Impact of Thermal Processing on Taste Development in Food

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Dedication

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Abstract

Despite the increasing demand, healthier foods suffer from lower consumer acceptability due to inferior flavor quality. The flavor of food is greatly affected by the food composition and thermal processing. This study specifically investigates the thermal processing impact on the positive and negative taste attributes of foodstuffs, which enables the optimization of processing strategies to improve the palatability and ultimately the consumption of ‘healthier’ formulated food products.

The overall goal of this work was to characterize the effect of thermal processing on the taste-active compounds or the generation of taste modulating compounds in foodstuffs. This work mainly focuses on the influence of three common thermal processing techniques on the resulting taste profiles in three food systems respectively: the roasting of cocoa, the extrusion of corn cereal, and the frying of potato chips. These three processing techniques are widely utilized by the food industry. Bitterness was investigated in roasted cocoa and extruded whole grain corn while umami was characterized in deep fried potato chips.

The influence of roasting on the taste attributes of cocoa was studied first. Roasting processes involve the Maillard reaction, which is a ubiquitous thermally catalyzed chemical pathway that is well-known to impact aroma development as well as taste, such as bitterness. Bitterness is a challenge for consumer acceptability, which is typically masked by adding sugar. The goal of this part of the research was to discover the impact of Maillard chemistry on endogenous bitter-tasting compound, catechin, in raw cocoa, and likewise on the resulting bitter taste profile of the roasted beans. Catechin-Maillard
reaction products were identified by stable isotope labeling techniques in model reactions using the simulated cocoa roasting conditions. Eight reaction products were identified and reported for the first time. One of the newly-identified compounds significantly suppressed the perceived bitter intensity of the caffeine solution, which is a novel bitter blocker. Further analysis revealed that this bitter blocking compound was present in both the raw and roasted cocoa beans; however its concentration was higher in roasted cocoa beans. A generation mechanism of the bitter blocker was proposed. The results of the first phase indicated that the bitter profile of cocoa beans was altered by thermal reactions of the endogenous bitter compounds.

In the second research phase of this study, the influence of extrusion on the taste profile of puffed whole grain corn products was examined. The goal of this phase is to identify the key bitter compounds and understand how they are influenced by extrusion. Three phenolic compounds (chaenorpine, coumaryl-spermidine, terrestribisamide) and one amino acid (L-tryptophan) were identified as the main bitter compounds in the extruded whole grain corn product. Based on sensory recombination analysis, chaenorpine was found to have the highest contribution to the bitterness intensity, based on the concentration of the bitter compounds reported in the saliva during mastication. Additionally, all of the identified bitter compounds were found to be degraded during extrusion, suggesting that the further optimization of extrusion could be utilized to suppress bitterness in order to improve the flavor quality of whole grain extruded products.
In the last phase of this research project, the role of the thermal process, deep frying, on the taste profile of potato chips was examined. Potato chips are a highly desired food product and the umami taste is well known to positively contribute to the taste profile. Initial analysis indicated that the umami taste attribute of potato chips increases with frying time thus the compounds that contribute to umami were characterized. A dehydration product of monosodium glutamate (MSG), monosodium L-pyroglutamate (L-MSpG) and monosodium D-pyroglutamate (D-MSpG) were identified for the first time as umami enhancing compounds that contributed to the umami flavor of potato chips. The generation of pyroglutamates was reported to be directly related to the frying time. Sensory time-intensity taste analysis of potato chips with topical added L-MSpG and D-MSpG revealed significantly higher umami intensity and the overall higher potato chip flavor intensity.

In summary, the impact of three thermal processes on taste profile was studied in three different food products. This study provides a novel basis for flavor optimization by investigating the thermal impact on taste chemistry. The ultimate goal of this study is to increase the market demand for health conscious foods, thus benefiting the food industry as well as promoting a healthy lifestyle.
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Chapter 1 Literature Review

Flavor

Humans have five fundamental senses (taste, smell, sight, sound, and touch) that are responsible for generating internal responses from the outside environment.\(^1\) Flavor is defined as a perceptual complex integration of all the contributing human senses at the time of food consumption.\(^2\) The perception of flavor is influenced by appearance, texture, sounds associated with mastication, and sensations derived from the two chemical senses: aroma and taste.\(^3\)

Aroma is perceived by the olfactory receptors in the nasal epithelium.\(^4\) The aroma receptors can detect more than 1000 different odorants with broad sensitivity range. The aroma compounds are volatiles or semi-volatiles that can be perceived in the nose during eating (retronasal) or smelling (orthonasal). The aroma chemistry is exceedingly complex as the aroma of a certain food may consist of hundreds of compounds.

Alternatively, taste is perceived by taste receptors on the tongue. There are about 50-100 taste receptor cells contained in a taste bud that are located on the surface of the tongue and the palate epithelium.\(^5\) The gustatory system utilizes taste receptor cells to generate chemical signals, which are then transmitted as neural information by a peripheral neural system and the central nervous system, where the sensory information is received and analyzed.\(^5\)

Although dietary trends are influenced by many factors, consumers report that flavor is the main impact on their food selection.\(^6\) As is shown in Figure 1.1, the integration of basic sensory information and other higher-order variables impact the perception of food within a complex background. When we consume food, the physiochemical characteristics of a food
product (determined by ingredients, preparation, and storage) interact with the sensory attributes, such as taste, aroma, texture, appearance. The sensory attributes are then integrated with economical (e.g., cost) and psychosocial influences (e.g., convenience) and all the information are processed in the brain, leading to a hedonic response of purchase or not. As illustrated, flavor, as well as cost and convenience, impacts food selection when consumers encounter any food product. Modifying the food ingredient (e.g., refined or whole grain) and controlling the processing parameters (e.g., processing time, temperature) will change the quality of food by altering the sensory attributes and influence the consumer choices. Flavor researches have been focused on aroma since early 1960s but the understanding of taste chemistry, especially for bitterness and umami, is lacking. Hence this thesis study mainly focuses on the chemical characterization of the key taste compounds in foods by understanding the processing impact on the taste development.

Figure 1.1 Consumer behaviors in regard of food consumption

2
Taste

The basic tastes that are generally recognized to exist are sweet, bitter, sour, salty, and umami. The perception of sweetness, bitterness, and umami involves the G-protein-couple receptors (GPCRs, Figure 1.2). The other two basic tastes, sourness and saltiness, involve the participation of ion channels. Receptor studies have shown that the five basic taste modalities are present in all regions of the tongue. The discoveries of taste compounds for bitterness and umami in foodstuffs are far less understood compared to the other basic tastes. This thesis is focused on chemical characterization of bitterness and umami in a food and thus this chapter only focuses on introducing the bitter and umami perception, and the thermal processing impact on the bitter or umami development.

Taste compounds are water-soluble or partially water-soluble molecules that can solubilize in saliva to get access to the taste receptors on the tongue in the oral cavity. The proteins on the apical surface of taste receptors provide the structure specificity to bind the taste-active molecules and receptor cells that results in a recognition signal transmitted to the brain. Even within one taste category, the taste stimuli might differ in hydrophilicity, compound structure, and molecular size. Alternatively, a pair of chemical isomers can elicit different tastes. For example, at equal concentrations, L-tryptophan is bitter while D-tryptophan is sweet.

As part of taste function, saliva plays an important role in transporting sapid compounds to the taste receptor. Secretion of saliva is also the first physiological behavior that is induced by food ingestion. Proteins are the major component in saliva and about 70% of the proteins consist mainly of proline, glycine, and glutamic acid. Some proteins in saliva have
been reported to bind tastants, especially some partially hydrophobic compounds, such as some bitter or astringent compounds, and facilitate the diffusion of tastants to the taste buds. The carrier function of saliva proteins also helps cleanse the bitter and astringent tastants on the taste buds reducing the long-lasting bitter aftertaste.\textsuperscript{11} Another function of saliva is to provide the background environment for taste stimuli during perception. The concentration of taste compound has to exceed the threshold concentration for detection in the background saliva to be perceived.\textsuperscript{10}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.2.png}
\caption{Receptors for umami, sweet, bitter, and sour\textsuperscript{1}}
\end{figure}

\textit{Bitter}

The bitter perception is complex and there is limited correlation between compound structure and bitterness. There are up to 30 different GPCRs identified that respond to known bitter compounds, which are classified in the T2R family (Figure 1.2). The structure of T2R is heterodimeric thus it can accommodate the broad chemical multiplicity of bitter compounds in foods (examples of dietary bitter compounds are shown in Figure 1.3).
Figure 1.3 Bitter compounds found in foods

Most of the phenolic compounds found in plants are bitter, including naringin found in grapefruit, quercetin found in wine, and catechin found in green tea infusion. Some triterpenes, such as limonoid aglycones (limonin, nomilin), contribute to the bitterness of lemon and orange. Quinine and caffeine are from the family of alkaloids, found in coffee. Summarized below are some molecular and stereo-chemical characteristics for bitter compound structure:

1. Functional group (nitro group, sulfur group, etc.): Compounds with three nitro groups are usually bitter, such as picric acid. As early as in 1895, Henry found compounds that share the structure, as in Figure 1.4, are bitter. Later in 1963, Pearson reported that bitterness is usually associated with ‘soft’ bases. At a receptor site, the interaction of a bitter compound and a receptor was viewed as a weak physical absorption between the bitter compound (soft base) and a counterpart protein (soft
acid) from the receptor. The softer the base, the more bitterness it elicits. In 1984, Saroli pointed out that the bitter compounds have the essential structure as a polar (electrophilic) group and a hydrophobic group. Very recently, a bitter compounds database was established by Wiener et al., which consisted of over 500 bitter compounds. However, none of the studies reported any structural requirements for bitter compounds.

![Chemical structure](image)

**Figure 1.4 The structure of some identified bitter compounds in 1895**

2. Sugar composition: the bitter taste depends on how the sugar moiety is attached. Neohesperidosides, a citrus flavanone glycoside, is bitter but nariginin glucoside is without taste. Sucrose tastes sweet but octa-acetyl sucrose tastes bitter with decreased hydrophilicity. β-D-mannose is the only hexose that has been found to taste bitter since the three oxygen atoms lie on the same plane, with O-O distance of 2.5 Å. The bitterness is associated with the hydroxyl methylene on the ring.

3. Stereochemistry: L-amino acids generally taste bitter whereas D-amino acids taste sweet. The stereochemistry difference of the compounds might also affect the sensory threshold of bitter compounds.

The detection of bitterness in foodstuffs is considered to be an evolutionary trait to signal for potentially poisonous materials. The range and types of foods in our diet is much broader
than our ancestors’. Bitter taste is found in 1) ‘traditional foods’ that have been consumed over centuries; 2) healthy foods that provide tailored nutrients and limited calories from sugars and saturated fats and sodium;\textsuperscript{17} 3) ‘functional foods’ that contain safe dietary ingredients that benefit specific targets in our body beyond providing nutrition.\textsuperscript{18} Currently significant pressures are placed on food producers to reduce the bitterness in food products to improve the product acceptability, while maintaining the nutritional value of the products. As many of the compounds that provide health benefits in functional foods are bitter, a purportedly ‘healthy’ food may fail in the marketplace due to excessive bitterness.

\textit{Umami}

As early as 1880, Ikeda first identified the taste umami in food.\textsuperscript{19} The umami taste was not listed as one of the primary tastes until the umami taste receptor was discovered in 2000.\textsuperscript{20} Umami means ‘delicious taste’ in Japanese and is known to have ‘savory’ or ‘meaty’ attributes. The taste transduction of umami taste entails stimulation of T1R1+3 receptor (see \textbf{Figure 1.2}), which binds to umami stimuli and elicits umami taste. Umami enhancement effect arises from the presence of purine-5’-ribonucleotides, including inosine-5’-monophosphate (IMP), guanosine-5’-monophosphate (GMP) which are shown in \textbf{Figure 1.5}. Monosodium glutamate (MSG) is one of the most commonly known umami taste compounds which is widely found in savory foods, \textit{e.g.} tomato products, parmesan cheese, meat, and soy sauce etc. The umami molecules have been used as ingredients and taste enhancers in culinary foods, such as meat broths and soups.
Figure 1.5 Common umami compounds found in foods

More unique characteristics of umami compounds have been reported such as the mutual taste synergy. An exponential increase in the perceived umami intensity is found within a MSG solution when 5'-IMP is added. Also, the addition of IMP significantly lowers the detection threshold of MSG by more than 50-fold.

Umami compounds have been reported to enhance the continuity, fullness, mildness, and thickness of the overall flavor character. For example, Morris et al. found that some umami compounds were able to enhance the overall potato flavor in potatoes. Additionally, umami has been shown to be effective in sodium reduction. The addition of umami compounds has been utilized to develop foods with 50% salt reduction while the acceptability was not lower. When soup contains the appropriate amount of glutamate, the salt content can be reduced from 0.75% to 0.4% without any decreasing of palatability. With the growing concern of hypertension caused by sodium, umami taste compounds have been explored as ingredients for salt mitigation strategies.

Other Basic Tastes
Sweetness is perceived by the G-protein-coupled receptors (GPCRs, shown in Figure 1.2). Different from bitterness and umami, sweetness is mediated by another family of GPCRs, the T1R2 and T1R3 receptor cells, abbreviated as T1R2+3. Sweet taste has been studied extensively.²⁴⁻²⁶ Shallenberger and Acree published the ‘AH-B’ theory in 1969 (Figure 1.6).²⁷ All the sweet tasting compounds were found having bifunctional group with an acidic group (AH) and a basic (B) group that are 3 Å apart. A sweet compound consists of two simultaneous hydrogen bonds with the receptor site. For example, β-D-fructose, saccharin, and D-amino acids are sweet and following the AH-B theory by shown in Figure 1.7.

![Figure 1.6 Scheme view of AH-B theory proposed by Acree and Shallenberger](image)
Figure 1.7 The AH-B units in sweet-tasting compounds

For the remaining two taste attributes, salty and sour, the taste transduction mechanism is through a specific ion channel in the apical membrane of taste receptor cells. For salt taste, the ion channel has selectivity for specific cations to pass through but some other cations that don’t fit the channel are not salty.\textsuperscript{28} Considerable amount of research has been using sodium
chloride (NaCl) for pure salty tasting however at very low concentration (below 0.04 M) the perception changes to sweet. On the other hand, potassium chloride is sweet at low concentration whereas at above 0.02 M it tastes bitter.\textsuperscript{29}

Sourness is elicited by acid-sensing ion channels, which are activated by protons. Previous studies suggested that the mechanism for sour perception is analogous to the titratable acidity that is a measure of both bound and free protons in solution.\textsuperscript{30} However, no relationship was found between pH, titratable acidity and sour taste intensity between several organic acids at both threshold and superthreshold concentrations.\textsuperscript{30} The transduction of protons and protonated acid species are responsible for sour taste.

**Taste Modulation**

A taste modulator is defined as a tasteless compound that modifies the taste of a food, albeit the definition has been broadened over time.\textsuperscript{31} More recent industrial interest in taste modulation includes masking bitterness and other negative taste attributes as well as enhancing positive taste attributes such as umami, which is utilized for the general understanding of food flavor as well as for the development of new flavors in the food and flavor industries.\textsuperscript{32,33}

The predominant identification work of novel taste modulators started in the early 2002. Hofmann \textit{et al.} first synthesized new glycoconjugates to structurally mimic the known umami enhancing compounds.\textsuperscript{34} The chemical structures were found to be dipotassium N-(D-glucos-1-yl)-L-glutamate (DPGG) and N-(1-deoxy-D-fructos-1-yl)-L-glutamate (Fru-Glu)
Figure 1.8). Fru-Glu is an Amadori product of DPGG and is a fairly stable amino acid glycoconjugate. Fru-Glu was found in dried tomatoes. In a follow-up study, the umami threshold of DPGG and Fru-Glu were reported as 1.6 mmol/kg and 1.8 mmol/kg, which are similar to that of monosodium glutamate (1.5 mmol/kg).\textsuperscript{35} However, DPGG is not stable under storage conditions, which limits its application in the food industry.

![dipotassium N-(D-glucos-1-yl)-L-glutamate](image1.png)  
**Figure 1.8 Umami enhancers identified by Hofmann et al. in 2002-2003**

Also in 2003, Hofmann and Ottinger identified alapyridaine, a sweetness and umami enhancer, from the heated aqueous solution of hexose and L-alanine. Alapyridaine was found native in beef broth in concentration as low as 419 µg/L, at which it enhances the sweet and umami character of beef broth and improves the overall brothy character.\textsuperscript{36,37} Alapyridaine is stable over 5 h of heating at 80 °C at a wide range of pH conditions and more than 80% can be recovered. Nevertheless, the achieved Maillard product is racemic, and the (+)-(S) enantiomer enhances sweetness, whereas the (-)-(R) does not affect sweetness or umami.

Another pyridinium betaine was identified as a taste modulator by Soldo and Hofmann in 2005.\textsuperscript{38} Instead of enhancing sweetness, the newly identified pyridinium betaine (1-carboxymethyl-5-hydroxy-2-hydroxymethylpyridinium salt) reduced the bitterness of L-phenylalanine, Gly-Leu, caffeine, salicin and naringin. To investigate the structure effect on
the sensory activities of the side chain of amino acid moiety, Hofmann et al synthesized a series of pyridinium inner salts and compared their bitter suppression abilities (Figure 1.9). The side structure-relationship activity of pyridinium betaines 2 to 4 was investigated. As shown in Figure 1.10, the bitter suppressing activity is inversely correlated with the length of the side chain. Additionally, the chirality of the amino acid side chain imparts only the sweet taste; the side chain affects the bitter modulating.

