USE OF STARCH INCLUSION COMPLEXES FOR IMPROVED DELIVERY OF DIETARY POLYPHENOLS TO THE ORAL CAVITY BY CHEWING GUM

DEBIE WESLEY BLAIR
Summer 2010

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Food Science
with honors in Food Science

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ABSTRACT

Starch inclusion complexes can increase the stability, dispersibility, and quite possibly bioavailability, of bioactive guest compounds encapsulated within a starch helix. It has long been speculated that these complexes, much like those between small molecule drugs and cyclodextrins, could be used for controlled release of bioactive compounds. Bhosale and Ziegler (unpublished) successfully encapsulated beta-carotene in a starch inclusion complex, which increases its dispersibility in water, protects the compound from degradative reactions, and also provides a means for controlled release of this sensitive hydrophobic compound. However, the effect on bioavailability was not determined. Like beta-carotene, green tea polyphenols, currently of interest for their therapeutic potential against oral cancer, are sensitive to oxidation. The main objective of this experiment is to make a starch inclusion complex with green tea polyphenols as the bioactive guest component, and then incorporate it into chewing gum as an oral delivery vehicle. This honors thesis comprises a review of the literature pertinent to this objective.
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ACKNOWLEDGEMENTS

First and foremost, I would like to thank my honors and thesis adviser, Dr. Ziegler, who has been my guidance counselor since day one and the one who has opened many doors for me throughout my college career.

I send out my gratitude to other food science professionals including my co-adviser, Dr. Lambert, who has been very helpful in teaching me outside the realms of my topic focus, Dr. Dudley who was my first thesis adviser and provided me with necessary building blocks of working in the lab setting, Rajesh Bhosale for his work in the lab, which helped develop my thesis topic, and to Dr. Ryan Elias, Vanesa Lay Ma, and the mouse team for their assistance throughout the development of my project.

Beyond the food science department, my college adviser Randi Congleton played a major role in keeping me focused during tough ups and downs that presented themselves throughout my stint in the IUG program.

Of course, the support and encouragement of my parents, my brothers, my sisters, and my close friends has influenced my school work even before my college years.

Lastly, without the graduate scholarship from National Starch and the funding and gum supplies from Wm. Wrigley Jr. Company, none of this would have been possible.
INTRODUCTION

Starch inclusion complexes, which are created by binding helical amylose to guest polymers (Kida et al. 2007), increase gelatinization temperature and decrease solubility and granule disruption of the starch (Putseys et al. 2010). A specific type of complex, with hydrophobic lipids as the guest polymer, was created with lipids of different chain lengths (Jovanovich and Añon 1999, Kida et al. 2007, Putseys et al. 2010). These lipids as part of the complex use the amylose helix as protection from the aqueous environment, increasing the dispersibility of the lipid, while also decreasing the solubility of starch.

Starch-lipid complexes may be useful in increasing the utilization and effectiveness of bioactive components, which may be attached to the lipids by esterification, shown by the successful encapsulation of beta-carotene done by Bhosale and Ziegler (unpublished). Beta-carotene, which is susceptible to oxidative reactions, had a reported increase in overall stability when bound to the complex. In addition, the encapsulation increases dispersibility in water of the bioactive compound, protects it from degradative reactions, and also provides a means for controlled release. These changes, however, could affect the bioavailability of the carotenoid, and the difference in bioavailability in free form and as part of the complex is unknown. This information is important to know, because although the complex may increase the stability of beta-carotene, if the component is not available in its new form, it is not useful.

The same type of complex may be made using polyphenols as the inclusion, which, like beta-carotene, are highly susceptible to oxidative reactions. More specifically, catechins found in green tea are targeted due to the beneficial effects of reducing the risks of oral cancer, cardiovascular disease, and tooth decay (Lee et al. 2004,
Koh et al. 2010). The beneficial effects of green tea catechins were investigated by Lee et al. (2004), who showed that chewing and/or holding tea leaves in the oral cavity as opposed to just drinking tea is an effective method in distributing tea polyphenols to the oral cavity. The direct contact and long exposure time to the buccal mucosa produced high salivary levels of polyphenols.

Since it is not practical to expect people to chew on tea leaves in order to obtain benefits from green tea, we considered using chewing gum as a delivery vehicle for these compounds. Besides needing to hold the tea leaves in the mouth without swallowing them, the polyphenols in the tea leaves are very bitter and astringent (Cheynier 2005). These unfavorable flavor characteristics could become unbearable to retain in the mouth. Putting the polyphenols in chewing gum as a powdered ingredient would decrease the intensity of the bitterness and astringency. Yet, the controlled release of the tea extract from the chewing gum matrix must be tested to see the concentration of bioactive components that would be released during the normal time span a person would chew one piece of gum.

This method seems feasible, but one road block in the release of polyphenols from the chewing gum and starch inclusion complexes would be the possible inhibition by green tea catechins on the activity of the digestive enzymes α-amylase and α-amylglucosidase. Inhibition of these enzymes would decrease the breakdown and release of the polyphenols from the chewing gum matrix if added as part of a starch inclusion complex. The inhibition of α-amylase was investigated in order to determine possible problems that may arise in the bioavailability of tea polyphenols when in the presence of starch inclusion complexes.
**Objectives**

The main objective of this experiment is to use chewing gum as a delivery vehicle for bioactive polyphenols found in green tea. By using this method, the release and absorption of the compounds should be higher than if merely consumed through drinking tea. The mastication time will increase the exposure time of the compounds in the oral cavity, which is an efficient way of absorbing the compounds into the body through buccal mucosa; it also provides direct exposure to potential cancerous cells in the case of oral cancer. We will conduct an experiment to evaluate the release of the green tea polyphenols from the chewing gum matrix to the saliva in free form and as a complex.

However, in order for this to work, the polyphenols must be bioavailable in the form in which it is introduced to the body. It may be beneficial to use the complex form to decrease the susceptibility of polyphenols to oxidation (Cheynier 2005), similarly to beta-carotene. Yet, if the starch inclusion complex as the ingredient in the gum is used, we must know that the compound will be released from the helix and freed from the matrix in which it is entrapped. Due to this, the bioavailability of beta-carotene in male mice when administered in free form and as an amylose-ascorbyl palmitate inclusion complex was compared in another experiment. The results will be related to the complexes that may be formed with polyphenols.

