b]	-13.27±0.41[c]	-2.27±0.40[a]	-0.04±0.02[a]	0.20±1.64[a]
					

MC is % moisture content of cookies, pH was measured in 10% cookie solution while Internal greening intensity (Hunter a* value) was averaged from two cookies after placing the spectrophotometer probe in the middle of the cookies after a lateral cut.

Same letters in each row are not significantly different (P>0.05).

Table 3. Carbonyl compounds (nmol/mg)[1] of dough and cookies using 2,4-dinitrophenylhydrazine (DNPH) derivatization.

Treatment	Maple syrup	Xylitol	Corn syrup	Agave syrup	Honey
Dough	0.16±0.00[b]	0.01±0.00[b]	0.37±0.00[b]	2.29±0.67[a]	0.56±0.11[b]
Cookie (0.25h)	7.61±0.21[b]	14.66±3.01[a]	4.40±0.05[d]	12.29±1.51[a]	5.82±0.52[c]
Cookie (24h)	4.77±1.05[a]	3.72±0.35[a]	3.31±0.47[a]	5.36±0.12[a]	4.14±0.02[a]

[1]Values are the average of four replicates ± standard deviation, same letter in each row means values were not significantly different (P>0.05)

Figure Captions

Fig. 1. Sunflower butter cookie formulation

Fig. 2. Schiff base fluorescence intensity at λ_{max}=475 nm (λ_{ex}=350 nm and λ_{em}=380-600 nm) between doughs and cookies made with different sweeteners 0.25 and 24 h post-baking; A.U. refers arbitrary unit. Error bars represent standard deviations of means from two cookies. Maple syrup Xylitol Corn syrup Agave syrup Honey

Fig. 3. Tryptophan intensity spectrum of sweeteners, doughs and cookies as a function of time. Black line refers to λ_{max}=356 nm, blue line indicates 6nm blue shift of cookies made with maple syrup and xylitol, λ_{max}=350 nm. Lines are means from two replicates.

Maple syrup Xylitol Corn syrup Agave syrup Honey

Fig. 4. Folin-Ciocalteu reagent reducing capacity/FRC (primary y-axis, bar graph) and trolox equivalent antioxidant capacity/TEAC (secondary y-axis, line scatter graph) of doughs and cookies made with different sweeteners.

Dough (2h) (FRC) Cookie (0.25h) (FRC) Cookie (24h) (FRC)
Dough (2h) (TEAC) Cookie (0.25h) (TEAC) Cookie (24h) (TEAC)

Error bars represent standard deviations of means from two cookies.

Same lettering above bars refers to treatments that were not significantly different (P>0.05) at the different time points

