January 2016

Macronutrient-Flavonoid Interactions, Effects in Model Food Matrices

Jennifer Louise Allen
Purdue University

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By Jennifer Louise Allen

Entitled
Macronutrient- Flavonoid Interactions, Effects in Model Food Matrices

For the degree of Doctor of Philosophy

Is approved by the final examining committee:

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Dr. Owen Jones

Dr. Gordon Smith

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Approved by Major Professor(s): Dr. Mario G. Ferruzzi

Approved by: Dr. Brian Farkas 10/19/2016

Head of the Departmental Graduate Program Date
MACRONUTRIENT-FLAVONOID INTERACTIONS, EFFECTS IN MODEL FOOD MATRICES

A Dissertation

Submitted to the Faculty

of

Purdue University

by

Jennifer L. Allen

In Partial Fulfillment of the

Requirements for the Degree

of

Doctor of Philosophy

December 2016

Purdue University

West Lafayette, Indiana
This dissertation is dedicated to my father, Chester Merritt Sr, though you may not be able to read these pages, my motivation has always been grounded in witnessing your strength and ability to persevere by any means.

To my siblings Derrick Merritt, Nicole Merritt, Bridget Merritt-Dabney, LaHarma Merritt, O’Hala Riddle, Patricia Allen-Robinson, Chester Merritt Jr, and Christine Allen. Your unwavering support, love, and prayers have been my guiding light through these years.

To all of my nieces and nephews, you are my inspiration. We have come a long way, and I want you to understand that it's never easy. Perseverance and hard work are key to being successful, and you have it in you.

To my extended family (friends since way back when), you ladies have been there for me through it all, always offering a positive opinion and supporting me through indecisiveness, skepticism, and apprehension, helping me realize my potential is only limited by my own self-conscience.

In memory of my mother Kathleen Allen and eldest brother Everett Merritt Sr, though you weren’t able to physically witness this dream come to fruition, I know you have been here every step of the way.
I would like to first thank my advisor, Dr. Mario Ferruzzi for his guidance and support. I probably never said this but to you but I think you are an awesome researcher and professor and I aspire to be as you are in your ability to communicate science so well. I would like to thank the members of my committee, Dr. Osvaldo Campanella, Dr. Gordon Smith, Dr. Owen Jones, and interim member Dr. Jonathan Gray for always offering immense knowledge and encouragement whenever I had questions.

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<tr>
<td>MPI</td>
<td>Macronutrient-Polyphenol Interactions</td>
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<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<tr>
<td>C</td>
<td>Catechin</td>
</tr>
<tr>
<td>EC</td>
<td>Epicatechin</td>
</tr>
<tr>
<td>ECG</td>
<td>Epicatechin 3-Gallate</td>
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<tr>
<td>EGC</td>
<td>Epigallocatechin</td>
</tr>
<tr>
<td>EGCG</td>
<td>Epigallocatechin 3-Gallate</td>
</tr>
<tr>
<td>OPC</td>
<td>Oligomeric Proanthocyanidins</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of Polymerization</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial Nitric Oxide Synthase</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic Guanosine Monophosphate</td>
</tr>
<tr>
<td>MRP</td>
<td>Multiresistant Proteins</td>
</tr>
<tr>
<td>GSE</td>
<td>Grape Seed Extract</td>
</tr>
<tr>
<td>GTE</td>
<td>Green Tea Extract</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>PRP</td>
<td>Proline-rich protein</td>
</tr>
<tr>
<td>PAC</td>
<td>Proanthocyanidin</td>
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<td>PLA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Phospholipase A&lt;sub&gt;2&lt;/sub&gt;</td>
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<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
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<td>CD</td>
<td>Circular Dichroism</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>TVG</td>
<td>Teavigo®</td>
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<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<td>LC-MS</td>
<td>Liquid Chromatography-Mass Spectrophotometry</td>
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<td>SM</td>
<td>Starting Material</td>
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<td>SCN</td>
<td>Sodium Caseinate</td>
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<td>Whey Protein Isolate</td>
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<td>KCGN</td>
<td>κ-carrageenan</td>
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<td>WS</td>
<td>Wheat Starch</td>
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<td>GEL</td>
<td>Gelatin</td>
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ABSTRACT

Allen, Jennifer L. PhD, Purdue University, December 2016. Macronutrient-Flavonoid Interactions, Effects in Model Food Matrices. Major Professor: Mario G. Ferruzzi.

Macronutrient-flavonoid interactions have been well documented through the years. Chapter 1 of this document provides an introduction of the literature documenting the theoretical and mechanical framework of their importance in human health, biological interactions, macronutrient interaction, and general bioaccessibility and bioavailability. Though macronutrient interactions have been characterized, often the levels utilized are above those that may be seen in processed food products due to undesirable effects. This document covers the effects two commonly used plant extracts, green tea extract and grape seed, may have on the macronutrient functionality in model food matrices. In Chapter 2 the effects of crude extracts (green tea and grape seed) and a purified green tea extract (composed primarily of EGCG) were examined for their effects on various protein functions within a gel, emulsion, and foam matrix. Due to their occurrence in many packaged food products the dairy proteins, sodium caseinate and whey protein isolate were utilized. These extracts were incorporated into each of the above stated matrices at levels that may be plausible in consumer packaged food products with minimal effects on the sensorial properties. It was determined that though level of incorporation were modest, an impact may still be had on the functional properties of these matrices. The addition of grape seed and green tea extracts may moderately alter gelation properties, including gel strength and gelation time. Likewise, emulsion stability was negatively modified while foam stability exhibited an inverse alteration.
Similar functional testing of functionality in carbohydrates were reviewed in Chapter 3, using wheat starch and κ-carrageenan, both commonly found in processed foods, representing a digestible and non-digestible carbohydrate, respectively. The crude green tea and grape seed sourced extracts were incorporated into the carbohydrate systems to determine the effects on starch pasting and polysaccharide gelation using dynamic rheology. In wheat starch pastes the incorporation of the plant extracts exhibited a dose dependent response that resulted in a decrease in gelatinization temperature and time however offered minimal overall impact on the final viscosity. Similarly the impact on the gelation of κ-carrageenan in the presence of green tea or grape seed extract was minimal in regards to its flow properties.

Chapter 4 evaluated how the bioaccessibility of flavan-3-ols from the plant extracts were impacted by their incorporation into the protein and/or carbohydrate matrices. Each of the crude green tea and grape seed extracts, along with the purified EGCG green tea extract were combined in the aforementioned food matrices and digested using a three stage static in vitro digestion model to determine the effects on bioaccessibility. These studies indicated that carbohydrate and protein matrices modulate the bioaccessibility of flavan-3-ols. Both types and structures of the macronutrients resulted in a modulated effect of overall relative bioaccessibility. The composition of the extract may have a modulating effect on bioaccessibility of the individual flavan-3-ols present. Chapter 5 reviewed the results of these studies to offer final overall conclusions and potential next steps for further research.
CHAPTER 1. REVIEW OF LITERATURE

1.1 Introduction

Processed foods have been arguably one of the greatest conveniences provided to consumers in the 20th century, allowing individuals to prepare meals and snacks in minutes rather than hours. As consumer’s needs and interest have changed, so have the nature and perceived value of these processed and packaged products. In a 2012 survey, nutritive value (61%) took precedence over convenience (53%) as a driver of consumer decision to purchase food and drink. Considering these trends, foods and beverages with nutritional or functional value, otherwise known as functional foods, are estimated to have a value of ~$38 billion in sales, representing approximately 27% of the US healthy foods category.

The term functional foods is often utilized in defining such foods that include ‘physiologically-active components’ which may provide additional health benefits beyond its nutritive value. Polyphenols are one category of ingredients that have been heavily leveraged in functional foods. Polyphenols are a plant secondary metabolite class which may have beneficial health effects in humans. As research has evolved on the role of these phytochemicals in human health, polyphenols as ingredients derived from plant extracts including tea, apple, cocoa and grape products has seen increased use in processed foods has expanded. However, as more foods are formulated with these ingredients the potential for interactions between polyphenols and food components altering both polyphenol bioavailability or physical/chemical properties of the foods are possible. These include interactions that promote cross-linking of proteins, gelatinization of starches, and potentiate the formation of macronutrient-polyphenol complexes with multiple effects possible. Macronutrient-polyphenol interactions (MPI) have, in fact,
been associated with enhancement of polyphenol stability through digestion, promoting delivery of active compounds for intestinal uptake \(^5\,6\). However, studies have also reported that indigestible or insoluble complexes between polyphenols and proteins or fiber may be formed, limiting beneficial nutritive value of foods, and potentially reducing polyphenol accessibility in the GI tract and ultimate bioavailability \(^4\,7\). Additional insights are needed to better leverage these ingredients across food matrices, particularly in a paradigm where multi-functionality of ingredients is now the norm.

Despite existing data, the extent to which the multi-functionality of polyphenols can be leveraged to modify food and health properties remains unclear. With consumers becoming more aware of product ingredient statements, a growing need for more transparency in product labeling is required. Polyphenols, by virtue of their structure and functionality in regards to color and flavor, provide potential alternative for certain functions in clean-label processed foods. Phytochemicals have been studied for their functionality in foods including their ability to impart natural color, flavor, preservative/antioxidant activity, and as stabilizing agents\(^8\,-\,10\).

Central to their activity in foods and health, polyphenols are well known for their ability to interact with macronutrients. These interactions have been cited as potential mechanisms involved in manipulating taste and textural properties of select foods \(^4\,11\,12\). Reports of polyphenol interactions influencing functional properties such as dough conditioning, foaming stability and haze formation in beer have been reported. While often reported as negative impacts, the question remains if these interactions can be leveraged as clean label innovations, with polyphenols provide both health promoting activities and functionality in foods. However, in order to achieve this, additional insights are needed to expand the application of polyphenol based ingredients into various food systems in a manner that can both promote the best functional properties in foods but also consider impact on ultimate bioavailability and efficacy of these health promoting compounds \(^13\,14\).
There are several factors associated with the polyphenol and/or the macronutrient, which govern the nature of their interaction including 15:

- Structure of the polyphenol (hydroxyl groups, galloyl groups, degree of polymerization)
- Nature and structure of the macromolecule (protein, carbohydrate, lipid)
- Environment Conditions (pH, ionic strength, temperature, concentrations)

Whole and processed foods are complex matrices, being composed of lipids, carbohydrates, and/or proteins of various sizes, structures, and binding attributes. Therefore, composition and interactions among matrix components govern the potential impact on physical and chemical attributes, digestibility, and subsequent absorption of these foods. In this review, the focus will be limited to flavan-3-ols, one of the most commonly consumed polyphenols in the US diet 16,17 and highly leveraged in food applications from tea and grape seed extracts. The objective of the review is two-fold, (1) highlight the impact of macronutrient-polyphenol interactions (MPI’s) on bioaccessibility of the flavan-3-ol and (2) discuss the role of MPIs on functional properties of foodstuff.
1.2 Classification of Polyphenols

Polyphenols are a class of phytochemicals that generally encompasses the large diverse classes of compounds which have at least one or more phenolic (aromatic) ring. Polyphenols are generally divided into 4 subclasses (phenolic acids, flavonoids, stilbenes, and lignans) according to the number of phenol rings and degree of substitution of the rings. Thousands of polyphenols have been identified in nature, and many of their effects extend beyond the plant to include potential health benefits to humans. Consumption of polyphenol rich foods has been associated through observational and experimental studies (preclinical and clinical) with prevention or protection against, heart disease, cancer, obesity, diabetes and neurocognitive disorders 18-22. Many of these benefits have been associated with the flavonoid class of polyphenols specifically and their ability to modify oxidative and inflammatory stress as well as their ability to interact with macronutrients including proteins 23-25. With that in mind flavonoids, and flavan-3-ols in particular, will be the focus of this review.

1.3. Flavonoid Classifications and Dietary Consumption

Flavonoids are chemical composed of 15 carbons and characterized by two phenolic rings, designated as A & B, with a C3 bridge that forms an oxygenated heterocycle (C-ring) between the aromatics. Flavonoids are further differentiated into sub-classes according to their substitution patterns and conformation 24. This differentiation includes the connectivity of the B-ring to the C-ring, oxidative state, and functional group substitution 26. Six major subclasses include flavan-3-ols, flavanones, flavonols, flavones, isoflavones, and anthocyanidins (Figure 1.1).

Flavonoids are one of the most extensively investigated subclasses of polyphenols due to their particular abundance in certain western diets and proposed role in human health. According to data analyzed from the NHANES (National Health and Nutrition Examination Survey) 2007-2010 study, tea, citrus fruit juices, berries, citrus fruits, wine and apples are the main sources of flavonoids in the US diet. Intake estimates in the US suggest that the daily intake of total flavonoids by US adults in 2007-2010 was ~ 200 mg/d, with an increase seen in the estimated intake of anthocyanins from previous years.
(1999-2002). The major group of flavonoids in the US diet are flavan-3-ols and flavonols \(^\text{16}\) derived from products identified above including plus cocoa based products. In nature, many flavonoid forms are primarily present conjugated to sugars (glycosides) or other conjugates, however flavan-3-ols are somewhat unique in that they are primarily found in free (aglycone) form.

\[
\begin{aligned}
\text{Flavone} & \quad \begin{array}{c}
\text{Flavan-3-ol} \\
\text{Flavonol} \\
\text{Anthocyanidin} \\
\text{Isoflavone} \\
\text{Flavonone}
\end{array}
\end{aligned}
\]

**Figure 1.1** Six Major Subclasses of Flavonoids

1.3.1 Flavan-3-ols

Flavan-3-ols, also referred to by their more common names flavanols or catechins, represent approximately 79% (158.4 mg/d) of flavonoid intake in the US diet \(^\text{16}\). The presence of these compounds in foods can affect many food quality parameters including taste (bitterness and astringency) and color formation \(^\text{19}\). Often used as functional ingredients, flavan-3-ols are formulated in foods from extracts derived from tea, grape seed and cocoa or purified into individual flavan-3-ols. These bioactives have been sold as dietary supplements and applied in the fortification of beverages and processed foods. Flavan-3-ols are also recognized as the most structurally complex group, ranging from simple monomers to polymeric proanthocyanidins \(^\text{27}\). The molecular structure of flavan-3-ols differ from other groups based on their lack of glycosylation in
the C ring and presence of a 3-hydroxyl or 3-O-gallate functional group on the C-ring. Flavan-3-ols are well known as traditional antioxidants and are believed to prevent oxidative damage decreasing the risk of certain chronic and degenerative diseases. The antioxidant capacity of many flavan-3-ols has often been compared to that of Vitamin C and E, as its concentration in our diets and antioxidant capacity is in fact comparable to vitamin C.

1.3.2 Monomeric Flavan-3-ols

Green tea is one of the most highly concentrated dietary source of monomeric flavan-3-ols in Western diets. The main monomers found in the diet (Figure 1.2) are (-)-epicatechin (EC), (-)-epicatechin 3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin 3-gallate (EGCG). (+)-Catechin, is also broadly distributed in foods, for example in apples, grape seeds and cocoa. Nevertheless, the monomeric flavan-3-ols are not limited to those aforementioned. Isomers including (-)-catechin, (-)-epiafzelechin, and (+)-epicatechin are found in foods but are considered rarer in nature or derived through process induced isomerization. This is the case for (-)-catechin in cocoa. Hydroxylation of either the (+)-catechin or (-) epicatechin allows for the formation of the gallocatechins, abundant in both green and black tea. Furthermore, esterification on the C-ring, for example with gallic acid will produce larger monomers (ECG and EGCG), as well as more complex structures such as proanthocyanidins.
1.3.4 Polymeric Flavan-3-ols Nomenclature and Characterization

Beyond monomeric forms in foods, larger oligomeric/polymeric forms make up the largest portion of dietary flavan-3-ol forms. Oligomeric (with a degree of polymerization (DP) 2-4) and polymeric (DP >4) flavan-3-ols or proanthocyanidins are often referred to as condensed tannins or larger procyanidins. Nevertheless, these terms define the broad groups of larger flavan-3-ols. Polymeric flavan-3-ols may incur a degree of polymerization (DP) up to 50 units, and may be divided into two categories based on their A ring classification, i.e. phloroglucinols and resorcinols\textsuperscript{32}. Among these groups there are two phloroglucinol proanthocyanidins most commonly represented in nature, procyanidins and prodelphinidins. Procyanidins have a 3’, 4’-dihydroxyl substitution (epi-/catechin), while prodelphinidins display a 3’, 4’, 5’-trihydroxy substitution (epi-/gallocatechin) pattern on the B ring\textsuperscript{19}. In this review, prodelphinidins will not be of focus therefore will not be further discussed.
Procyanidins are the most abundant type of proanthocyanidins found in plants. Proanthocyanidin rich dietary sources include, but are not limited to apples, blueberries, cocoa, cranberries, and grape seeds. These flavan-3-ols are mainly linked via C4→C8 bonds or C4→C6 bonds and are referred to as type- B procyanidins (Figure 1.2); the two bond types give rise to eight variations of the type-B procyanidins. Type A dimers exist as well via double interflavan linkages, C4→C8 and C2→O7 ether bonds. Other linkages are found in nature, but of the two types mentioned, B is the most commonly found in foods such as cocoa, grape and wine, while type A oligomers and polymers are found, to a large extent, in products such as cranberries, plums, peanuts, and cinnamon. Type C have also been referenced in literature in reference to trimeric forms, which are successive C4→C8 linkages of 3 monomeric flavan-3-ol units. Larger procyanidins occur as well, specifically in cocoa and grape seed extract, ranging from tetramers to decamers. A DP as high as 17 has been found in an extract of cider apple and may exist up to 50 DP.

Figure 1.3 Theaflavin Structures
1.3.4 Oxidized Forms of Flavan-3-ols

Oxidative reactions that impact flavan-3-ols are common in nature and through food processing. These reactions allow for the formation of derived compounds that are critical to the characteristic color and flavor of flavan-3-ol rich foods such as tea and cocoa. In black tea production, enzymatically facilitated reactions occur during the oxidative fermentation of green tea leaves lead to the formation of theaflavins and thearubigins. There are four principal theaflavins that have been identified, i.e. theaflavin, theaflavin-3-gallate, theaflavin-3’-gallate, and theaflavin-3-3’-digallate (Figure 1.3). Theaflavins are characterized as having an orange-red color in aqueous solutions and a benzotropolone ring occurs as the product of dimerization of a catechin and a gallocatechin. These oxidative products represent approximately 2% of the black tea leaf and as much as 6% of the solids content of brewed black tea. Additionally, other “further” oxidative products known as thearubigins and theabrownins have been described in black tea. These compounds have been only partially characterized to date but appear to be derived from theaflavins. Despite a lack of full chemical characterization, these compounds are speculated to comprise ~20% of black tea leaves and 60% of the solids content of brewed black tea.

1.3.5 Flavan-3-ols in Foods

Estimated flavan-3-ol content of common foodstuff has been compiled in several databases that are publically available. Phenol-Explorer was initiated in Europe and holds a database that incorporates over 500 different polyphenols found in over 400 foods. Similarly, the USDA Database for the Flavonoid Content of Selected Foods provides data on over 500 foods, specifically related to the flavonoid subclass of polyphenols (Table 1.1). As it may well be assumed, fruits and vegetables as well as tea and cocoa products commonly consumed in the US contain some of the highest concentrations of these compounds. For example, flavonoids can make up as much as 30% of the dry matter of certain tea leaves, which includes the major monomeric flavan-3-ols: EC, EGC, and their gallic acid esters ECG and EGCG. One serving of green tea (200 ml) may provide between 20-160 mg of the monomeric flavan-3-ols. A rich
source of the procyanidins is dark chocolate, with as much as 1635 mg/100 g (fw) total PC found, and over 50% of PC being > 4 monomeric units in size \(^{33}\). Grape seeds or grape seed extract (GSE), byproducts of grape juice and wine production are also a rich source of both monomeric and polymeric flavan-3-ols. Monomeric flavan-3-ols are as much as 660.3 mg/100 g, while polymeric units >10 DP represent 1100.1-mg/100 g of dry weight. The total PC content may be as much as 3532.3 mg/100 g dry weight \(^{33}\).

In most fruits and vegetables, the highest content of flavan-3-ols can be found in the skins or seeds, so peeling can significantly impact these levels of phenolics actually consumed \(^{39}\). Further industrial processing may also have a significant impact on the concentrations of flavan-3-ols in foods. Thermal and mechanical processing may lead to damaging of plant tissue that allows for oxidative or enzymatic degradation \(^{40}\). For example, cacao beans when processed for cocoa liquor or powder are subjected to drying, fermentation, and roasting steps. This results in the degradation of both (-)-epicatechin and (+)-catechin, with an increase in the formation of the enantiomer (-)-catechin, which has a lower bioavailability compared to the aforementioned compounds \(^{41},^{42}\). On the other hand, processing of grapes into juice and wine allows for an increase in the flavan-3-ol content of the finished wine as extraction of these phenolics remains a critical component of the overall flavor of the finished wines \(^{43}\). In red wine production, the increase of the compounds resveratrol, catechin, and epicatechin in the finished product is due to fermentation facilitate release from contact with skin and seeds of grapes; with both oxidative and enzymatic processes resulting in a higher general extraction of these compounds from grape skins.