Lastly, the release of polyphenols in chewing gum will be compared when in free form and when in the complex form. However, since the complex contains starch, the release from the matrix will be dependent on the activity of amylase. We will investigate the inhibitory effect that green tea catechins may have on amylase. Any inhibitory effects may lead to the decrease in effectiveness of the starch inclusion complex as the
bioactive ingredient in our chewing gum product. In this honors thesis, the literature pertinent to this objective will be reviewed in an attempt to evaluate the validity of the topic and potential future work.
BIOAVAILABILITY OF NUTRIENTS IN STARCH INCLUSION COMPLEXES AND FOOD MATRICES

Although starch inclusion complexes may protect the component that is entrapped in the amylose helix from degradative reactions and enhance its dispersibility in water, these characteristics provide no information to the bioavailability of the component in this form. The complex to be ingested, whether containing beta-carotene or polyphenols as the inclusion, is only useful if it can be broken down by human salivary and digestive enzymes. If the complex flows through the tract and is too stable and does not break down to release the component inside the helix, the bioavailability is extremely low and the complex is no better, and perhaps worse, than the free form of the component.

Typical starch inclusion complexes contain starch and a polymeric guest (Figure 1). Our beta-carotene complex contains three parts: starch, a lipid, and a bioactive component (Figure 2). Hence, any interactions involving these three types of compounds are integral to the bioavailability of the bioactive component.

![Figure 1. Schematic drawing of starch inclusion complex formation (Kida et al. 2007)](image1)

![Figure 2. Schematic drawing of beta-carotene starch inclusion complex (edited from Kida et al. 2007)](image2)
**Lipid interactions affecting bioavailability**

In general, proteins, lipids, and carbohydrates are absorbed through different systems in the body and at different rates. Also, depending on a person’s personal metabolism and ability to breakdown food components, these macronutrients are digested in different manners. For example, a person with a higher muscular content will have the tendency to break down caloric products faster than those with a higher fat content. Lipids in and among themselves are absorbed differently, with long chained fatty acids being transported through the lymphatic system while the medium and short chain fatty acids enter the circulation through the portal vein (Nelson and Cox 2005, Porter et al. 2007). This size and shape difference in lipids affects not only the route but also the rate of absorption. On top of that, the amount absorbed is different than the amount ingested, due to changes throughout the digestive tract. This refers to the bioavailability of a compound, or the “fraction of an ingested component that eventually ends up in the systemic circulation” (McClements et al. 2008). Bioavailability is a product of three main components: amount released from the matrix in which it may be entrapped; ability to be transported across intestinal epithelium; amount that reaches the systemic circulation without being metabolized, which depends on the pathway. Beyond these contributions to bioavailability, the rates of digestion and absorption both directly relate to bioavailability (McClements et al. 2008).

Molecular shape affects digestibility, but also the combination of compounds that are present together in the gut can alter the natural absorptive properties of a compound (McClements et al. 2008). For lipids, the state of the compound (liquid or solid) is important; higher digestion is seen with lipids of the same size in liquid form than solid,
and solid lipid particles can be used to purposely decrease digestibility and bioavailability by 15-20% (McClements et al. 2008). Additional structural changes occur along the GI tract, changing the absorptive patterns. The nature of the changes depends on original properties and organization within the ingested food, for example the layer around lipid droplets (McClements et al. 2008). Hence, the introduction of a compound through other forms (i.e. tablet, gum, gel) can hence change bioavailability.

**Starch interactions affecting bioavailability**

Along with lipids, structural characteristics of starch are also important for digestibility (Singh et al. 2010). And since the rate of digestion is an important factor in the bioavailability of a component (McClements et al. 2008), the structural characteristics of starch indirectly affect the bioavailability of starch. When it comes to starch inclusion complexes, bioavailabilities of both the starch and the included guest molecule are also integral, especially since the physical state of ingested starch has a major impact. Interactions with other compounds, including inhibitory effects, are possible, and the state of the compound and mode of introduction to the body becomes essential (Singh et al. 2010). Crowe et al. (2000) shows that glucose conversion of starch in amylose-lipid complexes ranges from as low as 48% and up to 71%.

Starch digestibility varies by type and modifications. With ranges from 67 to 100% digestibility in chemically modified potato starch to waxy maize starch, respectively, not only is there a difference in breakdown in the body due to the type and source of the starch (potato, corn, rice, or sorghum), but also the amount of amylose and amylopectin is a possible contributor to differences (Singh et al. 2010). Starch structure
changes kinetics, with the amount of branching of amylopectin acting as a self-inhibitor, due to both molecular weight and steric hindrance (Singh et al. 2010). Despite the branching inhibition, more amylopectin typically results in faster breakdown because of higher surface area. High amylose decreases digestibility from 96% in waxy maize starch to 77% in high amylopectin starch (Singh et al. 2010).

Interference of protein content has been seen in legumes, which tend to have a lower digestibility than starch products lower in protein content (Singh et al. 2010), due to interactions of proteins and starches in the food matrix, referring to conformational changes like the formation of disulfide-linked polymers or the protective protein barrier that can form surrounding starch granules (Singh et al. 2010). Both changes affect the access of starch to its environment, affecting the reactions that can take place. Legumes that were thermally processed not only saw a reduction in antinutrients such as tannins and phytic acid, but also, although to a lesser extent, a reduction in content and an increase in the digestibilities of both protein and starch (Rehman and Shah 2005).

Apart from protein-starch interactions, the addition of free lipids to starch can decrease digestibility of starch from 90% to 35%, mostly affecting amylose and less of amylopectin (Singh et al. 2010, Crowe et al. 2000). These formed lipid-starch complexes are more stable than either component in free form (Holm et al. 1983), using the hydrophilic amylose helix to protect the hydrophobic lipids from the environment. The protected complex is hydrolyzed and absorbed at a slower rate than free form amylose, resulting in the decreased digestibility (Singh et al. 2010). Complex formation is said to occur in the digestive tract and changes the glycemic index of the complexed starch. Certain free fatty acids like tauric, myristic, palmitic, and oleic acid significantly hinder
the percentage of glucose conversion by inhibiting hydrolysis by amylase (Crowe et al. 2000). These complexes can form outside the GI tract, but still at 37°C under physiological conditions (Singh et al. 2010).

