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NATURAL INHIBITORS OF MAILLARD BROWNING

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14. ABSTRACT This report documents research of a novel natural means of inhibiting Maillard browning, also known as non-enzymatic browning, a complex reaction which can lead to darkening of color, off-odors, off-flavors, and nutritive degradation in food. These efforts were performed by the US Department of Defense Combat Feeding Directorate (CFD) at the Natick Soldier Research, Development and Engineering Center between 2009 and 2012. This reaction has been a consistent problem for CFD's program for extended shelf-life ration components. Many ration components such as dairy based products, cheese spreads, bakery items, and other thermally and non-thermally processed items exhibit some level of Maillard browning over time in storage. This report details initial research that was performed in the laboratory on a model buffer system with glucose and glycine added and then scaled and incorporated in food formulations. Method development and chemical assays were performed to determine the color and chemical changes found in the model systems. The concentration of inhibitor was optimized, and the most effective inhibitors (rosmarinic acid and epigallocatechin gallate) were further studied in rations (baked rolls and sugar/protein-complexed applesauce). The natural inhibitory compounds were successful in limiting the Maillard browning in model systems and food items. The Hunter colorimeter, UV absorbance, pictorial display, and GC/MS confirmed the mitigation of the Maillard browning reaction. The addition of the inhibitor resulted in less browning.					
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PREFACE

This technical report documents the progress of a Science and Technology initiative for a novel, natural means of inhibiting Maillard browning in food, specifically in ration items. The US Department of Defense Combat Feeding Directorate (CFD) completed this work at the Natick Soldier, Research, Development and Engineering Center (NSDEC) under the Techbase project titled “Natural Inhibitors of Maillard Browning”, TB 10-12, funded by the Combat Feeding Research and Engineering Program Board from fiscal year 2010 to 2011.

The initial research that was performed in the laboratory on model buffer system with added glucose and glycine and was then scaled and incorporated in food formulations. Method development and chemical assays were performed to determine the color and chemical changes found in the model systems. The concentration of inhibitor was optimized on the benchtop, and the most effective inhibitors were further studied in rations such as baked goods and sugar/protein-complexed applesauce.

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This report is dedicated to Dr. William Porter. His guidance in writing the initial proposal was invaluable.

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NATURAL INHIBITORS OF MAILLARD BROWNING

1.0 Introduction

This report documents research performed by the US Department of Defense Combat Feeding Directorate (CFD) at the Natick Soldier Research, Development and Engineering Center (NSRDEC) from October 2009 to September 2011. The work was focused on finding a naturally occurring inhibitor that can prevent Maillard browning in combat rations over long periods in storage. The inhibition of Maillard browning via the addition of the most effective natural compounds could greatly improve overall organoleptic quality of the ration components during storage, enhance the Warfighters' nutritional intake, and decrease waste due to non-consumption of sensory degraded ration components.

1.1 Maillard Browning

Maillard browning, also known as non-enzymatic browning, can lead to darkening of color, off-odors, off-flavors, and nutritive degradation in food. This reaction has been a consistent problem for the CFD's program for extended shelf-life ration components. Many ration components such as dairy based products, cheese spreads, bakery items, and other thermally and non-thermally processed items exhibit some level of Maillard browning over time in storage. The most widely used commercial treatment method to inhibit Maillard browning is the addition of sulfites to food. However, the safety of adding sulfites to rations has been under scrutiny due to numerous complaints and hypersensitive reactions. As a result, sulfite usage is banned in combat rations, yet some foods require equivalent preservatives to meet the stringent shelf-life requirements of combat rations. For these ration items, it is imperative that an effective natural treatment method be developed (Kim and Taub 1987). Studies have indicated the potential to inhibit Maillard browning with methods utilizing such natural treatments as green tea epicatechins and other select flavonoid compounds (Schamberger and Labuza 2006).

Maillard browning is a very complicated series of reactions that occurs in foods containing reducing sugars and proteins. It is only in recent years that the reaction steps have been elucidated and scientists have begun to grasp the intricacy of this reaction. However, there are still some intermediary steps and species that are not fully understood. Totlani and Peterson investigated the mechanism of how epicatechin alters the pathway of the reaction in aqueous glucose and glycine models (2005).

The Amadori compound is an intermediary compound found in the early stages of the Maillard reaction. This compound has been heavily studied because it is one of the few which has been isolated from foods. The reducing sugar and amino acid condensation forms a Schiff's base which in turn forms the aminoketose, the Amadori compound. It is this compound that is broken

into numerous products. This action leads to the formation of the brown pigment which causes the dark color, off-flavor, and degradation of nutritional value (Fennema 1976).

The Maillard browning reaction consists of three stages: initial, intermediary and final. The intermediary and final stages have a strong ultraviolet (UV) absorption and an eventual darker pigmentation which can be monitored via spectrophotometer. However, the initial phase does not have UV absorption, yet this stage is critical to reaction monitoring because the Schiff base and Amadori products form during this stage (Schamberger 2006).

1.2 Flavonoids

Flavonoids are naturally occurring polyphenolic compounds that can be found in plants, vegetables, fruits, green tea, wine, and many other consumable items. They are known for their antioxidant properties and have varying molecular structures with a similar backbone (Figure 1). The position of the hydroxyl group and other characteristics of the chemical structures of the flavonoid are key to its antioxidant activities (Lee 1982). The theory behind this research is to introduce these flavonoid compounds before the onset of the Maillard browning reaction to inhibit the reaction from occurring.