Additional studies have determined that processing methods such as water and steam blanching may have differing effects on the flavonoid content as well. Though the processing of beans is regarded as beneficial for the increase in nutrient bioavailability, it has been shown to decrease the levels of flavonoids found in pinto and black beans. Xu and Chang determined that in black beans, flavan-3-ols (C, EC, and ECG) were all significantly reduced (p< 0.05) by regular and pressure boiling compared to regular and pressure steaming after all samples were standardized via freeze-dried method \(^{44}\).
However, overall, while flavan-3-ols remain present in both fresh and processed foods, their ability to react through processing and preservation suggest the potential for significant losses of these compounds through processing. Unfortunately, very limited data is available that discusses the impact of food formulation and processing on flavan-3-ol fortified products. Since consumers today receive most of their caloric energy from further processed foods, it would be of great insight to determine the impact of these processes on value-added flavan-3-ol recovery and delivery. Insight is therefore needed to enhance the preservation of these compounds through a broad range of processing operations including those formulated with high amounts of macronutrients capable of interacting with flavonoids (discussed later).
<table>
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<th>Description</th>
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<th>Monomer Flavan-3-ol</th>
<th>Content (mg/100g)</th>
<th>Edible Portion</th>
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1.4. Mechanisms of Flavan-3-ol Health Benefits

Research characterizing the potential health benefits of purified flavan-3-ols has grown significantly over the latest decades (Figure 1.4). Often relying on preclinical findings with purified compounds, their benefits as a part of a complex diet remain unclear. Early research hypothesized antioxidant activity as a primary driver of flavan-3-ol biological activity, but more recent evidence suggest other mechanisms are at play 27. Due to low oral bioavailability of flavan-3-ols (discussed later), it is less likely that their only means of activity is through antioxidant behavior. Furthermore, the metabolic process in the host or by the activity of the microbiota in the GI tract yields multiple metabolites including conjugated forms of these compounds. It is believed that this level of metabolic conversion may impact much of the scavenging activity of the hydroxyl groups 27,46. Still, flavan-3-ols, in their native forms, have the ability to chelate metal ions, and scavenge radical oxygen, nitrogen, and chlorine from pro-oxidant activity. In this section, some of the more recent advances of flavan-3-ol modulating effects on, oxidative and inflammatory stress, glycemic control, neurodegenerative diseases, cancer and cardiovascular disease will be described.

1.4.1 Control of Oxidative Stress

When tissue is damaged or deteriorated this can result in increased oxidative damage by process of metal-ion release, phagocyte activation, lipoxygenase activation, and disruption of mitochondrial electron transport chains, allowing the formation of $O_2^-$ 47. This may lead to an imbalance occurs in the antioxidant defense mechanism and the generation of free radicals. Although a natural process in normal cell function, this increase in reactive oxygen species may play a secondary role in the detrimental effects of many human diseases. Furthermore, enhanced oxidative stress is implicated in many disease processes such as atherosclerosis, arthritis, cancer, and neurodegenerative diseases. Flavan-3-ols, due to their structure are able to play an intricate role in this process. Flavan-3-ols ability to bind to pro-oxidative divalent metals may contribute to their antioxidant activity in vivo.
The -OH groups at the 3 position of the C-ring, the 3’, 4’ of the B-ring, and others on the galloylated moieties of some flavan-3-ols offer great antioxidant and chelating abilities.

Flavan-3-ols have also been shown to exhibit direct antioxidant activity against oxidative stress in human vascular endothelial cells. A study using (+)-catechin and (-)-epicatechin (0.5, 1, 10 µM concentration) along with the metabolites of (-)-epicatechin, showed that a free catechol was primarily responsible for the compounds ability to scavenge superoxide anion radicals. The results also indicate that the glucuronidated and methylated metabolites, although reduced, exhibited the same protective effects against oxidative stress however presumably via indirect antioxidant activities.

Indirectly flavan-3-ols are able to mitigate oxidative stress through induction of phase II enzymes that are responsible for the detoxification of xenobiotics that may be present in the system, which is likely their mechanism of action due to concentration that are actually available. The tea catechin EGCG has been reported to have effects on various pathways and receptors that are responsible for improved cardiometabolic function and other biological pathways. An example of this is the ability of EGCG to activate eNOS pathways that improve vasodilation and upregulates apoptosis by elevating cGMP through the 67LR receptor.

1.4.2 Inflammatory Stress

Inflammation is a natural response of the body that occurs as an attempt to repair damage due to infection or other stresses. Chronic inflammation can occur when this process is inefficiently regulated, and is recognized as precursor to several chronic degenerative diseases such as cardiovascular disease (CVD), cancer and diabetes. The mechanisms by which some flavan-3-ols have proven effective as anti-inflammatory agents include (1) acting as antioxidants and/or radical scavengers, (2) regulators of cellular activity of inflammatory cells, (3) modulators of arachidonic acid metabolism enzymes, (4) modulators of pro-inflammatory cytokine production and gene expression.
Figure 1.4  Potential Implications of Flavan-3-ols in Health
1.4.3 Neurodegenerative Disease

There are multiple forms of neurodegenerative processes including normal age-related cognitive decline, neuronal damage induced by traumatic brain injury as well as disease states such as Alzheimer’s disease (AD) and Parkinson’s disease (PD). Many are characterized by an increase in oxidative and inflammatory stress as well as other processes which leads to damage to neuronal tissues and function \(^{57}\). Flavan-3-ol rich foods and their purified forms have, by virtue of their abilities to chelate metals such as iron and modulate oxidative and inflammatory processes and antioxidant abilities, seen an influx of potential in their use in prevention or as therapeutic agents. For example, epidemiological studies have shown that elderly population that consume higher levels of green tea (high in flavan-3-ols) had a decreased prevalence of cognitive impairment \(^{48,58}\). Likewise, in a 13 year study drinking three cups of tea/day was associated with decreased risk of PD \(^{48,59}\). However, scarcely found are clinical data representing the efficacy of these flavan-3-ols in AD and PD. Animal models have also allowed for more mechanistic approach to determining the efficacy of EGCG in cognitive functions. Varying dosages and concentrations of EGCG in multiple animal models have resulted in extension of mean life span \(^{60}\), prevention of age related memory decline \(^{61}\), and protection against brain inflammation \(^{62}\).

1.4.4 Cancer

Tea derived flavan-3-ols, have shown considerable potential as anticancer agents in a number of epidemiological cell and animal studies with particular implications for cancers of the gastrointestinal tract. EGCG in particular has been the most studied of the flavan-3-ol compounds by virtue of its abundance in tea and reported biological activity. As a result, as noted by Wang and Jiang, clinical trials have been initiated to support further research on the therapeutic nature of the compound EGCG \(^{63}\). Clinical studies determining the efficacy of EGCG on colorectal and prostate cancers have demonstrated some potential, but more insights are needed to clarify the role of this flavan-3-ol in particular. In a pilot study, oral administration of green tea extract along with a tea drinking lifestyle decreased the incidence of colorectal adenomas in treatment group
(15%) compared to a control group (30%) after 12 months. Likewise, another clinical trial using green tea catechins for chemoprevention of prostate cancer indicated that tumors from neoplasias formed in only 3% of the treatment group, while incidence of tumors was 30% in the placebo group. While promising, more research is needed to better understand the potential benefits of flavan-3-ols from tea as well as other sources in cancer prevention.

1.4.5 Heart Disease

Epidemiological data supports the claim that diets rich in flavonoid-containing fruits and vegetables result in reduced risk of CVD. More specifically, cocoa and its flavan-3-ols have been the subject of various clinical trials investigating the mechanism behind their benefits. A meta-analysis of clinical trials including a collective n=1297, has drawn some consensus that flow-mediated dilation improvement, reductions in blood pressure, as well as reduced insulin resistance are likely primary mechanisms for flavan-3-ol, and epicatechin in particular, benefits toward cardiovascular protection.

Likewise, a meta-analysis of 14 randomized controlled trials (n=1136), demonstrated that green tea consumption has the ability to offer cardiovascular protection by decreasing LDL-cholesterol and total cholesterol concentrations with no regard to individual health status or treatment of dose of green tea flavan-3-ols.

1.4.6 Obesity & Diabetes

More than one-third of the US adult population is recognized as being obese. Obesity is caused by a disturbance of energy balance in which energy intake exceeds energy expenditure; while type II diabetes is typically a result of chronic overexposure to energy dense diets. Some flavan-3-ols, including tea catechins and grape seed procyanidins have demonstrated potential in weight management as well as anti-hyperglycemic effects. In a diabetic rat model, it was demonstrated that grape seed procyanidins might have insulin-like effects in insulin sensitive cells therefore allowing an anti-hyperglycemic effect. This effect was significantly increased when animals were administered a low insulin dose. Hsu et al. showed in their double-blind, placebo-
controlled crossover design study that a flavan-3-ol enriched oolong tea consumed along with a high fat food may increase lipid excretion by modulating lipid metabolism. Meta-analysis of 11 studies on weight loss and weight management after green tea supplementation indicated that flavan-3-ols might significantly reduce body weight and maintenance after weight loss. A smaller effect was seen in Caucasians as compared to Asians, while caffeine intake was noted to moderately alter these effects.

Strict glycemic control may prevent or reduce the risk of complications due to diabetes. Flavan-3-ols, in more recent years, have demonstrated the potential to modify acute blood glucose response by inhibition of starch digestive enzymes and interaction with intestinal glucose transporters. In a Caco-2 cell model, under both sodium dependent and independent conditions, Johnston et al. showed that the tea flavan-3-ols EGCG, EGC, and ECG were able to significantly (37%, 60%, and 65% respectively) reduce cellular glucose uptake compared to a control. Additionally, Cordero-Herrera et al. determined that cocoa extracts and (-)-epicatechin were able to enhance insulin receptors, activate key proteins in the insulin signaling pathway, and regulate glucose production through modulation of the AKT and AMPK proteins in HepG2 cells, decreasing glucose formed through hepatic gluconeogenesis. Though studies indicate many flavan-3-ols have the ability to mediate blood glucose and onset of obesity, their mechanisms of action are still under investigation. Studies have indicated modulation of enzyme activity, SNS activation, and mRNA as a few of the possible mechanisms. Both in vitro and clinical studies support potential modulation of lipid and glucose absorption via disruption of lipid micelle formation and competition for transport respectively.

1.5 Macronutrient Flavonoid Interactions

While flavan-3-ols are reported to have a variety of biological activities, it is perhaps by virtue of their interactions with macro and micronutrients that occur through the gastrointestinal tract and in the body that provide the greatest potential to impact health endpoints related to GI tract cancers, CVD and obesity/diabetes. In this regard, consideration should also be given to the interactions that may occur prior to digestion,
such as those occurring within the food matrix that affect food structure, function and digestibility and by extension availability of flavan-3-ols. In this section, protein/carbohydrate-flavan-3-ol interactions will be further discussed in regards to mechanisms, physical consequences in foodstuff, and modulations in metabolism and absorption.

1.5.1 Protein/Flavan-3-ol Interactions

Polyphenol-protein interactions are perhaps one of the most widely documented interactions that impact physical/chemicals properties of both polyphenols and proteins in foods and biological systems. Flavan-3-ols are well known to impart an astringent taste or “puckering effect” when consuming foods that contain them. This response is due to the chemical nature of flavan-3-ols, allowing them to bind at multiple sites of proline rich salivary protein eventually leading to their ultimate precipitation in the oral cavity \(^{81,82}\). This phenomenon is driven by non-specific interaction and occurs not only with the salivary PRPs, but also may be seen in food proteins as well, limiting their digestibility \(^{83}\). This phenomenon is best characterized by a process of flavan-3-ol and protein non-covalent interactions. During the course of the process, saturation of binding sites on the protein occurs by interactions with polyphenol (Figure 1.5). This saturation results in compaction of randomly coiled proteins and eventual cross-linking between itself and an additional protein-polyphenol unit. Finally, aggregation of the protein-polyphenol units result and subsequent precipitation is observed. Metastable colloid forms are possible as intermediates and can remains soluble in solution resulting in a visual haze formation but even these eventually result in precipitation \(^{12,84}\).

Haslam indicates that hydrophobic interactions are the central force driving flavan-3-ol/protein interactions. These interactions occur between the aromatic rings of the flavan-3-ol and the pyrrolidone rings of propyl residues in proteins \(^{85}\). These interactions are stabilized by hydrogen bonding between the –OH groups of the flavan-3-ols and the –H receptor sites of proteins \(^{85,86}\). As a result, polymeric flavan-3-ols and proline-rich proteins (PRP) such as gelatin, incur favorable conditions for complexation and eventual precipitation effects\(^{82}\).
It is possible that these types of interactions may impact the digestibility of the PRP and the eventual digestive release and bioavailability of the flavan-3-ol.

![Figure 1.5](Schematic of Polyphenol-Protein Interactions Leading to Precipitation)

Due in part to their ability to impart astringency, it is not surprising that flavan-3-ol rich foods (e.g. dark chocolate and black tea) are often formulated with milk to manage this response. It is also not surprising that these interactions have been the subject of several investigations over the years. Flavan-3-ols have been shown to weakly bind to β-lactoglobulin in solution and add to the protein’s structural stability through an increase in β-sheet and α-helix\(^87\). The order of binding was also shown to increase as the number of hydroxyl groups increased\(^87\). In a study with caseins (α- and β-caseins), similar results indicated the number of -OH groups of the flavan-3-ol being a driver of binding, however with casein proteins there was a decrease in α-helix and β-sheets with an increase in random coils leading to protein unfolding\(^88\).
Stronger binding was seen in β-casein when compared to α-casein which was attributed to the more hydrophobic nature of the protein (Table 1.2) 88.

Table 1.2  Binding and Secondary Structural Effects of Select Flavan-3-ols on Protein87,88

<table>
<thead>
<tr>
<th>Effects on Secondary Structure</th>
<th>Casein Proteins</th>
<th>Whey Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α- Casein</td>
<td>β- Casein</td>
</tr>
<tr>
<td>α-helix</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-sheets</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Random Coils</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Turns</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Overall Stability</td>
<td>Protein Unfolding</td>
<td>Protein More Stable</td>
</tr>
</tbody>
</table>

| Order of Binding by Flavan-3-ol | C < EC < ECG < EGCG |
| Order of Binding by Protein    | β- Casein > α- Casein > β- Lactoglobulin |

Binding of flavan-3-ols with proteins is known to impact secondary and tertiary structures of proteins, which may influence changes in their functionality. For example, green tea extract when added to a whey protein concentrate solution has been reported to increase the firmness and adhesiveness of the gels in addition to decreasing the gelation temperature 89. When using egg albumin proteins, the addition of tea flavan-3-ols enhanced the foaming and gelling properties 13. The foam stability was increased to 97-100% after 10 min of whipping compared to 34% in a control egg albumin solution 13. Gels were shown to have a significantly higher storage modulus compared to the control gel 13. These studies expand the knowledge of flavan-3-ol incorporation into protein matrices, as well as offer gaps in the translation of the results into actual food products. Unfortunately, most studies focus on a singular protein source or flavan-3-ol and utilize a
single concentration, often artificially high, resulting in a gap in the literature and ability to translate these findings to meaningful food products. Additional studies are necessary to allow a direct comparison of extract concentrations with multiple protein matrices and translation to multiple product forms desired by consumers. Also, most studies only investigate the effects of monomeric flavan-3-ol rich extracts. Effects of the polymeric or oxidized forms, such as those found in grape seed extract are minimally understood. Investigations that detail structure-function relationships through varying DP and multiple protein systems would offer a holistic understanding of these molecules and their interactions.

1.5.2 Carbohydrate/Flavan-3-ol Interactions

Flavan-3-ol interactions with carbohydrates are believed to be driven by similar forces as protein interactions. This is due to the hydrophilic/hydrophobic nature of the macromolecule \(^{24}\). The extent of the interaction is governed by the hydroxylation of flavan-3-ol and the structure of the carbohydrate, with differences being seen in the digestible and non-digestible carbohydrates, as well as differing ionic strengths. In fact, Le Bourvellec and colleagues were able to demonstrate a difference in apparent affinity among select polysaccharides and procyanidins found in apple juice, pear juice, and grape seed extract (citrus pectin > tamarind xyloglucan > commercially available starch > commercially available cellulose) \(^{90}\). Their investigation exhibited an increase in the affinity constant as procyanidins increased in DP. The only exception was cellulose. On the other hand, pectins demonstrated a greater affinity than all other tested polysaccharides possibly due to their ability to form hydrophobic pockets which encapsulated the procyanidin \(^{90}\). The structural conformation of both cellulose (microfibrils) and xyloglucans (globules) was thought to provide only surface adsorption and permit aggregation formation, hence limiting the further associations \(^{91}\).

The impact of these interactions is not limited to conformational changes in the structures of these carbohydrates. Some studies suggest that these structural changes potentiate physical changes in the carbohydrates functional properties as well. In a study that monitored the effects of tea flavan-3-ols on rice starch retrogradation, it was found
that higher concentration may induce gelatinization of the starch at lower temperatures with peak viscosity being reached at lower temperatures as well.\textsuperscript{92} Retrogradation enthalpy for the rice starch of samples inclusive of >10% tea flavan-3-ols did not appear until 20 days of storage, as such, were significantly lower than the control with no tea flavan-3-ols.\textsuperscript{92}

These effects have implications for formulation and process designs and new products that may find it necessary to delay the retrogradation process for extended periods. However, at the usage levels suggested, incorporation of flavan-3-ol extracts at these concentrations are well beyond levels that may be palatable in a commercial food product. The astringent factor imparted by the increased levels of the polyphenols deter usage at the upper limits of human sensorial detection, as evidenced by a study on the quality effects of green tea extract in bread formulations. Concentrations of green tea extracts at 1.5 and 5.0 g/kg flour both showed significant increases in the perception of astringency of both untrained (p<0.1) and untrained (<0.01) panelists\textsuperscript{93}. While promising, additional insights are needed to translate the multifunctional nature of the polyphenols into real food systems.

1.5.3 Mixed Matrix Interactions

Consideration of food physical properties and competition between interactions described above is also warranted in determining the effects of interactions and processing on food matrices fortified with flavan-3-ols. When green tea extract was incorporated in a bread formulation, differences were found in many of the physical properties associated with the product. At its highest concentration of 0.5% of the flour, significant differences were found in the hardness (p<0.005), crumb porosity (p<0.05), and stickiness (p<0.05) with instrumental analysis.\textsuperscript{93}

Macronutrient/flavan-3-ol interactions may also be modified by further interactions that occur when the matrix has multiple components, as would be expected in complex food systems. Particularly, many studies have determined that interactions with certain carbohydrates may alter the availability of flavan-3-ols to interact with proteins critical to structure. Mateus et al. described the action of xanthan gum, pectin, and gum
arabic as disruptive to the formation of insoluble complexes between grape seed procyanidins and bovine serum albumin (BSA) \(^{94}\). Their results indicate that these carbohydrates are very effective in blocking insoluble complexes of lower DP procyanidins compared to their larger counterparts. Ionic character and structure were hypothesized as drivers for the interactions. Xanthan gum exhibited the greatest solubility effects on the complexes, having a charged tri-saccharide side chain, allowing for higher potential of hydrophilic interactions \(^{94}\). Structurally, xanthan gum’s ability to form gel-like solutions may have allowed for encapsulation of the smaller procyanidins, however slightly ineffective with the larger DP procyanidins \(^{94}\).

Additional studies further implicate carbohydrates as disruptors of protein/flavan-3-ol insoluble complex formation. Liang et.al reported similar interactions in studying the effect of carbohydrates (sucrose and gum arabic) on procyanidin B3 and lysozyme interactions \(^{95}\). Initially the study determined that procyanidin B3 may spontaneously bind with lysozyme inducing insoluble aggregates with multiple structural changes to the protein. These modifications resulted in inhibition of lysozymes activity against \textit{M. lysodeikticus} \(^{95}\). However, the study distinguished that introduction of a simple carbohydrate (sucrose) and an acidic polysaccharide (gum arabic) had differential effects on complex formation with lysozyme.

Both sucrose and gum arabic were able to reduce aggregate formation and manipulate enzyme activity but assumingly by two different mechanisms (Figure 1.6):

- Gum arabic was assumed to form soluble ternary complexes, encapsulating the protein within the carbohydrate and procyanidin as described by Mateus and colleagues \(^{94}\). This allows for reduction in aggregate size however no changes in fluorescence activity, and inhibition of activity of the enzyme.

- Sucrose, on the other hand, reduced aggregate size but allowed recovery of fluorescence, suggesting that it may adsorb at the surface of the procyanidin, hence recovery of enzymatic activity \(^{95}\).
1.5.4 Interactions with Biological Proteins

In addition to interactions with food proteins it is important to consider the ability of flavan-3-ols, pre- and post-intestinal absorption, to interact with biological proteins. These interactions that may have an impact both on food digestion as well as other biological endpoints when consumed at different levels. Williamson discusses these impacts in an extensive review of intervention studies, indicating flavan-3-ols at varying dosages may have differing effects on biological biomarkers. Noting that green tea extracts dose-dependently increase plasma antioxidant activity (150, 300, 400 mL)\(^96\), fat oxidation (7.5 g and 15 g tea)\(^97\), and energy expenditure (90 mg EGCG)\(^98,99\). Green tea extract has also been shown to reduce starch digestion when incorporated in a gelatinized maize starch paste (10% based on starch content)\(^100\). It was indicated that a non-competitive interaction with the \(\alpha\)-amylase inhibited its action, allowing a decrease in maize starch breakdown\(^100\).
Perhaps most notable are flavan-3-ol abilities to associate with digestive enzymes and manipulate digestion of macronutrients central to disease risk such as lipids and starch. Lambert and colleagues determined that structural characteristics of proanthocyanidins (PACs), type and extent of DP in particular, have great influence on its ability to act as an inhibitor of phospholipase A2 (PLA2, responsible for fatty acid release from glycerophospholipids) and act as a potential mechanism of its action\textsuperscript{101}. In fact, results of their comparative enzyme testing showed that A-type PACs (found in cranberries) were less effective as inhibitors than B-type PACs (found in cocoa and grape seeds) on PLA2 (Table 1.3). Further, the in silico modeling studies showed that the B-type PACs were able to occupy the hydrophobic pocket of the PLA2 without altering the structure; blocking the active site from substrate binding. A-type PACs however render the hydrophobic pocket open, potentially available for further interaction\textsuperscript{101}.

