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Accepted Article

Acidulant effect on greening, reducing capacity, and tryptophan fluorescence of sunflower butter cookie dough during refrigerated storage

Running title Acidulant effect on greening and oxidation in sunflower butter cookie dough

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Abstract

BACKGROUND: Sunflower seed derived butter can be a source of protein and phenolic antioxidants in refrigerated dough. Chlorogenic quinone-amino acid induced greening can however occur at alkaline pH, which could result in less bioavailable conjugated phenol-amino acids. Acidulants were tested as potential anti-greening ingredients in refrigerated chemically leavened cookie dough. Effect of refrigerated storage time, leavening agents and acidulants on tryptophan fluorescence ($\lambda_{\text{ex}}=280\text{nm}$, $\lambda_{\text{em}}=300\text{-}500$), color (hunter LAB), reducing capacity (DPPH and Folin-Ciocalteu reagent reducing capacity/FCRC), and hydroxycinnamic acids were measured.

RESULTS: The pH range of acidified doughs was 4.83–6.98 compared to 7.65–9.18 in non-acidified leavened doughs after 24 days. Greening was higher in baking soda dough control ($a^*=-0.54$) than baking powder dough control ($a^*=2.98$) after 24 days, attributed to higher pH (9.18) of the former compared to pH 7.14 in the later. Tryptophan fluorescence intensity in baking soda dough decreased in the order: control > glucono-delta lactone H citric acid after 24 days. The DPPH and FCRC of acidified doughs were greater than corresponding control doughs.

CONCLUSION: The use of acidulants would prevent greening in sunflower dough without lowering its phenolic concentration, making use of sunflower butter in refrigerated dough for baked goods feasible.

Keywords: acidulants; baking soda; greening; hydroxycinnamic acids; sunflower butter; tryptophan

INTRODUCTION

Sunflower seed derived spreads and butter can be a source of protein and phenolic antioxidants in refrigerated dough. The protein content of sunflower butter (18g/100g) is similar to the estimated 21.88g protein /100g of peanut butter¹. However, peanuts are a source of major allergens in the West, and the leading cause of food allergenic death by anaphylaxis². Sunflower butter could thus be an alternative to peanut butter cookie dough for those with peanut sensitivities.

Sunflower seeds used to make butter contain 0.29 g/Kg-0.42 g/Kg and 0.004-0.009 g/Kg phenolic compounds in dehulled kernels and shells respectively. Chlorogenic acid (CGA) and its isomers 3- and 4 caffeoylquinic acids represent 61–93% of the total phenol content in sunflower kernels³. Interaction between phenolic compounds such as CGA and limiting amino acids such as lysine, methionine, and tryptophan at high pH can decrease free amino acid content and hence nutritional value of sunflower protein⁴⁻⁶. Chlorogenic acid (CGA) is oxidized to highly reactive electron rich quinone dimers at alkaline pH. These quinones bind to protein to form green pigments in the presence of oxygen⁷⁻⁹.

Acidulants were hypothesized to prevent greening in chemically leavened sunflower dough and increase free phenolic compounds. Baking acidulants like citric acid and glucono delta lactone (GDL) can react with leavening agents in aqueous solutions to improve dough leavening by CO₂ production¹⁰. Non-green refrigerated sunflower dough could be a phenolic rich alternative to ready to bake peanut dough since peanuts contains 2.42E-03g/Kg chlorogenic

acid, which is about 100 times lower than the chlorogenic acid (0.24-0.28g/Kg) content in sunflower kernel ^{3,11}.

Yabuta et al.,⁹ showed that ascorbic acid reversed the green pigmentation in chlorogenic acid-protein solutions to yellow, making acidulants potentially suitable for preventing or lowering greening in sunflower cookie dough. Despite a desire for alternative non-allergenic proteins, few studies have been published on ready to bake sunflower butter cookie dough⁸. This study was designed to analyze the effect of glucono delta lactone and citric acid on (i) greening (ii) hydroxycinnamic acid content and (iii) associated antioxidant capacity and tryptophan of sunflower butter cookie dough during 24 days of refrigerated storage at 8°C.

EXPERIMENTAL

Experimental design and sunflower butter cookie dough preparation

Two batches of sunflower cookie dough totaling 250 g for each treatment were prepared as described by Liang and Were⁸ with the following modifications: Leavening agents used were 1.5g of baking soda (Trader Joe's, USA) or baking powder (Clabber girl baking powder, IN, USA; UPC: 019900003332), xanthan gum (1.75g) added as recommended by Simsek¹². The acidulants: citric acid (0.522g) and GDL (1.154g) were added to dough except in the controls. The amount of leavening acids added was a function of the neutralization value of each acidulant ¹⁰.

$$\text{Mass of acid (g)} = \frac{\text{mass of leavening agent (g)}}{\text{Neutralization value}} * 100 + 0.3g \quad \text{Equation 1}$$

where leavening agent was either baking soda (NaHCO_3) or baking powder composed of NaHCO_3 , SALP and filler starch (universal product code: 041617007501). The neutralization value (amount of acid needed to neutralize bicarbonate) of citric acid and GDL was 159 and 52 respectively. Additional 0.3g of each acid from preliminary physical observation of dough was added to each acidified dough treatment to compensate for the alkaline pH of maple syrup. Another control without leavening agents and acidulant was used to assess the effect of both leavening agents and acidulants on tryptophan fluorescence intensity.