Figure 1.9 Identified taste modulating pyridinium betaines in 2003-2005

![Figure 1.9](image)

Figure 1.10 The structure of pyridinium betaine on bitterness suppression of caffeine solutions in the absence of additives (◊) and in the presence of alapyridaine (●), pyridinium betaine 2 (▲), pyridinium betaine 3 (×), and pyridinium betaine 4 (□).
In 2010-2014, a series of umami-enhancing compounds were identified as guanosine-5’-monophosphate (GMP)-Maillard reaction products.\textsuperscript{39-42} All of the identified (S)-configured isomers had higher umami-enhancing capacity than the (R)-configured isomers, suggesting the stereo-specificity of the umami taste receptor binding site (Figure 1.11). In the most recent study, the candidate umami enhancing compounds were tested with specific umami-tasting T1R-receptors. The expression activity of umami receptors matched well with that of the human sensory data.\textsuperscript{41} 

Figure 1.11 Identified 5’-ribonucleotides with umami enhancing property.
Thermal Processing & Food Flavor

Thermal processing is primarily used to improve food safety, preserve food, extend the shelf-life, and develop a desirable flavor profile. Conventional thermal processing involves in-container sterilization of foodstuff, which was developed in 1920 by Bigelow and Ball for calculating the minimum safe sterilization process.\textsuperscript{43} The conventional sterilization involves the application of a high-temperature thermal treatment for a sufficiently long time to destroy microorganisms of the public health and spoilage concerns. Nowadays, improvements in safety and shelf-life stability of processed foods have been achieved. Recent developments in food processing have aimed at reducing loses to nutrients and sensory attributes by ways of reducing heating time and optimizing heating temperatures. Three common thermal processing techniques (roasting, extrusion, and frying) in the current food industry are discussed on the taste properties of the processed food products in more details below.

Roasting - Cocoa

Roasting is a common practice in the food industry. Convective roasting is the most commonly used processing technique with a temperature setting of 130-150 °C for 15-45 min.\textsuperscript{44} For roasting of cocoa, the roasting parameters depend on the origin of the beans, harvesting period, postharvest treatment and the desired flavor. Roasting brings about the characteristic brown color, chocolate aroma, and texture of roasted cocoa beans. Cocoa beans are bitter, astringent, and acidic before roasting and roasting reduces volatile acids, such as acetic acid but the non-volatile acids are not affected. The flavor profile and taste of cocoa beans are significantly affected by roasting conditions, mainly the temperature utilized and
duration of the process. Roasting of cocoa is essential for developing chocolate flavor from the precursors.

Aldehydes and pyrazines are the major aroma compounds generated during roasting through Maillard reaction and Strecker degradation of amino acids and sugars. Aroma markers, including pyrazine, aldehyde, ketone, alcohol, and ester, have been found as indicators of optimum production. Based on the aroma profile, the optimal condition is at 160 °C for 30 minutes of roasting. However, for the final product evaluation, a lower temperature (150 °C) for 30 minutes is typically utilized which gives the lowest bitterness and low level of sourness and astringency. Most of the previous studies on cocoa is on aroma whereas very few studies have focused on the influence of roasting on the taste of cocoa products during roasting.

The identification of bitter compounds generated during cocoa roasting was first reported in 1975. Theobromine and a series of diketopiperazines (DKP) were found to contribute to the bitterness of cocoa. In 2000, Bonvehi and Coll found that DKP is mainly generated in the process of roasting. Contrary to some of the dipeptides that elicit brothy and nutty flavor, DKPs are generally bitter and when combining with theobromine, inducing a metallic bitter taste. Rizzi investigated the generation mechanism of DKP and argued that it is through intramolecular cyclization of linear peptide precursor. Two DKPs, cis-cyclo (Val-Phe) and cis-cyclo (Ala-Phe), were found in low amount in cocoa, but their bitter taste are similar to the bitterness perceived from aqueous cocoa solution. The chemical structures of taste-active compounds identified in roasted cocoa are illustrated in Figure 1.12.
Figure 1.12 Chemical structures of identified taste active compounds in roasted cocoa (adapted from 49)

In addition to DKPs, a series of phenolic compounds were reported to contribute to the bitter and astringent sensation in cocoa beans, including flavan-3-ols epicatechin, catechin, procyanidins, epicatechin dimer, quercetin, naringenin, luteolin, glycopyranosides, and some amino acid amides. Stark and Hofmann isolated the phenolic compounds and investigated the taste character. 50 As shown in Table 1.1, catechin and epicatechin have the highest bitter
threshold, whereas the glycosides of flavan-3-ols are not bitter but astringent. The human bitter thresholds for the phenolic compounds are in the range of 200 to 1000 μmol/L. The general trend is that the bigger the phenolic compound the lower bitter threshold, which confirms the earlier findings that the sensory quality of flavan-3-ol oligomers is dependent on the degree of polymerization. In comparison to the flavan-3-ols, the glycosides have lower astringent threshold. This matches to the other findings that the structures of the attached glycosides have an impact on the taste activity of threshold concentration of flavan-3-ol derivatives.

Table 1.1 Taste thresholds of bitter and astringent compounds isolated from cocoa. The taste threshold concentrations were determined by a series of triangle test in water.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Taste threshold (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bitterness</td>
</tr>
<tr>
<td>Flavan-3-ols (bitter, puckering astringent)</td>
<td></td>
</tr>
<tr>
<td>(+)-Catechin</td>
<td>1000</td>
</tr>
<tr>
<td>(-)-Epicatechin</td>
<td>800</td>
</tr>
<tr>
<td>Epicatechin-(4β→8)-epicatechin</td>
<td>500</td>
</tr>
<tr>
<td>Epicatechin-(4β→6)-epicatechin</td>
<td>900</td>
</tr>
<tr>
<td>[Epicatechin-(4β→8)]_2-epicatechin</td>
<td>400</td>
</tr>
<tr>
<td>[Epicatechin-(4β→8)]_3-epicatechin</td>
<td>300</td>
</tr>
<tr>
<td>[Epicatechin in-(4β→8)]_4-epicatechin</td>
<td>200</td>
</tr>
<tr>
<td>[Epicatechin-(4β→8)]_5-epicatechin</td>
<td>&gt;600</td>
</tr>
</tbody>
</table>
Extrusion – Corn based products

Extrusion cooking is one of the major thermal processing techniques used in the snack and breakfast cereal industry. Generally, a flour mix is quickly drawn and pushed by air and mechanical forces through a die at high temperatures to create puffed products. Specifically, the extruder acts as a mixer, heat exchanger, and pump, by joining several operations into a single unit. Cereal grits are gelatinized and swelled to form dough or paste in the presence of heat and moisture. Carbohydrates, such as moistened corn grits will melt when they are heated and become less viscous. Conversely, proteins become thicker and more viscous when they are heated.52 Hence, the mixture of carbohydrates and proteins becomes a complex system in which flavor develops. Cereal grits are then transported through a barrel by the screw rotation and heated by friction, direct steam injection and by heat transfer. Eventually, the mixture inside of the extruder goes through a cooling process applied by the barrel and
screw. However, the extrusion process can also generate off-flavors through oxidation, hydrolysis and other chemical reactions occurring in the high temperature short time (HTST) conditions.\textsuperscript{53}

Many studies have shown that extrusion processing can have a significant effect on the flavor profiles of food products.\textsuperscript{54} Non-enzymatic browning, lipid oxidation and oxidation of flavor compounds are important factors that influence overall flavor profile. The Maillard reaction can contribute to the development of color and flavor in extrusion products. For example, pyrazine is generated during extrusion cooking and contributed to the roasted aroma. Extrusion cooking also influences the lipid oxidation stage.\textsuperscript{55} When lipids bind to starches, the stability of the lipid will increase during extrusion cooking. However, the cis-unsaturated fatty acids can convert to trans-fatty acids during extrusion. Extrusion cooking can also inactivate lipase and other deteriorative enzymes.

Up to now, extensive studies have been conducted on the aroma (volatile) compounds released during the extrusion of corn flour.\textsuperscript{53} Among the over 100 key volatile compounds identified, furans, pyrazines, thiazoles, pyrrolizines, thiophenes, pyridines and certain proline-specific compounds were the major volatiles released at the die during extrusion. However, very few studies have looked into the impact of extrusion on bitter profile, such as in whole grain products. It is known that the endospermic part is mild in taste, while the strong bitterness is in the outermost bran layers. Thus refined flour based samples have lower bitterness than the whole grain samples due to the lack of the outer layers.\textsuperscript{56} The phenolic compounds\textsuperscript{57} and the small molecular weight peptide\textsuperscript{58} in the outer bran are thought to be responsible for the bitterness.\textsuperscript{59}
The thermal degradation of phenolic compounds by extrusion has been studied. Significant loss of phenolic content in corn maize were found after heating for one minute.\textsuperscript{60} For barley extrudates, the reduction of total phenolics is about 46 to 60\% compared to that of the unprocessed barley flour.\textsuperscript{5} Ferulic acid was found as a predominant phenolic acid in raw and processed whole grain.\textsuperscript{61}

**Deep-Frying - Potato Chips**

Deep-frying is one of the most commonly used processes to dry, cook, and formulate a wide range of food products at both the industrial and household scale.\textsuperscript{62} It has many advantages that are not offered by other technologies: efficient heat transfer, rapid drying, flavorful products, and crispy texture. However, deep frying also suffers from several limitations; one of which is the increased demand for low-fat products. During deep-fat frying, a large volume of oil is used to immerse the product during the frying process. Heat is transferred from oil to the external surface of the food product at a very high rate in order to vaporize water.\textsuperscript{63} Food slices or chunks are constantly in direct contact with the oil at 180 °C for various lengths of time. The heating fat is transferred into the food (5\%-40\% by weight).\textsuperscript{64} In general, the longer the frying, the more fat is absorbed by foodstuffs. Lipid reactions involve oxidation and decomposition at a rapid rate. Hydrolysis of triacylglycerols leads to free fatty acids which can further degrade to aldehydes, ketones and lactones.

In general, the effect of frying on taste development of potato products is less known, compared to aroma. In 1979, Solms and Wyler reviewed the taste components in potatoes.\textsuperscript{5} Sugars (e.g., glucose, fructose, and saccharide) were assumed to be flavor precursors for the
Maillard reaction but do not contribute to the potato taste. Solms also found that the raw potatoes contain trace amount of 5’-nucleotides. At around 50 °C and pH around 6, RNA is degraded to liberate the 5’-nucleotides. Thus the overall umami taste is enhanced after frying. The free amino acids and nucleotides were considered the major umami components in cooked potatoes. Glutamate is the most potent amino acid that eliciting umami taste while another major amino acid in potato, aspartate, shows only 7% of the umami activity of glutamate. The umami taste of glutamate is enhanced by salts (sodium, potassium) and succinate acid. Conversely, the most notable off-flavors of potato were thought to be bitterness and astringency, which were caused by glycoalkaloids. Another study showed that the glycoalkaloids, solanine and chaconine, were less affected by thermal processing compared to peeling or leaching.

The potato acceptability is strongly correlated with umami taste and umami is suggested as a key indicator of potato flavor. Currently, research on identifying new umami compounds through thermal processing of potatoes is limited. Research was focused on targeting known umami compounds with sensory profile changes of potato chips at different processing stages. In 2007, Morris et al. studied the free amino acids (glutamate and aspartate) and the 5’-nucleotides (GMP and AMP) during cooking process and confirmed the generation of 5’-nucleotides through cooking. The maximum concentration of 5’-GMP was derived after 5-10 min of cooking as the nucleases (the enzyme that breaks down RNA and liberates nucleotides) are active at around 50°C. Umami compounds 5’-nucleotides have also been reported to increase approximately by 14% and 31% from microwave cooking and pressure cooking, respectively.
Maillard Chemistry and Flavor: Impact of Phenolic compounds

The Maillard reaction is a ubiquitous food reaction that influences the flavor, color, and nutritional value of food. The chemical mechanism of the Maillard reaction was studied by Hodge in 1953. Briefly, reducing sugar condenses with a free amino group (from an amino acid or protein) to afford the condensation product which rearranges to generate the Amadori compound. At pH 7 or below, 1,2-enolisation is favored by forming the 3-deoxysone intermediate. On the contrary, at pH >7 the Amadori compound undergoes 2,3-enolisation by forming the 1-deoxysone (Figure 1.13). The dicarbonyl compounds, 3-deoxysome and 1-deoxysome, can undergo retroaldol or β-elimination to further generate more reactive C2, C3, C4, and C5 sugar fragments, such as glyceraldehyde, hydroxyacetone, and diketones. The sugar fragments are well-known to propagate the Maillard reaction in the generation of color and flavor compounds. For example, carbonyl compounds condense with free amine groups, which result in incorporation of nitrogen in the final flavor compounds. Strecker degradation occurs when dicarbonyl compounds react with amino acids, with the formation of aldehydes and aminoketones. Many reactions take place in the later stage of Maillard reaction, leading to the formation of melanoidins.
Phenolic compounds have been reported to inhibit the formation of certain Maillard reaction products by trapping the sugar fragments thus have an impact on the overall flavor profile. Some carbonyl intermediates, e.g. 3-deoxyosone and 1-deoxyosone, can undergo nucleophilic reaction with phenolic compounds or amino acids. Research attentions have been focused on the influence of phenolic compounds on the formation of aroma compounds. In 2000, Wang and Ho studied the impact of certain phenolic compounds (caffeic, ferulic, and chlorogenic acids) on the formation of some coffee aromas under roasting conditions. Caffeic acid was suggested to suppress pyrazines by serving as a radical scavenger trapping the dialkylpyrazine radical, which is an intermediate of pyrazine formation. Additionally,
caffeic acid and chlorogenic acid inhibit the formation of sulfur heterocyclic compounds, such as thiazoles and thiophenes. Ho et al. suggested that caffeic acid consumed the precursors of sulfuric compounds, which were thus less available to form other flavor-active compounds. In 2004, Counet et al. studied the catechin and epicatechin content of cocoa which was collected from different countries. The level of key aroma compounds in cocoa was found to be negatively correlated with the total level of catechin and epicatechin.

Besides the indirect findings of the negative correlation between phenolic and Maillard-generated aroma compounds, Totlani and Peterson suggested that phenolic compounds may function as a key reactant in the Maillard reaction. Using a model aqueous Maillard reaction system (a reducing sugar and an amino acid) reacted with epicatechin, they reported that the chemical structures of phenolic-Maillard reaction products were characterized as epicatechin-C2, -C3, or C4 sugar fragments adducts. Labeled sugars and amino acids were applied to elucidate the formation pathways of Maillard reaction products, which is known as carbohydrate module labeling (CAMOLA) approach. This technique utilizes a combination of unlabeled and 13C6-labeled glucose in 1:1 molar ratio to understand the fragmentation of the sugar skeleton and the subsequent generation of flavor molecules with the sugar fragments attached. The isotope labeling studies suggested that epicatechin directly reacted with the C2, C3, and C4 sugar fragments that were generated by Maillard reaction, which confirmed the previous findings that phenolic compounds were able to quench sugar fragments, thus inhibiting the formation of aroma compounds. The attached glucose fragments were identified as dicarbonyls or hydroxycarbonyls, such as glyoxal, hydroxyacetone, and erythrose, etc. The covalent bond was found to be formed between
the C1 position of the sugar fragment and either the C6 or the C8 position of the epicatechin ring A and was proposed to be generated by hydroxyalkylation and aromatic substitution (Figure 1.14).

![Figure 1.14 Proposed epicatechin (A)-Maillard reaction products structure](image)

Figure 1.14 Proposed epicatechin (A)-Maillard reaction products structure

The impact of epicatechin on Maillard chemistry was also investigated under a low moisture roasting condition. Similar to the aqueous conditions, phenolic compounds were found to suppress the generation of Maillard aroma compounds in the low moisture conditions. However, only epicatechin-C5 and -C6 sugar fragments were detected under low moisture conditions. The 3-deoxy-2-hexosulose was identified as the unique precursor for the epicatechin-Maillard reaction products based on matching LC/MS chromatographic fingerprints by directly reacting epicatechin with the intermediate 3-deoxy-2-hexosulose in a simple Maillard model system. By quenching the reactive dicarbonyl intermediates, 3-deoxy-2-hexosulose, the generation of the Maillard reaction products was inhibited.

The Maillard reaction is known to contribute to the generation of taste compounds in many food products. Free amino acids or peptides and reducing sugars participate in the Maillard reaction. It has been shown that the formation of some bitter compounds in food is due to Maillard reactions between reducing sugars and amino acids. The precursors that
generate the bitter compounds were studied extensively in the model Maillard reactions at low moisture conditions that simulated roasting.\textsuperscript{77} Table 1.2 summarizes the perceived bitterness intensity resulting from the reaction of a sugar (\textit{i.e.}, glucose, xylose) and a common amino acid (\textit{i.e.}, \textit{L}-proline, \textit{L}-lysine, \textit{L}-arginine, \textit{L}-alanine, glycine, \textit{L}-cysteine) under aqueous condition and low moisture conditions. The taste dilution (TD) factor was defined as the number of dilutions that bitterness is no longer detected in the next dilution.

\textbf{Table 1.2} The relative bitter activities of Maillard model system under aqueous condition and low moisture condition.

