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# Hydrolysis of Chlorogenic Acid in Sunflower Flour Increases Consumer Acceptability of Sunflower Flour Cookies by Improving Cookie Color

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### Recommended Citation

Verde, C. L., Pacioles, C.T., Paterson, N., Chin, J., Owens, C. P., & Senger, L.W. (2023). Hydrolysis of chlorogenic acid in sunflower flour increases consumer acceptability of sunflower flour cookies by improving cookie color. *Journal of Food Science*, 1–13. <https://doi.org/10.1111/1750-3841.16692>

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# Hydrolysis of Chlorogenic Acid in Sunflower Flour Increases Consumer Acceptability of Sunflower Flour Cookies by Improving Cookie Color

## Comments

This article was originally published in *Journal of Food Science* in 2023. <https://doi.org/10.1111/1750-3841.16692>

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## ORIGINAL ARTICLE

## Sensory and Consumer Sciences

# Hydrolysis of chlorogenic acid in sunflower flour increases consumer acceptability of sunflower flour cookies by improving cookie color

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**Funding information**

Research Corporation for the  
Advancement of Science Cottrell Scholars  
Award, Grant/Award Number: 2020-112;  
U.S. Department of Agriculture; Novel  
Foods and Innovative Manufacturing  
Technologies, Grant/Award Number:  
2020-67018-31261

**Abstract:** Sunflower meal, a byproduct of sunflower oil pressing, is not commonly used in alkaline baking applications. This is because chlorogenic acid, the main phenolic antioxidant in sunflower seeds, reacts with protein, giving the baked product a green discoloration. Our group previously demonstrated that a chlorogenic acid esterase from *Lactobacillus helveticus* hydrolyzes chlorogenic acid in sunflower dough cookie formulations, resulting in cookies that were brown instead of green. This study presents a sensory analysis to determine the acceptability of enzymatically upcycled sunflower meal as an alternative protein source for those allergic to meals from legumes or tree nuts. We hypothesized that the mechanism of esterase-catalyzed chlorogenic acid breakdown does not influence the cookies' sensory properties other than color and that consumers would prefer treated, brown cookies over non-treated cookies. Cookies made from sunflower meal were presented under green lights to mask color and tested by 153 panelists. As expected, the sensory properties (flavor, smell, texture, and overall acceptability) of the treated and non-treated cookies were not statistically different. These results corroborate proximate analysis, which demonstrated that there was no difference between enzymatically treated and non-treated cookies other than color and chlorogenic acid content. After the cookie color was revealed, panelists strongly preferred the treated cookies with 58% indicating that they "probably" or "definitely" would purchase the brown cookies, whereas only 5.9% would buy green, non-treated cookies. These data suggest that esterase-catalyzed breakdown of chlorogenic acid represents an effective strategy to upcycle sunflower meal for baking applications.

**KEYWORDS**

sensory analysis, sunflower cookies, chlorogenic acid Esterase, greening, color

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**Practical Application:** Sunflower meal is currently used as animal fodder or discarded. A major factor preventing sunflower meal use is its high chlorogenic acid content, which causes a green discoloration of baked goods made from sunflower meals under alkaline conditions. This study presents a sensory analysis in which panelists evaluate cookies made with sunflower flour that was treated with an esterase that breaks down chlorogenic acid. The results show that enzymatic treatment prevents greening and that panelists strongly prefer esterase-treated, non-green cookies, thus demonstrating the feasibility of utilizing sunflower flour in baking applications.

## 1 | INTRODUCTION

Sunflower oil accounts for about 8% of all vegetable oil produced in the world, resulting in large quantities of sunflower meal (SFM), a byproduct of sunflower oil production (USDA-Foreign Agricultural Service, 2023). The deoiled meal is non-allergenic and as reviewed by Wildermuth et al. (2016), SFM contains 27%–63% protein, 6%–9.5% ash, and <1.0%–5.6% oil on a dry weight basis depending on the dehulling and oil extraction method (Yegorov et al., 2019). The meal also contains 2%–5% antioxidant polyphenolics, in particular chlorogenic acid (CGA). The meal can be used to make protein isolates or ground to make sunflower flour (SFF) for baking applications. SFF is a well-suited flour alternative for individuals with gluten intolerances or those with allergies to tree nuts. Sunflower flour products also have the potential of being affordable since sunflower meal is cheaper than alternative protein meals from other oilseeds (USDA-Foreign Agricultural Service, 2023). However, most SFM is used as animal fodder, fertilizer, or soil compost and is rarely utilized in products intended for human consumption (de Oliveira Filho & Egea, 2021). Although there has been relatively little work on utilizing SFF in baking, a limited number of studies evaluated the sensory properties of baked goods containing SFF as a partial substitute for wheat flour. The sensory characteristics of SFF-baked goods have been tested in crackers, where the partial replacement of wheat flour with 15%–35% SFF contributed to increased acceptability by consumers for all sensory attributes (Man et al., 2017). In contrast, in cookies, the replacement of wheat flour with 30% SFF resulted in lower consumer overall acceptability (Puraikalan & Sabitha, 2014). Similarly, Grasso et al. (2019) reported changes to cookie taste and flavor when SFF replacement exceeded 18% of total flour. In muffins, the partial replacement of wheat flour with SFF increased protein, mineral, and fiber content, but also led to bitter flavors (Grasso et al., 2020, 2021). Furthermore, Nemš et al. (2022) demonstrated that par-

