

Furan Occurrence in Starchy Food Model Systems Processed at High Temperatures: Effect of Ascorbic Acid and Heating Conditions

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ABSTRACT: Furan, a potential carcinogen, has been detected in highly consumed starchy foods, such as bread and snacks; however, research on furan generation in these food matrixes has not been undertaken, thus far. The present study explored the effect of ascorbic acid addition and cooking methods (frying and baking) over furan occurrence and its relation with the non-enzymatic browning in a wheat flour starchy food model system. Results showed that furan generation significantly increased in the presence of ascorbic acid after 7 min of heating ($p < 0.05$). The strongest effect was observed for baked products. Additionally, the furan content in fried products increased with the increase of the oil uptake levels. As for Maillard reactions, in general, the furan level in all samples linearly correlated with their degree of non-enzymatic browning, represented by L^* and a^* color parameters (e.g., wheat flour baked samples showed a R^2 of 0.88 and 0.87 for L^* and a^* , respectively), when the sample moisture content decreased during heating.

KEYWORDS: Furan, starchy food model system, ascorbic acid, oil uptake, moisture, non-enzymatic browning

INTRODUCTION

Furan is a potential human carcinogen that can be formed in a broad range of foods processed at high temperatures, such as coffee, baby foods, bread, and snacks.¹ Although it is still unclear what the risks are associated with the current intake levels of dietary furan, furan mitigation in foods may be considered a challenge in the prevention of human diseases, such as cancer.² The presence of furan is common in foods processed at high temperatures, particularly in products packed in sealed containers (e.g., baby foods). Because of its low boiling point, furan generated during thermal processes easily vaporized, accumulating in the headspace of canned or jarred foods.³ However, despite its high volatility, furan has also been found in low-moisture foods processed in open containers, such as potato chips, crackers, crisp breads, and toasted breads.^{3–6}

The broad number of foods that have been shown to contain furan suggests that multiple pathways might be involved in its formation in foods.⁷ Thermal degradation and rearrangement of sugars was suggested as the primary source of furan in food;⁸ more recently, amino acids, polyunsaturated fatty acids (PUFAs), and ascorbic acid have also been implicated.^{1,3,6,8–11} The latter formed the highest amount of furan in aqueous model systems heated at high temperature.

It is worth noting that the furan content determined in foods was much lower than predicted from trials with pure ascorbic acid. Therefore, caution must be drawn about the plausibility of the proposed pathways for furan formation determined in model systems and their direct extrapolation to the more complex food products.¹²

Few authors have evaluated furan generation in more real systems that considered the interaction between potential precursors. Limacher et al.¹³ and Van Lancker et al.¹⁴ determined furan formation from the Maillard reaction in carbon module labeling (CAMOLA) model systems under both dry-roasting and pressure-cooking conditions. They concluded that glucose-derived furan was formed from the intact sugar skeleton and not from fragmentation and recombination mechanisms. However, some amino acids (especially alanine and serine) could provide an additional formation pathway, as previously proposed.¹⁵

The role of ascorbic acid and PUFAs on furan occurrence has recently been investigated¹⁶ in starchy model systems that mimic baby foods. The authors showed that, for CAMOLA model systems heated under roasting conditions, the furan formation from ascorbic acid was significantly reduced in binary mixtures (e.g., the presence of erythrose led to 80% less furan). These results agreed with previous findings, in which simple binary mixtures of ascorbic acid and amino acids, sugars, or lipids could reduce furan by 50–95%.¹⁷ Thus, more complex reaction systems result in lower furan generation, as compared to the individual precursors, most likely because of competing reaction pathways. Owczarek-Fendor et al.¹⁸ observed, however, that the presence of starch drastically enhanced furan formation from ascorbic acid. They hypothesized that furan synthesis was stimulated when ascorbic acid was incorporated in the starchy gel (inclusion complex);