### Table 1.3 Comparison of Procyanidin Effects on Inhibition of Phospholipase A2

<table>
<thead>
<tr>
<th>Procyanidin Type</th>
<th>Structure</th>
<th>Found in Nature</th>
<th>Mechanism of Protein Interaction</th>
<th>Inhibition of PLA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameritannin</td>
<td>[Diagram of Parameritannin structure]</td>
<td>Peanut Skins, Cranberries, Avocado</td>
<td>Open Hydrophobic Pocket, Active Site Available for Interaction</td>
<td>No</td>
</tr>
<tr>
<td>Aesculitannin</td>
<td>[Diagram of Aesculitannin structure]</td>
<td>Grape Seed, Cocoa</td>
<td>Blocks Active Site, Prevents Substrate Binding</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The extent to which the presence of carbohydrates may impact flavan-3-ols’ ability to interact with digestive enzymes have also been studied. Particularly, carbohydrates of differing ionic character were used to study their influence on procyanidin B3 and trypsin interaction. It was found that all ionic carbohydrates, upon addition, were able to disrupt trypsin/procyanidin B3 interaction by mode of competition, demonstrating an increase in fluorescence and loss of binding shown via
STD-NMR \(^{102}\). However, it is important to note that these studies have not taken into account the effect of the digestive process on potential interactions. Naz and colleagues suggest that while flavonoids such as epigallocatechin-3-gallate (EGCG) have inhibitory effects on \(\alpha\)-amylose, chymotrypsin, trypsin, and lactase, these effects are lessened in the presence of salivary proteins \(^{103}\). Even at varying physiological pH levels, in the case of \(\alpha\)-amylose, salivary PRPs were nevertheless able to alleviate the inhibitory properties of EGCG \(^{103}\). Therefore, consideration of the impact of the broader non-specific interactions that may be occurring between flavan-3-ols in food must be considered when placing these studies into physiological context. This includes both on activity in the GI tract and the ultimate release and bioaccessibility of flavan-3-ols during normal digestion of foods with complex interactions.

1.6 Bioavailability & Bioaccessibility

Bioavailability, as it applies to flavan-3-ols, can be defined as the extent to which flavan-3-ols are absorbed from the food matrix and becomes available at the site of action\(^{104}\). The term bioaccessibility describes the presence of the compound at the apical surface of the enterocytes \(^{104}\). Therefore the bioaccessible fraction of flavan-3-ols, is that level of compounds that are accessible post-digestion and available for intestinal uptake (Figure 1.7)\(^{104}\). The primary driver of the flavan-3-ols’ capacity to aid in therapeutic measures is its level of bioaccessibility, of which may be influenced by 3 important factors associated with the digestive process: (1) release of the flavan-3-ol from interaction within the food matrix, (2) their stability in the gastrointestinal tract, and (3) solubility in the aqueous phase of the gut lumen \(^{105}\).
1.6.1 Factors that Influence Bioaccessibility

As described previously, flavan-3-ols formulated in a food matrix, can interact with other components including proteins, lipids, or carbohydrates. These interactions may result in complexes that are soluble or insoluble through the extent of the digestive tract, however those deemed insoluble will limit the bioaccessibility and potentially the eventual availability of the flavan-3-ols. As Moser et al. reported, co-formulation of tea flavan-3-ols with select milk proteins in liquid solutions, resulted in direct binding interactions of the compounds. However effective GI digestion disrupted this binding by breaking the interactions between polyphenols and proteins and restoring their bioaccessibility. Therefore, interactions between macromolecules and phenolics may in fact be disrupted through normal digestion. However, this effect is not documented across different food matrices, so the full extent to which such interactions may impact bioavailability of biological effects remain to be investigated.

Figure 1.7  Schematic of Bioaccessibility & Bioavailability via Digestion
Interestingly, Schramm et al. administered cocoa flavan-3-ols to subjects in the presence of different foods in a crossover designed intervention study and determined a beneficial effect provided by protein and carbohydrates\textsuperscript{106}. When comparing the catechin and epicatechin in the plasma of the individuals, it was found that those whom consumed sugar and bread along with the cocoa flavan-3-ols had a 140\% increase in plasma flavan-3-ols over the control (cocoa flavan-3-ols consumed alone)\textsuperscript{106}. These results are also in agreement with other studies utilizing green tea\textsuperscript{107} and grape seed extract\textsuperscript{5} that determined the bioaccessibility and bioavailability of monomeric and oligomeric flavan-3-ols from a carbohydrate rich matrix through \textit{in vitro} and \textit{in vivo} models. Both studies found that carbohydrate-rich meals increased the bioavailability of the flavan-3-ols found in the extracts. Not only that, but both dimer and trimer procyanidins were absorbed and present in rat plasma, reaching a maximum concentration at 1 h post consumption, but also that those ingesting grape seed extract in the presence of a carbohydrate rich food had a higher concentration than those without\textsuperscript{5}. While these effects may be related to direct and indirect interactions of flavan-3-ols with macronutrients or key transport systems in the GI tract, these studies represent the importance of food matrix considerations on the bioaccessibility and bioavailability of flavan-3-ols.

Degree of processing is another variable that should be considered. Only a few studies mention or explore the potential compounding effects processing may have on bioaccessibility of flavan-3-ols. In the case of whole grains, processing has been previously documented as having a beneficial effect on the bioaccessibility of phenolic acids, allowing for the release of polyphenols from cell walls, increasing their availability\textsuperscript{108,109}. However less is known of the bioaccessibility of flavan-3-ols fortified into food products or model matrices. Processing may in fact drive certain interactions within the product either enhancing or limiting bioaccessibility. Exploration of both food processing and food matrix effects on bioaccessibility is an area of research for further growth as more concern is placed on the availability of these health-promoting compounds.
Additional consideration for bioaccessibility of flavan-3-ols is the successful stability of the compounds through the digestive process. In the oral cavity, flavan-3-ols have their first interactions with biological proteins and potential for formation of complexes under near neutral pH conditions. In particular, tannins have been shown to have an increased affinity for interaction with proline-rich proteins found in human saliva. Likewise both monomeric and polymeric flavan-3-ols have shown the ability to interact with salivary α-amylase. However, no modification or significant degradation to flavan-3-ols in the oral cavity has been reported.

In the stomach, the food matrix is subjected to exposure to the enzyme pepsin for protein hydrolysis and a highly acidic environment (pH 1-3). Flavan-3-ols, specifically monomeric ones have demonstrated greater stability under these acidic conditions. Particularly, Zhu et al. showed that the monomers catechin and epicatechin and the dimers B2 and B5 had differing stability to acidic conditions that simulated gastric pH conditions. The monomers were very stable however, both dimer B2 and B5 degrade to epicatechin and isomerize to B5 and B2 respectively.

The resulting chime then enters the small intestine where the pH is elevated to near neutral conditions and is combined with enzymatic secretions from the intestine, liver, and pancreas that further breakdown proteins, lipids, and carbohydrates. These intestinal juices may reach a pH of approximately 6.5-8.5. This is critical as flavan-3-ols are highly unstable in alkaline solutions (over pH 7). These conditions can result in oxidation mediated dimerization of EGCG, epimerization of (-)-epicatechin and (+)-catechin, and complete degradation of procyanidins B2 and B5. More specifically, following a 2-stage in vitro digestion (gastric and small intestine) it was found that losses of EGCG, EGC, and ECG being as much as 91, 100, and 61% respectively. More importantly perhaps, is the extent to which degradation of these compounds through the digestive process impacts their ability to provide a benefit in the body. Each of the homodimers (theasinsensins) formed as a result of the digestive process has markedly lower inhibitory effects on pancreatic lipase activity in vitro compared to its parent compound, EGCG. The small intestine is the site of majority of flavan-3-ol absorption. However, this occurs only if the flavan-3-ols are soluble in the aqueous
phase of the lumen, allowing diffusion across the unstirred water layer\textsuperscript{104}. Therefore, interaction with digestive enzymes or food matrix may modulate both stability of monomeric flavonoids and absorbable concentrations due to insoluble complexation\textsuperscript{112}.

1.6.2 Absorption and Metabolism of Flavan-3-ols

Upon absorption in the enterocytes, available monomeric flavan-3-ols may be distributed into the portal vein and found in the plasma at $C_{\text{max}}$ as soon as 0.8 hours post consumption\textsuperscript{117,119}. It was determined that dosages between 1 to 100 μM concentration of catechin resulted in an absorption rate of approximate one-third the initial dose, suggesting passive diffusion as a mechanism of transport, as this proportion is as expected for passive transport\textsuperscript{120,121}. Furthermore, monocarboxylic acid transporter (MCT) has been recognized as a transporter of flavan-3-ols, specifically ECG\textsuperscript{122}. Efflux transporters have been shown to be responsible for passage of flavan-3-ols back to the intestinal lumen for potential excretion. Specific transport of flavan-3-ols occurs as a result of action by multidrug resistant associated proteins (MRP) 2, MRP1, and to a limited extent P-glycoprotein\textsuperscript{104,122}. Phase II enzymes, are then responsible for the detoxification reactions that involve glucuronidation, sulfation, and methylation of flavan-3-ols respectively. Phase II metabolites, as well as some of the parent flavan-3-ols, may then enter the plasma for dissemination to other cells. According to Manach et al., EGCG is the only polyphenol found in plasma in its free (non-conjugated) form,\textsuperscript{18}

Degree of polymerization and galloylation seem to be drivers of the bioavailability of flavan-3-ols as procyanidins with a DP >3 are not absorbed in the small intestine\textsuperscript{123}. The oligomeric and polymeric procyanidins offer benefits yet and still potentially via their effects and absorption in the large intestine where microbiota may metabolize the compounds. Proanthocyanidins, along with those flavan-3-ols that are efflux back to the intestinal lumen, may flow to the large intestine where gut microbiota will determine their fate. The gut microbiota are assumed to be responsible for EGC and EC metabolites that are produced in renal excretion peaking at 7.5-13.5 h after ingestion and accounted for 6-39\% of the ingested total\textsuperscript{124}. However, in a follow-up study, it was determined that the amount of aromatic acids produced decreased as the degree of
polymerization increased, suggesting that larger highly polymerized procyanidins escape microbial metabolism. Thusly the metabolism of flavan-3-ols is an area of research to be further explored.

1.7 Specific Research Aims

In the context of the material presented in this review of literature, many avenues are yet to be explored involving macronutrient-polyphenol interactions. The interactions of flavan-3-ols with macronutrients such as proteins and carbohydrates are an inevitable consequence of the preparation, processing and consumption of foods. Therefore, a greater understanding of the potential modulation in macronutrient functionality as well as potential disruption or protection of the bioactive compounds in the context of typical food matrices is of great relevance to both the food industry and consumers. Current evidence suggests that structure-function relationship of macronutrient/flavan-3-ol interactions exist. However, this relationship post-processing and digestion are not clear. Likewise, much of the data available is restricted to the interactions that occur among liquid matrices, often utilizing single flavan-3-ol compounds or well defined protein ingredients. Therefore, consideration of further processed foods that are formulated with these compounds and are more representative of end use products i.e. foams, gels, pastes, and emulsions are warranted.

The overall objective of this project was to describe the implications of macronutrient/flavan-3-ol interactions on macronutrient functionality and flavan-3-ol bioaccessibility in specific model food matrices of differing physical properties (gels, foams and emulsions). The goal of these studies is to present a holistic view of the effects of interaction, processing, concentration, and flavan-3-ol profile on functionality and bioaccessibility within a model processed food system.

Aim 1 explored the effects of protein/flavan-3-ol interactions on the protein functionality in model food matrices. The working hypothesis is that interactions formed between the flavan-3-ols and specific proteins through processing will modulate the ability of each protein ingredient to provide typical functionality within the function
of the given matrix. Though protein/flavan-3-ol binding interactions have been researched, the practical implications of these interactions have not been comprehensively defined. Currently, limited data exists to implicate a positive or negative effect of protein/flavan-3-ol interaction on the ability of the protein to function in a gel, as an emulsifier or to create and stabilize foam. Studies performed investigated the impact of extracts (green tea and grape seed) on the determination of initial foaming and/or emulsion stability of sodium caseinate. Foaming properties including capacity, strength, and stability were measured. In determination of emulsion properties of proteins, characteristics such as capability and stability were measured. Exploration of the dynamics of protein/flavan-3-ol (gelatin and whey protein isolate) interactions in relation to gelation were assessed. Rheological properties such as small and large deformation characteristics (gel strength) were determined for all treatments.

Aim 2 demonstrated the effects of carbohydrate/flavan-3-ol interactions on the characteristics of select carbohydrate pastes and gel properties. The working hypothesis is that like protein matrices, flavan-3-ol incorporation into carbohydrate systems would modulate physical properties of these macronutrients. Specifically, wheat starch and κ-carrageenan are the carbohydrates to be explored, exhibiting commonly utilized digestible and indigestible carbohydrates found in processed food products. In aim 2, experimentation focuses on the changes in rheological properties similar to aim 1. Pasting temperatures, viscosity, and gelling properties were determined as necessary for each of the matrices.

Aim 3 determined the effects of macronutrient/flavan-3-ol interaction and processing on ultimate bioaccessibility of flavan-3-ols. The working hypothesis was that the interactions of the flavan-3-ols within food matrices were modulated through interactions with the macronutrients. These interactions may have a positive and/or negative effect on the stabilization of the flavan-3-ol through the digestive process. This aim specifies the various parameters associated with the digestion of macronutrient/flavan-3-ol complexes and seeked to determine which, if any, have influential roles in bioaccessibility. While in aim 1 & 2 the goal was to determine physical characteristics of the interactions, aim 3 was explore potential physiological
impacts of the macronutrient/flavan-3-ol interactions from the model-processed foods. The goal was to determine if the site of binding and/or manipulation in functionality of the proteins by the flavan-3-ols play a role in the bioaccessibility. The digestibility of flavan-3-ols was determined by comparing initial flavan-3-ol levels in the model systems to those in the final digesta. Bioaccessibility of these compounds was expressed as the fraction of polyphenols present in the final aqueous digesta as compared to the concentrations found in the initial extract.
CHAPTER 2. EFFECTS OF FLAVAN-3-OL RICH GRAPE SEED AND GREEN TEA EXTRACTS ON THE FUNCTIONALITY OF SELECT DAIRY PROTEINS

2.1 Introduction

Consumer interest in “better for you” products is driven by several factors including the growth of an aging population and increased awareness of research linking diet quality to the onset of chronic diseases. This market has provided opportunities for new functional foods focused on delivering health benefits beyond that provided by their basic nutritional value. This notion includes the potential physiological impact of nutrients such as functional proteins and non-nutrient bioactive plant based phytochemicals that have been associated with prevention of specific chronic and degenerative diseases.

It is important to consider that translation of new functional platforms is best accomplished on existing product platforms. This includes combinations of protein based or enriched products which are widely distributed in the market. Protein based snacks and beverages are commonly leveraged in products aiding performance and recovery, weight loss and management, as well as management of appetite and satiety. Dairy proteins in particular provide an established foundational matrix due to the structure and functionality in food systems. Their capacity to function as emulsification and foaming agents as well as their gelation properties make them uniquely suited as carriers of additional bioactives including phytochemicals. Whey proteins in particular are important in food products such as yogurt, puddings, and dairy smoothies as they provide the structure, viscosity, and characteristic mouth-feel for a variety of consumer products. Likewise, casein based ingredients offer diverse emulsifying and foaming capabilities with broad applications in liquid and dry products.
Additionally, these dairy proteins have also been studied for their ability to act as a natural carrier for phytochemicals, masking their taste and potentially facilitating their stability\(^{135}\). Interest in leveraging plant-based polyphenols in functional food products has continued to grow. Flavan-3-ols in particular are a key sub-group of the class of flavonoid polyphenols\(^{19}\) abundant in select fruits, cocoa, and tea as well as their extracts. Flavan-3-ols in particular have been associated with health benefits including the ability to prevent or ameliorate diabetes, neurodegenerative diseases, and cardiovascular disease\(^{48,66,72}\). Flavan-3-ols are the most widely consumed flavonoid in the western diet\(^{16}\), as such, extracts of tea, cocoa, and fruits are commonly formulated into functional food products\(^{136}\).

Many flavan-3-ol extracts have been leveraged in food products. Green tea and its extracts (GTE) are perhaps one of the most widely studied and applied in food and beverage systems. Green tea is abundant in monomeric flavan-3-ol forms including (-)-epicatechin (EC), (-)-epicatechin 3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG). A serving of green tea may provide as much as 238 mg of flavan-3-ols\(^{38,137}\). EGCG, the primary flavan-3-ol in green tea, has been implicated in many of tea’s biological activities\(^{37,56,57}\). As such, highly refined and purified EGCG ingredients have been developed as a means to enhance food content of this specific polyphenol without impacting the flavor or texture of products as GTE has been reported to do\(^{4,138}\). Grape seed extract (GSE), a byproduct of grape juice and wine production, is also a rich source of both monomeric and polymeric flavan-3-ols (proanthocyanidins) which have been associated through both preclinical and clinical studies to positively impact cardiovascular and neurodegenerative processes\(^{63}\). While monomeric flavan-3-ols may contribute as much as \(~\)660 mg/100 g of GSE dry weight, total proanthocyanidin content has been reported to be as high as \(~\)3500 mg/100 g dry weight\(^{33}\).

Considering the biological functionalities of both protein and polyphenols, it is not surprising that application of both in functional food systems has grown. Interactions between polyphenols and proteins have been well documented and reviewed over the years with significant impact on protein structure, function, and polyphenol stability/bioavailability from foods\(^{84,86,139}\). Considering that ultimate delivery of product
quality remains key to successful implementation of novel functional products in market, characterization and control of these interactions play a tremendous role in this outcome as such characterization of their interaction in foods is essential. Primarily driven by hydrophobic interactions, protein-polyphenol associations are stabilized by hydrogen bonding. These interactions may be strong enough to impact physical stability, functionality, and potentially even physiological delivery (bioavailability/bioactivity) of flavan-3-ols.

With the basis of protein-polyphenol interactions well documented in highly controlled systems, understanding of both phenolic and protein chemistry/functionality in more complex extract and food matrices is still somewhat lacking. Reports of polyphenol interactions influencing taste as well as functional properties do exist, including dough conditioning, foaming stability and haze formation in beer. However, the majority of observations have been made using highly purified model systems without systematic investigation of dose ranges or ingredient selection needed to expand the application of polyphenol based ingredients into broader consumer food systems. While these observations are promising, additional information is needed on the consequences of inclusion of relevant doses of flavan-3-ols specifically in dairy protein based food systems that more closely replicate usages in typical food systems including foams, emulsions and gels.

In the current study the impact three widely utilized flavan-3-ol ingredients (GTE, GSE and purified EGCG as Teavigo®) on dairy protein functionality was assessed in model food matrices including gels, foams and emulsions. As described above, these flavan-3-ol sources provide distinct forms of monomeric (GTE), polymeric (GSE) and purified (EGCG as TVG) flavan-3-ol presenting the opportunity to characterize, to a certain extent, the differences imparted by the structures found in each. The effects of these flavan-3-ol rich ingredients on the formation, physical properties and stability of whey protein (WPI) gels, sodium-caseinate (SCN) foams and emulsions were documented in broad ranges approximating those that could be practically applied in functional food systems.
2.2 Materials and Methods

2.2.1 Materials

Whey protein isolate (BiPro®, 95% protein) used in gels, was generously provided by Davisco Foods International (Eden Prairie, MN) and sodium caseinate (FB100, 90% protein), used in emulsions and foams, was provided by Farbest Brands. 92% pure EGCG (Teavigo®-TVG) was donated graciously by Taiyo International Inc. (Minneapolis, MN) green tea extract (GTE) was provided as a gift by Nestlé Product Technology Center (Marysville, OH) and Meganatural Gold grape seed extract (GSE) was purchased from Polyphenolics (Madera, CA). Acetic acid, acetonitrile, methanol, water (Mallinckrodt-Baker, Phillipsburg, NJ) and formic acid (Sigma-Aldrich, St. Louis, MO) used in HPLC analysis were certified HPLC-MS and ACS grade and purchased from Sigma-Aldrich. All phenolic standards were purchased from Sigma-Aldrich.

2.2.2 Preparation of Protein-Extract Solutions

Working protein solutions were prepared by hydrating whey protein isolate (WPI-18% w/v for gels) or sodium caseinate (SCN; 5.0% w/v for foams and 0.50% w/v for emulsions) in double distilled (dd) water. Protein-extract solutions containing green tea extract (GTE), Teavigo® (TVG), or grape seed extract (GSE) were prepared by adding each protein at their respective concentrations to an extracts dissolved in dd water at concentrations of 0.01-1.0 mg/mL for foam and emulsion experiments and as high as 5 mg/ml for gelation experiments. All solutions were stirred for 60 s to facilitate even mixing and allowed to hydrate for 24 h at 4°C prior to further analysis. Sodium caseinate solutions used in emulsion analysis were treated with 0.02% (w/v) sodium azide as an antimicrobial treatment.

2.2.3 Total Polyphenol Determination

Total phenolics of each of the extracts were determined by Folin-Ciocalteau assay as described by 141. Briefly, extracts were solubilized with double distilled water. An aliquot (10 μl) of the sample, 0.79 ml double distilled water, and 50 μl of Folin-Ciocalteau reagent (Sigma, St. Louis, MO) were combined in a 2-ml centrifuge tube and vortexed. Sodium carbonate was added (150 μl, 7.5% w/v aqueous solution) and held at
room temperature for 2 h. Samples were transferred into a 96 well plate and the absorbance read at 765 nm using a Spectra Max 190 (Molecular Devices, Sunnyvale, CA) spectrophotometer. Total phenolics was calculated using an external standard of gallic acid (0-0.75 g/l) prepared in 10% EtOH. Results were expressed as grams of gallic acid equivalent per gram of extract (mg-GAE/g).