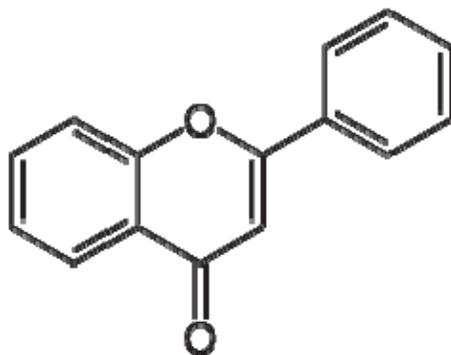


Figure 1: Molecular structure of a flavonoid backbone

1.3 Goal and Hypothesis

The goal of this research was to prevent the Amadori product from further reacting to create the degradative products. The hypothesis was that the introduction of flavonoids to the food matrix would prevent the reaction from continuing. The flavonoids would react with the active sites of the Amadori product, preventing further Maillard compounds from forming.

1.4 Tasks

The major tasks for this study included:

- Research and procure natural antioxidant compounds
- Test compounds at various concentrations in model glucose/glycine system
- Test compounds in model dairy based system

- Conduct chemical analysis to determine effectiveness
- Down select most promising compounds for treatment methods
- Determine best candidate ration components for “treatment”
- Conduct in-house production and storage of “treated” items
- Perform sensory, analytical and chemical analysis of items at pre-determined times throughout storage
- Compile successful results to develop formulation models for treatment of selected ration components

2.0 Experimental Approach

This inhibitory research focused on the parts of the reaction that were already understood, as cited in the literature discussion in Section 1.1. Therefore, initial research was performed in the laboratory on model system of aqueous buffer with glucose and glycine added. Green tea epicatechins were the primary compounds used in a model glucose/glycine system to determine initial concentrations for inhibitory effect. Secondary compounds included resveratrol, rosmarinic acid, and cranberry extract. Concentrated epicatechin and/or selected secondary compounds were incorporated into pre-selected candidate ration components for evaluation via storage, sensory and chemical analysis. The concentration of inhibitor was optimized on the bench top. The two most effective inhibitors were selected and further studied in more complex food matrices: sugar/protein-complexed applesauce and baked rolls.

The pro-oxidative effects of the flavonoid compounds were evaluated for both the model system and the food systems. Due to the lack of UV absorption in the initial stage of Maillard browning, that stage could not be monitored using the standard spectrophotometer. Instead it was monitored by tracking pyrazine formation with gas chromatography/mass spectrometry (GC/MS). The greatest technical challenges included determining the stability and usage levels of various natural compounds and extrapolating them from baseline models to determine treatment methods for various food items.

The proposed treatment method compounds were widely available at reasonable cost, and all of the necessary chemical analyses of the selected compounds and subsequent treated ration components were accomplished in house to provide significant program cost savings.

3.0 Model System

Preliminary evaluations were performed to determine the optimum methodology for the model system. Once the system was suitable, a 10-week storage study using 11 flavonoid concentrations was performed. Pyrazine formation was monitored during the initial phase of the Maillard browning reaction.

3.1 Method Development

For initial purposes, the model system needed to favor Maillard browning in a quick and consistent manner. There were many model systems in current published research, but the sugar, amino acid and heating methods varied. This model system development was based on results from Porter et al. (2006). Because 11 flavonoid concentrations were going to be tested prior to down selection, a rapid heating method was required. The majority of model systems used in published research allowed the model system to progress at its natural rate and remain in storage for up to 6 months. However, the down selection process needed to be rapid and completed in triplicate. It was experimentally determined that a system of glucose:glycine (2:1) in 8.0 phosphate buffer would be used. The cuvettes are shown in Figure 2. Each cuvette was heated for 30 s longer than the previous one. The initial heating apparatus was a microwave that allowed for rapid browning and measurements. Initial browning was detected via spectrophotometry at 5.0 min, as shown in Figure 3.



Figure 2: Cuvettes containing the glucose:glycine model system, heated from 0 s to 8.5 min (left to right) at 30 s intervals.

In order to study the effects of time on the reaction, it was determined that a more consistent thermal apparatus was required that would allow the selected products to heat consistently over an extended period of time. A Lab Armor bead bath (Figure 4) was chosen because Maillard browning is a function of temperature and time. The bead bath allowed study of the effects of heat over a prolonged time on the model system, while the microwave system simply allowed for study of the effects of temperature on the system. The bead bath maintained its heat regardless of environmental factors and sustained a consistent temperature for extended storage durations.