<table>
<thead>
<tr>
<th>Model system</th>
<th>Relative bitter activity (TD factor)</th>
<th>Aqueous condition $^1$</th>
<th>Low moisture condition $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td></td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>glucose/L-proline</td>
<td></td>
<td>1</td>
<td>2048</td>
</tr>
<tr>
<td>glucose/L-lysine</td>
<td></td>
<td>2</td>
<td>512</td>
</tr>
<tr>
<td>glucose/L-arginine</td>
<td></td>
<td>2</td>
<td>256</td>
</tr>
<tr>
<td>glucose/L-alanine</td>
<td></td>
<td>&lt;1</td>
<td>64</td>
</tr>
<tr>
<td>glucose/glycine</td>
<td></td>
<td>&lt;1</td>
<td>16</td>
</tr>
<tr>
<td>glucose/L-cysteine</td>
<td></td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>xylose</td>
<td></td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>xylose/L-proline</td>
<td></td>
<td>128</td>
<td>8</td>
</tr>
<tr>
<td>xylose/L-lysine</td>
<td></td>
<td>32</td>
<td>nd</td>
</tr>
</tbody>
</table>

\textsuperscript{77}
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>xylose/L-arginine</td>
<td>32</td>
<td>nd</td>
</tr>
<tr>
<td>xylose/L-alanine</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>xylose/glycine</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>xylose/L-cysteine</td>
<td>&lt;1</td>
<td>nd</td>
</tr>
</tbody>
</table>

\[ ^{1} \text{Aqueous condition: a solution of sugar and amino acid (100 mmol each) in water (200 mL, pH 6.0) was heated under reflux for 3 h.} \]

\[ ^{ii} \text{Low moisture condition: a mixture of 30 mmol sugar and 10 mmol amino acid was heated for 20 min at } 180 ^{\circ} \text{C in sand.} \]

Based on the observations in **Table 1.2**, the generated bitterness in low moisture conditions is generally higher than in aqueous condition, except for xylose-alanine, xylose-proline, xylose-lysine, and xylose-arginine. Also, compared to the absence of amino acids, sugar-amino acid reactions have significantly higher TD factor (higher bitterness), which suggests that the Maillard reaction, instead of sugar degradation, is responsible for the bitterness. The Maillard reaction products (MRPs) are tremendously complex but the number of compounds that contribute to the bitter taste is limited. The glucose/L-proline model generates the highest bitterness. The key resulting bitter compounds were identified as spirodiolone 1 (bitter threshold: 0.009 mmol/L), bispyrrolidinohexoses 2 (bitter threshold: 0.06 mmol/L) and 3 (bitter threshold: 0.1 mmol/L), the pyrrolidinohexose reductones 4 (bitter threshold: 0.5 mmol/L), 5 (bitter threshold: 6.8 mmol/L) and 6 (bitter threshold: 4.3 mmol/L) \[ ^{77} \text{, as well as the cyclopenta[b]azepin-8(1H)-ones 7 (bitter threshold: 0.08 mmol/L,} \]

\[ ^{78} \text{. The bitter generation pathways are shown in **Figure 1.15**.} \]
The identification and characterization of taste-active compounds

The level of taste active compounds in food sometimes are below the part-per-million level or even lower. The sensory threshold for some compounds is even at the part-per-billion level. In our daily food products, there are many compounds in large quantities but not contributing to the taste profile, thus complicate the analysis. Sensory-guided fractionation is an analytical tool to determine the compounds that have relatively high taste
activities by connecting sensory information with chemical molecules. Briefly, a food sample is fractionated by Multi-Dimensional Liquid Chromatography and the sensory active fractions are typically identified by Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR).

*Sensory directed analytical methods to determine ‘taste-active’ fractions*

The sensory-guided methods for screening taste active compounds have been developed since early 2000. In 2003, Hofmann et al. first introduced the comparative taste dilution analysis used in a model Maillard reaction.79 Similar to the aroma screening analysis, the fractions collected after HPLC separation were diluted stepwise 1+1 with pure water. Each of the serial dilution was presented to the sensory panel in the order of increasing concentrations. Each dilution was then evaluated for a specific taste attribute in a 3-Alternative Forced Choice (3-AFC) test. The taste dilution (TD) factor was defined as the dilution at which the taste difference could just be recognized between the test solution and the two blanks. Hence, a higher taste dilution factor indicates a higher contribution of that fraction to the taste attribute. For example, Fraction #3 in Figure 1.16 eluting out of the column at $R_t = 8$ min has the highest TD factor, suggesting the highest taste activity in that fraction.79 Instead of focusing merely on fraction of highest UV signal, the sensory guided fractionation methodology enables targeting on the fraction with highest taste activity. From the year 2003 up to the present, most work have used this TD factor concept to identify taste-active components generated during thermal processing.36,50,79,80
Figure 1.16 Taste dilution analysis of screening taste-active fraction generated from HPLC.

However, the sensory response to the concentration of an individual compound is not a linear relationship. Figure 1.17 shows the dose/response relationship of some identified bitter compounds generated during roasting, and most of them have a ‘S’ shape as opposed to a linear shape. Thus TD-factor cannot accurately represent the dose-response function. In 2005, the dose-over-threshold (DoT) factor was proposed by Hofmann et al. to evaluate the 2,5-diketopiperazines generated in roasted cocoa. The DoT factor was defined as the ratio of the concentration to the threshold concentration of the compound. The DoT factor above one is considered taste active. Hofmann et al. pointed out that the DoT factor indicated the taste
impact of each individual compound at the food level, thus telling the contribution of each compound to the overall taste profile. A number of papers have used this concept to identify taste active compounds between the years of 2005 and 2010.\textsuperscript{48–50}

![Diagram](image)

**Figure 1.17 Human dose/response function of identified 2,5-diketopiperazines.**

Direct rating is another method that has been used to guide fractionation. In this research approach, each fraction is tasted and compared to a reference solution for that taste attribute. A score is given based on the comparison and a trained panel is used. Schmiech \textit{et al.} used this method to identify bitter compounds in carrots in 2008.\textsuperscript{81} This method has been used to analyze taste-active component in a wide range of food matrixes.\textsuperscript{82–84} The illustration of direct scaling method is shown in **Figure 1.18**. Fractions 5, 7, 11, 17, 18, 19, and 23 were selected for further purification and identification based on bitterness intensity.
2D-HPLC

Due to the complexity of the food matrix, the resolving power for the separation system has to be enhanced in order to obtain the pure compound in a sufficient amount for identification. Two-dimensional HPLC separation can dramatically enhance the separation power by increasing the resolved peak numbers in a defined time window.\(^{85}\)

The optimization of the separation condition is important to enhance the separation power. The choice of column chemistry is essential for efficient separation as shown in Figure 1.19.\(^ {85}\) The peaks of System B were aligned at the diagonal of the separation space. This indicated that using the same column chemistry (C18) on the second dimension does not enhance the resolution. Besides the selection of column, many separation parameters (such as mobile phase, the separation pH, and pressure) have significant influence on the separation
power based on the analytes’ chemical properties. For example, the use of pH modifier was chosen for isolating bitter peptides in whey protein as some of the peptides are sensitive to the pH in the mobile phase.\textsuperscript{84}

Figure 1.19 Comparison of two 2D-HPLC separation systems. System A: First dimension: C18 column; Second dimension: Bonus RP column; System B: First dimension: C18 column; Second dimension: C18 column.\textsuperscript{85}
Nuclear Magnetic Resonance (NMR)

Due to the lack of structural database for non-volatile compounds, identification of unknowns is typically associated with multiple analytical techniques for structural elucidation. Liquid-state NMR is a spectroscopy technique in which most of the atom nuclei (e.g., $^1$H, $^{13}$C, $^{19}$F, and $^{31}$P) are oriented according to the absorbed radiation at characteristic frequencies by a strong magnetic field. In liquid state NMR, nuclei in different chemical environments result in distinct spectral patterns (chemical shift, multiplicities) which can be interpreted in terms of chemical structure, compound conformation, and so on.$^{86}$

Nuclear magnetic resonance is a universal tool for structural elucidation.$^{87}$ NMR gives molecular information about the stereochemistry, functional groups, the connectivity of the fragmentations, etc. The application of NMR in food science is mainly in two areas: (1) Food-omics (flavoromics, transcriptomics, proteomics, metabolomics) on non-targeted analysis of food metabolites with suitable statistical analysis$^{88,89}$. (2) Characterizing bioactive or sensory active food components or illustration of compound generation.$^{90,91}$ The present discussion focuses mainly on NMR characterization of compounds in food. For a detailed review of using NMR in metabolomics please see references.$^{88,89}$

In 2006, Totlani et al. using $^1$H NMR, first identified the structure of epicatechin-Maillard adducts isolated from epicatechin-Maillard reaction.$^{74}$ Peak broadening in Figure 1.20 (B) was reduced with cold temperature NMR (shown in Figure 1.20 (A)). Comparing the structures of EC in (A) and (C), the authors proposed that the structure of the EC-MGO adduct (Figure 1.21).
Figure 1.20 $^1$H spectra for (A) Epicatechin (EC) at room temperature; (B) EC-methylglyoxal (MGO) adduct at room temperature; (C) EC-MGO adduct at -25 °C.\textsuperscript{74}

Figure 1.21 The proposed structure of EC-MGO adducts and related HMBC C-H correlations by Totlani \textit{et al.} \textsuperscript{74}

For the carbon backbone information, heteronuclear multiple quantum correlation Spectroscopy (HMQC) is a method for correlating protons with attached carbons. In HMQC, the $^{13}$C frequencies are detected as echoes in $^1$H channel, which avoids the low sensitivity of $^{13}$C NMR. Each peak on the HMQC spectrum corresponds to a $^1$J (C-H) correlation, \textit{i.e.} a C-H bond, making it particularly useful in identifying inequivalent geminal protons. Figure 1.22 shows the HMQC spectrum of the EC-MGO product. In the HMQC spectrum, only proton-attached carbons have signals. With the assigned proton signal from $^1$H spectrum, the protons’ attached carbons could be identified using HMQC.
Figure 1.22 HMQC spectrum of EC-MGO adduct at -25 °C.

Totlani and Peterson used another heteronuclear multiple-bond correlation spectroscopy (HMBC) to identify long-chain correlation between carbon and proton (the correlations are shown in Figure 1.21). \(^{74}\) HMBC spectroscopy is useful to study \(^2\)J and \(^3\)J correlations between \(^{13}\)C and \(^1\)H that are multiple bonds away, and thus HMBC spectroscopy can be used to observe connectivity with or through nuclei without attached protons while \(^1\)J correlations are suppressed.

In addition to bonds correlation techniques, NMR can be applied to define through space correlation for identifying stereochemistry in taste compounds, including rotating-frame-
overhauser spectroscopy (ROESY) or nuclear-overhauser-effect-spectroscopy (NOESY). Spatial magnitude within $-1/r^6$ will appear as off-diagonal peaks on the spectrum. Jiang and Peterson reported the stereochemistry of Acortatarin A, a bitter marker identified in whole wheat crust, using ROESY. As shown in Figure 1.23, the correlation marked in red represents the $H_{3b}$-$H_5$ correlation indicating that $H_5$ and $H_{3b}$ are in the same planar. On the other hand, the $H_{3b}$ and $H_1$ correlation (in green) suggests that $H_{3b}$ and $H_1$ are in a planar. In summary, NMR is a power analytical tool to elucidate structures of taste compounds; utilizing 1D and 2D NMR techniques.

**Figure 1.23** ROESY spectrum of Acortatarin A. 82

**Research Objectives**

The overall objective of the study was to understand the effect of thermal processing on the taste profile of healthier formulated food, specifically low fat or polyphenol-rich products.
The thermal generation or degradation of taste compounds were characterized in three processed foods: roasted cocoa, extruded corn puffs, and deep-fried potato chips. By providing insight into the impact of thermal processing and food composition on flavor development, this study can lead to flavor optimization strategies for healthier food choices.

Increased health consciousness creates expectations for healthy and tasty food products in the market. According to the most current Dietary Guideline of Americans (2015-2020), more than 70% of the Americans consume exceed the recommendations for added sugars, saturated fats, and sodium (Figure 1.24). Generally, consumers are looking for food products with low sugar and low fat.
Figure 1.24 Dietary intakes compared to recommendations.¹⁷

Instead of adding sugar to improve the taste of phenolic-rich beverage, Chapter 2 is aimed at introducing a novel way to suppress bitterness in roasted cocoa by looking at how the roasting process modulates the endogenous bitter compounds. Endogenous taste compounds are known to be chemically modified by food reactions during thermal processing; however, how common food reactions could modulate these taste attributes is not well understood. Cocoa is rich in polyphenolic antioxidants, such as catechin, which
contributes to the bitterness of cocoa.\textsuperscript{92} The chemistry and fate of catechin was examined in two systems: roasted cocoa and simulated model Maillard reaction. The thermally generated catechin-Maillard reaction products were studied for their bitter modulation properties. This part of the project provides new insights to reduce the bitterness of phenolic-rich food products by altering a key bitter compound to a bitter blocker by thermal processing, which helps to improve the consumer acceptability with the demand of sugar reduction.

For a healthier diet, the US Dietary Guidelines recommend that ‘consuming at least three or more ounce-equivalents of whole grains per day can reduce the risk of several chronic diseases and may help with weight maintenance’.\textsuperscript{93} Whole grain cereals have been reported to provide potential health benefits and likewise the U.S. Dietary Guidelines recommend that 50\% or more of total grain intake should be whole grains. However, whole grain products suffer from low consumer acceptability. Bitterness is known to negatively impact the flavor quality and the consumption of whole grain food products. The compounds that cause the bitterness in whole grain corn extruded puffed cereal have not been identified nor characterized. In order to improve the taste of whole grain corn cereals, the taste difference between a refined formula and a whole grain formula was illustrated. Sensory-guided fractionation methodology was used to screen the bitter-active fraction for further structural elucidation. The processing and ingredient impact on the bitterness of extruded corn cereal is discussed in chapter 3.

Consumers also express their increasing demands for fat-free or low fat foods. Due to the high obesity rate in the US,\textsuperscript{94} many consumers choose foods according to lower fat intake and they move away from chips products because of the high-fat. As an alternative to salt
addition to improve flavor, chapter 4 proposes the generation of umami enhancing compounds in potato chips during frying. Considering shorter process time for lower fat potato chips, the goal of this study is to understand the frying impact on the development of the desirable taste. The taste fingerprint of lower fat potato chips was compared to the regular fat ones. The future application is to improve the taste of low fat potato chips by introducing thermally induced umami enhancing molecules.

The major research objectives of this study are:

1. To characterize the influence of thermally induced reaction products of a known dietary bitter compound on bitter modulation in cocoa roasting condition.
2. To investigate the impact of extrusion processing of whole grain corn puffed products on the bitterness profile.
3. To identify the differences of the taste profile between regular fat and low fat potato chips under the impact of frying
Chapter 2 Identification of Bitter Modulating Maillard-Catechin Reaction Products

Published in Journal of Agricultural and Food Chemistry, 2014, 62, 8470-8477

Introduction

Bitter taste is one of the basic gustatory taste sensations that can cause consumer dislike in foods and beverages. Common approaches for decreasing the bitterness of commercial foods often involve the addition of sugar or salt. However, with the increasing demand for healthier but tasty foods, the discovery of novel ingredients that is able to modulate bitterness is desirable. Consequently, the discovery of compounds with bitter modulator activity is of commercial interest to improve the palatability of food with bitter attributes, particular for reduced sugar and salt formulations.

Screening and identifying bitter modulating compounds is an analytically challenging task. Recently, a series of flavan-3-ol-C-glycosides isolated from cocoa were suggested to suppress bitter intensity of cocoa beverages. Additionally, β-D-glucopyranosides were reported to activate one of the human bitter receptors (T2Rs) with high specificity, and even stereoselectivity based on the conformation at the C1 position of the pyranose moiety. Therefore, slight changes of the compound structure (e.g. the pyranose moiety attached to a flavonoid) have been reported to influence the taste profile or even modulate the bitter perception.
Native taste compounds are known to be chemically modified by food reactions during food manufacturing; however how common food reactions could modulate these taste attributes is not well understood. Previously our group has reported that dietary bitter compounds, the flavan-3-ols, were chemically modified by reactive carbonyl species (i.e. dicarbonyls) generated by food reactions, such as Maillard pathways.\textsuperscript{75,73} The main goal of the current study was thus to investigate if the modification of known dietary bitter compounds, such as the flavanols, generated new chemical species that modulated the perception of bitterness intensity. These results were further extrapolated to cocoa beans to investigate if related phenolic-Maillard adducts existed.

**Materials and Methods**

*Chemicals.* D-glucose, D-xylose, D-galactose, glycine, (+)-catechin hydrate (≥98%), high-purity quartz sand, formic acid, and methanol-d4 (99.8% enrichment) were obtained from Sigma Aldrich Co. (St. Louis, MO, USA). High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Distilled deionized water was further purified through a Barnstead Nanopure Diamond water purification system (Thermo Scientific, Dubuque, IA). \textsuperscript{13}C\textsubscript{6}-glucose, \textsuperscript{13}C\textsubscript{6}-galactose, and \textsuperscript{13}C\textsubscript{1}-xylose were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA)

*Low-Moisture Maillard Reaction Model with Catechin.* Reactions were conducted according to those of Jiang.\textsuperscript{99} Briefly, the reaction apparatus consisted of a round-bottom flask (500 mL) attached with a vigreux column and a glass stirrer fitted with a Teflon blade (Ace Glass, Vineland, NJ). The reaction vessel was heated by oil bath and connected to a
rheostat (PowerStat, The Superior Electric Co., Bristol, CT) for temperature control. The reactants (reported in **Table 2.1**) were mixed with 15 g of quartz sand (previously cleaned and dried) and 1.5 g of water. The reactant mixture was mixed at 40-60 rpm in the round-bottom flask for 2-3 min. The apparatus was then placed in an oil bath maintained at 200 °C, and the reaction was conducted for 15 min, then immediately removed from the oil bath and prepared for further analysis.