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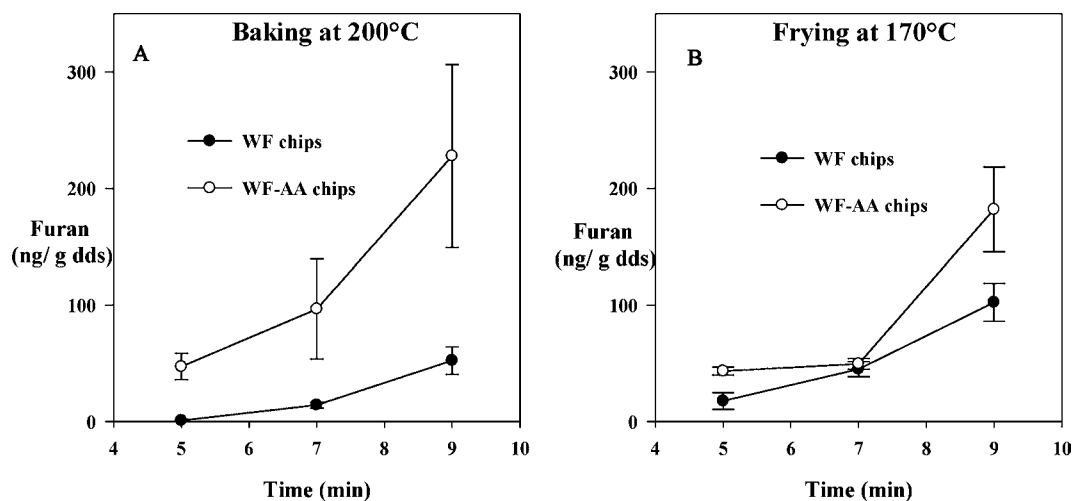


Figure 1. Role of ascorbic acid over furan formation in starchy food model systems processed at high temperatures. Error bars represent standard deviations ($n = 3$).

thus, its degradation was favored over the condensation with other compounds present in the reaction medium.

The furan formation from lipid oxidation was influenced by not only the fatty acid composition but also the interactions with other matrix ingredients.¹⁹ For example, while linolenic acid has been identified as responsible for furan generation in most research studies,^{15,19–22} the importance of the degree of fat oxidation is still unclear. Finally, the effect of different intrinsic and extrinsic factors, such as pH, matrix, and heating temperatures also considerably impact both furan generation and its retention.^{1,3,5,12,23,24}

Because high levels of furan were found in baby foods, most model systems focused in replicating as reliably as possible the physicochemical features of these matrices. To the best of our knowledge, research on furan generation in other food matrixes, such as bread, crackers, or potato chips, where its presence was demonstrated, has not yet been carried out. Considering the significant worldwide consumption of thermally processed starchy foods, in this work, we investigated the mechanisms involved in furan generation in these matrixes. The present study explored the effect of ascorbic acid and heating conditions (frying and baking) over furan occurrence, as well as the relationships between non-enzymatic browning and furan content in a starchy food model system.

Finally, because these low-moisture starchy food products are characterized by the development of non-enzymatic browning during high-temperature processing,²⁵ we explored if color development could be a good predictor of furan generation.

MATERIALS AND METHODS

Two different dough formulations with the same moisture content of 40% on a wet basis (wb) were prepared: (i) wheat flour (WF) and (ii) wheat flour and ascorbic acid (WF-AA). Then, both formulations were laminated and cut in circle slices to be either baked or fried. The furan concentration of the fried or baked slices on a dry defatted weight basis (ddb) was quantified by gas chromatography coupled with mass spectrometry (GC/MS). Finally, color development of the cooked samples was quantified in L^* , a^* , and b^* units using a colorimeter.

Materials. Dough formulations were prepared with the following materials: (i) wheat flour (moisture content of 15% on a wb), (ii) anhydrous ascorbic acid (<99%, Sigma-Aldrich, Steinheim, Germany), and (iii) Milli-Q water. For frying experiments, sunflower oil (Solsikkeolie, Copenhagen, Denmark) was used as a heating medium.

Chemical reagents for furan analyses were (i) furan (>99%, Sigma-Aldrich, Steinheim, Germany), (ii) d_4 -furan (98 atom % D, Isotec, Miamisburg, OH), (iii) methanol [high-performance liquid chromatography (HPLC) grade, Rathburn, Walkerburn, Scotland], and (iv) NaCl (>99%, Merck, Darmstadt, Germany). Finally, petroleum ether (>99%, Sigma-Aldrich, Steinheim, Germany) was used as an extraction solvent for oil determination by Soxhlet.

Dough Preparation. Dough formulations were prepared on the basis of the criteria that both formulations (with and without ascorbic acid) would have the same moisture content of $40 \pm 0.6\%$ wb before being fried or baked. To calculate the amount of water that had to be added to the solid materials, the exact dry solid content of wheat flour was determined experimentally by drying it until a constant weight. For WF-AA samples, anhydrous ascorbic acid was added in a concentration of 300 mg/kg of wheat flour. Then, the amount of wheat flour, ascorbic acid, and water necessary to prepare 500 g of each dough formulation was calculated on a dry basis (db). For WF and WF-AA formulations, 100 and 99.5% of wheat flour (db) was added. In WF-AA formulation near 0.5% (db) corresponded to the ascorbic acid necessary to reach the required concentration.