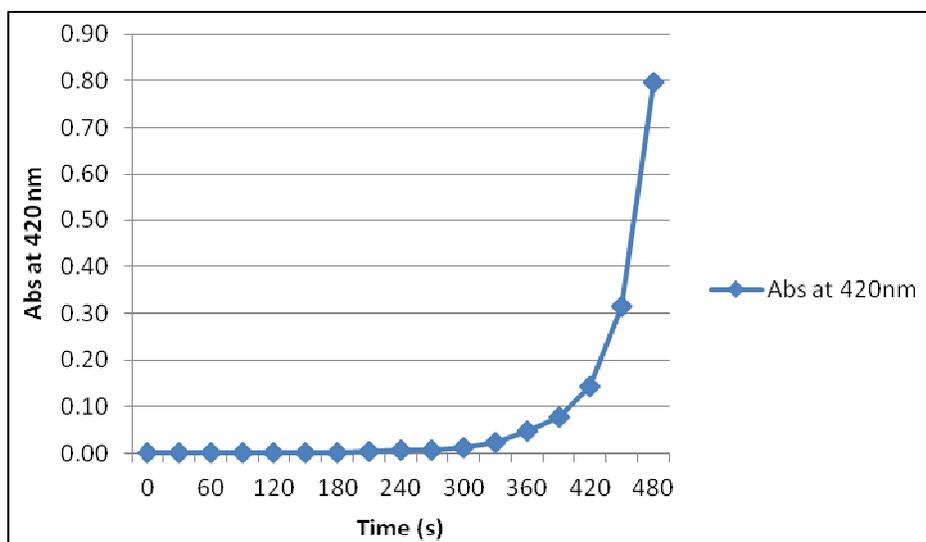


Figure 3: Spectrophotometer results of the microwave heated model system indicating an increase in darkness around 300 s (5.0 min).



Figure 4: Lab Armor bead bath containing various flavonoids added to the model system and stored for 10 weeks at 50 °C.

3.2 Model Storage Study Using Inhibitory Compounds

Once the model system was determined, bench top studies of the following 10 flavonoid concentrations, and a combination of those flavonoids (an 11th concentration), were performed to determine further inhibitory effects.

- Epigallocatechin gallate (EGCG)
- Polyhydroxytyrosine copolymerized with quercetin
- Rosmarinic acid

- Quercetin
- Epigallocatechin
- Resveratrol
- Cranberry extract
- Polyepicatechin
- Pomegranate powder
- Steeped green tea

The flavonoids were placed in the model buffer solution with an addition of sodium azide to prevent microbial growth for 10 weeks. The initial concentrations for study were between 0.1 mmol and 1.0 mmol in 25 mL of the glucose/glycine model system. However, the browning in all samples, except the control, was immediate before the addition of heat (Figure 5). It was discovered that high concentrations of the inhibitors promoted browning and much lower concentrations would be needed for the inhibitory effect. After testing concentrations that ranged from 0.25% to 1.0% of 0.1mmol stock solutions, it was determined that a 0.5% solution would be added for further analysis.

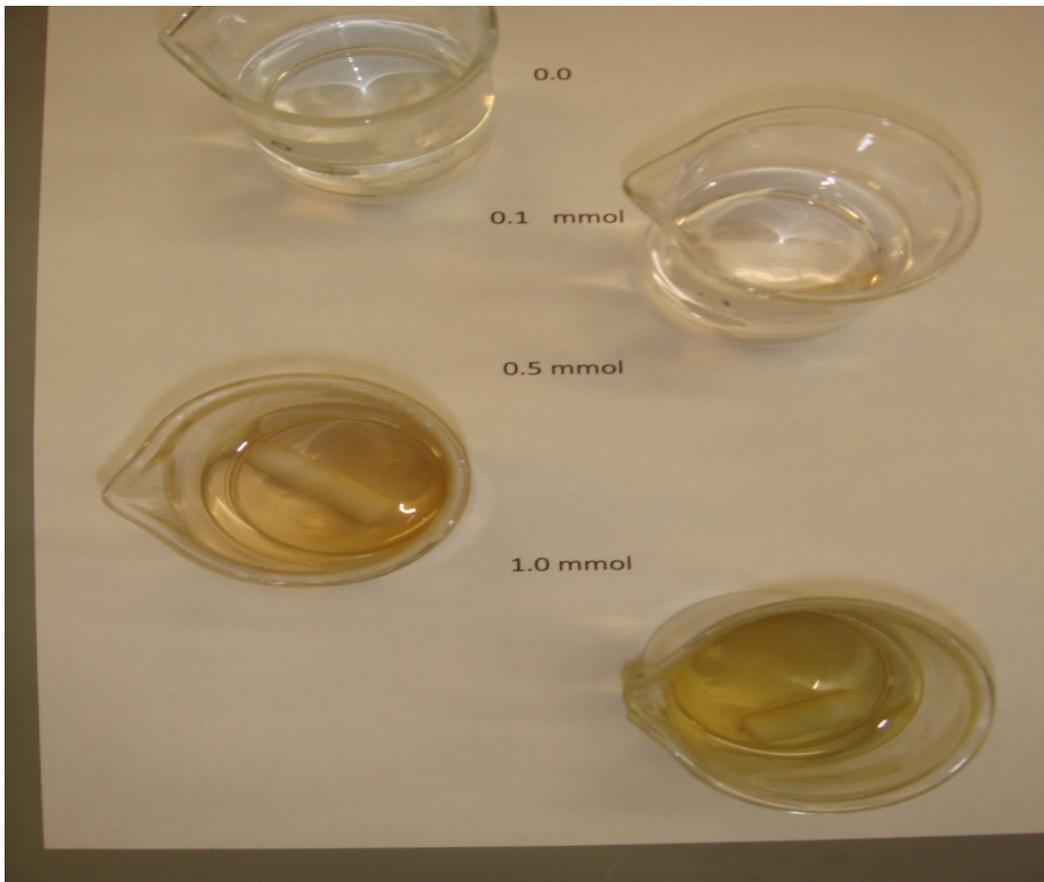


Figure 5: Pro-oxidative effect of high concentrations of EGCG in model system.