**Sample Preparation for Model Reactions.** Extraction was performed with methanol (3 × 30 mL), the isolate was filtered and the filtrate was separated by means of ultrafiltration equipped with 1 kDa MW cutoff membrane (8200 Series Stirred Cell, EMD Millipore, Billerca, MA). The permeate fraction was concentrated until precipitate appeared under vacuum (Buchi Rotavapor, model R110, New Castle, DE; 0.1 atm, water bath was maintained at 30 °C) and subsequently filtered through a 0.2 μm PFTE tip filter (Sigma Aldrich Co.) and analyzed by LC-MS.

**LC/MS-TOF Isotopomeric and Accurate Mass Analysis:** Maillard-Phenolic reaction products were characterized by identifying isotopomers as previously reported (**Table 2.1**, 75). Analysis was conducted with a Waters 2D UPLC Acquity iClass coupled with a Xevo™ G2 QTof system (Waters, Milford, MA). Accurate mass acquisition of the ion of interest was performed by chromatography injection using dual electrospray ion source. Reserpine (100 mg/L) was used as the lockspary injecting at 10 μL/min. The extracts (2 μL) were injected on ACQUITY UPLC BEH C18 1.7 μm column (2.1 mm × 50 mm) (Waters) maintained at a temperature of 25 °C. The mobile phase was maintained at a flow rate of 0.3 mL/min using a binary solvent system of 0.1 % formic acid in water (A) and methanol (B). The elution
gradient started at 10% B, linearly increased to 90% B (1-6.35 min), held at 100% B (6.35-8.35 min), and then held at 10% B (8.35-10 min). Mass spectrometric ionization conditions were as follows: desolvation temperature of 400 °C; source temperature of 110 °C; capillary voltage of 1.1 kV. For samples analyzed in both positive and negative sensitivity scan mode, the scan range was 50-1000 Da.

prep-LC. Target analytes were purified using a LC system that consisted of a binary pumping system (LC-10 ADvp), a degasser (DGU-14A), an autosampler (SIL-10vp), a variable-wavelength UV-Vis detector at 254 nm and 280 nm (Shimadzu, SPD-10A), a fraction-collector (Shimadzu, FRC-10A) and a RP-C18 column (21 mm × 250 mm, Pursuit 5, Varian, USA). The HPLC condition was as follows: the injection volume was 1.5 mL, the column temperature was 25 °C, binary mobile system A was 0.1% formic acid aqueous solution, pH 3.0 and B was 100% acetonitrile, column flow rate was 10 mL/min. Gradient was started with 5% B (0-10 min), then increased to 50% B (10-40 min), then increased to 100% B (40-50 min), and then decreased to 5% B (50-60 min). The total run time was 60 min. The effluent was collected with a fraction collector. Compounds 1 and 2, generated from model A, were collected at 19.5 min and 20.5 min respectively; compounds 3, 4 and 5 obtained from model B were collected at 20.3 min, 21.0 min, 22.0 min respectively; compounds 6, 7 and 8 were collected at 20.4 min, 21.4 min, 21.9 min, respectively (refer to Figure 2.1 for compound structures). Second dimension off-line liquid chromatography was conducted for further purification until at least 95% purity was achieved. The binary mobile phase for the second dimension was as follows: mobile phase A was water with pH=7; mobile phase B was methanol. The second dimension gradient was started with 5% B (0-5
min), then increased to 20% B (5-10 min), then slowly increased to 30% B (10-40 min), and then further increased to 100% B (41-50 min), and then decreased to 5% B (50-55 min). About 0.5-1.0 mg of Maillard-Catechin adducts was obtained by removing the solvent.

**Circular Dichronism (CD) Spectrum.** For CD spectrum, methanolic solutions of the samples were analyzed by means of a Jasco Model CD-1595 circular dichroism chiral detector (Easton, MD) (0.190 degrees ± 0.30 for D and L camphor sulfonic acid as references).

**Nuclear Magnetic Resonance Spectrum (NMR).** $^1$H, COSY, HMQC, HMBC, $^{13}$C, and NOESY NMR measurements were performed on a Bruker 700 MHz spectrometer with TXI cryoprobe and a Bruker 850 MHz spectrometer with TCI cryoprobe. Data were processed and analyzed with Topspin 3.1 and MestRenova 8.1. Methanol-d4 was used as the NMR solvent. The solvent residual peak at δ 4.78 (water) was set as the internal reference. NOESY correlations were verified within reasonable range (<4 Å) using ChemBio 3D software.

**Sensory Analysis.**

**Subjects and Training** A panel of eight judges (ages 23 to 45, 3 males and 5 females) from the Department of Food Science and Nutrition, St. Paul, MN evaluated the bitterness of the test solutions. Prior to analysis, all panelists participated in 12 training sessions of approximately 1 hour in duration over a period of 4 weeks. Panelists were initially trained on a 10-point scale with four references provided (0.03, 0.04, 0.08, and 0.15% w/w of caffeine in water), corresponding to bitterness intensity ratings of 2, 3.5, 5 and 10, respectively. Inconsistencies in the ratings were addressed with panelists as part of training.
**Evaluation** The test samples (Figure 2.1) were evaluated for bitterness modulation activity. Two separate sample evaluations were conducted. In the first session, all eight test compounds were evaluated followed by a second session consisting of two select compounds (numbers 2 and 7, Figure 2.1). For all evaluation sessions, the samples were evaluated in duplicate. Three reference levels were provided (0.03, 0.04, and 0.08 (w/w) of caffeine in water), corresponding to bitterness intensity ratings of 2, 3.5, and 5, respectively. All samples were prepared the morning of the evaluation. During the first evaluation session, each test compound (1.9 mg) was added to 32 mL of 0.04% caffeine solution. The ratio of test compound to caffeine was 1:7 (w/w), which was based on the quantification of these two analytes in roasted cocoa (Figure 2.3).

For the second evaluation session the concentrations of compounds 2 and 7 were doubled to 3.8 mg of each test compound in the 32 mL of 0.04% caffeine solution (ratio of 2:7 (w/w)). Solutions were stirred for 10 minutes and then 2 mL of each solution was dispensed in samples cups labeled with 3-digit codes. Panelists used a PTFE dropper to dispense 2 mL of solution on the center of their tongue. Water and unsalted crackers were used as palate cleansers.

**Data Analysis** Ratings were averaged and the differences among treatments were evaluated by analysis of variance (ANOVA) followed by Tukey’s HSD pairwise comparison. All analyses were performed with Statistix Software (version 9). Significance was established at $P < 0.05$.

**Sample Preparation of Cocoa Samples.** Fifteen grams of unroasted or roasted beans (135 °C for 15 min) were ground for 2 min then extracted with a mixture (2:1; v/v; 3 × 100 mL) of
ethanol and water for 2 hour at room temperature with stirring. The ethanol/water extract was added with methylparaben (100 ppm) as internal standard. Then it was concentrated until precipitate appeared under vacuum (Buchi Rotavapor, model R110, New Castle, DE; 0.1 atm, water bath was maintained at 30 °C) then filtered through a 0.2 μm PFTE tip filter (Sigma Aldrich Co.) and analyzed by LC-MS.

**LC-MS Identification of Compound 2 and 7 in Cocoa.** Cocoa isolates were analyzed on a Waters ACQUITY UPLC system interfaced with a Quattro Premier XE™ mass spectrometer (Waters, Milford, MA). Two microliters were injected on to an ACQUITY UPLC BEH C18 1.7 μm column (2.1 mm × 50 mm; Waters) at 25 °C. The mobile phase was maintained at a flow rate of 0.3 mL/min using a binary solvent system of 0.1 % formic acid in water (A) and methanol (B). The elution gradient started at 10% B, linearly increased to 90% B (1-6.35 min), held at 100% B (6.35-8.35 min), and then held at 10% B (8.35-10 min). The Quattro Premier XE™ mass spectrometer was equipped with an electrospray ionization probe and the operational parameters were positive ion mode, source temperature of 110 °C; desolvation temperature of 350 °C; and capillary voltage of 3.0 kV. Data were collected in multiple-reaction monitoring (MRM) mode. The ion transitions for the compounds were optimized according to the ion intensity of the product ion through full scan MS/MS. The MRM method was optimized by direct infusion of authentic compounds. The MS acquisition was collected as follows: Compound 2, m/z 435→283, 435→417, cone 35 V, collision 15 V, and Compound 7: m/z 435→247, 435→123, cone 30 V, collision 20 V.

**LC-MS Quantification of Compound 2 and Caffeine in Cocoa.** Analysis was conducted by LC-MS in MRM mode. The ion transitions for the compounds were as follows:
Compound 2, \( m/z \ 435 \rightarrow 283, 435 \rightarrow 417 \), cone 30 V, collision 15 V; caffeine, \( m/z \ 195 \rightarrow 138, 195 \rightarrow 110 \), cone 30 V, collision 20 V. The dwell time was 0.5 s for the analytes; the inter-scan delay was 0.1 s and inter-channel delay was 0.005 s. All samples were analyzed in duplicate. Quantification was conducted by internal standard (methylparaben, 0.3 M) and external 5-point calibration curve of Compound 2 and caffeine at \( 10^{-1} \) mM - \( 10^{3} \) mM that showed good linearity with regression coefficient ranging from 0.97 to 0.99.

**Results and Discussion**

In order to investigate the influence of thermal reactions on the modulation of bitterness in food, catechin a known dietary bitter compound was reacted in simple Maillard reaction model systems consisting of a simple sugar (glucose, galactose or xylose) and glycine (Table 2.1, Models A-C). It was expected the unique stereo-centers of the two hexose sugars would be transferred to catechin-Maillard reaction products, providing the ability to investigate structure-activity relationships on bitter perception. Additionally, the pentose sugar (xylose) was selected similarly to provide additional reaction products. Utilization of simple Maillard model systems provided the ability to enrich one of the reactants (in this example the sugar) with \(^{13}\)C-labeling for the identification of phenolic-Maillard reaction products (Table 2.1, Models D-F) as we have previously reported.\(^{75}\)

**Identification of catechin-C-spiro-glycoside adducts.**

Adducts were identified by LC-MS isotopomeric analysis of Models D-F, with the same unlabeled parent ion reported in Models A-C, respectively, at the same retention time.
Isotopomeric analysis of Models D-F indicated an intact sugar moiety was incorporated in the selected products. No reaction products were detected in Model H (glucose and glycine) and I (catechin only). Similarly reaction products were not identified in Model G (glucose and catechin). Overall, eight main reaction products were identified and isolated for structural determination.

Two reaction products (compound 1 and compound 2, Figure 2.1) were isolated from model A (catechin, glucose and glycine). The carbons from sugar moiety are noted as 1’’ to 6’’ while the carbons from catechin are marked as 2 to 9 and 1’ to 6’. For compound 1, majority of the $^1$H spectra of (+)-catechin (data not shown) were easily deduced. A pair of doublets at 3.30 and 2.82 with J-coupling of 16.8 Hz indicated a pair of geminal hydrogens is incorporated and connected directly to the A ring of catechin, which is unique compared with other flavan-3-ol-C-glycosides that have been reported.$^{97}$ According to NMR HMBC spectroscopy, the sugar moiety was connected through C-8. In considering the distinctive carbon chemical shifts, the 7-hydroxyl loses one molecule of water with the C-2’’ acetal group forming a spiro [4, 4] structure. The structure of sugar moiety is supported by references.$^{100}$ NOESY spectroscopy shows spatial correlation between H-1’’ and H-6’’ and correlation between H-1’’ and H-4’’. In consideration of the stereochemistry of the starting material as well, the stereochemistry of compound 1 is indicated in Figure 2.1.

Compound 2, based on the $^1$H spectrum (Figure 2.2), again shows a pair of geminal hydrogens connecting between catechin and the sugar moiety; compound 2 is distinctive in its HMBC correlations compared with the assignment of compound 1. The presence of $^3$J$_{H1''-C_7}$ and $^3$J$_{H1''-C_5}$ indicate that the sugar moiety is connected to the C-6 of catechin as shown in
Table 2.2. Spatial correlation is shown in Figure 2.1 according to NOESY spectroscopy. It is found that the volume ratio of H3’’-H4’’ cross-peak and H1’’a-H1’’b (1.8 Å) cross-peak is 0.0053, which shows H3’’ and H4’’ have a very weak NOESY correlation; thus H3’’ and H4’’ are not planar. The structure of the sugar moiety has been reported.¹⁰¹

For Model B (catechin, xylose, and glycine, Table 2.1) compounds 3, 4 and 5 (Figure 2.1) were isolated. According to the molecular mass (MW=422), compound 3 is different from other analytes isolated in model B (MW=404), which indicates one less dehydration step occurred. Moreover, no methylene moiety is found in connection with the A ring of catechin which makes compound 3 structurally different from others. The $^{13}$C chemical shift (82-68 ppm) indicates that no acetal group was incorporated. Additionally, a pyran ring formed for the distinctive chemical shift of C-3’’ and C-4’’ (<78 ppm). The J-coupling constants of H1’’-H2’’, H2’’-H3’’, H3’’-H4’’, and H4’’-H5’’b are 9~10 Hz while H4’’-H5’’a is 5 Hz, indicating all the hydrogens of the sugar moiety are axial hydrogens except H5’’a is equatorial.⁸⁶ Structure of the xyloside moiety is further supported by reference.¹⁰²

For compounds 4 and 5, HMBC spectroscopy shows a xyloside connected to catechin C-8 for compound 4 while the xyloside of compound 5 is attached to the C-6 of catechin. A typical furan carbohydrate carbon chemical shift is observed for compound 4 and compound 5. H-1’’ has NOESY correlation with H-3’’ in compound 4 and compound 5 supported by references.¹⁰³–¹⁰⁵

For Model C (catechin, galactose, and glycine) compounds 6, 7 and 8 (Figure 2.1) were isolated. For compound 6, COSY and HMBC spectrum followed the same trend as compound 2. However, the chemical shifts of sugar moiety at the 3’’, 4’’ and 5’’ and the
coupling constants are unique compared with compound 2; hence, the stereochemistry of sugar moiety of compound 6 is different from compound 2. NOESY shows H1’’-H6’’ and H3’’-H4’’ correlations of similar strengths, indicating that the stereochemistry of compound 6 at the 4’’ position is different from that at compound 2. For compound 6, H-3’’ is much closer to H-4’’ compared with compound 2 since the volume ratio of NOESY correlations H3’’-H4’’ and H1’’a-H1’’b is 0.88, much higher than that for compound 2. Therefore, compound 6 was reported as a diastereomer of compound 2.

The HMBC spectrum of compound 7 shows H-1’’ has correlations with C-7 and C-9; thus the reaction happens on the C-7 and the C-8 of catechin. Moreover, HMBC correlations confirm that there are correlations between H6’’-C2’’ corresponding to a pyran ring spiro-fused with a furan ring. Additionally, the chemical shifts of sugar moiety carbons belong to a [4,5] ring instead of a [4,4] ring structure. H1’’-H3’’ correlation in NOESY spectroscopy indicates the stereochemistry as shown in Figure 2.2.106

For compound 8, it is a C-8 substituted C-glycoside adduct. The 6’’ hydroxyl group losing one molecule of water to form the acetal with C-1’’ as confirmed by HMBC correlation (C1’’-H6’’). As illustrated above, the stereochemistry of compound 8 is discussed by both NOESY and J-coupling constants.107,108

**Plausible mechanisms for the spirocyclic catechin-Maillard products generation**

To gain insights into how the catechin-C-spiro-glycosides were generated in these simple model Maillard reaction systems, two mechanisms are proposed. In mechanism I (Figure 2.4), the Maillard derived imine rearranges to the keto form of the N-substituted 1-amino-2-
deoxy-2-ketone, which is then protonated and trapped by catechin at the alpha to the carbonyl group.\textsuperscript{109,110} The 5-hydroxyl group of the sugar moiety then attacks the carbonyl to form an acetal. According to LC-MS, one molecule of water is lost; hence the spirocyclic glycoside catechin is formed. For mechanism II, catechin traps the cation of the imine, then undergoes elimination following with tautomerization to afford the same catechin-Maillard intermediate and then dehydrated and ring-closed to generate the spiro-C-glycosides product.

\textit{Taste-Modifying Activity of Maillard-Catechin Products}

The eight isolated Maillard-catechin compounds (Figure 2.1) were screened for bitter modulation activity in a caffeine solution (see Table 2.3). Caffeine was selected because it is a highly ubiquitous dietary bitter compound and has been previously used to screen for bitter masking compounds.\textsuperscript{111,112} Additionally, caffeine is also a major bitter compound found in cocoa.\textsuperscript{112} To provide perhaps a more food relevant dosage, both compounds 2 and 7 were screened in cocoa beans. Only compound 2 was identified in cocoa and was subsequently quantified in addition to caffeine. The concentration of compound 2 is reported in Figure 2.3, for both unroasted and roasted cocoa. The concentration of compound 2 in roasted cocoa is approximately three times higher in comparison to unroasted cocoa, suggesting it was not only a natural product of microbial provenance in cocoa beans but also generated through roasting (thermal treatment) as well. Similar compound structures (C-spiro-glycoside flavonoids) as compound 2 have been previously reported as fungal products with anti-inflammation activities.\textsuperscript{113,114} Comparing the concentration of compound 2 (0.15 mg/g) to the concentration of caffeine (1.03 mg/g) in roasted cocoa, a concentration ratio of 7:1
(caffeine: test compound) was determined. At the same concentration level (caffeine: test compound=7:1, w/w) trends in the bitterness enhancement or suppression were observed among the different compound structures albeit there were no significant differences in bitter ratings between caffeine base and caffeine with test compounds added. Unfortunately, dose-response analysis was beyond the scope of this project due to the limited quantities of the reaction products available. However, based on the noted suppression of the average bitterness rating for both compounds 2 (generated from glucose, glycine and catechin) and 7 (generated from galactose, glycine and catechin), these compounds were further selected for evaluation at a higher dosage level. Subsequently, compound 2 was added at a double dosage level (caffeine: test compound=7:2, w/w) to further investigate potential modulation activity (see Table 2.4); compound 7 was also included for comparison. In the cocoa sample, an isomer of compound 2 consisting of epicatechin (as opposed to catechin) was also predicted by MS/MS fragmentation analysis and reported to be at a similar concentration (data not shown). This suggested epicatechin reaction product was not isolated from cocoa, nor was the taste activity tested. Given that compound 2 was a natural (fungal) product in the raw beans and also generated by thermal processing as well as other similar reaction products could be generated, the assessment of compound 2 at 2-fold the concentration reported in the beans used in the current study (Figure 2.3) was predicted to be within normal natural product variation. At this higher dosage level compound 2 was reported to significantly decrease the bitterness perception of the caffeine solution or compound 2 functioned as a bitter blocker (Table 2.4). This finding suggested the bitterness attributes of cocoa beans and
related products could be influenced not just by bitter compounds but also by compounds that modulate this taste attribute.

