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

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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. Bhosale and Ziegler (unpublished) successfully emulsified beta-carotene into a starch inclusion complex, which increased its dispersibility in water, protected the compound from degradative reactions, and also provided a means for controlled release of this sensitive hydrophobic compound. In this thesis, the overall effectivenesss of chewing gum as a delivery vehicle for bioactive polyphenols found in green tea was investigated. In order to fully understand this delivery method, the release of the catechins from three different gum treatments were measured by HPLC. The inhibitory effect that green tea catechins may have on amylase activity was tested to evaluate the effect of starch addition to the gum base. Lastly, the effect of the complex on bioavailability of bioactive components was investigated *in-vivo* by dosage in 72 male CF-1 mice and HPLC analysis of beta-carotene absorption in plasma and fecal samples. Overall, the results of these studies support the need for further investigation of the use of chewing gum and starch inclusion complexes as a delivery vehicle for bioactive components. There is no evidence of interference by tea polyphenols on the breakdown of starch by amylase and hence the release of the bioactive components into the body. Although the physical mixture of starch, tea polyphenols and ascorbyl palmitate into chewing gum possessed the highest C_{max}, the mixture of starch-ascorbyl palmitate complex and tea polyphenols could be the most effective treatment in relation to both possible organoleptic properties as well as total release of bioactive components when compared to gum with tea polyphenols alone.

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CHAPTER 1: INTRODUCTION

The practicality of using chewing gum as a delivery vehicle for bioactive compounds starts with its popularity in the United States and around the world. Chewing gum as we know it now has grown in popularity since World War I, and it has been used as a delivery vehicle for different flavors and functional components such as xylitol and nicotine (Ream and Moore 1979, Fritz 2008, Perfetti 1996). Encapsulation has been an effective method for controllinged release of these ingredients, extending sweetness by incorporation of polyvinyl acetate (Cherukuri *et al.* 1991, Yatka *et al.* 1992). Not only can flavors be encapsulated for controlled release, but other compounds may be incorporated into starch inclusion complexes, for example, to achieve the same effect.

Starch inclusion complexes, which are created by binding helical amylose to guest molecules (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.

Other inclusion complexes, like those created with cyclodextrin, alter the release rate of drugs from polymer films (Arakawa *et al.* 2005). The addition of β-cyclodextrin with lidocaine or ketoprofen to mucoadhesive buccal film dosage forms produced a reduction of 40% in the release of the drugs to artificial saliva when compared to the control. Complexes such as these could be potentially utilized in the controlled release of drugs or other bioactive components.

Similarly, starch-lipid complexes may also be useful in increasing the utilization and effectiveness of bioactive components, which may be emulsified to the complex, shown by the successful encapsulation of beta-carotene by Bhosale and Ziegler (unpublished). Beta-carotene (BC), which is susceptible to oxidative reactions, had a reported increase in overall stability when incorporated into the complex. The complex was characterized by X-ray diffraction, differential scanning calorimetry and iodine binding capacity, which was used to enumerate the degree of complex formation (80% compared to 74.14% for complex without BC). Enzyme digestion tests using pancreatin of the complex suggested possible starch breakdown and bioavailability of the bioactive, with maximal release of BC at 24 h.

In addition to alterations in stability, the encapsulation increased dispersibility of the bioactive compound in water, protected it from degradative reactions, and also provided a means for controlled release (Bhosale and Ziegler, unpublished). 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 might 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 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, I 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 potentially 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 if an effective concentration of bioactive components 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-amyloglucosidase. Inhibition of these enzymes could 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.

1.1 Objectives

The main objective of this experiment was 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 through the buccal mucosa 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 the buccal mucosa; it also provides direct exposure of potentially cancerous cells to tea polyphenols in the case of oral cancer (Arawaka *et al.* 2005). Experiments were conducted to evaluate the release of the green tea polyphenols from the chewing gum matrix to the saliva in free form, as a physical mixture with starch and ascorbyl-6-palmitate, and as a mixture with starch-ascorbyl-6-palmitate complex.

Since the complex contains starch, the release from the matrix may be dependent on the activity of amylase. Therefore, the inhibitory effect that green tea catechins may have on amylase activity was investigated. Any change in the activity of the enzyme due to presence of polyphenols may in turn alter the effectiveness of the starch inclusion complex in a chewing gum product.

It may be beneficial to use a complexed form to decrease the susceptibility of polyphenols to oxidation (Cheynier 2005), in a manner similar to beta-carotene. Yet, if the active ingredient is in the form of a starch inclusion complex, the compound must be released from the helix and freed from the matrix in which it is entrapped to be effective. Therefore, the bioavailability of beta-carotene in male mice when administered in free form and as incorporated into an amylose-ascorbyl palmitate inclusion complex was

compared in another experiment, and the results related to the complexes that may be formed with polyphenols.

In this master's thesis, these three areas were studied to develop an overall validity and efficacy of using chewing gum as a delivery vehicle for bioactive polyphenols.

CHAPTER 2: LITERATURE REVIEW

2.1 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*) may provide consumers with benefits beyond mitigation of thirst or stimulation. 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). 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).

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, I considered the practicality of including the bioactive components as a powdered 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 not known whether the polyphenols would be better delivered in free form or as a starch inclusion complex.

Chewing gum is an attractive and practical delivery method because of its popularity in the United States. When gum first became popular after World War I, it was used mostly for mitigation of thirst, but as time has passed, use has expanded to include 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).

Introduction of green tea extract into chewing gum is not a completely new concept. Chewing gum products have already been created containing green tea polyphenols, but the release of the bioactive compounds from the gum has yet to be analyzed. 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 a proprietary tea extract blend per one piece of gum (1.3 g). Knowing that EGCG is approximately 40% of the extract by weight, each piece of gum would contain 24 mg of the active catechin. Yet typically, one cup of green tea is considered 2.0 g of tea leaves, which would comprise 600 mg of extract, much more 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 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 proposed here.

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 began during World War I 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 war in the Far East and the nationalization of the gum industry in Mexico both 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 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. $CaCO_3$), 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 labeled "gum base" on the nutritional label, while the water-soluble ingredients are labeled separately and known to dissipate over time through chewing (Grey *et al.* 2007). The composition of an example of chewing gum is shown in Table 1. 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)

Ingredient	Percentage by Weight
Gum base (elastomers, resins, etc.)	22
Sweeteners (pulverized)	52
Corn syrup	23
Flavorants	1
Emulsifiers	1.5
Colorants	0.05

Polyvinyl acetate (PVAc), a major component of gum bases typically at about 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). 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 sugar 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 such as reduction in bitterness and enhanced flavor profile (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 has been observed 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 a decreased release rate 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 related to flavor profiles. 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 to 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 bioactives 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 compounds 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, given that the bioactive components experienced the same retention as the ingredients tested in the patent.

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 change 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, Cherukuri *et al.* 1992).

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 cause the product to 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 hydrophilic 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 (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). These methods help the flavor and sweetness last for the 10-60 min for which consumers, on average, chew a piece of gum (Mackay and Schoenholz 1977, Fritz 2008).

Encapsulation: In various patents, the delayed release of sucrose and other components (*e.g.* flavorants, aspartame) was accomplished 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 acesulfame K instead, the effective results were not seen (*i.e.* the acesulfame 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 acids like ascorbic and citric acids (Cherukuri *et al.* 1991), which provide sour flavor to the product and characteristic fruitiness. 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 is dependent 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.* (2007), a compressed granulated chewing gum was prepared so it could be compressed into tablet and optionally coated with these materials or others. 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 undesirable 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 could be an effective 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 potential of this product.

2.2 Inhibition of salivary and gastrointestinal enzymes by polyphenolic compounds

The salivary components that come into contact with chewing gum could affect the effectiveness of the gum as a delivery vehicle of bioactive components. As the most abundant enzyme in saliva, α-amylase contributes to 30% of all salivary enzymes (Soares *et al.* 2007). If polyphenols are added to chewing gum as a bioactive ingredient, the interactions that are possible between salivary proteins and polyphenols could affect salivary levels.

The interest in green tea as a bioactive component stems from linkages to reduction in risk for cancer, cardiovascular disease (CVD), obesity, and diabetes (Koh *et*

al. 2010). The associated decreased risk of obesity and diabetes may be related to inhibition of digestive enzymes by 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).

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 tea polyphenols 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 ug/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 inhibit 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).

Inhibition of lipase by polyphenols. The associated decreased risk of obesity with supplementation of polyphenols may be related to inhibition of lipase (Nakai *et al.* 2005). 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.* 2006, Nakai *et al*. 2005). Matsumoto *et al.* (1993) showed a decrease in enzyme activity in rat intestine and Wolfram *et al.* (2006) showed a decrease in adipose weight in Young Wistar rats when administered green tea. 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 proanthocyanidins present in oolong tea had lower activity than EGCG.