3.2.1 Model Storage Results

Based on the results of the storage study, it was determined that a 0.5% solution of EGCG and rosmarinic acid (mutually exclusive) presented the greatest levels of browning inhibition to the model system, without the pro-oxidative effect. Both the quick microwave model system and the Lab Armor model system presented similar results. The results from the microwave testing for those two compounds and the control are compared in Figure 6. The results from the first 5 weeks of storage in the Lab Armor model system for those two compounds, two others, and the control are compared in Figure 7; pomegranate powder showed the greatest inhibition during that period. The results for the control for the entire 10 weeks of storage are shown in Figure 8; the browning began to present at Day 9.

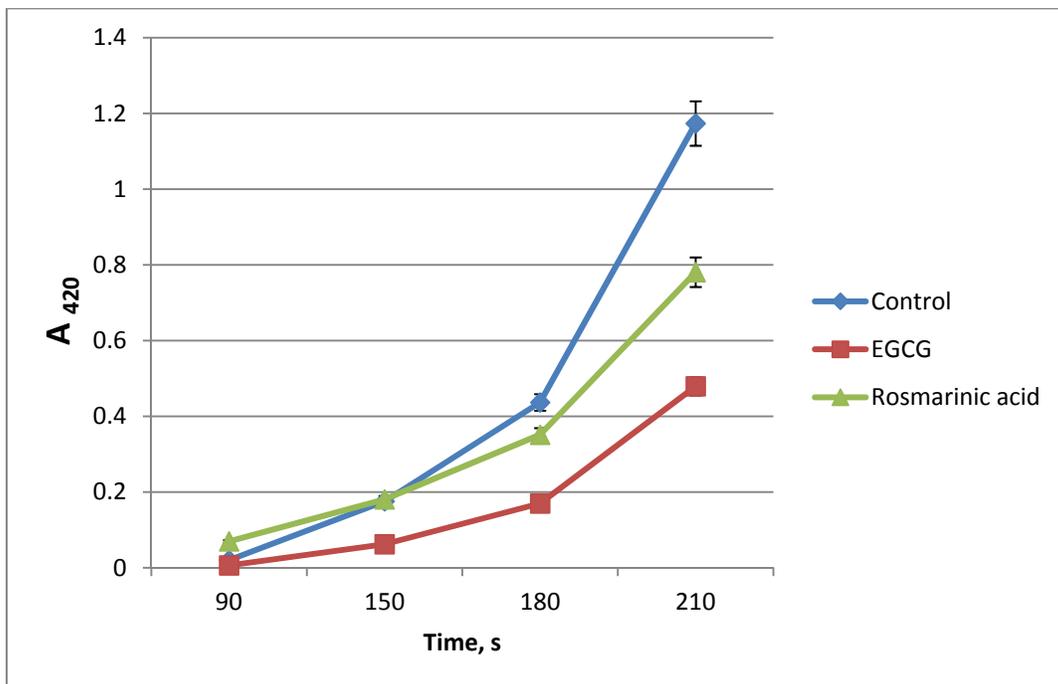


Figure 6: Comparison of inhibition of Maillard browning of rosmarinic acid, EGCG, and the control to the microwave model system (high heat, short storage time).

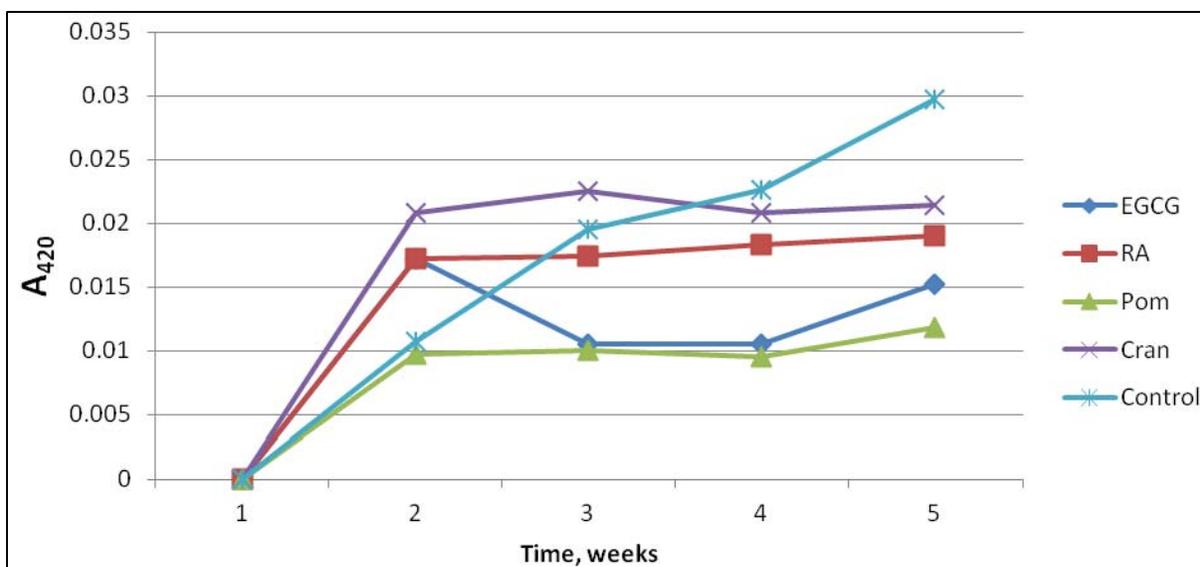


Figure 7: Comparison of inhibition of Maillard browning of rosmarinic acid, EGCG, pomegranate powder, cranberry extract, and the control to the Lab Armor heated model system (low heat, extended storage).