WF and WF-AA dough formulations were prepared using a food mixer (Teddy Bear Varimixer, Copenhagen, Denmark), and water was added according to the protocol previously described.²⁶ Half of the water was gradually added at 15°C while mixing for 1 min. After mixing for 1 extra min, the remaining water previously heated at 90°C was added to the dough and then all of the ingredients were homogenized for 2 min. The resultant dough was then wrapped in a plastic bag and left for 1 h at room temperature (20°C). Then, the dough was kneaded to ensure homogeneity, laminated to obtain the required thickness using a dough sheeter (Rollmatic, Vicenza, Italy), and cut into 40 mm diameter circles. The exact thickness of the resultant dough slices ranged from 2 to 2.3 mm. Approximately 500 g of dough was prepared for each batch of the experiment.

Thermal Processing of Dough. The resulting samples were fried and baked at 170°C and 200°C for 5, 7, and 9 min.

Frying Conditions. The samples were fried in a 20 L capacity deep-fryer (FKI, Copenhagen, Denmark). The fryer was filled with 15 L of oil that was preheated for 2 h prior to frying²⁷ and was discarded after 90 min of frying time. The chip/oil mass ratio was maintained as low as possible to keep a constant temperature of frying. Throughout the frying process, 10 chips of 3.7 ± 0.03 g were placed in a basket and held in position with a wire grid to prevent them from floating. The fried chips were drained over a wire screen for 5 min.²⁸ After that, drained samples were homogenized and refrigerated for 30 min. Then, chemical and color analyses were carried out.

Baking Conditions. The samples were baked in a forced-air oven (drying rate of 1 m/s; Heraeus, Copenhagen, Denmark) preheated for