Inhibitory effects on amylase and amyloglucosidase. Obesity does not only involve lipids but also the storage of glucose in the body. Hence, inhibition of dietary amylase can have an effect on weight loss. The catabolism of amylose involves three major enzymes, pancreatic α-amylase and α-glucosidase found in the intestine, and salivary α-amylase found in the mouth (Koh *et al.* 2010). Essentially, any inhibition of one or all of these enzymes can affect the conversion of starch 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).

Koh *et al.* (2010) found that the concentration of green tea that was needed for inhibition of α-amylase was higher than the amount needed to see an inhibition of α-glucosidase. The study also showed that a higher concentration of EGCG in pure form

was needed to inhibit α-amylase compared to α-glucosidase, indicating that α-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 α-amylase and α-glucosidase than blueberry, black currant, and red cabbage where as strawberry extract had the highest inhibitory activity among all polyphenol extracts against pancreatic amylase. Possibly due to anthocyanin content, inhibition of α-glucosidase was most effective with blueberry and blackcurrant extracts while strawberry and raspberry were lower. Raspberries that were treated to be essentially anthocyanin free had no inhibitory effect; 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).

Although inhibition of all three enzymes involved in breaking down starch is possible (pancreatic α-amylase and α-glucosidase, and salivary α-amylase), more focus is put on α-amylase because of its status as an action-limiting step; in sequence of conversion to glucose, starch 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 salivary 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 increased in the normal model whether or not administered green tea catechins while amylase activity decreased in diseased rats only when administered green tea catechins. These results show either that enzyme activity may not be affected by the catechins, but the cerulein could play a larger role, or that catechins only have an effect on amylase in the diseased state. 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 rats 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 or the disease that was given to the rats. 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 this *in vitro* study, EGCG (0.1 mM) enhanced the enzyme activity after 120 minutes of exposure, with a final peptide 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 inhibition caused by maltose and further alter the rates of catabolism between amylose and amylopectin.

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, results vary. 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 of salivary amylase (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 α-glucosidase 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 (1998) 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 guest molecule 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. There is a possibility that the compounds that are present inhibit enzymatic breakdown, which is needed to release the compounds. 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.

2.3 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 on the bioavailability of the component in this form. The complex to be ingested, whether containing beta-carotene or polyphenols as guest molecules, 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 from within the complex, the bioavailability is extremely low and the complex is no better, and perhaps worse, than the free form of the component. On the other hand, bioavailability may also increase due to the presence of lipid, increase in dispersibility, and reduction in particle size.

Typical starch inclusion complexes contain starch and a hydrophobic guest (Figure 1). The beta-carotene complex contains three parts (Bhosale and Ziegler, unpublished): starch, a lipid, and a bioactive component (Figure 2). In the complexation process, water is used as the dispersive solvent, and due to the high hydrophobicity of BC, direct complexation of BC with the amylose was not achieved. With low contact, the BC was not incorporated into the amylose helix; as seen in Figure 2, the successful complexation of BC involved emulsification of the bioactive component into the complex as opposed to complete entrapment (Bhosale and Ziegler, unpublished). No matter entrapment or emulsification, any interactions involving the components of the complex (starch, lipid, and bioactives) are integral to the bioavailability of the bioactive component.

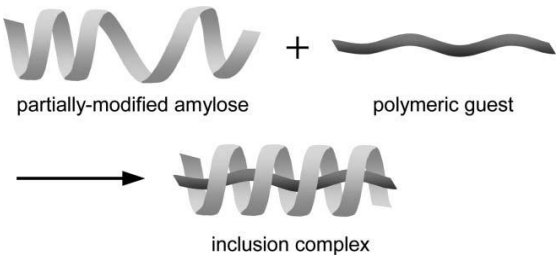

Figure 1. Schematic drawing of starch inclusion complex formation (Kida *et al.* 2007)

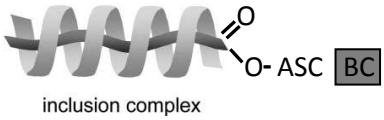

Figure 2. Schematic drawing of beta-carotene starch inclusion complex (adapted from Kida *et al.* 2007)

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 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-chain 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. 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 change bioavailability.

Starch interactions affecting bioavailability. Along with lipids, structural characteristics of starch are also important for digestibility (Singh *et al.* 2010). 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, bioavailability 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) showed that glucose conversion of starch in amylose-lipid complexes ranged 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 cooked waxy maize starch to 77% in cooked high amylose maize starch (Singh *et al.* 2010).

Protein content may play a role in starch digestion 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 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^{o}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 may also change 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 dehulling, soaking, and germination provides a loss of phytic acid, tannins, and polyphenols that normally inhibit α-amylase activity (Rehman and Shah 2005, Singh *et al.* 2010). 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 loss in the content 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 *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 when in gum than when in tablet form. Kamimori *et al.* (2002) showed a significantly faster rate of absorption of caffeine from 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_{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_{max} in the study done by Noehr-Jensen *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 *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 of iron from the acidity of gastric fluid; 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 the 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 guest molecules in starch inclusion complexes would be helpful to determine whether the complexes could be used for bioactive compound supplementation.

CHAPTER 3: MATERIALS AND METHODS

3.1 Chewing gum study

Chemicals and reagents. Sweetened, unflavored gum was provided by Wm. Wrigley Jr. Company, green tea extract 80% (from *Camellia sinensis*, decaffeinated, Lot 0161510) was provided by Nature's Sunshine Products, Inc. (Spanish Fork, UT), ascorbic acid 6-palmitate (A1968, USP grade) was purchased from Sigma-Aldrich Company (St. Louis, MO). Hylon VII starch is a product of National Starch (Ref E748-35). Pure undenatured ethanol (200 proof, Cat 111000200PL05) was received from PharmCo, and dimethyl sulfoxide (D1258, USP grade) was received from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ).

Complexation of amylose and ascorbyl palmitate. A mixture of 95% dimethyl sulfoxide in water (10 mL) and Hylon VII starch (500 mg) was stirred for two hours then heated to 90^{o}C before the addition of 50 mg ascorbic acid 6-palmitate (A6P) to create the inclusion complex. The solution was held at 90^{o}C for one hour, then added to 90^{o}C water (25 mL) with vigorous stirring. The water bath was shut off, and the complex was slowly cooled to room temperature in the water bath undisturbed for 72 hours.

The complex was then recovered by centrifugation (903 × g, 20 min). The pellet was washed with an ethanol:water solution (1:1) and centrifuged at the same parameters as previously described. This washing step was completed three times, after which the pellet was washed with pure ethanol and recovered on #2 filter paper using vacuum. The remaining pellet was dried for 24 hours in a dessicator and pulverized into a fine powder.

This procedure was repeated until enough complex was made for the formulations in Table 2.

Complexation of amylose, ascorbyl palmitate, and tea extract. The same complexation method was used to incorporate polyphenols into the system; however, the method was not 100% efficient. The high solubility of tea polyphenols prevented incorporation to the hydrophobic amylose helix. Hence, to ensure the gum treatment contained the same amount of tea extract in the final formula, the polyphenols were not complexed to the starch-A6P, but were merely added as a separate powder ingredient. The starch-A6P complex was tested as an additional treatment against those ingredients used in the physical mixture (starch, A6P, and tea extract all in free, powder form).

Ingredient preparation for chewing gum. Three gum treatments were made including a control, a physical mixture, and a complex mixture. The former two treatments contained 4.0 g of gum per stick. The latter treatment weighed 5.15 g/stick due to the difference in consistency upon addition of the ingredients. A lower amount of unflavored gum would not have allowed for a chewable piece of gum without premature dissolution in the oral cavity when the complex mixture was incorporated. Although the total weight of the piece of gum is different, the amount of additional ingredients/stick remained constant. The formulations are shown in Table 2.

Table 2. Formulations for gum treatments per 4.0 g and 5.15 g stick of gum. Values for complex mixture are based on the weights of A6P and starch used to make the inclusion complexes.

Treatment	Gum (g)	Tea extract (g)	A6P (g)	Starch (g)
Control	3.950	0.050	-	-
Physical Mixture	3.400	0.050	0.050	0.500
Complex Mixture	4.550	0.050	0.050	0.500

Addition of complex to gum base and formation of chewing gum. The Brabender Farinograph-E was preheated to 55^{o}C. The unflavored gum (40.0 g) was added to the mixer and mixed for two minutes before any extract was added. The dry ingredients (Table 2) were added in 30 second intervals over a two minute period in order to allow the powder to disperse evenly throughout the gum. Once the entire amount of dry ingredients were added, the gum was mixed for another four minutes then removed from the mixer.

The chewing gum was rolled out on a marble slab using a metal roller and metal T-form to help mold the gum to a 1.0 mm thickness. The slab and the roller were coated with $CaCO_3$ to prevent sticking of the gum. Once shaped, the gum was cut into pieces (approximately 4.0 g and 5.15 g each), wrapped in aluminum foil, and stored at 4^{o}C to inhibit oxidation.