Color and pH changes of sunflower cookie dough

Dough samples (50g) were weighed into plastic cups and the surface color (hunter L^* , a^* , b^*) was measured as outlined by Liang and Were⁸. To determine pH, distilled and deionized water (DDW) was boiled to 100°C for 20 min and cooled to room temperature to eliminate carbon dioxide from water. Dough samples (0.6 g) were dissolved in 10.0 mL DDW and homogenized using a ProScientific, Multi-prep Homogenizer 01-01620 (USA) for 180 sec at 11963* g. The homogenized samples were incubated for 120 mins at room temperature, centrifuged at 4000 g for 20 mins (Fisher, AccuSpin IR centrifuge) before pH of each sample supernatant was measured using a LabQuest[®] 2 pH meter (Vernier Software & Technology).

Phenolic compounds, Folin-Ciocalteu and DPPH reducing capacity of sunflower cookie dough

Dough (0.6g) in 10ml methanol (Sigma-Aldrich, USA) was homogenized with a ProScientific, multi-prep homogenizer. Mixtures were then incubated for 12h at 8 °C and

centrifuged at 4000* g for 20 min. The supernatant was used for hydroxycinnamic acid and reducing capacity measurements.

Hydroxycinnamic (HA) compounds of 1:7 dough: methanolic solutions were quantified by measuring absorbance at 320nm in quartz cuvettes using a USB-650 Red tide Spectrometer (Ocean optics, Winter Park, FL USA) against CGA concentrations of 0.086- 0.69mM.

The Folin-Ciocalteu reducing capacity of 1ml of 6% methanolic extract of cookie dough was determined as described by Liang and Were⁷. Radical-scavenging capacity of dough supernatants after 1, 4, 12 and 24 days of refrigerated storage was also measured against 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical (Sigma-Aldrich, USA)¹³.

Tryptophan fluorescence in sunflower cookie dough

Tryptophan fluorescence (λ_{EX} 280nm, λ_{EM} 300-500nm) was determined as described by Liang and Were⁷ using a Fluoromax-4 Spectrofluorometer (Horiba Scientific, NJ, USA). A 1:19 dough: DDW, and 1:19 solutions of the major contributors of Trp: wheat flour, sunflower butter and egg ingredients were prepared. Next, Trp FI at λ_{max} of 359nm for egg (1.23×10^7), sunflower butter (1.98×10^6) and wheat (3.12×10^6) was multiplied by 39.4%, 24.3% and 13% respectively as these were the percentage of these ingredients in dough to estimate their contribution to FI in dough. The Trp fluorescence from sunflower butter dough without leavening was 4.8×10^6 . To determine which acidulant quenched Trp most, the Trp FI was also recorded at 359nm after increasing volumes of each acidulant was added to pure Trp solution.

Since FI of dough without leavening was 4.8×10^6 , a Trp solution was prepared to have a similar FI as the Trp FI of unleavened control dough. The Trp solution was diluted till the FI was

close to 4.8×10^6 and the concentration of this solution was 2.45mM. The FI of the 2.45mM L-tryptophan (Sigma- Aldrich, USA) solution was 4.29×10^6 CPS. This was used to evaluate changes in Trp FI with acidulants prepared at 9.23–10mM to correspond to the percentage of acid in dough. The FI and pH of acidulant-tryptophan solution were recorded after incremental amounts (0-200 μ l) of acidulant solution were added to Trp (Table 3).

Statistical Analysis

Two batches of dough with 2 acidulants (citric acid and glucono-delta lactone) and 2 leavening agents (baking powder and baking soda dough leavened) were used to assess greening prevention and oxidation stability at 4 storage times (1, 4, 12, and 24 days) at 8 °C. Changes in dough color, pH, phenolic content, Folin-Ciocalteu, DPPH reducing capacity, and tryptophan were determined by analysis of variance (ANOVA) followed by post hoc LSD test using SAS statistical analysis software¹⁴. The level of significance used was $\pm = 0.05$. Correlations between Folin-Ciocalteu reducing capacity, phenols, greening (a^*) and pH were also determined.

RESULTS AND DISCUSSION

Sunflower cookie dough color, and pH changes as a function of acidulants and storage time

Baking powder leavened dough had higher a^* values (Figure 1) attributed to its lower pH than baking soda leavened dough with higher pH and lower a^* values. Baking soda control had the lowest a^* value on all days. Light greening in baking soda control (BSC) was observed within 4-6 hours, and its pH was 7.65 on day 1. The a^* value of BSC decreased while its pH increased during refrigeration with a final pH of 9.18 on day 24. Liang & Were⁸ reported that alkaline pH favored greening in cookies formulated with baking soda. Sodium bicarbonate

(baking soda) in the presence of heat produces CO₂ and sodium carbonate, which has a higher pH than sodium bicarbonate and accounts for increased pH with time¹⁵. Formation of sodium carbonate with accompanying pH increase usually occurs with heating. Our results however showed that long term storage of dough was also accompanied by greening and pH increase at refrigerated storage. Increased dough volume was however not measured over time to determine CO₂ production. This would be further confirmation that the pH increase was due to baking soda producing CO₂ and sodium carbonate during refrigerated storage.

The increased pH of BSC resulted in a 62% increase in greening (-a*) after 12 days because alkaline pH favors green pigment formation (Table 1, Figure 1, Table S1). This green pigment has been identified to be a trihydroxybenzocridine (TBA) derivative, which is produced when oxidized dimers of CGA react with most amino acids, except cysteine, tryptophan, proline, serine, and threonine^{9, 16}. As expected, added acids lowered pH of both baking soda and baking powder acidified doughs to prevent greening. The decrease in pH was due to intrinsic differences in acidulants. These acidulants can also produce carbonic acid when acids react with bicarbonate, but carbonic acid can further degrade to CO₂ and water¹⁷. The pH of acidified baking soda dough ranged from 6.32-6.79 on day 1 and decreased to 5.53-6.98 on day 24. In both baking powder and baking soda leavened doughs, citric acid lowered pH more than GDL. The parts by weight of each acid needed to neutralize available bicarbonate depends on the reactivity of the acidulant, so residual acid would decrease pH in the dough but if no acid is present, then pH increases overtime.