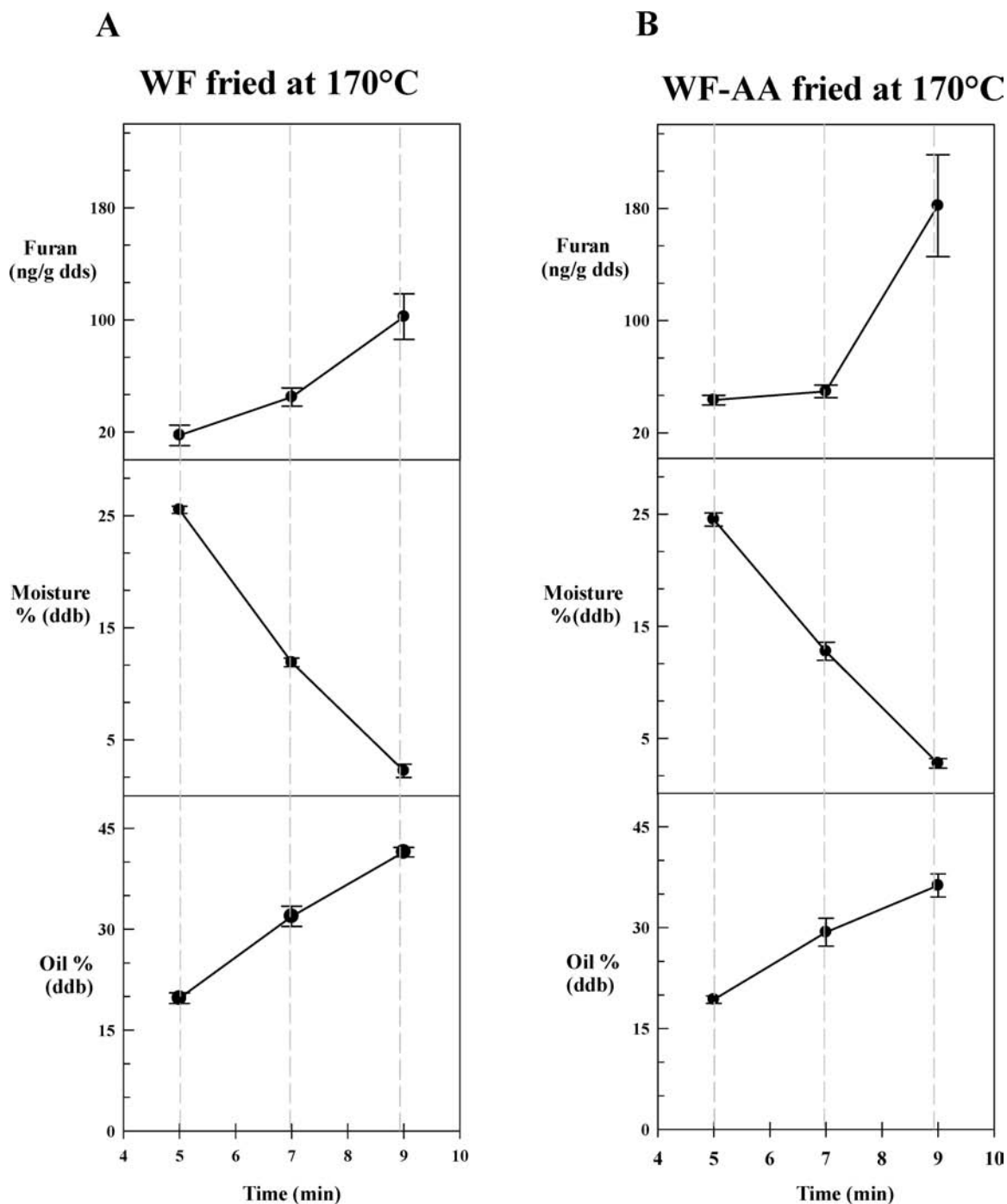


Figure 2. Influence of oil uptake and moisture over the final furan content in fried starchy food model systems. Error bars represent standard deviations ($n = 3$).

1 h prior to baking. Throughout the baking process, 10 chips were cooked, and then the baked samples were homogenized and refrigerated for 30 min.²⁹ Chemical and color analyses were then carried out.

Analytical Methods. Solid Content. Raw material (wheat flour) was placed in a Petri dish, dried in a forced air oven at 105 °C to a constant weight, and cooled in a desiccator.³⁰ The solid content of baked samples was determined using the same procedure. For fried products, the solid content was determined in an extracted, oil-free sample. The solid content of samples was used to calculate their furan concentration on a defatted dry weight basis (ddb). Hence, changes in these concentrations with high-temperature processing times were not influenced by changes in moisture and fat contents.

Oil Content. The total oil content of fried chips was determined gravimetrically by Soxhlet extraction with petroleum ether.³⁰

Furan Quantification. Furan was quantified according to the methodology of the National Food Institute of DTU. This method is a revised version of previous methodology.³¹

A total of 0.5 g of fried sample and 0.75 g of baked sample, both previously pulverized, were weighed into headspace vials, diluted with 5 M NaCl solution. After the internal standard (d_4 -furan) was added, the vials were sealed. Automated headspace sampling followed by GC/MS analysis was used to detect furan and d_4 -furan in the scan mode. Furan was quantified using a standard addition curve, where the concentration of furan in the fortified test portions was plotted versus the furan/ d_4 -furan response factors. To construct the calibration curve, seven vials were used, of which four vials were fortified with furan, as follows: two vials at ca. half of the expected concentration of furan in the sample, one