Experimental design. The protocol for human subjects was based on the salivary collection methods conducted by Lee *et al.* (2004) and the altered version was approved by the Institutional Review Board for the Protection of Human Subjects (Protocol no. 32273) at The Pennsylvania State University (University Park, PA). Seven volunteers

who did not smoke, drink alcohol, nor drink tea for at least two days prior to the start of the study were included. Commencement of the experiment was with a thorough brushing of the teeth and oral cavity to remove any polyphenols that may be present. A baseline sample of the saliva was taken prior to placing the chewing gum into the oral cavity. Over a span of one hour, volunteers chewed a piece of the control gum while holding their saliva in the oral cavity. At 0, 2, 5, 10, 15, 20, 30, 45, and 60 min the saliva was collected in tubes, to which a solution of 20% ascorbic acid and 0.1% EDTA was added at 0.1% vol/vol. Samples were frozen at -80^{o}C until HPLC analysis. This process was repeated for the remaining treatments.

Analysis of polyphenols in samples by HPLC. HPLC analysis was conducted using the method described by Lee *et al.* (2004). Sample concentrations were determined by comparison to the standard (60 ng/mL).

3.2 Inhibition study

Chemicals and reagents. Potato starch (#E748-36) was purchased from National Starch (Bridgewater, NJ), and EGCG (93% pure) was purchased from Taiyo Green Power Company (Jiangsu, China). α-amylase (Type VI-B from porcine pancreas, Lot 092K0850) and amyloglucosidase enzymes (from *Aspergillus niger*, Lot 045K7450) were purchased from Sigma-Aldrich Company (St. Louis, MO).

The potato starch was pulverized following pregelatinization and drying in a 215^{o}C oven for 60 minutes. The EGCG was prepared in a stock solution (100 mM) and stored in -80^{o}C. For use in the experiment, the solution was heated to 37^{o}C for 2-3

minutes for pipetting. The phosphate buffer was prepared in stock solution (20 mM phosphate, pH 6.9, and 10 mM NaCl).

Preparation of solutions. The α-amylase solution was prepared by adding 8.9 mg enzyme to 10 mL of phosphate buffer. The amyloglucosidase solution was similarly prepared by adding 8.75 mg to 10 mL of stock phosphate buffer. Both solutions were centrifuged for 10 min at 1500 × g, and supernatants were used for digestion.
Three sample solutions were prepared for testing of glucose production from pregelatinized starch. In the control solution, EGCG (14 µL, 100mM) was added to potato starch (35 mg) and phosphate buffer (7.0 mL). The treatment solutions each contained potato starch (35 mg), α-amylase (1.0 mL), amyloglucosidase (1.0 mL), and phosphate buffer (5.0 mL). To test inhibition of glucose production by EGCG, the catechin was added to one of the treatments (14 µL, 100mM), while the other contained no EGCG.

Glucose production. Immediately upon addition of the enzymes to the sample solutions, treatments were incubated in 37^{o}C water bath for 110 min while shaken with a VWR DS-500 Orbital Shaker. At 10, 20, 30, 40, 50, 60, 90, and 110 min, glucose production was measured from each sample using an Accu-Check® Aviva glucometer (Roche Diagnostics, Indianapolis, IN) in four replicates, with two observations for each replicate.

3.3 Bioavailability study

Chemicals and reagents. Guar gum (Lot 508518, pretested 8/22 powder) was purchased from TIC gums (Belcamp, MD). Beta-carotene (Lot 22040) and ascorbic acid 6-palmitate (A1968, USP grade) were purchased from Sigma-Aldrich Company (St. Louis, MO). Hylon VII starch is a product of National Starch (Ref E748-35). Pure undenatured ethanol (200 proof, Cat 111000200PL05) was received from PharmCo, and dimethyl sulfoxide (D1258, USP grade) was received from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ). All other reagents were of the highest grade available.

Inclusion complex. The inclusion complex was created based by the procedure of Bhosale and Ziegler (unpublished). A mixture of 95% dimethyl sulfoxide in water (10 mL) and Hylon VII starch (500 mg) was stirred for two hours then heated to 90^{o}C before the addition of 50 mg ascorbic acid 6-palmitate (A6P) and 50 mg beta-carotene to create the inclusion complex. The solution was held at 90^{o}C for one hour, then added to 90^{o}C water (25 mL) with vigorous stirring. The water bath was shut off, and the complex was slowly cooled to room temperature in the water bath undisturbed for 72 hours.

The complex was then recovered by centrifugation (903 × g, 20 min). The pellet was washed with an ethanol:water solution (1:1) and centrifuged at the same parameters as previously described. This washing step was completed three times, after which the pellet was washed with pure ethanol and recovered on #2 filter paper using vacuum. The remaining pellet was dried for 24 hours in a dessicator and pulverized into a fine powder. The resulting beta-carotene content was 10% in the starch-A6P-BC complex (Bhosale and Ziegler, unpublished). Presence of complex was confirmed by X-ray diffraction.

Animals and treatment. Male CF-1 mice (n=72, 4 wks old) were purchased from Charles River Laboratory (Wilmington, MA). Mice were housed 5 per cage. After a two week acclimation period on corn cob bedding and given ad libitum access to food (Purina Rodent Chow 5001) and water, mice were fasted for 16 h and divided into two treatment groups, complexed beta-carotene (CBC) and free beta-carotene (FBC). The CBC mice were given a single dose through gavage method of beta-carotene inclusion complex (20 mg beta-carotene/kg body weight) suspended in 0.25% guar gum (Huang *et al*. 2008). The FBC group was given a single dose through gavage method of beta-carotene (20 mg/kg body weight), Hylon VII amylose (1.5%), and ascorbyl palmitate (0.15%) suspended in 0.25% guar gum. The dosing solutions contained 2 mg beta-carotene/mL.

At baseline and 20, 50, 90, 180, and 360 min after treatments, six mice per treatment were euthanized. Blood, kidney, lung, liver, spleen, heart, small intestine, colon, and fecal samples removed from the colon were collected. Tissue and fecal samples were immediately frozen to -80^{o}C for later analysis. Blood samples were centrifuged at 900 × g for 15 min to prepare plasma and subsequently frozen to -80^{o}C.

Extraction of plasma. Plasma (100 µL) was combined with deionized water (140 µL) and ethanol (150 µL). After addition of hexane (1 mL), the mixture was vortexed and centrifuged for 10 min at 14462 × g. The hexane layer (800 µL) was removed and transferred to a new tube. The remaining aqueous phase was extracted again with hexane. The combined hexane extract was dried under vacuum, and the residue dissolved in absolute ethanol (100 µL) for HPLC analysis.

Extraction of feces. Sample (10 mg) was combined with deionized water (140 µL) and ethanol (150 µL). Fecal samples were vortexed and tissue samples were homogenized with mechanical dounce homogenizer, followed by centrifugation of the sample for 4 min at 14462 × g. The supernatant was removed and transferred to a new tube. Samples were then extracted in an analogous manner to plasma. The hexane extract was dried under vacuum, and the residue dissolved in absolute ethanol (100 µL) for HPLC analysis.

Analysis of beta-carotene in samples by HPLC. HPLC analysis was conducted on the residues of the hexane extractions after dissolution in ethanol with a Supelcosil C_{18} reversed-phase column (150 x 4.6 mm; 5 um particle size) and an isocratic mobile phase of 82% ethanol in water containing 20 mM ammonium acetate (pH 4.4). The mobile phase flow rate was 1.2 mL/min and the analyte was monitored by electrochemical detection with potentials set at 100, 200, 500, and 800 mV.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Chewing gum study

Lee *et al*. (2004) found that chewing tea leaves led to higher salivary levels of catechins 2-4-fold as opposed to holding tea leaves in the mouth without chewing, for the same duration of time. This led to the assumption that chewing gum would be a great practical method for distributing green tea catechins to the oral cavity. In this study, the release of green tea catechins from a chewing gum matrix over a 60 min period of time was measured by administering the chewing gum to humans, and the catechin content of the saliva was analyzed by HPLC. Three different chewing gum formulas were tested and compared to determine if the use of starch inclusion complexes altered their release.

Over time, the complex treatment released more catechins from the matrix to the saliva compared to both the control and the physical mixture (55560 ug mL^{-1} min), shown by the area under the curve of the concentration vs. time (Tables 4, 8), however not significantly (determined by two-way ANOVA, p=0.096)). As suspected, exposure (AUC) to EGCG was significantly highest (p=0.000) of all catechins in the chewing gum treatments, at 25052, 30102, and 30400 ug mL^{-1} min for the control, physical, and complex treatments, respectively (Table 8). In the first two minutes of release, the physical mixture showed an EGCG C_{max} of 2992 ug/mL, while the control and complex respectively showed a significantly smaller response of 2539 and 2097 ug/mL (p=0.004, Tables 3, 7). The same trend between the treatments was seen in the release of EGC, EC, and ECG. Despite the low C_{max} of the complex treatment, the effectiveness of this formulation should not be diminished, based on the combination of a high amount of total release plus the possible hindrance of undesirable organoleptic characteristics such as

astringency, which could possibly be seen in the physical mixture as a result of quick release.