Figure

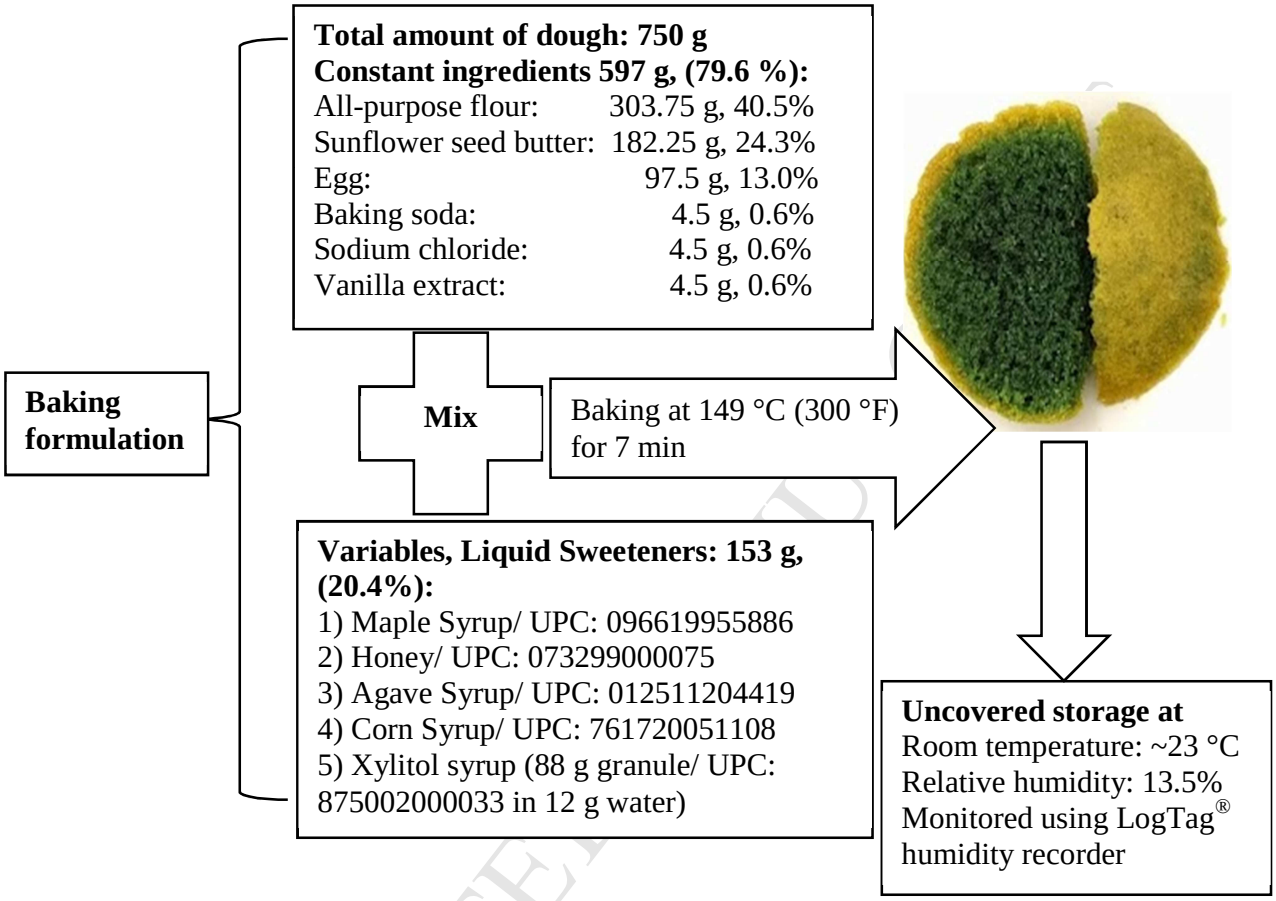


Fig. 1.