2.2.4 LC-MS Flavan-3-ol Profiling in Extracts

Individual flavan-3-ol profiles were determined by LC/MS of each extract. GTE, TVG, and GSE each were each prepared in a water/formic acid (95/2; v/v) solution at 0.5 mg/ml. Extract solutions were filtered through 0.45 μm PTFE filter prior to injection (10 μl) on to a Waters 2695 Separation Module (Waters, Milford, MA) equipped with a Xbridge® BEH Shield RP18 2.5μm, 2.1 x 100 mm column heated to 40°C. A gradient with a flow rate of 0.25 mL/min with mobile phase A, 0.2% formic acid in MS grade water, and mobile phase B, 0.1% formic acid in acetonitrile was used. Solvent composition was 95%, 65%, 50%, 95%, 95% mobile phase A at 0, 15, 17, 18, 22 min respectively. The capillary and cone voltage were set to 3000 and 40 volts respectively. Desolvation temperature was set to 250°C while the source temperature was set to 150°C. The electrospray ionization was set to negative mode with C, EC, EGC, ECG, EGCG, dimers, and trimers detected at 289, 305, 441, 457, 577, 865 m/z. Individual flavan-3-ols and proanthocyanidin compounds were quantified using calibration curves constructed with authentic standards of flavan-3-ol monomers with Procyanidin dimers and trimers being reported as equivalents of Procyanidin B2 (mg-PB2E/g).

2.2.5 Extraction of Flavan-3-ols from Matrix.

Flavan-3-ols within the starting materials were determined to understand the potential loss of flavan-3-ols through preparation of protein systems. The extractions performed were as described by Ferruzzi and Green with modifications142. For emulsions, a 2 ml aliquot of starting material (SM) was defatted via hexane extraction. Subsequently, 100 μl of a 2.7 mM L-ascorbic acid and 2.2 mM Na2-EDTA in water solution and 6 ml of pepsin solution (45 mg/ml in 0.1 N HCl). The mixture was blanketed
with nitrogen, capped and placed in a water bath at 37°C for 30 min at 120 opm. Flavan-3-ols were further extracted from the digested solutions with 3 ml of ethyl acetate 3 times. Samples were vortexed for 3 min then centrifuged at 4255 x g at 4°C for 5 mins. The ethyl acetate layer was collected, dried under nitrogen and resolubilized in 2 mL of 0.4% formic acid prior to injection on the LC-MS system.

2.2.6 Assessment of Protein-Polyphenol Binding and Interfacial Tension

Study of the binding interactions between the proteins and the extracts were measured using fluorescence spectroscopy following a procedure outline by He et al. with modifications143. Using a Perkin-Elmer LS55 Spectrophotometer (Woodbridge, ON, Canada) fluorescence measurements were taken for each protein and its extract complex. The fluorescence emission (λ<sub>exc</sub>) spectra was recorded from 300 nm to 500 nm with the excitation wavelength (λ<sub>exc</sub>) set to 280 nm. The excitation and emission slit width was maintained at 10 nm and the intensity recorded at 350 nm (Trp). Samples were prepared fresh for each analysis by combining a working solution of the protein (1.0 mg/ml) and each extract (0.1 mg/ml) in Tris-HCl buffer solution (pH 6.5). The protein concentration for each was held consistent among all measurements with the concentration of extracts changing in accordance with the levels found to have the greatest impact on functionality. The final ratios of protein to extract were WPI (0.010 mg/ml) 1:0, 18:1, 36:1, 72:1 and SCN (0.010 mg/ml) 1:0, 20:1, 10:1, 5:1, 2:1, 1:1, 1:2. The exception is with solution prepared with GSE, in which the concentration of protein were lowered (0.005 mg/ml) were lower due to its high fluorescence.

Changes in the surface pressure caused by individual flavan-3-ols were determined using a KRÜSS DSA30b drop shape analyzer (Krüss, Hamburg, Germany) using a method described by Murphy et al. with modifications 144. Dynamic interfacial tension (IFT) was calculated through shape analysis of a drop of the aqueous complex solution, suspended from a needle syringe measuring 1.826 mm in diameter using pendant drop setup. IFT was measured for a period of 3600 s allowing samples to reach an equilibrium. Standards representing the smallest and largest monomeric flavan-3-ols EC and EGCG as well as a procyanidin dimer B<sub>2</sub> (B<sub>2</sub>) were first combined with a fixed
concentration (100 mg/L) of SCN solution. Protein/flavan-3-ol complexes were prepared fresh for each replicate and allowed to react for 10 min before each measurement. The surface pressure was calculated as follows,

$$\Pi = \gamma - \gamma_0$$

where $\Pi$ equals surface pressure and $\gamma$ is the interfacial tension at a given time, and $\gamma_0$ is the initial surface pressure. Ratios of SCN: flavan-3-ol (1:0, 20:1, 10:1, 5:1, 2:1, 1:1, 1:2) used for drop shape analysis offer a range of potential surface pressure effects caused by incorporation of the monomeric and polymeric flavan-3-ols in which may be used in a food matrix.

2.2.7 Small Amplitude Oscillatory Rheological Measurements.

Rheological experiments were performed on a Discovery Hybrid Rheometer (TA Instruments, New Castle, DE) equipped with a peltier solvent trap cover to minimize evaporation and a peltier plate for temperature regulation. A 1.25 ml sample of each WPI (18% w/v) solution was placed at the center of the plate and measured within a 1mm geometry gap. The flow properties and gelling characteristics were determined at a constant strain of 0.5%, which was determined to be within the linear viscoelastic region in preliminary experiments and a frequency of 1.0 Hz. Protein solutions were pipetted on to the plate at 15°C and heated from 15°C to 75°C at a rate of 3°C/ min. Upon reaching 75°C, the temperature was held for 10 min, then reduced to 15°C at a rate of 3.0°C/min, and held at 15°C for 10 min. The storage modulus ($G'$), loss modulus ($G''$), phase angle ($\delta$) were continuously measured throughout the procedure. All rheological measurements were done in triplicate on different samples.

2.2.8 Foam Formation and Stability.

Foams were produced utilizing the 5% (w/v) SCN solutions and evaluated for overrun and stability as described by Phillips et al with modifications. A volume of 150ml of SCN solution was placed into the mixing bowl of a KitchenAid Ultra Power 300-watt countertop mixer. Solutions were whipped at a speed of 8 for a total of 15 min with samples taken at 5 min interval (n=3) for overrun measurements. At each interval, foam samples were collected into polystyrene cups (100 ml capacity) and leveled, prior to
recording weight of the foam and dish. Percent overrun was calculated using the following equation:

\[
\% \text{ Overrun} = \left( \frac{\text{wt of 100 ml protein solution}}{\text{wt of 100 ml foam}} \right) \times 100
\]

Foam stability was measured upon termination of whipping by collecting foam into polystyrene cups with precut bottoms on top of 50 ml graduated cylinders and allowing drainage to flow. Stability of each sample was measured in triplicate and calculated based on time needed for a given volume (10 mL) of foam to collapse.

2.2.9 Emulsion Formation and Characteristics.

Protein solutions of 0.5% SCN in dd water with 0.02% sodium azide (antimicrobial) and 25% canola oil were coarse homogenized with a Polytron high shear mixer at 11K rpm for 20s followed by high pressure homogenization at 5K psi using the NanoDeBEE lab scale homogenizer (BEE International Inc.; South Easton, MA) to produce an O/W emulsion. Coarse emulsions were passed through the homogenizer twice and analyzed for particle size diameter (PSD) using a dynamic light scattering (DLS) Malvern Zetasizer Nano (Malvern Instruments Ltd.; Worcestershire, UK). Emulsions were diluted to a concentration of 0.04% (v/v) in dd water to avoid a multi-scattering effect of the particles. The refractive index ratio used to calculate particle size was 1.46. Particle size is reported as the intensity harmonic mean, which is indicated as z-average mean size (Z-avg) of 10 readings made in triplicate. Zeta potential was determined using the same instrument with further dilutions to 0.02% (v/v) with dd water.

Creaming index was used to determine droplet aggregation propensity. Samples were placed in polystyrene tubes and centrifuged using a Beckman-Coulter Optima L-90K Ultracentrifuge at 18,000 x g for 7 min. The height of the emulsion before centrifugation (He) and creamed layer (Hu) was measured with calipers, with the emulsion stability (ES) determined based on the following equation, where:

\[ ES = \frac{Hu}{He} \times 100 \]
This index provides data based on the cream layer versus the serum layer because there was no measurable difference between the serum layer and the middle (emulsion) layer. Therefore, a decreased index value indicates a decrease in stability.

2.2.10 Data Analysis.

All rheological tests were performed in triplicates and analyzed using the TA Instrument Software Trios®. Results are reported as mean of three independent experiments ± standard deviation. Emulsions and foams were prepared in triplicate. Individual WPI solutions were prepared for the analysis of foam formation (% overrun) and stability (sec/ml), each replication represents a single solution. Statistical analysis for main effects on gelation temperature, storage and loss modulus, overrun, foam stability, particle size, zeta potential, and emulsions stability were accomplished by one-way ANOVA and Dunnett’s post hoc test ($\alpha=0.05$) on JMP®10 (SAS Institute, Cary, NC).

2.3 Results and discussion

2.3.1 Flavan-3-ol profile of GSE, GTE, and TVG

Three extracts GSE, GTE and TVG were chosen with the expectation that they would yield distinct flavan-3-ol profiles allowing for different effects on protein functionality. Total phenolic content measured by FC method was 390.5, 859.09, and 937.4 mg GAE per g extract for GTE, TVG, and GSE respectively. The higher total phenolics content of both GSE relative to GTE was expected as similar extracts have been previously documented to have similar values$^{146,147}$. GSE is typically a product with additional refinement of the extract and therefore a higher proportion of phenolics$^{148}$ relative to the GTE which more closely resembles powdered green tea that is less refined to concentrate phenolics. Similar to previous reports, the monomeric C and EC as well as six flavan-3-ol dimers and several trimers and larger oligomers and polymers were detected in GSE $^{149}$. Dimers of C/EC were identified tentatively and quantified based on response of Procyanidin B2 with a content of 48.5 mg/g. Trimers were estimated represented 29.0 mg-PB$_2$E/g. In contrast to GSE, GTE composition was principally, monomeric flavan-3-ols including EGCG and ECG (170.3 mg/g and 54.2 mg/g) followed
by EC and EGC at 10.6 mg/g and 14.2 mg/g respectively. TVG is composed primarily of
the monomeric flavan-3-ol EGCG with trace levels of ECG, with the previous being the
most prevalent flavan-3-ol 961 mg/g.

2.3.2 Protein Polyphenol Binding

The ability of flavan-3-ol rich plant extracts to quench fluorescence of both WPI
and SCN’s fluorophore, tryptophan, was assessed as a measure of interaction among the
proteins and compounds within the extract. A reduction of fluorescence is an indication
of interaction between the fluorophore and its quencher. The type of interaction may be
dynamic or static, resulting from collision of the fluorophore with the quencher and/or
formation of a complex of the two, respectively. The quenching mechanism is
typically described by the Stern-Volmer equation:

\[
\frac{F_0}{F} = 1 + k_q \tau_o [Q] = 1 + K_{SV}
\]

where \(F_0\) is the initial fluorescence intensity, \(F\) is the intensity post quencher
addition, \(k_q\) is the bimolecular quenching constant, \(\tau_o\) is the average lifetime of the
fluorophore absent of the quencher, \(K_{SV}\) is the Stern-Volmer constant derived from the
linear regression of \(\frac{F_0}{F}\) vs \([Q]\), the quencher concentration. A linear regression of the
Stern-Volmer plot may be an indication that only one of the quenching mechanisms
occurred (dynamic or static).

Figure 2.1 displays the raw fluorescence spectra and the Stern-Volmer plot for
SCN and WPI in response to the addition of each extract. All of the spectra shown have
been corrected for fluorescence presented by the quenchers (extracts) at equal
concentrations used in experiments. As shown, each of the extracts are able to quench the
fluorescence intensity of both SCN and WPI as the concentration of extract increased,
indicating the quenching effect is concentration dependent. The ability of individual
flavonoids to quench the Trp is consistent with those results previously published. TVG
exhibited the largest fluorescence intensity quenching effects with near complete
quenching (99.9%) in samples prepared as SCN: TVG 1:2. The greatest quenching effect seen within GTE was 78.4% and 26.6% in GSE at their highest concentration. The Stern-Volmer plots for SCN in the presence of all extracts and WPI in the presence of GTE and TVG were linear, indicating that only one interaction occurs\textsuperscript{150}. The observed $K_{SV}$, which is derived from the linear regression indicates the differing quenching effects among the 3 extracts with each respective protein.

Typically, the $k_q$ is used to determine if the interaction is dynamic or static. A $k_q$ value lower than the diffusion-limited rate in an aqueous medium indicates a dynamic quenching effect, while static quenching is indicated by a 2-fold increase.
Figure 2.1 Raw Fluorescence Spectra and Corresponding Stern-Volmer Plots of SCN with the Addition of Plant Extracts. Each spectra represents SCN (1st row) in the presence of increasing concentrations of GTE (1st column), TVG (2nd column), and GSE (3rd column) displayed as emission wavelength (x-axis) vs. intensity (y-axis). Both WPI and SCN with the addition of GSE were prepared at reduced concentrations to account for the increase in fluorescence of the GSE at higher concentrations.
2.3.3 Dynamic Interfacial Tension Measurements

The ability of flavan-3-ols to interrupt the interfacial tension and contribute independently to emulsion stabilization was explored. SCN is a known surfactant that provides excellent stability to O/W emulsion by lowering the interfacial tension of the water. The change in surface pressure of proteins has been characterized as having three distinct periods, the induction period in which gradual change in surface pressure occurs as the protein adsorbs, the second- displaying rapid changes in the surface pressure, and finally a stabilization of the surface pressure. Determination of surface pressure with SCN:flavan-3-ol mixtures was considered to offer further insight on the effects flavan-3-ol size, structure, and concentration may have on this attribute. Flavan-3-ols alone had relatively no effect on the interfacial tension of water in the absence of surface active molecules (Figure 2.X). This is consistent with other reports on flavan-3-ols. When singular flavan-3-ol compounds, EC, EGCG, and PB2 were combined with SCN, a variance in effects was noticed across each compound (Figure 2.2). SCN solution that included EC, the lowest molecular weight compound tested but characteristic of the monomer fraction of GSE, displayed lengthening of the induction period, delaying the increase in surface pressure. This may have been due a combination of effects, including competition with the protein to adsorb at the interface due to interaction with the compound. Alternatively, binding of EC to SCN may have delayed SCN adsorption at the interface, a hypothesis that remains to be assessed. It is hypothesized that the eventual increase in surface pressure may have been a result of the protein reaching and unfolding at the interface. Previous work utilizing β-casein and EC, C, or gallic acid indicated no changes in the final pressure with minimal changes in induction time. The greatest of this impact of this was seen at a ratio of SCN:EC 5:1, with less steep increases in surface pressure, above and below this point. The resulting surface pressure of the solutions at all concentrations were higher than that of SCN.

SCN:EGCG solutions all had similar responses compared to EC (Figure 2.2B). The general trend was seen to be a delay in the increase of surface pressure. However, at higher concentrations, EGCG was able to prevent adsorption of SCN at the interface. Incorporation of the EGCG at the higher concentration may have mitigated the ability of
the SCN to reach the interface. At higher concentrations of EGCG (greater than the protein) it has been shown that the polyphenol will begin to act as a bridge, having multiple sites of interaction were the protein may have only one\textsuperscript{155}. Following this theory, EGCG may have been able to interact with all potential points, reducing its mobility to the interface. Likewise, the interactions at the higher concentration of SCN:TVG 1:2 (93% EGCG) in our fluorescence study showed a complete quenching effect with SCN, further indicating this as a potential cause of the reduced surface pressure. Furthermore, proteins such as SCN are able to increase the surface pressure through its ability to change conformation, exposing its hydrophobic region at the interface. Since it has also been shown that this is the same region that allows for hydrophobic interactions with polyphenols\textsuperscript{156}, this may likely be the mechanism.

The eventual increase in surface pressure near the end of the experiment, however may have been due to the oxidation of the EGCG over time. Specifically, as EGCG becomes oxidized there is a C-C bond that forms on the B-ring resulting in a net loss of two hydrogens, generating homodimers\textsuperscript{117}. These larger oxidized compounds have been shown to have lower antioxidant properties\textsuperscript{157}. Larger dimers, PB\textsubscript{2}, exhibited similar trends as the EC at all levels of incorporation (Figure 2.2C). PB\textsubscript{2} is larger than EGCG, however structurally more compatible with EC, as it is an EC/EC dimer. This difference along with studies indicating an increased affinity for those flavonoids that are galloylated to interact with protein\textsuperscript{158} further explain the similarity in their surface pressure.
Figure 2.2 Effects of Select Flavan-3-ols on Surface Pressure of SCN Solutions

Graphs are an average (n=3) surface pressure for each flavan-3-ol with their corresponding structure. Graph (A) represents SCN:Epicatechin, (B) represents SCN:EGCG, and (C) represents SCN:Procyanidin B2.
2.3.4 Effect of extracts on rheological properties of 18% WPI gel

Gelation of globular proteins can be described as a heat-induced protein denaturation process resulting from two main steps. The first is a change in the matrix resulting in an increase in intermolecular interactions, and the second involves the aggregation of the protein molecules into a fixed network\(^{159}\). A WPI concentration of 18% (v/v) was used in experiments, as this concentration yielded satisfactory level of protein to form a heat induced gel as determined through preliminary testing. The gelation time (sec) was determined to be the point (within the procedure step time) in which storage and loss modulus intersect, continuing with a rapid increase in G’, and phase angle equal to 45°. In Table 2.1, the control sample (18% WPI) had a mean gelation time of 397.4 sec at the hold temperature of 75°C. These results are within range of those previously reported in the literature\(^{160}\). No general trends were observed in the analysis of the gelation times among each treatment extract type as concentration increased (0.01-5 mg/mL). Within the concentration range examined, flavan-3-ol rich GSE and TVG extracts demonstrated modest but non-significant decreases in gelation time 323.0 (0.01 mg/ml) and 326.0 sec (1 mg/ml) without further decreases at higher concentrations of flavan-3-ols. However, inclusion of monomer rich GTE at 3 mg/ml decreased gelation time significantly to 300.3 sec (p=0.026).

A decrease in the time to form a WPI gel in the presence of an extract containing larger flavan-3-ol oligomers and polymers was expected, but, was not observed to reach significant levels. This may be due to the documented ability of larger polymeric flavan-3-ols to interact to a greater extent with protein and form aggregates\(^{161}\). Flavan-3-ols of a higher DP have been shown to have a higher affinity for globular proteins than that of smaller flavan-3-ol structures. Isothermal titration of larger procyanidins (DP=7.4), with α-lactalbumin suggests that this is due to their multidentate character allowing simultaneous binding at several sites\(^{162}\). In a study on the effects of monomer rich green tea on whey protein concentrate gels, it was reported that at a pH of 6.0 there was a reduction in the temperature at which gels were formed at concentrations of 0.5% and 1%\(^{89}\). At pH 6, with its higher concentration of GTE, oxidation of monomeric flavan-3-ols is likely factor. As reported by Song et al., monomeric flavan-3-ols found in GTE (pH
6.3) oxidized in the presence of heat, both mild (37°C) and harsh (62°C), forming larger oxidized products\textsuperscript{163}. These products would potentially give rise to an increase in protein/flavan-3-ol interactions. While our observations were not significant at most concentrations, it is important to note that a trend toward decreasing gelation time was also observed with GTE treatments at higher concentrations of 1 and 5 mg/ml. Furthermore, the model applied in the present study was designed to limit gelling temperature as a dependent variable and optimize the strength of the complex modulus data, based on the matrix not the procedure model. Therefore, it may not have been optimal to detect subtle effects brought on by monomeric flavan-3-ols in GTE and TVG.

**Table 2.1** Effects of Extracts on the Gelation Characteristics of 18% WPI Gels

<table>
<thead>
<tr>
<th>Extract Type</th>
<th>Concentration (mg/ml)</th>
<th>Gelation Time (sec) At 75°C</th>
<th>G' (Pa) At 75°C</th>
<th>G&quot; (Pa) At 15°C (after heating)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>397.4 ± 31.18</td>
<td>2732 ± 308.1</td>
<td>424.3 ± 29.83</td>
</tr>
<tr>
<td>EGCG</td>
<td>0.01</td>
<td>396.7 ± 13.83</td>
<td>2671 ± 214.2</td>
<td>419.1 ± 32.59</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>436.3 ± 10.87</td>
<td>2148 ± 157.6</td>
<td>389.3 ± 15.33</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>394.3 ± 10.47</td>
<td>2394 ± 42.47</td>
<td>389.4 ± 14.48</td>
</tr>
<tr>
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<td>440.2 ± 14.00</td>
<td>3154 ± 767.7</td>
<td>487.4 ± 93.68</td>
</tr>
<tr>
<td></td>
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<td>326.0 ± 11.90</td>
<td>4208 ± 307.6</td>
<td>582.3 ± 39.72</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>446.6 ± 64.80</td>
<td>2146 ± 626.7</td>
<td>443.6 ± 71.29</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>367.1 ± 82.90</td>
<td>2191 ± 222.2</td>
<td>462.8 ± 22.41</td>
</tr>
<tr>
<td>GSE</td>
<td>0.01</td>
<td>323.0 ± 90.74</td>
<td>3309 ± 720.0</td>
<td>448.6 ± 39.56</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>365.0 ± 5.85</td>
<td>2924 ± 306.9</td>
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<tr>
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<td>420.6 ± 19.22</td>
<td>3072 ± 293.3</td>
<td>471.3 ± 55.46</td>
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<tr>
<td></td>
<td>0.5</td>
<td>355.3 ± 31.99</td>
<td>3646 ± 323.0</td>
<td><strong>517.1 ± 30.49</strong></td>
</tr>
<tr>
<td></td>
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<td>430.2 ± 20.34</td>
<td>2598 ± 189.5</td>
<td><strong>410.5 ± 30.12</strong></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>364.6 ± 21.32</td>
<td>3452 ± 42.35</td>
<td><strong>514 ± 16.31</strong></td>
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<td></td>
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<td>461.8 ± 19.25</td>
<td>2768 ± 253.1</td>
<td><strong>508.1 ± 36.50</strong></td>
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<tr>
<td>GTE</td>
<td>0.01</td>
<td>351.3 ± 1.55</td>
<td>3100 ± 227.5</td>
<td>424.6 ± 24.80</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>354.2 ± 31.03</td>
<td>3067 ± 613.6</td>
<td><strong>412.9 ± 10.87</strong></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>422.4 ± 44.75</td>
<td>2809 ± 565.0</td>
<td><strong>427.2 ± 56.74</strong></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>398.4 ± 48.70</td>
<td>2537 ± 246.5</td>
<td><strong>412.9 ± 10.87</strong></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>343.2 ± 63.02</td>
<td>5834 ± 899.9</td>
<td>***700 ± 71.37 ***</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>300.3 ± 17.81</td>
<td>4678 ± 387.9</td>
<td>**568.9 ± 28.59 **</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>350.5 ± 8.690</td>
<td>4911 ± 958.1</td>
<td>**635.8 ± 129.2 **</td>
</tr>
</tbody>
</table>

Data shown is expressed as the mean ± standard deviation. Significant differences include *p< 0.05, **p<0.005, and ***p<0.0005 when compared to the control 18% WPI gel.
Storage modulus ($G'$) represents the solid-like state while the loss modulus ($G''$) represents the fluid-like state of the sample. The effects of the flavan-3-ol rich extracts on $G'$ and $G''$ of the 18% WPI gels were determined by comparing both parameters at the conclusion of the time sweep to that of a control. In all cases, $G'$ of the samples was found to be higher than that of the $G''$. This is indicative of a more solid or elastic characteristic than liquid, and is common in protein gels. The most significant impacts were observed at 1 mg/ml and higher in treatments prepared with monomer rich GTE, in which case samples had a significantly ($P<0.05$) higher $G'$ than the control gels. These results are similar in nature to that previously reported by von Staszewski, Jogus, and Pilosof\textsuperscript{89} and Wu, Clifford, and Howell\textsuperscript{13}, in which an increase in $G'$ was observed as concentration of GTE was increased in various protein gel systems. Interestingly, treatments with TVG, representing a highly purified EGCG fraction of green tea, and GSE, rich in oligomers and polymers of flavan-3-ols, exhibited significantly higher $G'$ at lower concentrations than that of monomer rich GTE. Proanthocyanidin-rich GSE had a significantly ($p=0.031$) higher $G'$ at 0.5 mg/ml and TVG had a significantly ($p=0.0021$) higher $G'$ at 1 mg/ml compared to control and GTE at similar concentrations.