In summary, the chemical modification of a dietary bitter compound by common thermally induced food reactions was reported to modulate this taste attribute. The role of reaction products of taste compounds on the flavor properties of foodstuffs is not well understood; the study provides a novel basis to develop food processing strategies for flavor optimization by suppressing or enhancing specific taste attributes of interest.
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Figure 2.1 Structure and key NOESY correlations of the successfully isolated Maillard-catechin products
Figure 2.2 $^1$H spectrum of Compound 2.
Figure 2.3 Mean concentrations of Compound 2 and caffeine in roasted and unroasted cocoa. (Error bars represent standard deviation from duplicate extraction.)
Figure 2.4 Proposed mechanism for generating the spiro-C-glycosides
Table 2.1 Maillard-Catechin Model Reaction under Low Moisture Condition $^a$

<table>
<thead>
<tr>
<th>Model System</th>
<th>Reactant (mMoles)</th>
<th>Hexose</th>
<th>Pentose</th>
<th>Amino Acid</th>
<th>Phenolic Catechin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>glucose</td>
<td>galactose</td>
<td>xylose</td>
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<tr>
<td>A</td>
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<td></td>
<td></td>
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<tr>
<td>I</td>
<td>5</td>
<td></td>
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$^a = 10\%$ moisture, $200\degree C$ for $15\text{min}$
Table 2.2 Assignment of 1H and 13C of Compound 2 generated from glucose-glycine-catechin model reaction \(^{a,b}\)

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<tr>
<th>Assignment</th>
<th>(\delta_H)</th>
<th>(\delta_C)</th>
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<td>glucose</td>
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<td>1''a</td>
<td>3.42 (d, J=16.8 Hz)</td>
<td>33.8</td>
<td>C5, C6, C7, C2’’, C3’’</td>
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<tr>
<td>1''b</td>
<td>2.89 (d, J=16.8 Hz)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2''</td>
<td>/</td>
<td>120.8</td>
<td>H1’’a, H1’’b, H3’’</td>
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<td>3’’</td>
<td>4.18 (d, J=3.5 Hz)</td>
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<td>152.5</td>
<td>H4a, H4b, H1’’a, H1’’b</td>
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<td>104.6</td>
<td>H1’’a, H1’’b</td>
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<tr>
<td>2’’</td>
<td>6.80 (d, J=1.4 Hz)</td>
<td>115.2</td>
<td>C1’, C3’, C4’, C6’</td>
<td></td>
</tr>
<tr>
<td>3’’</td>
<td>/</td>
<td>146.3</td>
<td>H2’, H5’</td>
<td></td>
</tr>
<tr>
<td>4’’</td>
<td>/</td>
<td>146.3</td>
<td>H2’, H5’, H6’</td>
<td></td>
</tr>
<tr>
<td>5’’</td>
<td>6.72 (d, J=8.4 Hz)</td>
<td>116.2</td>
<td>H6’</td>
<td>C4’, C6’, C1’, C3’</td>
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<tr>
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<td>6.68 (dd, J=8.4, 2.1 Hz)</td>
<td>120.0</td>
<td>H5’</td>
<td>C1’, C5’, C2’, C4’</td>
</tr>
</tbody>
</table>

\(^{a}\) \(^{1}\)H NMR experiment was conducted on 700 MHz cryoprobe. Samples were dissolved in CD\(_3\)OD.

\(^{b}\) The identified structure is matched with reference.
Table 2.3 Mean bitterness intensity ratings of 0.04% caffeine solution with and without Maillard-catechin (MC) compounds at a 7:1 mass ratio

<table>
<thead>
<tr>
<th>Samples (7:1 Mass Ratio)</th>
<th>Bitterness ratings$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine : MC 5</td>
<td>3.95$^a$</td>
</tr>
<tr>
<td>Caffeine : MC 6</td>
<td>3.77$^a$</td>
</tr>
<tr>
<td>Caffeine : MC 1</td>
<td>3.60$^a$</td>
</tr>
<tr>
<td>Caffeine : MC 3</td>
<td>3.51$^a$</td>
</tr>
<tr>
<td>Caffeine : MC 4</td>
<td>3.45$^a$</td>
</tr>
<tr>
<td>Caffeine : MC 8</td>
<td>3.40$^a$</td>
</tr>
<tr>
<td>Caffeine</td>
<td>3.31$^a$</td>
</tr>
<tr>
<td>Caffeine : MC 7</td>
<td>3.06$^a$</td>
</tr>
<tr>
<td>Caffeine : MC 2</td>
<td>3.05$^a$</td>
</tr>
</tbody>
</table>

$^1$ Bitterness ratings are based on 15-point rating scale; a rating of 2, 3.5 and 5 corresponded to 0.03 %, 0.04 % and 0.08% caffeine solution, respectively; Mean ratings with letter superscripts in common do not differ significantly (p>.05)

$^2$ Adapted from Hofmann et al.97
Table 2.4  Mean bitterness intensity ratings of 0.04% caffeine solutions with and without Maillard-catechin (MC) Compounds 2 and 7 at a 7:2 mass ratio

<table>
<thead>
<tr>
<th>Test Samples</th>
<th>Bitterness rating(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(7:2 Mass Ratio)</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>3.50(^a)</td>
</tr>
<tr>
<td>Caffeine : MC 7</td>
<td>3.19(^{ab})</td>
</tr>
<tr>
<td>Caffeine : MC 2</td>
<td>2.75(^b)</td>
</tr>
</tbody>
</table>

\(^1\) Bitterness ratings are based on 15-point rating scale; a rating of 2, 3.5 and 5 corresponded to 0.03 %, 0.04% and 0.08% caffeine solution, respectively; Mean ratings with letter superscripts in common do not differ significantly (p>.05)
Chapter 3 Identification of Bitter Compounds in Extruded Corn Puffed Products

Introduction

Dietary Guidelines of Americans recommend making half of all grains consumed be whole grains and corn is one of the principal grains used for breakfast cereal based foods.\textsuperscript{115} Whole grain corn consumption is associated with potential health benefits, e.g. regulate body-weight, reduce the risk of chronic disease, lower blood glucose and so on.\textsuperscript{116–118} Extrusion cooking is one of the major thermal processing techniques used for the production of breakfast cereals such as puffed-based whole grain corn products. Many studies have shown that extrusion cooking has significant effects on the flavor profiles of food products.\textsuperscript{54} Non-enzymatic browning and lipid oxidation are considered important reaction pathways that influence overall volatile flavor profile of extruded corn puffed products.\textsuperscript{53}

Even though the consumption of whole grain food is associated with a healthy lifestyle, less than 5% of Americans consume the recommended intake (48 g/day) and up to 20% consume no whole grain products.\textsuperscript{93} Food choices are mainly driven by flavor, cost and convenience. Negative flavor attributes associated with whole grains have been reported as one of the most influential factors limiting consumption.\textsuperscript{119} Higher perceived bitterness in whole grain products was suggested to contribute to poorer consumer acceptability especially for children who have lower tolerance to bitterness.\textsuperscript{6} According to a recent study on cereal consumption, families with children consume much more breakfast cereals than those without any children, suggesting children are a key driving force of purchasing.\textsuperscript{115}

Traditionally, food producers have incorporated flour mixing (51% whole grain and 49% refined flour) and bitterness masking ingredients (sugar and salt) to their formulation in order to improve
the flavor profile. To accommodate the taste preference of children or even adults, almost all of the commercially available whole grain cereals are coated with sugar or salt to mask the bitterness. An important step to improve the palatability of whole grain cereals is to identify the origin of bitterness. Numerous flavor studies on whole grain foods have focused on the aroma compounds generated during extrusion\textsuperscript{120} but less is known regarding the effect of extrusion on taste attributes.

Therefore the objective of this study was to characterize the changes in the taste profile of puffed corn cereal made with whole-grain and refined corn flour and quantitatively monitor the taste compounds from the flour to the finished products in order to better understand their formation pathways.

**Materials and Methods**

**Materials and chemicals.** Trisodium phosphate, calcium carbonate, sodium chloride, d4-methanol, methylparaben, HPLC grade methanol were purchased from Sigma Aldrich (St. Louis, MO). Innovasure refined corn flour and Maizewise whole grain corn flour samples were received from Cargill, Inc. (Wayzata, MN).

**Twin-screw corn extrusion.** Extrusion conditions were designed to yield uniform cell structure throughout each puff.\textsuperscript{52,54} Briefly, extrusion processing was carried out using a Buhler DNDL-44 twin-screw extruder. Two formulations were designed: the refined corn flour formulation and the whole grain corn flour formulation. The refined corn flour formulation consisted of 1000 g (100%) Cargill Innovasure refined corn flour with 10 g (1%) trisodium phosphate, 10 g (1%) calcium carbonate and 10 g (1%) sodium chloride. The whole grain corn formulation consisted of 480 g (48%) Cargill Innovasure refined corn flour and 520 g (52%) Maizewise whole grain
corn flour with 10 g (1%) trisodium phosphate, 10 g (1%) calcium carbonate and 10 g (1%) sodium chloride. The ingredients and the flour were added to a ribbon mixer and mixed for 10 min. The flour mixture was introduced to the extruder and processed using the following extrusion parameters: computer controlled shaft speed of 350 rpm, measured die pressure of 10.1 ± 0.5 bar, die temperature of 160 ± 1 °C, dry material throughput of 50.8 ± 0.1 kg/hr, water addition of 7 kg/hr water, and a cutter speed of 1200 rpm resulting in 1/4 inch puffs. Due to differences in the physical and chemical characteristics of the refined and the whole grain flour mixes, the refined corn flour formulation showed an increased shaft torque of 224 NM over the whole grain corn flour formulation which had a shaft torque of 215 NM; the specific mechanical energy for the refined corn flour formulation was 164 kw/h while for the whole grain corn flour was 159 kw/h. All other parameters were constant across both formulations. The resulted puffed products were dried on a liquid air bed, packaged in high-density polyethylene bags and stored at -40 °C prior to analysis.

**Solvent extraction of corn puffed products.** Corn puffs (300 g) were ground in a blender for 2 min, and then extracted using a 75% ethanol-25% water solution (500 ml). Extraction was performed at room temperature for 3 h and was repeated three times. The extracts were pooled and centrifuged at 4000g for 15 min at 4 °C. The resulting precipitate (Figure 3.1, FI) was collected and the solvent-removed for sensory evaluation. The supernatants were combined, and the ethanol was removed using rotary evaporation and the aqueous mixture was then frozen and freeze-dried twice to yield fraction II (Figure 3.1, FII).

**Ultrafiltration.** Fraction II was dissolved in 20% ethanol aqueous solution and subsequently underwent ultrafiltration using Amicon 8200 ultrafiltration cells (Millipore, Bedford, MA) with cutoff membrane at 3 kD, under a nitrogen pressure of 200 kPa. Upon completion, the membrane
was rinsed by passing through deionized water. The resulting permeate and retentate, underwent rotary evaporation separately for the removal of ethanol and were then frozen and freeze-dried. The bitter intensity of each fraction was determined and the primary bitter fraction FII-Uf1 was then selected for additional purification by LC fractionation.

**First dimensional liquid chromatography fractionation.**

The dried bitter fraction FII-Uf1 was dissolved in 95% water-5% ethanol solution (40 ml). Aliquots were filtered through a 4.5 μm hydrophilic syringe filter and then separated by HPLC using a preparative RP C-18 column (21.2 x 250 mm, pursuit 5, Varian, USA). Chromatography was performed with flow rate of 10 ml/min and a gradient starting with a 95% of aqueous formic acid (0.1%, pH 3) and ethanol with formic acid (0.1%, pH 3). Initial conditions were held for 5 min, then linearly increased the ethanol content to 50% within 20 min, and then to 100% within 1 min, finally maintained the ethanol content for another 10 min. The eluent was collected in 18 fractions from 3.5 to 21.5 min in 1 min intervals (**Figure 3.2**). Each fraction was evaporated and freeze-dried twice to remove the solvent. Subsequently each fraction was rehydrated in 2 ml of water (dosage level was 30 g puffed cereal) and a trained sensory panel evaluated the bitterness intensity of each fraction.

**Second dimensional liquid chromatography fractionation.** The HPLC fractions from the first dimension (**Figure 3.2**) with the highest bitterness intensity (FII-Uf1-10, 11, 13 and 16) were subsequently further fractionated using a Zorbax Bonus-RP column (21.2 x 100 mm, 5 μm), and a mobile phase consisting of methanol and water at a flow rate of 10 ml/min. Chromatography was performed using a gradient starting with 95% water and 5% methanol. Initial conditions were held for 3 min, then linearly increased the methanol content to 60% within 25 min, and then to 100% within 1 min, finally maintained the methanol content for another 4 min. Methanol was
then decreased to 5% within 1 min and was held there for 4 min. Each isolate was collected into 16 fractions (shown in Figure 3.3) and the solvent was removed under vacuum and resulting aqueous solutions were freeze-dried twice. The resulting residues obtained were dissolved in water for sensory evaluation of bitterness.

**LC-MS Accurate Mass.** Bitter isolates were analyzed using a Waters Xevo G2 ToF MS equipped with electrospray ionization (ESI) probe and coupled with a 2D UPLC ACQUITY iClass (Waters, USA). Highly accurate mass acquisition of the ion of interest was performed by chromatography injection using reserpine as internal standard. The analyte (2 µL) was injected on a WATERS ACQUITY UPLC BEH C18 1.7 µm maintained at a temperature of 30 °C. Chromatography was performed with flow rate at 0.3 ml/min starting with a 95% of aqueous 0.1% formic acid and methanol, then increasing the methanol content to 95% within 6.45 min, finally maintaining the methanol content for another 1 min. Elemental composition prediction was based on < 5 ppm mass accuracy and > 50% i-Fit confidence.

**NMR.** Spectra were acquired with a Bruker Zod 850 spectrometer. Data processing and analysis was performed using MestRenova and Top spin 2.1 software.

*Purity and Spectra of Bitter Compounds.* The purity of chaenorpine, coumaryl-spermidines, terrestribisamide was 90%-95% based on $^1$H NMR.

**L-Tryptophan.** LC-TOF/MS C$_{11}$H$_{12}$N$_2$O$_2$; LC-MS (ESI$^+$), m/z = 205.0977 (-2.4 ppm). NMR spectra ($^1$H, $^{13}$C) were as previously reported by Yamaji *et al.*$^{121}$

**Chaenorpine.** LC-TOF/MS C$_{28}$H$_{36}$N$_4$O$_4$; LC-MS (ESI$^+$), m/z = 491.2658 (0.4 ppm). The NMR assignment of chaenorpine was listed in Table 3.1., which matched to those reported.$^{122,123}$
N¹, N⁵-Di-[(E) p-coumaryoyl]-spermidine LC-TOF/MS C₂₅H₃₁N₃O₄; LC-MS (ESI), m/z=436.2233 (-0.7 ppm). The ¹H NMR is attached in Figure 3.4. NMR spectra (¹H, ¹³C) were as previously reported.¹²⁴,¹²⁵

*Terrрестribisamide.* LC-TOF/MS C₂₄H₂₈N₂O₆; LC-MS (ESI), m/z=439.1868 (-0.2 ppm). ¹H NMR (850 MHz, CD₃OD): δ 7.44 (d, 2H, J=15.6 Hz, H-C(8/8’)), 7.12 (d, 2H, J=2.0 Hz, H-C(2/2’)), 7.03 (dd, 2H, J=8.2, 2.0 Hz, H-C(6/6’)), 6.79 (d, 2H, J=8.2 Hz, H-C(5/5’)), 6.43 (d, 2H, J=15.7 Hz, H-C(7/7’)); 3.88 (s, 2H, H-C(12/12’)), 3.36-3.19 (m, H-C(10/10’), 1.66-1.60 (m, H-C(11/11’)). ¹³C NMR (212.5 MHz, CD₃OD): 169.2 (C-9/9’), 149.8 (C-4/4’), 149.3 (C-3/3’), 142 (C-8/8’), 128.2 (C-1/1’), 123.2 (C-6/6’), 118.6 (C-7/7’), 116.4 (C-5/5’), 111.4 (C-2/2’), 56.3 (C-12/12’), 40.2 (C-10/10’), 28 (C-11/11’). Structure matched spectra reported by Wu et al. and Iwasa et al.¹²⁶,¹²⁷

**LC/MS/MS - Quantification of Bitter compounds**

**Saliva Analysis.** Three subjects (1 male and 2 females; ages 25-35) were instructed to chew 2 grams of extruded corn cereals for 30 s without swallowing and then expectorate in a 15ml Eppendorf centrifuge tube. Saliva samples were then centrifuged at 4000 g at 4°C for 20 min and the top layer (1 ml) was collected and underwent solid phase extraction (SPE) with C18 cartridge (1 g). The cartridge was preconditioned by 2 ml methanol. After saliva sample is loaded, elution was performed with 70% methanol aqueous solution (4 ml) which was then filtered by 0.22 um Nylon filter tip and analyzed via LC/MS/MS for quantification.