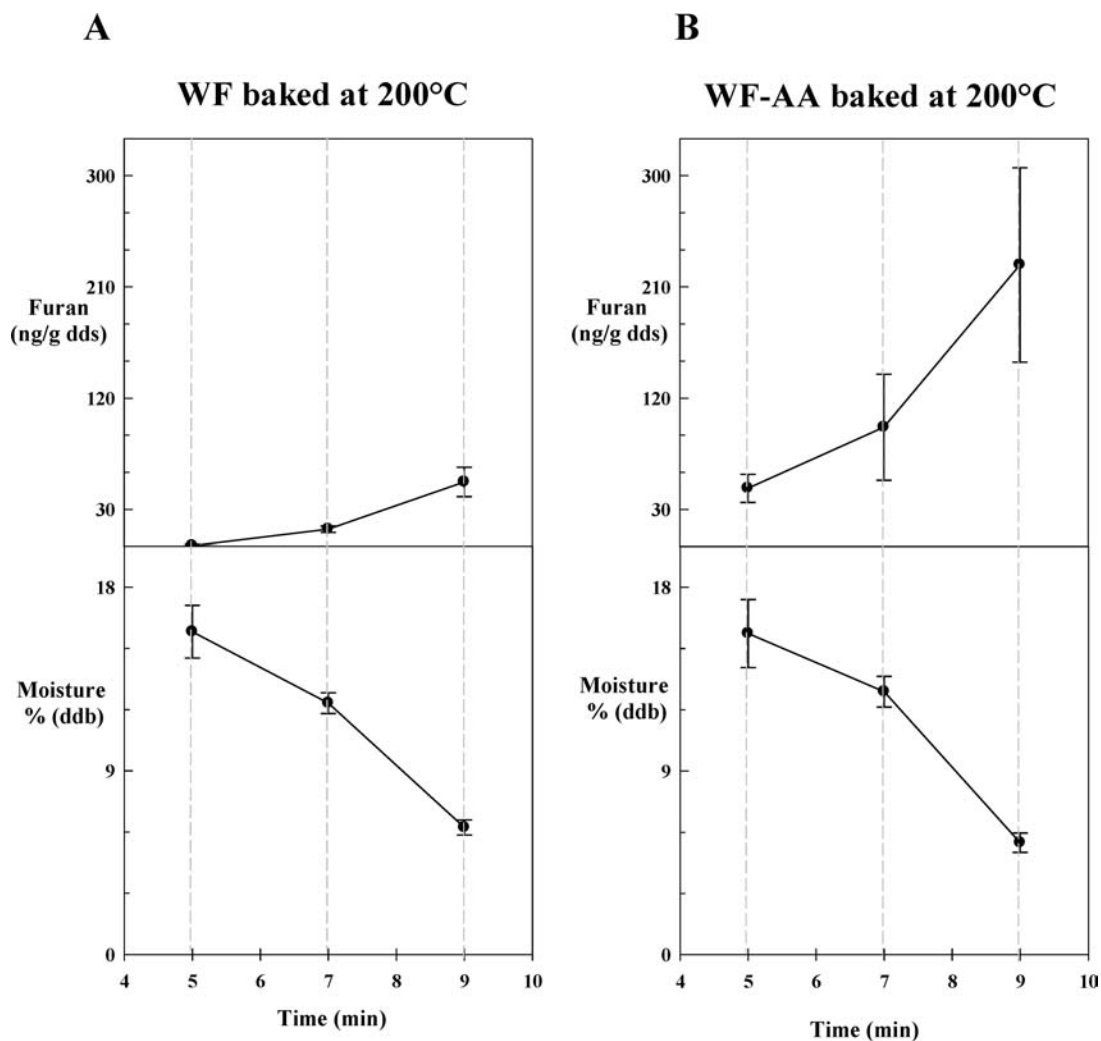


Figure 3. Effect of the moisture content over furan generation in baked starchy food model systems. Error bars represent standard deviations ($n = 3$).

vial at ca. the expected concentration of furan in the sample, and one vial at ca. twice the expected concentration of furan in the sample.

The analyses were performed on a gas chromatograph–mass spectrometer (GC–MS) (Agilent 6890N GC with Agilent 5973 N MSD, Palo Alto, CA) fitted with a CTC CombiPAL static headspace autosampler. The syringe was heated to 70 °C, and the sample vial was heated at 60 °C for 30 min. A total of 1 mL of the headspace from a 10 mL headspace vial was injected splitless on the gas chromatograph. A 15 m × 0.32 mm × 20 μm HP-Plot Q column was used. The following conditions were set: helium flow, 1.7 mL/min; injector temperature, 200 °C; oven temperature, 50 °C (1 min), with a temperature ramp of 10 °C/min to 130 °C, then a temperature ramp of 3 °C/min to 157 °C, and finally, a temperature ramp of 20 °C/min to 260 °C and held for 2.5 min. The MS source temperature was 230 °C, and the MS quad temperature was 150 °C, with a dwell time of 50 ms. The MS was operated in electron ionization mode. Furan was detected using single-ion monitoring of the fragments m/z 68 and 39. The internal standard d_4 -furan was detected by monitoring the fragments m/z 72 and 42. This method has a limit of quantification of 2.4 ng/g for all samples, except for potato chips, where the limit of quantification is 2.9 ng/g. All analytical determinations were carried out in three replicates.

pH Estimation. The pH of the dough was calculated considering that pure water was used, and also, the pH of wheat flour was 7.0. For WF formulation, the pH corresponded to $-\log[\text{H}_3\text{O}^+]$, where the molar concentration of H_3O^+ was 10^{-7} g/mol; thus, pH_{WF} is 7. For the case of WF-AA formulation, the Henderson–Hasselbach equation was used considering that the ascorbic acid solution used has a molar concentration of 0.003 65 mol/L (which corresponded to the added ascorbic

acid amount of 300 mg/kg of wheat flour) and a pK_a of 4.17; thus, $\text{pH}_{\text{WF-AA}}$ is 3.3.

Color Development. The color of baked and fried samples was measured using a colorimeter (Minolta Chromo Meter CR 200b, Ramsey, NJ) attached to a data processor DP-100 using the CIE L^* , a^* , and b^* color scale. Triplicate readings were carried out at 20 °C on each three equidistant locations of each chip, and the mean value was recorded. Color changes were followed by the lightness (L^*) and redness (a^*) parameters, because these color components presented the highest and significant variations during high-temperature processing because of non-enzymatic browning reactions.³²

Statistical Analysis. The experiments were replicated 3 times. Statistical analysis was carried out using Statgraphics Centurion XV software (Manugistic, Inc., Rockville, MD). One-way analysis of variance was performed to confirm that there were no significant differences between measurements of a sample processed under the same specific conditions. Differences between treatments were analyzed by a least significant difference (LSD) test using the linear general model. All significant differences were determined with a confidence level of 95%.