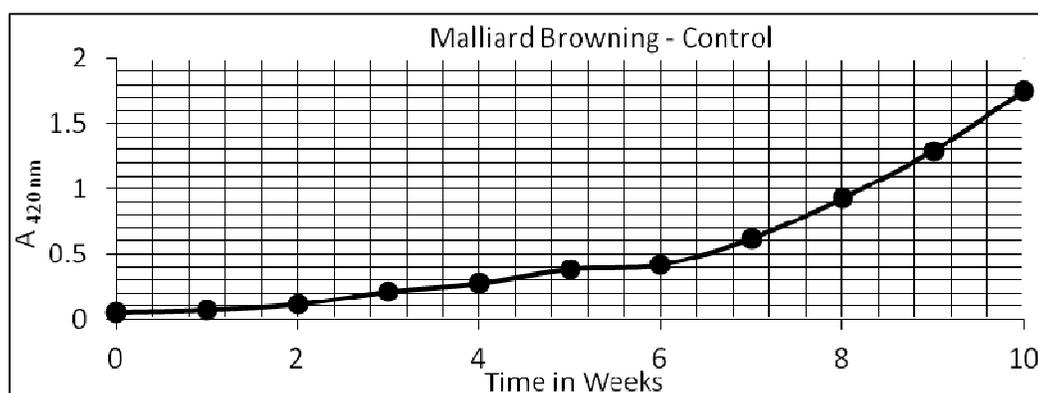


Figure 8: Lab Armor heated model system without added inhibiting compounds. .

3.2.2 Pyrazine Detection in Model System

The early stage of the Maillard reaction produces volatile flavor compounds that include pyrazines. The presence of pyrazines is important in the overall flavor and quality of cooked foods, as it is indicative of the Maillard reaction. Pyrazine detection was performed by solid phase micro extraction (SPME) followed by GC/MS. An Agilent 6890/5975 GC/MS equipped with a CombiPal autosampler was used to complete the pyrazine analysis. A gray Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber was used for the extraction, with a 1200 s incubation time at 70 °C, 500 rpm agitation speed and 1200 s extraction time. The inlet was set to 250 °C in splitless mode, and helium was used as the carrier gas. A Supelcowax 10 column was used in an oven set to 60 °C and a ramp up program of 4.0 °C/min to 250 °C. Figure 9 compares examples of pyrazine chromatograms with and without inhibitors.