Subsequently, a decrease in $G'$ was seen as the extract concentration increased.

These results can again be attributed to several factors including the differences in flavan-3-ol composition between extracts. TVG by virtue of the EGCG structure and GSE with its higher proportion of oligomeric and polymeric flavan-3-ols likely provide an increase in potential interaction points (hydrogen binding) between the flavan-3-ol and the proteins at lower concentrations (Figure 2.3). The concentration of both the protein and polyphenol, as well as the ratio of the two has been reported to affect the binding and eventual loss of solubility, manifested as protein haze\textsuperscript{155}. Given the profile of both the TVG and GSE, the decrease in $G'$ is likely due to a limitation of active protein binding sites by these phenolics, therefore limiting protein-protein interactions, decreasing the $G'$. At higher concentrations the likelihood of hydrophobic interactions occurring due to increased presence of aromatic rings is increased\textsuperscript{110,164}. A peak change in storage modulus was reached sooner, with GSE reaching its peak $G'$ at half the concentration of
TVG. The subsequent decrease is likely due to the ability of these molecules to saturate the binding sites of the protein.

Previously, binding studies utilizing isothermal titration calorimetry indicate that as the protein becomes saturated at its points of interaction, the energy of interaction will plateau and return to an equilibrium. This occurrence resulted when similar concentrations of both galloylated and non-galloylated monomeric flavan-3-ols were titrated with L-(poly) proline. Though the proteins utilized represent a differing structure, hydrophobic interaction may still occur with hydrophobic residues of WPI as the protein is denatured.

![Epicatechin-3-gallate](image1)

![Procyanidin Oligomers](image2)

**Figure 2.3** EGCG and Oligomeric Procyanidin Structure.

2.3.5 Effect of extracts on foam formation and stability of SCN

GSE, GTE, and TVG were individually formulated into 5% SCN solutions between 0.01 and 1.0 mg/ml to determine the impact of flavan-3-ol rich ingredients on foam formation, measured as overrun, and foam stability, a function of time (sec/mil collapse). Percent (%) overrun of 5% SCN solutions was consistent with that previously reported for similar SCN foams. Inclusion of flavan-3-ol rich ingredients resulted in only modest changes in overrun relative to control over the given 15 min mixing period (Table 2.2). These results agree in principle with previous work with GTE as reported by
Wu, Clifford, and Howell using egg albumin. Increasing concentrations of GTE, a general trend of reaching an optimal effect prior to a decrease in overrun, as extract concentration increased, was exhibited. While most effects in the present study did not reach statistical significance, inclusion of EGCG rich TVG at concentrations of 0.1 mg/ml and 0.5 mg/ml did demonstrate a significant increase in overrun at each time point, including the final 15 min with 1043.3% (p= 0.044) and 1031.5% (p= 0.038) respectively. The effectiveness of TVG may be related to the properties of EGCG. Factors including size of the flavan-3-ol, i.e. degree of polymerization, as well as number of hydroxyl groups may be of great influence to these interactions. EGCG, being a galloylated flavan-3-ol with increased -OH groups, would be more effective at driving interactions that could facilitate foam formation, in that they extend the protein network, allowing faster formation of the foams (Figure 2.2). Still, these results indicate that the addition of flavan-3-ol rich extracts may not have a meaningful impact on the formation of foams produced by SCN when used at levels that could practically be incorporated into foods.

In regards to foam stability, measured at 15 min post whip cycle, collectively at 0.01 mg/ml concentration of extract, none of the treatments were significantly different than the control (Figure 2.4). However, at higher but still practical levels of 0.5 mg/ml and 1.0 mg/ml extract, both TVG and polymer rich GSE demonstrated a significant (p<0.0001) increase in foam stability 907.22 and 1005.7 sec/mL or 132.9%, and 158.3% and 1040.8 and 1050.5 sec/mL or 167.3% and 169.8% improvement respectively. Inclusion of monomer rich GTE at all concentrations tested had no significant impact on foam stability. Similarly, Wu et al reported that various types of GTE when applied with egg albumin had modest impacts to foam stability. The lack of a direct impact from monomer rich GTE on foam stability may be due to the combination of monomeric flavan-3-ol profile compared to that of polymer rich GSE and distribution of galloylated (EGCG, ECG) to non-galloylated (C, EC and EGC) compared to TVG which is primarily EGCG. In contrast to GTE, TVG (essentially a fractionated GTE) displayed significant increases in stability over all concentrations with the exception of 0.01 mg/ml level, with the most significant (p<0.0001) increase in stability at 0.5 mg/ml and 1.0 mg/ml.
Table 2.2  Effects of Extracts at Different Concentrations on SCN Foams

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (mg/ml)</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>685.2%</td>
<td>775.0%</td>
<td>831.8%</td>
</tr>
<tr>
<td>GTE</td>
<td>0.01</td>
<td>691.7%</td>
<td>792.4%</td>
<td>848.7%</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>725.3%</td>
<td>780.5%</td>
<td>809.9%</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>625.6%</td>
<td>770.7%</td>
<td>831.1%</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>673.4%</td>
<td>761.7%</td>
<td>813.6%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>663.3%</td>
<td>729.1%</td>
<td>787.4%</td>
</tr>
<tr>
<td>EGCG</td>
<td>0.01</td>
<td>692.4%</td>
<td>787.7%</td>
<td>833.1%</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>688.8%</td>
<td>825.1%</td>
<td>887.0%</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>792.8%</td>
<td>932.4%</td>
<td>1043.3%</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>801.2%</td>
<td>929.3%</td>
<td>1031.5%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>741.4%</td>
<td>848.1%</td>
<td>893.3%</td>
</tr>
<tr>
<td>GSE</td>
<td>0.01</td>
<td>723.4%</td>
<td>801.2%</td>
<td>898.0%</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>743.7%</td>
<td>822.5%</td>
<td>915.2%</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>719.7%</td>
<td>806.2%</td>
<td>892.1%</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>747.1%</td>
<td>812.7%</td>
<td>889.0%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>727.9%</td>
<td>781.4%</td>
<td>846.5%</td>
</tr>
</tbody>
</table>

Significant differences are denoted as **p< 0.005 and *p< 0.05 when compared to the control 5% SCN foams.

It is hypothesized that because flavan-3-ols have characteristics favorable for both hydrophilic and hydrophobic interactions, these compounds are able to extend the protein network by being active at the interface. The fact that TVG is primarily composed of EGCG may provide increased bridging interactions compared to that of the crude monomer rich GTE (45.4 % EGCG). For example, flavan-3-ols may hydrogen bond with water and the protein to increase the thickness of the adsorbed layer of the foam film, consequently increasing the stability of the foam as the extract concentration increases. Smaller flavan-3-ol molecules and non-galloylated compounds such as C, EC, and EGC that collectively make up a majority of the GTE assessed in this study have been reported to have lower protein binding potential than that of the larger galloylated EGCG.
Specifically, C showed little change in enthalpy when added to globular and proline-rich proteins, while EGCG was reported to reach a maximum energy shift before plateauing at higher concentrations\textsuperscript{161,162}. Therefore, in a crude system such as GTE, limited benefits to stability are potentially offered via increased aggregation of the proteins by flavan-3-ols to form bridges. By comparison a more refined system such as TVG, it may have improved effectiveness due to the increase in % galloylated flavan-3-ols and by extension due to its multiple binding sites. Potential stacking of these smaller flavan-3-ols could also block protein-air interface at the hydrophobic sites of the protein inhibiting the stabilizing effect\textsuperscript{168}.

![Figure 2.4](image.png)

**Figure 2.4** Stability of 5% SCN Foams in the Presence of Extracts at a Range of Concentrations. Statistical differences are as compared to the control 5% SCN foam. *p< 0.05, **p< 0.0005, and ***p< 0.0001. Error bars indicate ± s.d. of the means.

When comparing the ability of the two extracts to quench the fluorescence of SCN at varying concentration, EGCG rich TVG was shown to have a higher % quenching effect compared to the more crude GTE at all like concentrations. This gives indication of the
decreased interaction, among smaller GTE flavan-3-ols compared to that of its galloylated counterpart. The GTE used in this system, as previously stated, contains 45.42% EGCG therefore smaller non-galloylated compounds like EGC make up over half the composition, potentially limiting the extended interactions.

Similar to the current findings, Wu et. al also note GTE with a higher concentration of EGCG improved foam stability. Limited studies are available that utilize pure EGCG in protein functionality testing. As such mechanism of its effects are only subjectively characterized. In the current study the fluorescence intensity and analysis of surface pressure as it relates to single flavonoids was conducted to offer further insight into the mechanism of these effects. The results offered minimal conclusions on mechanism, however brought additional points of interest for further follow up. The studies indicated an interaction occurs with the hydrophobic residues of Trp as the extracts are added and a delay in adsorption of SCN to act the interface in most cases with the exception of higher ratios of EGCG. This suggests that the binding may allow for complex formation, but they do not alter adsorption, which could be an indication that of an alternative mechanism of stability of the foams with TVG and GSE. Therefore, the previous suggestion of an extended network formed through the interaction of the extracts (bridging) with the protein is plausible.

A similar study reported no significant impact on foam overrun in foams made with purified β-lactoglobulin and GTE with faster liquid drainage and the persistence of foam height as a dry foam. This may have been a result of foams generated from a purified protein model or conformational changes in the protein that were not assessed in the current study. A previous study on the conformational changes of β-LG when complexed with various tea polyphenols reported an increase in the β-sheets and α-helices with a decrease in the random coils. This contributed to greater stabilization of the protein, which may also have been a factor in the stabilization shown in the foams produced using flavan-3-ol rich extracts.
2.3.6 Effects of GTE and GSE on emulsification properties of 0.5% SCN in O/W emulsions

Homogenization of the SCN o/w emulsion was used to generate a test emulsion for screening potential effects of flavan-3-ol rich extracts on stability. Preliminary studies utilizing a high shear blender provided only mildly stable emulsions with increased particle size and polydispersity (data not shown). These provided inconclusive results on the effects of the extracts. Mean particle size (Z-avg measured as d.nm) was performed on treatments throughout a 30 day period, however due to the increased rate of creaming and initial size of particles, particle size distribution (PSD) resulted in decreasing values over time. Therefore, only initial PSD data is shown in (Table 2.3). The control emulsion of 0.5% SCN had a Z-avg of 549.68 d.nm, consistent with previous data reported by Hu, McClements, and Decker\textsuperscript{170} of a 0.5% SCN stabilized corn oil emulsion with a diameter of 0.49 μm. All flavan-3-ol treatments were found to have a significant impact on the Z-avg when compared to the control emulsion, with an increase in Z-avg as concentration of flavan-3-ol extracts increased. As such, the most significant (p< 0.0001) increase in Z-avg of each flavan-3-ol rich extract was seen at higher concentration of 0.5 mg/ml and 1.0 mg/ml concentrations of each extract as well as 0.05 mg/ml and 0.1 mg/ml in samples prepared with GTE and TVG. Similarly an increase in PSD was previously reported in solutions with β-LG and green tea extract when compared to a control β-LG and caseinomacropeptide, indicating the formation of larger complexes\textsuperscript{171}. It is proposed that the uniformity of TVG’s incremental increase in Z-avg is due to the purity of the extract (95% EGCG).

Zeta potential is another indirect measurement of the colloidal stability. Generally, the magnitude of the zeta potential value (mV) is an indication of the potential stability of the suspension. Particles that are largely negative or positive values will have the tendency to repel flocculation. The zeta potential of each treatment was determined 24 hrs after homogenization. Values ranged from -69.25 to -30.60 mV (Table 2.2). Most all samples formulated with flavan-3-ols from specific extracts were significantly different compared to control. With the exception of both TVG and GTE systems at 1.0 mg/ml and 0.01 mg/ml; likewise, the zeta potential was largely negative. Treatments
with polymer rich GSE at 0.01 mg/ml and 0.05 mg/ml were significantly (p < 0.0001) lower than the control, while all others were significantly higher (p < 0.0129 or better). TVG treated emulsions at 0.05 mg/ml and below displayed a significantly (p < 0.0001) lower zeta potential than the control, while only GTE at 0.1 mg/ml showed a significantly (p < 0.01) lower zeta potential than the control. The measurement of zeta potential did not give direct indication of the colloidal stability of the emulsions formed considering in most cases the degree of difference compared to the control is never greater than 15 mV. Moreover, the zeta potential of the treatments in the present study were not indicative of the stability observed during accelerated stability testing. Although not indicative of stability, the range of zeta potential displayed were consistent with other published literature in which protein-polyphenol mixed systems were analyzed in colloidal stability 89.
Table 2.3  Effects of Extracts on Emulsion Properties of SCN Stabilized O/W Emulsions

<table>
<thead>
<tr>
<th>Extract Type</th>
<th>Concentration (mg/ml)</th>
<th>Z-Avg (d.mm) ± s.d.</th>
<th>Zeta Potential (mv) ± s.d.</th>
<th>Emulsion Stability (%) ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>549.68 ± 6.32</td>
<td>-56.1 ± 0.58</td>
<td>34.77 ± 3.44</td>
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<tr>
<td>TVG</td>
<td>0.01</td>
<td>814.28 ± 14.72</td>
<td>-47.55 ± 0.26</td>
<td>33.73 ± 3.51</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>910.85 ± 33.14</td>
<td>-52.6 ± 0.62</td>
<td>37.86 ± 2.16</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1126.3 ± 19.45</td>
<td>-51.2 ± 0.57</td>
<td>37.96 ± 1.58</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1473.3 ± 81.26</td>
<td>-63.13 ± 1.02</td>
<td>31.77 ± 0.94</td>
</tr>
<tr>
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<td>1</td>
<td>1545.3 ± 204.1</td>
<td>-57.35 ± 0.91</td>
<td>26.73 ± 2.80</td>
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<tr>
<td>GSE</td>
<td>0.01</td>
<td>867.45 ± 53.78</td>
<td>-46.2 ± 0.73</td>
<td>35.88 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>982.18 ± 57.44</td>
<td>-45.2 ± 0.34</td>
<td>33.80 ± 2.84</td>
</tr>
<tr>
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<td>0.1</td>
<td>1028.8 ± 93.36</td>
<td>-61.65 ± 0.59</td>
<td>39.59 ± 1.71</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1394.3 ± 153.6</td>
<td>-57.93 ± 1.17</td>
<td>32.97 ± 3.01</td>
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<tr>
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<td>1483.5 ± 238.2</td>
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<td>GTE</td>
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<td>704.68 ± 10.71</td>
<td>-54.23 ± 0.5</td>
<td>31.95 ± 0.40</td>
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<td>34.42 ± 2.00</td>
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<td>1064.5 ± 64.51</td>
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<td>33.79 ± 3.16</td>
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<tr>
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<td>33.93 ± 4.22</td>
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<tr>
<td></td>
<td>1</td>
<td>1238.5 ± 132.5</td>
<td>-57.28 ± 0.56</td>
<td>31.41 ± 0.66</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± s.d. Letters indicate statistical difference (p< 0.05 or better) when compared to the control 0.5% SCN stabilized O/W emulsions.

An accelerated emulsion stability test provides insight into potential issues with colloidal system stability. While no significant differences in stability were seen among the treatments when compared to the control, a trend of decreasing emulsion stability was observed as concentration increased for all extracts (Figure 2.5). In comparing emulsion stability among each extract, at concentrations at and above 0.5 mg/ml, a decreasing trend was observed. However, samples showed a dramatic difference in the opaqueness of the serum layer indicating that at higher concentrations the extracts may be initiating or facilitating denaturation of the protein to the point where solubility is impacted and emulsification properties of protein and phenolic are both lost. The increased Z-avg as concentration increased noted in the PSD determination, along with decreased emulsion stability are consistent with the rules of Stokes law. The increase in particle size
decreases the surface area of the oil droplets, which supported the coalescence of the oil droplets in the presence of larger, higher concentrations of monomeric or polymeric flavan-3-ols.

In emulsions, flavan-3-ols may modify the stability by interacting with the emulsifying agent, in this case, SCN, or by its association with the lipid layer specifically. Di Mattia, Sacchetti, and Pittia 172 demonstrated that catechin may partition as much as 42% in the aqueous phase and the remaining 58% resulted in interaction with the surfactant Tween 20 and interaction with the dispersed phase. Interaction with either SCN or the interface of the dispersed phase may occur due to the hydrophobic/hydrophilic nature of the flavan-3-ol. It is likely that the association with SCN is due to hydrogen bonding of the hydroxyl groups of the flavan-3-ols with the protein. Furthermore, the interaction with the interface may occur through the ability of flavan-3-ols to compete with SCN for placement at the hydrophilic/hydrophobic interface. In the case of the GSE, GTE, and TVG at higher concentrations, this becomes detrimental to the stability of the droplets and increases the rate of creaming.

It should also be noted that while very little research has been published confirming the effects of these differing flavan-3-ol rich extract profiles as emulsifying agents, Luo et al. has reported that a trend exists among pure flavonoids, their partition coefficients, and emulsification activity. Those flavonoids with coefficient values -0.6 < log10P < 0 are either active or not as emulsifiers, however those removed have no activity 173. Specific flavonoids that have no activity include, but are not limited to catechin (0.32, 0.53), epicatechin (0.13, 0.6), and EGCG (0.39, 0.26) 173-175. Therefore, the modest effects observed in this study in extracts and food systems may be simply related to the activity predicted by individual compounds that make up the complex but well defined extracts.
2.4 Conclusions

Functionality studies with both whey protein isolate and sodium caseinate based ingredients suggest that proteins and flavan-3-ol may interact in ways that have direct impact on formation and strength and quality of gels, emulsions and foams within ranges commonly added to foods. The greatest impact on protein functionality was demonstrated with the use of the extracts in emulsion formation and stability. The degree of association is potentially affiliated with the concentration of the extract and its flavan-3-ol profile, with high concentrations of larger molecule and highly galloylated flavan-3-ol extracts having the greatest impact on functionality. Protein-polyphenol ratio plays a role in the effects these extracts have on the gelation characteristics. Overall while these data suggest that flavan-3-ol rich extracts can be applied in protein based systems, both positive and negative impacts on protein functionality, and by extension, product quality are likely. As such, application of these ingredients in combination requires a measure of consideration in the application. However, complexation of the selected proteins with the flavan-3-ols as well as the processing may further impact the delivery of these flavan-3-ols in biological systems. These outcomes will be discussed further in Chapter 4.
CHAPTER 3. EFFECTS OF FLAVAN-3-OLS ON PHYSICAL PROPERTIES OF CARBOHYDRATES IN MODEL FOOD SYSTEMS

3.1 Introduction

Carbohydrates make up as much as 50% of the energy intake in the US and approximately 54% in developing countries\textsuperscript{176}. After maize and rice, wheat is the third most widely consumed cereal grain, presenting itself mostly in the form of processed foods\textsuperscript{177}. Starch based ingredients are very commonly used in foods as thickener/gelling agents and stabilizers. Similarly, carrageenan is an anionic polysaccharide that extracted from red marine algae characterized by its multidentate, sulfated, structure that allows it to form thermoreversible gels\textsuperscript{178}. The structure of $\kappa$-carrageenan specifically consists of linear chains of (1-3) linked $\beta$-D-galactose and (1-4) linked $\alpha$-D-galactose units (Figure 3.1). It is known for its ability to gel products at near neutral pH, thicken beverages through binding water and oils, and stabilize crystals of frozen desserts\textsuperscript{179}. Carrageenan is often combined with starch in select foods to act as a fat mimetic, giving non-dairy frozen dessert the desired mouthfeel\textsuperscript{179}.

![Chemical Structure of $\kappa$-carrageenan](image)

Figure 3.1  Chemical Structure of $\kappa$-carrageenan
With a growing interest placed on “natural” and “clean” labeling, native wheat starch and carrageenan are both excellent substitutions of semi-synthetic texturants. However, a challenge exists in reformulation of products in alignment with clean label strategies. With such changes, modulation of their activities is possible but may prove detrimental to the end product quality parameters. In addition to these clean labeling needs, a greater emphasis has also been placed on nutritional quality of processed foods, including both nutrients and bioactive phytochemicals. It is therefore not surprising that plant based ingredients, including phenolic extracts, are becoming more broadly used. In particular, polyphenol or flavonoid rich extracts including tea, cocoa, grape seed are widely used in functional products.