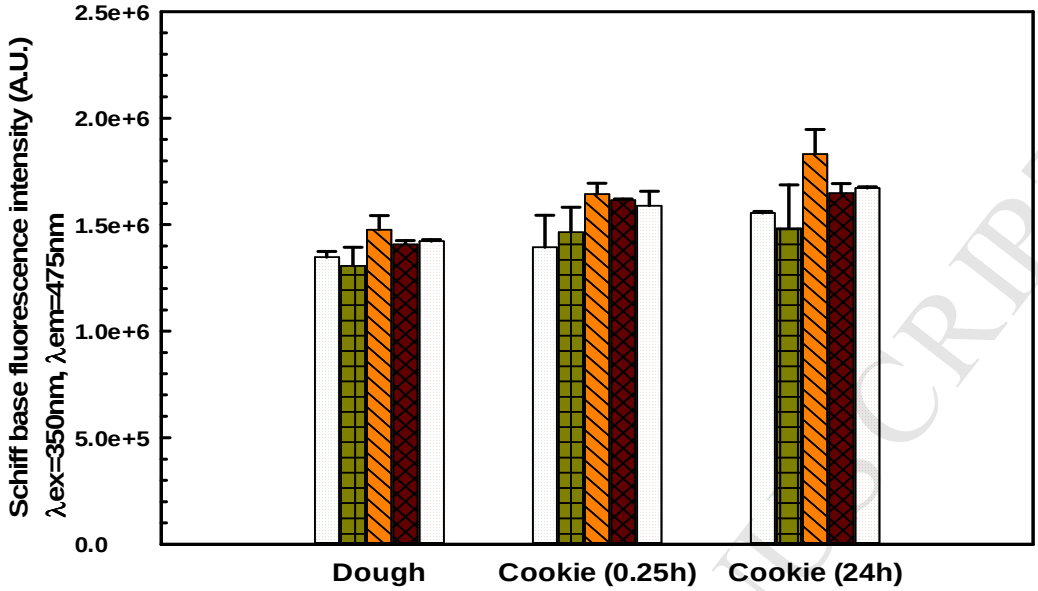
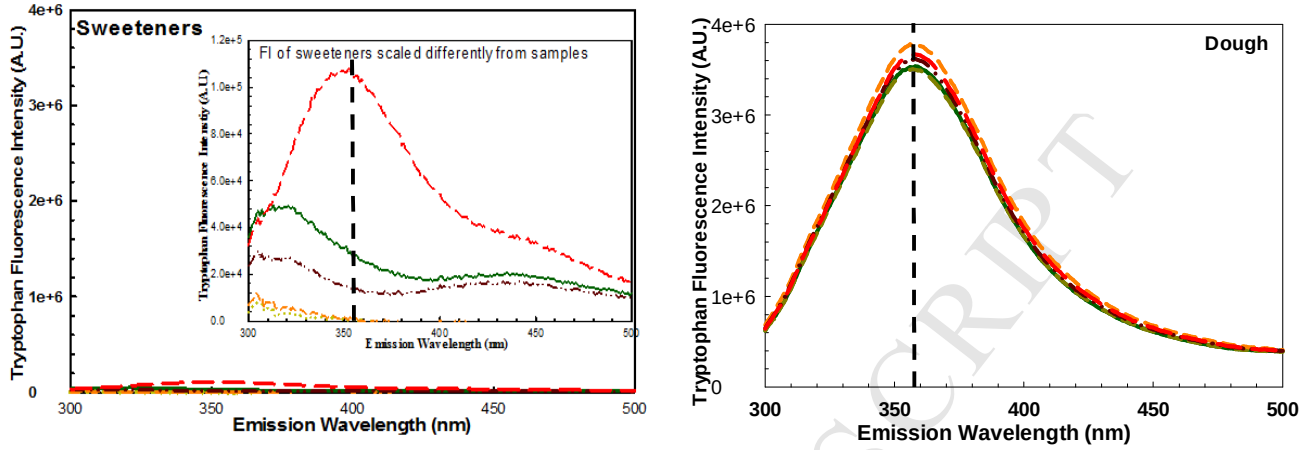


Fig. 2.

a) Sweeteners

b) Dough



c) Cookies after 0.25h of uncovered storage

d) Cookies after 24h of uncovered storage

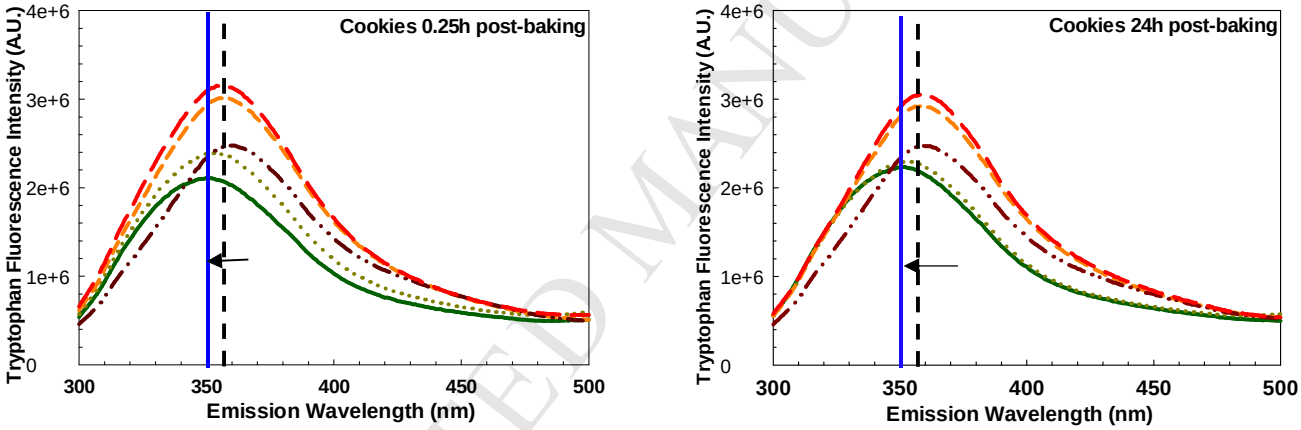


Fig. 3.