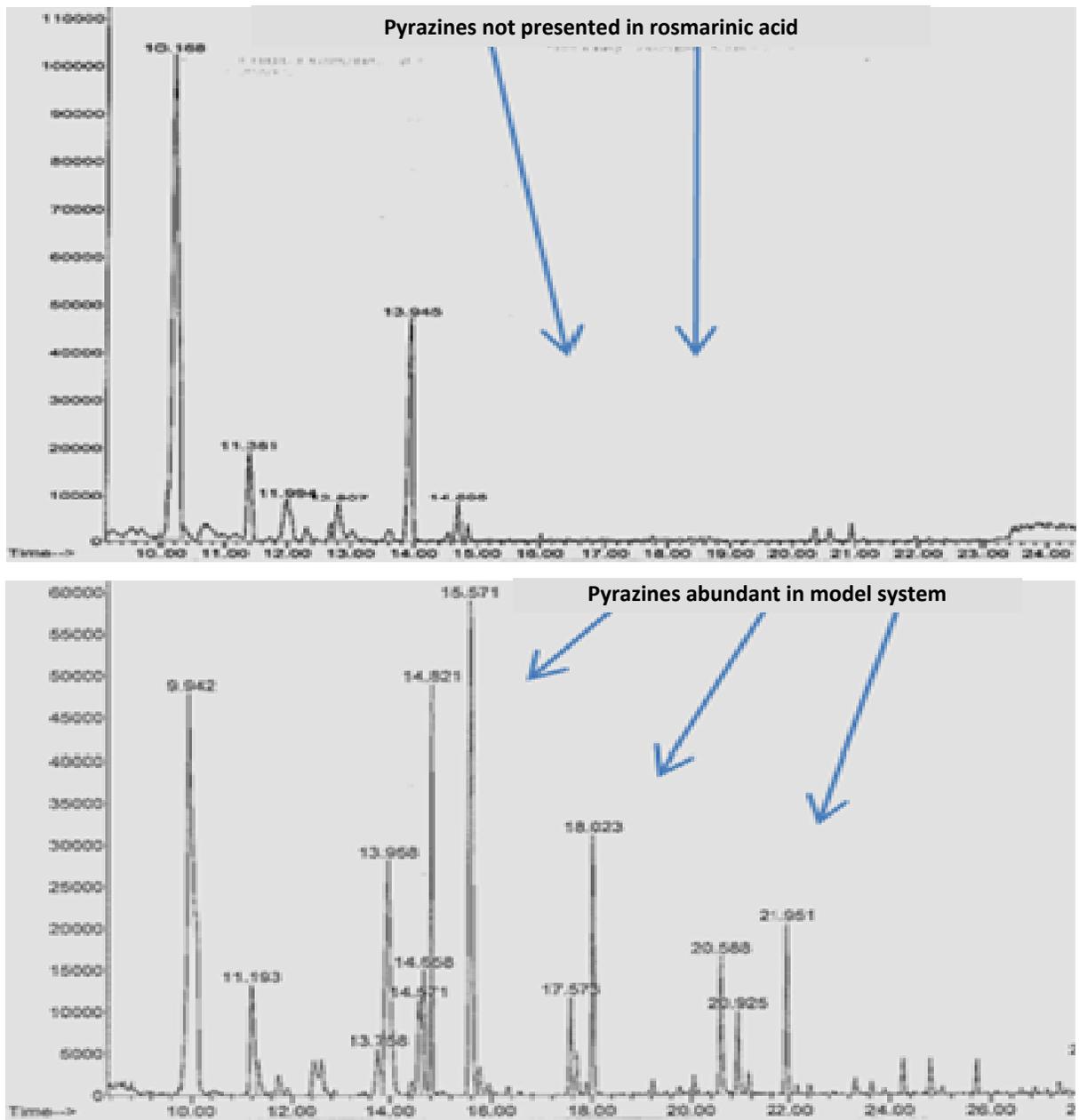


Figure 9: Comparison of pyrazine presentation in GC/MS chromatograms of model system samples. Top: With rosmarinic acid; Bottom: Without inhibitory compounds.

4.0 Storage Study Using Inhibitory Compounds in Foods

Based on the results of the model storage study, rosmarinic acid and EGCG were selected to be added to food and ration items for 4-week storage studies to determine their effectiveness in food. A combination of the two flavonoids (in equal parts) was also studied to determine if a synergistic effect existed. The ration items tested were sugar/protein-complexed applesauce and bakery rolls.

4.1 Applesauce

The rosmarinic acid, EGCG, and the combination of the two were added to applesauce at two concentrations: 1% and 1.5% of a 0.01 mmol solution. The applesauce was stored for 28 d at an accelerated temperature of 50 °C. The samples were pulled weekly, and color analysis was performed on a Hunter colorimeter. The L,a,b results from the colorimeter were measured and recorded. The L-value was the most significant value because an increase in L means that the sample is lighter than the control. The L-values for each compound at each concentration at each pull are shown in Figure 10; the lower the value, the darker the sample. The, L, a, and b values are compared for each compound at each concentration at the end of the storage period in Figure 11. The rosmarinic acid concentrations produced the highest L-values; the control had the lowest L-values.

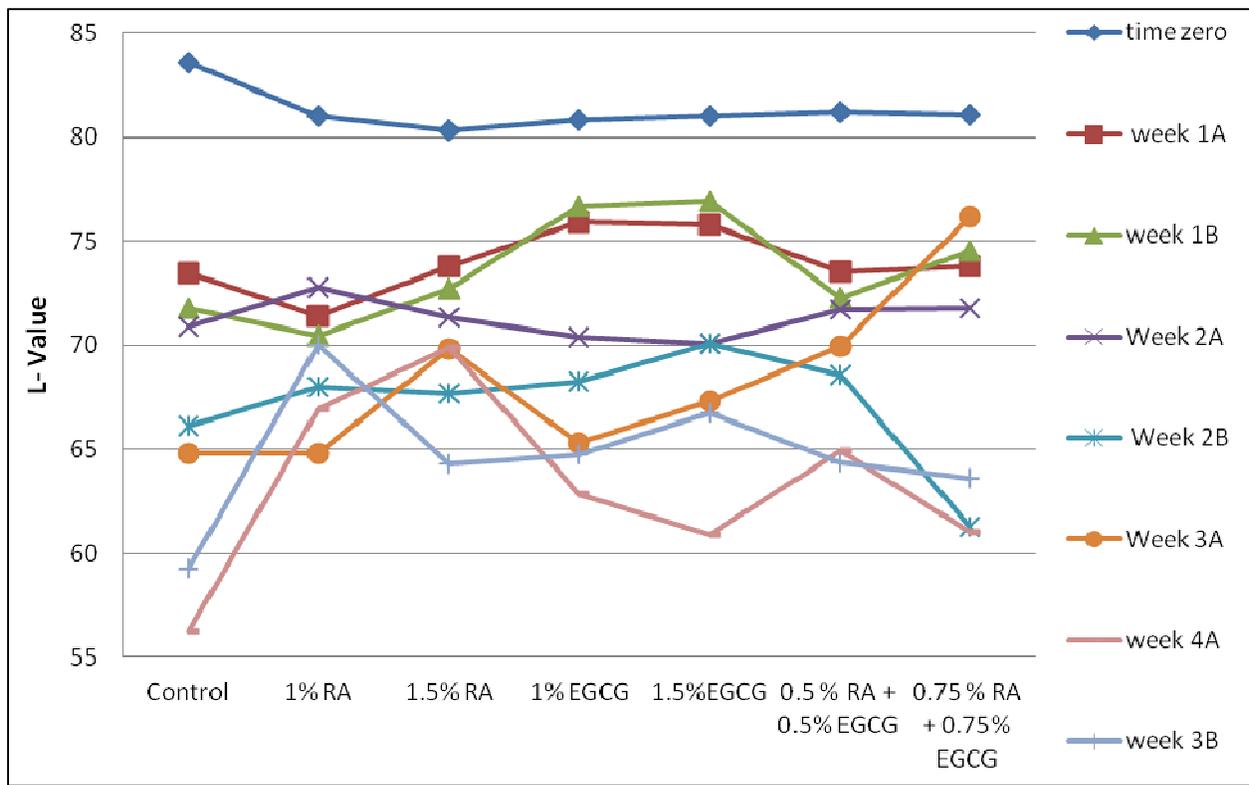


Figure 10: L-values of the stored applesauce over the 4-week period.