RESULTS AND DISCUSSION

The occurrence of furan and its relation with some intrinsic factors (ascorbic acid content, oil uptake, and moisture) and non-enzymatic browning were explored in a starchy food model system simulating frying and baking conditions, to elucidate a main furan pathway for this kind of product.

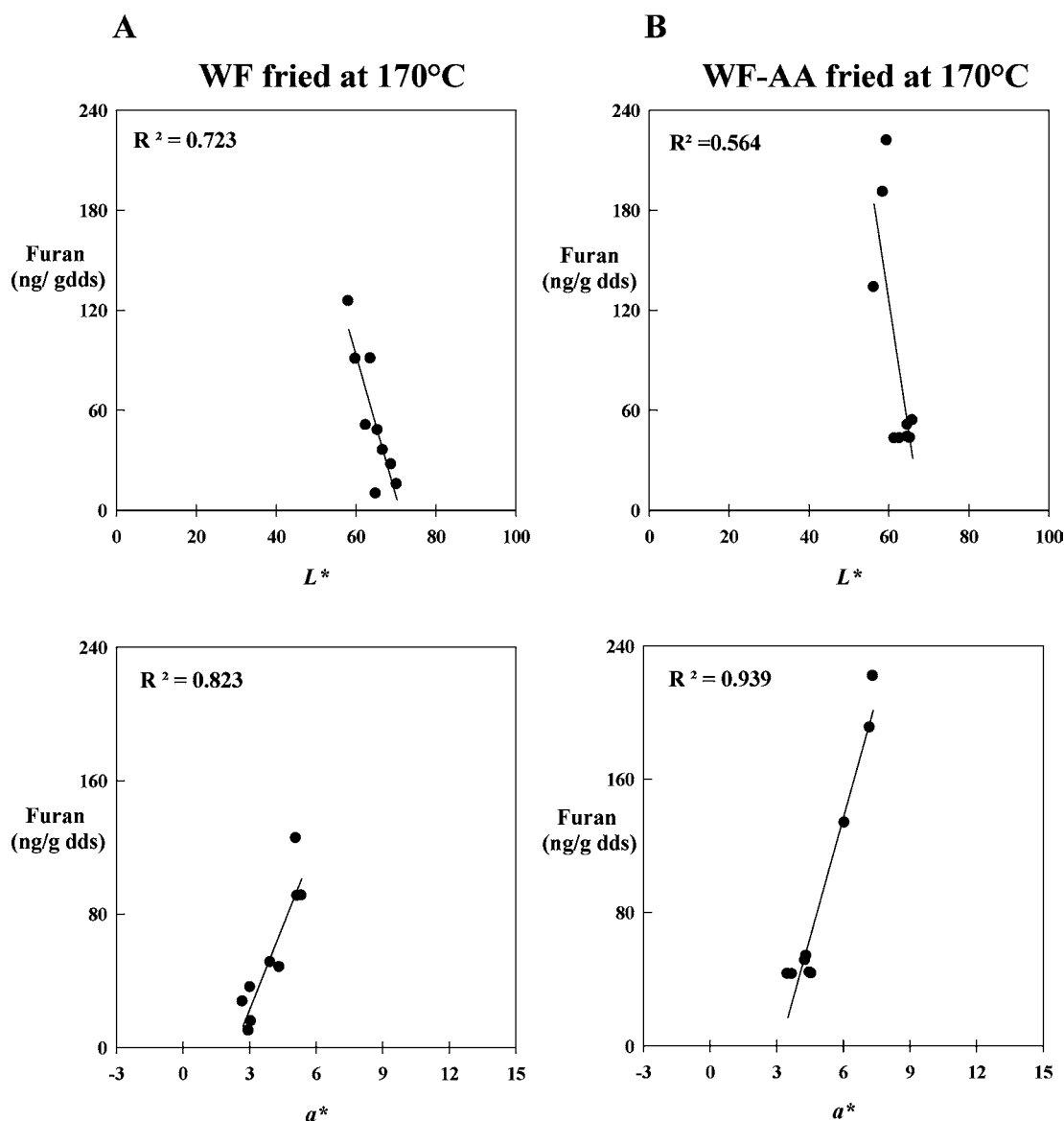


Figure 4. Relationship between color development and furan content in fried starchy food model systems.