**Corn puffed sample analysis.** Corn puffed samples (10 g) were ground and extracted using 75% ethanol aqueous solution (20 ml x 2). The combined extracts were underwent rotary evaporation to remove ethanol and the resulting aqueous solution (5 ml) was collected and underwent SPE with a C18 cartridge (2 g) using the same SPE condition as described in Saliva Collection.
session. Elution was performed with 70% methanol aqueous solution (6 ml) which was then filtered by 0.22 um Nylon filter tip and analyzed via LC/MS/MS for quantification.

**LC/MS/MS analysis.** Quantification was conducted on a Waters ACQUITY UPLC system interfaced with a Quattro Premier XE™ triple quadrupole mass spectrometer (Waters, Milford, MA, USA). Analyte (2 µL) was injected on an ACQUITY UPLC BEH C18 1.7 um column (2.1 mm × 50 mm) set at 30°C. The mobile phase was maintained at a flow rate of 0.3 ml/min using binary solvent system of 0.1 % formic acid in water and 0.1 % formic acid in methanol. Elution gradient started at 10% methanol, then linearly increased to 50% methanol in 6.45 min and maintained for 1 min. The Quattro Premier XE™ mass spectrometer was equipped with an electrospray ionization probe and the operational parameters in positive ion mode were as follows: source temperature 110 °C; desolvation temperature 350 °C; and capillary voltage 30 v. Data were collected in multiple-reaction-monitoring (MRM) mode. The ion transitions for the five bitter compounds along with the cone voltages (CV) and collision energies (CE) were as follows: tryptophan: 205→188, CV: 30v, CE: 25v; coumaryl-spermidine: 438→292, CV: 30v, CE: 20v; terestribisamide: 441→265, CV: 30v, CE: 15v; chaenorpine: 493→265, CV: 30v, CE: 25v. Methylparaben (1 M) was used as internal standard: 153→121. All samples were analyzed in duplicate. The quantification results were evaluated by a pairwise comparison statistical analysis with level of significance was set at 0.05.

The recovery efficiency of each of the bitter compound was calculated by standard addition techniques, using a control sample as well as a sample spiked with known amounts of bitter compounds. The total free bitter markers were calculated by using a six point calibration standard curve and the recovery rates were tryptophan (95%), coumaryl-spermidine (104%), chaenorpine (114%), and terestribisamide (102%), respectively.
Sensory analysis

Precaution Taken for Sensory Analysis of Food Fractions and Taste Compounds. All fractions from food samples for taste analysis, prior to sensory testing, were liberated from solvent by rotary evaporation and were subsequently freeze-dried twice. GC/MS or $^1$H NMR revealed that fractions treated by the above protocol are free of solvents and suitable for sensory analysis. Approval of the sensory evaluation protocol was granted by the Ethics Committee, University of Minnesota (IRB #1505E70948).

Sensory screening of fractions. Four experienced descriptive analysis panelists (staff and students from the Flavor Research and Education Center, St. Paul, MN) evaluated the fractions for bitterness intensity. Bitterness intensity was rated on a line scale from 0 to 10. The following caffeine references were provided to panelists: 0.03, 0.08, 0.12 % w/w in water corresponding to bitterness intensities of 2, 5 and 10, respectively. Fractions were presented at room temperature and nose clips were used during evaluation to eliminate aroma sensory inputs.

Evaluation of bitterness intensity of identified compounds at saliva concentration. Using a pairwise ranking test, thirteen trained panelists (ages 23-45, 6 males and 7 females, staff and students from the Flavor Research and Education Center, St. Paul, MN) evaluated the bitterness intensity of aqueous solutions spiked with the identified bitter compounds at concentrations reported in saliva (four samples were tested: tryptophan 236.8 mg/L, N$^1$,N$^5$-di-[(E)p-coumaryl]-spermidine 245.5 mg/L, terrestribisamide 250.6 mg/L, chaenorpine 150.9 mg/L). Each panelist was presented with a total of 8 pairs of samples over a period of two sessions (one hour break between sessions). The samples were presented in a randomized balanced order in cups coded with 3-digit codes. Panelists were asked to pipette 1 mL sample onto the tongue while wearing a
nose clip and select the sample that is more bitter within each pair. The rank sum for each sample was computed by adding the sum of the ‘less bitter’ frequencies to twice the sum of the ‘more bitter’ frequencies. The results were evaluated by a Friedman-type statistical analysis with level of significance was set at 0.05.

Results and Discussion

Identification of key bitter compounds in extruded corn puffs

The influence of refined versus whole grain flour on the taste attributes of extruded corn puffed products was investigated. A comparative LC-taste profile analysis for both products was conducted. The taste fingerprint was generated from a taste-active isolate obtained by an aqueous ethanol extraction followed by ultrafiltration (Figure 3.1) that was further fractionated by HPLC and subsequent sensory evaluation (Figure 3.2). Two taste attributes, bitter and salty were observed, along with the mouthfeel attribute, astringency (data not shown). Direct comparison of the taste profiles between refined and whole grain extruded products revealed that the only difference was the perceived bitterness intensity (see fractions #10, #11, #13 and #16, Figure 3.2).

As the chemical composition of a food matrix is complex, a second-dimension separation is typically necessary to achieve the purity required to identify the taste compounds of interest. The corresponding second-dimension chromatograms for each bitter fraction from the first-dimension separation (#10, #11, #13, #16, Figure 3.2) are shown in Figure 3.3 (a), (b), (c), (d), respectively. Based on further fractionation and sensory evaluation, the bitter regions were identified (corresponding peaks are shaded).

For fraction #10 (2nd dimension shown in Figure 3.3 (a)), L-tryptophan was identified as the bitter compound based on comparison of authentic compound using LC/MS/MS and 1H NMR.
Tryptophan has been widely used as an indicator of protein quality for corn products. The tryptophan content in cereals is important especially for people consider cereals as major source of protein. However L-tryptophan has been identified as a major bitter compound that is responsible for the bitterness in both the crust and crumb of whole wheat bread crust and crumb.

Evaluation of the compound identity in the bitter fraction #11 (2nd dimension in Figure 3.3 (b)) identified chaenorpine as the bitter compound. The detailed NMR assignment is shown in Table 3.1. $^1$H-NMR spectrum shows two resonances at 6.5 and 6.0 ppm with coupling constant $J = 11.9$ Hz supports the presence of cis-alkenyl hydrogens. H-16, H-17 and H-20 at δ 6.88, 6.92 and 7.15 ppm with characteristic coupling patterns represents 1,2,4-tri-substituted aromatic ring. For another four protons in the aromatic region δ=7.29 ppm and 6.94 ppm, the integration and the coupled two sets of protons reveals a para-substituted aromatic ring. Aliphatic protons are in the region of δ 2.5-3.8 ppm and 1.4-2.0 ppm, One of the two carbon signals at low magnetic field, C-12, has HMBC correlation with the alkenyl hydrogens, suggesting an amide group is connected with a cis-alkene. Both of the low field carbons have correlations with aliphatic protons. The unsaturation number of the molecule is 13, which comes from two aromatic rings, two carbonyls, one alkene group, and two macro-rings. Hu and Hesse first reported this compound in the plant *Chaenorhinum minus* (L.) Willk. Et Lge. (*Scrophulariaceae*). To the best of our knowledge this is the first time that chaenorpine is reported to contribute to the bitterness of corn products.

For fraction #13 (purified in Figure 3.3 (c)), N$^1$, N$^5$-dicoumaryl-spermidine was identified as the bitter compound. The $^1$H NMR spectrum is shown in Figure 3.4. Two coumaric acid moieties were incorporated in the structure: Two pairs of trans-alkenyl protons are connected to para-aromatic rings. The distinctive chemical shifts of amide carbonyl carbon are observed at δ 169.5
and 160.9 ppm. Based on the chemical shifts, H-2, H-4, H-6, and H-9 are attached to nitrogen, among which H-9 links to an amine group (lowest $\delta$) whereas H-2, H-4, H-6 are attached to the amide group (higher $\delta$). Hence the coumaric-acid moieties are connected to the aliphatic chains via N$^1$ and N$^5$ instead of N$^{10}$. Due to the lack of free rotation of the N-C bond in the secondary amide (N$^5$), a pair of conformers was observed as 2:1 ratio, for $\delta_{H4}$ in the major conformer is larger than $\delta_{H6}$ and vice versa for the minor conformer. This is because the protons that are closer to the carbonyl oxygen have higher chemical shift. Coumaryl-spermidine has been found in corn but the taste activity was unknown. They are major pigments in plants and photoisomerization would occur with alteration of the alkene geometry.$^{130}$ They are pollen fertility markers and known to function as protection agents against pathogenic bacterial, virus, and fungus.$^{130}$ Finally, terrestribisamide was found as the bitter compound in fraction #16 (secondary dimension in Figure 3.3(d)). Because the number of carbons in the molecular formula is twice as the carbon signals shown in the $^{13}$C spectrum; the molecule has $C_2$ symmetry. Similar to chaenorpine, the protons at $\delta$ 7.12, 7.03, and 6.79 ppm have characteristic coupling constants as 1,2,4-tri-substituted aromatic protons. The coupled H-7 and H-8 have a much larger coupling constant as $J=15.6$ Hz, suggesting a trans-alkenyl protons in the structure. The alkenyl protons are connected with an amide group based on HMBC spectrum. To our knowledge this is the first time this compound is reported to have a bitter taste.

The bitter compounds chaenorpine, N$^1$, N$^5$-dicoumaryl-spermidine, and terrestribisamide are hydroxycinnamic acid amides (HCAA), which are a widely distributed group of plant metabolites. The spermidine amides were found as derivatives of hydroxycinnamic acids (i.e., coumaric, ferulic, caffeic, or sinapic acid). The hydroxycinnamic derivatives have been observed as linear conjugates (coumaryl-spermindines and terrestribisamide) or macrocyclic lactams,
especially in 13- or 17- membered lactam rings (chaenorpine). HCAA has been reported to constitute a major fraction of phenolic acids in corn. \(^{61}\)

**Quantification of bitter compounds in extruded corn puffs**

All of the identified bitter compounds are endogenous molecules in corn and processing degrades them albeit they are responsible for the bitterness of the final products (Figure 3.5). Also, whole grain samples have significant higher level of the bitter compounds compared to refined grain samples, which correlates well with the bitter profile of each fraction shown in Figure 3.2. L-tryptophan was found the highest in whole grain flour and thermal process significantly lowers the tryptophan level in the final products, although the decrease of processing on refined samples are not obvious, which indicates that tryptophan in whole grains is likely consumed during extrusion processing, as expected by participating in Maillard reaction or is thermally degraded. Similar trend applies to chaenorpine indicating degradation occurs during extrusion. For N\(^1\), N\(^5\)-dicoumaryl-spermidine and terrestribisamide, the thermal impact on degradation is significant for both refined and whole grain samples found to be higher in concentration in the flour than the product suggesting they are native to the flour compounds and degrade during processing. It is possible that the free amine group of N\(^1\), N\(^5\)-dicoumaryl-spermidine makes it moderately reactive with sugars or other carbonyls in flour thus it is more vulnerable to the thermal degradation.

**Sensory evaluation of bitter compounds - pairwise ranking**

Flavor compounds need to be released from a food matrix to be perceived. The release of taste compounds from food during mastication is complex and matrix dependent. Additionally, saliva extraction is typically less efficient compared to organic solvent extraction due to the hydrophobicity of many bitter compounds. Hence a thorough understanding of saliva extraction
efficiency and oral concentration after mastication is advantages to understand the sensory contribution of taste active compounds to the overall taste profile. In the current study, the concentrations of the bitter compounds were monitored in the expectorated saliva of three panelists after mastication. A sensory panel ranked recombination aqueous solutions of each bitter compound at the reported saliva concentrations (shown in Table 3.2). Chaenorpine had the highest contribution of bitterness, followed by coumaryl-spermidine and terrestribisamide. Tryptophan had the lowest bitter rating compared to the other identified bitter compounds. Based on the quantified saliva concentration and the concentration determined in food samples after solvent extraction, the efficiency of bitter compound extraction in saliva after mastication was found to be in the range between 11.9 % and 27.8% (Table 3.2); terrestribisamide being the least extracted at 11.9%, whereas N^1, N^5-Di-[(E)-p-coumaryl]-spermidine being the highest yield at 27.8%. Owing to the lack of quantitative extraction efficiency of the bitter compounds into the saliva from food matrix, evaluation of the perceived bitterness of compounds based on the concentration of food is with error. Thus this study provides novel perspectives to connect sensory relevance with instrumentation data.

In summary, key bitter compounds were identified in extruded corn puffed cereals. All of the four bitter markers are endogenous to flour and were reported to degrade during extrusion cooking. The identified key bitter compounds are higher in concentration in whole grain samples compared to refined samples. The noted degradation of bitter compounds during extrusion indicates optimizing processing strategies can be applied to decrease negative bitter attributes for flavor optimization.
Table 3.1 Assignment of 1H NMR of Chaenorpine.\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>$\delta_C$</th>
<th>$\delta_H$</th>
<th>COSY</th>
<th>HMBC</th>
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<tbody>
<tr>
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<td>24, 26, 28a</td>
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<tr>
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<td>47.5</td>
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<td>4a</td>
<td></td>
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<tr>
<td>4</td>
<td>28.6</td>
<td>a: 1.94-1.87 (m); b: 1.84-1.74 (m)</td>
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<td></td>
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<tr>
<td>5</td>
<td>51.7</td>
<td>a: 3.30-3.25 (m); b: 3.09 ($J = 12.6, 7.3, 3.3$ Hz)</td>
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<tr>
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<td>49.9</td>
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<tr>
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<td>a: 1.61-1.58 (m); b: 1.40-1.33 (m)</td>
<td>10</td>
<td>10a</td>
</tr>
<tr>
<td>10</td>
<td>50.9</td>
<td>a: 3.65 (ddd, $J = 14.4, 8.9, 6.1$ Hz); b: 3.06-2.99 (m)</td>
<td>9</td>
<td>--</td>
</tr>
<tr>
<td>12</td>
<td>171.3</td>
<td>--</td>
<td>--</td>
<td>13, 14, 10a, 33a, 33b</td>
</tr>
<tr>
<td>13</td>
<td>121</td>
<td>5.93 (d, $J = 12.8$ Hz)</td>
<td>14</td>
<td>--</td>
</tr>
<tr>
<td>14</td>
<td>134.1</td>
<td>6.43 (d, $J = 12.8$ Hz)</td>
<td>13</td>
<td>13, 16, 20</td>
</tr>
<tr>
<td>15</td>
<td>128.1</td>
<td>--</td>
<td>--</td>
<td>13, 17</td>
</tr>
<tr>
<td>16</td>
<td>129.8</td>
<td>6.92 (dd, $J = 8.5, 2.2$ Hz)</td>
<td>17, 20</td>
<td>14, 20</td>
</tr>
<tr>
<td>17</td>
<td>118.1</td>
<td>6.88 (d, $J = 8.3$ Hz)</td>
<td>16</td>
<td>--</td>
</tr>
<tr>
<td>18</td>
<td>151.2</td>
<td>--</td>
<td>--</td>
<td>16, 17, 20</td>
</tr>
<tr>
<td>19</td>
<td>144.3</td>
<td>--</td>
<td>--</td>
<td>17, 20</td>
</tr>
<tr>
<td>20</td>
<td>119.8</td>
<td>7.15 ($J = 2.2$ Hz)</td>
<td>16</td>
<td>14, 16</td>
</tr>
<tr>
<td>22</td>
<td>158.6</td>
<td>--</td>
<td>--</td>
<td>(23, 27), (24, 26)</td>
</tr>
<tr>
<td>23, 27</td>
<td>117.7</td>
<td>6.94 (d, $J = 8.6$ Hz)</td>
<td>(24, 26)</td>
<td>(23, 27), (24, 26)</td>
</tr>
<tr>
<td>24, 26</td>
<td>128.4</td>
<td>7.29 (d, $J = 8.9$ Hz)</td>
<td>(23, 27)</td>
<td>(23, 27), (24, 26)</td>
</tr>
<tr>
<td>25</td>
<td>134.6</td>
<td>--</td>
<td>--</td>
<td>1, (23, 27), 28a</td>
</tr>
<tr>
<td>28</td>
<td>39</td>
<td>a: 2.83 (dd, $J = 14.3, 3.7$ Hz); b: 2.75 (dd, 14.3, 4.0 Hz)</td>
<td>1</td>
<td>--</td>
</tr>
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<tr>
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<td>---</td>
</tr>
<tr>
<td>29</td>
<td>172.6</td>
<td>--</td>
<td>--</td>
<td>1, 28, 31</td>
</tr>
<tr>
<td>31</td>
<td>38.4</td>
<td>a: 3.13 (dd, J = 13.5, 9.1, 2.6 Hz); b: 2.59 (dd, J = 13.4, 8.7, 8.7 Hz)</td>
<td>32</td>
<td>--</td>
</tr>
<tr>
<td>32</td>
<td>26.8</td>
<td>a: 1.84-1.74 (m); b: 1.66-1.60 (m)</td>
<td>31, 33</td>
<td>--</td>
</tr>
<tr>
<td>33</td>
<td>44.9</td>
<td>a: 3.06-2.99 (m); b: 2.80 (dd, J = 13.2, 11.6, 3.7 Hz)</td>
<td>32</td>
<td>10a, 31</td>
</tr>
</tbody>
</table>

\[a\] The NMR assignment of chaenorpine is matched to previous reports.\(^{(122, 123)}\)
Table 3.2. Sensory ranking of bitterness at oral saliva concentrations and percent extraction during mastication