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Unlike BSC, acidified doughs did not green with time (a^* of 3.17 to 4.27) except for baking soda leavened dough with GDL (GS) whose a^* decreased from 3.95 to 1.54 on day 24 resulting in slight greening in GS dough (Figure 1, Table S1). Slight greening was observed in GS. The greening observed on day 24 in GS, was from slow GDL hydrolysis during refrigeration. GDL hydrolysis to gluconic acid increases with temperature (20-70°C)¹⁸, and slower hydrolysis could thus have occurred at refrigeration temperature. The pH remained close to neutral (6.6 - 6.9) on days 12 and 24, which could also account for decrease a^* and some greening in GS dough (Figure 1; Table S1). The increase in greening may have been faster than the pH lowering when GDL was added in baking soda dough, resulting in the same sample (GS) forming the same green TBA pigment as BSC but at a lower intensity. Compared to baking soda leavened dough, the pH and a^* of baking powder leavened dough was higher. Baking powder control had an initial pH of 7.02, which increased to pH 7.13 on day 24, resulting in numerical non-significant decrease of a^* (2.71 to 2.02); and slight greening at this neutral pH on day 24. Our results are consistent with studies by Yabuta et al.⁹ who reported that greening could occur slowly at neutral pH.

With extended storage after 12 days, the color of BSC turned blue (Figure 1c) with b^* values progressing to negative values while baking soda control (BSC) was not blue (Table S1). The b^* of BSC decreased from 5.15 to -0.55, on day 24, and did not change back to green (Figure 1c, Table S1). This blue TBA pigment formed after prolonged storage⁹. As expected blueness only occurred in BSC which was also the only sample that was green from day 1. The

blueness observed in BSC was absent in acidified baking soda and baking powder leavened doughs (Figure 1, Table S1) whose b^* values were positive.

The b^* of citric acid and GDL acidified baking soda leavened dough ranged between 3.65 and 6.76 after 24 days of storage compared to lower b^* of -0.55 in BSC. The b^* of acidified doughs with baking powder ranged between 5.78 and 8.66 compared to 4.65 in BPC on day 24 (Figure 1). On day 12 and 24, baking soda leavened doughs acidified with citric acid had lower pH and higher b^* values when compared to GDL acidified doughs. In baking powder dough, pH was not significantly different ($p>0.05$) on most days, but was lower than that of the control. Overall, these acidulants could have prevented interaction between CGA quinone and amino acids in dough at the low pH resulting in either low greening or non-green dough, as observed by positive a^* and b^* values in acidified doughs.

Hydroxycinnamic acid content and reducing capacity of sunflower dough with time

Acidulant effect on hydroxycinnamic acid content in sunflower dough with storage time

The hydroxycinnamic acids (HA) containing ingredients in sunflower dough were wheat, maple syrup, and sunflower butter. Both BSC and baking powder control (BPC) had lower HA than dough with added acids. The initial HA in BSC of 11.58 mg/g, decreased by 27.62% after 24 days of refrigerated storage. In contrast to decreased HA in BSC, the HA of BPC increased from 25 to 62.76 mg/g (Table 2). This higher HA in BPC could be related to higher hunter a^* values at neutral pH (7.13) compared to higher greening in BSC at alkaline pH (9.18). The correlation between HA and pH ranged between -0.19 to -0.83 over time. Since greening only

occurs at alkaline pH^{9, 16}, acidic pH did not favor interaction between CGA quinones and amino acids, resulting in higher soluble HA (Table 2).

The HA of acidified baking soda doughs was greater than that of BSC on day 1. On the other hand, the HA of baking powder leavened dough with added GDL and citric acid were similar to those of BPC (Table 2). After 24 days, HA of baking soda and baking powder acidified doughs was higher than that of BSC and BPC controls. Compared to their initial values on day 1, the HA of all samples with BP and acidified samples with BS increased significantly ($p < 0.05$) with storage time (Table 2). This trend could be related to the extent of quinone reduction to parent phenols as well as pH decrease in the presence of acids (Figure 1, Table S1-S2). Although no acid was added to BPC, baking powder used contains sodium aluminum phosphate leavening acid. Acidulants in dough could have prevented CGA oxidation by either competing with primary amines, reducing *o*-quinones hence regenerating CGA by donating protons to phenoxyl radical, or by lowering pH^{19, 20}. Iglesias et al.,²¹ for instance reported that caffeic acid was regenerated in the presence of ascorbic acid, while Sastry and Rao²² found that CGA-protein binding sites decreased when pH decreased with less binding at pH 5.5 and 7.0 compared to alkaline pH. Our LC chromatographs (Figure S1) indicated that CGA was greater in acidified in acidified doughs, compared to BSC controls consistent with Table 2. Liang & Were⁸ likewise reported less greening in a 10% (w/v) solution of sunflower butter cookies made with honey whose pH was lower (honey pH 3.9) compared to cookies with maple syrup (pH of maple syrup 6.3), because of honey's higher acidity stemming from gluconic and other acids.