Role of Ascorbic Acid in Furan Formation. The impact of ascorbic acid over furan generation in model systems mimicking complex foods has not yet been completely understood. Some results have suggested that furan formation from ascorbic acid is negatively affected by the presence of additional molecules, such as carbohydrates, lipids, and amino acids, which may increase the fragmentation rate or change the redox status of the reaction system.^{15,17} However, in our model conditions, ascorbic acid significantly increased furan generation ($p < 0.05$) after 7 min of processing, having a stronger effect in baking, as shown in Figure 1 (e.g., WF-AA samples baked and fried for 9 min contained ~74 and 33% more furan than their WF counterparts). Interestingly, not only the ascorbic acid concentration but also the processing time were critical factors for furan formation, suggesting a synergistic effect between the ascorbic acid added and the reducing sugars and amino acids naturally present in wheat flour, at least under the dry heating conditions applied in this work. The latter could be explained because, under similar dry heating conditions (180 °C), ascorbic acid behaves like reducing sugars in the Maillard reaction.³³

On the other hand, despite ascorbic acid increasing the final content of furan in WF-AA fried samples, this effect was weaker

than in baked samples. Because furan formation strongly depends upon the heating temperature, these results could be attributed to the fact that frying experiments were performed at lower temperatures (170 °C) than the baking temperatures (200 °C). It is worth mentioning that processing temperatures for both unit operations were selected according to common requirements in the food industry.

Furthermore, pH significantly affects the yield of furan generation in Maillard model systems;^{8,15,17,22} the higher the pH of the system, the higher the formation of 1-deoxyosones, which is the most effective furan synthetic route in the Maillard reaction. However, for our model conditions, the final furan content was similar in both WF-AA and WF samples, despite their differences in the pH (WF-AA and WF samples presented a pH values of ~3.3 and ~7, respectively).

This is in accordance with a previous study in starch-based model systems with and without ascorbic acid of the relationship between pH (range of 3.5–6.5) and furan formation, showing that the highest formation was found in model systems with ascorbic acid at pH 3.5.¹⁸ This fact may be explained because these experiments were performed under more drastic heating