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ranking Sum (HSD=13.34)$^{1,2}$</th>
<th>Saliva Concentration (mg/L)</th>
<th>Extraction Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Tryptophan</td>
<td>59 a</td>
<td>236.8</td>
<td>23.6</td>
</tr>
<tr>
<td>N$^{1}$, N$^{5}$-Di-[(E)-p-coumaroyl]-spermidine</td>
<td>82 bc</td>
<td>245.5</td>
<td>27.8</td>
</tr>
<tr>
<td>Terrestribisamide</td>
<td>75 b</td>
<td>250.6</td>
<td>11.9</td>
</tr>
<tr>
<td>Chaenorpine</td>
<td>94 c</td>
<td>150.9</td>
<td>25.2</td>
</tr>
</tbody>
</table>

$^{1}$ Letter in common is not significant difference.
$^{2}$ The higher the ranking sum, the more bitter
Figure 3.1. Sensory-guided fractionation methodology (BI = bitterness intensity rating of fraction; bitter scale 0-10).
Figure 3.2. 1st Dimension LC-Taste fractionation of a) refined and b) whole grain extruded corn puffed product. Fractions FII-UF1-(#1-#18) were collected from retention time 3.5-21.5 min in 1 min intervals.
Figure 3.3 Second dimension HPLC-UV of fraction #10 (a), #11 (b), #13 (c), #16 (d) from first dimension RPLC fractionation; highlighted regions were reported as main bitter region. The proposed structures of bitter compounds were attached with atom numberings.
Figure 3.4 1H spectrum of N¹, N⁵-dicoumaryl-spermidine conformers (major: minor=2:1)
Figure 3.4 Concentrations of \( L \)-tryptophan, chaenorpine, coumaryl-spermidine terrestribisamide in refined flour, refined product, whole grain flour and whole grain product. Letter in common is not significant difference. Significance was established at \( \alpha=0.05 \).
Chapter 4 Identification of a Novel Umami Compound in Potatoes and Potato Chips

“Confidential - international patent application in progress”

Introduction

Potato chips are a popular salted snack worldwide. As with most savory snacks, the flavor profile of potato chips is a key driver of consumption. A current goal of the food industry is to offer savory snacks to provide healthy choices for consumers by looking at reducing fat or salt. The manufacture of these reformulated products is challenged however by lowering the overall palatability of the food. For example, regular potato chips typically exhibit a more desirable flavor profile with higher consumer liking and acceptability when compared to their reduced fat counterpart. Moreover, an appropriate level of salt in a product has been found to positively influence the taste profile and enhance overall flavor intensity. Consequently, the successful development of lower fat or salt content snacks requires great technical effort to retain the positive flavor attributes.

Flavor studies on potato chips have been predominantly focused on aroma. Very little is known about the taste profile and how it might be affected by different processing treatments. A better understanding of the taste profile of potato chips can facilitate development of reformulation approaches as well as discovery of desirable tastants, such as compounds with umami attributes typically associated with fried potato
products. Additionally, with the increasing demand for reduced sodium alternatives, the discovery of novel food-derived umami enhancing compounds is highly desirable because they have been shown to improve overall flavor perception of savory foods and snacks.\textsuperscript{22,134}

Umami, described as savory or meaty, is one of the primary tastes.\textsuperscript{9} The pleasant savory taste has been discovered in a wide range of foods such as meat, seafood, soy sauce and some processed foods. As early as 1909, monosodium glutamate (MSG) was identified in seaweed as the key compound responsible for meaty and savory taste.\textsuperscript{135} Since then more molecules have been discovered that demonstrate umami characteristics. These compounds include small organic acids (e.g. succinic acid, aconitic acid, gluconic acid),\textsuperscript{136} L-amino acids (L-glutamic acid, L-aspartic acid, L-aromatic amino acids, \textsuperscript{137} peptides,\textsuperscript{138,139} nucleotide derivatives, as well as Maillard-reaction products.\textsuperscript{42} In addition to savory characteristics, these compounds have shown synergic taste effects.\textsuperscript{9} For example, a mixture of L-glutamate and purine-5’-ribonucleotides exhibits an additive effect on the umami taste intensity when compared to just L-glutamate or purine-5’-ribonucleotides alone. Studies have also revealed that overall potato flavor is boosted when umami enhancing compounds are added.\textsuperscript{22}

The main objective of this work was to investigate how different processing treatments might affect the taste profiles of potato chips. More specifically, the goal was to assess the influence of frying time on the taste compounds of potato chip samples in order to provide guidelines for product flavor optimization.
Materials and Methods

Chemicals: L-glutamic acid ≥99% (L-GA), D-glutamic acid ≥99% (D-GA), L-pyroglutamic acid ≥99% (L-PGA), α-solanine ≥95%, α-chaconine ≥95%, chlorogenic acid ≥95%, and D-quinic acid 98% were purchased from Sigma Aldrich, Inc (St. Louis, MO). D-pyroglutamic acid ≥98% (D-PGA) was obtained from TCI Co, Ltd (Portland, OR). HPLC grade ethanol and methanol, USP sodium hydroxide and sodium chloride were obtained from Fisher Scientific (Pittsburgh, PA).

Potato Chip Samples: Unsalted and salted potato chips fried for either 170s (termed shorter fry time potato chip or ‘SFT-PC’) or 210s (termed regular fry time potato chip or ‘RFT-PC’) were obtained from Frito-Lay (Plano, TX). L- and D-MSG plus L- and D-MSpG were prepared using aqueous solutions of GA or PGA that were neutralized with 0.1N NaOH to yield a high purity powder liberated by freeze-drying (purity verify by LC-MS).

Taste and Astringency Profiling of Potato Chip Isolates and HPLC Fractions

Sample preparation. Unsalted potato chips (200g) fried for 170s (SFT-PC) and 210s (RFT-PC) were individually ground and extracted with hexane to remove fat (200 mL × 2). The hexane extracts for each sample were separately combined and solvent was removed under vacuum. The resulting isolate (Isolate I) was rehydrated, frozen and freeze dried twice to ensure food grade levels of hexane prior to taste activity screening. The defatted potato chips (Isolate II) were allowed to air dry in a fume hood and were further extracted (2 × 200 ml) using an ethanol: water mixture (1:1) for a total extraction time of 6 h (2 × 3 h). The aqueous ethanol solvent extracts from each sample were
separately pooled (Isolate III) and centrifuged. The supernatant was collected and the organic solvent was removed under vacuum. The resulting aqueous isolate was then loaded onto a C-18 solid phase extraction cartridge, and eluted with 70 % methanol aqueous solution (Isolate V). The resulting eluent isolate was subsequently filtered using a 0.22 um Nylon tip filter and stored at -80 °C prior to preparative LC fractionation (see Figure 4.1). Extracted potato chips (Isolate IV) were also collected for each sample separately in order to screen for residual taste activity. The solvent was removed under vacuum and potato chips were then frozen and freeze dried twice prior to tasting. When reconstituted in water, Isolates I and IV were found to be practically tasteless thus they are not the focus of the study. Isolate V (Figure 4.1) was found to be taste-active and was subsequently selected for HPLC fractionation and sensory evaluation.

Preparative HPLC Fractionation-Sensory Screening. Fractions were collected using a LC binary pump system (Shimadzu, MD) configured with a autosampler, a variable-wavelength UV-VIS detector at 254 and 220 nm, a fraction collector and a Zorbax RP-18 column (150 mm × 21.2 mm, Varian, USA). The sample injection volume was 5 mL and the binary mobile phase system used consisted of 100% water (solvent A) and 100% ethanol (solvent B), the column flow rate was 10 mL/min. The gradient elution started with 5% B (0-3.5 min), then linearly increased to 50% B (3.5-12 min), then increased to 100% B (12-13 min), then held at 100% B (13-16 min), then finally decreased to 5% B (16-16.5 min), and held at 5% B (16.5-20 min) in order to equilibrate to initial gradient conditions. Fourteen fractions (F1-F14) were collected (Figure 4.2) and screened for taste activity. Prior to sensory screening, the organic solvent was
removed under vacuum (rotary evaporation) and subsequently fractions were frozen and freeze-dried twice. The resultant powdered isolates from each fraction were rehydrated in 5 mL of water for tasting and initial sensory screening. The screening sensory session includes 4 panelists. The panelists rated the intensity of all taste attributes perceived as well as astringency using a categorical scale (threshold, weak, moderate, strong and extremely strong). Fractions with the highest observed intensities, namely those with moderate to strong ratings, were selected for further purification using secondary dimension HPLC fractionation. Second dimension fractionation was conducted using a Zorbax SB-AQ column (150 mm × 21.2 mm, Agilent, USA) and a binary mobile phase system consisting of 100% water (solvent A) and 100% ethanol (solvent B) at a flow rate of 10 mL/min. Three different taste attributes with moderate to strong intensities were identified among the first dimension fractions and were further separated in order to isolate and identify tastants of interest. Fractions F1 and F2 ([Figure 4.2](#)) were umami and were each further separated into ten additional fractions using the following mobile phase gradient conditions: 0% B (0-5 min) initially, then increased to 100% B (6-9 min), and finally held at 100% B (9-13 min). Fractions F3 and F4 ([Figure 4.2](#)) were sour and were each further separated into ten additional fractions using the following mobile phase gradient conditions: 0% B (0-3.75 min) initially, then increased to 2% B (3.75-10 min), then linearly increased to 100% B (10-11 min), and finally held at 100% B (11-13 min). Lastly, fractions F11-F14 ([Figure 4.2](#)) were bitter and were each further separated into twelve additional fractions using the following mobile phase gradient conditions: 10% B (0-4.49 min) initially, then linearly increased to 100% (4.5 min-16 min), then
held at 100% B (16-20 min), and finally decreased to 10% (20-21 min), and held at 10% B (21-26 min). The resultant fractions containing the corresponding tastants were subsequently analyzed by LC-MS-ToF and NMR analysis for structural characterization and identification.

**Accurate Mass Analysis (LC/MS-ToF).** The fractionated samples (≥ 1 mg each) were analyzed on a Waters 2D UPLC Acquity iClass system consisting of a binary solvent delivery module, autosampler, and column heater and coupled with a Xevo G2 QToF system (Waters, Milford, MA, USA). Injections were performed on Agilent Zorbax UPLC SB-Aq RRHD column (2.1 x 100 mm, 1.8 um) maintained at a flow rate of 0.3 ml/min using a binary solvent system of 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B). The elution gradient started at 5% B, linearly increased to 100% B (1-6 min), held at 100% B (6 min-7 min), and then held at 5% B (7-9 min). Mass spectrometric ionization conditions were as follows: desolvation temperature of 400 °C; source temperature of 110 °C; capillary voltage of 1.1 kV; leucine enkephalin was used as the lockspray mass calibrant. Samples were analyzed in both positive and negative mode, with a scan range of 50-1000 Da.

**Nuclear Magnetic Resonance Analysis**

The samples after accurate mass analysis were solvent-removed and rehydrated in Deuterium oxide. Measurements were performed on a Bruker 700 MHz spectrometer with TXI cryotube. Data were processed and analyzed with MestReNova 8.1. Deuterium oxide was used as the NMR solvent. The solvent residual peak of deuterium oxide at 4.80 was set as the internal reference.
Spectral data of taste active potato chip isolate. **L-PGA** \(\text{C}_5\text{H}_7\text{NO}_3\), 128.0351 (-2 ppm).

\(^1\text{H} \text{NMR (700 MHz, D}_2\text{O): } \delta 2.19 \text{ [ddd, } 1\text{H, } J = 13.0, 9.8, 6.3, 4.9 \text{ Hz, H-C(3a)}], 2.41 \text{ [ddd, } 1\text{H, } J = 17.4, 9.8, 6.3 \text{ Hz, H-C(4a)}], 2.45 \text{ [ddd, } 1\text{H, } J = 17.5, 9.5, 7.0 \text{ Hz, H-C(4b)}], 2.56 \text{ [ddd, } 1\text{H, } J = 13.2, 9.6, 9.6 6.9 \text{ Hz, H-C(3b)}], 4.40 \text{ [dd, } 1\text{H, } J = 9.4, 5.1 \text{ Hz, H-C(2)}. \text{13C NMR (175 MHz, D}_2\text{O): } \delta 27.5 \text{ [C-3], 32.3 [C-4], 59.1 [C-2], 179.7 [C-5], 185.0 [C-1]. The NMR data of PGA matches to the spectrum of the authentic compound.}

**D-Quinic acid** \(\text{C}_7\text{H}_12\text{O}_6\), 191.0560 ([M-H]⁻, 1 ppm). NMR spectrum matches to that of the authentic compound and published data.\(^89\) **Chlorogenic acid** LC-MS (ESI-) 353.0375 ([M-H]⁻, -3 ppm). The NMR data of chlorogenic acid matches to the spectrum of the authentic compound. **Chaconine** \(\text{C}_{45}\text{H}_{73}\text{NO}_{14}\), 850. 4960 ([M-H]⁻, 2 ppm);\(^67\) **Solanine** \(\text{C}_{45}\text{H}_{73}\text{NO}_{15}\) 866.4910 ([M-H]⁻, 3 ppm). The NMR spectrum matches to that of the authentic compounds.

**Analysis of Umami Compounds During Frying**

Russet potato and a potato slicer were purchased from local grocery store (Minneapolis MN). The potatoes were washed, peeled and sliced with a thickness of less than 0.1 inches. The potato slices were fried in vegetable oil (Crisco Pure All Natural\(^\circledR\)) at 173 °C for 170 s for reduced fat potato chips and 210 s for regular fat potato chips. The moisture content of the raw potato and potato chips was determined using the method from Ghadge, A. D. *et al* (Ghadge, Britton, Jayas, 1989); raw potato was 74.6%, low fat potato chips was 25.1%, and regular fat potato chips was 6.1%. Fat content was measured based on Krokida M.K. *et al.*,\(^140\) raw potato 0.17%, low fat potato chips 25.4%, and regular fat potato chips 30.1%.
Quantification of Taste Compounds and Sodium in Potato, Potato Chips and in Saliva During Potato Chip Mastication

Potato and Potato Chip Taste Extraction. Raw potato, salted SFT-PC, and salted RFT-PC were individually ground and extracted with an ethanol: water mixture (1:1) for three hours. The final extracts were centrifuged and the supernatant was run through solid phase extraction (C18 cartridge) and eluted with a 70:30 methanol: water solution for LC/MS/MS analysis.

Saliva Collection and Preparation. Three subjects were instructed to chew 2 g of Lay’s potato chips for 20 s without swallowing and then expectorate in a collection tube. This process was duplicated and both saliva samples for each subject were combined and centrifuged. The top layer (saliva liquid, 0.5 ml-1 ml) was subsequently collected and underwent SPE cleanup using a C18 cartridge (0.5 g). Elution was performed with a 70:30 methanol:water solution (4 ml) which was then filtered by a 0.22 um Nylon filter tip. The isolate was analyzed via LC/MS/MS for the quantification of taste compounds and by Atomic Absorption Spectrometer for the quantification of sodium.

LC/MS/MS analysis. Isolates from raw potatoes, potato chips and from human saliva collected after potato chip mastication were analyzed with a Waters ACQUITY UPLC system interfaced with a Quattro Premier XE mass spectrometer (Waters). Samples were diluted and injected on a Zorbax RRHD HILIC Plus column (2.1 x 150 mm, 1.8 um). The mobile phase was maintained at a flow rate of 0.35 ml/min using a binary solvent system of 0.1% formic acid in water (A) and acetonitrile (B) at isocratic 80% B for 10 min. The Quattro Premier XE mass spectrometer was equipped with an electrospray
ionization probe, and the operational parameters were positive ion mode, with desolvation temperature of 350 °C, source temperature of 110 °C, and capillary voltage of 3.0 kV. Data were collected in multiple-reaction monitoring (MRM) mode. The MRM methods were optimized by direct infusion of authentic compounds. The ion transitions for the compounds of interest were as follows: L-PGA and D-PGA (ESI⁺), m/z 130.05 → 112.01 and 130.05 → 101.99, cone 27 V, collision 9 V; L-GA and D-GA (ESI⁺), m/z 148 → 130 and 148 → 102, cone 30 V, collision 10 V; chlorogenic acid (ESI⁺), m/z 355→276 and 355→163; D-quinic acid (ESI⁻), m/z 191.02→126.93 and 191.02→110.93, cone 51 V, collision 21 V. The dwell time was 0.1s. Quantification was conducted by internal standard (methylparaben, 0.3 M) and an external five-point calibration curve of L-MSpG at 4×10⁻⁴-2×10⁻² g/L, D-MSpG at 10⁻³-4×10⁻² g/L, L-MSG at 10⁻⁴-5×10⁻³ g/L, D-MSG at 10⁻⁵-3×10⁻⁴ g/L, chlorogenic acid 10⁻³-0.4 g/L, D-Quinic acid at 10⁻³-0.2 g/L showed good linearity with regression coefficients ranging from 0.90-0.999. The quantification data for the panelists was averaged as the basis for future sensory study use.

**Sodium Analysis.** Saliva samples were diluted and analyzed using AAnalyst 800 (PerkinElmer Inc., Waltham, MA) equipped with K-Na Lumina hallow cathode lamp. Sodium standards were prepared with concentrations ranging from 0.2-20 mg/L and a seven-point calibration curve was prepared with good linearity and a regression coefficient of 0.999. The sodium level was averaged among the three panelists and provided the basis for the sensory recombination study.