Besides alterations that are made in the digestive tract, food processing procedures that are completed before a food is ingested also changes how food components are absorbed (Parada and Aguilera 2007). While pregelatinization can increase digestibility, incomplete gelatinization can decrease postprandial carbohydrate absorption due to an increased resistance to enzyme hydrolysis (Singh et al. 2010). Processing like pressure cooking, roasting, baking, frying, and malting can increase digestibility by increasing rapidly digestible starch and decreasing slowly digestible starch (Roopa and Premavalli 2008). These changes can be both good and bad; with some cereals, processing like denulling, soaking, and germination provides a loss of phytic acid, tannins, and polyphenols that normally inhibit α-amylase activity (Rehman and Shah 2005). This would increase breakdown and absorption of carbohydrates.

A good example of starch processing that affects bioavailability is the nixtamalization process of corn to hominy and masa, which can change flavor, texture, and color of corn (Inglett 1970) while also increasing availability of proteins and certain vitamins found in raw corn. The lye treatment step in this process has been found to alter amino acid composition and correct the imbalance and release of niacin from a bound form (Harper et al. 1958). Although nixtamalization produces a total loss of food nutrients like niacin, thiamin, and amino acids in tortillas compared to raw corn, animals fed on tortillas grow better than when simply fed raw corn, shown by higher weight gains in the tortilla-fed groups (Bressani et al. 1958, Cravioto et al. 1952, Harper et al. 1958).
These data indicate a change in bioavailability of nutrients in corn as a result of lye treatment, increasing weight gain despite a reduction in total essential nutrients.

**Micronutrient availability**

Not all matrices are artificially produced (e.g. chewing gum matrix), but natural matrices are often composed of micronutrients entrapped in a starch or lipid matrix, or in the matrix of the plant, including cell walls, starch granules, proteins, water and oil droplets, fat crystals, and gas bubbles, among other food components, which act in the same way to protect food components as inclusion complexes and gel matrices (Parada and Aguilera 2007). Encapsulation of micronutrients requires disruption of the compartmental microstructure to release the nutrients and make them available to the body upon consumption. These microstructural elements affect the uptake and presence of all types of food components, including beta-carotene. Castenmiller *et al.* (1999) showed that consumption of spinach with an intact matrix produced blood serum with less beta-carotene than liquefied spinach. The disruption of the matrix allowed release of beta-carotene and increased uptake. It was also shown that beta-carotene from raw vegetables gives a different plasma response than when in a fat matrix. The response is higher in fruits than in vegetables, supporting that both cooking and fat-accompaniment increases bioavailability of carotenoids (Castenmiller *et al.* 1999), ranging from 10 to 50% in raw vegetables and oil and commercial products, respectively (Parada and Aguilera 2007). Additionally, Courtney *et al.* (2003) reported a significant difference in bioavailability of posaconazole when fed a high-fat meal versus a nonfat meal at 290% and 168%, respectively. It is possible that prolonged GI tract residence time caused by
the high-fat diet may play a major role in the change in bioavailability and increase the
time available to absorb the drug (Courtney et al. 2003). Hence, beta-carotene absorption
is dependent on the surrounding environment in which it is consumed.

Similarly to starches and lipids, carotenoids like lycopene and beta-carotene are
more available when processed (e.g. grinding and fermentation), and processing
conditions and interactions with other components are important factors to take into
consideration when talking about bioavailability. The intended mode of action and route
of administration are more important than the total nutrient composition in the food
because of differences in bioavailability (Parada and Aguilera 2007, Pottenger et al.
2000). Parada and Aguilera (2007) also state that for carotenoids, the two most important
factors for digestibility are the food matrix in which it may be entrapped and the
solubilization in digesta (this is increased by cooking and processing).

The matrix is not so much an issue with polyphenols, but source, structure, and
protein interactions are (Hollman et al. 1997, McDougall and Stewart 2005, Parada and
Aguilera 2007). Depending on the compound structure and conjugation, bioavailability
ranges from 0.1 to 50% in polyphenols like tannins and catechins (Parada and Aguilera
2007). The flavonoid quercetin has a 30% higher bioavailability from onions than from
either apples or pure quercetin supplements. Peak plasma levels of quercetin were seen
at 0.7, 2.5, and 9.0 hours in onions, apples, and pure quercetin-3-rutinoside
supplementation, respectively, indicating a difference in absorption rates depending on
the source (Hollman et al. 1997). Further still, fried onions were used in the experiment,
introducing fat as a confounding variable, which may have affected the results. Protein
interactions, like those with polyphenols and pancreatic enzymes, have an effect on
stability. However, the protein interactions that cause astringency in the mouth with salivary proteins play little to no effect to the stability of the polyphenols (Parada and Aguilera 2007).

**Matrices and interactions with guar gum**

It is important to know the effects of a matrix system on the bioavailability of a nutrient, especially when it comes to starch inclusion complexes. The complex protects the included compound from environmental factors, changing the reactivity of the compound and quite possibly the digestibility and availability as well. Encapsulation, whether chemically produced or in a food matrix, is one of the main factors involved in availability of nutrients (Castenmiller et al. 1999).

McClements et al. (2008) summarized that matrix encapsulation is often used as a specific technique to change the bioavailability of lipids. This can be done by either using one of three methods, including interfacial engineering, food matrix encapsulation, or solid lipid particles. Though the encapsulation increases stability, it is however, necessary to disrupt the matrix before digestion can occur, and incomplete or incorrect digestion of the matrix can decrease bioavailability and absorption of the nutrients (Parada and Aguilera 2007, McClements et al. 2008).

Due to resistance to breakdown in the primary end of the GI tract, fibers, as opposed to other starches and proteins, are often used in carrier complexes and coatings (McClements et al. 2008, Singh et al. 2010, Roopa and Premavalli 2008). It was also found that duration and intensity of mastication of food matrices are integral to the completion of structural changes that are necessary for digestion of compounds
(McClements et al. 2008). Especially in an inclusion complex or a chewing gum matrix, this takes effect, where the mastication period is much longer for chewing gum than other foods, increasing time to disrupt the matrix and release that which is encapsulated.