tial replacement of wheat flour with SFF led to decreased consumer acceptability and lower ratings of cookie color. Several properties of SFF hinder its more widespread use in baking; for instance, SFF can impart a bitter taste (Grasso et al., 2020), possibly attributed to CGA lactones that are formed during heating (Gigl et al., 2021; Kraehenbuehl et al., 2017). Another problem with using SFF is that under alkaline baking conditions, such as those encountered when using baking soda, CGA reacts with proteins to form a dark green trihydroxy benzacridine pigment (Pepra-Ameyaw et al., 2022). Many attempts to prevent greening have been proposed, including the addition of reducing thiols to the SFF dough or the extraction of phenolics from SFF using organic solvents. These methods, however, have limited effectiveness, are costly, or remove beneficial phenolics from the flour. Our group recently demonstrated that a recombinantly expressed CGA esterase from *Lactobacillus helveticus* rapidly hydrolyzes CGA into caffeic acid (CA) and quinic acid (Lo Verde et al., 2022). CGA esterase treatment prevented greening in alkaline-extracted sunflower protein isolates (Lo Verde et al., 2022) and cookies made with SFF (Pepra-Ameyaw et al., 2022). While this demonstrated that SFF can be used to produce non-green cookies while retaining beneficial phenolics, the questions of whether SFF cookies are appealing to consumers and whether they would accept enzymatically treated SFF cookies were left unanswered. Moreover, our previous studies did not examine if CGA esterase treatment has an effect on the cookie's sensory properties and composition. We hypothesized that the sensory characteristics and composition of treated cookies would be identical to non-treated cookies, with the exception of the cookies' color since CGA esterase is not expected to react with any other components in the flour other than CGA. We further hypothesized that color would be an important factor in consumer acceptance, with consumers preferring the CGA esterase-treated cookies to the green, non-treated cookies. Thus, the main objectives of this study were to compare consumer acceptance between enzymatically treated and

non-treated cookies using a nine-point hedonic scale, to examine the cookie's proximates, and to determine to what extent the color of cookies influenced consumer intent to purchase.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

Sunflower kernels (Lyric Wild Bird Food) were obtained in 2022 from Home Depot. Unsalted butter, eggs, light brown sugar, grade A maple syrup, baking soda, salt, and vanilla extract were purchased from local grocery stores. All reagents were ACS or HPLC grade and purchased from Thermo Fisher Scientific (Waltham, MA, USA) or Sigma (St. Louis, MO, USA). For baking, the following items were used: spatulas, mixing bowls, electric hand-mixer, weighing scale, measuring spoons, 1000  $\mu$ L pipettes and tips, metal whisks, parchment paper, baking trays, and a convection oven (Rational Commercial Cooking Appliance model no. SCC WE 61, Landsberg am Lech, Germany).

### 2.2 | Chlorogenic acid esterase preparation

*Lactobacillus helveticus* CGA esterase was expressed and purified according to procedures described in Lo Verde et al. (2022) with the modification that the enzyme was dialyzed into 20 mM potassium phosphate buffer, pH 8.0 prior to being used in baking as phosphate salts are generally recognized as safe for human consumption (21CFR182, n.d.). After being dialyzed, CGA esterase was sterile-filtered using a 0.2  $\mu$ m filter.

### 2.3 | Sunflower flour preparation

Cold-pressed SFM was prepared by modification of the method used by Pepra-Ameyaw et al. (2022). Sunflower seeds were ground using a coffee grinder (Model BCG111, KitchenAid Blade) in two 30 s increments to make SFM. Then, samples of 45–50 g of SFM were pressed in a Carver Hydraulic Press (Model 3912 3852–0; Wabash, IN, USA). In each round, the flour was brought to a pressure of 9000–10,000 psi and was allowed to slowly release to 0–1000 psi. This was completed four times before the sample was removed, re-crumbed with a knife into a powder inside the Carver cylinder, and then re-pressed for another two rounds. The final mass of each pressed SFM cake was measured to determine the percentage of extracted oil. The percent oil removed after six rounds represented at least 40% of the starting SFM weight. Cold-pressed sun-

flower cakes were then ground with a mortar and pestle and passed through a 500- $\mu$ m sieve to achieve a fine flour for baking.

### 2.4 | Sunflower flour cookie formulation, baking, and cookie storage

The formulation described in Pepra-Ameyaw et al. (2022) served as the basis for improvements. Initial, qualitative testing of untreated cookies by the study's researchers had indicated that the cookies were nutty, lacked sweetness, and were dry. Thus, ingredients were systematically replaced or added/removed, and cookies iteratively tasted qualitatively. This led to a formulation in which dairy butter was used instead of almond butter to decrease the ingredients that impart a nutty flavor. Furthermore, the amount of maple syrup was lowered, while light brown sugar was added to increase sweetness. The final cookies were thus formulated with cold-pressed SFF (39.0%), unsalted butter (21.6%), egg (13.5%), light brown sugar (13.4%), grade A maple syrup (10.8%), baking soda (0.6%), salt (0.6%), and vanilla extract (0.6%). The percentages in parentheses refer to the percentage of each ingredient in the final dough. For baking, all ingredients were brought to room temperature before being mixed together. For every batch, unsalted butter, brown sugar, and maple syrup were mixed using a hand mixer on low until combined. The sides of the bowl were scraped with a rubber spatula; then, egg and vanilla extract were added and mixed with a hand mixer on low for ~1 min until combined. In a separate bowl, SFF, baking soda, and salt were combined and mixed until they were homogenous. Then, 0.02 mg of CGA esterase per gram of flour was added to the wet ingredients and gently mixed. This was followed by the dry ingredients that were mixed into the wet ingredients by gently folding the dough with a rubber spatula until a well-combined and sticky dough formed. It should be noted that for one batch of cookie dough, no more than a total of 3 mL of the buffered enzyme was added to the wet ingredients to minimize dough stickiness. Dough was then placed in between two pieces of labeled parchment paper and flattened into a ~5.08 cm square, and then placed into a freezer for 10 min to harden slightly. After 10 min, the dough was placed into a refrigerator. The dough was rolled out to a thickness of 6.35 mm, cut out with a cookie cutter, and transferred directly to a baking tray. The cookies were baked at 177°C (350°F) for 8 min. The baking trays were rotated after 4 min to ensure even baking. Once done, the cookies were left to cool to room temperature on a baking sheet for at least 2 h before moving them. Cookies were then stored for 24 h at room temperature before sensory analysis. CGA esterase is denatured during baking since the baking temperature of 177°C (350°F) is well above the 65°C denaturation temper-

ature of CGA esterase (Lo Verde et al., 2022). The mass of the cookie was between 14 and 17 g.

## 2.5 | Proximates, water activity, texture analysis, and phenolic compounds

### 2.5.1 | Proximate analysis

Cookie samples were dried in a vacuum oven for 24 h at 70°C at a pressure of 25 bar. Moisture and crude fat extraction were determined as described in Pepra-Ameyaw et al. (2022). Samples ( $2 \pm 0.5$  g) were dry ashed in pre-dried porcelain crucibles for 6 h at 600°C in a Thermo Scientific Lindberg/Blue M Moldatherm Box Furnace (AOAC 923.03-1923). Nitrogen content was determined by Kjeltac™ 8100 Foss apparatus, and crude protein was calculated using a nitrogen factor of 6.25 noted in AOCS method Ai 4–91 (AOCS, 2017).