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Acidified doughs had lower pH and higher HA than doughs without acid (Table 1), confirming the hypothesis that acidic pH could prevent phenol oxidation and reduce quinones formed. Acidulants also possibly increased soluble phenols by releasing CGA and ferulic acid from the cookie dough resulting in increased soluble HA. Shchekoldina and Aider⁴ reported increased solubility of phenols when succinic acid was used to extract phenols from sunflower, and similar effects could have been observed in the current study. The extent to which release of bound phenolics using the leavening acids compared to succinic acid needs to however be confirmed. In addition, Bau et al.,²³, reported higher stability of soluble phenolic compounds in sunflower kernels which had been soaked in citric acid, because of decreased phenol oxidation due to citric acid's chelating ability. Adding acids may thus have stabilized the phenols^{19, 20}, and without acidulants, the alkaline pH also promoted the interaction between CGA quinones and amino acids^{9, 16}. A negative potential nutritional effect of CGA quinone and amino acid binding could be decreased bioavailability of amino acids²⁴ and lowered free phenolic compounds.

Acidulant effect on Folin-Ciocalteu and DPPH reducing capacity of sunflower butter dough during refrigerated storage

Since greening could decrease free CGA in dough, the reducing capacities of phenols was measured to assess the effect of added acidulants. Compared to baking soda doughs, baking powder doughs had higher Folin-Ciocalteu reducing capacity (FCRC) values. The higher FCRC in baking powder leavened dough compared to baking soda leavened dough was attributed to higher HA in baking powder doughs. The FCRC of BSC was significantly lower ($P < 0.05$) than acidified doughs and increased in the order CSHGS > BSC on day 24 (Figure 2a).

Baking powder control had an initial FCRC of 3.61mg/ml which decreased by 12.06% on day 24. In acidified BP dough, the initial FCRC was 23.62%, and 5.83% higher in CP and GP respectively compared to BPC (Figure 2b). In acidified sunflower doughs, acidulants lowered pH which prevented phenol oxidation and may also have reduced quinone dimers to their parent phenolic compounds, which is consistent with previous studies¹⁹.

There was no change in FCRC in baking powder doughs on day 24 compared to their initial concentrations, an indication that the reducing capacity of phenolic compounds was stable with time. In addition, the FCRC of BSC remained lower than that of acidified dough on day 24 attributed to the instability of phenolic compounds at alkaline pH, resulting in CGA quinones reacting with amino groups in protein⁹. The pH of BSC increased from 7.65 on day 1 to 9.18 over 24 days (Figure 2). The correlation between FCRC and pH ranged between -0.38 and -0.77, in leavened controls, and GS, an indication that a decrease in pH resulted in a moderate increase in FCRC.

Comparable to FCRC, the DPPH reducing capacity of baking soda leavened dough was lower than that of baking powder leavened dough (Figure 2), with BSC having the lowest DPPH attributed to the greening reaction⁹. Liang & Were⁸ reported that chlorogenic acid-protein interaction lowered CGA content, consistent with our results where the FCRC/phenol content of BSC decreased (Table 2) with higher greening. Over time, the DPPH in BSC, CS and GS increased by 3.95%, 23.82%, and 63.94%, respectively after 24 days compared to their initial DPPH values. In general, the increase in DPPH over time was greater with baking powder

doughs, since baking powder contains SALP leavening acid, hence the lower pH compared to baking soda (Table 1). The DPPH of BPC, CP and GP increased by 91.81%, 51.72% and 18.43% after 24 days compared to their initial concentration (Figure 2). This increase in DPPH over time could be attributed to the reduction of quinones back to their reduced forms or release of soluble phenols with acidulants¹⁹. The soluble phenol compounds increased at acidic pH resulting in higher DPPH reducing capacities (Table 2). Many antioxidant in-vitro assays are sensitive to pH, and differences in pH of the doughs in addition to the intrinsic properties of the acidulants may have also affected the results. It has been reported^{25,26} that when methanol is used as solvent, pH is not a concern for DPPH analysis.

The DPPH reducing capacity of BSC was significantly lower ($p < 0.05$) compared to acidified doughs with baking soda. The DPPH of doughs formulated with baking soda and acidified with citric acid and GDL were 65%, and 30% higher on day 1 and 97%, 104% higher on day 24 compared to BSC on those days respectively. CGA quinone binding to amino acids could have been responsible for the lower reducing capacity in BSC⁸. Increased free phenols in acidified doughs accounted for higher DPPH reducing capacity compared to non-acidified controls.

There was a weak negative correlation (-0.15 to -0.31) between FCRC and DPPH in all samples. The DPPH reducing capacity of all acidified baking powder doughs was not significantly different from that of BPC on all days of analysis (Figure 2). Baking powder control contained SALP leavening acid which could have also prevented phenol oxidation, accounting

for DPPH reducing capacity that was comparable to the reducing capacity of acidified doughs. The lower pH of baking powder doughs could decrease phenol oxidation and hence higher DPPH reducing capacity when compared to the baking soda leavened dough. Altunkaya & Gokmen¹⁹, reported that citric and ascorbic acid prevented phenolic oxidation by reducing quinone and thus low pH prevented phenol oxidation.

Acidulant effect on tryptophan fluorescence of chemically leavened dough

Tryptophan is one of the amino acids besides cysteine, proline, serine, and threonine which does not produce green trihydroxybenzacridine (TBA) derivatives upon reacting with oxidized dimers of CGA^{9, 16}. It was however important to monitor Trp fluorescence as an indicator of changes in this essential amino acid due to reactions with oxidized lipids and quinones²⁸, since polyphenols can lower its bioavailability in the dough²⁴. The Tryptophan FI of both baking soda and baking powder leavened doughs was lower than that of non-leavened and non-acidified control dough. Tryptophan Fluorescence intensity (FI) of baking soda dough was however higher than that of baking powder dough (Figure 3) and this could be because of differing interactions between the leavening agents with acidulants and Trp. In addition to lower Trp FI, a red shift was observed in leavened doughs when compared to non-leavened and non-acidified controls. The maximum Trp FI wavelength in non-leavened and non-acidified control was 330 nm. Compared to non-leavened dough solutions, a red shift of 24–31nm occurred in doughs formulated with baking soda (Figure 3a) on day 1 while red shift of 27–30 nm was observed in doughs made with baking powder (Figure 3b) on day 1. The red shifts were an indication that stronger conformational changes occurred in leavened doughs. Similarly, on day

24, a red shift of 20–33 nm was observed for baking soda doughs (Figure 3c), while a red shift of 29–31nm was observed in baking powder doughs (Figure 3d), compared to FI at »max (330 nm) in non-leavened dough control on day 1. This red shift also indicated that Trp residues were in a more hydrophilic environment as the protein conformation changed after acidification^{5, 27}.