While inclusion complexes entrap compounds in a matrix, gels tend to surround compounds in a matrix, and chewing gum can do both, depending on the method of production. Using chewing gum as a mode of transport allows a dual entry system through buccal mucosa cells and passage through the GI tract via salivation (Noehr-Jensen L et al. 2006, Kamimori et al. 2002). The buccal cavity pathway produces a faster absorption rate of the compound, although release and absorption is still dependent on pH, volume, and composition (Kamimori et al. 2002). Studies show faster absorption rates of compounds faster when in gum than when in tablet form. Kamimori et al. (2002) showed a significantly faster rate of absorption of caffeine in gum than tablet, and bioavailabilities at 50, 100, and 200 mg doses of 75, 87, and 90%, respectively. Within the first 90 minutes, the gum formulations at 200 mg had a significantly lower $T_{\text{max}}$ (time after administration of drug at which maximum plasma concentration is reached) than the tablet formulation, showing a quicker onset and release from the matrix (Kamimori et al. 2002). On the other hand, the chewing gum formulation had a higher $T_{\text{max}}$ in the study done by Noehr-Jensen L et al. (2006). Plasma concentrations of loratadine were higher with the gum formulation than in tablet form. The bioavailability increased by a factor of 2.68 using gum as a transport system as opposed to tablet (Noehr-Jensen L et al. 2006). It is important to note that not all chewing gums are formulated the same, so although the two papers showed different release responses, differences in the gum base formula may play a role in the results.
The gel matrix of guar gum or sodium alginate can provide encapsulation and protection from gastric fluid of iron; different release profiles from the gels were seen depending on the microstructure in which the chemical was entrapped (Parada and Aguilera 2007). Not surprisingly, guar gum not only increases viscosity as a food thickener, but also it increases the viscosity of digesta within the GI tract, leading to a decreased rate of digestion and absorption of glucose (Singh et al. 2010, Ellis et al. 1995). This polysaccharide delays digestion of glucose by entanglement of galactomannan in the GI tract, increasing the viscosity of the digesta and acting as a physical barrier to starch hydrolysis. In a swine study, after four hours of administration of guar in their diet, there was a significant difference in glucose absorption at 20 and 40 g guar/kg BW (Ellis et al. 1995).

**Bioavailability and product feasibility**

The bioavailability of the compound included in the starch inclusion complexes is yet unknown. Beta-carotene, for example, may be more stable as part of a complex instead of in free form, but it may not be broken down enough and released from the matrix in order to be beneficial if ingested by a human. The investigation into the bioavailability of starch inclusion complexes would be helpful to determine whether the complexes could be used for bioactive compound supplementation.
USING CHEWING GUM AS A DELIVERY VEHICLE OF BIOACTIVE GREEN TEA POLYPHENOLS

As one of the most popular beverages in the world, tea (*Camellia sinensis*) provides consumers with benefits beyond mitigation of thirst. Black, oolong, and green teas have been linked to the reduction of risks of oral cancer, cardiovascular disease, and tooth decay (Lee *et al.* 2004, Koh *et al.* 2010). As a result of the fermentation processes of the fresh tea leaves, compounds such as polyphenolic catechins and theaflavins are abundantly present in these teas, with the former being more abundant in green teas and the latter in black tea. Possibly the most important catechin involved in the beneficial effects of green tea is epigallocatechin-3-gallate (EGCG), which is the most copious polyphenol found in green tea extract, at 40% w/w, half of all total polyphenols present, as reported by Nature’s Sunshine Products, Inc. (2008).

In an attempt to investigate methods to consume these beneficial compounds, Lee *et al.* (2004) showed that chewing and/or holding tea leaves in the oral cavity as opposed to just drinking tea may be an effective method in distributing tea polyphenols to the oral cavity, which may be beneficial to human health. However effective this method may be, it is not very practical. Despite the difficulty of holding the tea leaves in the mouth without swallowing them over a period of time, the polyphenols in the tea leaves are very bitter and astringent. These flavor characteristics are not favorable to a consumer and at high concentrations—as would be seen in pure tea leaves—could become unbearable to retain in the mouth. Hence, we considered the practicality of including the bioactive components as a powder ingredient in chewing gum.

If we find a way to successfully add the polyphenols in chewing gum, the slow
and direct release would increase the absorption into the buccal tissue and may be more effective than simply drinking tea or taking a supplement due to the increased time spent in the oral cavity. However, it is unknown whether the polyphenols would be better delivered in free form or as a starch inclusion complex.

Chewing gum is a practical delivery method because of its popularity in the United States. When gum first became popular after WWII, gum was used mostly for mitigation of thirst, but as time has passed, usages have expanded to more medicinal and nutraceutical purposes. For athletes the dual function of chewing gum includes the sense of relaxation similar to that provided by chewing tobacco, as well as the increase in salivation and decrease of thirst that can easily replace the need for a bottled beverage on the playing field (Ream and Moore 1979). For the general public, ingredients like xylitol and nicotine have made chewing gum a dental aide and a non-smoking aide, respectively (Fritz 2008, Perfetti 1996).

Due to popularity, even skeptic consumers would be tempted to consider the product. As a matter of fact, there are already chewing gum products that have been created containing green tea polyphenols. CCA Industries has marketed Mega-T Green Tea Gum, claiming that "each piece delivers all the healthy super antioxidants of a cup of green tea". According to the label, this brand has 60 mg of proprietary blend tea extract per one piece of gum (1.3 g). Knowing that EGCG is approximately 40% of the extract by weight, each piece of gum contains 24 mg of the active catechin. Typically, one cup of green tea is considered 2.0 g tea leaves, which totals 600 mg of extract, much less than the claim from this product (Zhang and Kashket 1998). However, the composition of the proprietary blend of antioxidants is unknown, so the exact concentration of polyphenols
cannot be deduced. Other products including Spry Green Tea Gum by XClear, Inc. and Unique Sweet® Gum with Xylitol and Green Tea is are targeted to the same market, and they are examples that show the product idea is a possibility, although these products contain much less green tea extract in each piece of gum than we would put in our product.

**Growth of the gum industry**

The practicality of chewing gum as a delivery vehicle starts with its popularity in the United States and around the world. Since early civilizations, chewing gum has been used by humans as a salivatory stimulant as well as a breath enhancer. Mayan natives used *chicle* while ancient Greeks used *mastic bark* to clean their teeth and sweeten their breath. (Fritz 2008). In current times, the chewing gum of which we know evolved from the use of chicle, which was brought to this country from Mexico by Antonio de Santa Anna in the 1860s. In his desire to create a new type of rubber tire, he asked entrepreneur Thomas Adams for his help. After many failed attempts, Adams finally popped the natural resin in his mouth, and he enjoyed the first prototype of chewing gum (Fritz 2008).