### 2.5.2 | Water activity, color, and texture analysis

Water activity was determined, as outlined by Pepra-Ameyaw et al. (2022). The internal colors of non-treated and esterase-treated cookies were measured at room temperature 24 h post-baking. White tiles were used for calibration before color measurement. The color (CIE  $L^*a^*b^*$  values) was determined using a CM-2500D spectrophotometer (Konica Minolta, Inc.) by averaging the color of three cookies per treatment.

A compression force test was conducted on the SFF cookies using a Stable Micro Systems Texture Analyzer (Model Plus Upgrade) with a trigger force of 15 g and a load cell of 50 kg. The following parameters were used: 0.5 mm/s pretest, test and post-test speed, and 3 mm target value as stated by AMETEK Brookfield, Inc. (Brookfield, 2019). The texture analyzer cycle speed of 0.5 mm/s, and a distance of 3 mm using a 2 mm diameter cylinder stainless probe were used. The average force was calculated using three cookies per treatment with four pseudoreplicate measurements per cookie.

### 2.5.3 | High-performance liquid chromatography (HPLC) and ATR-FTIR spectroscopy

High-performance liquid chromatography was conducted as outlined by Pepra-Ameyaw et al. (2022) with no

modifications. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was performed as described by Ishii et al. (2021) with no modifications using dehydrated-defatted SFF cookies that were finely ground with a mortar and pestle and passed through a 500- $\mu$ m sieve.

## 2.6 | Sensory analysis

### 2.6.1 | Panelist selection

The study was reviewed and approved by Chapman University Institutional Review Board (IRB# 23-14). Panelists from Chapman University and the local community were recruited through flyers, word of mouth, and in courses. Before participating in the study, informed consent was obtained from each subject. Participants were excluded if they had any food sensitivities (allergies or intolerances) and were not screened for regular cookie consumption. A total of 153 panelists participated in this study, of whom 39.2% were male, 59.5% were female, and 1.3% were non-binary. The age interval of panelists ranged from 18 to 64 years old, with 85.6% of participants aged 18–22, 5.9% were 23–29 years old, and 8.5% were 30 years old and above.

### 2.6.2 | Surveys used for sensory evaluation

The RedJade platform (<https://redjade.net/>) was utilized to electronically provide the questionnaire for all sensory evaluation tests, with each panelist having access to a computer at their designated station. At the beginning of the sensory evaluation test, panelists were shown the concept card (Figure 1) with characteristics of SFF and the nutritional label for the SFF cookie they would be consuming. Panelists were asked to indicate their acceptance of an SFF cookie based on the information on the concept card using a five-point scale from 1 (“would definitely not buy”) to 5 (“definitely would buy”).

A tetrad difference test, a hedonic rating test, and purchasing intent were used to evaluate treated and non-treated SFF cookies. For the tetrad difference test, the panelists were provided with four coded samples of cookies and were asked to group the cookies into two groups, placing two cookies in each group based on their evaluation of similarity between the cookies (Ennis, 2012). The panelists were not instructed in the questionnaire to consume the cookies to differentiate them. For the acceptance test, panelists were asked to evaluate the cookies' texture, smell, flavor, and overall acceptability using nine-point hedonic scale survey questions, where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. The purchase

Nutrition Facts	
servings per container	
<b>Serving size</b>	(23g)
Amount per serving	
<b>Calories</b>	<b>90</b>
% Daily Value*	
<b>Total Fat</b> 5g	6%
Saturated Fat 3g	15%
Trans Fat 0g	
<b>Cholesterol</b> 25mg	8%
<b>Sodium</b> 95mg	4%
<b>Total Carbohydrate</b> 8g	3%
Dietary Fiber 0g	0%
Total Sugars 4g	
Includes 4g Added Sugars	8%
<b>Protein</b> 5g	
Vitamin D 0mcg	0%
Calcium 15mg	2%
Iron 1mg	6%
Potassium 12mg	0%

\*The % Daily Value tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

**INGREDIENTS:** Sunflower seed flour, unsalted butter, egg, brown sugar, maple syrup, baking soda, salt, vanilla extract.

**CONTAINS:** Egg.

## Upcycled sunflower flour for use in baking



Sunflower seeds → Sunflower flour

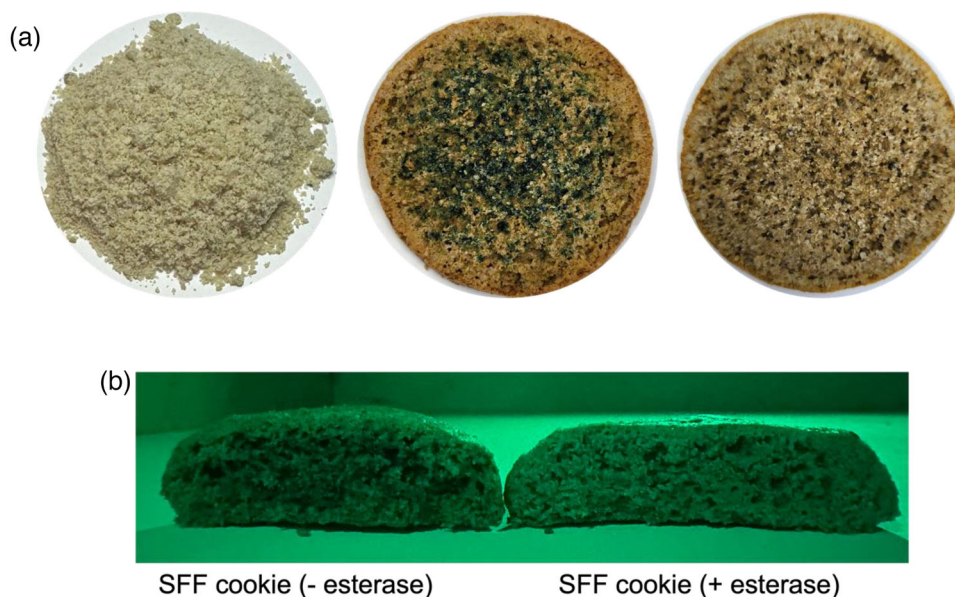
Sunflower flour is a byproduct of sunflower oil production and is mainly discarded or used as animal feed. However, sunflower flour is packed with nutrients and is allergen-free. Instead of discarding the flour, we are upcycling it to make baked foods such as the cookies you are about to taste.