Within the flavonoid class, flavan-3-ols are widely found naturally in foods and are perhaps the most commonly consumed group among Westernized diets\(^16,29\). They are present in products of tea, cocoa, select fruits and their seeds\(^29\) that are leveraged as extracts in foods. While these flavonoids are well known to impart astringency, bitterness, sourness, and to some extent color\(^12\), additional functional effects are likely. In particular, flavan-3-ols are well known to interact with macromolecules including protein and carbohydrates through non-covalent hydrophobic interactions and hydrogen bonding. When combined in food products it is likely that these compounds alter the physical properties of processed foods. However, the extent of these effects are not well characterized at levels that are feasible within consumer food products. Expanding on insights gained through protein interactions, information on interaction effects between flavonoids and carbohydrates is needed to improve translation of these extracts into quality products.

The current study considered the impact of select flavan-3-ol plant extracts (GTE and GSE) on the physical properties of carbohydrate matrices utilizing wheat starch and \(\kappa\)-carrageenan based foods as model systems. The plant extracts that were evaluated represent potential sources of flavan-3-ols that contain either high levels of monomeric (GTE) or polymeric (GSE) forms of these compounds. This allowed for the characterization of their effects on commonly used carbohydrates in processed foods and
represent near maximum levels that are practical, in terms of sensorial properties, and potential health benefits.

3.2 Methods & Materials

3.2.1 Materials

Wheat starch (WS) Midsol™ 50 from MGP Ingredients, Inc® (Atchinson, KS). κ-carrageenan (kCGN) (Formula Name) was provided by FMC BioPolymer (Philadelphia, PA). Green tea extract (GTE) was provided as a gift by Nestlé Product Technology Center (Marysville, OH) and Meganatural Gold grape seed extract (GSE) was purchased from Polyphenolics (Madera, CA). Acetonitrile, water (Mallinckrodt-Baker, Phillipsburg, NJ) and formic acid (Sigma-Aldrich, St. Louis, MO) used in HPLC analysis were certified HPLC-MS and ACS grade and purchased from Sigma-Aldrich. Phenolic standards were purchased from Sigma-Aldrich.

3.2.2 Starch Pasting & Flow Properties

The effects of the flavan-3-ol rich plant extracts on the physical properties of WS pastes were determined by measuring changes in the rheological characteristics. A pasting study was initially conducted for the WS to determine changes in the pasting properties using a AR-G2 (TA Instruments, New Castle, DE) controlled stress rheometer equipped with a starch pasting cell and spindle designed to minimize water loss during the cook cycle. WS (8% w/v) was added to 25 ml of dd water (control) or a 1 mg/ml and 5 mg/ml plant extract solution and placed in the canister of the pasting cell. The instrument was set to condition the sample at 50°C with a preshear of 25 rad/s for 10s to all for suspension of the starch particles before a continuous angular velocity of 16.76 rad/s was used for the duration of the experiment. The temperature was then held at 50°C for 60 s before a temperature ramp to 95°C at 6°C/ min was performed. Upon reaching 95°C the temperature was held for 300 s and a final temperature ramp to 50°C was done. Finally, the temperature was held at 50°C for 60 s. The time, temperature, and viscosity was measured for the duration of the experiment. The starch pasting was characterized by the following parameters: peak viscosity, peak viscosity temperature/time, holding viscosity, breakdown, final viscosity, and setback.
Following the pasting of the starch, additional rheological experiments were performed on a Discovery Hybrid Rheometer (DHR-3) (TA Instruments, New Castle, DE) equipped with a peltier solvent trap cover to minimize evaporation and a peltier plate for temperature regulation. A 1.5 ml sample of each WS solution was placed at the center of the plate and measured within a 1 mm geometry gap. The flow properties were determined using a 40 mm parallel plate geometry at a constant strain of 0.5%, which was determined to be within the linear viscoelastic region in preliminary experiments and a frequency sweep 0.1 to 10 Hz. WS solutions were pipetted on to the plate at 25°C. The storage modulus (G’), loss modulus (G’’), were continuously measured throughout the procedure. All rheological measurements were done in triplicate on different samples and reported as Pa.

3.2.3 Rheological Properties of κ-Carrageenan Gels

Potassium chloride (0.125 mol/L) was first added to dd water under heated stirring conditions (80°C), followed by addition of individual extracts and κCGN (1% w/v). Dynamic viscoelastic properties were measured on the DHR-3 (TA Instruments, New Castle, DE). Measurements were taken using a 40 mm diameter parallel plate geometry with 100 grit sandpaper on the parallel plate. The solvent trap was utilized to prevent evaporation of water from the samples while peltier plate was preconditioned to 80°C. Solutions were loaded on to the plate with a geometry gap set to 1 mm and allowed to condition to 80°C for 600 s. The sample was then conditioned to 25°C for 300 s with a frequency sweep conducted between 0.01 to 10.0 Hz with a 0.5% strain using the same previous listed parameters. All rheological measurements were done in triplicate on different samples with storage modulus (G’) and loss modulus (G’’) recorded throughout the experiment.

3.2.4 Data Analysis

All experiments were performed in triplicate and prepared fresh daily for analysis. Rheological measurements were analyzed using TA Instrument software, Trios® (New Castle, DE). An analysis of variance (ANOVA) was conducted for flavan-3-ol
concentrations and pasting parameters. Significant differences were calculated using the Tukey method for differences ($\alpha = 0.05$) with JMP® 12 (SAS Institute, Cary, NC) software.

3.3 Results & Discussion

3.3.1 Effects of Plant Extracts on Wheat Starch Pasting

Traditionally, analysis of the pasting properties of starch has been done utilizing the Rapid Visco Analyzer, which reports the pasting properties of starch in RVA units (RVU). In this study, a controlled stress rheometer (AR-G2) equipped with a pasting cell was used, with data reported as Pa.s. Similar to the RVA, a pasting curve may be obtained by manipulating the temperature and angular velocity of the spindle producing data that is highly reproducible. The pasting curve provides important information on the behavior of the starch. As the starch is heated to a temperature at or above its gelatinization temperature, the starch granules begin to swell and take up water, ultimately reaching a peak viscosity. When the solution is continually heated at a given temperature, a holding viscosity or holding strength is obtained. This is the viscosity at the end of the heating period. The starch paste is then cooled and a final viscosity measurement is recorded. Additional characteristics that are determined include the breakdown and setback. The breakdown represents the difference in viscosity between the peak viscosity and holding strength; while setback represents the degree of retrogradation and is determined as the difference in final viscosity and the holding strength.

The pasting trends of the wheat starch with GTE and GSE (Figure 3.2) against the control indicate an impact of their addition at each concentration tested. During the heating and holding cycle, an increase in peak viscosity is achieved with the addition of both the GTE and GSE at concentrations of 1.0 mg/ml (GTE 1 and GSE 1) and 5 mg/ml (GTE 5 and GSE 5) (Table 3.1). A viscosity of 0.692 Pa.s was achieved in the control sample which was significantly lower ($p< 0.01$) than pastes with added plant extracts. Though no significant differences among or between the GTE and GSE, GSE 5 displayed a greater increase in viscosity (0.884 Pa.s). The lowest peak viscosity was found in the
GTE 5 (0.820 Pa.s). In considering the onset of peak viscosity of the current study, both the time and temperature exhibited a dose-dependent decrease, i.e. in order of concentration and level of polyphenol content (GSE 5 < GTE 5 < GSE 1 < GTE 1 < Control). Each of the extracts displayed a significantly different time and temperature of the peak viscosity, with the control being the highest. GSE 5 exhibited the greatest reduction of temperature and time, from 95.0°C and 523.4 sec in the control to 91.9°C and 490.8 sec, respectively, a decrease of 3°C and 32.6 sec. This indicates faster swelling of the starch granule and leaching of amylose. The holding viscosity of the samples indicated no significant difference among and between the extracts, again as with the peak viscosity, a significant difference from the control (p<0.005). The control holding viscosity reached 0.344 Pa.s and the highest extract holding viscosity was 0.466 Pa.s found in the samples prepared with GSE 5.

The increases in peak viscosity and decreases in peak temperature and time upon the addition of plant extracts are consistent with other studies that report the effects of GTE on these properties of starch pastes. While Wu et al looked at changes in the gelatinization of rice starch, consistencies are present in the ability of the polyphenols to impact the peak viscosity onset temperature. Concentrations of 6%, 10%, 14%, and 20% tea extract resulted in a consistent decrease in the peak temperature of rice starch gelatinization, with a total decrease of 6.5°C as concentration of the extract increased. Likewise, Zhu et al, demonstrated a modest increase in the peak viscosity upon the addition of green tea extract (13.60 g/100 g total polyphenol concentration) from 230 to 238 RVU. Contradictory to the results seen in the previous studies, Li et al showed an inverse relationship in the peak viscosity with the addition of green tea powder when incorporated into wheat flour, a dose-dependent decrease was seen in the samples. These differing effects may be due differences in green tea and/or starch type and structure as well as concentrations used. It is interesting to note that the concentrations used in the present study were far lower than those of previously discusses investigations. This suggest that these effects may be possible even at lower levels of phenolic present in consumer foods.
Figure 3.2  Effects of Plant Extracts on Wheat Starch Pasting Curve Trend

The number that follow extract name represent concentration of extract, 1- 1.0 mg/ml and 5- 5.0 mg/ml.
Flavonoids are also known for their abilities to interact non-covalently carbohydrates which may be a potential mechanism for their ability to modulate onset of peak viscosity at a decreased time/temperature\textsuperscript{184}. Few studies actually investigate the potential mechanisms of the early onset of peak viscosity in the presences of flavonoids though it has been hypothesized that compounds with increased hydroxyl groups cause close packing of the starch granules\textsuperscript{185} and inhibitory effects of hydration\textsuperscript{186}, similar to that of sucrose\textsuperscript{181}.

During the cooling period a final viscosity is reached and the level of setback can be determined. These attributes give further understanding of the retrogradation properties (reorganization of starch granules) of the starch paste. A lower degree of setback is indicative of a reduction in the retrogradation capacity of the starch.
Table 3.1  Characteristics of Wheat Starch Pastes with Plant Extracts

<table>
<thead>
<tr>
<th></th>
<th>Peak Viscosity (Pa.s)</th>
<th>Peak Viscosity Temperature (°C)</th>
<th>Peak Viscosity Time (s)</th>
<th>Holding Viscosity (Pa.s)</th>
<th>Final Viscosity (Pa.s)</th>
<th>Breakdown (%)</th>
<th>Setback Region (%)</th>
<th>Total Setback (Pa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.692 ± 0.07 A</td>
<td>95.0 ± 0.23 A</td>
<td>523 ± 3.58 A</td>
<td>0.344 ± 0.04 B</td>
<td>0.952 ± 0.06 C</td>
<td>54.2% ± 0.06 A</td>
<td>26.0% ± 0.01 A</td>
<td>0.008 ± 0.07 AS</td>
</tr>
<tr>
<td>GTE 1</td>
<td>0.836 ± 0.02 A</td>
<td>94.6 ± 0.08 B</td>
<td>517 ± 0.93 B</td>
<td>0.446 ± 0.01 A</td>
<td>1.12 ± 0.02 C</td>
<td>59.0% ± 0.01 A</td>
<td>28.7% ± 0.01 A</td>
<td>0.677 ± 0.01 A</td>
</tr>
<tr>
<td>GTE 5</td>
<td>0.829 ± 0.02 A</td>
<td>93.0 ± 0.02 C</td>
<td>500 ± 0.446 A</td>
<td>0.426 ± 0.01 A</td>
<td>0.989 ± 0.017 BC</td>
<td>39.3% ± 0.01 A</td>
<td>17.9% ± 0.02 C</td>
<td>0.563 ± 0.01 BC</td>
</tr>
<tr>
<td>GSE 1</td>
<td>0.863 ± 0.01 A</td>
<td>94.0 ± 0.03 B</td>
<td>511 ± 0.282 C</td>
<td>0.448 ± 0.01 A</td>
<td>1.09 ± 0.01 AB</td>
<td>41.9% ± 0.01 A</td>
<td>22.4% ± 0.01 B</td>
<td>0.659 ± 0.00 AS</td>
</tr>
<tr>
<td>GSE 5</td>
<td>0.881 ± 0.04 A</td>
<td>91.9 ± 0.08 B</td>
<td>491 ± 0.272 B</td>
<td>0.467 ± 0.02 A</td>
<td>1.01 ± 0.04 BC</td>
<td>41.8% ± 0.02 A</td>
<td>12.3% ± 0.06 D</td>
<td>0.540 ± 0.02 C</td>
</tr>
</tbody>
</table>

Data in the table is expressed as the mean ± s.d.  GTE is green tea extract and GSE is grape seed extract.  The number that follow represent concentration of extract, 1- 1.0 mg/ml and 5- 5.0 mg/ml.  Columns with different letters indicate a significant difference as compared to the control (p< 0.05 or better).
Both the setback region (difference in final viscosity and peak viscosity) and total setback exhibited trends similar to that of the changes seen in the onset of the peak viscosity, that being a dose-dependent response (Table 3.1). There was no significant difference (p=0.1001) seen between GTE 1 and the control, however all other treatments showed a significant decrease in the setback region, with the greatest decrease seen at GSE 5 at 12.27% (p<0.0001). This dose-dependent decrease in the retrogradation is in accordance with previous studies that indicate the ability to tea flavonoids have similar effects in rice starch. Wu et al. determined that not only was retrogradation decreased, but it was delayed over time as the tea polyphenol increased to 20% of the rice starch formula. However, when comparing the total setback these significant differences were negligible compared to the control. Significant differences were seen among GTE concentrations (p=0.0122), GSE concentrations (p=0.0297) and between extracts GTE 1 and GSE 5 (p=0.0035).

Retrogradation is considered to have a negative effect on the quality of starch based processed foods so additives are often studied for their inhibition abilities. Flavonoids, as previously mentioned have been shown to have these inhibitory effects but little consideration has been given to their action. There is potential for the flavonoids to interact with the starch molecules preventing or blocking further reordering of the structure upon cooling. According to Wu et al, in a study using H NMR and FTIR, they determined via H NMR that the incorporation of tea polyphenols prior to gelatinization as opposed to after gelatinization resulted in an increase in the coupling constants from 1 to 2, indicating a difference in the H-H interactions. They go on to describe that in the sample gelatinized in the presence of the tea polyphenols, the smaller coupling constant may be due to a transfer of electrons from the –OH bond as a result of the hydrogen bonding. This interaction is an indication of a potentially stronger affiliation of the polyphenols due to heating versus addition afterwards. Though intriguing, additional studies are warranted across an array of starches to indicate potential mechanisms of interactions.
Figure 3.3  Frequency Dependence of G’ (filled) and G” (open) of Wheat Starch Pastes made with GTE (A) and GSE (B) at 1 mg/ml extracts (■) and 5 mg/ml (▲) Compared to a Control Wheat Starch Paste (×).
3.3.3 Effects Plant Extracts on Mechanical Spectra of Wheat Starch

After the starch pastes were prepared, dynamic oscillatory measurements were done for each of the samples (Figure 3.3). This category of analysis allows for the determination of sensitive changes in the flow properties in response to chemical changes in the product. The frequency sweep is most commonly used in determining the linear viscoelastic range of a material, as well as indicating changes in the storage and loss modulus of the material under the application of stress\textsuperscript{187}. In analysis of the wheat starch pastes formed, the storage modulus (G’) was found in all samples to be higher than that of the loss modulus (G’’) over the entire frequency range, but begin to approach each other at the higher frequencies. Likewise, the data shows that there is a frequency dependent behavior (slope > 0.05) of G’ and G’’ with both modulus increasing as the frequency increased in all of the pastes, indicating a viscoelastic behavior. In samples prepared with GTE and GSE, the storage and loss modulus was consistently higher at 1 mg/ml concentrations compared to the control, while those prepared with 5 mg/ml of extract were slightly lower. In general, the wheat starch formulated with the plant extracts followed similar trends in response to frequency changes, therefore addition of extracts at the tested levels did not have a detrimental impact on the flow properties of the wheat starch pastes.

3.3.4 Effects of Plant Extracts on κ-Carrageenan Mechanical Spectra

The dynamic frequency sweep was conducted within the linear viscoelastic region of the 1% κ-CGN gels (Figure 3.4). It was determined that G’ was significantly higher than G’’ and constant in all samples across the frequency ranged utilized. The consistent, higher G’, and tan <0.01 are all indicators of gel formation. In comparing the G’ of each of the individual samples it appears that control is significantly higher than all of the samples treated GTE and GSE. In the samples treated with GTE the frequency sweep showed similar consistent trends across the spectrum, however at an overall lower G’, this indicates an effect on the solid-like properties of the gel formed in the presence of GTE.
Figure 3.4  Frequency Dependence of $G'$ (filled) and $G''$ (open) of k-carrageenan gels made with GTE (A) and GSE (B) at 1 mg/ml extracts (■) and 5 mg/ml (▲) Compared to a Control k-carrageenan gel(●).
The effects tend towards a concentration dependent interaction, as the storage modulus decreased when concentration of GTE increased. Similarly, the GSE exhibited a decrease in the gel strength when formulated with the extract however the effect as concentration increased was not significant and exhibited an inverse reaction compared to the GTE. The results shown here are in agreement with previous studies of 1% κ-CGN gels, in which the G’ and G” are consistent throughout the sweep, with G’ being larger than G” across the frequency spectrum. The addition of the plant extracts resulted in a decrease in the G’, indicating potential interaction that may have decreased the κ-CGN network. Though currently no studies are available on the effects of plant extracts on κ-CGN gel, the effects of other molecules have been determined. For example, Nishinari and Watase determined that the incorporation of polyols decreased the elastic modulus (G’) of the κ-CGN gels when added at excessive concentrations. The shift in G’ was attributed to relative distance between junction zones and the polyols. At a lower concentration these junction zones became shorter so at the higher concentration the junction zones may be increased, decreasing the G’189. Furthermore, concentrations or amount of hydroxylation is seen as a factor in the effects on the polysaccharide’s rheological properties. Structurally, polyols may be made comparison to polyphenols in that both have a relatively higher degree of hydroxylation that occurs in the side chain position on the molecules. The presence of the hydroxyl groups, in the previously described study, are an attributing factor in the cross-linking points in the κ-CGN gels, as may be the case with the plant extracts utilized in this study. The degree of hydroxylation as well as the concentration of the polyphenols are an ever important factor in determining the effect of physical characteristics of these gels.

In factoring the ability of κ-CGN to interact with the flavan-3-ols in a more significant manor than the wheat starch, there must be consideration of the structural characteristics of these compounds. As an anionic polysaccharide, polyphenols have a greater affinity for these molecules. Anionic polysaccharides have been shown to decrease the formation of insoluble complexes with proteins as they are able to more effectively cross-link the flavan-3-ol within its matrix; this effect was not seen in carbohydrates with a neutral charge, presumably due to low solubility and hydrogen
bonds that exist to stabilize the molecules\cite{190}. Therefore, the wheat starch interactions may only be a surface as a result of hydrogen bonding of the hydroxyl groups, whereas the κ-CGN effect may be due to encapsulation within the formation of its double helix in the gelation process\cite{190}.

3.4 Conclusion

GTE and GSE when incorporated into wheat starch pastes at lower levels feasible in food systems, resulted in minimal changes to the rheological properties of the matrix. Therefore, their incorporation into processed foods, from a physical standpoint, does not warrant concern in matrices leveraging these phenolics at reasonable concentrations. However when included in a κ-carrageenan gel system the gelling abilities of the texturants may be altered to a certain extent. Previous studies have viewed the effects of these extracts on gelatinization, but at levels much higher than those that may be incorporated without having a significant impact on quality parameters of the product. In this study it was shown that at levels of 1 mg/ml and 5 mg/ml, there may be a provided benefit of lowering the time of gelatinization onset however the effects on final viscosity and flow properties are negligible. The effects of these extracts on κ-carrageenan based gels however decreased the gel strength, additional studies are necessary to access the total impact of plant extracts on the characteristics of the polysaccharide gels.
CHAPTER 4. FLAVAN-3-OL BIOACCESSIBILITY FROM MODEL FOOD MATRICES

4.1 Introduction

Flavonoids are phytochemicals with potential human health impact. While often associated with anti-cancer effects, flavonoid benefits appear to extend beyond chemopreventative activities to broader inflammatory mediated diseases and include effects that are linked to obesity and diabetes including inhibition of starch digestion and glucose absorption\textsuperscript{191, 192}. A recent study on the enzyme inhibitory properties of 30 commonly consumed fruits indicated that lingonberry offered inhibition of pancreatic lipase IC\textsubscript{50} 0.72 mg/ml, while α-glucosidase was inhibited by blue honeysuckle IC\textsubscript{50} 39.91 mg/ml, and red gooseberry inhibited α-amylase IC\textsubscript{50} 1.04 activities\textsuperscript{193}. The growing evidence base related to flavonoid biological activities has already translated into inclusion of these compounds in both dietary supplements and novel functional food products. This is particularly true for flavan-3-ols with established supplements based on apple, grape seed and green tea. While promising for consumer, it remains unclear the extent to which benefits from these new products are actually delivered.

One of the most important factors concerning delivery of functional benefits from such plant based phenolics is consideration of their bioavailability from the diet. Disintegration of the food matrix and release of phenolics through normal digestion is an integral step in this process often referred to as bioaccessibility. The terms bioaccessibility and bioavailability are related terms that refer to the ultimate availability of a specific nutrient through the course of digestion and intestinal absorption. Specifically, bioaccessibility refers to the proportion of a nutrient or phytochemical that is released from a food matrix and available for subsequent uptake via the small intestine. Bioavailability is a broader term that defines the fraction of a nutrient or phytochemical
that is ultimately absorbed and made available to target tissue within the body. Overall bioaccessibility is a pre-requisite to bioavailability and may be impacted by several factors that begin prior to the digestive process, including both food matrix and processing. Consideration of factors that may influence bioaccessibility is therefore critical in defining food matrix effects on phytochemical absorption. This is particularly important for flavonoid-protein interactions which may alter both protein digestion and by extension release/bioaccessibility of flavonoids.