Tryptophan FI of acidified dough differed with added acids, compared to non-leavened dough. Compared to BSC, Trp FI was not significantly different ($p>0.05$) on day 1. On day 1, Trp FI decreased in the order: non-acidified and non-leavened dough<GS<CS<BSC. In baking powder dough, Trp FI on day 1, decreased in the order non-acidified and non-leavened dough<BPC<GP<CP compared to the control (Figure 3). After 24 days, the same trend was observed, although Trp of baking powder dough was lower than Trp in baking soda doughs. Baking powder doughs were more acidic than baking soda doughs, and thus quenched Trp more than less acidic baking soda doughs. The correlation between Trp and FCRC ranged from -0.30 to -0.73 in baking soda dough. This correlation could be because oxidized CGA dimer and monomer can react with Trp, so an increase in free phenol could decrease Trp.

At the same ingredient concentration in the dough, the Trp FI of ingredients containing Trp was ranked as egg>wheat flour>sunflower butter with the Trp FI of egg being 74.63% higher than Trp FI of wheat flour, and 83.90% higher than Trp FI of sunflower butter. The Trp FI of non-acidified dough was 4.8×10^6 , while the sum of Trp from egg, wheat and sunflower butter was less (3.38×10^6). The maximum FI of Trp solution at 359nm of 4.29×10^6 was diluted to be similar to the FI of non-acidified dough.

To further elucidate the effect of acidulants on Trp, acidulants were added to a 2.45mM, Trp solution and Trp fluorescence was monitored (Table 3). The pH of 2.45mM Trp was 4.82 and decreased to 2.86 and 2.77 with 120 μ l added gluconic acid and citric acid respectively (Table 3). Increasing the volume of each acidulant decreased pH and Trp FI. After adding 200 μ l of each acidulants, Trp FI decreased by 15% and 18% in Trp solution with added citric and gluconic acid (Table 3). The moderate negative correlation (-0.26 to -0.69) between pH and Trp of dough solutions at λ_{EX} 280nm, λ_{EM} 359nm (Figure 3), could indicate that decreased Trp was attributed to quenching by acidulants or crosslinking with CGA in addition to Trp oxidation²⁸. Tryptophan is an essential amino acid susceptible to oxidation, so lower Trp as a function of acidulant could signal some loss during storage.

CONCLUSION

Acidulants prevented greening in baking soda and baking powder leavened sunflower dough by decreasing pH since chlorogenic acid quinone and amino acid do not bind to each other at acid pH. In addition to lowering pH, which prevents greening, acidulants contribute to leavening in sunflower dough but does not degrade phenols. The phenolic compounds in acidified dough still maintained their reducing capacity over time as assessed by FCRC and DPPH. The reducing capacities of acidified doughs were significantly higher than that of non-acidified controls. In contrast to reducing capacities, the Trp fluorescence of acidified doughs was lower than that of non-acidified controls. Further investigations could include sensory

testing of cookies made with acidified dough to compare balance between lowered greening with taste.

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List of Tables

Table 1: Changes in pH of baking soda and baking powder leavened dough as a function of added acids and time.

Time (days)	Doughs with baking soda			Doughs with baking powder		
	BSC	BS + CA	BS + GDL	BPC	BP + CA	BP + GDL
1	7.65±0.08 ^a _B	6.79±0.12 ^c _A	7.53±0.01 ^a _A	7.02±0.03 ^b	6.32±0.13 ^d _B	6.45±0.03 ^d _A
12	9.10±0.35 ^a _A	6.19±0.09 ^{cd} _B	6.60±0.23 ^c _C	7.85±0.07 ^b	4.83±0.21 ^c _A	5.57±0.45 ^d _B
24	9.18±0.09 ^a _A	6.17±0.09 ^c _B	6.98±0.05 ^b _B	7.135±0.11 ^b	5.46±0.31 ^d _{AB}	5.36±0.06 ^d _B

BS=baking soda, BP= baking powder, BPC= baking powder control, BSC= baking soda control,

CA= citric acid, GDL= glucono delta lactone.

Treatments with the same superscript letter in the same row (same day) are not significantly different on that day.

Values with the same capitalized letter in the same column (same treatment) are not significantly different with storage time.

Table 2: Hydroxycinnamic acid concentration (g/Kg) in baking soda and baking powder leavened dough with added acids

Time (days)	Baking Soda/BS leavened doughs			Baking powder/BP leavened doughs		
	BSC	BS + CA	BS +GDL	BPC	BP + CA	BP + GDL
1	11.56±3.57 ^c _A	28.56±5.40 ^b _B	36.32±2.36 ^b _B	25.49±9.69 ^{bc} _B	39.32±4.57 ^b _B	59.62±7.31 ^a _B
4	13.11±3.41 ^c _A	75.71±2.80 ^{ab} _A	73.18±2.15 ^{ab} _A	56.59± 8.83 ^b _A	85.98±16.51 ^a _A	78.2±4.37 ^a _A
12	9.02±0.78 ^d _A	64.00±14.44 ^{ab} _A	43.33±5.38 ^a _B	48.61±3.76 ^a _A	74.06±1.44 ^a _A	66.40±7.83 ^a _{AB}
24	8.36±0.84 ^b _A	69.07±1.42 ^a _A	68.96±7.68 ^a _A	62.76±5.10 ^a _A	74.83± 5.52 ^a _A	73.72±0.67 ^a _{AB}

BP=baking powder, BS=baking soda BSC= baking soda control, BPC= baking powder control,

CA= citric acid, GDL= glucono delta lactone.