## Benefits of sunflower flour

- High in protein
- High in Antioxidants
- Gluten & soy free
- Allergen-free
- Nut-free
- GMO free/Organic



**FIGURE 1** Concept card provided to the panelists at the beginning of the study. The nutrition label was made using Genesis R&D.



**FIGURE 2** (a) Sunflower flour (SFF) and sunflower flour cookies with and without esterase enzyme. (b) Cross section of a non-treated cookie (left) and enzymatically treated cookie (right) under green light, as panelists experienced during the sensory test. Although the green color of the non-treated cookie is not perceivable, the cookie is darker than the treated counterpart.

intent was evaluated at the end of the sensory evaluation test. After completing the tetrad test and the acceptance tests, panelists were also asked their purchase intent of the green non-treated SFF cookie and pale brown-treated SFF cookie using a five-point scale from 1 (“would definitely not purchase”) to 5 (“definitely would purchase”) after seeing photos of the green non-treated SFF cookie and pale brown-treated SFF cookie, which are shown in Figure 2a.

### 2.6.3 | Sensory station design

All evaluations were carried out in a testing room where a total of 14 individual panelist stations were set up. Each station had a three-walled cardboard booth where the samples were placed. A computer was located outside of the booth for panelists to fill out the questionnaire in Red-Jade. The test room was completely dark, except for a single

green light located in each testing booth. As each panelist entered the room, they were provided a four-digit panelist code that they input into their station computer before beginning the test. Prior to sensory testing, each cookie was cut in half vertically (Figure 2b), and half a cookie was placed in each cup, before sealing it with a lid. To begin the tetrad test, four 2 oz, clear containers labeled with different three-digit codes were provided to panelists. Two of cups contained enzymatically treated cookies, and the other two cups contained non-treated (control) cookies.

Once the tetrad test was completed, the four cookies were taken away, and panelists were instructed to cleanse their palate with water and a non-salted cracker before beginning the hedonic test. For the hedonic test, each panelist evaluated one treated and one non-treated cookie, provided in a random order to each panelist. Lastly, panelists completed an intent-to-purchase questionnaire. Panelists were then shown a side-by-side picture of the non-treated green cookie and the treated brown cookie and asked to rate how likely they would buy either cookie.

## 2.7 | Statistical analysis

All statistical analyses were conducted using R studio (R Core Team, 2022). Data for the tetrad test were analyzed through a binomial exact test to determine if the proportion of the correct grouping was significantly greater than 1/3. A chi-squared test of proportion was used to determine if there were statistically significant differences in panelists' likelihood of purchasing the treated and non-treated cookies based on the concept card and based on the intent to purchase questionnaire. A chi-squared test of independence was used to determine if there were associations between consumer ratings of characteristics and cookie type (enzymatically treated and non-treated cookies). Throughout the study, a test was considered statistically significant if  $p$ -value was  $<0.05$  level of significance.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Chlorogenic acid hydrolysis and prevention of greening in SFF cookies

The physical properties of the SFF used are listed in Table 1. To hydrolyze CGA, cookie dough was treated with CGA esterase from *L. helveticus* as described by Pepra-Ameyaw et al. (2022). Internal greening in the cookies was measured 24 h post-baking. Enzymatic mitigation of greening was evidenced by significantly higher CIE  $a^*$  values in

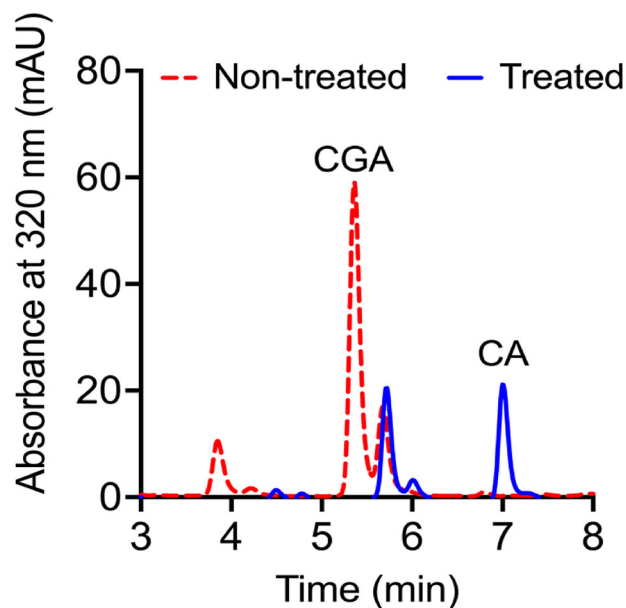


FIGURE 3 High performance Liquid chromatograms of cookies formulated with and without chlorogenic acid (CGA) esterase.

esterase-treated cookies, as higher  $a^*$  values indicate less greening (Figure 2a and Table 1). These results were validated by HPLC analysis that measured the concentrations of CGA and CA in cookies (Figure 3). The esterase-treated cookies had approximately 90% less CGA than non-treated cookies. These results are similar to those described by Pepra-Ameyaw et al. (2022), further indicating that CGA esterase is an effective way of hydrolyzing CGA in a cookie matrix.

### 3.2 | Proximate and textural analysis of SFF and SFF cookies

The proximate composition of treated cookies, non-treated cookies, and SFF is shown in Table 1. The data indicate that moisture, protein, carbohydrate, ash, lipid, and texture (hardness and fracturability) between esterase-treated and non-treated cookies were not statistically significant. As expected, the addition of CGA esterase to the SFF neither affected the macronutrient composition of the flour nor the texture of the cookies.