	C_{max} (ug/mL)			
Treatment	**EGC**	**EC**	**EGCG**	**ECG**
Control	915 ± 380	694 ± 161	2539 ± 515	639 ± 134
Physical	1168 ± 308	836 ± 96	2992 ± 273	731 ± 135
Complex	704 ± 304	515 ± 134	2097 ± 420	306 ± 68

Table 3. Maximum salivary concentration of bioactive components from control, physical, and complex treatments over 60 min period of time.

	Area under curve (ug mL^{-1} min)				
Treatment	**EGC**	**EC**	**EGCG**	**ECG**	**Total**
Control	8152 ± 2814	6710 ±1851	25052 ± 8559	5654 ± 2003	45568[A]
Physical	9091 ± 2923	7494 ± 1557	30102 ± 7661	7185 ± 2363	53872[A]
Complex	11112 ± 4208	8642 ± 2200	30400 ± 5033	5406 ± 1617	55560[A]

[A] *Means that do not share a letter are significantly different, Fisher Method.*

Table 4. Total salivary levels of bioactive components in control, physical, and complex treatments over 60 min period of time.

	Elimination rate constant (min^{-1})			
Treatment	**EGC**	**EC**	**EGCG**	**ECG**
Control	0.0481 ± 0.0076	0.0434 ± 0.0065	0.0540 ± 0.0100	0.0478 ± 0.0085
Physical	0.0489 ± 0.0081	0.0431 ± 0.0064	0.0333 ± 0.0110	0.0267 ± 0.0102
Complex	0.0464 ± 0.0072	0.0388 ± 0.0065	0.0362 ± 0.0190	0.0218 ± 0.0195

Table 5. Elimination rate constant of bioactive components in saliva from control, physical, and complex treatments over 60 min period of time.

	Half life (min)			
Treatment	**EGC**	**EC**	**EGCG**	**ECG**
Control	14.8 ± 2.7	16.3 ± 2.4	13.2 ± 2.2	14.9 ± 2.5
Physical	14.5 ± 2.4	16.4 ± 2.6	22.6 ± 6.3	28.8 ± 9.1
Complex	15.2 ± 2.2	18.3 ± 3.1	25.7 ± 17.3	260.2 ± 574.3

Table 6. Half life of bioactive components in saliva from control, physical, and complex treatments over 60 min period of time.

Table 7. Two-way ANOVA table: C_{max} versus treatment and catechin.

Source	DF	SS	MS	F	P
Treatment	2	555988	277994	16.00	0.004
Catechin	3	7662710	2554237	147.04	0.000
Error	6	104229	17371		
Total	11	8322926			

S = 131.8 R-Sq = 98.75% R-Sq(adj) = 97.70%

Table 8. Two-way ANOVA table: Area under curve versus treatment and catechin.

Source	DF	SS	MS	F	P
Treatment	2	14303819	7151909	3.55	0.096
Catechin	3	990687561	330229187	164.00	0.000
Error	6	12081401	2013567		
Total	11	1017072781			

S = 1419 R-Sq = 98.81% R-Sq(adj) = 97.82%

Table 9. Two-way ANOVA table: Elimination rate constant versus treatment and catechin.

Source	DF	SS	MS	F	P
Treatment	2	0.0003700	0.0001850	3.92	0.081
Catechin	3	0.0003770	0.0001257	2.66	0.142
Error	6	0.0002832	0.0000472		
Total	11	0.0010301			

S = 0.006870 R-Sq = 72.51% R-Sq(adj) = 49.60%

Table 10. Two-way ANOVA table: Half life versus treatment and catechin.

Source	DF	SS	MS	F	P
Treatment	2	10371.2	5185.59	1.12	0.385
Catechin	3	15870.5	5290.17	1.15	0.404
Error	6	27686.8	4614.46		
Total	11	53928.4			

S = 67.93 R-Sq = 48.66% R-Sq(adj) = 5.88%

Despite the initial high response, the control, physical, and complex treatments did not have significantly different EGCG elimination rate constants of 0.0540, 0.0333, and 0.0362 min^{-1}, respectively (p=0.081, Tables 5, 9). The half life of the catechins was not significantly different in the complex treatment compared to all cases of the control and the physical mixture (p=0.385, Tables 6, 10). Still, the expected trend of an immediate increase at 2 min and slow decrease of polyphenol concentration over the remaining 60 min period was seen in all treatments and for all catechins (Figures 3-6).

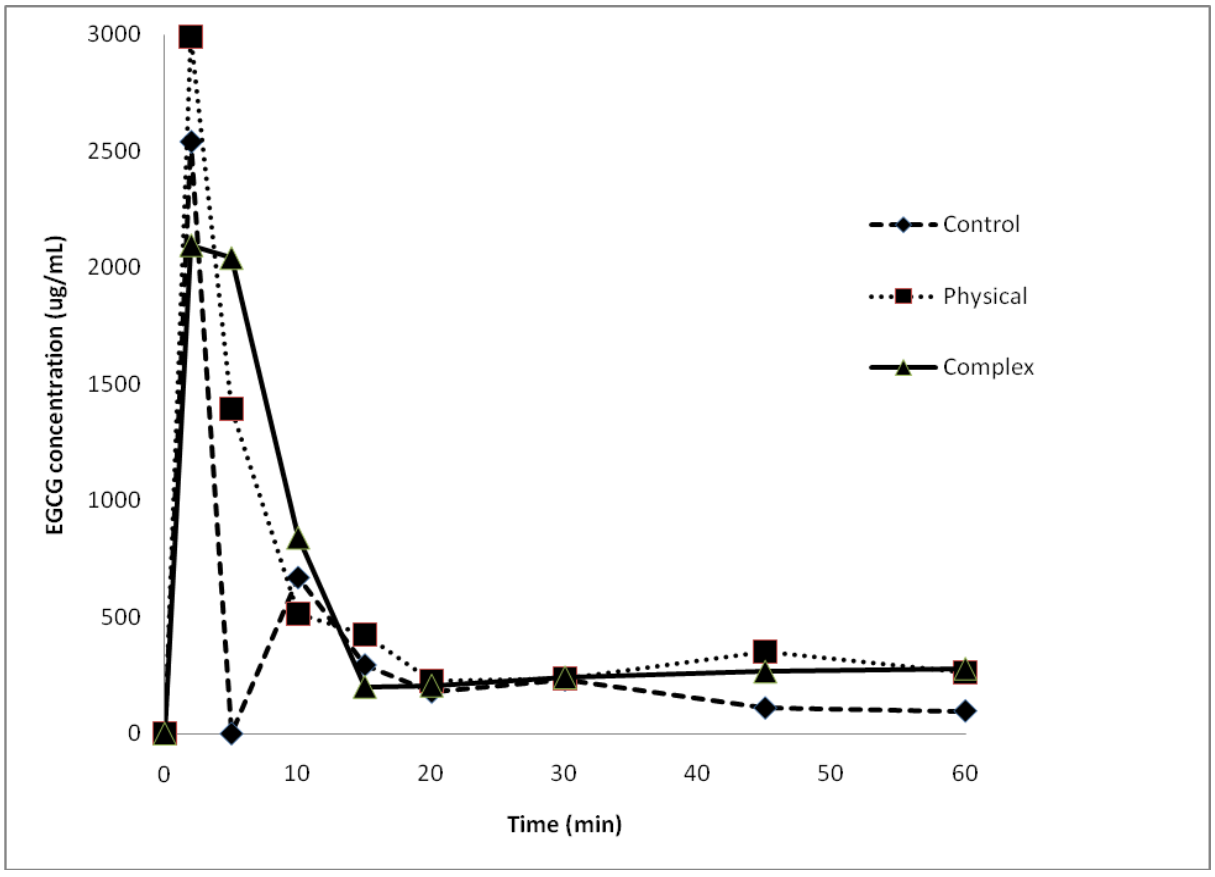

Figure 3. Average salivary concentration of EGCG in all test subjects released from control, physical, and complex treatments over a 60 min period of time.