The popularity of chewing gum resulted from World War II when soldiers used it to quench their thirst when beverages were not always readily available. Global spread of the chewing gum trend was due to the soldiers visiting other countries, and upon their return to the United States, the popularity spread beyond the soldiers for mitigation of thirst as well as relief of nervous tension (Hoar 1924). Around this time, the nationalization of the gum industry in Mexico and the Far East War contributed to the
increase in price and decrease in supply of the natural chicle used for chewing gum (Fritz 2008, IBAA 1909). The need for synthetic materials for gum bases has skyrocketed due to the fact that they are cheaper and easier to obtain, and the use of other resins resulted in gums of inferior quality (Fritz 2008, Hoar 1924).

**Gum composition**

The effectiveness of chewing gum as a delivery vehicle is all dependent on the composition of the chewing gum matrix. Since the shift to synthetic polymers, chewing gum has become a more complex entity. In the beginning, it was just a mixture of chicle with sugars and flavors. However, chewing gum has evolved into a complex mixture composed of two main parts: the soluble and insoluble portions. The insoluble gum base consists of elastomers, resins, fats and oils, waxes, softeners, plasticizers, inorganic fillers (e.g. CaCO₃), and optional components such as antioxidants, colorants, and emulsifiers (Cherukuri et al. 1992, D’Amelia et al. 1984, Mackay and Schoenholz 1977, Cherukuri and Mansukhami 1988, Yatka et al. 1992). As for the soluble gum base components, additional softeners such as glycerin are used, as well as sweeteners (e.g. sorbitol, sucralose, and maltose) and flavorants (Cherukuri et al. 1992, Mackay and Schoenholz 1977, Cherukuri and Mansukhami 1988, Yatka et al. 1992). The water-insoluble portion is typically the reference to the nutritional labeling ingredient “gum base” while the water-soluble portion is labeled separately and is known to dissipate over time through chewing (Grey et al. 2007). The composition of an example of chewing gum is shown in Table 1. As is noticed, most of the final formulation consists of gum base and
sweetener—in both pulverized and syrup form—with miniscule additions of flavorants, emulsifiers, and colorants.

Table 1. Chewing gum composition (D’Amelia et al. 1984)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage by Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gum base (elastomers, resins, etc.)</td>
<td>22</td>
</tr>
<tr>
<td>Sweeteners (pulverized)</td>
<td>52</td>
</tr>
<tr>
<td>Corn syrup</td>
<td>23</td>
</tr>
<tr>
<td>Flavorants</td>
<td>1</td>
</tr>
<tr>
<td>Emulsifiers</td>
<td>1.5</td>
</tr>
<tr>
<td>Colorants</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Polyvinyl acetate (PVAc), which comprises a majority of gum bases at 55% composition, is a hydrophilic type elastomer that aides in absorbing saliva, which causes the cud to become slippery when chewed (Cherukuri and Mansukhami 1988, Kehoe et al. 1981). There are multiple types of PVAc, ranging from low to medium to high molecular weight, and the correct combination of the low and medium is essential for the correct viscosity needed for bubble gum function and correct elasticity. High molecular weight PVAc can also be incorporated into the product, but at small levels (Cherukuri and Mansukhami 1988, D’Amelia et al. 1984). Further yet, polyisobutylene can be used in combination with PVAc with additional fats, oils, and fillers to achieve the necessary non-tacky base (D’Amelia et al. 1984).

With a shift away from sucrose as the sweetening agent, intense sweeteners such as sucralose and aspartame have replaced sucrose in low-calorie gum, providing longer lasting sweetness along with synergistic effects (Cherukuri et al. 1992). With the invention “Chewing gum having longer lasting sweetness”, the effect of an advanced
initial burst and prolonged sweetness can be achieved. It is seen that higher amounts of PVAc with enhanced hydrophilic character retains sweetness and slowly releases the soluble components in the base; this is better than bases with lower amounts of PVAc in the insoluble portion of the gum. With the PVAc, the hydrophilic characteristics of the base increase and will bind sweetness, which leads to inadequate sweet release, and hence longer retention of sweetness (Cherukuri et al. 1992).

Not only are sugar alcohols used to sweeten chewing gum, but other non-caloric sweeteners are incorporated together for their synergistic effects. Depending on the sweetness profile desired, combinations of the following sweeteners are often used by the industry for increased sweetness release and longer lasting sweetness: sucralose, aspartame, alitame, saccharin, acesulfame K, maltitol, bulk sweeteners (fructose, flucose, maltose, xylitol, mannitol, sorbitol), polydextrose, and maltodextrin (Cherukuri et al. 1992).

Longer lasting sweetness and flavor are two very important properties of a chewing gum base not only on consumer enjoyment, but for product shelf life as well. While most inventions focus on retaining these two profile components, the same concepts can be used to retain or further alter the release of starch inclusion complexes into the oral cavity. It is also helpful in that if the sweetness lasts longer in the chewing gum, the mastication period increases, and the time for release as well as the total amount of the beneficial inclusions to the oral cavity increases. For instance, one invention by Mackay and Schoenholz (1977) retains sweetness due to the specialized coating, resulting in a chewing gum with lasting sweetness for 60 minutes, a decreased bitter aftertaste, and an increased overall shelflife of 3-6 months. This would provide a full hour for the
bioactive components to release from the matrix before the consumer would be tempted
to spit out the piece of gum.

**Texture**

Depending on the types of the above ingredients that are used and the ratios in
which they are mixed, the properties of a chewing gum can vary greatly (Fritz 2008). By
modifying the ingredients, the texture and the flavor profile can be maintained and
effectively customized (Cherukuri and Mansukhami 1988). For example, the fillers used
in low-calorie gum are different than those in sucrose-sweetened gum and changes the
overall mouthfeel (Cherukuri *et al.* 1992). Additionally, these properties are what
distinguish a chewing gum from a bubble gum. A chewing gum is typically tougher and
less elastic than a bubble gum, whereas the gum bases that are good for making large
bubbles have a high elasticity and often a larger insoluble cud portion (Fritz 2008,

In order to make a homogenous phase with proper resiliency, nearly miscible
components like certain elastomers and resins are required. These polymers have
different internal mobility and bonds, and hence provide different characteristics in a gum
base (D’Amelia *et al.* 1984). For example, elastomer solvents in too high of a proportion
will result in a gum that is too sticky and when put in bubble gum can get stuck to the
face after popping a bubble (Cherukuri and Mansukhami 1988).

Texture is an important determinant for the release time of soluble components in
a gum base. The elastomers, fillers, plasticizers and other ingredients have varying
hydrophobicities, which contribute to the texture and in turn contribute to the release
properties. Using PVAc and low molecular weight waxes will increase the hydrophilic character of the base (Cherukuri et al. 1992). Both types of elastomer are often used concurrently, with the percentage of incorporation into the base requiring a balance to find the desired viscosity for chewing and release properties. Most importantly, since the elastomers make up the majority of the insoluble cud, the larger the size of the incorporated base, the more hydrophilic character the overall gum base has (Cherukuri et al. 1992).