After the removal of approximately 42% of the total weight as fat during cold pressing, the lipid content of SFF after Soxhlet extraction was  $7.56 \pm 0.64\%$ . As shown in Table 1, the protein content for SFF was  $35.69 \pm 0.31\%$ . The protein content was higher than that found (27.80%) by de Oliveira Filho (2021) and is higher than that of wheat flour which contains about 11.50% protein (Man et al., 2017). The protein content for treated and



**TABLE 1** Effect of chlorogenic acid (CGA) esterase on proximates, texture, color, pH, and water activity.

	Non-treated cookies	Treated cookies	Sunflower flour
Color			
<i>L</i> *	35.55 ± 4.29	46.76 ± 0.47	82.94 ± 1.26
<i>a</i> *	-2.40 ± 2.93	6.77 ± 0.27	1.33 ± 0.09
<i>b</i> *	13.10 ± 3.78	20.13 ± 0.80	10.42 ± 0.54
Phenolic content (mg/g flour)			
Chlorogenic acid	1.00 ± 0.04	0.13 ± 0.02	2.35 ± 0.24
Caffeic acid	bdl**	1.40 ± 0.13	bdl**
Water activity	0.54 ± 0.01	0.55 ± 0.01	0.45 ± 0.01
pH	7.69 ± 0.01	7.18 ± 0.02	n/a
Proximates (%)			
Moisture	7.12 ± 0.14	7.44 ± 0.09	7.32 ± 0.01
Lipid	23.03 ± 0.83	22.73 ± 0.14	7.56 ± 0.64
Protein	21.13 ± 3.80	20.80 ± 1.66	35.69 ± 0.31
Ash	3.51 ± 0.53	3.88 ± 0.01	6.05 ± 0.27
Carbohydrate	45.21	45.15	43.38
Texture			
Hardness	1373.19 ± 46.04	1332.65 ± 21.52	n/a
Fracturability	1378.79 ± 20.70	1367.49 ± 18.94	n/a

\*\*Below detection limit

non-treated cookies was  $20.80 \pm 1.66$  and  $21.13 \pm 3.80$ , respectively.

Since Liang and Were (2018) showed high water activity and pH increase greening, they were also measured. Water activity was not significantly different in treated and non-treated cookies; however, the pH of esterase-treated cookies was lower by about 0.5 pH units, which is within the pH difference range reported in Pepra-Ameyaw et al. (2022) between enzymatically treated and non-treated cookies. CGA esterase-treated cookies are slightly less basic than non-treated cookies, most likely because CGA hydrolysis produces two acidic products, quinic acid and caffeic acid, which have pKa values of 3.4 and 4.5, respectively. In contrast, CGA has a single acidic pKa of 3.6 (Kabir et al., 2014). While esterase-treated cookies were less basic than non-treated cookies, our previous work demonstrated that these small pH differences do not noticeably influence the greening, as there is greening in cookies at pH as low as 6.5 (Pepra-Ameyaw et al., 2022).

### 3.3 | FTIR of ground-up cookies

The structural properties of the macromolecules within the cookies were analyzed by ATR-FTIR (Ishii et al., 2021). The ATR-FTIR spectra of esterase-treated and non-treated cookies are shown in Figure 4. The spectra are indistinguishable, indicating that esterase treatment does not alter the structure of lipids, proteins, or carbohydrates within

the cookie. These data confirm previous results that *L. helveticus* CGA esterase is specific and does not act on biomolecules other than CGA to a noticeable degree (Lo Verde et al., 2022).

### 3.4 | Concept card

One hundred fifty-three untrained panelists participated in the study. Initially, panelists responded to a concept card describing the benefits of using SFF in baking (Figure 1). This allowed us to gauge the initial consumer interest in cookies formulated with SFF. The responses from the concept card indicated that 40.9% of consumers would either “probably buy” or “definitely buy” an SFF cookie based on the nutritional information and/or the idea of “upcycling” (Figure 5). Overall, the average rating was  $3.27 \pm 0.88$  on a five-point scale. We did not, however, study which concept was the most important to panelists as all the attributes were placed within the same concept card. We realize that it would have been possible to determine the motivation behind panelists’ decisions by providing one concept at a time as done by other researchers (Levis & Chambers, 1997; Mohayidin & Kamarulzaman, 2014). However, such an investigation would not have been insightful in the current study since our exclusion criteria omitted any panelists with food sensitivities (allergies or intolerances to gluten, egg, etc.). This means that we would not have been able to gauge interest in SFF as a baking ingredient from this

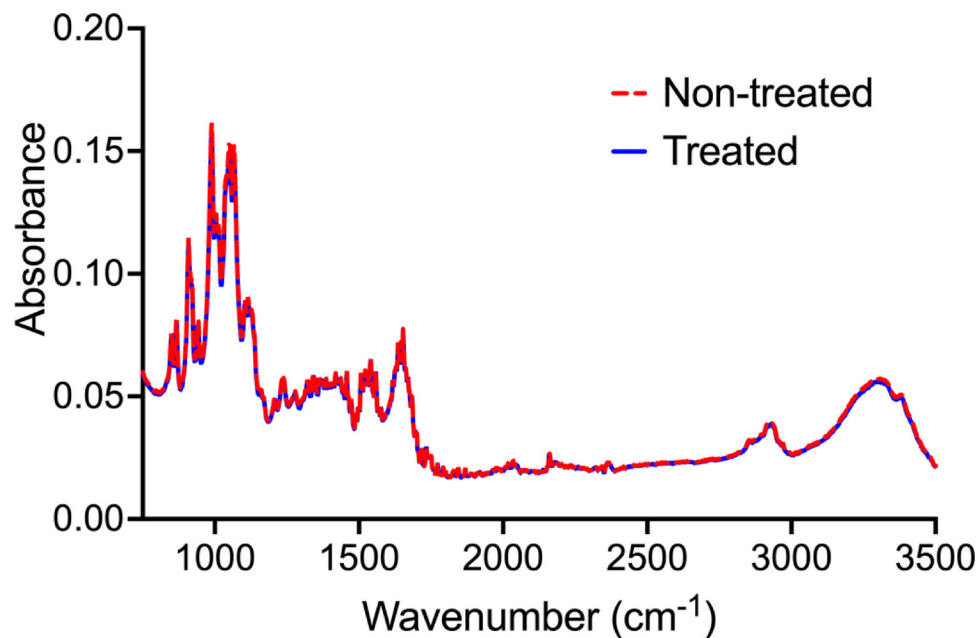


FIGURE 4 Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra of non-enzymatically treated and enzymatically treated cookies made with sunflower.