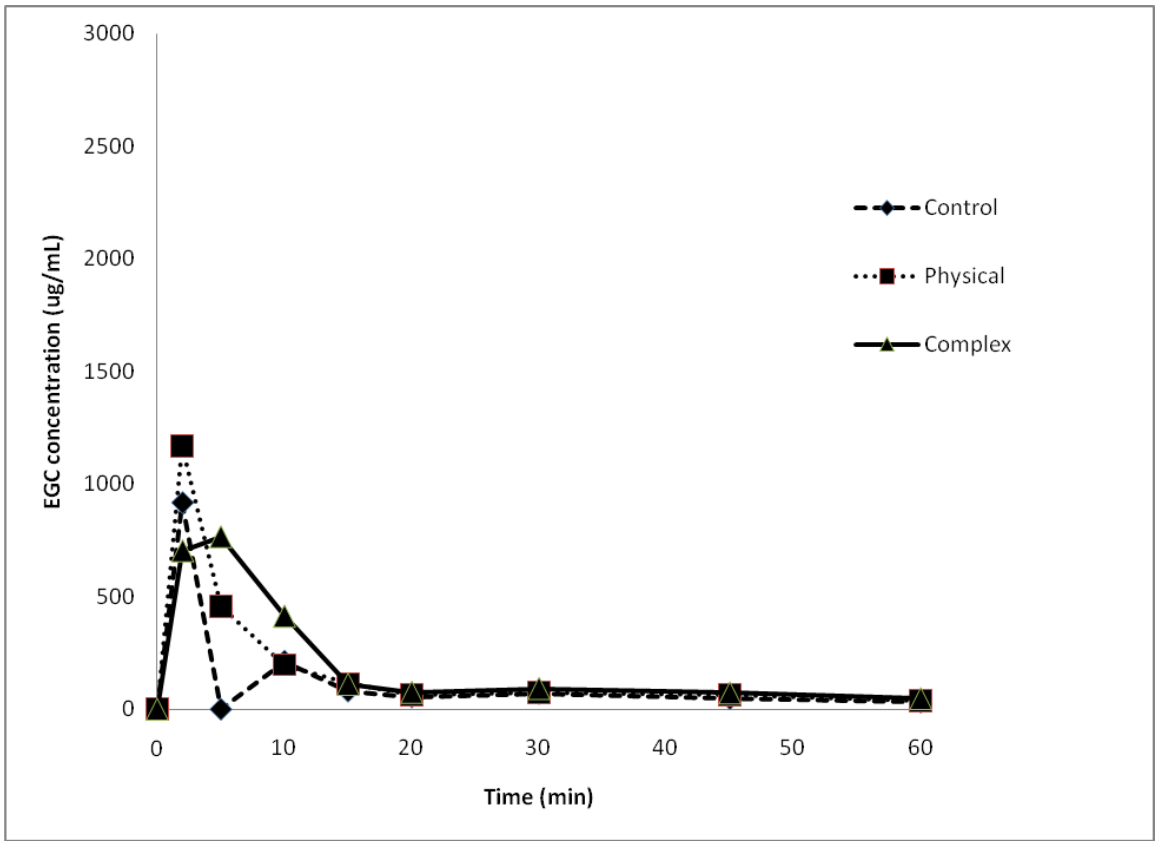

Figure 4. Average salivary concentration of EGC in all test subjects released from control, physical, and complex treatments over a 60 min period of time.

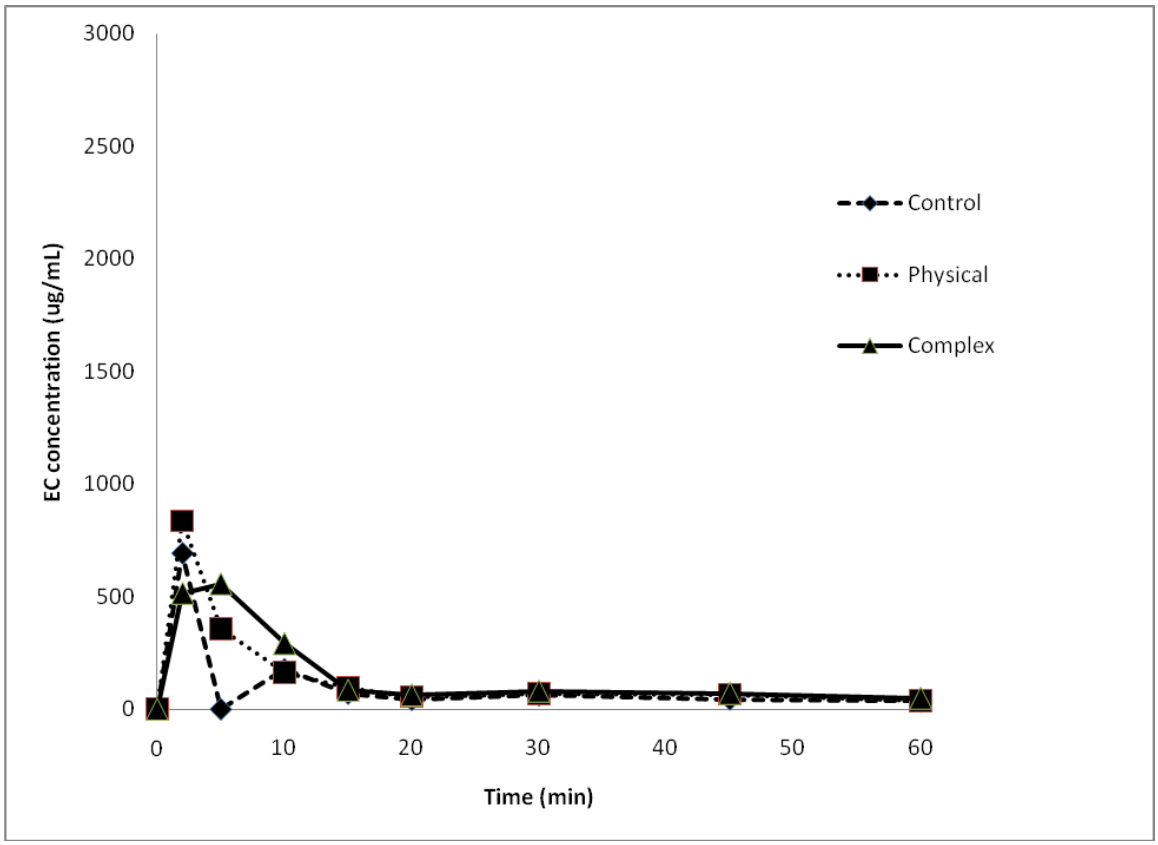

Figure 5. Average salivary concentration of EC in all test subjects released from control, physical, and complex treatments over a 60 min period of time.

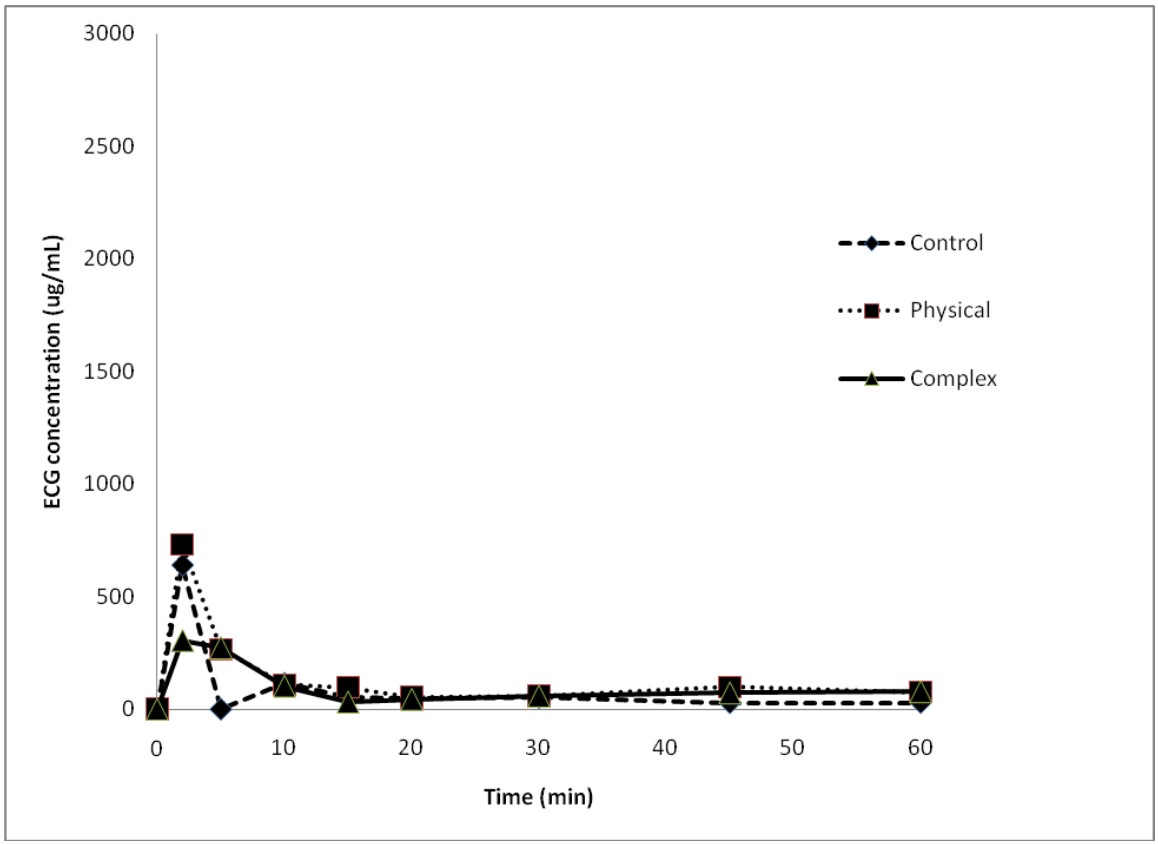

Figure 6. Average salivary concentration of ECG in all test subjects released from control, physical, and complex treatments over a 60 min period of time.