As mentioned before, while whole foods including fruits, vegetables, cocoa and tea products are rich source of flavan-3-ols in the diet, new processed foods are increasing being seen as vehicles for the delivery of these compounds. However, the translation of flavonoids into processed foods is not without challenges including the effect of the flavonoids on the food matrix itself and by extension, delivery of efficacy of flavan-3-ols themselves. As described in the previous chapters, the ability of macronutrients to interact with flavonoids, and flavan-3-ols in particular, through non-covalent interactions can impact several quality attributes of food products. These often non-specific hydrophobic interactions and hydrogen bonds help govern the stability of flavonoids, the protein and the overall food matrix (as described in Chapter 2 and 3).

In more recent years, research has begun to determine the effects of these compounds on the physical properties in processed food matrices. However, consideration for the matrix effect on bioaccessibility of the compound due to interaction and processing is also critical to determine how a complex system may alter delivery and potential efficacy of the ultimate food product. The occurrence of these interactions not only affect physical and chemical properties of the macronutrient but may also modulate the bioaccessibility of both the macronutrient and the flavonoid. An example of this can be seen in the case of green tea. On one hand, a cup of green tea consumed by itself, may deliver as much as 200 mg of flavan-3-ols however in terms of what is bioaccessible <20% of that is recovered following simulated digestion. On the other hand, when co-formulated with milk proteins (dairy, soy, and rice) the bioaccessibility was observed to increase to 52-69% of the oral dose. Such modifications in the delivery of flavan-3-
ols offer insight into how food matrix may be leveraged to positively impact bioaccessibility. While Green et al, focused on beverages, additional insight is needed in consideration of levels that are achievable in a more complex food matrix with minimal effects on product quality, i.e texture, flavor, and color attributes and still provide a nutritional benefit to the end consumer.

A number of clinical studies have also examined the role of food matrix on bioaccessibility of these compounds in relation to their health effects as well. It has been seen in vivo that the incorporation of dairy and soy protein sources may negatively impact bioavailability of galloylated flavan-3-ols from green tea. In a randomized four-armed, diet-controlled cross-over design with 1-week intervals between treatments, participants consumed like amounts of the various proteins in conjunction with 1.75 g of green tea extract in 300 g of solution. Results suggest that in the presence of any proteins, relative bioavailability is reduced for the galloylated flavan-3-ols. Specifically, bioavailability of EGCG was reduced by 68%, 63%, 76% and for ECG was reduced by 68%, 66%, and 77% when formulated with milk, caseinate, and soy proteins, respectively. This further suggest that protein effects may be specific to galloylated flavan-3-ols and that food matrix, concentrations, and interaction effects must be determined in order to leverage beneficial effects from flavan-3-ols.

While in the previous chapters, our focus was to investigate the impact of flavan-3-ol rich plant extracts on functionality and physical properties of protein and carbohydrate food matrices, the implications on physiological delivery of phenolics remain unknown from these matrices. In the present study we investigated the bioaccessibility of flavan-3-ols from green tea extract (GTE) and grape seed extract (GSE) in model protein and carbohydrate food systems using an in vitro digestion model that simulates oral, gastric and small intestinal phases of digestion. These experiments begin to develop insights into how food matrix interactions may alter in the initial stages of flavan-3-ol absorption in the GI tract.
4.2 Methods & Materials

4.2.1 Solutions, Solvents, Standards, and Starting Materials

Green tea extract was a gift from Nestlé Product Technology Center (Marysville, OH), Meganatural Gold grape seed extract (GSE) was purchased from Polyphenolics (Madera, CA), and 92% pure EGCG (Teavigo®-TVG) was donated graciously by DSM Nutritional. For the protein matrices, whey protein isolate (BiPro®, 95% protein) used in gels, was generously provided by Davisco Foods International (Eden Prairie, MN), sodium caseinate (FB100, 90% protein), used in emulsions and foams, was provided by Farbest Brands Inc. (Montvale, NJ). For carbohydrate matrices, wheat starch (WS) Midsol™ 50 was gifted by MGP Ingredients, Inc® (Atchinson, KS) and κ-carrageenan (kCGN) was provided by FMC BioPolymer (Philadelphia, PA). Enzymes and salts used including porcine α-amylase, pepsin, pancreatin, lipase, and bile were acquired from Sigma-Aldrich (St. Louis, MO). Acetic acid, acetonitrile, methanol, water (Mallinckrodt-Baker, Phillipsburg, NJ) and formic acid used in HPLC analysis were certified HPLC-MS and ACS grade and purchased from Sigma-Aldrich.

4.2.2 In vitro Digestion

A three phase (oral, gastric, and intestinal) in vitro digestion model was used to assess the bioaccessibility of flavan-3-ols from the different matrices. This model was previously described by Moser et al⁶ and modified through preliminary experiments to best digest model food products used in these studies. Starting materials were prepared according to the methods described previously in Chapters 2 and 3. Both protein and carbohydrate matrices were utilized for this study (Table 4.1).
Table 4.1  Protein and Carbohydrate Systems for in vitro Digestion

<table>
<thead>
<tr>
<th>Macronutrient</th>
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<tr>
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<td>Solution</td>
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<tr>
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All starting materials were formulated with individual extracts of green tea (GTE), grape seed (GSE), or Teavigo (92% EGCG) (TVG) at 1 mg/ml of the total volume of the solution. Starting materials that were prepared into gels were sheared into smaller portions with a cheese grater to reduce particle size (1/8") prior to digestion. Foams were prepared using the KitchenAid mixer then quick frozen at -20°C and cut into pieces before being added to the digestion vessel.

The oral phase base was prepared by combining potassium chloride, sodium phosphate, sodium sulfate, sodium chloride, and sodium bicarbonate into 1 L dd water, and stored at 4°C. The oral phase solution was prepared daily by adding urea (40 mg), uric acid (3 mg), mucin (5 mg), and α-amylase (1.56 g) into 100 ml of oral base. A small aliquot (20 g) of each starting material (SM) was then added to a 50 ml conical tube, followed by 6 ml of the oral phase solution. Tubes were then vortexed, blanketed with nitrogen, sealed with parafilm, and incubated in a shaking water bath at 37°C for 10 min (120 opm). Samples were placed on ice upon removal from the water bath and diluted with 30 ml of a 0.9% NaCl solution. The gastric phase was initiated by adjusting the pH of the samples to 3.0± 0.1 with a 1.0 M HCl solution prior to the addition of 2 ml porcine pepsin solution (30 mg/ml in 0.1 M HCl). Samples volumes were brought up to 40 ml with saline solution, blanketed with nitrogen, sealed with parafilm, and then incubated in
a shaking water bath at 37°C for 60 min (120 opm). Again, samples were removed from the water bath and placed on ice to initiate the small intestinal phase. The pH was first adjusted to 5.0 ± 0.1 with 1.0 M NaHCO₃ solution followed by the addition of small intestinal enzymes and digestive juice. A 2 ml aliquot of porcine pancreatin (30 mg/ml) and lipase (15 mg/ml) in 0.1 M NaHCO₃ and a 3 ml aliquot of bile (120 mg/ml) in 0.1 M NaHCO₃ was added to each digestion tube. A final pH adjustment to 6.5 ± 0.1 with 1.0 M NaHCO₃ solution was completed followed by a volume adjustment to 50 ml with saline solution. The samples were then blanketed with nitrogen, sealed with parafilm, and incubated in a shaking water bath at 37°C for 120 min (120 opm). Digested materials were then centrifuged at 10,000 x g for 60 min at 4°C to recover the aqueous “bioaccessible” fraction, filtered with 0.22 µm filters to remove microparticulated materials and aliquots were frozen until analysis for flavan-3-ol content (previously described in Chapter 2).

4.2.3 Data Analysis

In vitro digestions were repeated in triplicate, with starting materials prepared fresh daily. Bioaccessibility refers to the percent of each individual flavan-3-ol remaining in the aqueous fraction compared to that of the original plant extract material added to the starting material. Losses through preparation were not considered in bioaccessibility calculations at this time due to interferences in sample extraction and analysis. A one-way ANOVA was completed using JMP®12 (SAS Institute, Cary, NC) with significant differences and comparisons with a control evaluated using Dunnett’s method for differences. Bioaccessibility data is represented as means ± standard deviations.

4.3 Results and Discussion

4.3.1 Bioaccessibility of Flavan-3-ols from Protein Based Systems

The impact of model protein rich food matrices on bioaccessibility of flavan-3-ols from plant extracts was assessed. As previously discussed in Chapter 1, both protein type and concentration may have an effect on the interaction capacity of the flavan-3-ol with the protein, as such, the results are primarily discussed in relation to the control. Each system may be compared in terms of overall trends within the matrix, i.e. emulsion,
gel, or foam. However due to the varying protein concentration necessary to provide function (Table 4.1), significant differences were not assessed statistically between food matrices.

Protein foods were formulated with SCN, WPI and gelatin allowing for a broad representation of different protein structures i.e. relatively open, globular, and random structures, respectively. In samples prepared with SCN (aqueous 5% SCN, 5% Foam, and 0.5% Emulsions), bioaccessibility followed a similar trend among all of the flavan-3-ols, suggesting that SCN concentration used to achieve proper function in foams and emulsions may not be a limiting factor on flavan-3-ol bioaccessibility. Specifically, in the aqueous GTE bioaccessibility ranged from 61.3% to 63.84% for EGCG, 68.2% to 98.3% for ECG, 61.5% to 89.9% for EGC, and 27.5% to 30.4% for EC within all SCN matrices (aq. solution, foam, and emulsions).

In the presence of SCN in its aqueous state, as a foam or an emulsion, there were significant increases in bioaccessibility of EGC (61.5% to 89.9% p= .00139 or better) and ECG (68.2% to 98.3% p=.0009 or better) (Figure 4.1A). These results conflict with those presented by Moser et al, which reported a significant decrease in bioaccessibility with the addition of SCN. However, in the present study different protein/polyphenol ratios (5:1 and 50:1) and processing techniques such as homogenization and whipping were employed which may have altered the digestive stability of the compounds. Previously, Song et al. did report that protein binding can stabilize flavan-3-ol at higher protein to polyphenol ratios.

Interestingly, EGC (3.4% to 12.9% p=0.0040 or better) and EGCG (12.3 to 16.8% p<0.0001) from GTE in WPI matrices were significantly less bioaccessible than the control. In the presence of gelatin in a gel form, the bioaccessibility of EC (6.6%) and EGCG (21.8%) was significantly (p<0.0001) lower than the control (49.5%) and (80.1%) respectively, where ECG was significantly (p<0.0001) improved from 21.2% to 80.7%. The significant decrease in bioaccessibility in gelatin matrices may be attributed to interaction effects of the flavan-3-ols in the presence of gelatin that limited the release through normal digestion. Lui et al determined that EGCG’s presence in gelatin gels formed hydrogen bonds with the molecule, however the interactions between adjacent
gelatin molecules was strengthened via N-H bonds forcing compaction of the gelatin molecules\cite{Liu:2014tm}. This aligns with Jöbstl’s theory that cross-linking of proline-rich proteins may be forced by interactions with the polyphenols\cite{Zhu:1997cg}. The occurrence of cross-linking may potentiate the formation of insoluble complexes among these molecules and therefore may result in further loss of flavan-3-ols, which may be a potential mechanism in the case of bioaccessibility among the gelatin gels.

Comparing samples of aqueous 5% SCN and its subsequent foam, the flavan-3-ols follow along similar trends of bioaccessibility, with the mechanically prepared foam having a slightly higher bioaccessibility of EC (27.81% compared to 30.4%), EGC (69.0% compared to 89.6%), ECG (68.2% compared to 71.8%), and EGCG (61.3% compared to 63.8%) respectively. Likewise, 18% WPI gel showed a similar non-significant trend to increase bioaccessibility compared to the unprocessed liquid sample. Bioaccessibility from WPI L as it compares to WPI G was as follows, EC (49.8% compared to 51.8%), EGC (3.4% compared to 12.9%), ECG (31.2% compared to 40%), and EGCG (12.3% compared to 16.8%) respectively. This suggests that compared to the aqueous solutions, protein denaturation may play a subtle role in enhancing bioaccessibility of GTE flavan-3-ols within the protein based food matrix.

Stability through processing of test samples remains to be defined completely. EGC and EGCG are unstable at pH conditions >5.5 and high temperatures\cite{Zhu:1997cg}. It is therefore possible that a potential decrease in absolute bioaccessibility post processing was observed through loss of flavan-3-ols. The degree of difference between the samples formulated with SCN, compared to those of WPI and gelatin suggest that protein type being a more significant factor in bioaccessibility as well. Gels formed with both WPI and gelatin exhibited a significant decrease in bioaccessibility. However, a similar decrease was observed in the unprocessed WPI sample as well, further indicating that WPI may form more or stronger insoluble complexes through the digestive process that serve to limit digestive release of flavan-3-ols.
In order to better understand how these effects play out in a more refined sample, studies were conducted with TVG, a derivative of green tea extract that is highly purified. Like the EGCG present in the more crude GTE, a similar impact to the bioaccessibility of EGCG was observed in regards to SCN (Figure 4.1B). The SCN aqueous, foam, and emulsions (22.7% to 27.5%) had a higher bioaccessibility than those in the gel matrix of WPI (8.3% to 15%) or gelatin (7.0%). However, in the Control samples (15.0%), EGCG bioaccessibility was significantly lower than SCN L 27.46% (p<0.0001), Foam 27.5% (p<0.0001), and Emulsions 22.7% (p<0.0001). The mechanical processing into foam appeared to produce only minimal effects to the relative bioaccessibility of EGCG compared to the aqueous solution. Likewise, homogenization of the emulsion also had only minimal effect on bioaccessibility of EGCG from TVG. Considering these observations, other considerations may be given to the concentration of protein and its ability to provide stability with higher concentrations of EGCG.

Gel matrices made with either WPI 8.3% (p=0.001) or gelatin 7.0% (p<0.0001) had a significantly lower bioaccessibility compared to the Control 15.0%. Formation of gels occurred as a result of heating therefore the reduction in bioaccessibility seen between the WPI L 15.0% and its gel 8.3% may have been due to a combination of factors including the instability of EGCG through processing or perhaps the increased interactions between the flavonoids and proteins through this heating step. As seen in the GTE samples, TVG prepared in SCN matrices had higher bioaccessibility (22.7% to 27.5%) than WPI (8.3% to 15.0%) matrices. This overall reduction is seen in both unprocessed and processed WPI samples, giving indication that WPI may be an inhibitor of bioaccessibility or likewise there is a greater ability EGCG to interact with digestive proteins inhibiting their further breakdown and subsequent digestion. EGCG is known for its ability to bind with proteins, having a higher binding affinity than other flavan-3-ols, so it is likely that some insoluble complexes may have been formed in the process. This decrease in bioaccessibility is consistent with similar studies utilizing non-fat dry milk (NFDM) at varying concentrations. Though no significant differences in bioaccessibility of the galloylated GTE flavan-3-ols were seen across the concentrations
of NDFM (10%, 20%, and 40%) however bioaccessibility was reduced (16.1%) to levels similar to the present study (GTE- WPI L 12.3% and TVG-WPI L 14.96%) 6.

To determine the impact to larger oligomeric and polymeric flavan-3-ols known to more effectively bind protein, studies were conducted flavan-3-ol rich grape seed extract. Within the samples made with GSE, the flavan-3-ols that were quantified for relative bioaccessibility included CAT, EC, ECG, and Procyanidin B (Total Dimers). For protein samples (Figure 4.1C), ECG is not shown (see appendix), co-elution of protein residues in WPI samples with resulted in inaccurate reporting of bioaccessibility of the compound. Relative bioaccessibility of the total dimers indicated that a 0.5% SCN stabilized emulsion significantly (p<0.0001) increased the bioaccessibility from 55.1% in the control to 76.1%. However, in further processed WPI and gelatin matrices, total dimer bioaccessibility was significantly decreased to 35.2% (p< 0.0001) in WPI L, 40.3% (p= 0.0001) in WPI G, and 26.9% (p<0.0001) in gelatin. A significant (p < 0.0001) descending trend of bioaccessibility was seen in EC in the order of WPI L (21%) > WPI G (11.4%) > Gelatin (2.7%) compared to the 49.4% in the Control. EC was lower, though not significantly (p= 0.3928) compared to the control in GSE formulated in SCN Emulsions (41.5%). Similar trends were also found for CAT. Emulsions had a higher CAT bioaccessibility that other matrices, however, this not significantly different from the control. Both WPI and Gelatin matrices had a negative impact on CAT bioaccessibility with significantly (p< 0.0001) lower levels found compared to the control. Bioaccessibility from Gelatin matrices was determined to be 28.0%, while gelation of WPI decreased bioaccessibility slightly from 18.6% in WPI L to 17.33% in WPI G.

Overall among all of the flavan-3-ol and protein matrices, the trends indicate that SCN in minimally processed matrices may have beneficial and/or minimal detrimental impact on bioaccessibility. The greatest impact being an increase in bioaccessibility of the dimers and galloylated compounds, EGCG and ECG that are typically reported to have the lowest bioavailability in vivo 5,107,199. Conversely, WPI (liquid and gels) and Gelatin may have a detrimental impact on the bioaccessibility of the larger and galloylated flavan-3-ols from these extracts across product formats tested (liquid and gel).
The results seen here are consistent with many of the other studies on bioaccessibility of tea and grape seed flavan-3-ols. Though the trends varied, these matrices generally significantly lowered the bioaccessibility compared to the control. However, these results are inconsistent as to whether or not thermal processing of these matrices plays a role in the bioaccessibility.

WPI and gelatin represent different orders of structure, globular and random coiled, respectively. Therefore, the similarities in their effects are notable. WPI exhibits a similar structure to that of digestive enzymes in which flavan-3-ols are known to provide an inhibitory effect through binding interactions. Binding interactions with the WPI may prevent degradation of the flavonoid and protein through the gastric phase potentially leaving the complex intact after gastric and intestinal digestion. Stojadinovic et al describes the interaction of β-lactoglobulin with green tea flavonoids at pH 7.2 as having a greater binding capacity than that of lower pH, which was also highly correlated with a decrease in the antioxidant activity of the flavonoids potential due to protein masking. Though the concentrations of flavan-3-ols were not quantified post-digestion in that study, the reduction in antioxidant capacity and subsequent reduction in β-lactoglobulin digestion may coincide with degradation or formation of insoluble complexes.
**Figure 4.1** Relative Bioaccessibility of Flavan-3-ols from Plant Extracts in Protein-Based Matrices. GTE-Crude Green Tea Extract (A), TVG- Green Tea Extract (92% EGCG) (B), and GSE-Crude Grape Seed Extract (C). Bars represent mean ± s.d. (n=3). EC= epicatechin, ECG= epigallocatechin, ECG=epicatechin gallate, EGCG= epigallocatechin gallate.
4.3.2 Bioaccessibility of Flavan-3-ols from Carbohydrate Based Systems

In a manner similar to the investigation of protein matrices, carbohydrate interactions were also assessed. This is particularly important considering the notable positive impacts of digestible carbohydrate sources (starch and sugar) on flavan-3-ol bioavailability in vivo\textsuperscript{117}. To better understand this effect, the current investigation leveraged a digestible (wheat starch) and indigestible (k-carrageenan) in gel formulations. Both WS pastes and kCGN gels were made in the presence of each of the flavan-3-ol extracts (Figure 4.2). Across both WS and kCGN, ECG in GTE was statistically the same as the control. However, EC bioaccessibility was significantly (p<0.0001) increased in WS pastes compared to the control (49.4%) by 227% to 162%. Serra et al. reported similar increases when procyanidin rich grape seed extract was digested in the presence of a carbohydrate rich food, significantly increasing the levels of catechin and epicatechin after the gastric step of the digestion process\textsuperscript{5}.

In contrast, kCGN gels were found to significantly (p<0.0001) reduce bioaccessibility of EGC relative to the control, 93.3% to 39.3% respectively. EGCG in both GTE and TVG treated WS pastes had a higher bioaccessibility compared to kCGN gels suggesting a difference in binding or in release through digestion of WS. Similar to proteins, the larger molecules of kCGN may have been able to encapsulate and bind EGCG limiting their accessibility following digestion. The likeliness of this occurrence is due in part to the ability of kCGN to escape the digestive process within the oral, gastric, and small intestinal phases. As such digestion may not effectively break established interactions formed through preparation of the kCGN samples.

TVG in WS pastes had a significant (p=0.0004) increase in bioaccessibility of EGCG to 22.02% compared to the control 15.04%. Though not significant, a similar (p=0.08) effect was observed with kCGN gels that decreased bioaccessibility of EGCG to 11.38%. This decrease is in agreement with the previously noted changes seen in the EGCG from GTE as well as protein systems. A likely result of instability of the galloylated compounds to the environment\textsuperscript{117,163}.

In GSE samples kCGN was able to decrease bioaccessibility across all of the flavan-3-ols, however only significantly (p=0.0002 and p<0.0001) in CAT 56.01% and
Total Dimers 31.78% compared to the control 79.52% and 55.08% respectively. Modulation of flavan-3-ol bioaccessibility formulated with WS showed no significant effects on the non-galloylated compounds however a significant increase (p<0.0001) in bioaccessibility was seen in ECG 46.41% and decrease (p= 0.0269) in total dimers 31.78% compared to the control. Wheat starch is complex system being both a stable and unstable mix in dilute solutions and of a hydrophobic nature. The nature of wheat starch lends itself to interactions with the galloylated compounds for potential stabilization. The galloyl moiety may allow for hydrophobic interaction that allow for stabilization within the wheat starch paste, increasing its bioaccessibility.