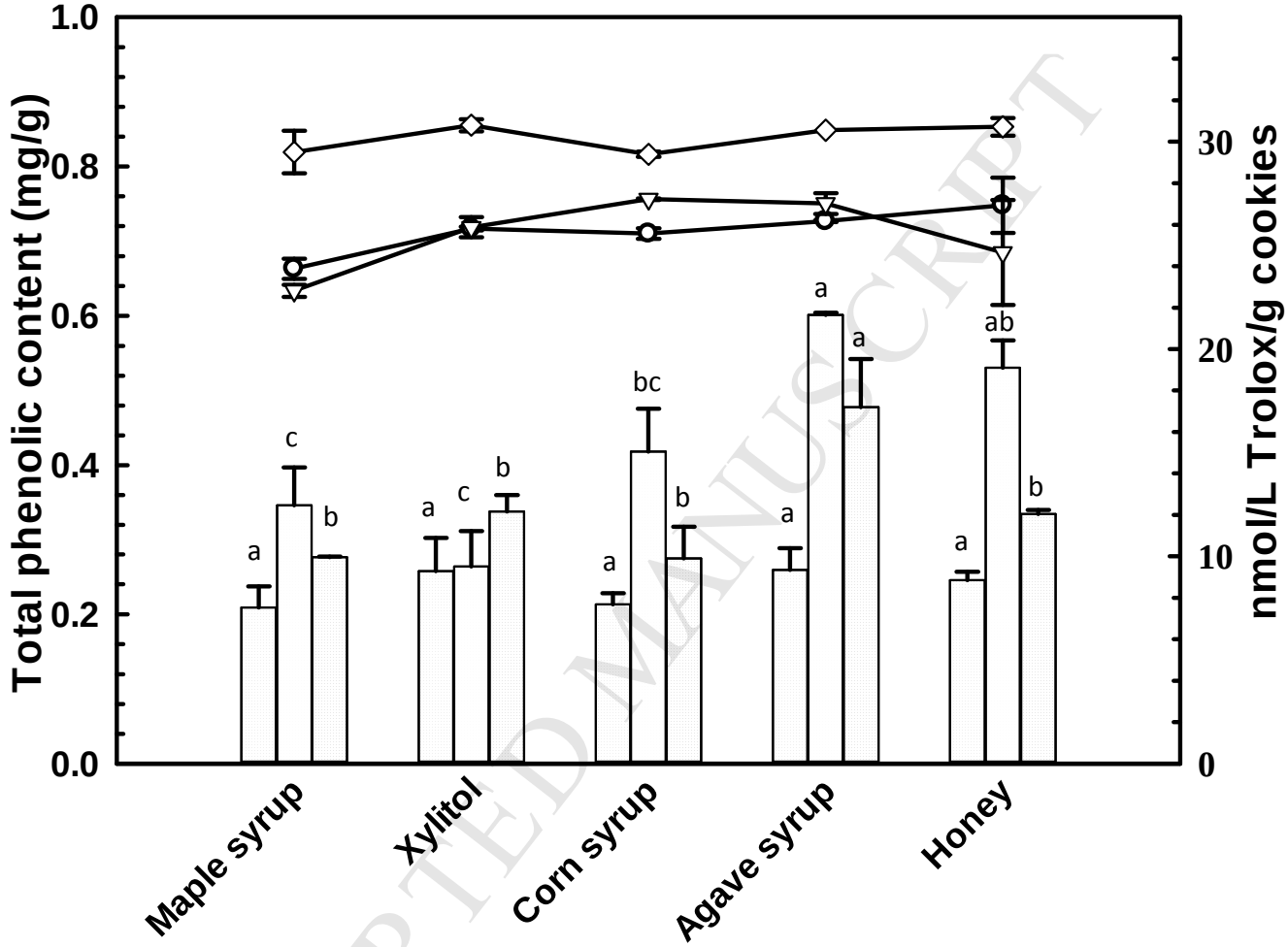


Fig. 4.

Highlights:

- Chlorogenic acid is a reactant in both greening and browning reactions
- Greening reaction lowered tryptophan fluorescence and total phenols
- Greening reaction did not affect the antioxidant capacity in cookies
- Schiff bases from oxidation were not affected by sweeteners and greening