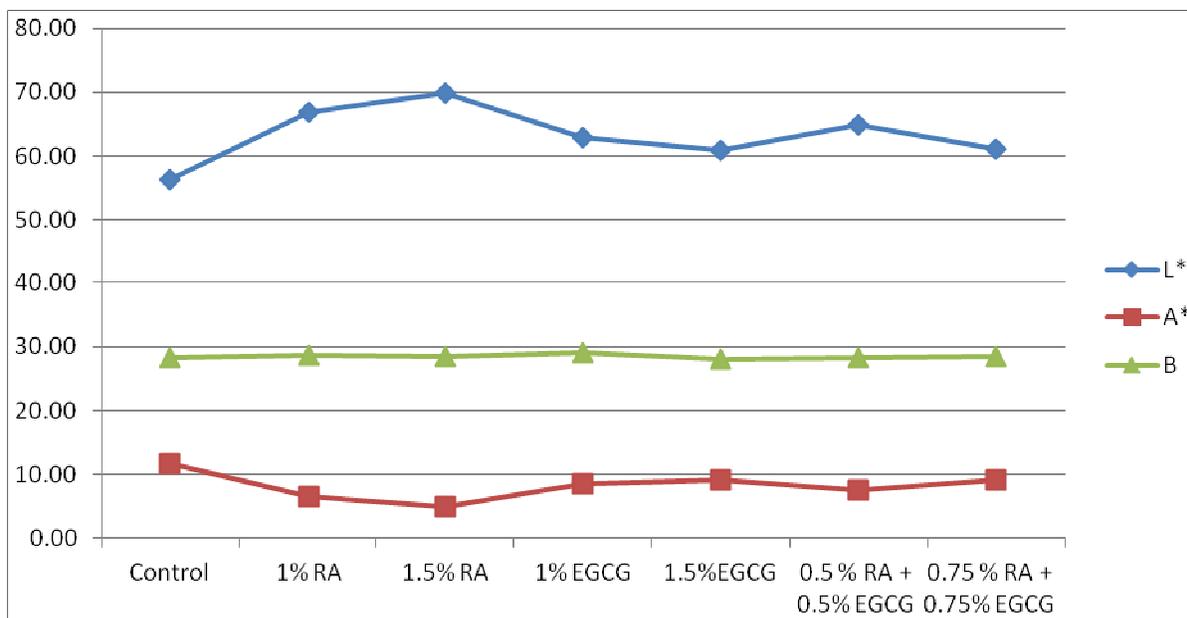


Figure 11: L,a,b color values of the stored applesauce at end of 4-week storage period.

4.2 Bakery Rolls

The rosmarinic acid and EGCG compounds were added to the standard CFD bakery roll formulation at varying concentrations determined from the previous bench top work and in combination. The bakery rolls were individually packaged, sealed, and stored for 4 weeks at 49 °F. Hunter colorimeter values were taken at the weekly pulls. The L-values at each pull for each compound at 0.5% of a 0.1 mmol concentration in water and the control are compared in Figure 12. The bakery rolls with a 0.5% EGCG/rosmarinic acid combination solution in water presented the least amount of darkening while in storage. In all storage studies, the control rolls were darkest after storage and had the lowest L values. As shown in Figure 13, the control rolls (right) were significantly darker than the combination solution at the end of storage.

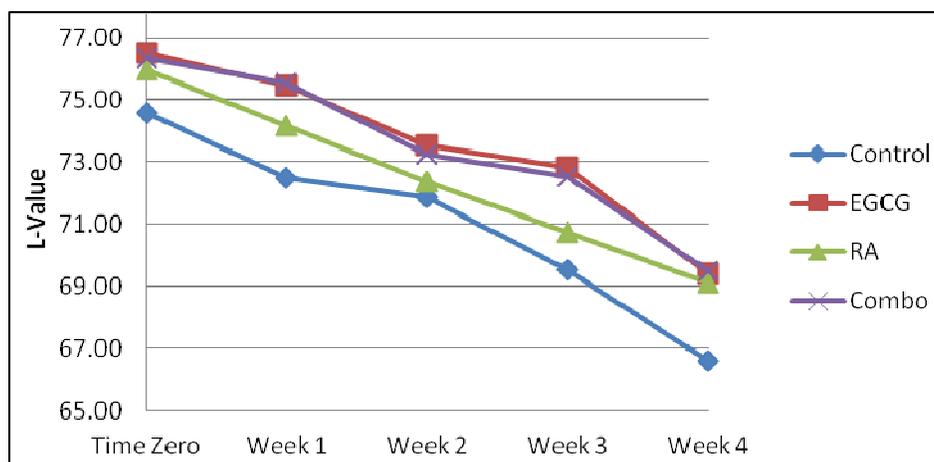


Figure 12: L-values of the bakery rolls stored for 4 weeks at 120 °F.