A note that must be made in the results is that one of the test subjects missed the 5-minute sampling period for the complex treatment, and the 10-min sampling period was a combination of both. Hence, their value at 10 min was higher than all other subjects for this treatment. This data was still included in the statistical analysis.

Although all treatments had the same amount of tea extract added (refer to Table 2), the complex treatment required an additional amount of gum base, and thus had a higher overall weight. This was done to increase elasticity and to hinder the disintegration, which was prone to this particular treatment. This treatment may have needed more unflavored gum than the control because the amount of powder ingredients were greater, decreasing the gum base:powder ingredient ratio. As for the treatments, the complex may have needed more gum base because of a change in physical properties of the powder ingredients. Although starch and A6P were added to both treatments, the form in which they were added was different.

The same alterations that were made to the complex treatment may have been necessary for the physical mixture due to the experience by one of the test subjects of a breakdown of the treatment in her mouth after less than 10 min of chewing (data not included in analysis). This disintegration in one subject represents the many differences that exist between chewing gum bases, as well as among consumers of those gums. The salivary components of each individual test subject are different and play a role on the flavor and release of products from gum (Fritz 2008). The salivary amylase of this particular test subject may have acted in excess on the carbohydrates in the product, interrupting the continuity of the matrix. Carbohydrate ingredients such as corn syrup

and sweeteners, and polysaccharides in the gum base such as pectin and carrageenan may have been the target for amylase activity (Mackay and Schoenholz 1977).

On the same note, the variation of elimination rate constants and maximum salivary levels that was seen between the three treatments could be due to the gum itself as opposed to the use of starch inclusions. Although the gum was the same in each sample, the consistency of the gum was altered depending on the ingredients that were added, as was noted by the volunteers during the study. The same amount of active components were included in each treatment, but the amount of gum (3.44 to 4.55 g), and total size of the final product varied (4.00 and 5.15 g). The polymers, resins, and waxes that are part of the gum base provide the final product with a certain consistency and elasticity that changes depending on the concentration of gum base that is used in a piece of chewing gum (Fritz 2008). Addition of other ingredients (like starch and other powders) may decrease the elasticity of the product undesirably. The difference in overall cud size is a confounding variable that could contribute to the release of the components. The percentage of powder ingredients in the complex treatment (11.7%) was lower than that of the physical treatment (15%). If polyphenols preferentially partitioned into the gum phase vis-à-vis the saliva, the larger cud of the complex treatment may have reduced C_{max}.

In the control gum, only tea extract was added, giving it certain textural, organoleptic, and release properties. In the treatments, it was noted by the volunteers that the gum was more "crumbly" with the physical mixture than both the control and the complex treatment. Due to the addition of high amounts of starch and ascorbyl palmitate as well as tea extract, the elasticity decreased, and the homogeny and continuity of the

matrix was interrupted, leading to a possible increase in the release of the ingredients into the saliva. This also led to a decrease in the desirable texture of the final product, in one case, falling apart in a test subject's mouth. For this reason, the complex treatment required a higher amount of gum base and hence overall size in order for the final product to remain as a chewable gum.

The patent for *Chewing gum with improved sweetness profile incorporating finely ground bulk sweetener without starch* (Patel 1992) describes how starch that is used in sweeteners as anti-caking agents, "tends to dry gum out, absorb a portion of the flavor, and adversely affects the mouth feel." This mirrors our findings of the treatments that contained the starch inclusion complexes and the starch and ascorbyl palmitate. Yet, in the patent for *Chewing gum flavor ingredient* (Patel *et al.* 1992), the addition of a flavor ingredient, which was prepared by addition of elastomers and aqueous starch solution before reduction to a fluffy white powder, increased the flavor quality and intensity of the chewing gum, and also provided a long-lasting flavor. The starch solution contained between 20-50% starch (68% w/w of ingredient) and the rest water. It is unknown the exact type of starch that is used in the invention, except that it may include any or all of the following: starch, starch mixture, of modified starch (*e.g.* maltodextrin, corn syrup solids, gum Arabic, cellulose). An investigation into this method could present an alternate to the starch used for our starch inclusions, and a possible retention of desired flavor qualities.

In regard to the release of all incorporated tea polyphenols, it would be undesirable for these catechins to remain in the insoluble base for too long. The retention in the cud would result in an undesirable taste due to the earlier release and solubilization

of the masking flavors and sweeteners. Also, retention in the base would decrease salivary concentrations and uptake of bioactive components by the buccal mucosa. However, if this does occur in a particular treatment, it may be viable to add poorly water-soluble sweeteners and poorly extractable food acids. This method would decrease the observation recorded by Mackay and Schoenholz (1977) that "after about five minutes of chewing all that remains in the mouth is an essentially tasteless wad which provides little in the way of flavor, aroma or sweetness." Their invention in the patent for *Long-lasting flavored chewing gum including chalk-containing gum base* extends flavor and decreases bitter aftertaste in chewing gum by "incorporating a solid poorly extractable or poorly water-soluble sweetener in particulate form or food acid in particulate form" (Mackay and Schoenholz 1977).

The total salivary level of EGCG for the control treatment is above the efficacious dose reported by Shimizu *et al.* (2005), who reported inhibition of colon cancer cells with as little as 10.4 ng/mL EGCG per hour. The control treatment, which had the lowest salivary levels, released 418 ug/mL EGCG into the saliva over one hour. The physical and complex treatments released even higher concentrations of EGCG into the saliva (502 ug/mL and 507 ug/mL, respectively), representing possible higher efficacy than the control treatment.

4.2 Inhibition study

While starch inclusion complexes may be a beneficial delivery system, their breakdown is dependent on the activity of amylase. The possible inhibitory effect of green tea catechins on amylase was investigated by subjecting starch to catabolism via amyloglucosidase and amylase enzymes, while in the presence and absence of EGCG.

After 110 min of incubation, the enzyme digestion was completed and samples were removed from the water bath. To analyze the data, two replicate observations were averaged at each time point from each of four replicated experiments, shown in Figure 7. For each time point, the glucose concentration for the sample with EGCG was higher than that of the sample without EGCG.

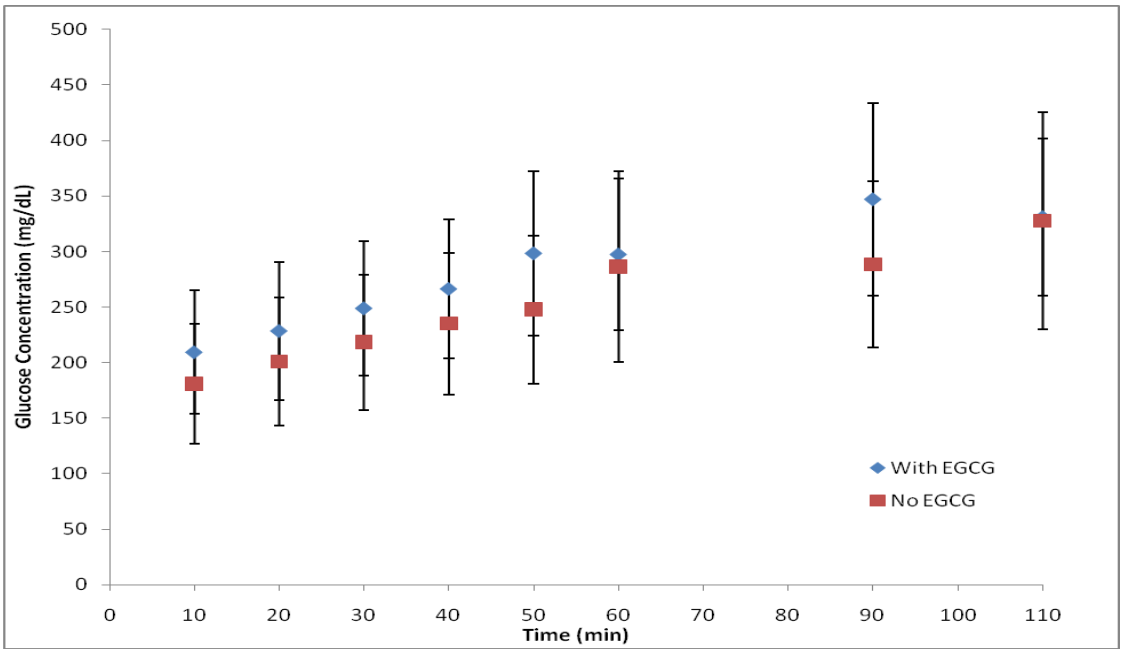

Figure 7. Glucose production as a function of time over 110 min in samples with and without EGCG (200 uM). Standard error bars are shown.