Treatments with the same superscript letter in the same row (same day) are not significantly different on that day.

Values with the same capitalized letter in the same column (same treatment) are not significantly different with storage time.

Acid (ul) Added to 5ml Trp (2.45mM L- tryptophan)	Trp Fluorescence Intensity (a.u)		pH	
	Trp + citric acid	Trp+ GDL	Trp+ citric acid	Trp + GDL
0 (control/no acid)	4.29E+06	4.29E+06	4.82	4.82
40	4.21E+06	3.90E+06	3.32	3.25
80	3.75E+06	3.63E+06	2.9	3.04
120	3.74E+06	9.42E+06	2.77	2.86
200	3.66E+06	3.51E+06	2.71	2.8

Table 3 Acidulant effect on tryptophan (Trp) and pH changes

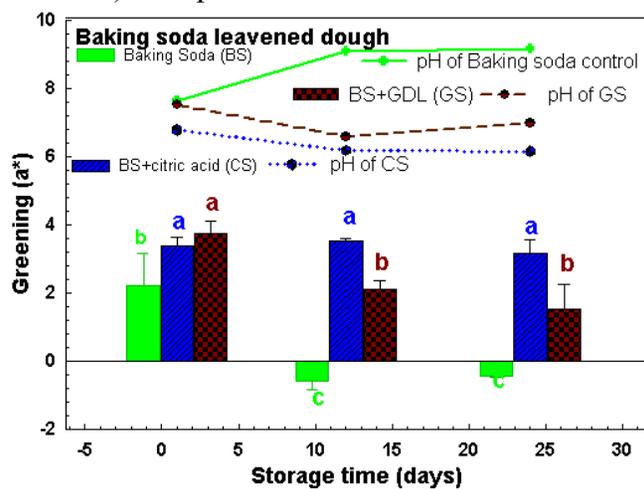
Figures Captions

Figure 1: Surface greening (-a*) changes of baking soda (a) and baking powder (b) leavened sunflower butter cookie dough; Changes in (b*) of baking soda (c) and baking powder (d) leavened sunflower butter dough as a function of acidulant and refrigeration time. Treatments with the same letter on the same day are not significantly different on that day.

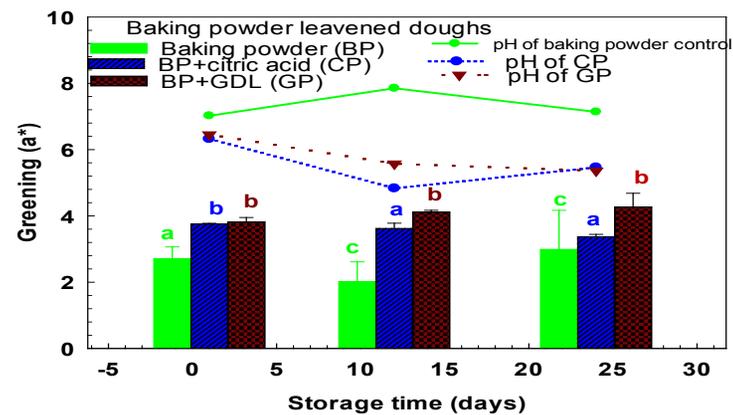
Figure 2: Folin-Ciocalteu reducing capacity changes of baking soda (a) and baking powder (b) and changes in DPPH reducing capacity changes in baking soda (c) and baking powder (d) leavened sunflower butter cookie dough as a function of refrigeration time and acidulant. Treatments with the same letter on the same day are not significantly different on that day.

Figure 3: Tryptophan fluorescence intensity of baking soda dough on day 1(a) and day 24 (b) and baking powder dough on day 1(c) and day 24 (d), as a function of added acidulants.

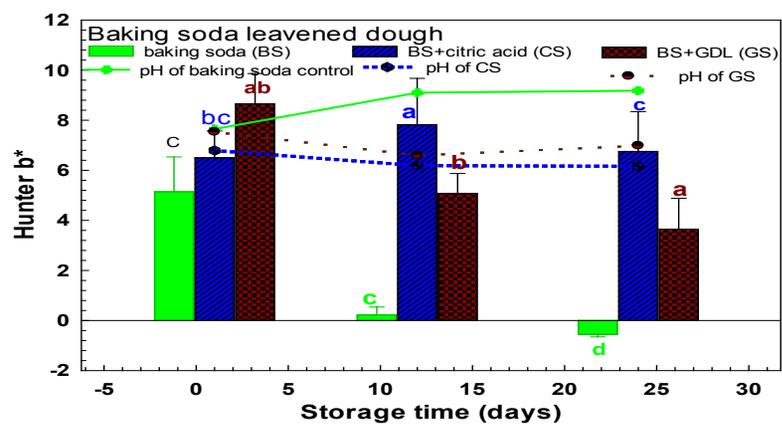
a) Baking soda leavened dough greening and correlation (-0.59 to -0.80) with pH