**Sensory Analysis for Recombination and Application Study**
A panel of eight judges (4 males and 4 females) evaluated the salty and umami intensity of all the following test samples. Prior to evaluations, all panelists participated in 12 training sessions of approximately 1 hour over a period of 4 weeks. For all of the following sensory analysis, panelists were trained on a 10-point scale using a salty reference (5, 0.35% NaCl) and an umami reference (5, 0.225% MSG+0.2% L-MSpG+0.025% D-MSpG).

Water, unsalted crackers and apples slices were used as palate cleansers. An unsalted potato chip sample was used as a reference for potato chip flavor, intensity 5.

**Evaluation of umami compounds in aqueous models.** The panelists evaluated the salty and umami intensity of the compounds of interest (Table 4.1) in duplicate. All samples were prepared the morning of the evaluation by adding 0.35 g/L of each compound to a tasting solution containing 3.41 g/L NaCl. The concentration of D-MSpG in saliva (data from *Saliva Collection and Preparation* session) was chosen as the test concentration of each compound in order to evaluate the umami intensity of all compounds at the same concentration. Sodium level and pH can affect the umami perception, thus the sodium levels for all samples were adjusted to be the same as the sodium concentration quantified in saliva after mastication of 2 g of salted potato chips. The pH of each sample was adjusted to 6.5 ± 0.5 with 0.1 N NaOH. Samples were assigned three-digit codes and presented to panelists in randomized order. Panelists used PTFE droppers to dispense 2ml of solution on to the center of their tongue and were asked to rate the umami and salty intensity of the samples using a linear 10-point scale.
In the recombination study, the panel rated the salty and umami intensity of salted potato chips and an aqueous recombination model containing the umami compounds in Table 4.1 formulated at concentrations quantified in the saliva (Figure 4.7). For the aqueous model, panelists used PTFE droppers to dispense 2ml of solution on the center of their tongue. For the potato chip sample, 2 gram of crushed chips were provided in 1 oz. cups. The panelists were asked to masticate the chips for 20s and subsequently rate the intensity of the attributes of interest. For both the recombination model and the chips, panelists rated the umami and salty intensity using a linear 10-point scale.

*Time-intensity analysis of potato chips with and without the topical addition of the novel umami compound.* L- and D-monosodium glutamates were topically added to potato chips. Compounds were added at 2-fold the concentration of the amount quantified in the RFT-PC sample. For sample preparation, Frito Lay’s salted potato chips were divided into batches of 60.0 ±0.1g and placed in a polyethylene food-grade transparent bag (Ziploc). The chips were crushed to ≤ 0.5 mm in diameter. For the topical addition sample (treatment) 0.20g of L-monosodium pyroglutamate and 0.03g of D-monosodium pyroglutamate powder was applied topically on the 60g crushed potato chips. Both the control and treatment bags were sealed, flushed with nitrogen, and shaken at 285rpm for 1h (30 min each side). The coefficient of variation was monitored for the pyroglutamate topically applied and reported to be less than 5.5%.

The panelists were presented with three-digit coded samples in a randomized order. They were asked to masticate each potato chip sample (2g) for 20s and then swallow. Panelists rated the intensity of saltiness, umami and potato chip flavor at the following
Results and Discussion

Identification of taste active compounds

In order to investigate the influence of frying time on the taste attributes of potato chips, a comparative HPLC taste profile analysis was conducted between the RFT-PC and SFT-PC samples using sensory-guided fractionation techniques. The taste ‘fingerprint’ for both Frito Lay’s potato chip samples obtained by HPLC sensory-guided fractionation is shown in Figure 4.2. Direct comparison of the two taste profiles revealed two main differences in the taste profile; the RFT-PC sample was higher in umami intensity (fractions 1 & 2) and lower in sourness intensity (fraction 3 & 4) as compared to the SFT-PC sample. For the remaining fractions, only minor differences were observed for bitterness (fractions F11-F14) and no difference were observed for astringency which was reported to be at threshold levels (fractions F5-F10). Consequently, fractions F1-F4 and F11-F14 were chosen for further purification and targeted for taste identification; however, the umami fractions were selected as the primary focus of investigation due to the well-known positive role of umami flavor in savory snack foods.

Each fraction (F1-F4 and F11-F14, Figure 4.2) underwent a secondary dimensional separation and further sensory-guided fractionation to identify the principal taste-active compounds. An example of the chromatogram for the second-dimensional separation of
fraction F2 obtained from first-dimension (Figure 4.2) and the corresponding sensory active region is presented in Figure 4.3. Further analysis (MS-accurate mass and 2D NMR) of the taste active fractions, resulting from second dimension separation revealed PGA and GA as the main components. Sensory analysis further confirmed that the salt forms of both compounds (sodium pyroglutamate and sodium glutamate) were the primary taste active compounds in fractions F1 and F2 (Figure 4.2) responsible for the umami character. Similarly, quinic acid was identified as the primary sour compound in fractions F3 and F4. Chaconine and solanine were reported as the bitter compounds in fractions F11-F14 (Figure 4.2). The identified taste compounds are illustrated in Figure 4.4.

Though pyroglutamyl peptides have been previously reported^{142}, the salt forms of L- and D- PGA (MSpG) has never been reported, to our knowledge, to have umami taste attributes. The origin of PGA in potato chips was further investigated. The concentration of L- and D-GA as well as L- and D-PGA in raw potato and in samples that were subsequently fried for 170s (SFT-PC) and 210s (RFT-PC) were quantified and are shown in Figure 4.5. PGA was identified as both an endogenous natural product of the raw potato and also a thermal reaction product generated during frying. As suggested by the structure of PGA, PGA is a thermal reaction product of GA generated during frying. A proposed thermally catalyzed generation pathway for PGA is shown in Figure 4.6. GA underwent a ring-closure and a subsequent dehydration step to yield PGA. During frying water molecules in the pore site of the potato matrix are replaced by oil molecules, facilitating dehydration reactions. The stereochemistry of PGA is the same as
the GA. The noted decrease in GA concentration with the corresponding increase in PGA with frying time (Figure 4.5) was in agreement with this reaction mechanism. The acidic compounds identified in fractions F3 and F4 (Figure 4.2; shown in Figure 4.4) were also quantified in the SFT-PC and RFT-PC samples and the results are shown in Figure 4.7. The concentration of quinic acid did not change, however the content of chlorogenic acid was degraded by approximately 35% in the RTF-PC sample (compared to SFT-PC) supporting the noted decrease in sourness intensity observed in these fractions.

**Quantification of PGA and GA in Saliva During Consumption of Potato Chips**

To investigate the sensory relevance of the identified PGA compounds, their concentrations were determined in saliva collected during the mastication of potato chips (see Figure 4.8). Factors that govern the dissolution of tastants in saliva during food mastication are complex and not easy to predict (i.e. solvent accessibility, solubility). The direct quantification of the taste compounds in saliva during mastication provides an effective concentration for sensory analysis. This approach allows testing the sensory effect of compounds at levels consumers experience during mastication. The concentration of L- and D- PGA reported in expectorated saliva during consumption of the SFT-PC and RFT-PC samples are shown in Figure 4.8. The noted higher concentration of PGA in the RFT-PC sample (versus SFT-PC) was also in agreement with the sensory data collected after taste activity screening of the HPLC fractions F1 and F2 in Figure 4.2.
The concentration of L-GA and D-GA were also quantified in the saliva for the RFT-PC sample and were 0.82 and 0.08 g/L, compared to 2.12 and 0.35 g/L for L-PGA and D-PGA, respectively. Thus, in human saliva after mastication of RFT-PC L-PGA was approximately 2.6× higher in concentration when compared to L-GA, even though L-GA was slightly higher in concentration than L-PGA in the actual RFT-PC sample (Figure 4.5). This observation further supports the importance of monitoring tastants in saliva in order to determine their sensory relevance as extractability from the food matrix will determine what consumers perceive. Based on the chemical structures, L-GA would be predicted to be slightly more water-soluble than L-PGA. Consequently, L-PGA was suggested to be at a higher concentration on the surface of the potato chip (presumably because of thermal conversation) and therefore was primarily extracted during mastication.

**Sensory Analysis of Umami Compounds**

The pH value of the RFT-PC sample (1 part chip: 3 parts water) was reported to be 6.5, similar to the pH of the oral cavity. Consequently during mastication of salted potato chips under these pH conditions, GA and PGA would exist as the salt forms MSG and MSpG; which are the umami taste active forms. To establish the taste activity of L-MSpG, D-MSpG, L-MSG and D-MSG each compound was reconstituted in water at 2.7 mM (equivalent to 0.35 g/L PGA) in NaCl tasting solutions at iso-concentration of sodium content (equivalent to 4.4g NaCl/L or 75.2 mM). The test compound concentration of 2.7 mM was selected because it was the lowest amount of PGA
quantified in saliva during consumption of RFT-PC samples (Figure 4.8); whereas the sodium level was also based on the amount quantified in the saliva during consumption of salted potato chips. The sensory results are shown in Table 4.1. Both the L- and D-MSpG were found to have significantly higher perceived umami intensity when compared to the pure salt solution. The L-MSG sample, which is well known to have umami taste, received the highest rating for umami intensity at iso-concentration; D-MSG had the lowest umami intensity as based on previous findings. The salt intensity of all solutions was also evaluated. Interestingly, only the perceived salty intensity of the L-MSG solution was found to be significantly lower than the control solution suggesting saltiness was suppressed. Others have reported the use of L-MSG as a strategy to reduce the sodium content of the food by 30% without decreasing the palatability of foods; loss of saltiness was offset by increased umami attributes. However, based on the unique loss of saltiness perception with added L-MSG (Table 4.1), L-/D-MSpG may have unique functional advantages for food formulation strategies for sodium reduction.

**Reconstitution study for identified umami compounds**

A taste recombination study was also conducted in order to evaluate the overall intensity of the identified umami compounds at the reported concentration in saliva during mastication of the RFT-PC sample. The concentrations of L-GA, D-GA, L-PGA and D-PGA in the saliva were 0.82, 0.08, 2.12, and 0.35 g/L; whereas sodium level was 29.7mM (equivalent to 4.4g/L NaCl). A trained panel evaluated the umami and salty intensity of salted potato chips and the taste recombinant consisting of the salt (sodium)
form of each compound, D-MSG, L-MSG, D-MSpG and L-MSpG (see Table 4.2). Overall, the perceived saltiness and umami intensity were not significantly different between the salted potato chips and the taste recombination model, indicating the primary umami compounds were identified.

**Potato Chips Application and Time-Intensity Study**

The effect of L-/D-MSpG on the time-intensity flavor profile of salted potato chips was also investigated. Both compounds were applied topically at a dosage of 2-fold the concentration of the amount quantified in the RFT-PC sample. Initial sensory screening of samples indicated this dosage level provided unanimous detectable differences in the flavor properties compared to regular salted potato chips. The time intensity results are shown in Figure 8 and indicated the topical application of the MSpG compounds on potato chips significantly enhanced the umami and potato chip flavor intensity as well as a single time point for saltiness intensity. Review of the temporal profile for umami perception illustrated both samples reached an average maximum at 15s but the sample with added MSpG compounds was rated as significantly higher umami intensity at most time points throughout the mastication process and after swallowing. The perceived intensity of the potato chip flavor was also significantly higher during the initial stages of the mastication process (5, 10, 15s time points) while after swallowing (20s) the difference between samples became less apparent. For saltiness, the sample with added MSpG was characterized by an overall higher average saltiness intensity, however only the middle time point (20s) was found to be significantly different.
In conclusion, the umami-enhancing tastants L-MSpG and D-MSpG were reported for the first time in potato chips and further generated during frying. Sensory recombination studies also indicated the identified compounds contributed to the characteristic umami taste of regular potato chips. Topical application found that L- and D-MSpG enhanced the umami and the overall potato chip flavor intensity. The identification of the umami enhancers would inevitably benefit food industry on improving the taste of shorter fried (lower fat) potato chips.
Table 4.1. Mean salty and umami ratings of NaCl solution with and without test compounds at the concentration of D-MSpG reported in human saliva.\(^a\)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Salty</th>
<th>Umami</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>75.2 mM NaCl</td>
<td>6.09a</td>
<td>0.27</td>
</tr>
<tr>
<td>72.5 mM NaCl + 2.7 mM D-MSG</td>
<td>6.09a</td>
<td>0.45</td>
</tr>
<tr>
<td>72.5 mM NaCl + 2.7 mM L-MSpG</td>
<td>6.03a</td>
<td>0.49</td>
</tr>
<tr>
<td>72.5 mM NaCl + 2.7 mM D-MSpG</td>
<td>5.91ab</td>
<td>0.48</td>
</tr>
<tr>
<td>72.5 mM NaCl + 2.7 mM L-MSG</td>
<td>5.47bc</td>
<td>0.77</td>
</tr>
</tbody>
</table>

\(^a\)Mean ratings with letters in common do not differ significantly (5% significance level); MSG = monosodium glutamate, MSpG = monosodium pyroglutamate.

\(^b\)The sodium level is equivalent for all samples.
Table 4.2. Mean salty and umami ratings of the recombination model and potato chips

<table>
<thead>
<tr>
<th>Samples</th>
<th>Salty</th>
<th>Umami</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Umami Compound Aqueous Mixture⁴</td>
<td>5.38a</td>
<td>0.71</td>
</tr>
<tr>
<td>Potato Chips</td>
<td>5.46a</td>
<td>0.61</td>
</tr>
</tbody>
</table>

⁴ Mixture consisted of 16.4 mM of L-MSpG, 2.7 mM of D-MSpG, 5.6 mM of L-MSG, 0.5 mM of D-MSG in NaCl concentration adjusted to 29.7mM sodium (equivalent to 75.2mM NaCl); MSG = monosodium glutamate, MSpG = monosodium pyroglutamate. The concentrations were derived from quantification of the analytes (including sodium) in human saliva.
Figure 4.1 Sensory-guided fractionation for screening umami active compounds in potato chips.
Figure 4.2 Taste HPLC-fingerprints of short fry time potato chip (SFT-PC; top) and regular fry time potato chip (RFT-PC; bottom) samples. Ratings were based on the following categorical scale: threshold, weak, moderate, strong. F1-14 indicates fractions as eluted and collected from HPLC column. F5-F10 had low taste activity, were pooled for sensory analysis.
Figure 4.3 Second-dimension HPLC chromatogram of fraction 2 collected from first-dimension separation. The shaded fraction illustrated the taste active region for L-pyroglumatic acid.
Umami Compounds

L-Pyroglutamic Acid

D-Pyroglutamic Acid

L-Glutamic Acid

Sour Compound

D-Quinic Acid

D-Chlorogenic Acid

Bitter Compounds

Chaconine

Solanine

Figure 4.4 Select key taste active compounds identified in potato chips
Figure 4.5 Mean concentrations of L-glutamic acid (GA) & L-pyroglutamic acid (PGA) (top) and D-GA & D-PGA (bottom) in raw potato and potato chips. Error bars illustrated triplicate analysis.
Figure 4.6 Proposed generation pathway of pyroglutamic acid from glutamic acid during frying.
Figure 4.7 Mean concentration of Chlorogenic acid and D-Quinic acid in SFT-PC and RFT-PC samples. Error bars illustrated, triplicate analysis.
Figure 4.8 Mean concentration of L- & D- pyroglutamic acid (PGA) reported in human saliva for short fry time potato chip (SFT-PC) and regular fry time potato chip (RFT-PC) samples. Error bars illustrated, triplicate analysis.
Figure 4.9 Time-intensity ratings of saltiness, umami and potato chip flavor for potato chips with topical addition of L- & D-monosodium pyroglutamate (treatment) and potato chips (control); * indicates significant difference in mean value (α = 0.05).
Chapter 5 Suggested Future Work

The results presented here on bitter and umami attributes of food as influenced by thermal processing techniques suggest further research in 1) the screening of the bitter modulators generated from polyphenol-Maillard reaction; 2) the investigation of extrusion temperature and duration to reduce the bitterness in whole grain corn products; 3) the further identification of umami enhancers from amino acid derivatives.

First, the screening of the bitter modulators generated from polyphenol-Maillard reaction can be expanded to other polyphenols that are active in cocoa beans or other foods. Additionally, glycine was the only amino acid used for screening bitter modulators in the work of Chapter 2 (based on time constraints) however other amino acid such as L-proline showed further potential for screening bitter modulators from polyphenol-Maillard reaction products based on the literature (Table 1.3, 77). The identified catechin-Maillard reaction products are also a basis for studying the structure-activity relationship of bitter modulation. Previous research into taste modulators shows that when a taste modulator has a structure similar to a taste compound, it has a higher potential for modulating that taste.

Second, further investigation into the relationship between the processing temperature and the duration of extrusion is needed in order to reduce the bitterness in whole grain corn extruded products. As the major bitter compounds in the final products are hydroxycinnamic acid amides, controlling processing temperatures and durations could reduce the bitterness by making them react with Maillard intermediates. Additionally, in
the formulation of the extruded products, trisodium phosphate and calcium carbonate were added for texture and appearance of the product. However, the role of phosphate on flavor development and taste profile needs further discussion since it affects the pH during the extrusion process. Therefore, the taste profile of the final product could be changed as the pH is known to have an impact on the mechanism of the Maillard reaction.

Finally, the umami enhancers generated from nucleotide-participated Maillard system have been studied by Hofmann et al.\textsuperscript{41} but the thermal impact of amino acids in potatoes is not known. The thermal degradation products of basic amino acids can be screened and investigated. The taste profiles of the amino acid derivatives could be studied to understand the impact of this mechanism on the overall flavor quality of fried potatoes.

In summary, the discovery of processing conditions that modulate the taste profile, as illustrated in this study, opens up future opportunities to improve the palatability of food products.
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