Encapsulation and coatings

When the mere consistency and properties of the cud itself is not sufficient enough to produce a long-lasting sweetness and flavor, other methods may be used to increase the barrier to release. Encapsulation and coatings are two such methods that have been used to make a controlled release product, which could increase the stability of an added ingredient and aide in abating any taste issues that might be presented by the ingredient (Song et al. 2003).

Encapsulation: In various patents, it was seen that the delayed release of sucrose and other components (e.g. flavorants, aspartame) was done through complexing and entrapment in matrices. Controlled release is a result of encapsulation in the gum matrix, an effective delay release mechanism where size is a major factor in the slow release kinetics. The preferred size of particles for optimum slow release is between 80 and 400 microns. The “fine particles may lose some of the slow release benefits” because of complete entrapment in the matrix, while larger particles may be too large and not be able to be retained in the matrix (Yatka et al. 1992). With any controlled release ingredient,
the target component must be in correct proportion in the water-soluble and the insoluble portions of the gum base in order to control release to the mouth.

Encapsulation is used in the invention for “Polyvinyl acetate encapsulation of crystalline sucralose for use in chewing gum” (Yatka et al. 1992) to extend sweetness in chewing gum using sucralose, but when using this invention with aspartame instead, the effective results were not seen (i.e. the Ace K did not respond with delayed release). In addition to the release kinetics, it was found that this invention can be used as a stabilization method in that the crystalline sucralose is stable when incorporated in the PVAc, but not when the dry ingredient is alone under certain conditions (Yatka et al. 1992). Cherukuri et al. (1991) found that there is a synergistic salivary effect when acids and sweeteners are added together to a base, and for the best release kinetics of the acid during mastication is when there is a direct addition of acids to the water-soluble portion of the gum base. Low molecular weight PVAc should be used to deliver these acids (Cherukuri et al. 1991), which provide sour flavor to the product and characteristic fruitiness like ascorbic and citric acids. Encapsulation can also be used for these acids and sweeteners, instead of the free form, but in formulation, encapsulation must be completed through complete dissolution in PVAc (Cherukuri et al. 1991). Co-encapsulation of sweeteners and caffeine were also seen to work in decreasing poor taste qualities of active ingredients in chewing gum products (Song et al. 2003). These attempts to prolong sweetness and flavor or to stabilize aspartame are both important concepts for addition of tea polyphenols into a gum base for nutraceutical use. It is known that stabilization of certain core materials are variable on the shape (e.g. aspartame is rod-shaped) and wettability, which would alter the use of these concepts
with polyphenols since they are different compounds than the sweeteners and acidulants that have been discussed (Cherukuri et al. 1991). Stabilization, however, has been seen to increase with usage of the encapsulation technique (Song et al. 2003).

A controlled release gum base with tea polyphenols could be used to decrease the perceived bitterness from the compounds and allow the consumer to withstand the released flavorings over the desired exposure period for which the consumer would need to receive benefits from the antioxidants (Song et al. 2003). Yet, it is important to know that polyphenols are not fully stable compounds in certain conditions, most importantly due to their high reactivity and temperature-sensitivity (Cheynier 2005). Hence, these characteristics must be considered when making a green tea chewing gum, because of the consumer liking properties and shelf stability.

**Coatings:** Instead of or in conjunction with encapsulation, edible coating materials can also be helpful in producing a control release gum product. The coating materials can be a part of either the insoluble gum portion or water soluble portion; the former would include types of hydrocolloids or waxes like carnauba wax and polyvinyl alcohol, while the latter would include materials similar to sodium alginates or pectins. With any of these materials, the methods of either coating or encapsulation can be used to increase the mastication time of chewing gum (Mackay and Schoenholz 1977). In the invention by Grey et al., a compressed granulated chewing gum was prepared so it could be compressed into tablet and optionally coated with these materials or others (2007). Further patents have created coatings for controlled release of active ingredients and compounds such as caffeine and nicotine (Song et al. 2003). Song et al. added the active ingredients to the coating, while others added the compounds to the gum and used the
coating as a barrier to release. Similarly to tea polyphenols, caffeine is a bitter compound that would not normally be able to be added at effective doses due to the discomfort in flavor, however the multiple methods of coating plus encapsulation that was used in the invention by Song et al. could be exactly what is needed to both hinder bitterness in green tea chewing gum and increase stability of the product containing sensitive ingredients.

**Chewing gum as an oral delivery vehicle**

Whether using encapsulation, coating, or other methods to create a chewing gum containing polyphenols, the final product would be beneficial to oral health. The chewing gum would be a great method to deliver the bioactive compounds because of the large product market in the United States and around the world. Yet, the rate and amount of release of the compounds from the matrix still needs to be determined to evaluate the complete effectiveness of this product.
INHIBITION OF SALIVARY AND GASTROINTESTINAL ENZYMES BY POLYPHENOLIC COMPOUNDS

Tea in general has been used as a preventative measure for cancer, cardiovascular disease (CVD), obesity, and diabetes (Koh et al. 2010). While effectiveness against cancer and CVD can be attributed to the antioxidant properties of tea catechins (You and Bergman 1998, Lambert and Yang 2003), the associated decreased risk of obesity and diabetes is related to the proposed enzymatic inhibitory properties of tea polyphenols (Takabayashi and Harada 1997, Wolfram et al. 2006, Zhong et al. 2006). Green tea in particular has been beneficial against CVD and cancer due to the polyphenol content (He et al. 2006, Wolfram et al. 2006). The slow release of these polyphenols into the oral cavity through chewing of green tea leaves could decrease risks of oral cancer and tooth decay by extract interactions (Lee et al. 2004).