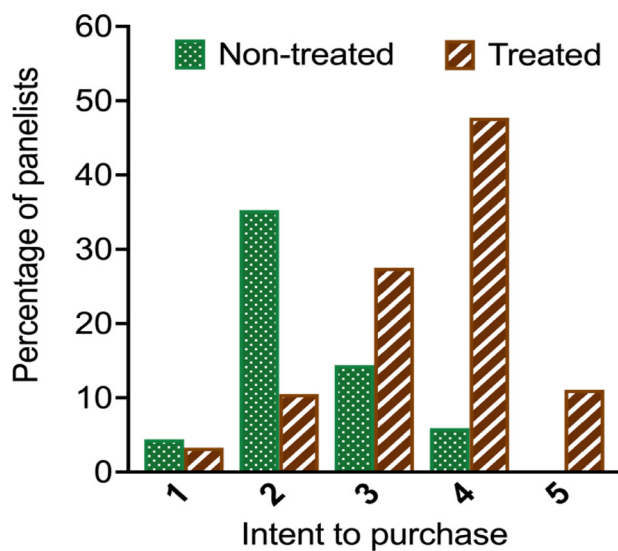


FIGURE 5 Panelists' responses to the concept card. Panelists rated their intent to purchase on a five-point scale where 1 = "definitely would not buy," 2 = "probably would not buy," 3 = "might or might not buy," 4 = "probably would buy," and 5 = "definitely would buy."

key demographic group, who may find certain attributes in the concept card, for example, being gluten- and soy-free, particularly important.

### 3.5 | Tetrad discrimination

The tetrad discrimination test assessed panelists' ability to differentiate between treated and non-treated SFF cook-

ies based on sensory characteristics other than color. The test was conducted under green light so that panelists could not perceive the green color of the non-treated cookie (Figure 2b). The tetrad results demonstrate that 42.5% of panelists correctly paired the treated and non-treated cookies. The percentage of correct grouping was significantly greater than 33.3% ( $p$ -value = 0.01) using the binomial exact test, indicating that panelists could discriminate between the treated and non-treated cookies. This result was unexpected since green lighting was used. These results are most likely explained by the fact that the interior of non-treated cookies was darker than that of enzymatically treated cookies (Figure 2b), allowing some participants to group cookies correctly. Panelists were not given any instructions on which sensory characteristics to use to group samples together since this may create an expectation that samples may be different (Ennis, 2012). Therefore, panelists may have compared the cookies differently (i.e., some panelists may have grouped cookies based on appearance, whereas others may have grouped them based on a combination of factors). We do not believe that comparison testing in the tetrad test influenced panelists in the subsequent hedonic test since they were not given information on what samples they were comparing so they would not have been able to infer whether samples given to them in the tetrad and hedonic tests are the same.

### 3.6 | Consumer acceptance

A nine-point hedonic rating test was used to determine panelists' liking of enzymatically treated versus non-

**TABLE 2** Hedonic test results for non-treated and treated sunflower flour cookies.

	Smell		Texture		Flavor		Overall acceptability	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
1 = Dislike extremely	1.31%	1.31%	0.65%	1.96%	1.31%	0.65%	0.65%	0.65%
2 = Dislike very much	0.00%	1.31%	1.31%	1.31%	0.65%	0.00%	0.65%	0.65%
3 = Dislike moderately	0.65%	0.00%	7.84%	7.19%	5.88%	5.23%	3.92%	4.58%
4 = Dislike slightly	11.11%	3.92%	18.95%	15.69%	15.03%	10.46%	15.03%	7.84%
5 = Neither like nor dislike	13.07%	13.73%	9.80%	6.54%	14.38%	9.15%	13.73%	10.46%
6 = Like slightly	22.22%	22.22%	18.95%	20.92%	21.57%	32.03%	22.22%	24.84%
7 = Like moderately	24.84%	29.41%	26.14%	24.18%	27.45%	26.80%	29.41%	30.72%
8 = Like very much	24.84%	23.53%	13.07%	18.30%	12.42%	11.11%	13.07%	17.65%
9 = Like extremely	1.96%	4.58%	3.27%	3.92%	1.31%	4.58%	1.31%	2.61%
Chi-squared test of independence (p-value)	0.23		0.84		0.23		0.63	

Note: The values are percentages (%) out of the 153 responses for each of the nine-point hedonic scale.

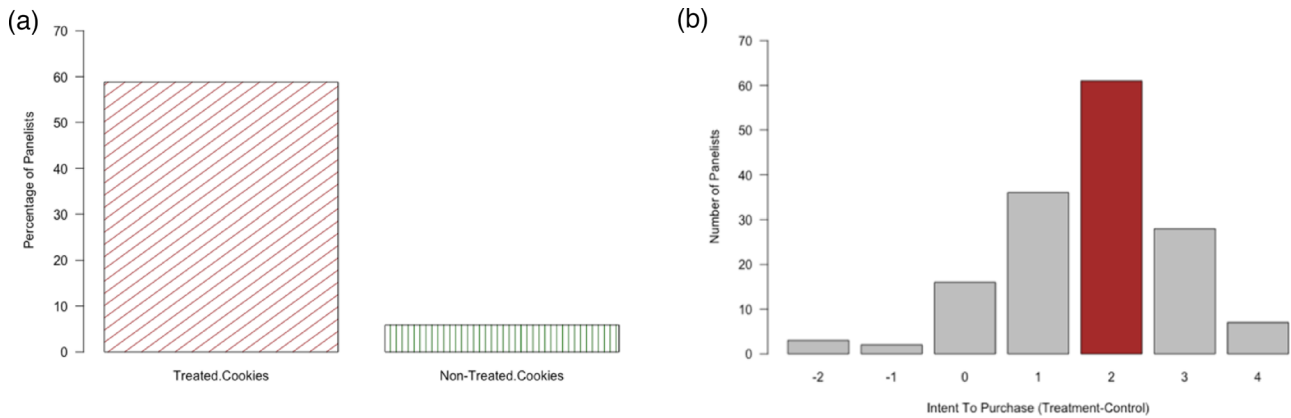
treated cookies. The recipes for the non-treated and treated cookies were identical, except for adding 0.02 mg of enzyme per gram of flour in treated cookies. Table 2 shows the distribution (in terms of percentage) of panelists' responses to how much they like or dislike the smell, texture, and flavor and the overall acceptance of the cookie. We observe in Table 2 that the distribution of the percentage of panelists who like or dislike the sensory properties of the cookies was similar for both esterase-treated and non-treated cookies. For instance, about 74% of the panelists gave a rating of "6 = like slightly" to "9 = like extremely" for the smell of non-treated cookies, while about 80% of the panelist gave a rating of "6 = like slightly" to "9 = like extremely" for the smell of the esterase-treated cookie. On the other hand, for the texture of the cookie, about 29% rated the non-treated cookie as "1 = dislike extremely" to "4 = dislike slightly," while 26% rated the enzymatically treated cookie as "1 = dislike extremely" to "4 = dislike slightly." This led to the hypothesis that the panelists do not like one cookie more than the other and that, generally, the two types of cookies received indistinguishable acceptance ratings. To verify this hypothesis, we used a chi-squared test of independence to determine if there was an association between the consumers' ratings and the type of cookie for each sensory characteristic. This test was used since the type of cookies and the nine-point hedonic scale are both categorical variables and may not meet the normality assumption needed for a *t*-test (Lim, 2011; Voong et al., 2019). The results of the chi-squared tests of independence (Table 2) supported our hypothesis that the panelists' responses in their liking of flavor, texture, smell, and overall acceptability are independent of the type of cookie they consumed ( $p > 0.05$ ). This suggests that the panelists like treated and untreated cookies equally. Fur-