Table 11. Two-way ANOVA table: Response (concentration) versus treatment and time.

Source	DF	SS	MS	F	P
Treatment	1	14535	14535.3	0.85	0.361
Time	7	130189	18598.4	1.08	0.386
Error	55	943852	17160.9		
Total	63	1088576			

S = 131.0 R-Sq = 13.29% R-Sq(adj) = 0.68%

The commencement of glucose production at 10 minutes recorded glucose values for EGCG and no EGCG of 209 and 181 mg/dL, while the progression of enzymatic digestion concluded at 110 min with values of 331 and 328 mg/dL, respectively. At time 60 and 110, the values for the two samples had the smallest difference at 11 and 3 mg/dL, respectively, while the other time points had an average difference of 38 mg/dL between samples. This difference was determined to be not significant by two-way ANOVA (Table 11), showing that the enzymatic production of glucose from starch in the two samples, while consistently higher in the presence of EGCG, is not significantly different. In all of the experiments in the absence of α-amylase and amyloglucosidase, no glucose was found during 110 min of digestion (data not shown).

A possible explanation for inhibition of amylase by catechins is catechin binding to proteins (He *et al*. 2006), in particular the binding of polyphenols to salivary proteins (amylase for the majority), which is responsible for creating sediment and haze, or protein precipitation. This phenomenon is also responsible for the astringency experienced upon consumption of polyphenols.

While most studies show evidence for enzyme inhibition of proteins, Tagliazucchi *et al.* (2005) found that pepsin activity was in fact enhanced by the presence of polyphenols *in vitro*. According to the study, EGCG (0.1 mM) enhanced the enzyme

activity after 120 minutes of exposure, with a final peptide concentration of 165.4 ug/mL in the presence of EGCG versus 120.3 ug/mL in its absence (Tagliazucchi *et al.* 2005).

4.3 Bioavailability study

In this part of the study, the complex beta-carotene group (CBC) were given a single dose of beta-carotene inclusion complex (20 mg beta-carotene/kg body weight) suspended in 0.25% guar gum (Huang *et al*. 2008). The free beta-carotene group (FBC) was given a single dose of beta-carotene (20 mg/kg body weight), Hylon VII amylose (1.5%), and ascorbyl palmitate (0.15%) suspended in 0.25% guar gum. The bioavailability of the beta-carotene in complex form and in free form was compared.

All HPLC chromatograms of the plasma for all mice were similar to Figure 9, lacking peaks at the suspected location for presence of beta-carotene (retention time=12 min) as seen in the standard chromatogram (Figure 8).

The chromatograms for FBC fecal samples, however, did result in a peak at 20 to 360 min (Figures 10 and 11) while those for CBC fecal samples resembled that of Figure 9. The FBC group had a maximum response at 180 min (46.8 nA). After this time point, there was a decrease in absorption at 360 min (20.7 nA).

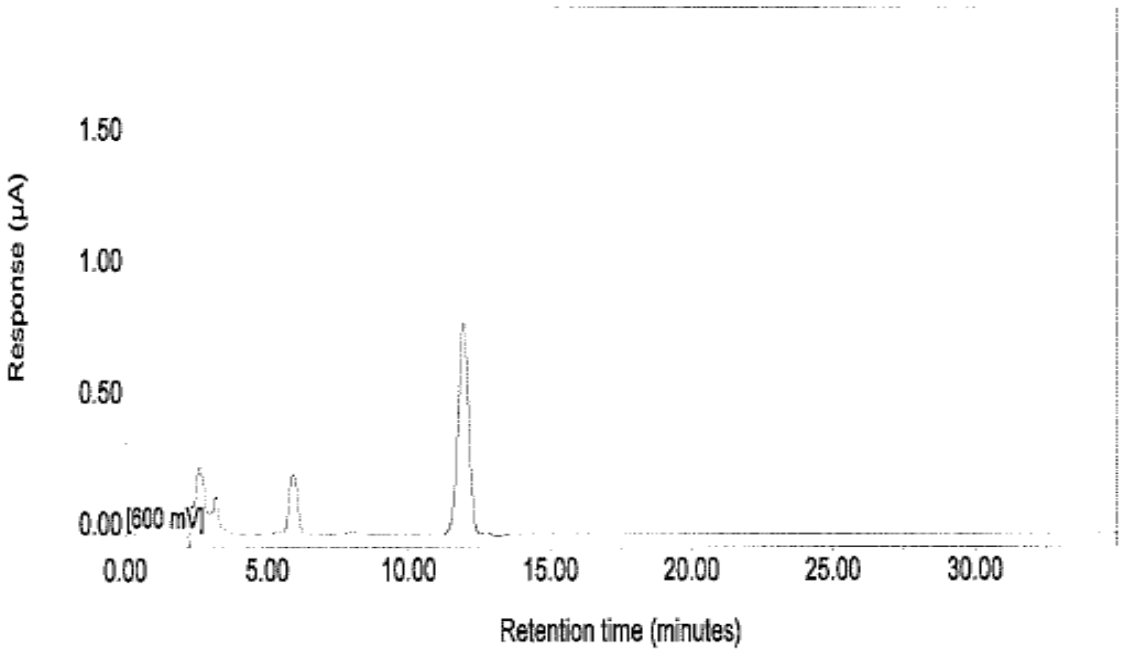

Figure 8. HPLC chromatogram of plasma beta-carotene standard.

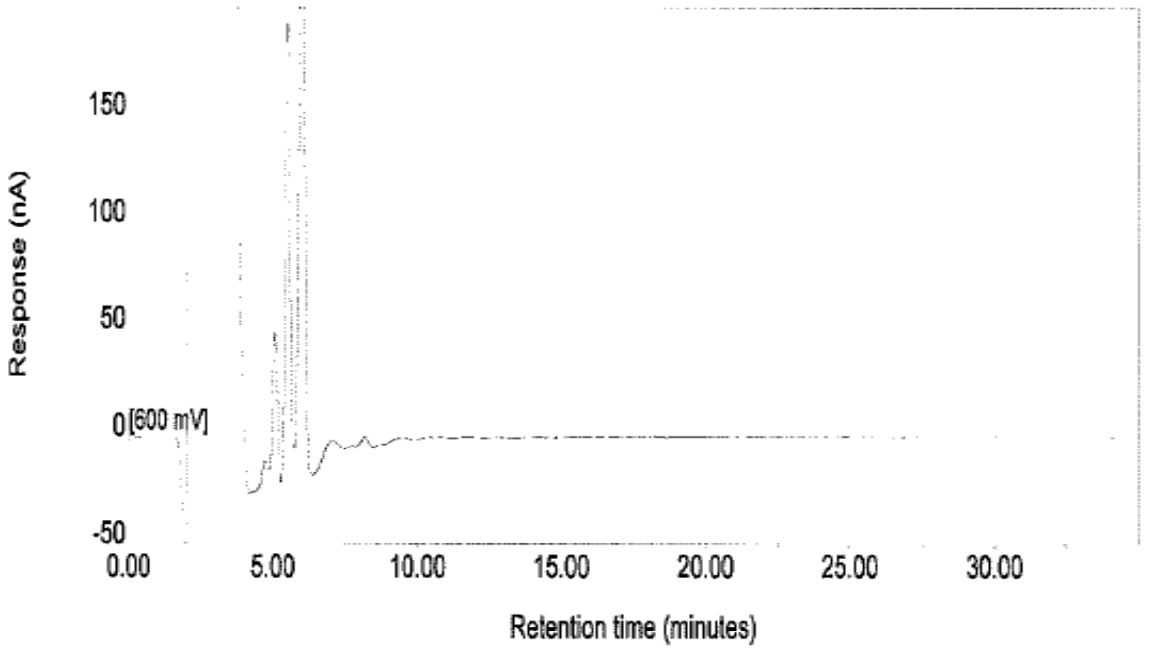

Figure 9. HPLC chromatogram of fecal sample from CBC group.