b) Baking powder leavened dough greening and correlation (-0.80 to 0.80) with pH



c) Baking soda leavened dough Hunter b* changes with time



d) Baking powder leavened dough Hunter b* changes with time

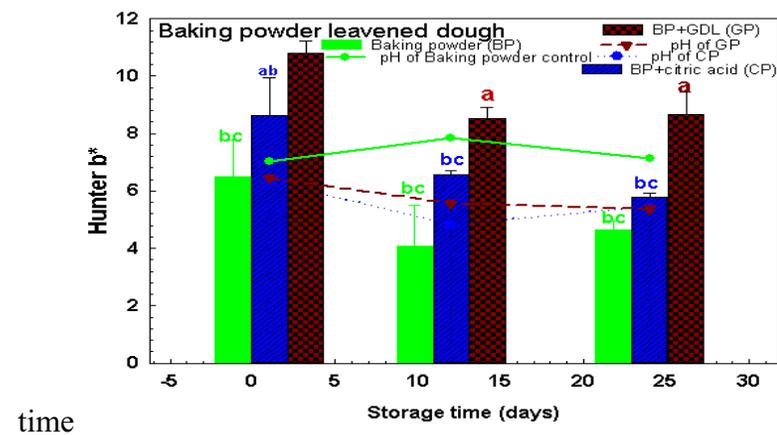
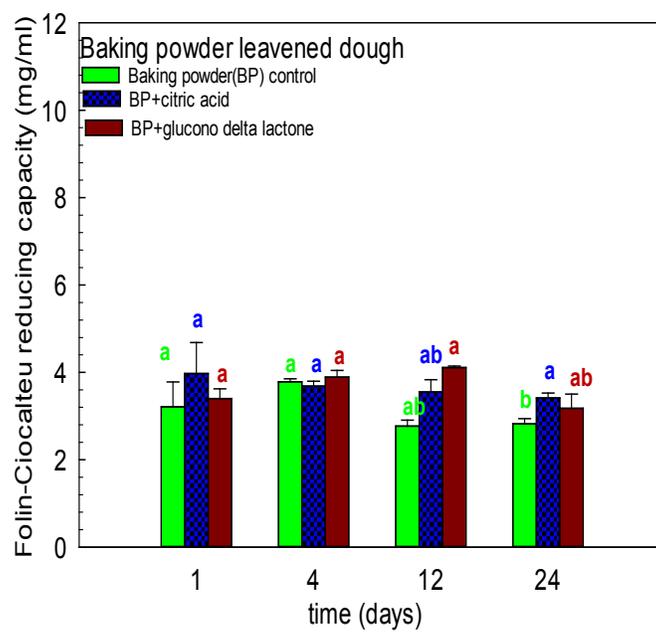
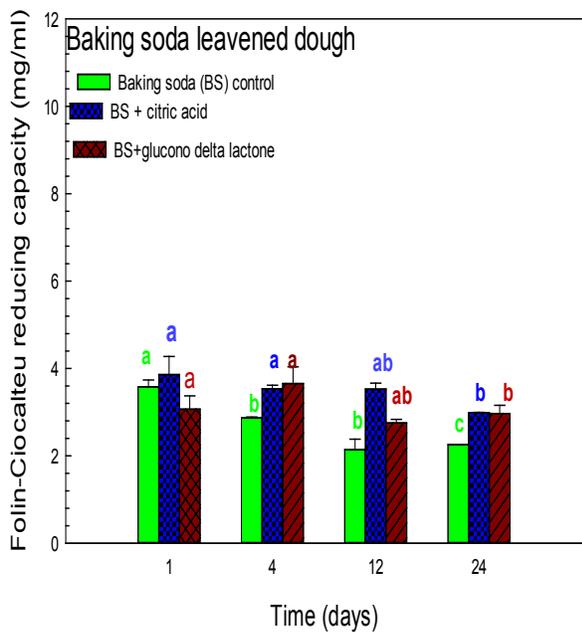


Figure 1

a) Folin-Ciocalteu reducing capacity of baking soda leavened dough

b) Folin-Ciocalteu of baking powder leavened dough



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c) DPPH reducing capacity of baking soda leavened dough