Figure 13: Comparison of bakery rolls after 4 weeks storage at 120 °F. Left: 0.5% EGCG/rosmarinic acid 0.1 mmol solution: Right: Control.

To continue the research regarding the pro-oxidative effects of the EGCG flavonoid compound, a large amount of EGCG (1.5%) was added to the bakery roll formulation. The L-value with the elevated EGCG concentration was much higher than the L-values for the control or the other samples with the optimized amounts (0.5%) of flavonoids. This comparison is shown in Figure 14. The color of the bakery roll containing the high concentration of EGCG was darker than the control, which was darker than the rolls with the optimized concentrations..

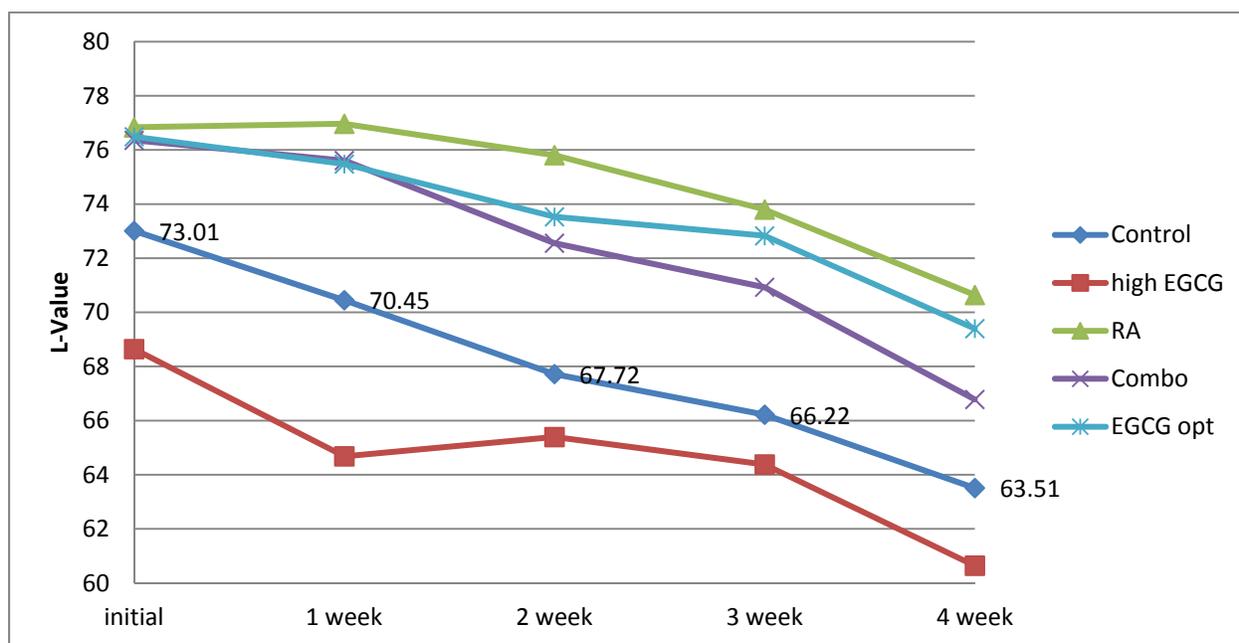


Figure 14: Pro-oxidative effects of EGCG in food -- comparison of L-values for rolls with optimized inhibitor concentrations (0.5%), the control, and rolls with high concentration (1.5%) of EGCG.

5.0 Conclusions and Future Research

Determining a sulfite alternative that prevents Maillard browning in foods has been a historic problem that has perplexed food technologists for decades. The natural inhibitory compounds studied in the project reported here were successful in limiting the Maillard browning in model systems and food items. The Hunter colorimeter, UV absorbance, pictorial display, and GC/MS confirmed the mitigation of the Maillard browning reaction. The addition of the inhibitor resulted in less browning (Figure 15). This research will be useful in future product development for ration and commercial items that deteriorate from Maillard browning effects while in storage.



Figure 15: Avocado 96 h after being cut and processed on the Nova 2200. Left: Without flavonoids added; Right: With a water solution of 0.5% combined EGCG/rosmarinic acid added.

The data from this research was immediately transitioned to the Techbase project “Innovative Ration Preservation via Supercritical Carbon Dioxide (SCCO₂)” and the JSN project “Next Generation Bakery Items”. The EGCG and rosmarinic acid were added to the SCCO₂ processing method to halt an initial browning process that was believed to occur as a function of Maillard browning and not enzymatic browning.

The information in this study will continue to be used for future product development. It is unlikely that a single compound will be a panacea for all developmental products, but this research will provide a starting point for food technologists. That is, the end result of this work determined baseline usage levels of selected compounds for inhibition of the Maillard browning reaction in existing ration components and future developed prototypes. Furthermore, the determination that high quantities of many compounds may cause a browning effect could prove to be invaluable information.

This document reports research undertaken at the U.S. Army Natick Soldier Research, Development and Engineering Center, Natick, MA, and has been assigned No. NATICK/TR- 14/005 in a series of reports approved for publication.

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