Overall the presence of kCGN had a slightly negative impact on the relative bioaccessibility of the larger flavan-3-ols and galloylated compounds. With the exception of EGCG from TVG and EC from GTE, WS displayed similar effects on bioaccessibility. The digestion of carbohydrates begins in the oral phase upon the interaction with α-amylase, so it is plausible that a matrix comprised of wheat starch would have minimal effects on the flavan-3-ols from any of the plant extracts. Therefore, digestion of the carbohydrates may have potentially left the flavan-3-ols susceptible to the conditions of the gastrointestinal tract including elevated pH and initiation of auto-oxidative processes detrimental to flavan-3-ol stability. So any enhancement of bioaccessibility must be balanced with the susceptibility to digestion.

Besides not being digestible in the upper GI, kCGN’s structure allows for its flexibility which may favor encapsulating effects on flavan-3-ols. Once entrapped, the indigestibility would potentially serve to physically limit the flavan-3-ol intestinal bioaccessibility. These compounds may pass bound to the fiber into the large intestine where they may potentially be released through the action of specific microbial communities. As the lower intestine was not modeled in this study the potential to quantify the true effects of this carbohydrate remains to be further explored.
Figure 4.2 Theoretical Bioaccessibility of Flavan-3-ols from Plant Extracts in Carbohydrate-Based Matrices. GTE-Crude Green Tea Extract (A), TVG- Green Tea Extract (92% EGCG) (B), and GSE-Crude Grape Seed Extract (C). Bars represent mean ± s.d. (n=3). EC= epicatechin, EGC= epigallocatechin, ECG=epicatechin gallate, EGCG= epigallocatechin gallate.
4.4 Conclusions

From these studies it may be concluded that at the levels tested within protein and carbohydrate food systems, only modest impacts were observed on the bioaccessibility of flavan-3-ols from select plant extracts. The extent of the effects are based on estimated starting levels of flavan-3-ols and thus includes but does not separate potential losses through processing of the matrix. Therefore, additional experiments are required to further identify the extent to which interactions in foods alter the stability of flavan-3-ols and their bioaccessibility in vivo. In any case, the greatest impact was observed for galloylated flavan-3-ols EGCG and ECG which are known to have the strongest interactions with proteins and carbohydrate as well as lowest bioavailability in vivo. However, as these compounds are often indicated as having the most beneficial health benefits, strategies are required to better understand factors that may limit their absorption from foods.
CHAPTER 5. CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Introduction

The research discussed in this dissertation presented the effects of select flavan-3-ol rich plant extracts on the functionality of specific protein and carbohydrate ingredients utilized in development of functional foods. As a secondary objective the impact of these interactions on flavan-3-ol bioaccessibility from model food systems was examined in order to determine if there were any specific systems that may be more appropriate to leverage these functional ingredients in. While previous investigation had documented the impact of flavonoids and plant extracts more broadly may impact various functional properties macronutrients, many of these studies relied on flavonoid levels far beyond that which may be practically be added into finished products due to cost and impacts to sensorial properties (color and flavor specifically). The focus of this research was to deliver on matrix interaction at practical levels (at or below 5 mg/ml) which would in essence represent levels more typical in single servings of fruits, vegetables as well as common tea products. These concentrations are also at levels that may be added to processed food without hindrance of the quality of the products. Levels beyond or at 5 mg/ml have been shown to have various effects on the sensory and textural\textsuperscript{189,92,183}, however the extent of benefit at modest concentrations has been minimally studied.

In this study, models of protein and carbohydrate systems were used as they encompass the physical properties of most processed foods consumed. For that purpose, protein gels, emulsions, foams and carbohydrate pastes and gels were utilized to determine the effects of these extracts and their flavan-3-ols on bioaccessibility, physical, and chemical properties. Dairy proteins such as whey protein and sodium caseinate are widely used to increase protein content of foods for a wider audience, while gelatin exhibits the profile of proline-rich proteins, which are known to have interactions with
the flavan-3-ols. Likewise, wheat starch k-carrageenan are found in many products, specifically k-carrageenan is used in many of the same products that incorporate the above mentioned proteins. For these reasons, model systems that incorporate these ingredients deserve greater attention as to the effects of addition of such functional ingredients.

In Chapter 2 the focus was on the role flavan-3-ol rich plant extracts on modulating protein functionality as an emulsifier, foaming agent, and gel formation. In addition, some assessment of potential mechanisms behind these impacts was explored. It was determined that addition of these flavan-3-ol extracts had direct impacts to functionality but these effects were largely specific to individual systems and protein source. Both positive and negative effects were found in the protein systems and all dose dependent according to the concentration of the extract and size of the flavan-3-ols present. In agreement with previous reports, it was confirmed that flavan-3-ol interaction with hydrophobic residues of the protein and the resulting instability/conformational changes in protein structure are likely responsible for many of the effects observed in these studies.

In Chapter 3 the ability of flavan-3-ol extracts to modify the physical properties of carbohydrate pastes and gels was explored. These interactions, though thought to be governed by similar forces as proteins, were interestingly not as pronounced. Flavan-3-ol ingredients, in the dose ranges assessed (1 mg/ml and 5 mg/ml) were not as effective in modulating final pastes physical properties of wheat starch for example. However, the effects of addition of these plant extracts even at the low level may affect the gelatinization temperature and retrogradation properties, with structure playing a big role in the effects. For example, the order of effect in most cases followed along with increased procyanidin content and concentration. These effects were found to be significantly affected indicating that while final properties may not be impacted, the processing of starches may be impacted by presence of flavan-3-ols. This hold great importance to the potential processing conditions of functional foods, as the products quality may potentially be effected if over processed due to these changes. In terms of the effects on gelation of κ-carrageenan significant changes were exhibited in gel strength
however these changes did not alter the flow properties of the gel. This allows the product to function under similar means as prior to addition of the plant extracts. Likewise, processing flow rates and viscosities are likely to be unaffected upon the addition of these extracts.

Finally, in Chapter 4, these matrices were subjected to a 3-stage *in vitro* digestion to determine how the matrices impacted bioaccessibility of the flavan-3-ols from the plant extracts. At the concentrations utilized in this study (1mg/ml), bioaccessibility was significant impacted by the presence of proteins and carbohydrates in model systems. Specifically, these effects were more pronounced for flavan-3-ol dimers (from GSE) and galloylated compounds (in tea) in most matrices. Whey protein isolate and gelatin exhibited the most significant impact on the bioaccessibility of these flavan-3-ols, indicating that a higher degree of affinity for these compounds with proline rich proteins and globular proteins as well may be the cause. These effects have already been seen with the affinity of flavonoid compounds to interact with enzymes (also globular) and salivary proteins (proline rich)\textsuperscript{11,110,205}. Therefore, if health benefits are of particular interest when formulation is considered with these materials, the ability to deliver these compounds may be affected and thus direct assessments of these effects in vivo are warranted.

While others have reported on similar effects of protein and carbohydrates on flavan-3-ol bioavailability, the benefit of the current investigation is the ability to demonstrate significant differences in effects between specific model food matrices. These data provide a general baseline on the effects of flavan-3-ol extracts on model food matrices including gels, foams and emulsions that ultimately encompass most food products. Though these studies answered many questions of the effects, greater understanding of the interactions and processing of such products is warranted.
5.2 Future Direction for Flav-3-ol and Macronutrient Functionality

The results of the physical and chemical effects of the plant extracts on protein and carbohydrate functionality found in Chapter 2 and 3 provided unconvincing data as to the true effects of these extracts on model systems. Though similar results were achieved in other studies \(^{13,89}\), of protein matrices, the contradictory results in systems of similar nature i.e sodium caseinate stabilized emulsions and sodium caseinate foams, warrant further analysis. Both emulsions and foams are governed by the principles of hydrophobic interactions, in that the proteins must be oriented to interact with the non-aqueous phase. In an emulsion this refers to the oil interaction and in the foam, that is the protein’s interaction with the gas, so it is plausible that similar effects of stabilization or destabilization would be seen in these systems. However, this study rendered differing results. Consequently, additional understanding is necessary to address the mechanism of each of these effects. Competitive binding studies may be a potential resource deployed to determine if competition at the interface is a deciding factor in the stability of the system. Likewise, additional analysis of conformational changes in the protein structure could confirm the orientation of the protein and may offer insight into these changes. Similarly, these techniques could be applied to allow greater understanding of the effect of the extracts in the carbohydrate systems. Wheat starch pasting temperatures and processing times were decreased likely due to self-aggregation of the starch molecules but confirmation of this is necessary. Additional analysis of the surface wettability of the starch with the aqueous extract solution could also determine if this was a potential mechanism for the early onset gelatinization times and temperatures. Though the effects of addition were only modest in the modulation of physical properties at the level incorporated, they provide great understanding of the consequence of their interaction with these macromolecules. Also, because k-carrageenan is often used in dairy systems, it would be interesting to understand if known principles of protein/carbohydrate/polyphenol ternary complexes has a role in interactions of the 3 molecules in a processed sample.
5.3 Future Directions for Flavan-3-ol and Macronutrient on Flavan-3-ol Bioaccessibility

In these studies, the bioaccessibility was determined using relative values as it relates to the plant extract itself. Unfortunately, this does not provide a holistic understanding of the true bioaccessibility of the flavan-3-ols from each of the plant extracts after processing. Determination of the relative bioaccessibility, as it relates to processing, would give a greater knowledge of the true effects of each matrix. However, whey protein isolate, gelatin, and κ-carrageenan matrices posed difficulty in extracting the flavan-3-ols from the matrix. Further development of an extraction process is warranted to allow for greater extraction from these solid-like matrices. In determining the difficulty of the extraction of these compounds from such matrices, supplementary studies on the changes of the proteins tertiary structure may offer insight on this phenomenon.

There were three types of protein utilized in the bioaccessibility study however the levels necessary to provide the various functions limited the ability to cross-wise compare their effects on bioaccessibility. Studies on different protein levels and extract concentrations could then suggest with better discretion the effects each protein may have on bioaccessibility. It would also be interesting to determine if there may be a competing interaction effect of individual flavan-3-ols on bioaccessibility. Results from the green tea extract and Teavigo® produced conflicting bioaccessibility trends for EGCG, therefore it is reasonable that this may be true. Competition in binding of the individual flavan-3-ols may alter the bioaccessibility of the compounds in a mixed system such as green tea extract.

Digestions were optimized based on the control protein samples (exclusive of extracts). Upon completion of the digestion process it was observed that there was greater undigested material in these samples compared to control with no extracts. This is an indication that there may have been insoluble complexes formed limiting their digestibility or interaction with the digestive enzymes that limited further digestion. Therefore, analysis of the undigested pellet would offer further insight into effects the flavan-3-ols have on macronutrient digestion, likewise measuring the enzyme inhibition.
would determine that as a factor as well. Greater understanding of the effects of processing of these matrices warrant further studies. Analysis of each of the proteins under similar treatments could help distinguish if the processing was a factor in driving the bioaccessibility in a particular direction.
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APPENDIX
APPENDIX

Extraction procedure for Starting Materials

This procedure should be used for the preparation of protein matrices in the analysis of phenolics.

1) Cut or grate protein gels into small pieces using a small grate cheese grater to facilitate digestion. For SCN foams, freeze in -80°C freezer for 10 min and cut into pieces with a knife.
2) Add 100 µl of 2.7mM L-ascorbic acid and 2.2mM Na₂EDTA in water into each 15-ml centrifuge tube.
3) Add 2 ml or 2 g of sample into each tube.
4) Add 5 ml of Pepsin solution (45mg pepsin/ml of 0.1 M HCl). For 8 samples, 35 ml of 0.1 M HCl and 1575mg pepsin.
5) Vortex samples for 30s, flush with N₂, parafilm, and place in a water bath at 37°C for 45 min with 120 rpm.
6) Remove samples from water bath.
7) Add a 1:1 ratio of 2% Acetic Acid solution and vortex.
8) Add 1.5 ml of sample to a microcentrifuge tube and centrifuge at 14K x g for 5 min.
9) Syringe filter samples with .45 µm filters into HPLC vials for further analysis.
### Three Stage In Vitro Digestion Procedure

#### Oral Phase Base Solution: (As Needed) Makes 1L

- Potassium Chloride- 1.792g
- Sodium Phosphate- 1.776
- Sodium Sulfate- 1.140g
- Sodium Chloride- 0.596g
- Sodium Bicarbonate- 3.388g

#### Other Solutions (As needed)

- 0.9% NaCl Solution: 9g NaCl in 1L ddH2O
- 0.1M HCl- 2.5ml of 36%HCl up to 300ml ddH2O
- 1.0M HCl- 20.83ml of 36% HCl up to 250ml H2O
- 0.1M NaHCO3- 5.04g NaHCO3 in 600ml ddH2O
- 1.0M NaHCO3- 21g NaHCO3 in 250ml

#### Daily Prep for Oral Phase

In 100ml of Base Solution (16 reps):

- Urea- 40mg
- Uric Acid- 3mg
- Mucin- 5mg
- A-amylase- 1.06g

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<th>Step Description</th>
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<tbody>
<tr>
<td>Weight 20ml or g of sample into 50-ml Centrifuge tubes</td>
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<td>Collect Additional 10g for Raw Materials-15ml tube</td>
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<td><strong>ORAL PHASE</strong></td>
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<tr>
<td>Add 6ml of Complete oral phase to each tube</td>
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<tr>
<td>Vortex 1min</td>
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<tr>
<td>Blanket with nitrogen, cap, seal w/ parafilm</td>
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<tr>
<td>Place in 37°C water bath OPM: 120 for <strong>10 min</strong></td>
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<tr>
<td>Calibrate pH meter, Get Ice, Make 360mg pepsin in 18ml 0.1 M HCl <strong>Record Time</strong></td>
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#### GASTRIC PHASE

- Remove from Bath, Place On Ice
- Bring to 30ml with Saline (~14 ml)
- Adjust pH to 3.0 +/- 0.1 w 1.0M HCl (**Record vol**)
- Add 2 ml of Pepsin
- Bring to 40ml w Saline (acct. for pH adjustment) ~7-8ml
- Blanket with nitrogen, cap, seal w/ parafilm
- Place in 37°C water bath OPM: 120 for **1hr Record time**

#### ISOLATION OF MICELLULAR FRACTION

- Prep Intestinal Phase enzymes: 540mg Pancreatin + 270mg Lipase in 18ml of 0.1 M NaHCO3; Make first 3000mg Bile in 25 ml of 0.1 M NaHCO3 (Stir for 30min before use)

#### INTESTINAL PHASE

- Remove from Bath, Place On Ice
- Adjust pH to 5.0 +/- 6.1 w 1.0M NaHCO3 (**Record vol Ice**)
- Add 2ml of Pancreatin/Lipase and 2.5ml Bile Solution
- Check pH and adjust pH to 6.5 +/- 0.1 as necessary
- Bring to 50 ml w Saline solution (acct for pH adj.) ~2.5ml
- Blanket with nitrogen, cap, seal w/ parafilm
- Place in 37°C water bath OPM: 120 for **2hr Record time**

#### TRANSFER TO INTESTINAL PHASE

- Collect 10ml of DM add 5 ml of 2% aq Acetic Acid in 15ml tube; Nitrogen, Cap, Parafilm
- Transfer 30ml of digesta to polycarb tubes, Centrifuge @ 10,000 x g for 1 hr
- Filter 10ml of aqueous fraction w 0.22mm filters into 15ml tubes; add 5 ml of 2% acetic acid, flush w N2, Cap, Parafilm
VITA
EDUCATION

Doctor of Philosophy
Food Science- Foods for Health
Purdue University, December 2016

Master of Science
Food Science- Nutritional Biochemistry & Functional Foods
Alabama A&M University, August 2012

Bachelor of Science
Food Science & Human Nutrition- Food Industry & Business
University of Illinois at Urbana-Champaign, December 2004

RESEARCH & TEACHING EXPERIENCE

Graduate Research Assistant Purdue University 2012—2016
- Analyze the relationship between macronutrient-flavan-3-ol interaction and effects of processing on bioaccessibility of the flavan-3-ols
- Determine specificity of flavan-3-ol-protein binding interactions and mechanism of effect on functionality changes
- Determine effects of macronutrient-flavan-3-ol interactions of functionality of macronutrient
- Mentor and train undergraduate and graduate students in laboratory techniques and skills

Teaching Assistant Purdue University Fall 2015
- Coordinate & facilitate labs for undergraduate students in food processing II
- Provide laboratory instruction for individual labs section
- Grade lab reports and exams
Graduate Research Assistant | Alabama A&M University 2011—2012
- Determined synergistic effects of soybean flour, flaxseed meal, and a probiotic on azoxymethane induced aberrant crypt foci in male rats
- Analyzed the effects of soybean flour, flaxseed meal, and a probiotic on hepatic antioxidant and detoxification enzyme activity
- Developed a functional food product using soybean flour, flaxseed meal, and a probiotic

USDA Summer Scholar Mentor | Alabama A&M University Summer 2012
- Mentor to high school students from under-represented minority groups and low-income areas
- Coordinate lab experiments for high school students on product development process and food safety
- Assisted students in developing a functional food product, marketing, and persuasive presentation skills

PROFESSIONAL EXPERIENCE

Intern | ConAgra Foods Inc. 2013
- Developed 5 frozen breakfast items under nationally recognized brand
- Successfully amended 2 breakfast items to fit within FDA definition of “Healthy”
- Assisted in pilot/production trials as needed

Food Technologist | Northern Star Potato Co. -Michael Foods Inc. 2010—2008
- Lead Developer on several food service and retail refrigerated potato products
- Designed and tested new process for several new microwaveable cut potato products
- Provided research and testing protocols that led to the acquisition of new processing equipment
- Provided recommendations on packaging material/processing equipment based on research
- Developed risk assessments associated with new refrigerated, processed potato products related to microbiological spoilage
- Assisted in commissioning of a new potato processing facility

Food Technologist | Michael Foods Inc. 2008—2005
- Lead Developer on a private label competitor match product that was successfully launched in 2008
- Formulated egg substitute product to match leading retail brand in category
- Established manufacturing procedures for plant production of products
- Coordinated and supervised plant trial productions for shelf life studies
- Designed and executed research for the development of a new preservative system
- Performed extensive shelf life testing to verify increased shelf life
- Assisted customer in home repair and maintenance projects in various departments including seasonal, hardware, paint & home décor, and customer service centers.
- Cash office attendant- secured previous days’ business transactions for safe count and deposit
- Assisted in inventory counts at year to ensure accurate account of products on hand
- Solved customer complaints and facilitated meetings with local businesses to ensure positive relationships with customers

**Intern** | OSI Industries Inc.  2003
- Provided data to increase production yields according to specifications
- Conducted plant trials testing validity of hypothesis of projects
- Assisted in training QC technicians on safety and quality issues

**PRESENTATIONS & PUBLICATIONS**


INTERNATIONAL PROJECT WORK

**Farmer-to-Farmer East Africa** | Catholic Relief Services | 2016
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Project: Development of new nutritious products for Stawi Foods & Fruits Company-Nairobi, Kenya

- Refresher training for factory employees on HAACP and GMP practices for producing safe, quality foods
- Develop and facilitate training materials on the basics of food product development
- Developed a prototype of a ready-to-eat breakfast biscuit to increase nutrition as opposed to current available biscuits
- Conducted sensory evaluation of the 2 versions of a breakfast biscuit for consumer tasting during the Baby Banda Baby Expo located at the Sarit Center, Nairobi, Kenya
- Provided recommendations for process facility equipment and efficiency in processing

**International Engagement and Development Strategies** | Purdue University | 2016
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Developed an intervention strategy for Northwestern Tanzanian villages to adopt orange fleshed sweet potatoes as a source of income & improve nutrition with the communities.

**World Food Problems** | Purdue University | 2015

**Discovering Caribbean Agriculture Systems** | University of Illinois | 2004

AWARDS & HONORS

1st Place Graduate Research Poster Competition-MANRRS 31st Annual National Conference, 2016
Farm Credit/ MANRRS VIP Scholar, 2016
Purdue University- Alliance for Graduate Education and the Professorate (AGEP) Scholar Award, 2016
2nd Place Graduate Research Poster Competition- MANRRS 30th Annual National Conference, 2015
Graduate Poster Finalist, Food Chemistry Division, IFT Annual Meeting & Expo, 2014
Purdue University Doctoral Fellowship, 2012
Purdue University Industry Fellows Fellowship, 2012
IFT Southeastern Section Graduate Leadership Award, 2012
Alabama A&M University USDA Graduate Food Science Scholarship, 2011-2012
PROFESSIONAL SOCIETIES & ACTIVITIES

Purdue University-College of Agriculture Graduate Student Advisory Board
Graduate Student Member 2015-2016
- Liaison between under-represented minority graduate students and Asst. Dean of Graduate Students
- Reviewer of nominations for various faculty and student awards
- Provide input on matters affecting graduate student courses and activities

Minorities in Agriculture, Natural Resources, and Related Sciences (MANRRS)
Graduate Student Representative 2015-2016
- Lead recruitment seminars for parents and incoming undergraduates/graduate students
- Serve as a peer graduate student recruiter for the College of Agriculture
- Provide peer mentoring to undergraduate students

Institute of Food Technologist (IFT)
Midwest Area Student Representative- Fun Run Committee 2014-2015
- Encouraged and increased student participation in the IFT15 Fun Run Event
- Collaborated with other IFT members to enhance the experience of the IFT15 Fun Run Event
- Solicited sponsorship for the IFT15 Fun Run from IFT Sections and Industry affiliates

Vice-President of Student Chapter (AAMU) 2011-2012
- Facilitated monthly meetings and plan agenda
- Coordinated social and professional networking activities
- Volunteered for the Southeastern Section Supplier’s night

Phi Tau Sigma Honor Society of Food Science
Vice-President of AAMU Student Chapter 2011-2012
- Led a workshop entitled “Networking Skills & Etiquette: Surviving the IFT Supplier’s Night”
- Coordinated the induction of new honorees
- Coordinated organizational activities and fundraising events

SKILLS & TRAINING

Purdue University Aseptic Processing & Packaging
General Mills Graduate Leadership Seminars
Dale Carnegie Leadership Training
How to Develop Products for Microwave Use