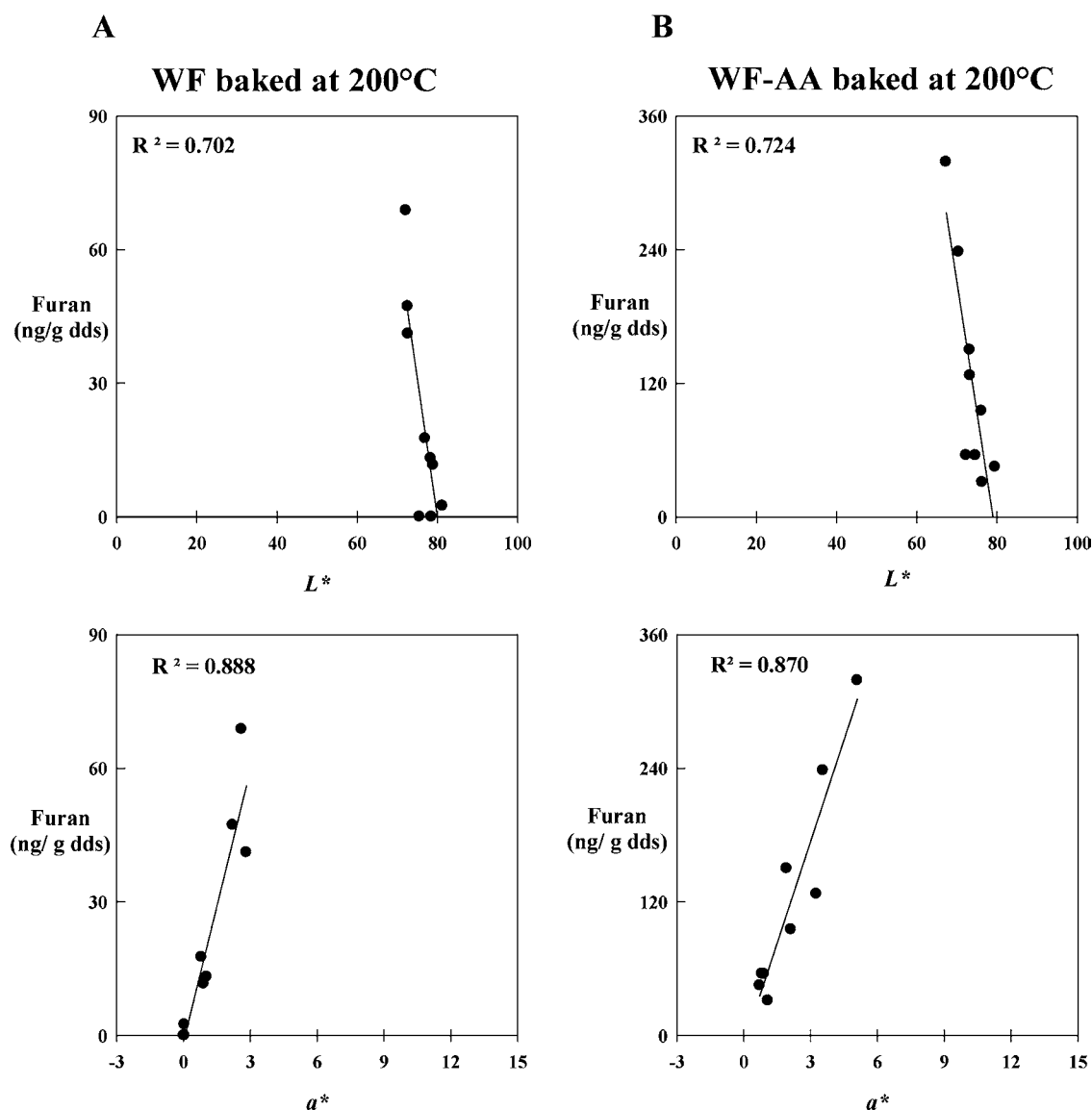


Figure 5. Relationship between color development and furan content in baked starchy food model systems.

conditions (~ 190 °C) than previous studies (~ 120 °C) performed in simple model systems of sugars.³⁴

For the present model system the addition of ascorbic acid decreased the furan occurrence under the low moisture conditions and higher processing temperatures applied.

Influence of Oil Uptake over the Final Furan Content.

In addition to the role of ascorbic acid and specifically for fried products, the influence of oil uptake could also affect the final furan content of the product, because at high temperatures commonly used during atmospheric frying, the penetrated oil³⁵ can easily be oxidized and forms furan.¹¹

On the other hand, considering that furan is a nonpolar compound, the penetrated oil could also have a retention effect over the furan generated from the other precursors, originally present in these samples (sugars, amino acids, and ascorbic acid).

In this respect, our results showed that, not only for WF but also for WF-AA fried products, the amount of furan generated increased during frying, similar to oil uptake (Figure 2).

Some authors have suggested that the overall role of lipids in furan formation was restricted in practice, because it is necessary

to significantly oxidize the oil, which is sensory unacceptable by the consumer.³⁶ Moreover, recent studies have shown that, for PUFAs, only α -linolenic fatty acid is a precursor for furan, because of its unique potential among fatty acids to produce 2-butenal upon oxidation.²⁰

Because our frying experiments were performed with commercial sunflower oil, which contains antioxidant [butylated hydroxyanisole (BHA)] but lacks α -linolenic fatty acid, the retention effect of penetrated oil could be considered as the major contribution of oil in the final furan occurrence of the tested model system.

Effect of the Moisture Content over Furan Generation.

As for the Maillard reaction, in general, for all samples, an exponential increase in the furan level was observed when the moisture content decreased (Figures 2 and 3). The furan concentration of fried and baked samples (both WF and WF-AA) did not significantly change ($p > 0.05$) until the moisture content was below 12% (ddb), achieving the highest values at moisture levels of 2.23 and 2.77% (ddb) for WF (102.32 ng/g of dds) and WF-AA (182.04 ng/g of dds) fried samples and 6.22 and 5.48% (ddb) for WF (52.36 ng/g of dds) and WF-AA (227.00 ng/g of

dds) baked samples, respectively. Similar results were found in carrot slices³⁷ and hazelnuts,²¹ which were dried at temperature ranges of 113–133 and 50–150 °C, respectively.

Relation between the Furan Content and Non-enzymatic Browning. The presence of furan in foods is related to the thermal degradation of carbohydrates, ascorbic acid, amino acid, and PUFAs.⁹ Considering that non-enzymatic browning in low-moisture starchy foods is also a consequence of these reactions,³⁸ we determined the color development of WF and WF-AA formulations processed at high temperatures. Figures 4 and 5 show that color represented by the parameters L^* and a^* followed linear correlations with the furan content, for both frying (WF formulation, R^2 of 0.72 and 0.82 for L^* and a^* values, respectively; WF-AA formulation, R^2 of 0.56 and 0.94 for L^* and a^* values, respectively) and baking (WF formulation, R^2 of 0.70 and 0.88 for L^* and a^* values, respectively; WF-AA formulation, R^2 of 0.72 and 0.87 for L^* and a^* values, respectively) experiments. These results suggest that preliminarily color development could be considered as a good predictor of furan formation in starchy matrixes. Lower correlation values obtained for lightness in fried products could be attributed to distortion effects caused by oil located in the fried sample surface.

L^* values tended to decrease with the processing time because the samples become darker on the surface as a result of non-enzymatic browning reactions. On the other hand, the a^* value showed an increase during the tested cooking processes because the sample surface obtained more red coloration as a result of the reactions mentioned before. Interestingly, these results agreed with those obtained by other researchers when they studied acrylamide formation phenomena in real systems, such as potato chips and French fries.^{39–41} The Maillard reaction might be the main route of formation of furan in low-moisture starchy foods.

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Notes

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