d) DPPH reducing capacity of baking powder leavened dough

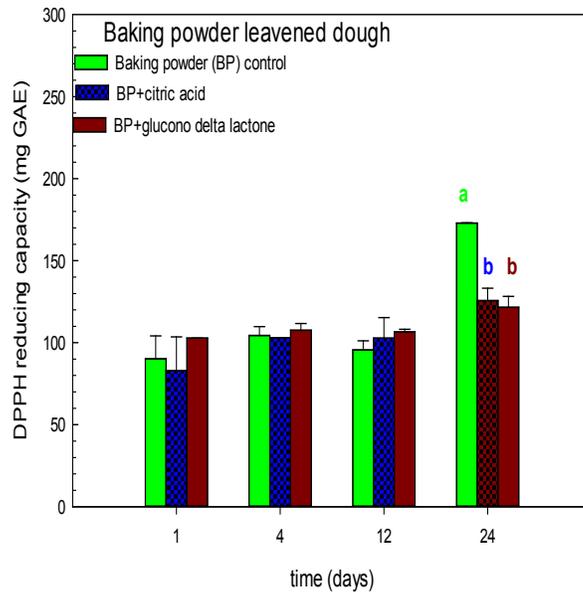
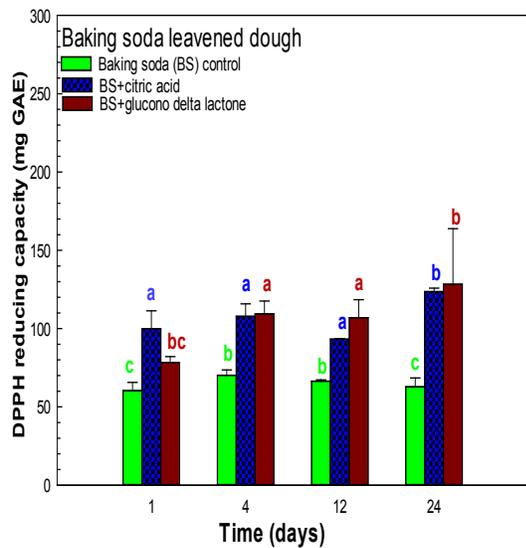
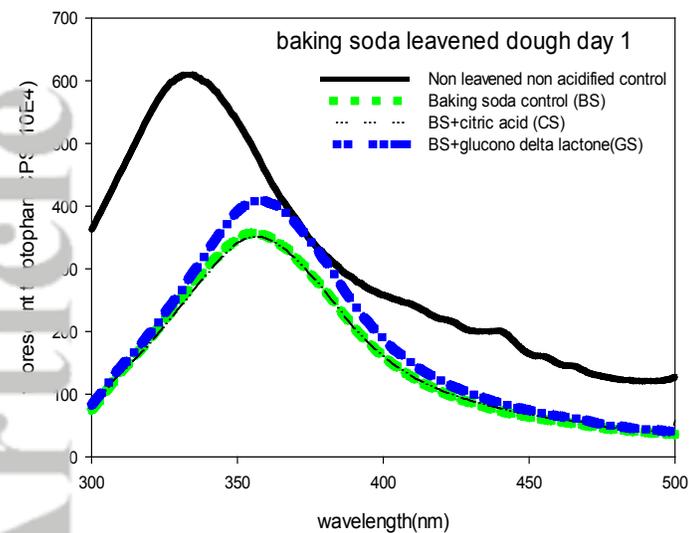
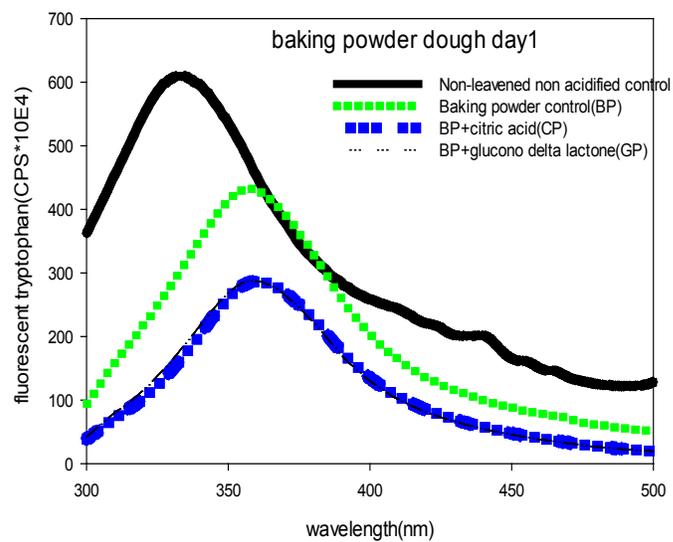


Figure 2

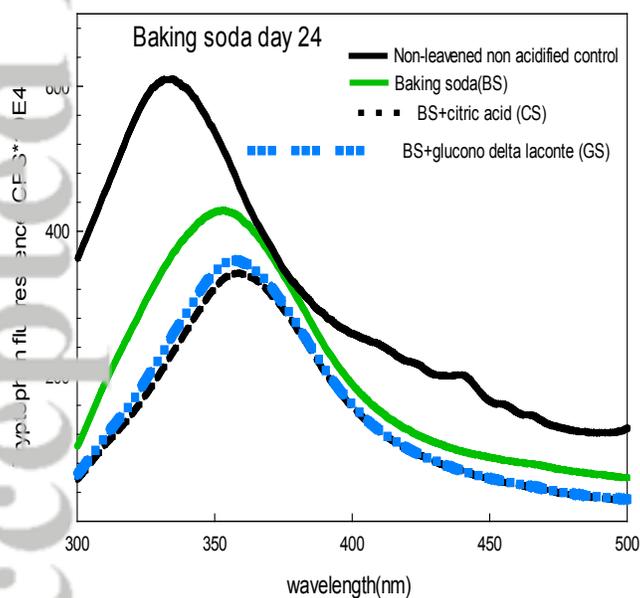
a) Baking soda tryptophan fluorescence day1



b) Baking powder tryptophan fluorescence day1



c) Baking soda dough tryptophan fluorescence day 24



d) Baking powder dough tryptophan fluorescence day 24

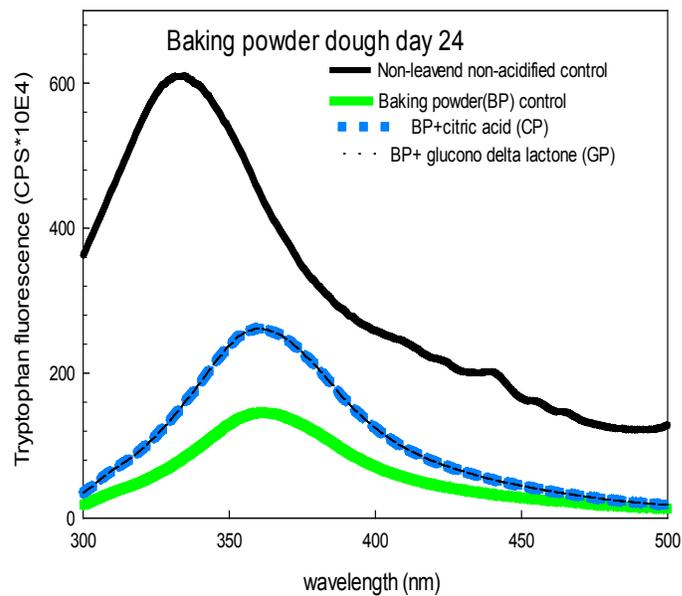


Figure 3