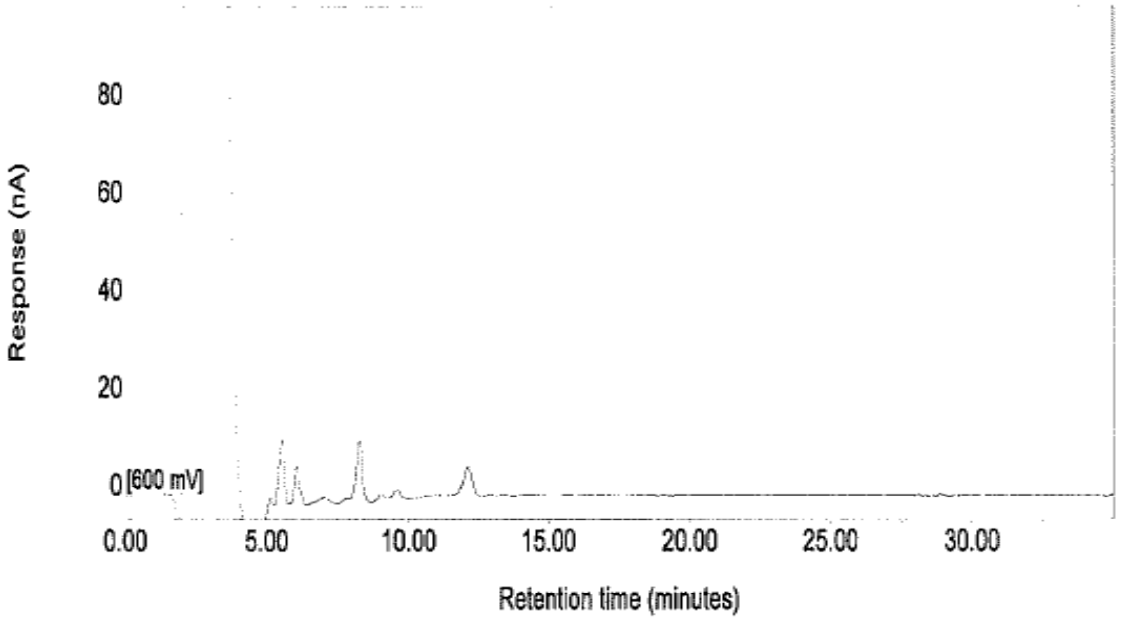

Figure 10. HPLC chromatogram of fecal sample from FBC group.

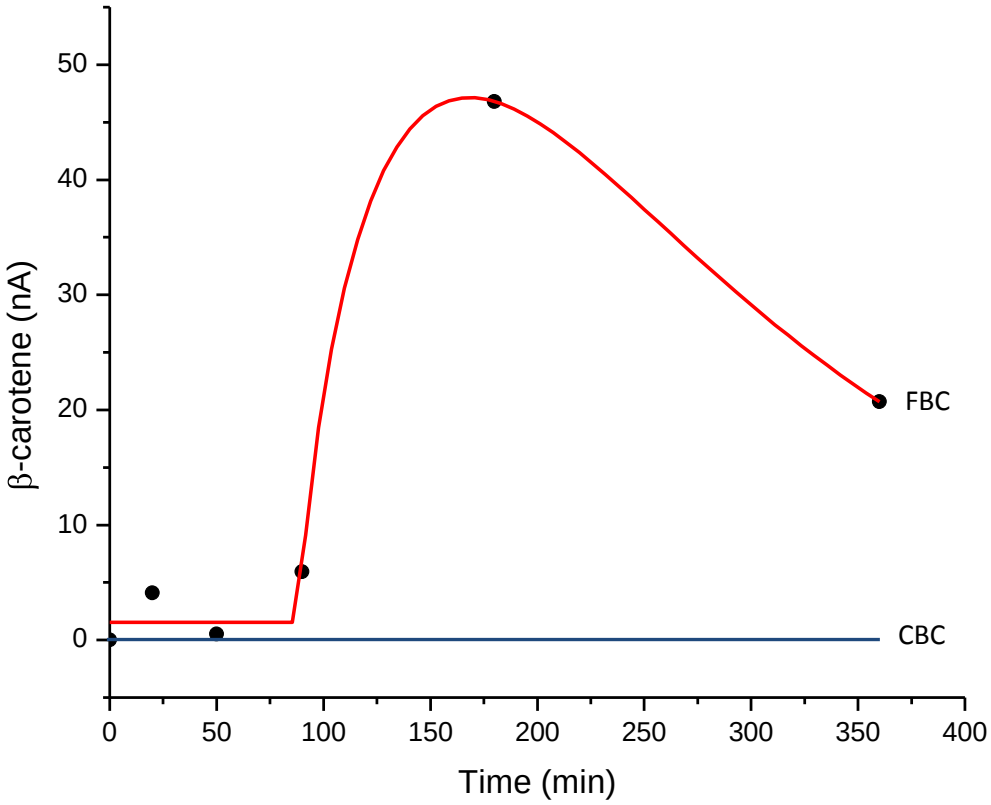

Figure 11. Response values of fecal samples at all time-dosage points in FBC and CBC groups at 12 min retention time.

The FBC samples contained beta-carotene in the feces, indicating passage through the body and lower absorption into the body. The lack of beta-carotene in the feces by the CBC group indicates either absorption into the body or slower passage through the body (longer than the maximum 360 min in this study). Since there were also no measured responses in the plasma samples for the CBC, there is a possibility that the bioactive components were absorbed and accumulated in other parts of the body (*e.g.* liver) before having a chance to reach the blood.

Huang *et al.* (2008) used the same concentration as was in our study (20 mg beta-carotene/kg BW) with corn oil as the delivery vehicle. Both lycopene and beta-carotene were effective as chemopreventative actions, showing that the bioactive components are indeed bioavailable to an extent at this concentration.

CHAPTER 5: CONCLUSIONS

There were a few important factors observed in the release study on green tea extracts in chewing gum. First off, the release results of EGCG, ECG, EGC, and EC were typical of other flavorants and ingredients often added to chewing gum base with C_{max} seen early in the mastication process and a slow drop off over the extended period of time (Fritz 2008). Total salivary concentration of EGCG was the highest among the catechins, while the highest total salivary concentration among treatments was seen in the complex mixture, but the quickest release was seen in the physical mixture, yet none of these calculations were statistically different.

It is still important to remember that all the ingredients found in chewing gum base play a role in the release of flavorants and bioactive components from the gum. Possible interactions between ingredients could not only alter the release rate but also the flavor profile, overall liking, and the continuity of the finished product.

In future work, any acceptable treatments should be subjected to sensory testing. Despite the desire for a complete release of polyphenols from the matrix, the quick release as in the physical mixture is not desired organoleptically. The quick release interferes with the flavor profile and may not provide a long-lasting flavor. In addition, the crumbliness of the cud in the mouth is not desirable in a gum, so alteration of the base to optimize the textural properties may change the release characteristics. For this reason, the complex treatment may be a good candidate for overall acceptability as well as adequate release of the bioactive components into the saliva.

Also, as was seen in one test subject of our study, not all consumers have the same reaction to the same chewing gum product. In the future, a larger sample size

would allow an accurate assessment of the overall acceptability and functionality of the gum product.

Most importantly, complexation of polyphenols into the starch inclusion complex should be further investigated. Methylation and esterification could be considered as possible methods for incorporation (Bhosale and Ziegler, unpublished).

In the inhibition study, there was no significant evidence for either enhancement or inhibition of the enzyme by tea polyphenols. Although other studies support the inhibitory properties of tea polyphenols and one illustrated a possible enhancement, our enzymatic digestion study did not support either side conclusively.

In the bioavailability study, the plasma samples of the CBC and FBC groups showed no beta-carotene peaks. However, fecal samples did show a difference, with the FBC group showing beta-carotene peaks at 20 to 360 min and the CBC samples not showing any peaks. Absorption into the body was higher in the CBC samples, indicating a higher possible bioavailability due to the presence of starch inclusion complexes.

In future work, the liver and other tissues should be measured for presence of beta-carotene. The overall testing period could be increased from 360 minutes to perhaps 720 min, in case the CBC group was retained longer in the digestive system.

5.1 Overall conclusions

Overall, the results of these studies support the need for further investigation of the use of chewing gum and starch inclusion complexes as a delivery vehicle of bioactive components. There is no evidence of interference by tea polyphenols on the breakdown of complexes by amylase and hence the release of the bioactive components into the

body. Although the physical mixture treatment of chewing gum reported the highest release (C_{max}), the complex treatment could be the most effective treatment in relation to both organoleptic properties as well as release of bioactive components.

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V I T A

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EDUCATION

2010 B.S., M.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

2010-present Associate Field Quality Specialist
Kraft Foods Global, Inc., East Hanover, NJ
Project: Maintenance of food quality standards in Snacks division

2007-2010 Research and Development Summer Intern
Kraft Foods Global, Inc., Tarrytown, NY
Projects: Crystal Light, Bull's-Eye, Oreo, and Ritz products

LABORATORY EXPERIENCE

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 avocadoes

ORGANIZATION INVOLVEMENT

2009-present Sigma Lambda Gamma National Sorority, Inc., Expansion Committee member
2007-2010 Schreyer Honors College Diversity Committee, Undergraduate Representative
2006-2010 Mary Beaver White Building, Fitness Center Desk Attendant

SELECTED AWARDS

2009-2010 National Starch Food Innovation Scholarship
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