thermore, these results strongly suggest that the difference identified in the tetrad test (Section 3.5) did not influence participants' ratings.

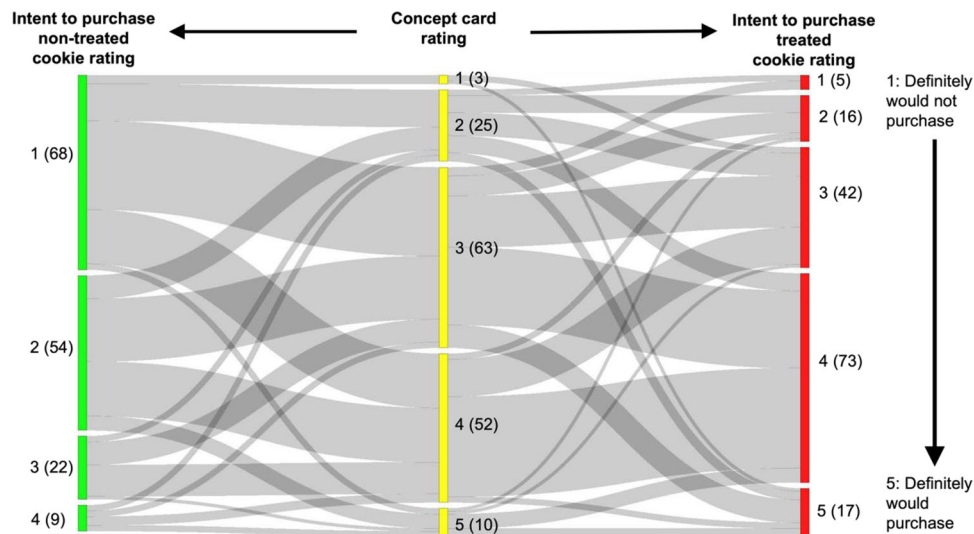
The hedonic rating test results for texture are consistent with texture analyzer measurements, which determined that the enzyme did not impact hardness and fracturability (Table 1). Furthermore, these results are consistent with the proximates and ATR-FTIR measurements suggesting that CGA esterase does not participate in side reactions with other macromolecules that could negatively affect the cookie's sensory properties. Overall, these results confirm that enzymatic treatment does not influence consumer perceptions of the cookie regarding texture, flavor, smell, and overall acceptability.

### 3.7 | Intent to purchase

The main goal of this study was to determine if greenening prevention by CGA esterase in SFF cookies affected a consumer's intent to purchase based on cookie color. Figure 5 indicates that color influences purchasing intent, as 79.7% of panelists stated that they "probably would not" or "definitely would not" purchase untreated, green-colored cookies. In contrast to untreated cookies, only 13.7% of panelists "probably would not" or "definitely would not" buy the enzymatically treated, pale brown colored cookies. Furthermore, 58.8% of panelists indicated that they "probably would purchase" or "definitely would purchase" esterase-treated SFF cookies, while only 5.9% of panelists indicated that they "probably would purchase" or "definitely would purchase" the non-treated SFF cookies (Figure 6a). A test of equality of two proportions showed that the proportion of panelists that "probably would" or



**FIGURE 6** (a) Percentage of panelists who responded either “probably would purchase” or “definitely would purchase” on the intent-to-purchase question after seeing the photos of the treated and the non-treated cookies. (b) Distribution of differences in intent-to-purchase responses for the treated (esterase-treated cookies) and the control (non-treated cookies). A difference of 2 (maroon colored bar) was the most common difference in the intent-to-purchase responses for the treated and for the non-treated cookies.



**FIGURE 7** Sankey plot showing the shift in panelists' rating between the concept card and the treated and non-treated cookies. The breakdown of concept card responses is shown in the center in yellow. The gray lines depict shifts in rating between the concept card and non-treated cookies on the left and between concept card and treated cookies on the right. The width of the line corresponds to the number of panelists who shifted their opinion. The numbers in parentheses represent the number of panelists who chose that ranking. The five-point scale provided for both concept card and “intent to purchase” is as follows: 1 = “definitely would not buy,” 2 = “probably would not buy,” 3 = “might or might not buy,” 4 = “probably would buy,” and 5 = “definitely would buy.” The number in parentheses represents the number of panelists in a specific category.

“definitely would” purchase esterase-treated SFF cookies is significantly greater than the proportion of panelists that “probably would” or “definitely would” purchase the green non-treated SFF cookies ( $p < 0.05$ ). These data strongly suggest that enzymatic hydrolysis of CGA in SFF increases general consumer acceptability.

We were also interested in understanding how the initial concept card responses compared to the responses once panelists had tasted and seen the color of the cookies.