The inhibition that is related to polyphenol content can also be attributed to antioxidant activity. However, biotransformation, which decreases bioavailability of catechins, is also a part of inhibition of enzymes and other proteins (Lambert and Yang 2003). Tea polyphenols are known to form complexes with proteins which will precipitate out of solution, make sediment, and cause haziness (He et al. 2006). Some enzymes are denatured by the tea polyphenols and become anti-nutritional compounds when ingested in excess; it is suggestive that they may bind to digestive enzymes (He et al. 2006, Soares et al. 2007). He et al. (2006) reported that binding of tea polyphenols and enzymes in the presence of hydrogen affects inhibitory properties, and the hydrophobic association often results in changes in enzyme activities; a 61% inhibition of amylase (9 µg/mL) was seen when in the presence of 0.05 mg/mL tea polyphenols.
Besides being an antioxidant, polyphenols like acarbose and miglitol can moderate blood glucose and insulin levels, acting as a treatment for diabetes (Funke and Melzig 2005, Koh et al. 2010). Inhibition of α-amylase and α-glucosidase decreases digestion and absorption of glucose into the blood (Koh et al. 2010). Multiple compounds are known to be effective against α-amylase but acarbose had the lowest IC$_{50}$ among fifteen tested compounds at 23.2 uM while quinic acid and dihydrocaffeic acid had the highest IC$_{50}$ at 13 and 14 mM, respectively (Funke and Melzig 2005).

**Effects of lipase on obesity development**

The associated decreased risk of obesity with supplementation of polyphenols is a result of inhibition of lipase (Nakai et al.). Similar to the mechanism for diabetes treatment, the compounds inhibit digestion and absorption of lipids and starches, causing a decrease in weight and obesity rates (Zhong et al., Nakai et al.). Most specifically, green tea catechins have no obvious effect on lipolysis, but are thought to decrease lipogenesis (Wolfram et al. 2006). In support of all this, (Matsumoto et al. 1993) showed a decrease in enzyme activity in rat intestine and 53 wolfram showed a decrease in adipose weight in Young Wistar rats. Similar effects have been seen with consumption of oolong tea. Nakai et al. (2005) showed in an *in vitro* study that EGCG and GCG inhibited lipase strongly while the proanthocyanadins present in oolong tea had lower activities than EGCG.
**Inhibitory effects on amylase and amyloglucosidase**

Obesity does not only involve lipids but also the storage of glucose in the body. Hence, breakdown of amylose can have an effect on weight loss. The catabolysis of amylose involves three major enzymes, pancreatic $\alpha$-amylase and $\alpha$-glucosidase found in the intestine, and salivary $\alpha$-amylase found in the mouth (Koh *et al.* 2010). Essentially, any inhibition of one or all of these enzymes can affect the conversion of amylose to glucose, which would affect the risk of obesity as well.

Despite their bodily locations, pancreatic and salivary amylases are similar in nature and were found to have comparable results in breakdown of glucose (Koh *et al.* 2010). Yet, there are some sensitive variants of the enzymes themselves (e.g. variants of glucosidase) and processing variations in manufacturers as well that result in different levels of inhibition (Koh *et al.* 2010).

Koh *et al.* (2010) found that the concentration of green tea that was needed for inhibition of $\alpha$-amylase was higher than the amount needed to see an inhibition of $\alpha$-glucosidase. The study also showed that a higher concentration of EGCG in pure form was needed to inhibit $\alpha$-amylase compared to $\alpha$-glucosidase, indicating that $\alpha$-glucosidase is a more sensitive factor when it comes to enzymatic interactions with green tea and its components.

Polyphenol-rich extracts from fruits, berries, and green tea were tested for inhibition of these enzymes by McDougall *et al.* (2005). Green tea showed greater inhibition of $\alpha$-amylase and $\alpha$-glucosidase than blueberry, black currant, and red cabbage where strawberry extract had the highest activity against pancreatic amylase. Possibly due to anthocyanin content, inhibition of $\alpha$-glucosidase was most effective with blueberry
and blackcurrant extracts while strawberry and raspberry were lower. Raspberries have zero anthocyanin content and also had no effect in this situation; hence the anthocyanins in particular may play a large role in this inhibition study (McDougall et al. 2005).

Whether due to the inhibition of α-amylase or α-glucosidase, Zhong et al. (2006) found through measuring breath-hydrogen concentrations that ingestion of tea extract beverage increased the release of hydrogen through the breath, suggesting an induced malabsorption of carbohydrates (Zhong et al. 2006).

**Inhibitory effects on amylase**

Although inhibition of all three enzymes involved in breaking down amylose is possible, more focus is put on α-amylase because of its status as an action-limiting step; in sequence of conversion to glucose, amylose must first be broken down by amylases to shorter chained saccharides such as maltose and maltohexose before amyloglucosidase can finish the job by finally catabolizing the disaccharides to the monosaccharide glucose (Singh et al. 2010). The α-amylase found in the mouth is the main enzyme in saliva, contributing to 30% of all enzymes. It is well known that polyphenols interact with salivary proteins, causing astringency, yet it is unknown how many other proteins interact with polyphenols as well (Soares et al. 2007).

According to Takabayashi and Harada (1997), green tea catechins in the form of Polyphenon 100 decreased cerulein-induced acute pancreatitis in rats, showing a protective effect of catechins against the disease. In addition, amylase activity was decreased in diseased subjects but increased activity in the normal model with or without addition of green tea catechins. These results show that enzyme activity may not be
affected by the catechins, but the cerulein could play a larger role. Also, in comparing
the groups that were administered catechins in drinking water and those that were given
unadulterated water, there was a significantly lowered amylase activity in the diseased
subjects given catechins compared to those diseased but given water. However, in the
normal model, the catechins did not make a significant difference. This shows a possible
interaction of the catechins with the cerulein that was given to the subjects. However,
these results are also contrary to other studies that show inhibition of amylase by green
tea extract. The authors were unsure how much of a role the cerulein played in the
enzyme activity, but in these rats, inhibition was noted in one group and not in the other
(Takabayashi and Harada 1997).

Another study that showed evidence against enzyme inhibition in general was
Tagliazucchi et al. (2005), who found that pepsin activity was in fact enhanced by the
presence of polyphenols. According to the study, EGCG (0.1 mM) enhanced the enzyme
activity after 120 minutes of exposure, with a final glucose concentration of 165.4 ug/mL
in the presence of EGCG versus 120.3 ug/mL in its absence. The low absorption in the
GI tract of polyphenols could be a possible explanation for this outcome (Tagliazucchi et
al. 2005)

Despite these two studies, most other studies support the inhibition of enzymes by
polyphenols. Besides inhibiting the action of the enzyme directly, Soares et al. (2007)
suggests decreased enzymatic activity due to complex formation. In this study, test
compounds all showed a decrease in fluorescence, suggesting formation of polyphenol-α-
amylase complexes. With the knowledge that lower emission values correspond to lower
amounts of enzyme present, it would be a possible mechanism for decreasing the
availability of amylase and decreasing the conversion of starch to glucose (Soares et al. 2007).