Figure 7 (Sankey plot) shows the shift in each panelists' rating between the concept card and the intent to purchase for treated and non-treated cookies. For non-treated cookies, the intent to purchase declined for 81.7% (125 out of 153) of panelists after seeing and tasting the untreated cookies, while 3.9% (six out of 153) of panelists had an increased intent to purchase rating. This suggests that although panelists may be interested in SFF cookies based on their nutritional value and/or the idea of “upcycling,” the green

color deters them from purchasing. In contrast, for the treated cookies, the intent to purchase decline was only 22.2% (34 out of 153), whereas 34.6% (53 out of 153 panelists) were more inclined to purchase the enzymatically treated SFF cookies after tasting and seeing them. This indicates that for treated cookies, the intent to purchase increased by a net of 12.4% ( $= 34.6\% - 22.2\%$ ) by the end of the sensory test.

We next tracked how individual panelist's intent to purchase differed between the non-treated and treated cookies. To do so, we viewed the five category responses for the intent-to-purchase questionnaire as numerical ratings, with 1 representing "would definitely not purchase" to 5 representing "definitely would purchase." The average rating for all panelists of the intent to purchase was  $3.53 \pm 0.94$  for enzymatically treated cookies, whereas the non-treated cookies' average rating was  $1.82 \pm 0.89$ . We then took the differences between the intent-to-purchase responses for the treated cookie and the intent-to-purchase for the non-treated cookie for each panelist. For example, if a panelist rated treated cookies as a "definitely would" purchase (5) and the non-treated cookie as a "may or may not" purchase (3), the difference would be 2. Figure 6b shows that the most common difference (40% of panelists) in intent-to-purchase ratings between the treated and non-treated cookies was 2. Since hedonic testing results revealed no difference in rating between treated and untreated cookies, it can be concluded that cookie color influences a consumer's preference, on average, by 2 points on a five-point scale.

This study is, to the best of our knowledge, the first that investigates the sensory properties of baked SFF products in which wheat flour was fully replaced with SFF. We are, nevertheless, able to compare our results with work on partial SFF substitution in baked and cooked foods. Hedonic testing by Nemš et al. (2022) who made up to 30% SFF substituted cookies indicated that 30% SFF substitution lowered overall acceptability and color scores by approximately 1 and 4 points on a nine-point hedonic scale, respectively, compared to wheat flour cookies. Similarly, research on pasta demonstrated that substituting wheat with 3%–9% sunflower meal resulted in a darker pasta. Darkening was dependent on sunflower meal concentration and correlated with lower sensory scores with respect to color (Grasso et al., 2021; Zaky et al., 2022). These results are consistent with our observations that panelist acceptance dropped in untreated cookies after the cookie color was revealed and suggest that effective greening prevention will be essential to enable the utilization of sunflower meal in foods where a light color is desired.

## 4 | LIMITATIONS AND FUTURE DIRECTIONS

As mentioned in Section 3.5, it is possible to undertake a more detailed investigation of consumer's interest in SFF products. A check-all-that-apply ballot at the end of the sensory test may provide information on which attribute is most important to panelists. Such a test should furthermore include panelists with food sensitivities (food intolerances or allergies) to compare if consumer ratings differ between panelists with and without food sensitivities.

Furthermore, research efforts should be devoted to testing utilization of SFF in other baking formulations and non-alkaline processing conditions. Most research, to date, has focused on partial replacement of wheat flour with SFF (Nemš et al., 2022, Zaky et al., 2022). While adding sunflower would enhance the fiber, protein and antioxidant content compared to wheat-only foods, such products will not be beneficial to people with wheat sensitivities. We hypothesize that SFF could act as an antioxidant-rich component in gluten-free compound flours where it can be mixed with other nonallergenic flours (Beltrão Martins et al., 2020; Sakač et al., 2011).

## 5 | CONCLUSION

We concluded from the sensory and proximate analyses that treatment of SFF with CGA esterase improved consumer acceptance of SFF cookies. This improvement was entirely due to CGA esterase treatment that resulted in non-green cookies as there were no sensory or compositional differences aside from color. Based on initial concept card responses, we conclude that SFF is a potentially attractive alternative protein source and ingredient in baking. Esterase treatment further enhanced the desirability of SFF, as consumers rated cookies higher after comparing the color differences. This research demonstrates the utility of CGA esterase in foods formulated or processed under alkaline conditions and provides a new method for "upcycling" SFF. As such, this research adds to the evidence that food side streams are an excellent but underutilized source for nutritious and useful baking materials. Side stream utilization is often hindered by negative sensory properties of the resultant ingredients. Enzymatic treatment is being increasingly used to enhance the properties of side streams and create upcycled ingredients (Hoang et al., 2022; Nguyen et al., 2021). The physical properties of CGA esterase would make this enzyme well suited for commercial application since it is stable and displays com-

parable activity similar to other enzymes that are currently used in the food industry (Lo Verde et al., 2022).

## AUTHOR CONTRIBUTIONS

**Christine Lo Verde:** Investigation; Methodology; Writing—original draft; Writing—review and editing; Visualization. **Criselda Toto Pacioles:** Software; Methodology; Validation; Visualization; Formal analysis. **Natalie Paterson:** Investigation. **Jamie Chin:** Investigation. **Cedric P. Owens:** Conceptualization; Investigation; Funding acquisition; Writing—review and editing; Project administration; Supervision. **Lilian W. Senger:** Conceptualization; Investigation; Funding acquisition; Writing—review and editing; Project administration; Supervision.

## ACKNOWLEDGMENTS

The authors would like to thank Dr. Pepra-Ameyaw for assistance with the proximate analysis, Charlie Drucker for help in making figures, Kellie Omori, Mitch Underdahl, Alex Mayorga, Josh Arellano, Nate Carl for helping set up the sensory lab, and all the panelists for participating in the sensory panel. We would also like to thank the reviewers for useful suggestions. This work was funded by a USDA-NIFA Novel Foods and Innovative Manufacturing Technologies grant 2020-67018-31261 to Lilian W. Senger and Cedric P. Owens and a Research Corporation for the Advancement of Science Cottrell Scholars Award to Cedric P. Owens.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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**How to cite this article:** Verde, C. L., Pacioles, C. T., Paterson, N., Chin, J., Owens, C. P., & Senger, L. W. (2023). Hydrolysis of chlorogenic acid in sunflower flour increases consumer acceptability of sunflower flour cookies by improving cookie color. *Journal of Food Science*, 1–13. <https://doi.org/10.1111/1750-3841.16692>