Interestingly, some starches may act as their own inhibitor by the nature of their size and shape. As previously stated, amylopectin and amylose are cleaved at different rates due to steric hindrance produced by the amylopectin branches and molecular weight (Singh et al. 2010). Singh et al. noted noncompetitive inhibition by maltose of α-amylase. Structural differences changed the rheology and viscosity of the food matrix, and hence changed the velocity of hydrolysis (Singh et al. 2010). With this in mind, a question arises whether EGCG and other catechins and polyphenols interfere with the natural inhibition caused by amylopectin and smaller saccharides like maltose.

Despite inhibition or enhancement of salivary α-amylase by polyphenols, neither situation would normally be a problem because of the short time exposure of the food matrix in the mouth. However, in the case of chewing gum, this becomes progressively more integral due to the increased mastication time and hence increased exposure time to enzymes.

**Inhibitory effects of black tea compounds**

Green tea is not the only type of tea that has been attributed to beneficial antioxidant and anti-obesity properties. When it comes to tea, as is with most products, they vary among themselves. Teas are very different depending on their source and location, showing different inhibition patterns (Lee and Chambers 2010, Zhang and Kashket 1998); green tea from China acts quite differently than Korean tea in relation to inhibitory abilities (Lee and Chambers 2010). A comparison between North India tea and
Mali tea (400 uL) resulted in 75% versus 20% inhibition (Zhang and Kashket 1998). Hence, it makes sense that there is variety between black, green, and oolong teas as well. For example, Koh et al. (2010) found that the concentration of tea that was needed for inhibition of α-amylase was higher for green tea than either black or oolong, suggesting higher inhibitory ability in black and oolong teas rather than in green tea. Yet, there are still ranges in effectiveness due to the tea source.

In a study by Kusano et al. 2008, the inhibitory activities of black tea were investigated. With the polyphenol concentration at 0.04 mg/mL, the inhibitory activity of EGCG (more abundantly found in green tea) to pancreatic lipase activity was found to be 14.5%, which was much less than theaflavin, found in black tea. The compound theaflavin-3,3’-di-O-gallate was 48.9% inhibitory against lipase and 81.6% against α-amylase (Kusano et al. 2008). In accordance, Koh et al. (2010) reported relatively low inhibitory activity from green tea and stated that theaflavins inhibit at a higher percentage than catechins, hence making black tea a possibly more effective inhibitor against these enzymes because it contains more theaflavins (Koh et al. 2010). While black tea seems to have the highest effectiveness, oolong and green tea have shown similar results. Still, there seems to be a summation effect of polyphenols and inhibition and despite green tea containing more EGCG and ECG than oolong, there is a possibility that green tea may not be the more effective tea (Koh et al. 2010).

While different teas have different polyphenols and different activities, different polyphenols also have different bioavailabilities depending on their source (McDougall et al. 2005, McDougall and Stewart 2005). The polyphenols that are extracted from tea are not the same in all aspects as those extracted from berries (McDougall et al. 2005).
McDougall and Stewart (2005) reported inhibition of α-glucosidase by anthocyanins in berries that act similarly to the diabetic treatment, acarbose. Both compounds inhibit in a competitive manner, in the former case having structural similarity to the glucosidase substrate, maltose.

Just as starch is important in obesity and diabetes, its breakdown to smaller sugars can induce development of dental caries (Zhang and Kashket 1998), and the inhibition of salivary amylases may decrease incidence of caries, which has been shown in rats (Mormann et al. 1983). Zhang and Kashket (1998) tested the inhibitory properties of tea polyphenols in bacterial and salivary amylase. In 600 uL of tea, black teas showed higher inhibition than green teas (80 to 90% versus 20 to 25%), most likely due to the higher tannin content found in black tea. Zhang and Kashket found a strong positive correlation between amylase inhibition and tannin concentration (r=0.93, p<0.001). Tannic acid produced higher inhibition than (+)catechin, which was only inhibitory at a concentration higher than 2 mg/mL. Not surprisingly, inhibition increased with an increase of the concentration of the compounds (Zhang and Kashket 1998).

**Overall inhibitory effect and product feasibility**

Even if a starch inclusion complex can be successfully made using polyphenols as the inclusion and then added to chewing gum as the delivery vehicle, the enzymatic processes to follow ingestion are important to the bioavailability and effectiveness of the product. The very compounds that are to be added to the complex have a possibility of inhibiting itself. The inhibition of amylase activity by EGCG must be investigated to determine whether the creation and utilization of such a chewing gum product is feasible.
REFERENCES


# Vita

**Debie Wesley Blair**

## Education

<table>
<thead>
<tr>
<th>Year</th>
<th>Degree/Program Details</th>
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| 2010 | B.S. in Food Science, Schreyer Honors College IUG Program  
*The Pennsylvania State University, University Park, PA*  
Thesis: Use of starch inclusion complexes for improved delivery of dietary polyphenols to the oral cavity by chewing gum |

## Work Experience

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| 2009 | Quality Assurance Summer Intern  
*Kraft Foods Global, Inc., East Hanover, NJ*  
Project: Oreo Cookie and Ritz Cracker products |
| 2008 | Productivity Summer Intern  
*Kraft Foods Global, Inc., Glenview, IL*  
Project: Bull’s-Eye Barbecue Sauce |
| 2007 | Research and Development Summer Intern  
*Kraft Foods Global, Inc., Tarrytown, NY*  
Project: Crystal Light Ready-to-Drink product |

## Laboratory Experience

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| 2008-2010 | Food Chemistry Undergraduate/Graduate Research  
*The Pennsylvania State University, University Park, PA*  
Topic: Analyzed textural properties of chewing gum products and ingredients |
| Spring 2008 | Food Microbiology Undergraduate Research  
*The Pennsylvania State University, University Park, PA*  
Topic: Examined growth patterns of *E. coli* O157:H7 in agricultural arenas |
| Summer 2007 | Food Microbiology Undergraduate Research in Studies Abroad  
*The Pennsylvania State University, Los Teques, Venezuela*  
Topic: Evaluated prevalence of foodborne illness in a foreign country based on their harvesting methods and sale venue |
| Spring 2006 | Food Science Undergraduate Research  
*The Pennsylvania State University, University Park, PA*  
Topic: Investigated identity of seed pigments in avocados |

## Organization Involvement

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## Selected Awards

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